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Abstracts
of the First FEPS Congress

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Preface

In this abstract book you will find the abstracts as submitted to the Organizing Committee of the First Congress of the Federation of European Physiological Societies (FEPS). No selection was made regarding the scientific quality of the abstracts. All abstracts have been reproduced without editorial modifications. Some abstracts were retyped for better reproduction.

The decision to accept the abstracts as submitted was made by the Organizing Committee and the Executive Committee of FEPS to fulfill one of the main aims of FEPS, that is, to stimulate the collaboration and exchange of ideas between physiologists in Europe. Therefore, we have also decided to publish the abstracts of our colleagues from Central and Eastern Europe, who were not able to attend the meeting for financial reasons.

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DISSOCIATION OF CARDIAC OXYGEN CONSUMPTION AND ADENOSINE PRODUCTION FROM CARDIAC ENERGY STATUS IN THE GUINEA PIG HEART. *U.K.M. Decking, G. Schlieper, S. Arens, and J. Schrader*

The cardiac energy status, in particular the concentration of free ADP or AMP, has been suggested to control both the rate of oxidative phosphorylation and adenosine production. We have employed ^{31}P NMR spectroscopy to test this hypothesis in the intact, saline perfused, working guinea pig heart. Cardiac output, coronary flow, external heart work, oxygen consumption (MVO_2) and adenosine release (AR) were continuously monitored together with the cardiac energy status (ATP, PCr, P_i, pH (NMR)); free ADP and AMP were subsequently calculated.

Under basal conditions (preload 1 kPa, afterload 11 kPa) external cardiac work was 10.6±2.9 mW, MVO_2 7.8±1.0 µmol*min^{-1} and AR 226±179 pmol*min^{-1}; free ADP was 40.8±11.5 µM and AMP 297± 189 nM (n=29). Decreasing arterial PO_2 by 50% reduced cardiac work (-50%) and MVO_2 (-24%) without any change in PCr/ATP ratio; a minor increase in AMP (+29%) was associated with a 3-fold augmentation of AR (n=5). When afterload was raised from 7 kPa to 15 kPa, MVO_2 increased (+45%) and AR decreased (-60%) despite no change in free AMP or ADP (n=6). Switching substrates from glucose+pyruvate to glucose only deminished external work (-43%) and MVO_2 (-19%) while doubling ADP. In spite of a 4-fold increase in AMP, adenosine release remained constant (n=4). There was no change in total adenosine production, as assessed by inhibition of AR kinase and AR deaminase (iodotubercidin/ EHNA), either (n=4). These results indicate that the cardiac energy status is not the prime regulator of oxidative phosphorylation or adenosine formation in the well oxygenated guinea pig heart.

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EFFECT OF SURGICAL CARDIAC DENERVATION ON TISSUE LEVELS OF CATECHOLAMINES, ß-ADRENOCEPTOR SUBTYPES AND ENZYMES INVOLVED IN CARBOHYDRATE AND FAT METABOLISM. G.J. van der Vusse*, M.L. Dubelaar, W.A. Coumans, M. Steinfath, A. Drake-Holland, M.I.M. Noble.

Cardiac denervation has shown to result in loss of efficiency of external myocardial work. Experiments were performed on adult Beagle dogs to elucidate the cause of loss of efficiency. Time interval between cardiac denervation and harvesting of biopsies of the heart amounted to 4-5 weeks. Data in table indicate that catecholamine stores are virtually depleted. The total number of ß-adrenoceptors remained unchanged. A shift from ß1 to ß2-adrenoceptor occurred, reflecting an adaptation towards the lack of cardiac noradrenaline. The maximal activity of enzymes involved in glucose conversion (hexokinase, phosphofructokinase, aldolase, pyruvate kinase) and fatty acid oxidation (carnitine acyltransferase I and II) remained unaffected. Glutamate oxidation rate in mitochondria isolated from denervated hearts did not change. Also values of markers for mitochondrial coupling (P/O and RCI) were not significantly different between control and denervated dogs. The present findings failed to provide an indication that loss of cardiac efficiency due to denervation is primarily caused by alterations in enzymatic activity and mitochondrial functioning.

	Sham			Denervated		
Catecholamines (pmol/mg protein)						
Noradrenaline	26.4	±	11.9	0.12	±	0.12
adrenaline	0.3	±	0.11	0.01	±	0.02
dopamine	2.25	±	1.09	0.15	±	0.03
ß-adrenoceptors (fmol/mg protein)	50.3	±	6.5	48.4	±	4.3
ß1 subtype (%)	80.6	±	3.0	70.2	±	6.9
ß2 subtype (%)	19.4	±	3.0	29.8	±	6.9

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THE MYOCARDIAL MITOCHONDRIAL AEROBIC CAPACITY IS PARTIALLY LIMITING FOR CARDIAC OXYGEN CONSUMPTION AND CONTRACTILITY. J.H.G.M. van Beek, J.B. Hak, M.H. van Wijhe, M.H.J. Eijgelshoven and N. Westerhof

The myocardial mitochondrial capacity for ATP synthesis was generally considered non-limiting at submaximal cardiac workloads. To challenge this idea we partially inhibited the mitochondrial enzyme directly responsible for ATP synthesis, F_0F_1-ATPase, by infusing varying amounts of oligomycin in 12 isolated rabbit hearts at 28 °C; 4 hearts formed the control group without oligomycin. Before and after infusion of oligomycin we determined the systolic pressure in a water-filled balloon in the left ventricle (LVSP), cardiac O_2 consumption and the response time of mitochondrial oxygen consumption to heart rate steps (t_{mito}; corrected for vascular transport and diffusion delay; Van Beek and Westerhof, *Am.J.Physiol.* 260:H613, 1991). After these physiological measurements at submaximal cardiac O_2 consumption, mitochondria were isolated and the inhibition by oligomycin of mitochondrial aerobic capacity (MAC) was determined from the maximal O_2 consumptions during stimulation with ADP and during uncoupling with carbonyl cyanide p-(tri-fluoromethoxy)phenylhydrazone. LVSP and cardiac O_2 consumption decreased linearly with the fractional inhibition of MAC, and were reduced significantly by, respectively, 2.4±0.5% (SE) and 2.6±0.7% per 10% reduction in MAC, independent of heart rate. For heart rate steps between 60 and 80 beats/min, t_{mito}, which was 7 s at baseline, was increased by 7.9±2.9% per 10% decrease in MAC. For heart rate steps between 60 and 120 beats/min t_{mito} was 11 s and did not change with MAC. We conclude that only at low heart rate the mitochondrial aerobic capacity partially limits the dynamic response of mitochondrial O_2 consumption. We further conclude that, even at submaximal cardiac workloads, about 25% of the limitation of ATP turnover and contractility in the rabbit heart is due to mitochondrial ATP synthesis, with the remainder probably largely attributable to the state of activation of the myofibrillar ATPase.

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CONTROL OF CARDIOMYOCYTE GLUCOSE TRANSPORT BY ALTERNATIVE ENERGY SUBSTRATES. Y.Fischer, U.Böttcher, M.Eblenkamp, J.Thomas, G.D.Holman‡, I.J.Kozka‡, and H. Kammermeier.

Metabolic fuels such as lactate or fatty-acids are known to counterregulate the utilization of glucose in heart and skeletal muscles, thus having a glucose-sparing action. The basic mechanisms of this inhibition have been clarified at the level of glycolysis and pyruvate oxidation ('Randle-cycle'). We have now detected and explored inhibiting effects of substrates on glucose transport, another important rate-limiting step in the utilization of the sugar, in isolated cardiomyocytes from adult rats. In these cells, various energy substrates such as pyruvate (0.4-3 mM), lactate (0.4-3 mM), β-hydroxybutyrate (0.25-1 mM), propionate (1-4 mM), acetate (0.5-3 mM), or α-ketoglutarate (3-10 mM) reduced the basal, phenylephrine- or insulin-stimulated rate of glucose transport by 20% to 60%. The middle- and long-chain fatty-acids (octanoate, palmitate) were not or poorly effective. This inhibition was rapid (being maximal after 7 min). No correlation was found between changes in glucose transport, on the one hand, and the intracellular level of glucose-6-phosphate, on the other hand, in the presence of substrates. In contrast, inhibition of glucose transport significantly correlated with the anaplerotic action of substrates, as evidenced by changes in the intracellular malate concentration. Aminooxyacetate or cycloserin, inhibitors of aminotransferase reactions (which are coupled to the metabolism of malate) stimulated glucose transport and counteracted its inhibition by pyruvate. As previously described, alanine (the 'aminated' form of pyruvate), as well as valine were also stimulatory. This stimulation was paralleled by an decrease in the malate concentration. Finally, pyruvate reduced the phenylephrine- or the insulin-dependent increase in the number of glucose transporters (GLUT1, GLUT4) at the cell surface, as assessed by using the specific photolabel 2-*N*-[4(1-azi-2,2,2-trifluoroethyl)benzoyl]-1,3-bis-(D-mannos-4-yloxy)propyl-2-amine.

In conclusion, this is the first report on the metabolic control of myocyte glucose utilization at the level of glucose transport. This control probably involves one or several intermediary metabolites participating in reactions linked to malate formation (and degradation). The regulating mechanism affects the number of glucose transporters at the cell surface (supported by the Deutsche Forschungsgemeinschaft).

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VASODILATATION AFFECTS CORONARY VENOUS RESISTANCE AS WELL. I. Vergroesen, J.W.G.E. VanTeeffelen and J.A.E. Spaan.

The effect of physiological and pharmacological vasodilatation on the coronary venous resistance was compared to the vasodilatating effects on the coronary arterial resistance.

In 12 anaesthetized dogs the left main coronary artery was cannulated and perfused under controlled pressure. A small coronary vein was cannulated retrogrately to measure coronary venous pressure (Pv) Coronary perfusion pressure (Pp), left ventricular pressure (Plv), and coronary blood flow (CBF) were measured as well. Venous pressure after 15 s coronary occlusion ($P_{f=0}$) was used as back pressure of the venous system. Coronary venous resistance (Rv) was calculated by (Pv-$P_{f=0}$)/CBF. Coronary arterial resistance (Ra) was calculated by (Pp-Pv)/CBF. The protocol consisted of a control measurement followed by several periods of reactive hyperemia (RH), induced by a coronary occlusion of 15 s. Pharmacological vasodilatation was induced by infusion of adenosine directly into the coronary vascular bed in a supramaximal dose. Significance was tested using paired t-test and significant results are presented as mean ± SEM.

During RH Rv dropped to 7.0 ± 1.1 mmHg.s/ml compared to control 13.1 ± 1.8 and Ra changed from 93.3 ± 9.9 at control to 18.8 ± 1.2 at RH. Vasodilatation with adenosine decreased Ra to 17.1 ± 1.3, while Rv dropped to 5.6 ± 1.2 in comparison to control. During control 14% ± 1% of total coronary resistance is located in the coronary veins, during RH the contribution of the venous resistance doubles to 28% ± 2% and after maximal vasodilatation with adenosine 36% ± 4% is venous resistance. Plv was not significantly changed by RH and dropped to 90% of control during adenosine infusion.

It is concluded that vasodilatation affects coronary venous resistance significantly, which implies that regulation of coronary flow is for some extent located in the venous vessels.

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CORONARY VASOREACTIVITY IN THE DOG.

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Blood flow in the critically stenosed coronary artery with endothelial damage declines with accumulating platelet thrombosis. After embolisation of the thrombus, flow is restored, but falls again cyclically. Distal coronary pressure behaves similarly but on a different time scale. In the open-chest pentobarbitone anesthetized dog, after embolization, flow increased more rapidly than pressure distal to the stenosis (reactive vasodilatation) in a first period of low distal resistance. In the next period, distal pressure rose while flow fell, (increasing distal pressure/flow [DP/F]). In the third period, flow and distal pressure declined together (constant DP/F), due to thrombus growth. We observed transient complete occlusions of the critically stenosed artery, with (n=6) and without (n=4) thrombus growth. Stenosis resistance was equal in both groups before and after the second period of 65±36s (mean±SD).

Distal coronary DP/F was lower without thrombosis ($p<0.005$), but rose similarly ($p<0.05$) during the second period, albeit to a lower level ($p<0.025$).

It is concluded that a condition analagous to reactive hyperæmia persists with a critical proximal coronary stenosis. At any given time after release of occlusion, DP/F was higher in the presence than in the absence of thrombus, suggesting thrombus induced distal vasoconstriction.

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EVIDENCE FOR TONE-DEPENDENT CLOSURE OF CORONARY RESISTANCE VESSELS AT ZERO FLOW. D.Merkus,

J.W.G.E.VanTeeffelen, J.Dankelman, I.Vergroesen, J.A.E.Spaan

We hypothesized that coronary arterioles can close at zero flow and that the pressure at which this closure occurs depends on vascular tone. In order to test this hypothesis we instantly ceased the perfusion of the heart. Without closure, coronary arterial pressure will decay until it equals venous pressure; with closure, coronary arterial pressure will fall until it equals the arteriolar closing pressure. However, the occlusion induces metabolic vasodilatation making an arteriolar closing pressure undetectable. Administration of glibenclamide decelerates the rate of dilatation making closing pressure measurable.

The experiments were performed in sixteen open-chest anaesthetised goats. The left main coronary artery was cannulated and perfused via an extracorporeal perfusion circuit with controlled pressure. Perfusion pressure (Pp), left ventricular pressure (Plv), venous pressure (Pv), arterial inflow (Qa) and venous outflow (Qv, in 10 goats) were measured and averaged per beat. The perfusion line was occluded several times for 15s. Pp before (0s) and 2, 5, 8, 11 and 14 s after occlusion in control and after administration of glibenclamide were compared (paired t-test).

Pp in control was significantly lower compared to Pp with glibenclamide after 8s (16.2±1.8 vs. 19.5±1.6 mmHg), 11s (13.7±1.5 vs. 17.6±1.5) and 14s (12.3±1.7 vs. 16.7±1.5) of occlusion. Furthermore, at the end of the occlusions, Pp was 10.0±1.7 mmHg(control) and 14.9±1.5 mmHg (glibenclamide) higher than Pv and Qv was zero.

We conclude that in the absence of flow, arterioles with tone can close and that the pressure at which they close is tone-dependent.

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NONINVASIVE APPROACH OF CORONARY HEMODYNAMICS IN MAN : DOPPLER ULTRASOUND OF INTERNAL MAMMARY ARTERY GRAFT DURING ISOMETRIC EXERCICE.

A. W. Kedra, C. Eiferman, I. Azancot, C. Kucharski, S. Dunica, C. Bazzi-Grossin, P. Bonnin, E. Savin, O. Bailliart, P. Beaufils, J.P. Martineaud

OBJECTIVES: 1° to study the usefulness of duplex Doppler echography (DDE) of internal mammary artery (IMA) graft for evaluation of coronary hemodynamics (CH), 2° to analyse CH variations during isometric exercice of upper extremity (handgrip - HG). METHOD: DDE of IMA from the supraclavicular fossa was performed in 14 subjects who underwent coronary artery bypass surgery using IMA to the left anterior descendig coronary artery (LAD). No patient had myocardial infarction or stenosis of IMA or LAD below the anastomosis. Heart rate (HR), arterial blood pressure (BP) and proximal IMA velocity patterns were recorded at rest and at the end of 3 min. HG at 30-40% of maximal force. Peak velocities: maximal systolic (MSPV), maximal diastolic (MDPV), average systolic (ASPV), average diastolic (ADPV), and velocity integrals: systolic (SVI), diastolic (DVI) and total (TVI) were measured. RESULTS: While mean BP increased significantly during HG (mean +/- SD was 90.4 +/- 12.4 to 106.6 +/- 11.1 mmHg; p< .001) there was no change of HR (65.5 +/- 18.8 to 69.1 +/- 19.4 beats/min; NS), MSPV (73.8 +/- 23.3 to 69.6 +/- 20.1 cm/s; NS), MDPV (50.3 +/- 16.3 to 55.4 +/- 22.6 cm/s; NS), ASPV (36.2 +/-14.3 to 28.7 +/- 6.1 cm/s; NS), ADPV (21.9 +/- 12.2 to 20.1 +/- 8.3 cm/s; NS), SVI (11.9 +/- 5.3 to 12.1 +/- 4.5; NS), DVI (20.6 +/- 8.8 to 18.4 +/- 5.5; NS) or TVI (32.5 +/- 13.4 to 30.5 +/- 9.5; NS). CONCLUSIONS: 1° Duplex Doppler echography of internal mammary artery graft from the supraclavicular fossa is an interesting method for noninvasive approach of coronary hemodynamics. 2° Isometric exercice increases mean arterial blood pressure but does not modify the coronary flow velocities.

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ACETYLCHOLINE INDUCES VASOCONSTRICTION IN PERFUSED RAT HEART WITH INTACT ENDOTHELIUM. L. Dézsi, I. Schiszler and M. Szekeres

According to Furchgott endothelium plays an obligatory role in acetylcholine (ACh) induced vasodilatation and damage of the endothelium, due to a decreased nitric oxide (NO) production, causes paradoxical vasoconstriction. This finding can be observed in various vascular beds, but rat coronaries behave differently. The aim of the present study was to compare the effects of endothelium independent and dependent agents on the coronary flow (CF) in rat Langendorff hearts, and to determine the mechanism behind the paradoxic ACh-induced constriction. Hearts were perfused with constant pressure (80 mmHg) and the CF was recorded continuously. Infusions of adenosine (ADO, 2.5X10-5 M) and sodium niroprusside (SNP, 10-5 M) as well as bradykinin (BK, 10-6 M) and ACh (5.5X10-7 M) were given into the coronary perfusion line (n=18). Endothelial NO production was blocked using NG-nitro-L-arginine (LNA, 10-4 M) perfusion. Baseline CF was 10.5 ± 1 ml/min. ADO increased CF in all cases (by 30 % in av.). A similar dilatation (by 21 %) was observed during SNP infusion. BK induced effects were not uniform: In one group BK increased CF (by 22 %), in the other group BK decreased CF (by 35 %). However, ACh without exception caused strong vasoconstriction (45 % of CF reduction in av.) in the perfused rat hearts. Blockade of NO production by LNA (10-4 M) significantly reduced basal CF (by 45 %). This blockade of NO-mediated vasodilatation could not be considered as a selective treatment, because it also suppressed ADO and SNP-induced dilatations partially. These data clearly demonstrate the presence of paradoxic vasoconstriction by ACh in the rat coronaries with intact endothelium. The mechanism of this seems complex, and requires further elucidation. We suppose, that in addition to NO-mediated and direct smooth muscle effects of ACh, other constrictor factors (eg. constrictor prostaglandins, superoxide anion) may be involved in it.

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PRESSURE DEPENDENCY OF RESISTANCE DISTRIBUTION IN THE VASODILATED CORONARY BED. J.A.E.Spaan, J.M.Cornelissen, C. Chan, J. Wentzel, F.C.P. Yin.

The distribution of coronary resistance, R, and compliance, C, in the coronary circulation determines the dynamic and static response of coronary arterial flow, Qa, to pressure, Pp. Because microvascular volume, MV, can not be measured dynamically the role of microvascular C remained unclear. An isolated septum preparation was used in which a septal artery was perfused with perfluor carbon using a pressure controlled system. MV variations were indexed by tissue thickness, measured by sonomicrometry. This method was validated using "iodine (added to the perfusate) thickness" as measured by digital subtraction angiography. Linear fits were found with correlation coefficients over .98. Pressure was varied sinusoidally with an amplitude of 7.5 around a mean of 30, 50 and 70 mmHg and frequency F ranging from 0.03 to 10 Hz. Modules, M, and phase, φ, of the transfer between Qa and Pp (adm.) and thickness (vol.) and P were compared with those of linear and non-linear 2 compartmental models.

Low frequency M of adm. (F <.5Hz) was lower than steady state Qa/P, only explainable by volume dependency of R's. F-φ relations peaked at F=1Hz with a Pp dependent max. (10^0, 20^0, 40^0 at P=70, 50, 30 mmHg) only explainable by increasing relative proximal resistance with Pp. However, F-M relations demonstrate a decrease in absolute R. M of Vol. decreased from F= 0.1 Hz well explained by a distal C 5 times higher than proximal C.

This study demonstrates that distribution of absolute values of resistance is pressure dependent and can not be determined by linear signal analysis not withstanding the absence of higher harmonics in responses.

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RELAXATION OF UPPER AIRWAY MUSCLES REDUCES THE MAGNITUDE OF THE REFLEX RESPONSE TO NEGATIVE LARYNGEAL PRESSURE - MECHANISM LEADING TO UPPER AIRWAY OBSTRUCTION. **W. A. Janczewski, P. H. Janczewski**

Respiratory efforts following obstruction of the upper airway (UA) may considerably (up to -120 cmH_2O) decrease laryngeal pressure. Negative *(i.e. subatmospheric)* pressure (NP) in the UA markedly increases activity of the UA dilating muscles but not the diaphragm. Such a reflex response should restore the balance between the suction pressure generated by contraction of the diaphragm and the tension developed by UA muscles opposing this pressure. Therefore, we address the question, why this potent reflex fails to prevent UA obstruction during sleep in some human subjects. **We hypothesized** that the loss of skeletal muscle tone, always occurring during sleep, suppresses the reflex response to laryngeal NP. **Methods:** we recorded activities of the hypoglossal (n.XII), facial (n.VII) and phrenic (n.Ph) nerves together with electromyogram (EMG) of the genioglossus muscle in 12 decerebrate rabbits. Muscle tension was reduced or eliminated several times during each experiment by means of a short acting neuromuscular blocking agent (vecuronium bromide – 0.03 mg/kg i.v.). Animals were mechanically ventilated via tracheostomy tube inserted below the larynx. Larynx was converted into a closed system. This enabled to apply selectively to larynx either atmospheric or negative pressure ranging from -30cmH_2O to -50cmH_2O.
Results: muscle relaxation eliminated EMG of genioglossus muscle and significantly (17%) reduced activity of n.XII. The amplitude of n.VII was reduced less (7%) and n.Ph remained unchanged. Before muscle relaxation, immediately after application of NP, n.XII activity increased to 197% and n.VII to 137% of their control values. After muscle relaxation these figures were reduced to 146% and 117%, which was significantly less. **We conclude** that muscle relaxation selectively decreases respiratory activity of the UA muscles and compromises reflex response to NP in the larynx. This findings indicate that the loss of skeletal muscle tone during sleep impairs mechanisms preserving UA patency. This negative effect may be enhanced by ethanol, benzodiazepines, barbiturates and other drugs diminishing muscle tone.

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MECHANISM UNDERLYING THE LIMITED GENIOGLOSSUS RESPONSE TO UPPER AIRWAY NEGATIVE PRESSURE IN NEWBORN LAMBS S. Duara, A. Bonnet, K. Mizuno, J. Lin, R. Everett, N. Claure. Dept. of Pediatrics, University of Miami School of Medicine, Miami, FL.

The neonatal genioglossus (GG) muscle was recently recognized to be relatively refractory to moderate amounts of upper airway negative pressure (NP), at levels previously reported to be effective in adult subjects (JAP 77:37-42, 1994). Underlying mechanisms were evaluated in the isolated upper airways (IUA) of 7 chronically prepared awake lambs (mean±SD weight 5.0±1.3kg, age 11±3 days). End-expiratory NP (-5, -10, -20 and -40 cm H_2O) was randomly applied to the IUA (3 breaths, or 3 sec if apnea was induced), and repeated after intrathoracic slow adapting receptor (SAR) blockade with sulphur dioxide gas (SO_2, 300ppm) administered through a tracheal stoma. The moving time average (MTA) of phasic GG activity was quantified as average activity (AvAc, area MTA/burst duration) and burst duration (T_{Ac}). Results obtained during baseline (BL) and NP (first GG burst) were ($\bar{x}$ values):

		NP (cm H_2O)							
		RA				SO_2			
		-5	-10	-20	-40	-5	-10	-20	-40
AvAc au/ms	BL	0.2	0.3	0.2	0.4	0.3	0.2	0.3	0.4
	NP	0.2	0.4	0.8	1.4	0.4	0.5	1.0	1.9
T_{Ac} ms	BL	632	785	760	830	732	760	788	766
	NP	896	1219	2073	2700	955	1329	2358	3151

There was a highly significant linear relationship between the percent increase above baseline in AvAc and T_{Ac} and the magnitude of NP, independent of an effect from SO_2 ($p<0.001$). We conclude that a high NP threshold, rather than reflex vagal inhibition through SAR activation, underlies the poor EMG response of the neonatal GG to moderate amounts of NP in the UA.

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CARBON MONOXIDE TRANSFER DURING MECHANICAL VENTILATION IN PIGS. *F.C.A.M. te Nijenhuis, J.R.C. Jansen, and A. Versprille.*

Objective of study. We evaluated the transfer of carbon monoxide between alveolar gas and capillary blood (DL_{CO}) during mechanical ventilation at different alveolar volumes (V_A).

Method. In 14 anaesthetized, paralysed and mechanically ventilated pigs, we used an Inspiratory Pause (IP) procedure, i.e. a simulated single-breath technique, to estimate DL_{CO}. The IP procedure consisted of an inspiratory pause in between an inflation and an expiration both at a constant flow rate. The test gas contained 0.3% $C^{18}O$, 5 or 10% He and balance air. In a first series of 5 pigs, V_A was varied by increasing positive end-expiratory pressure (PEEP) from 2 to 10 cmH_2O. Inflation volume was 25 $ml.kg^{-1}$ and IP time ranged from 1 to 8 s. In a second series of 9 pigs, DL_{CO} was estimated at four different V_A's by inflating 10, 15, 20, and 25 $ml.kg^{-1}$ at 2 cmH_2O PEEP using an IP of 7.2 s. The expired fractional $C^{18}O$ and He concentrations were measured with use of a mass spectrometer. These concentrations in the expired gas volume, after discarding the dead space wash out volume, were averaged. In our calculations of DL_{CO}, an effective diffusion time and an effective alveolar volume of which $C^{18}O$ was taken up was used [1]. Corrections were made for CO back pressure.

Results. In the first series, an exponential decay of $C^{18}O$ with effective diffusion time was found at both PEEP levels. In both series, DL_{CO}/V_A decreased and DL_{CO} did not change with increasing V_A.

Conclusions. The IP procedure can be used as a simple and well standardized technique to measure DL_{CO} in mechanically ventilated subjects. Increasing V_A decreases DL_{CO}/V_A, whereas DL_{CO} remains constant, which we ascribe to a decrease in alveolar blood volume, if alveolar membrane diffusing capacity is assumed to increase [2].

[1] DJ Cotton et al. J. Appl. Physiol. 1979;46:1149-56
[2] H Stam et al. Bull. europ. Physiopath. resp. 1983;19:17-22

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Haemodynamic conditions during alternating and synchronous ventilation of both lungs. *A. Versprille, M. van Oosterhout and J.R.C. Jansen.*

Objective. We tested the hypothesis that during alternating ventilation (AV) (i.e. mechanical ventilation of each lung with a phase difference of half a ventilatory cycle) inflation of one lung causes compression of the other lung, leading to a lower mean lung volume during AV compared to synchronous ventilation (SV) of both lungs. As a consequence intrathoracic and central venous pressure will be lower and cardiac output higher.

Methods. Separate ventilation of the lungs was obtained by airtight fixation of an endo-bronchial tube in the left main bronchus via a trachea cannula. In eight anaesthetized piglets SV and AV were alternately applied for respectively four and three periods of 10 minutes. Minute ventilation at a rate of 10 bpm was the same during AV and SV and adapted to normocapnia. Two series of observations were performed. The first series (n=8) with normal intact thorax and monitoring of oesophageal pressure, the second series (n=6) after perforation of the sternum and insertion of a pericardial catheter, followed by airtight closure and evacuation of air.

Results. In both series mean lung volume (280 $\pm$ 71 ml, during SV) was about 15-20 % lower and central venous (4.5 $\pm$ 1.3 mmHg, SV), oesophageal (1.0 $\pm$ 1.4 mmHg, SV) and pericardial pressure (1.5 $\pm$ 1.1 mmHg, SV) were about 0.5-0.7 mmHg lower during AV (all $p < 0.001$). In series 1 aortic pressure (95 $\pm$ 7 mmHg, SV) was about 5 mm Hg and cardiac output (1.89 $\pm$ 0.32 $ml/s^{-1}kg^{-1}$], SV) about 8 % higher during AV (both $p < 0.001$). In series 2 cardiac output was 5 % higher during AV ($p < 0.001$), but aortic pressure did not change ($p = 0.07$).

Conclusion. Our data verified the hypothesis. The lower oesophageal, pericardial and central venous pressure during AV compared to SV could be explained by the lower mean lung volume. We attributed the lower mean lung volume during AV to an expansion of the inflated lung at the expense of the volume of the opposite lung during its expiration.

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RELATIONSHIP BETWEEN MINUTE VENTILATION, CO_2 RELEASE AND RECIRCULATION TIME AT CONSTANT EXERCISE
C.P.M. van der Grinten, A.J.B. Wanders and S.C.M. Luijendijk.

During incremental exercise (IE) minute ventilation (MV) is well correlated to CO_2 release ($\dot{V}CO_2$). Removing a dead space (DS) also decreases $\dot{V}CO_2$, when excess CO_2 is washed out from all body stores. In the present study we analyzed responses of MV to changes in mid-tidal PCO_2 at intervals determined by the recirculation time (t_{RC}). Changes in $\dot{V}CO_2$ were brought about by removing a DS of 1 l and by IE tests on a bicycle ergometer (0,50,100,150,200W). All tests were done during hyperoxia (F_I-O_2=40%) to reduce contributions from carotid bodies. On day 1 t_{RC} of 2 female and 7 male subjects were determined at 4 levels of exercise (0,50,100,150 W) using the washout of a bolus of halothane added during one inspiration. On day 2 IE test was done. On day 3 responses of MV and $\dot{V}CO_2$ to removing DS were determined at the same levels of exercise used for t_{RC}. Latter experiments were repeated using an additional inspiratory CO_2 load ($\approx$2%) to block contributions from lung receptors.

The results showed that removing DS caused an almost perfect step in mid-tidal PCO_2 of on average 1.1 ($\pm$0.36) kPa. During IE test correlation between MV and $\dot{V}CO_2$ was high (r=0.97-0.99) with a slope of 23.8 ($\pm$3.4). During removal of DS correlations were also high (r=0.80-0.98) but slopes were much steeper (30.0$\pm$4.0 and 45.7$\pm$7.6 for DS and DS+CO_2, respectively). The table shows that removing DS decreased MV significantly by 28% at $0.5t_{RC}<t<t_{RC}$. ΔMV is expressed as the decrease in MV for that interval of t relative to the overall change in MV 3 min after the step change.

	$0.25t_{RC}$	$0.75t_{RC}$	$1.25t_{RC}$	$1.75t_{RC}$	
ΔMV	0.15	0.28*	0.43*	0.53*	*P<0.05

The larger CO_2 sensitivity for DS than for IE may be attributed to the contribution of arterial chemoreceptors. However, the large and rapid decrease in MV is inconsistent with the known properties of these receptors in hyperoxia.

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PULMONARY FUNCTION AND RESPIRATORY SYMPTOMS IN A POPULATION OF BLACK SOUTHERN AFRICAN ADULTS.
M.H. Shamssain.

We studied 1566 black southern Africans from the Transkei aged 20-60 years living in modern 2-3 bedroomed houses. They spent part of their life in ill-ventilated traditional huts where the level of indoor pollution is high. Pollution in these huts is due to utilising firewood for heating and cooking and also to grinding maize. Subjects were interviewed using the MRC respiratory questionnaire and they underwent ventilatory function (compact Vitalograph) in order to assess the prevalence of respiratory symptoms and ventilatory function. Compared with normal ranges in Western countries, ventilatory function in Southern Africans were lower. Very few Transkeian women smoke. There were no significant demographic differences between smokers and non-smokers. The prevalence of respiratory symptoms (morning cough, breathlessness and wheeze) were higher than those reported in Europeans for both smokers and non-smokers. Non-smoking females had a significantly higher incidence of breathlessness compared to non-smoking males. Morning cough was highest in non-smoking males. Smokers had poorer ventilatory function than non-smokers. This study suggests a high prevalence of respiratory symptoms in this population.

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RESPIRATORY RESPONSES TO CHEMICAL ACTIVATION OF BÖTZINGER COMPLEX NEURONS IN THE RABBIT. F. Bongianni, D. Mutolo and T. Pantaleo.

Expiration-related (ER) neurons located at the rostral pole of the ventral respiratory group (VRG), i.e. the so-called Bötzinger complex (Böt. c.), are a source of postsynaptic inhibition for both inspiration-related medullary neurons and phrenic motoneurons in the cat. These neurons also send axonal projections to the ER bulbospinal neurons of the caudal VRG (cVRG). Excitatory as well as inhibitory functions of these projections have been reported. Recently, we have shown in the cat that chemical activation of Böt. c. neurons exerts strong depressant effects on the inspiratory motor output as well as excitatory effects on the activity of both ER neurons of the cVRG and abdominal motoneurons. Little information is available on the role of Böt. c. neurons in the rabbit. We investigated the respiratory role of Böt. c. neurons in α-chloralose-urethane anesthetized rabbits, either spontaneously breathing or vagotomized, paralysed and artificially ventilated, by means of microinjections of 160 mM DL-homocysteic acid which excites cell bodies, but not axons of passage. Microinjections (5-30 nl) were performed through glass micropipettes. Tungsten microelectrodes were employed for extracellular recordings of neuronal activity. Unilateral microinjections into the Böt. c. caused mild depressant effects on inspiratory activity as revealed by decreases in peak amplitude and frequency of integrated phrenic nerve activity. Stronger depressant effects up to complete apnea were seen on few occasions, and more consistently in response to bilateral microinjections. Concomitant depressant effects on the activity of both expiratory motoneurons and ER neurons of the cVRG were observed. The results indicate that Böt. c. neurons exert inhibitory influences on inspiratory activity, although less pronounced than in the cat. Further, unlike in the cat, these neurons appear to be an important source of inhibition for ER bulbospinal neurons and, hence, for abdominal motoneurons.

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RHYTHM GENERATION AND CALCIUM OSCILLATIONS IN ORGANOTYPIC MEDULLARY CULTURES

D.Bingmann[1], R.E. Baker[2], D. Jones[3], D. Ballantyne[4]

In organotypic medullary cultures derived from 300 µm thick slices of the obex level of 5 days old rats many neurons at the side of the nucleus ambiguus discharge rhythmically (5-60/min) and are excited by CO_2 as would be expected from respiratory neurons. The present studies aimed to analyze the mechanisms underlying the generation of this rhythm They revealed: (1) An excitatory drive was a prerequisite for rhythmic discharges as TTX (0.2 µM), CNQX (50 µM) or APV (50 µM) blocked periodic biolectric activity. A blockade by CNQX could be delayed by adding nicotine (50µM) to the bath saline. (2) Exposure to the inorganic and organic calcium antagonists cobalt (2 mM), magnesium (6 mM) verapamil (50-100 µM) and flunarizine (50-100 µM), suppressed periodic discharges and reduced the excitatory action of CO_2. (3) Thapsigargine (2 µM) which blocks pumping of calcium ions into intracellular stores and dantrolene (10 µM) which inhibits intracellular calcium induced calcium release weakened and eventually abolished the periodic activity.(4) After loading the medullary tissue with FURA-2AM imaging technology showed large oscillations of the intracellular calcium concentrations in many cells. The mean frequency of these oscillations was about 30/ min and thus was in the range of the periodicity of the bioelectric events. The higher the calcium concentrations the higher were the amplitudes of the calcium oscillations. The calcium signals in neighbouring cells often seemed to occur in a constant phase relation. After exposure to cobalt the calcium concentrations declined and the oscillations were suppressed.

The effects of drugs affecting transmembrane calcium fluxes, intracellular calcium release and extrusion on bioelectric activity indicate that calcium is involved in the generation of rhythmic discharges in these neurons.

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Role of Glutamate in the K⁺ channel blocker mediated ventilatory response to hypoxia. C. Suguihara, J. Huang, X. Yu, C. Devia, D. Hehre and E. Bancalari. Dept. of Pediatrics, University of Miami School of Medicine, Miami, FL.

We have previously observed that the depressed neonatal ventilatory response to hypoxia was reversed by K^+ channel blockade (Pediatr Res 37:342A, 1995). In order to determine whether the K^+ channel blockade mediates the ventilatory response to hypoxia by increasing CNS glutamate (Glu) levels, 8 sedated newborn piglets <7 days of age were studied before and 40 min after the administration of a K^+ channel blocker, 4-aminopyridine (4-AP; 0.50 mg/kg, IV). 4 animals were also studied before and after saline infusion. CSF Glu levels were obtained from cisterna magna sampling and analyzed using HPLC with an electrochemical detector. Glu levels, minute ventilation ($\dot{V}_E$), arterial blood pressure (ABP), heart rate (HR) and blood gases were obtained during room air (RA) and after 10 min of hypoxia (FiO_2=0.10).

	$\dot{V}_E$ (ml/kg/min)		Glu (µM)	
	RA	10%O_2	RA	10%O_2
Pre- 4-AP	359±24	362±26	8.1±2.3	8.0±2.0
Post-4-AP	319±22	451±51*+	19.1±6.2	35.7±9.1*+

x̄±SEM; *p<0.05(Pre vs Post); +p<0.05(RA vs 10%O_2)

A significant increase in $\dot{V}_E$ and Glu levels with hypoxia was observed after the 4-AP infusion, while $\dot{V}_E$ and Glu levels during hypoxia were similar to RA in the pre-4-AP state. Changes in ABP, HR, pH, PaO_2 and BE were similar pre- and post-4-AP infusion. However, a significant decrease in $PaCO_2$ ($p<0.02$) with hypoxia was observed after 4-AP administration. Changes in cardiorespiratory and Glu values with hypoxia were similar pre- and post-saline infusion. These data demonstrate that the increase in the ventilatory response to hypoxia induced by the K^+ channel blockade is in part related to the elevated levels of Glu in the CSF.

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RESPIRATORY RESPONSES TO GLUTAMATE INJECTIONS IN THE PONTINE A5 REGION OF THE RAT. J.P. Lara, M.S. Dawid-Milner, K.M. Spyer and S. González-Barón.

The pontine A5 region, containing mainly catecholaminergic neurones, is known to be involved in regulation of the cardiovascular system and modulation of sympathetic activity. Little is known of its possible role in respiratory control. We therefore used microinjections of glutamate to investigate the effects of activating the A5 region on respiratory rhythm. Experiments were carried out in twenty-three spontaneously breathing anaesthetized rats (Pentobarbitone, 60 mg kg^{-1}, i.p., initial dose). Multibarreled electrodes were positioned stereotaxically in the A5 region to inject glutamate (10-30 nl, 100 mM, pH 7.4 ± 0.1) and to mark the sites of the stimulation. Control saline injections were also made. Airflow, respiratory volume, pleural pressure, phrenic nerve activity and arterial pressure were recorded. Glutamate activation of cell bodies located in the A5 region produced an expiratory facilitatory response. This consisted of a decrease in respiratory rate ($p<0.01$), due to an increase of expiratory time ($p<0.01$) as measured by observing phrenic nerve activity. Postinspiratory activity was also observed occasionally (eight animals). No changes were observed in inspiratory time. In most cases, increases in both blood pressure and heart rate were observed also. The expiratory facilitatory response was independent of the concomitant cardiovascular changes since it persisted after guanethidine (10 mg Kg^{-1}) that abolished the changes in blood pressure and heart rate. Our results show that activation of A5 neurones modulates respiratory activity, facilitating expiratory mechanisms as well as postinspiratory activation. The pneumotaxic role mainly attributed to the rostral pons, particularly involving the parabrachial complex, can thus be extended to other pontine areas, including the A5 region.

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CONTROL OF INTERSTITIAL FLUID VOLUME. G. Miserocchi.

The overall hydration state of the body is fairly constant, however within organs and tissues it may change according to functional conditions. The need to control interstitial volume varies considerably in the tissues. For example interstitial volume is not tightly controlled in the muscle, while for other interstitial spaces like the lung parenchyma and the pleural space, it is kept at its minimum value. Interstitial fluid volume depends upon several factors that include the transcapillary water and solute transport, the physicochemical and mechanical properties of the interstitial space and the efficiency of the lymphatic drainage. This rather complex interaction depends upon the pressure gradients causing fluid and solute transport, the water and solute permeability coefficients, the mechanical arrangement of the macromolecules of the interstitial matrix and, finally, the conductance and efficiency of the lymphatic removal. Interestingly, when a condition of relative dehydration exists, this may imply either a relatively high (like the lung interstitium) or low protein concentration (like for pleural fluid). Autoregulation of interstitial fluid volume is operated by readjustment of the transcapillary Starling forces and increase in lymph flow rate. The buffering action of Starling forces to prevent edema formation is favoured by low interstitial tissue compliance. Lymphatics operate as a passive negative feedback control tending to offset the perturbation induced on the set point of the controlled variable (either interstitial volume or pressure). When a tight control on interstitial volume is required, relatively wide variation in transcapillary water fluxes or protein concentration of the filtrate result in minor deviation of the controlled variable relative to the steady-state physiological condition.

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INTERSTITIAL FLUID PRESSURE.

R.K. Reed.

Interstitial fluid pressure (P_{if}) is the pressure measured at the end of a fluid filled column inserted into a tissue. P_{if} is an important determinant of interstitial fluid volume since it participates in control transcapillary fluid filtration and serves as filling pressure for the initial lymphatics. P_{if} is normally slightly subatmospheric (0 to -1 mmHg) in peripheral tissues like skin and skeletal muscle. Interstitial fluid pressure and volume are linked together in the term compliance which is defined as the ratio between a change in interstitial fluid volume divided by the corresponding change in P_{if} ($\Delta IFV/\Delta P_{if}$). Compliance in loose connective tissues is normally 10-15% per mmHg and this implies that when capillary fluid filtration increases an increase in P_{if} and a reduction of interstitial fluid colloid osmotic pressure (through dilution of interstitial proteins) are of equal importance in maintaining a constant interstitial fluid volume. In contrast to this normal role of P_{if} in maintaining constant IFV, we have recently observed that under several inflammatory reactions in skin and trachea an increased negativity of P_{if} may enhance, rather than limit the transcapillary fluid filtration. Thus, in burn injuries in skin P_{if} has been observed to fall to -120 mmHg. Furthermore, P_{if} falls to between -5 and -10 mmHg within 10 minutes in skin and trachea in anaphylaxis, following mast cell degranulation and in neurogenic inflammation. Similarly, blockade of the fibroblast adhesion receptors towards matrix molecules (β_1-integrins) results in lowering of P_{if} with a magnitude and time course similar to that described above. Perturbation of the β_1-integrin function is therefore believed to be the final event in the inflammatory reactions described above. The increased negativity of P_{if} can only to a small extent be blocked by conventional anti-inflammatory drugs. The experimental anti-inflammatory drug α-trinositol (D-*myo*-inositol-1,2,6-trisphosphate; Perstorp Pharma, Lund, Sweden) has successfully attenuated the lowering of P_{if} in the inflammatory reactions described above, likely via intracellular modulation of β_1-integrin function.

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INTERSTITIAL PRESSURE AND MATRIX STRUCTURE.

D.Negrini and A. Passi.

Tissue matrix plays a key role in controlling fluid and solute exchanges between microcirculation and interstitium. We have studied the relationship between pulmonary interstitial pressure and the structure of the lung tissue matrix during development of lung edema. Lung tissue matrix dysplays normally a very low compliance of about 0.5 ml/(mmHg·100 g wet weight). Experiments were carried out in 30 anesthetized rabbits. Pulmonary interstitial pressure (Pip) was measured by micropipettes connected to a servo-null pressure measuring system. Micropuncture of in situ lung was performed through a "pleural window". Pip was measured in control condition (C) and during the development of interstitial (IE) and alveolar (AE) edema induced by 0.5 ml/(Kg·min) intravenous saline infusion (hydraulic edema) or by an intravenous single bolus of 60-200 µg of elastase (lesional edema). Samples of lung tissues were taken, processed and analyzed for biochemical matrix structure and for determination of the wet weight to dry weight ratio (W/D) in C, IE and AE. In both hydraulic and lesional edema, pulmonary interstitial pressure increased from -10 cmH_2O to + 4 cmH_2O going from C to IE and subsequently declined towards zero in AE. W/D was 4.91 ± 0.04, 5.2 ± 0.23 and 6.51 ± 0.7 in C, IE and AE, respectively. Biochemical matrix analysis revealed no basic differences in matrix structure going from C to IE, but a considerable increase in free proteoglycan fragments in AE. The decrease in interstitial pressure concomitant with the alteration in matrix structure going from IE to AE suggests that the major mechanism leading to alveolar fluid invasion is an increase in interstitial matrix compliance.

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ELECTRICAL PROPERTIES OF THE INTERSTITIUM.

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The role of the extracellular matrix (ECM) in determining the structure and mechanical properties of tissues and influencing the movement of water and solutes is well recognised. The physiological implications of the anionic nature of many of the ECM macromolecules have received less attention.

Glycoaminoglycans carry 1-2 carboxyl or sulphate groups per disaccharide and so, at the concentrations found in tissue (1-10% w/v), contribute a fixed charge of 0.05 - 0.2 mMol/g wet tissue which ensures that the concentration of cations is much greater than that of anions in dense connective tissue. Cells such as chondrocytes may have adapted to this environment by developing unusual ion channels for osmoregulation. Electrostatic interactions are shielded at physiological ionic strength so that the diffusivities of ionic solutes in biopolymers are similar to those of neutral solutes. Electrostatic interactions are, however, important in increasing the interstitial concentrations of solutes ranging from Ca^{++} to proteins and growth factors, though interactions are frequently stabilised by non-electrostatic forces. The high interstitial concentration of ions generates an osmotic pressure of up to 8 atmospheres. The osmotic pressure depends nonlinearly on concentration in isolated proteoglycans and is, like the pK's of the charge groups, weakly dependent on the biochemical composition. Approximately 20% of the observed pressure is due to non-electrostatic effects. Osmotic forces make a major contribution to the hydration and mechanical properties of dense connective tissues.

Convection of ions through the charged interstitium generates an electric current, the streaming current. The currents generated by flow over the endothelial cell glycocalyx or through interstitium are comparable with theoretical estimates, providing confidence in theoretical predictions that electrokinetic effects are important in flow in confined geometries, such as in the interendothelial junction and around blood cells in small capillaries. Electrokinetic effects may also be important in tissue growth and remodelling.

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FLUID AND SOLUTE TRANSPORT IN SEROUS MEMBRANES. B. Rippe

The peritoneal membrane is the largest serous membrane in the body, employed as an endogenous dialysis membrane in conjuction with peritoneal dialysis (PD). The main barrier to the transfer of solutes and water between the plasma and the peritoneal cavity during PD is represented by the capillary wall. For small solutes the relative resistance of the interstitium as compared to the capillary wall is relatively large, but for solutes larger than inulin the capillary wall is more or less the sole determinant of the blood-peritoneal transport. There is good evidence that the peritoneal mesothelium plays just a marginal role in the overall transperitoneal exchange during PD.

Like continuous capillary walls, the peritoneal membrane shows a bimodal selectivity towards molecules of graded molecular size. Small solute transport can be described as occurring by diffusion through numerous small (≈ 45Å) radius pores whereas large solute transfer is consistent with blood-peritoneal convection through a very low number of large pores (radius ≈ 250Å). Furthermore, peritoneal sieving data are compatible with the presence of a large number of water-exclusive pores (aquaporins). Approximately one-half of the osmotic fluid-flow induced by high intraperitoneal glucose osmotic gradients can occur through these pores in PD.

Transport across the peritoneal membrane is asymmetric. Solutes larger than 20Å are drained from the peritoneal cavity almost exclusively via the lymphatics, of which the diaphragmatic lymphatics dominate. If a macromolecular tracer is placed in the peritoneal cavity only ≈ 20% of the tracer disappearance is accounted for by a lymphatic absorption under non-steady state conditions, while the majority of tracer molecules are entering the peritoneal interstitium by convection owing to the local pressure gradients existing between the peritoneal cavity and the peritoneal interstitium.

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PULMONARY INTERSTITIAL PRESSURE AFTER HYPOXIA EXPOSURE. M. Del Fabbro and G. Miserocchi.

We aimed at measuring pulmonary interstitial pressure after hypoxia exposure that has longtime been known as a possible cause of pulmonary edema. Experiments were done in three groups of anesthetized spontaneosuly breathing rabbits (n=20): control (C), 3h and 6h of hypoxia exposure induced by 12% O_2 breathing (3H and 6H, respectively). Arterial PO_2 averaged 88.7±1.6 in C and 39.2±16 torr in the hypoxic groups. After inducing paralysis with pancuronium bromide, pulmonary interstitial pressure (P_{ip}) was measured in in-situ lung with glass micropipettes connected to a servonull system, directly inserted into the lung parenchyma through a "pleural window". Interstitial pressure in intercostal muscles (P_m) was also measured. P_{ip} was -10±1.5 (SD) cmH_2O in C and significantly increased to-1.8±2.1 and 3.5±2.5 cmH_2O in 3H and 6H, respectively. P_m was -2.4±0.6 cmH_2O in C and increased significantly to 2.8±1.1 cmH_2O in 3H. The wet weight to dry weight ratio (W/D) of the lung was 4.9±0.1, 4.9±0.2 and 4.8±0.1 in C, 3H and 6H, respectively (no significant change), while the corresponding values for the muscle were 3.3±0.1, 3.5±0.2 and 3.8±0.1 (a significant $P<0.05$ increase) in the 3 groups, respectively. Plasma protein concentration was 4.78±0.4 g/dl in C and decreased significantly ($P<0.05$) to 4.2±0.4 g/dl in 3H and to 4.1±0.5 g/dl in 6H. The increase in P_{ip} and in P_m after hypoxia exposure and the decrease in plasma protein concentration indicate that low oxygen tension causes an increase in filtration rate from microcirculation into interstitial spaces. The fact that the W/D of the lung does not increase despite a marked increase in interstitial pressure confirms that the pulmonary interstitial matrix displays a very low compliance that appears adequate to withstand interstitial fluid accumulation.

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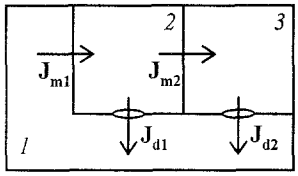
FLUID AND SOLUTES EXCHANGES ACROSS THE PLEURAL SPACE DESCRIBED BY A THREE-COMPARTMENT MODEL. D.Venturoli, D.Negrini, M.Del Fabbro, and G. Miserocchi

We developed a mathematical model for a three compartment biological system based on the mass conservation principle. The compartments are arranged as sketched in figure: they can be identified with the systemic microcirculation (1), the subpleural interstitium (2) and the pleural space (3). The membrane separating compartment 1 and 2 is the capillary endothelium, that between compartment 2 and 3 is the pleural mesothelium. The drainage flows are generated by interstitial (2→1) and pleural (3→1) lymphatics that can increase the flow rates proportional to the increase in liquid pressure. J_{m1} and J_{m2} represent flows through sieving membranes whereas J_{d1} and J_{d2} are non-sieving drainage flows. Using the balance equations for fluid and solutes one can perform a sensitivity analysis obtaining the influence of the parameters appearing in the equations (namely, the permeability properties of membranes and the operative features of lymphatics) on the state variables (hydraulic pressures and protein concentrations in compartments 2 and 3). The analysis shows that compartment 2 (the subpleural interstitium) acts as a buffer to prevent an increase in pleural liquid volume and protein concentration. Normal pleural lymphatics function assures a minimum pleural liquid volume: pleural effusion may result by reducing lymphatic drainage efficiency.



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INTRA-ARTICULAR HYALURONAN & FLOW ACROSS SYNOVIAL LINING OF RABBIT KNEE. PJ Coleman, RM Mason & JR Levick.

The interstitium of the synovial lining is exposed to synovial fluid. Synovium is permeable to saline solution, yet retains synovial fluid within the joint cavity; so we investigated the effect of hyaluronan (chief glycosaminoglycan of synovial fluid, 2-4 $g.l^{-1}$) on trans-synovial flow. Rate of fluid absorption from the joint cavity in the steady state $\dot{Q}_s$ was measured as intra-articular pressure (P_j) was raised in steps to >20cmH_2O, with normal saline or 4 $g.l^{-1}$ hyaluronan in the joint cavity (method, Levick (1979) J Physiol 289, 69). Hyaluronan preparations from human umbilical cord (low viscosity) and rooster comb (high viscosity) were used. Analysis by HPLC indicated molecular mass of $1.1x10^6$ and $2.5x10^6$ daltons respectively.

For saline $\dot{Q}_s$ increased steeply as P_j was raised. By contrast, hyaluronan led to the development of an almost flat relation, i.e. $d\dot{Q}_s/dP_j \rightarrow 0$. Thus by >20 cmH_2O, hyaluronan $\dot{Q}_s$ was an order of magnitude smaller (~8 $\mu l.min^{-1}$, umbilical; ~4 $\mu l.min^{-1}$, rooster comb) than saline $\dot{Q}_s$. Hyaluronan also reduced $\dot{Q}_s$ to a very low level after prior infusion of saline. Similar results were obtained when microvascular blood flow was stopped by killing the animal, indicating that the phenomenon was related to material properties of synovial interstitium.

The increasing resistance to flow, as indicated by the failure of flow to increase with pressure, could be due to partial molecular sieving of hyaluronan by the synovial lining, creating a resistive 'filtercake'. Deliberate perforation of the lining led to a sharp rise in hyaluronan solution $\dot{Q}_s$ to 12-26 $\mu l.min^{-1}$, confirming that synovial lining is the main hydraulic resistance in the system. Stirring the intra-articular hyaluronan by repeated flexion/extension of the joint was quickly followed by restoration of low $\dot{Q}_s$, so the putative filtercake may be either within the lining or else rapidly reformed. When hyaluronan was washed out by saline and a saline infusion begun, $\dot{Q}_s$ increased markedly (e.g. 22 $\mu l.min^{-1}$ at 20 cmH_2O), though not to the expected level for saline.

Further work is in progress to evaluate the above phenomena & to assess their dependence on hyaluronan chain length.

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SOLUTE TRANSPORT THROUGH GLYCOSAMINOGLYCAN SOLUTIONS AND INTACT HUMAN ARTICULAR CARTILAGE. PM Gribbon, *A Maroudas, #MT Bayliss, KH Parker and CP Winlove.

Articular cartilage is an avascular tissue and its homeostasis depends on the efficient exchange of nutrients, metabolites and signal molecules between chondrocytes, blood and synovial fluid. Impaired nutrient delivery has been implicated in cartilage degeneration. The highly charged proteoglycan (PG) component of cartilage is thought to present the major barrier to the permeability of larger macromolecules and charged solutes.

The diffusion of solutes in the intricate network formed by PG's and their glycosaminoglycan components, was measured using a capillary diffusion tube technique. The diffusivity of rhodamine (Rh) and Rh labeled bovine serum albumin (Rh.BSA) in solutions of hyaluronan (HA), chondroitin sulphate (CS) and PG, was determined over a range of polyion (C_P) and counterion concentrations (C_C). In PG and HA solutions, tracer diffusivities (D_t) reduced and C_P increased, and the reduction was greater at low C_C, demonstrating the effects of electrostatic interactions. In CS solutions, D_t was essentially independent of both C_P and C_C.

The diffusion of Rh.BSA through full depth plugs of normal and osteoarthritic (OA) [N=6] human articular cartilage was examined using quantitative digital fluorescence microscopy. An apparent D_t was calculated using a moment analysis of the spatial distribution of fluorescence. Diffusion in the articular surface region was significantly greater that elsewhere, reflecting the higher distribution volume and lower fixed charge density of this region. Both trypsin digestion and OA cartilage showed a higher available space and D_t than normal tissue due to their increased water content. The pericellular matrix surrounding the chondrocytes was more fluorescent than the surrounding intercellular matrix suggesting either BSA binding to the cell membrane or a higher available space for BSA. These observations confirm that regional and pathological variations in the composition of the cartilage ECM influence the transport and distribution of solutes.

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IN VIVO INHIBITION OF TRANSCELLULAR WATER CHANNELS (AQUAPORIN-CHIP) DURING ACUTE PERITONEAL DIALYSIS (PD) IN RATS. O. Carlsson, E. R. Zakaria and B. Rippe

According to the three-pore model of peritoneal permselectivity a major portion of the osmotically induced transmembrane water flow in PD occurs through transcellular water-selective pores rejecting solutes. Recently a 28 kDa "channel forming integral membrane protein" (CHIP-28, AQP1) has been recognised as the molecular correlate to the plasma membrane water channels in endothelial cells. This channel can be inhibited by $HgCl_2$.

In the present study $HgCl_2$ (10 ml, 0.1 mM) was locally applied to the peritoneal cavity (PC) in rats after short term tissue fixation (using 1% glutaraldehyde). 3.86% glucose in lactated Ringer's was employed as dialysis fluid and ^{125}I-albumin (RISA) as an intraperitoneal (IP) volume indicator. ^{51}Cr-EDTA (constantly infused i.v.) was used as a marker for small solute permeability-surface area product (PS or MTAC).

The $HgCl_2$ seemed to rather effectively reduce transperitoneal water flow and to inhibit the sieving of sodium during hypertonic dwells without causing any untoward changes in microvascular permeability, as compared to rats where the PC was exposed to tissue fixation alone. After tissue fixation alone the mean IP volume increased from 20.5±0.15 ml to 25.0± 0.52 ml in 60 minutes, whereas in fixed and $HgCl_2$ treated rats the increment was from 20.7±0.23 ml to 23.5±0.4 ml (60 min). In $HgCl_2$ treated rats the sodium concentration was unchanged between 0 and 40 minutes, whereas in control rats it fell from 135.3±0.97 mM to 131.3±1.72 mM. There were no significant changes in either PS for Cr-EDTA or transperitoneal RISA-clearance following $HgCl_2$ treatment.

In conclusion it seems to be possible to inhibit endothelial CHIP-28 using $HgCl_2$ *in vivo* after moderate tissue pre-fixation during experimental PD. A major portion of the osmotically induced water-transport across the peritoneum during PD seems to occur through water-only pathways.

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MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL ANALYSIS OF CORTICOFUGAL INFLUENCES ON PREOPTIC NEURONS. P.Kravtsov, A.Tereschenko, V.Andreeva and I.Kuznetsov

The physiological significance and morphological organization of inputs from the limbic cortexes to the preoptic area (RPO) is still unclear. Distribution of neurons in the proreal, cingulate, piriform cortexes and hippocampus that send axons to the preoptic region (RPO) were studied on cats by horseradish peroxidase tracing. The proreal cortex was found to send the maximal number of fibers to RPO, while the density of units forming such connections was maximal in the cingulate cortex. The field potentials (FP) and neuron reactions were studied on ketamine anesthetized cats. The most pronounced FPs were generated by RPO neurons in response to the piriform and cingulate cortexes stimulation. The majority of neurons responding to cortical stimuli were located mainly in the lateral RPO division (LPO), where the larger amount of primary excitatory reactions were registered. Medial RPO division (MPO) contained a smaller number of neurons prevailingly generating primary inhibitory reactions. The ratio between inhibitory and excitatory reactions to all cortical simulation for LPO neurons was 0.6:1, but for MPO - 5.8:1. In the RPO division adjoining the bed nucleus of the stria terminalis the primary inhibitory reactions considerably prevailed over the primary excitatory, while in the RPO division adjoining the supraoptic nucleus primary excitatory reactions slightly prevailed (the ratios were 4.9:1 and 0.7:1 correspondingly). RPO was found to be a zone of pronounced convergence of cortical inputs to single cells, where 75% neurons responding to the cortical stimulation have two, three or four cortical inputs.

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CONVERGENT PROPERTIES OF THE VISCEROSENSITIVE NEURONS IN THE PREOPTIC AREA. V.Kazakov and I.Kuznetsov.

It is well ascertained that the preoptic area (RPO) play special role in thermoregulation and reproductive behavior control. On the other hand, it was shown recently that RPO neurons are sensitive to shifts in such homeostatic constants as plasma osmolarity, glucose concentration, systemic pressure etc. The present study was designed to analyze reactions of single RPO neurons to thermal and non-thermal stimuli. The experiments were performed on cats using mixed ketamine + nitrous monoxide anesthesia. Local and generalized heating and cooling of the skin, infusion of small doses (up to 0.3 ml) of hypoosmotic (0.2%), hyperosmotic (3.0%) NaCl and isoosmotic (5.5%) glucose solutions into homolateral carotid artery, and i/v phenylephrine-evoked systemic pressure elevation were used to test RPO responsiveness. High responsiveness to all visceral stimuli applied and especially to glucose and pressure influences was found to be of RPO neurons characteristic. Neuron reactions were of threshold nature. Responses were subdivided into 4 types: 1 - monophase excitatory that were the most frequent (41% of reactions), 2 - monophase inhibitory (15%); 3 - biphase excitatory-inhibitory (6%), and 4 - inhibitory- excitatoty (3%) respectively. Certain specificity were found in localization of viscerosensitive RPO neurons. It was found the pronounced convergence of visceral inputs to single neurons - 86% of the cells responded to more than one stimulation. About 50% of neurons were sensitive to shifts in thermal, glucose, osmotic and pressure homeostasis. Analysis of RPO neuron convergent properties allow to conclude that thermal (cold) and glucose sensitivity as well as pressure and osmotic (hyperosmolarity) sensitivity are frequently combined.

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PROCESSING OF SHORT-TERM AUDITORY SPATIAL MEMORY IN UPPER LEVELS OF THE AUDITORY SYSTEM AND HIPPOCAMPUS (NEUROPSYCHOLOGICAL AND ELECTROPHYSIOLOGICAL STUDY)
J.Altman

Electrophysiological study of single neurons from the thalamic (medial geniculate body) and cortical levels of the auditory system showed pronounced afterdischarges.These responses arise in seconds following sound signal off-set and are of several seconds in their duration. Using sound signals which produce auditory image movement, it was possible to establish that these afterdischarges,in their intensity and time structure,are connected with parameters of the auditory image movement (with the movement direction and velocity). Investigations of neurons from hippocampus revealed neurons with similar afterdischarges which also depended on the auditory image movement parameters.Neuropsychological investigation of patients with temporal epilepsy, after surgical ablation of cortical and/or hippocampal epileptic foci,revealed a pronounced deficit in sound source localization. It was found that removal of either temporal cortex or hippocampus was followed by a mild deficit in localization of a moving auditory image, whereas destruction of both the temporal cortex and hippocampus produced more severe disorders in localization,up to full loss of ability for localization of a moving sound source. The same gradient of disorders was observed when testing the patients for reproducing a set of successive sound signals with different spatial characteristics. A hypothesis is suggested, that in the brain there is a complex "higher auditory centers - hippocampus" which play an important role in memorizing spatial position of a sound source.

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SENSITIVITY TO CRUCIFORM FIGURES IN THE CAT STRIATE CORTEX. I.Shevelev, R.Novikova, N.Lazareva, A.Tikhomirov, G.Sharaev

The responses and orientation tuning in 48 from 62 (77.4%) neurons of the cat striate cortex (area 17) significantly, but with different sign, changed at stimulation by specific cross-like figure flashing in receptive field as compared with single light bar of preferred orientation. Neurons of the first group (19 units from 62, 30.6%) were found to increase the responses by 3.3 times (limits 2.4-12.9 times) if stimulated by cross-like figure of a certain, specific for each cell configuration and orientation. In 12 of these neurons the selectivity index increased in 9 times (limits 1.7-34 times), while general tuning quality (directly proportional to selectivity and inversely proportional to tuning width) increased in 14 units in 8 times (limits 1.9-43 times). Tuning sharpened in 9 neurons (tuning width decreased by $66.7 \pm 14.3^{\circ}$). Under the same conditions, neurons of the second group (29 from 62 or 46.8%) revealed a 3-fold decrease of the responses and worsened all tuning characteristics. Among them 8% of total amount of cells showed bimodal or double orientation tuning when stimulated by some configurations of crosses due to an angle specific inhibition. The effects revealed can be based on excitatory convergence from neurons with different orientation tuning, on inhibitory influences from end-stop and side-zones of receptive field. Possible functional implication of the first group neurons for an angle and line-crossing detection is discussed.

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EFFECTS OF TRANSPLANTATION OF FETAL PREOPTIC AREA ON ESTROUS CYCLES, LOCOMOTORY ACTIVITY AND BODY TEMPERATURE OF ADULT FEMALE RATS.
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The influence of the medial preoptic area (MPA) grafts on estrous cycles, locomotory activity (LA) and body temperature (BT) was in the focus of the present study. The transplantation of neural tissue blocks approximately 0.5 mm^3 were performed into the third ventricle of androgenised female rats or into the third ventricle of MPO-lesioned female rats. These animals were characterized persistent vaginal estrous. LA and BT rhythms had 4-5 day period. The tissue for transplantation was obtained from 16-18-day-old fetuses from the same colony strain. It was found that after the transplantation the MPA-lesioned animals estrous cycles and sexual behavior were recovered. But in contrast with intact rats the locomotory activity and body temperature did not increased in the estrous phase, though their parameters were higher than those of MPA-lesioned rats without the grafts. The transplantation of MPA tissue into the third ventricle of androgenized rats did not recover estrous cycles. Moreover, the number of epithelia's cells in the vaginal smears was much higher than that in the androgenised rats without the grafts. The period of LA and BT rhythms had been shifting from 2 to 6 day. It is conclude that shifts of the hormonal content during the estrous cycle, in control of which MPA participates, rather form the amplitude of LA and BT rhythms than initiate it. It is also suppose that shift of estrogen concentration in the androgenized rats orients development of grafts accordingly to man sexual type.

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PLASTICITY OF THE INJURED SPINAL CORD IN PHYLOGENESIS. L.Matinian, A,Andreasian, V.Mirzoyan, Kh.Nahapetian, Sh.Grigorian

Investigations were being carried on amphibians, reptiles, birds, mammals in the spinal cord organic injuries. Electrophysiological, clinical, histological, histochemical, biochemical, morphometric, statistical methods of investigation have been used. Phylogenetic pecularities of the plasticity mechanism making have been stated and its limit in different injuries of the spinal cord, species and structural-functional pecularities, various expressiveness of scar processes and hystochemical picture of the spinal cord and depending on it - the conducting ways nervous fibers regeneration expressiveness. In animals with more highly developed nervous system in comparison with the less developed ones the rehabilitation degree is more perfect, the plasticity limit is much higher and more expressed morphological revelation of compensation. So, in rats the function rehabilitation takes place even in the existence of one segment between two cuttings, meanwhile in frogs, turtles the compensation is absent even in the presence of 4-5 segments. So, the plasticity of the central nervous system increases in the phylogenetic process, reaching in mammals a high level. The obtained data allow to suggest a new approach to the treatment of spinal injuries considering the changes taking place in the spinal cord and corresponding the influences of both enzyme preparations, preventing the scar tissue development, blocking the nervous fibers growth, and in the further application of physical and other factors (laser radiation, alternating magnetic field, diadynamic currents, hormonal preparations et al.), stimulating the nervous fibers growth.

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STRUCTURAL AND FUNCTIONAL CHANGES RELATED TO THE HIPPOCAMPAL GRANULAR LAYER LESION

J.Pokorný, J.Mareš and S.Trojan

Functional stability of a neuronal system depends on the features of its internal structure and on its ability to recover the structure after a degenerative or traumatic process. Any intervence into the structure which brings an imbalance in inputs to the nerve cells, may result in a functional pathology. Interruption of hippocampal local circuits was achieved by a fluid injection into the infragranular cleavage plane of the dentate gyrus dorsal blade which brakes axons and causes disintegration of the granule cells. Five days or two months later, neuromorphological and electrophysiological observation was performed. We found a decrease of the number of cells in the hilar area, loss of spines (mossy terminals) on shafts of the CA3 pyramidal cells apical dendrites, and an increase of the spine density in proximal segments of granule cells in the ventral blade. Morphological changes were not fully expressed in the earliest stage after the operation. Acute kindling effect was present with no regard to type of lesion. Animals with acute lesions localized only into hippocampus more often prevailed serrated waves during seizures. 60 days after surgery it was reversed. Acute lesions in dentatum caused slight decrease in prevalence of serrated waves. Almost inverted picture shows the ranking of spike-and-wave discharges during seizures. We thus conclude that the damage of the part of the granule cell layer results in a profound rearrangement of the internal neuronal circuits both in the hilus and in the neighboring areas. At the same time damage of the main "interface" between neocortex and limbic structures - dentatum - possibly decelerates generalization of seizures originating in neocortex during acute phase after surgery. After 60 days the lesion in dentatum increases the seizure susceptibility.

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RESOURCES OF THE BRAIN AND THEIR MOBILIZATION

N. N. Lyubimov, S. N. Lyubimov

In previous years the new concept of the brain (coexistent mechanisms of conduction, steering and conditioning) was presented by us on the basis of experimental and clinical investigations. A number of ideas (about multichannel organization of afferent conduction, hierarchy of central mechanisms of conditioning on the basis of interreaction between different afferent channels of sensory systems, reorganization of sensory system after different forms of deafferentation et cetera) were formulated (N. N. Lyubimov, 1980,1986) and seemed to be fruitful for study and mobilization of the reserves of the brain. The Transcendental meditation (TM) and TM-Sidhi program created by Maharishi Mahesh Yogi has great perspectives in this respect.

In our work we tried to estimate the distribution of the cortical EP with peak latency to 100ms (N27-28, P40-41, N59-60) associated with sensory input which have wider distribution in additional brain structures of the cortex, in contralateral and ipsilateral hemishere. These results testify that during TM there is an increase in the area of the cortex taking part in the perception of specific information, an increase in the functional relationship between the two hemisheres and an increase in functional activity of the cortical structures of the anterior regions of both hemispheres. This phenomenon correlates well with the subjective experience of expansion of consciousness that takes place during TM. The activation of areas of the cortex at some distance from the primary response center can be seen as a result of a more integrated functioning of the brain involving more comprehensive processing of the received stimuli.

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INHIBITION OF 5-LIPOXYGENASE IMPROVES REGIONAL MYOCARDIAL FUNCTION AFTER REPETITIVE ISCHEMIA IN THE RAT HEART

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Background. Cardiac ischemia causes activation of the enzyme 5-lipoxygenase with the subsequent production of leukotrienes thus inducing activation of neutrophils. Therefore, 5-lipoxygenase may play a role in reperfusion injury after myocardial ischemia. Several studies have shown a reduction in infarct size after pretreatment with leukotriene antagonists. However, no such studies have been performed employing repetitive, brief episodes of myocardial ischemia as would occur, for example, in patients with unstable angina. The aim of this study was, therefore, to investigate the effect of a selective 5-lipoxygenase antagonist ZM 230487 (ZM) (10mg/kg, orally), on the reduction of regional myocardial function and myocardial blood flow (MBF) occuring after both repetitive ischemia (RI) and myocardial infarction (MI). *Methods.* In an in vivo rat model, the regional myocardial function was determined as the fraction of systolic thickening (FT) by pulsed doppler and the MBF determined by hydrogen clearance technique. In the RI group, the left anterior descending coronary artery (LAD) was ligated 5 times for 10 min each time followed by 20 min reperfusion. In the MI group the LAD was ligated for 50 min followed by 60 min reperfusion.

		Untreated group		ZM 230487	
		Baseline	After ischemia	Baseline	After ischemia
RI	FT (%)	21.9±1.4	8.9±2.0	22.1±1.1	12.2±0.6 *
	MBF (ml/min/g)	3.7±0.8	1.9±0.9	4.0±0.8	1.9±0.4
MI	FT (%)	22.1±2.4	7.9±1.9	19.7±1.2	9.3±1.3
	MBF (ml/min/g)	3.5±0.7	1.3±0.9	3.5±0.7	1.9±0.6

Results. ZM treatment had no effect on baseline values. After RI, FT measured in the treated group was significantly higher than in the control group. MBF remained unaltered. In the MI group, there were no significant differences in the reduction of MBF and FT under control conditions compared to the ZM treated group. *Conclusion.* Pretreatment with the 5-lipoxygenase inhibitor ZM 230487 significantly improves the reduction in regional myocardial function after repetitive brief episodes of ischemia in the rat heart. It is suggested that inhibition of 5-lipoxygenase attenuates leukotriene production leading to reduced activation and extravasation of neutrophils and thus preservation of myocardial tissue.

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EFFECT OF ATRIAL PACING ON SYSTOLIC SHORTENING, VELOCITY OF SHORTENING, AND POWER OF INTACT AND STUNNED MYOCARDIAL AREAS.

H. Schad, W. Heimisch and N. Mendler

Isolated healthy myocardium responds to an increased rate of contraction with an increased force. Myocardium from failing hearts has lost this property. Stunned myocardium as characterized by a disturbed post-ischaemic contraction after pre-ischaemic coronary blood flow is restored, possibly also shows an impaired force-frequency relationship. This was tested by comparing the effect of right atrial pacing on systolic shortening (MSS), velocity of shortening (VSS), and power index (MPI) of stunned and intact left ventricular myocardium in 16 anaesthetized, open chest pigs. Stroke volume (SV electromagnetically), ejection time (T), aortic blood pressure (AoP), heart rate (HR), left descending (LAD) and circumflex (LCX) coronary artery blood flow (Q, ultrasonic transit time) and wall movement (sonomicrometry) were monitored. Q_{LAD} was obstructed for 40min to reduce MSS_{LAD} to 20±2% of control followed by repetitive LAD occlusion (5 times 1min occlusion, 1min perfusion); then the LAD stenosis was removed. 90min later, Q_{LAD} was 118±6% of control, MSS_{LAD} was 44±6%, MPI_{LAD} = $MSS \cdot AoP \cdot HR/T$ was 53±4%, and VSS_{LAD} = MSS/T was 53±4%, which indicates stunning; LCX area showed increased MSS (106±3%), VSS (122±3%), and MPI (125±6%); HR increased to 89±4 min^{-1} (from 75±3min^{-1} before stunning), SV and AoP were 90±2% of control, global ventricular power was 106±5%. **Pacing to 145 min^{-1} before stunning** caused a decrease in MSS (LAD: -42±3%, LCX: -39±3%) at almost maintained VSS (LAD: -14±4%, LCX: -10±4%), and an increase in MPI (LAD: +68±10%, LCX: +71±11%). **After stunning, pacing** caused a decrease in MSS (LCX: -25±3%, LAD: -62±3%), VSS_{LAD} remained unchanged (-1±2%), VSS_{LCX} decreased (-49±4%), MPI_{LCX} increased (+62±10%), MPI_{LAD} did not change significantly (-6±9%). Obviously, stunned myocardium is not able to maintain VSS and to increase MPI during pacing in contrast to intact myocardium. This is cannot be explained by an inadequate Q_{LAD} during pacing after stunning (105±7% of Q_{LAD} during pacing before stunning), but is possibly due to an impaired Ca^{++}-availability or -sensitivity of the myofilaments.

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INTERSTITIAL CHANGES IN CHRONIC HIBERNATING MYOCARDIUM.

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Cardiomyocytes in biopsies obtained from 20 patients with chronic hibernating myocardium show depletion of contractile elements and sarcoplasmic reticulum, accumulation of glycogen, dispersion of nuclear chromatin and mitochondrial size reduction. These alterations were not reminescent of degeneration, but were interpreted as cell de-differentiation as a result of prolonged hypo/akinesia. The cellular changes were accompanied by a marked increase of the interstitial space. In order to find out whether this increase is related to either fibrosis consequent to the loss of intercellular cohesion, to proliferation of interstitial cells, or enhanced production of extracellular material, the following parameters were assessed: 1) the surface of inter-connecting areas (desmosomes, gap junctions) in cardiomyocytes; 2) the percentage of interstitial tissue; 3) the amount of non-cellular connective tissue (collagens I, III and fibronectin); and 4) the number of mesenchymal (vimentin positive) cells. We found that the number of interconnections between cardio-myocytes had markedly decreased, that the amount of collagen and fibronectin had strongly increased, along with a highly significant increase in the number of mesenchymal cells which produce these extracellular matrix proteins. When these quantitative data were confronted with data of functional recovery after coronary bypass surgery, it could be concluded that the increase in interstitial tissue is one of the main determinants for the lack of immediate recovery of contractile function after restoring blood flow to the affected (chronic hibernating) myocardial segments.

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Systolic function of the left ventricle in hyperthyroid patients on long term ß – blocker therapy

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45 hyperthyroid patients treated with propanolol were studied by M mode, 2 D and Doppler echocardiography and compared to a similar sized group of matched controls.

Both groups were formed of males and females aged between 20 and 60 years. The hyperthyroid patients were all diagnosed in the endocrinologycal service and were suffering of Graves Basedow, toxic adenome or polinodular hyperthyroidised goiter. T3, T4 measurements were performed for each and TSH in 16 patients. All the patients received also specific antithyroid therapy.

The following indexes were calculated: ejection fraction, shortening fraction, E/A ratio of the mitral valve. EF in hyperthyroid patients (mean ± ϑd = 80 ± 1,8) was significantly higher than in controls (mean ± ϑd = 60 ± 5,3, p < 0,01) and in chirotic patients (mean ± ϑd = 61 ± 3,8).

38% of the hyperthyroid patients were diagnosed as having mitral valve prolapse (cooptation of the mitral valve on the atrial side of the mitral anriulus) versus 6,66% in the control group.

Propanolol does not decrease EF in hyperthyroid patients.

The study showed hypercontractility in the hyperthyroid patients and an unexplained high incidence of mitral valve prolapse without important mitral regurgitation.

The fact that β-blockers do not reduce the ejection fraction could suggest that in patients with a high T3-4 level such drugs do not decrease cardiac performance and can safely be used.

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TRANSMITRAL FLOW IN LEFT VENTRICULAR HYPERTROPHY

Alexandra Grigorescu, Cornel Tudorică, Adrian Ionescu, Andreea Iancu

50 patients with known left ventricular hypertrophy were studied by M Mode, 2d and Doppler echocardiography and were compared to a similar sized group of matched controls in which the left ventricular mass (LVM) was less than 100g/sqm (in females) and 110 g/sqm (in males). In the control group, hypertension, ischemic heart disease, cardiomiopathies, rheumatic fever or any other cause that could induce false results were excluded.

The patients studied were 50 males and females aged between 35 and 80, with hypertension or hypertension and ischemic heart disease. The left ventricular mass was a minimum of 160 g/sqm in males and 145 g/sqm in females. The patients with a left ventricular mass (LVM) of more than 220 g/sqm (females) and 250 g/sqm (males) or with a left ventricular diastolic diameter (LVDD) of more than 69 mm were also excluded. The only criterium for admission was the echocardiographic measurement of LVM. To evaluate the diastolic function of the left ventricle, we have studied the E/A ratio, which ranged between 0,48–1,75 and isovolumic relaxation time (IVRT) between 68–135 ms. 44 of the patients had a E/A ratio less than 1,2 and also a IVRT less than 70 ms or more than 110 ms.

The global results, presented as averages, are the following: age 59,77 years, LVM 207,89 g/sqm, E/A ratio 0,85, IVRT 96,2, LVDD 55,13 mm.

In the 18 patients with major left ventricular diastolic disfunction, the average results were: age 63,64 years LVM 201,05 g/sqm, E/A ratio 0,78, LVDD 57,27 mm.

In the 32 patients with normal IVRT (average age 55,53 years), the average results found were: E/A ratio 0,90, LVDD 53,89 mm.

Severe diastolic disfunction was poorly correlated with the duration of illness or LVM, but it was correlated well enough with age and LVDD.

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VECTORCARDIOGRAPHIC AND ECHOCARDIOGRAPHIC CORELATIONS IN LEFT VENTRICULAR HYPERTROPHY.

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The present study tries to find corelations between left ventricular mass (LVM) and some vectorcardiographic parameters: the QRS loop surface (SQRS), the QRS loop perimeter (PQRS) and the maximal vector magnitude of QRS loop (MQRS), with intention to improve the electrocardiographic diagnosis of left ventricular hypertrophy (LVH). We used a group of patients with various forms of LVH diagnosed by echocardiographic and electrocardiographic criterions. LVM calculated with echocardiographic parameters--interventricular septal and posterior parietal thickness--and the SQRS,PQRS and MQRS from computerized vectorcardiographic analisis were introduced in a statistic study.We found a mean corelation between LVM and SQRS, and a better corelation between LVM and PQRS. There was no corelation between LVM and MQRS--the wellknown parameter in electrocardiographic diagnosis of LVH. These results demonstrate that the PQRS is the most useful parameter in electro- and vectorcardiographic diagnosis of LVH as a measure of electrical abnormalities in various forms of left ventricular overload and emphasize the necessity of vectorcardiographic study corroborated with echocardiography in LVH.

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R56865 inhibits the development of phenylephrine- and endothelin-1-induced hypertrophy of neonatal rat ventricular cardiomyocytes. J.A.A.M. Claes, J.E. de Vries, G.J. van der Vusse, R.S. Reneman and M. van Bilsen.

To gain more insight into the possible role of intracellular Na^+ and Ca^{2+} in the development of cardiac hypertrophy, the effect of R56865, a compound with putative Na^+ and Ca^{2+} overload inhibiting properties, on agonist-induced hypertrophy of cardiac cells was studied. Neonatal rat ventricular cardiomyocytes in which hypertrophy was induced by phenylephrine (PE, 10^{-5} M) or endothelin-1 (ET-1, 10^{-8} M) were used as a model system. 48 Hours of treatment with PE resulted in an increase in cell size and a marked induction of ANF mRNA and upregulation of MLC-2 mRNA levels. Co-administration of compound R56865 (10^{-8}-10^{-6} M) reduced the increase in cell size and both ANF and MLC-2 expression dose-dependently. In the presence of 10^{-6} M R56865 PE-induced ANF expression was abolished. Cardiomyocytes transfected with a plasmid containing the luciferase gene under the control of part of the ANF-promoter (638 bp) underwent the same protocol as described above. At 10^{-7} M R56865 completely inhibited PE-induced ANF-promoter activity. At 10^{-6} M R56865 partly reduced ET-1-induced ANF-promoter activity. From these results it can be concluded that R56865 inhibits the development of cellular hypertrophy suggesting that alterations in intracellular Na^+ and Ca^{2+} levels may contribute to the development of myocardial hypertrophy.

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RELATIONSHIP BETWEEN POST-ISCHAEMIC HEART POWER OUTPUT AND MODELLED REPERFUSION RESPONSE. R.J.F. Houston, B. Oeseburg, A. Heerschap and S.H. Skotnicki
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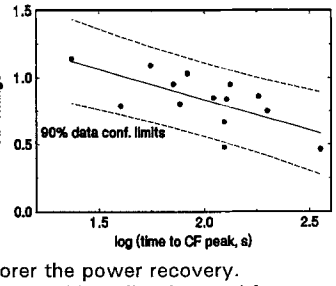
Background and objective: To meet more exacting patient requirements, new cardioprotective strategies are being introduced, though seldom based on fundamental scientific research. Isovolumic isolated animal heart models cannot predict the post-arrest ability of the heart to support a physiological workload. Our main objective was to develop, test and calibrate a model for assessment of these new cardioprotective protocols.

Method and results: We have further developed our blood-perfused, ejecting, isolated rat heart model for use inside the bore of an NMR magnet. This allows on-line haemodynamic and ^{31}P metabolic monitoring. To calibrate the model, 15 hearts were subjected to normothermic ischaemic insults of varying duration. On reperfusion, coronary flow increased rapidly to a peak, then decayed to a baseline value. Only in a blood-perfused model is there the reserve coronary flow to allow such an overshoot.

Analysis: The reperfusion response was modelled, and the resulting equation fitted to the data for each experiment. The relationship between log of modelled time to peak flow, and fractional left ventricular power output recovery, the ultimate test of a cardioprotective strategy, is shown here. The longer the time to reach peak flow, the poorer the power recovery.



Conclusion: Reperfusion response could predict the need for post-operative patient support. This research was possible thanks to the financial support of the Dutch Heart Foundation (grant 902-19-115).

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BRIEF ISCHAEMIA OR HYPOXIA RESULT IN DECREASED MITOCHONDRIAL FUNCTION AND CONTRACTILITY IN ISOLATED RABBIT HEART, BUT NOT IN ITS RIGHT VENTRICULAR PAPILLARY MUSCLE. C.J. Zuurbier and J.H.G.M. van Beek

The purpose of this study was to investigate *in vivo* mitochondrial function in stunned rabbit heart. Isolated isovolumic rabbit hearts were perfused with a constant flow of Tyrode solution (11mM glucose) at 37 °C, maximally vasodilated with 10 μM adenosine. The response time of mitochondrial oxygen consumption (t_{mito}; Van Beek et al, Am. J. Physiol. 260:H613, 1991) to a step in heart rate (from 100 to 200 beats/min) was determined before ischaemia (I, n=8) or high-flow hypoxia (H, n=8) and after 20 min reperfusion following ischemia or reoxygenation following hypoxia. A control group (C, n=8), with the same timing but no I or H, was also examined. Developed left ventricular pressure (DLVP) decreased to 79 ± 3% (mean ± SEM), 45 ± 3% and 59 ± 6%, whereas t_{mito} (appropriately corrected) increased with 22 ± 11%, 44 ± 12% and 47 ± 15% for C, I and H, respectively. The decrease in DLVP and the increase in t_{mito} were significantly different between C and I or H. Subsequently, the time constant of the recovery heat production following a twitch train of 10 contractions (0.2 Hz) of papillary muscles, isolated from the right ventricle of the same hearts, was determined using thermopiles at 20 °C (Mast et al, Pflüg. Arch. 411:600, 1988). No significant differences in tension (41 ± 6 mN/mm^2 (C, n=7), 51 ± 6 mN/mm^2 (I, n=6) and 39 ± 4 mN/mm^2 (H, n=8)), or time constant (38 ± 4 s (C), 35 ± 3 s (I) and 40 ± 3 s (H)) among the three groups were observed. We conclude that 15 min of ischaemia or hypoxia in the intact isolated rabbit heart, resulting in stunning, is associated with decreased *in vivo* mitochondrial function. Because stunning or decreased mitochondrial function is not observed in its right ventricular papillary muscle, the relevance of the latter as a model for stunning is doubtful. *Supported by Netherlands Heart Foundation, grant no. 91.120.*

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TIME-RELATED EFFECTS OF HEAT STRESS ON CARDIAC PROTECTION AGAINST ISCHEMIA AND REPERFUSION IN THE ISOLATED RAT HEART. R Cornelussen, M Vork, G van der Vusse, R Reneman and L Snoeckx.

Experimental findings indicate that within 3 hours after heat stress, heat shock proteins (HSP) are expressed in cardiac tissue. In this study we investigated the time-lag between heat stress-induced protection against ischemia and the published time-lag of HSP expression in order to delineate a direct causal relationship between HSP expression and cardioprotection. To this end, post-ischemic functional recovery of isolated, ejecting rat hearts was investigated 0, 3, 6 and 24 hours after heat stress (HS 0-3-6-24; 42°C for 15 min). Hearts of non-heated rats served as controls (C). During reperfusion after 45 min of global ischemia, the recovery (% of preischemic value) of cardiac output (CO), left ventricular developed pressure (LVDP), and positive and negative dPdtmax showed a gradual improvement, depending on the time-lag between heat treatment and ischemia. Only after 24 hours the differences with the control group reached statistical significance.

Treatment	R_{CO}	R_{LVDP}	$R_{+dPdtmax}$	$R_{-dPdtmax}$
Control (n=7)	58±15	82±4	71±9	71±10
HS 0 (n=4)	59±19	79±10	70±12	73±16
HS 3 (n=6)	63±14	81±9	68±14	79±14
HS 6 (n=5)	68±14	87±6	80±5	84±7*
HS24 (n=6)	75±7*	94±9*	96±3*	94±8*

R= recovery; * significantly different from control values. Mean ± SD

These findings indicate that the time-lag between heat shock and cardioprotection does not correspond with that of the expression of HSP, suggesting that the mere presence of HSP is not the only factor of heat stress-induced cardiac protection.

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CLONING AND SEQUENCE DETERMINATION OF RAT HEART MEMBRANE-ASSOCIATED LOW-MOLECULAR WEIGHT PHOSPHOLIPASE A_2. L.J. De Windt, M. Van Bilsen, G.J. Van der Vusse, R.S. Reneman.

The degradation of membrane phospholipids by phospholipase A_2 (PLA_2) appears to play an important role in the development of irreversible cell damage during myocardial ischemia and reperfusion. Although cardiac tissue contains different types of PLA_2, several arguments like substrate preference, calcium dependency, and the fact that antibodies raised against snake venom group II PLA_2 reduced phospholipid degradation in ischemia/reperfusion experiments of isolated rat hearts, favor a role of a type II PLA_2. In order to delineate the potential role of this type of PLA_2 in cardiac tissue, it was first attempted to clone the cardiac representative of this type of PLA_2. To this end we designed two oligonucleotide primers against the coding region of rat spleen calcium-dependent membrane-bound type II PLA_2. With these primers a PCR reaction was performed on RT-mRNA from total heart, and the resulting 275 bp fragment was positively identified as a group II PLA_2 by DNA-sequencing. The PCR-product was subsequently labeled and used to screen a rat heart cDNA library and to perform Northern blotting. DNA-sequencing of a positive candidate from the library revealed a PLA_2 cDNA with a coding region homologous to rat type II PLA_2, and predicts a low-molecular weight protein with a signal peptide at the 5'-end for secretion. Northern blotting showed a 0.9 kb signal of low intensity that was found along the digestive tract and in the heart. RT-PCR and Northern blotting showed that the group II PLA_2 is present in cardiomyocytes. It remains to be established whether this group II PLA_2 is located intracellularly and/or can be secreted.

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NITRIC OXIDE PRODUCTION IN THE ACUTE PERIOD OF EXPERIMENTAL MYOCARDIAL INFARCTION. L.M.Belkina, E.B.Manukhina, V.D.Mikoyan, L.N.Kubrina and A.F.Vanin

Acute myocardial infarction (MI) is accompanied by severe alterations in vascular tone which may seriously aggravate the patient's condition and even result in death. Such a hazardous complication of MI as cardiogenic shock is known to be associated with a fall of peripheral vascular resistance and blood pressure. However the role of nitric oxide (NO), the principal mediator of vasorelaxation, in the pathogenesis of acute MI remains unclear. The aim of the present study was to follow the time course of NO production in the heart and liver of rats during the acute period of MI. MI was induced by ligation of the left coronary artery. The presence of infarct was detected by ECG. The NO content in tissue was assayed by the electron paramagnetic resonance (EPR) method using the NO trap diethyl dithiocarbamate which specifically binds NO giving rise to a characteristic triplet EPR spectrum. It was shown that the NO production remained unchanged in both organs within the initial 2 hours following MI. At 3 h after MI, a transient but a significant fall of NO production was observed, then a rapid increase in NO content began so that by the 5th hour the NO production was almost twice as high as in control. This enhanced NO level persisted to the 24th hour after MI. The result suggests that the increased NO production may play a role in the fall of peripheral vascular resistance observed in acute MI.

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VIOLATION OF FUNCTIONING OF SARCOPLASMIC RETICULUM IS ACCOMPANIED WITH MYOCARDIUM INSUFFICIENCY A.I.PLISKA, S.S.SHAVARAN.

Having investigated contractive activity of isolated trabecular muscles of the right nodule of human heart, they studied the functional condition of sarcoplasmic reticulum. With this purpose they increased intracellular concentration of calcium ions by means of Strophantine K and Bay K 8644. In dependence on functional condition of the heart, the patients were divided in two groups. The first conditionally control group consisted of patients without myocardium hypertrophy and without pre- and postoperative cardiac insufficiency. The second group included patients with myocardium hypertrophy and without preoperative cardiac insufficiency in the rest. In dependence upon postoperative period run they were divided in two subgroups: a) without this and b) with this. For stimulation of contractive activity the isolated trabecular muscles were irritated with over threshold rectangular electric current pulses with frequency 0.5 Hz. Perfusion of trabecular muscles of conditionally control group with solution of Bay K 8644 (10^{-7}-10^{-5} mol/l) and Strophantine K (10^{-6}-10^{-3} mol/l) caused dose-related increase of contraction strength. But within subgroup 2a Bay K even in minimal concentration caused paradoxical decrease of contractions strength its alternation and contracture. At the same time sensitivity of myocardium to Strophantine K have being increased. It resulted in increase of as contractions strength in response to perfusion of muscles with preparation of respective concentration so the expansion of sensitivity to pharmacological agents from 10^{-7} mol/l to 10^{-4} mol/l. Within subgroup 2b both preparations even in minimal concentrations cause paradoxical decrease of contractions strength, its alternation and contracture.

Thus with development of myocardium insufficiency functioning of sarcoplasmic reticulum is violated more and more.

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HYPERTROPHY AND REGRESSION OF THE MYOCARDIUM: THE ROLE OF FREE RADICAL RELATED MECHANISMS.

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Active oxygen species play a dual role in the organism: participate in biosynthetic and destructive processes. Hypertrophy of rat hearts caused by repeated (6 hours daily for 30 days) exposure of Wistar rats to a simulated altitude (6000 m above the sea level) was accompanied by accumulation of lipid peroxidation (LPO) products and increasing rate of LPO induced in myocardial homogenates by the Fe^{2+}+ascorbate system *in vitro*. The activity of antioxidative protection enzymes was also increased thereby. After adaptation to hypobaric hypoxia, the hypertrophied myocardium was subjected to rapid regression: within 8 - 10 days the heart mass returned to the control level. The first 3 days of regression were marked by additional activation of LPO which by day 10 reached the basal level. The activity of antioxidative protection enzymes also gradually returned to control values. These findings suggest that LPO activation in the hypertrophied myocardium is compensated for by elevation of activity of antioxidative protection enzymes. During regression free radical reactions take part in the disassembly of excessive membrane and other structures of the cell. Therefore during regression (postoperative, after long physical loading, living in the mountains and so on) the myocardium is particularly sensitive to injuries caused by LPO activation (ischemia, reperfusion, cardiosclerosis, stress).

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REDUCED BLOOD PRESSURE AND HEART RATE VARIABILITY IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION. A. Kardos, L. Halmai, Z. Gingl, J. Simon, L. Rudas.

A decreased heart period variability (HRV) and baroreflex sensitivity in patients with acute myocardial infarction (MI) have already been proofed. Since one of the major determinants of HRV is blood pressure variability (BPV), it would be important to know the characteristic of blood pressure regulation in this setting. The changes in BPV during the acute phase of MI has not yet been studied. As a part of a larger MI follow up study we investigated the BPV and the HRV in patients with acute MI.

Methods: Twenty two patients were enrolled into this analysis. Nine patients with MI and 13 age matched patients served as controls (54.1+10 years vs. 51.2+10 years). Post MI patients were studied 72-94 hours after admission. We used the frequency domain indexes of short term measurement of BPV and HRV. The spectral powers for both HRV and BPV were divided into three major components: total frequency (TF, 0.01-0.4 Hz), low frequency (LF, 0.04-0.15 Hz) and high frequency (HF, 0.15-0.4 Hz) components.

Results:

	Acute MI	p	Control
TFHRV (ms^2)	400±115	ns	2624±1255
LFHRV (ms^2)	106±26	ns	4928±2213
HFHRV (ms^2)	48±24	0.032	432±132
TFBPV($mmHg^2$)	87±23	ns	3521±1609
LFBPV($mmHg^2$)	16±6	0.013	105±26
HFBPV($mmHg^2$)	36±9	0.032	620±210

Conclusion: Striking differences were seen in BPV parameters, indicating a restricted BP fluctuation in post MI patients. The same tendency is seen in HRV parameters, although the differences did not reach statistical significance. The reduced BPV in the acute phase of MI could be related to a relative invariance of stroke volume as a consequence of reduced left ventricular compliance. This is the first study demonstrating abnormally low BPV during the acute phase of MI. The study was supported by the grant of OTKA F 012709.

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CEREBROCORTICAL CAPILLARY FLOW DURING SYSTEMIC HYPOTENSION AND INTRACRANIAL HYPERTENSION G. Fehér, A.G. Hudetz, C.G. Weigle, D. Knuese, J. Kampine

Objective of the study: Although autoregulation of cerebral blood flow is well established, the response of cerebral capillary circulation to reduced cerebral perfusion pressure is unclear. The objective of this study was to determine if red cell flow velocity in individual capillaries of the cerebral cortex is maintained during acute decreases in cerebral perfusion pressure.

Method: Microcirculation of the superficial parietal cerebral cortex of adult, barbiturate-anesthetized, artificially ventilated rats was visualized using a closed, perfused cranial window and epi-fluorescent, intensified video-microscopy. Fluorescein-isothiocyanate labeled red blood cells (FRBC) injected intravenously were used as markers of capillary flow. Cerebral perfusion pressure, defined as mean arterial pressure minus intracranial pressure, was reduced by controlled hemorrhage or by stepwise elavation of local intracranial pressure. The movement of FRBC in the parenchymal capillary network was video-recorded at each pressure level and FRBC velocity in each capillary was measured off line using the dual window, digital cross-correlation technique. FRBC flux in the capillaries was measured by automated cell counting.

Results: FRBC velocity at normal perfusion pressure was 1.47± 0.58 mm/s (SD) and changed little in the perfusion pressure range of 70 to 120 mmHg. The autoregulatory index in this pressure range was 0.0049 mm/s/mmHg. Opening of previously unperfused capillaries was not observed. FRBC flux correlated with FRBC velocity but the latter was maintained in a narrower range than FRBC flux suggesting a decrease in capillary diameter or hematocrit with decreasing perfusion pressure.

Conclusions: The result suggest that flow autoregulation is associated with tha maintenance of capillary flow velocity and that capillary recruitment does not not contribute to flow autoregulation in the rat cerebral cortex.

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THE STUDY OF CARDIO-VASCULAR DIMENSIONAL AND FUNCTIONAL PARAMETERS TO ATHLETES LOT. S. Gusti, G. Raca, A. Dunarintiu-Gusti

The human body study in effort condition reveals additional information comparatively with the repose condition and permits the assessment of cardiovascular system challenge degree. The autors have investigated the cardiovascular dimensional and functional parameters in athletes lot, 18-27 years old, using noninvasive methods. The cardiac volumes and the cardiac performances were determined using radiologic and noninvasive methods to calculate the systolic time intervals, corected in relation with cardiac frequency, on phono-cardiogram, pulse carotidiene and EKG made at the same time to the polyinscriptor 6 NEK - 4 - Germany. We estimated the cerebral and the lower limbs circulation by the Döppler ultrasound method, using a Sonopan U.D.P. - 10, apparatus of 8 MHz with pulsatile emission. Analysing the results and the statistic data compared with data from tests on healthy people, 18-30 years old too, who do not practice any sports, we found an increase of cardiac volumes of 9% closely correlated (r=0,89) with the increase of 27% in left ventricular ejection period due to the long time aerobic effort in case of the studied lot. We reveal also an increase of the lower limbs circulation and a reduction with 24% of resistance index of both the carotides in the athletes lot.

A conclusion, the utilisation of cardiovascular exploration noninvasive methods has permitted the assessment of cardiac performance, cerebral and lower limbs irrigation in the studied group. We think that the studied group has been well selected, and the sportive training has achieved a very good adaptation to the specific effort.

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REDUCED TOLERANCE TO LOW FORCE STATIC LOAD AT ESSENTIAL HYPERTENSION A.Vītols, D.Liepiņa

In order to characterize the peculiarities of vasodilatation, energetic metabolism, potassium (K^+) balance and myoelectric activity related with reduced fatigue resistance of skeletal muscle to static load at essential hypetension (EH), 23 pts (stage II WHO, men, age 48±4 yrs) and 19 sex and age matched controls (C) were studied. Forearm blood flow (FBF, by venous occlusion plethysmography), oxygen consumption (VO_2), lactate (L, by enzymatic method) and K^+ efflux and myoelectric signal power spectrum median frequency (MF) during handgrip with force 5% of maximal voluntary contraction till exhaustion as well as K^+ concentration (by flame photometry) in m. vastus lateralis biopsy samples were analysed.

In EH pts comparing to C, load endurance is reduced to 13±0,6 min. vs. 20±0,4 min. ($p<0,05$) and FBF, VO_2, L and K^+ efflux increase while MF decreases more rapidly at the 10th min. of load achieving the following values: Mean ± SE ♦ $p<0,001$; * $p<0,05$

	+ FBF ml/min.dm^3	+VO_2 mM/ min.dm^3 x10^{-2}	+L mM/ min.dm^3 x10^{-3}	+K^+ efflux mM/ min.dm^3 x10^{-3}	-MF Hz
EH	109±6*	62±3♦	140±12♦	46±2♦	22±2*
C	59±3	36±3	44±9	21±2	12±2

MF decreases and K^+ efflux during the load is inversely related to K^+ level in a resting muscle (r= - 0,69, r= - 0,76; $p<0,05$) being reduced in a number of EH patients. Thus, the changes in transmembrane electrolyte transport and with it related modifications in motor unit recruitment and microvessel dilatation mechanism may be possible causes of reduced fatigue resistance of skeletal muscles at EH.

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ESSENTIAL HYPERTENSION AGAINST BINDING AND DEGRADATION OF ^{125}J-INSULIN BY ERYTHROCYTE RECEPTORS Ł. Szczęśniak, T. Rychlewski, F. Banaszak, J. Głuszek.

The work presents the results of researches of binding of ^{125}J-Insulin by erythrocyte receptors in the patients with essential hypertension (n=23) against control group of healthy persons (n=21). Binding and degradation of ^{125}J-Insulin by erythrocyte receptors was assayed with the method described by Gambhir et al (1977) with own modification introduced by the authors. The modification consisted in usage of constant iodized insulin concentration (0.9 pg/0.1 ml and bovine insulin (2.3 j.m./0.1 ml). Anthropometric factors and the parameters of glucose-lipid metabolism were registered as well, with the view to obtain full description of investigated individuals.

The results obtained were compared by means of the Student's t-test for two independent trials. It was found that binding of ^{125}J-Insulin by erythrocyte receptors is statistically significantly lower as compared with the values obtained in the control group ($p<0.01$). Moreover, in hypertensive patients, higher total cholesterol and immunoreactive insulin concentrations in serum is observed. In healthy persons negative correlation between degradation of ^{125}J-Insulin by intact blood cells and age, and HDL cholesterol fraction was found, while positive correlation was observed with binding of ^{125}J-Insulin by blood cell receptors. The above relations were not observed in the patients with essential hypertension.

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BINDING OF ^{125}J INSULIN IN PATIENTS WITH ESSENTIAL HYPERTENSION DURING ORAL GLUCOSE LOAD T. Rychlewski, Ł. Szczęśniak, P. Michocki , F. Banaszak .

The work presents binding of 125J-Insulin by erythrocyte receptors during oral glucose load (75 g of glucose diluted in 300 g of water) in the persons with essential hypertension (n=11) compared with a control group (n=9).

Measurements of the glucose, insulin, C-peptide concentrations and ^{125}J-insulin binding were effected at fasting before glucose administration and after 30, 60 and 120 minutes since the glucose intake. The insulin level was assayed by means of the RIA-INS test, manufactured by the Research & Development Centre of Isotopes in Świerk, C-peptide level was determined by the Biodato-Serono test, glucose level was measured with the method described by Gambhir et al (1977) with own modification introduced by the authors. The modification consisted in usage of constant iodized insulin concentration (0.9 pg/0.1 ml and bovine insulin (2.4 j.m./0.1 ml)

The results show that glucose load in the patients with essential hypertension results in higher insulin secretion from the beta-cells of pancreas that is manifested in higher serum concentration of C-peptide, time delay of glycemic curve and insulin resistance, measured in terms of ^{125}J-Insulin binding by erythrocytes compared to the control group.

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THE EFFECT OF BRLP-42 ON THE BIOMECHANICAL PROPERTIES OF SAPHENOUS ARTERY AND VEIN IN SPONTANEOUSLY HYPERTENSIVE STREPTOZOTOCIN-DIABETIC RATS. G. L. Nádasy, M Szentiványi Jr., L. A. Szirmai, M. Tóth, V. Kopcsányi, A. Jednákovits, E. Monos.

OBJECTIVE: The present study was performed to demonstrate a potential direct effect of BRLP-42 (a compound developed by the BIOREX Co to treat diabetic vasculopathy) on the passive and active biomechanical properties of extremity vessels in rats subjected to hemodynamic and metabolic stresses. METHODS: Control SHR rats and streptozotocin-diabetic SHR rats were given BRLP-42 orally (20 mg/kg body weight, daily) for 3 months. Then saphenous artery and vein were excised and cylindrical segments were subjected to in vitro biomechanical test. Intraluminal pressure was continuously changed and outer diameter was continuously measured by computerized microangiometry. Outer diameter, inner diameter, wall thickness, distensibility, elastic modulus were computed and plotted against intraluminal pressure for vessels in spontaneous tone, in methoxamine induced contraction (10^{-6} moles/lit) and in papaverine relaxation. RESULTS: In nondiabetic SHR rats BRLP-42 decreased the vascular distensibility at certain pressures, decreased the spontaneous tone, and increased the methoxamine induced tone of saphenous artery. In saphenous veins, there was an increase in elasticity. In diabetic rats, oral BRLP-42 treatment partially restored the geometrical changes induced by streptozotocin treatment in saphenous arteries: wall thickness and outer radii were increased. E.g. at 170 mmHg (close to in vivo pressure) streptozotocin treatment decreased wall thickness of arteries in spontaneous tone from 62±5 micrometer to 43±2 micrometer which latter value was increased up to 53±3 micrometer ($p < 0.05$) by oral BRLP-42 treatment. In saphenous veins oral BRLP-42 treatment induced a decrease in distensibility in diabetic rats. CONCLUSIONS: The present study indicates that some characteristic changes induced by streptozotocin diabetes in the biomechanical properties of blood vessels can be improved by oral BRLP-42 treatment. (Supported by grants ETT 291 93/94, OTKA 1113-91/94, and BIOREX Research and Development Co.)

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DISTRIBUTION OF CGRP IN RELATION TO GAP-43 AND SYNAPTIC VESICLE MARKERS IN THE DEVELOPING RAT ATRIUM. J.Slavíková, A. Dahlström

The developmental pattern and distribution of calcitonin gene-related peptide- (CGRP) containing neurons in the rat heart have been studied by indirect immunofluorescence. Antibodies against CGRP were applied to the whole-mount stretch preparations of the right atria from hearts of newborn to 40-day-old animals. Comparison with growth associated protein (GAP-43) and synaptic vesicle markers, synaptophysin (p38) and SV2 in nerve axons and terminals was done in double immunoincubation studies. Immunofluorescence was examined with confocal laser scanning microscopy. Nerve fibres with CGRP-, p38-, and SV2-like immunoreactivity (LI) were present throughout the atria already at birth with the highest density around the sino-atrial node. An extensive network of all antigens-LI was also seen along the coronary vessel examined. A gradual increase in the density and intensity of fluorescence was observed up to the age of 40 days. CGRP-LI was not colocalized in the same preparations with either SV2 or p38, which are mainly present in small synaptic vesicles of the classical autonomic innervation. In contrast, nerve axons strong in GAP-43-LI colocalized with CGRP-LI. Also GAP-43-LI was present in nerve axons and fibers at birth, but its density has not changed substantially from newborn to 10-day-old animals. The results show, that CGRP-positive nerve fibres are present already at birth, and that the pattern of innervation is qualitatively similar to that observed in adults. Distinct location from classical autonomic innervation supports nonadrenergic noncholinergic origin of CGRP, matrix peptide, which seems to be located in both small and large synaptic vesicles. Strong GAP-43-positivity at birth could be linked to axon growth and development.

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ARTERIAL HYPERTENSION DUE TO OCCLUSION OF THE CENTRAL ADRENAL VEIN IN THE RAT IS GENETICALLY DETERMINED. P. Abramczyk, J. Przybylski, K. Papierski, A.Lisiecka and B.Ciszek

The purpose of the study was to evaluate the impact of an increased blood flow through the direct adrenal-renal vascular connection (ARVC) on the blood pressure in different strain of rats: (I) - Glaxo Wistar rats, (II) - Lewis rats, (III) - outbred Wistar rats, (IV) - WKY bred for low systolic blood pressure (118 mmHg ± 2 SE), and (V) - WKY bred for high systolic blood pressure (133 mmHg ± 2.5 SE). The rats were anesthetized and bilaterally the central adrenal veins were ligated. In the control group in addition the vessels connecting the kidneys and respective adrenal glands were eliminated. Systolic blood pressure (SBP) was measured every fourth day up to 12 weeks after the surgery. In group I, II, III, IV SBP remained on unchanged level throughout the observation period. Whereas in V group twelve days after the occlusion of adrenal veins, systolic blood pressure was significantly higher in the experimental group than in the respective control group, reaching the maximal value (160 mmHg +/- 5.5 SE) one week later. We postulate that in group V direct vascular connection is better developed and/or adrenal gland is more active as compared to previous groups.

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EXCITABILITY OF SCIATIC NERVE AFFERENT FIBRES IN CONDITIONS OF HEMORRHAGIC HYPOVOLEMIA

I. Bračoková, M. Murín, Š. Kujaník, P. Švorc.

Somatic afferentation, which is of little importance for cardiovascular homeostasis in normal conditions, may cause marked hemodynamic changes if the conditions in the organism change. The aim of the study in cats was to analyze the hemodynamic and respiratory changes during the stimulation of with the sciatic nerve afferent fibres different intensities (0.5-3.5 mA) both in norm and immediately after the loss of 10-30 % of total blood volume calculated from the animal weight. Our study concentrated on the estimation of the blood pressure changes and their latent times as well as on the breathing changes, excitability threshold of the sciatic nerve afferent fibres and threshold of the blood pressure response conversion (the change of hypotensive response to the hypertensive one). Our results show that character of the blood pressure response during the stimulation of the sciatic nerve afferent fibres in normal conditions depends on the stimulation parametres. The low intensity current (1 mA) evokes a hypotensive response, whereas the high intensity current (3.5 mA) a hypertensive one. In conditions of hemorrhagic hypovolemia the excitability threshold of the sciatic nerve is reduced, the threshold of the blood pressure conversion is decreased to 1 mA, and the latent times of the hemodynamic responses are changed. After the sciatic nerve stimulations the tolerance of animals against hemorrhage decreased.

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THE REDUCTION IN SYMPATHETIC VASOCONSTRICTOR RESPONSE OF BLOOD VESSELS OF ACUTELY INFLAMED RABBIT KNEE JOINT IS NOT POST-SYNAPTICALLY MEDIATED. *H. Najafipour, and **W.R. Ferrell.

It has been shown that the effectiveness of the sympathetic nerves in regulation of blood flow is reduced by inflammation. Experiments were performed on 20 NZW rabbits (2.2-3.5 Kg) under pentobarbitone anaesthesia (0.25-0.5 mg/min, slow IV infusion) to assess the effect of carrageenan induced acute inflammation on nerve mediated vasoconstictor responses of articular blood vessels. Electrical stimulation (10V, 5Hz, 1ms, 90s trains) of posterior articular nerve resulted in 33.9±3.2% reduction in posterior capsular blood flow of the normal and 21.8±6% reduction of the inflamed group measured by laser Doppler flowmetry technique ($p < 0.05$). Close intra-arterial injection of 2.2 nmol α_1-agonist phenylephrin and 250 pmol of α_2-agonists UK-14304 and clonidine reduced the blood flow of the normal joint by 19±3.5%, 40±5.3% and 13.2±2%, and of the inflamed joint by 25.2±6.4%, 38.8±6.8%, and 17.7±5.8% respectively. None of the values between the two groups were significantly different. The results of this study showed that, alteration of nerve mediated responses of articular blood vessels due to inflammation was not related to changes in number or responsiveness of postsynaptic α-adrenergic receptors, and probably had a pre-synaptic mechanism.

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BLOOD PRESSURE VARIATIONS IN NEWBORNS. J. Dušek, J. Siegelová, B.Fišer, R. Nekvasil, G.Cornelissen, F. Halberg

The aim of the study was to prove the hypothesis that in newborns blood pressure has 7 day instead of 24-hour variability (Halberg). Long lasting blood pressure (BP) monitoring was applied using the sphygmonanometer every hour. Fifty six premature newborns (850 to 3250 g b.w.) hospitalized in the intensive care unit from 7 to 35 days after birth were examined. We computed power spectral density of systolic (SBP) and diastolic BP (DBP) and heart rate (HR). We computed also the cosinor analysis of SBP, DBP, HR. Slow oscillations with a different period between 5 to 10 days were found either in HR or in BP in all newborns. The peak of 1 day in HR was found in 50% of newborns, in SBP and DBP only in 43%. The peak of 1 day periodicity was always smaller than those found with circaseptan rhythm. Mean power spectra revealed a significant peak ($p<0.05$, Student t-test) at 0.16 cycles per day in HR, SBP and DBP. Cosinor analysis proved that the double amplitude of 7 day period is much more prominent in SBP, DBP, HR then 1 day period ($p<0.001$). Our results support the concept that the week period is not a mere feature of culture, but a part of nature.

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POSSIBLE INVOLVEMENT OF RENIN-ANGIOTENSIN SYSTEM IN THE ARTERIAL HYPERTENSION INDUCED BY L-NAME.

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Like others (Chyu et al., 1992; Wang et al., 1993) we observed an increase in the arterial blood pressure in rats chronically subjected to Nitro-L-arginine methyl ester (L-NAME) treatment (25 mg /kg body wt., i.p.). In rabbits we applied the same chronic treatment as above (for 6 days) and observeda decrease of renin activity (RIA) in blood plasma, together with an increase in the angiotensin converting enzyme (ACE) activity measured by Depièrre and Roth technique (1976). In turn, in vitro experiments on rat thoracic aorta revealed a stimulation of vascular reactivity in response to either angiotensin I or II in the presence of L-NAME (10^{-6} M). Angiotensin I induced vasoconstriction was abolished by captopril (2mM), but reappered if the rat aorta rings were pretreated with L-NAME. Quite different from captopril, saralasin (5-10 µM) inhibited both the angiotensin I-induced contraction and the desinhibition of ACE by L-NAME .
The observed increase of the in vitro vascular reactivity to angiotensin I induced by L-NAME suggests either a desinhibition of angiotensin receptors (AT), or the formation of angiotensin II using another enzymatic pathway.

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INFLUENCE OF OPEN AND CLOSED ENDOTRACHEAL SUCTIONING ON CEREBRAL OXYGENATION AND HAEMODYNAMICS IN PRETERM INFANTS. L.Kollée, K.Liem, J.Klaessens, W.Geven and B.Oeseburg

OBJECTIVE: To compare the effect of closed and open endotracheal suctioning (ETS) on changes in cerebral oxygenation and haemodynamics in preterm infants. METHODS: In 8 ventilated preterm infants (28-34 weeks, 750-2050 g) open and closed method (Trach-care, Ballard) ETS was performed randomly. During the procedure cerebral concentration changes of oxyhaemoglobin (ΔcO_2Hb), deoxyhaemoglobin ($\Delta cHHb$) and total haemoglobin ($\Delta ctHb$) were continuously measured with Near Infrared Spectrophotometry. ΔcO_2Hb and $\Delta cHHb$ reflect changes in cerebral O_2 supply, while $\Delta ctHb$ reflects changes in cerebral blood volume (CBV). Changes in mean cerebral blood flow velocity (ΔCBFV) of the internal carotid artery was also recorded continuously using pulsed Doppler ultrasound, as well as changes in heart rate, pulse oximetric saO_2, $tcpO_2$ and $tcpCO_2$. RESULTS: The use of the closed ETS device resulted in an increase of $tcpCO_2$ (7.1 $\pm$ 3.3 mm Hg), CBFV (3.1 $\pm$ 1.6 cm/s) and $ctHb$ (0.55 $\pm$ 0.34 µmol/100 g). Maximal changes in the measured variables during open and closed ETS are shown in the table.

Values = mean $\pm$SD	open	closed
ΔcO_2Hb (µmol/100 g)	-0.83$\pm$0.30*	-0.38$\pm$0.38*
$\Delta cHHb$ (µmol100 g)	0.56$\pm$0.14*	0.27$\pm$0.21*
$\Delta ctHb$ (µmol/100 g)	-0.27$\pm$0.40	-0.10$\pm$0.33
ΔCBFV (cm/s)	-4.0$\pm$3.1*	-3.2$\pm$3.0*
ΔsaO_2 (%)	-10.6$\pm$5.4*	-6.2$\pm$4.3*
$tcpO_2$ (mm Hg)	-14.7$\pm$4.4*	-6.5$\pm$6.1*

CONCLUSION: Closed ETS resulted in less alterations in cerebral oxygenation and haemodynamics, but the large dead space of the device caused increased $tcpCO_2$, resulting in cerebral hyperperfusion. Reduction of dead space of the closed ETS device would be necessary.

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BIOMECHANICS OF SAPHENOUS VESSELS FROM SHR AND WKY RATS. M. Szentiványi Jr., G.L. Nádasy, L.A. Szirmai, M. Tóth, V Kopcsányi, A. Jednákovits, E.Monos

OBJECTIVE: Geometrical, elastic and contractile properties of isolated saphenous arteries and veins from male spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats were compared. METHODS: Outer diameter of cylindrical saphenous artery and vein segments was measured in vitro. Intraluminal pressure (IP) was changed continuously. Pressure-diameter plots for resting, methoxamine ($1.06x10^{-5}$ mol/lit) contracted and papaverine ($2.8x10^{-4}$ mol/lit) relaxed segments were recorded. Biomechanical parameters were computed as a function of pressure. RESULTS: Saphenous arteries of SHR showed larger contractile response to methoxamine, than those of WKY, thus active strain was higher (e.g. 100 mmHg IP: 7.12±4.1 vs. 0.35±0.46 %). Resting incremental distensibility was higher (e.g. 100 mmHg IP: $3.4\pm0.4x10^{-6}$ vs. $1.2\pm0.3x10^{-6}$ cm^2/dyne), elastic modulus lower (e.g. 100 mmHg IP: $3.7\pm0.6x10^6$ vs. $27\pm7.6x10^6$ dyne/cm^2) in the arteries from SHR in pressure range of 60-110 mmHg. After papaverine the artery became more rigid, thus the increased elasticity of SHR artery might be due to the enhanced smooth muscle tone. In geometrical parameters there were no differences between the two groups. Saphenous vein of the SHR had larger external and internal radii, than those of WKY, while in the wall thicknesses no difference was found (e.g. external radius-wall thickness ratio at 8 mmHg IP: 16.8±2.9 vs. 8.5±0.7). Lumen capacity was also higher in SHR than in WKY (e.g. 8 mmHg IP: 0.43±0.04 vs. 0.27±0.02 mm^3/mm), but elastic parameters did not exhibit significant differences. CONCLUSION: Hypertension is accompanied with increased reactivity and elasticity of the saphenous artery without changes in the passive geometry. Opposite alterations were found in veins: increase in the passive lumen capacity without changes in elasticity. These findings support the hypothesis that peripheral arterial responsiveness and venous capacity is enhanced in hypertension. (Supported by grants ETT 291-93, OTKA 1113-91/94 and BIOREX Res. & Dev. Co. Budapest, Hungary.)

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EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON VASCULAR BIOMECHANICS IN SHR RATS. L. A. Szirmai, G. L. Nádasy, M Szentiványi Jr., M. Tóth, V. Kopcsányi, A. Jednákovits, E. Monos.

OBJECTIVE: This work was aimed at testing whether streptozotocin (STZ)-induced diabetes altered the active and passive biomechanical properties of saphenous artery and vein in hypertensive (SHR) rats. METHODS: Diabetes was induced by a single dose of STZ (40 mg/kg i.v.) followed by three months housing period. Cylindrical segments of saphenous vessels were excised and subjected to a large-deformation mechanical test in vitro. Intraluminar pressure (IP) was continously changed between 0-200-0 mmHg in arteries and 0-30-0 mmHg in veins. Outer diameter was monitored by an automatic image-analysing video-microangiometer. Stress-strain relationships were obtained in control, methoxamine (10^{-6} M) contracted, and papaverine (10^{-4} M) relaxed segments from STZ-SHR and from control-SHR groups. RESULTS: STZ treatment resulted a significant decrease in the wall thickness of saphenous artery in SHR rats throughout the pressure range studied (e.g. 68±5 µm vs. 45±3 µm at 80 mmHg IP). External diameter was also smaller in STZ-SHR than in SHR rats, particularly at lower pressures. Lumen capacity and elastic properties (incremental distensibility, elastic modulus) of the saphenous artery were not different between the two goups. Adrenergic reactivity of the artery was larger in STZ-SHR than in SHR group (active tangential strain in response to methoxamine at 80 mmHg IP: 15.5±3.8% vs. 6.8±1.7%). Basal tone (indicated by the degree of papaverine-induced relaxation) was relatively small and did not differ in the two groups. Saphenous vein from STZ-treated rats showed smaller external diameter and lumen capacity than that from SHR animals through the whole pressure range (e.g. external diameter at 8 mmHg of IP: 655±33 µm vs. 750±51 µm). However, no difference was found in the wall thickness of STZ-SHR vs. SHR rats. Elastic parameters of the vein were not different between the two groups either. Methoxamine-induced contractile responses and spontaneous tone of the saphenous vein were similar in the two groups. CONCLUSIONS: STZ-induced diabetes causes wall thinning and enhanced constrictor reactivity of the artery and reduced venous capacity in the saphenous vascular bed of SHR rats. Assumingly, such changes of the artery result increased passive and active wall stresses wich may contribute to further pathological alterations of the vessel wall common in diabetic-hypertensive subjects. (Supported by grants ETT 291-93, OTKA 1113-91/94, and BIOREX Research and Development Co.)

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No implication of UTP in the neurogenic vasomotricity of the rabbit ear artery

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The aim of this study was to evaluate the possible role of UTP in the neurogenic vasomotricity of the superfused rabbit ear artery (REA). We have studied: i) the effect of UTP on the evoked and spontaneous ^{3}H NA release and ii) the uptake, evoked and spontaneous release of ^{3}H UTP. Proximal segments of REA were preincubated with ^{3}H NA or ^{3}H UTP for 90min. The preparations were left to equilibrate (90 min) with an initial tension of 0.5-1g and superfused (1ml/min) by a Kreb's solution at 37°C. They were stimulated (using parallel wire electrodes) twice (S1 and S2), 26 min a part at 30V, 5Hz, 0.3ms for 90 sec. Contraction was measured as changes in the isometric tension. The superfusate was collected during 2 min each time, and ^{3}H-radioactivity was measured. At the end of experiment, the ^{3}H radioactivity of the whole tissue was also measured. We found that the adding UTP (10^{-4}M) 10 min before S2 induced no modification of the spontaneous and/or evoked release of ^{3}H NA. On the vessel preincubated with ^{3}H UTP, we observed a 8% uptake of ^{3}H which was greatly reduced by dipyridamole (10^{-4}M), an ^{3}H UTP spontaneous release but any ^{3}H UTP evoked release.

These experiments suggest that in the REA:

1) UTP does not influence spontaneous or evoked release of ^{3}H NA.
2)UTP is taken up by the vascular wall but is not released by nerve terminal stimulation.
3) UTP uptake was probably not effective at the nerve terminal level, but in the vascular smooth muscle or in the endothelial cells (1).

It can be proposed that UTP is not involved in the neurogenic regulation of REA vasomotricity.

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LIMITATION OF BARORECEPTOR REFLEXES BY β_1-BLOCKER ESMOLOL AND α_2-ADRENERGIC AGONIST DEXMEDETOMIDINE.

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A stressfull response, resulting in tachycardia and hypertension, can be dangerous in certain groups of patients. β–blocking drugs are used in order to attenuate these responses, but the central sympatholysis achieved with α_2-adrenoceptor agonists could also be useful. Our goal was to compare the effects of the α_2-agonist dexmedetomidine (DEXMED) and β_1-blocker esmolol (ESMO) on baroreceptor reflexes. In 10 open chest dogs, anesthetized with chloralose, changes in heart rate and blood pressure were measured after bicarotid artery occlusion (BCO). Also the heart rate response during changes in aortic pressure, induced by caval vein and aortic balloon inflation (Baroreflex Sensitivity Test, BST), was determined. These interventions were performed at baseline, during infusion of ESMO (300 $\mu g.kg^{-1}.min^{-1}$ IV), again at baseline, and during IV infusion of DEXMED (target plasma concentration 0.5 $ng.ml^{-1}$). At baseline BCO significantly increased aortic pressure (32±13%) and heart rate (8±4%). During ESMO the increase in aortic pressure (23±8%) and heart rate (2±3%) was significantly lower compared to the preceding baseline. During DEXMED the increase in aortic pressure (11±6%) was also lower whereas the increase in heart rate (20±17%) was not diminished. The BST showed a significant decrease in baroreflex sensitivity after ESMO, whereas Dexmed maintained baroreflex regulation, be it at a lower heart rate. So, during baroreceptor reflex testing both DEXMED and ESMO limit the increase in aortic pressure, but only DEXMED preserves the heart rate response.

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SELECTIVE bNOS INHIBITION REDUCES CORTICAL INFARCTION FOLLOWING FOCAL CEREBRAL ISCHEMIA.

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Nitric oxide (NO) plays a multifunctional role in cerebrovascular regulation both in physiological and in pathological conditions. NO is synthesised in the central nervous system by different isoforms of the nitric oxide synthase (NOS). The constitutive NOS of the endothelium and neural elements of the brain (ec-bNOS) participates in the maintenance of normal cerebrovascular tone and acts as a neurotransmitter. The inducible form of NOS is activated by ischemia/hypoxia. In the early phase of focal cerebral ischemia the amount of NO significantly increases, but the source of the NO elevation is unclear. This study was focused on the dose dependent effects of a selective bNOS blocker 7-nitro-indazole (7-NI) in focal cerebral ischemia on rats. Morphological changes (infarct size, measured in tetrazolium red stained coronal sections) and cardiovascular parameters (blood pressure, cardiac output, cortical blood flow) were recorded in control and in 7-NI pretreated (10-20-30-40 mg/kg i.p.) rats. Infarct size was significantly reduced by the 20 mg/kg treatment to 6.27±1.82% of the total brain volume, compared with controls (8.58±1.36%). On the contrary, the 40 mg/kg dose caused a significant increase of infarction volume to 10.83±1.26%. Furthermore, this dose of 7-NI significantly increased the blood pressure (by 22±9 %) and reduced the cortical blood flow (by 25±8 %), suggesting a non-selective action of this high dose of 7-NI on ecNOS. The selective blockade of NOS isoforms, applied in different doses and proper timing, can be a promising tool in stroke therapy. Supported by NIH, NS RO1: 31429 and OTKA: 3111

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EFFECT OF SYMPATHO-VAGAL INTERACTION ON HEART RATE VARIABILITY IN ANAESTHETIZED DOGS.

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The function of the autonomic nervous system underlying changes in heart rate variability (HRV) are not fully understood. The objective of this study was to study how the interaction between sympathetic and parasympathetic inputs can affect the high frequency (HF) component of HRV. That component is generally thought to reflect solely cardiac parasympathetic control. We developed an animal model, in which neural outflows to the heart were eliminated but pure sympathetic and vagal effects could be produced by electrical stimulation. We stimulated cardiac sympathetic and vagal nerves in anaesthetized, vagotomized, spinal anaesthetized dogs and analysed the resulting changes in heart rate variability by power spectral analysis. At the control the mean R-R interval (RRI) was 637±31 ms and HRV at a low level: total HRV and the HF component of HRV were 188±130 ms^2 and 316 ms^2,respectively. When the right vagus nerve was stimulated with a pattern in which the instantaneous stimulation frequency oscillated at the frequency of 0.20 Hz with the mean frequency of 10 Hz, the mean RRI increased to 828±42 ms and there was a significant ($p<0.05$) increase in HRV corresponding to the frequency of modulation in vagal stimulus. The total HRV and the HF component of HRV increased to 31303±16183 ms^2 and to 26656±14662 ms^2, respectively. However, when a constant sympathetic stimulation (20 Hz) was combined to the vagal stimulation, the mean RRI deceased to 562±35 ms and the total HRV and the HF component of HRV decreased significantly ($p<0.05$) to 2709±1022 ms^2 and to 2263±986 ms^2, respectively. The results were similar also if the change in the mean RRI was taken into account. Our results suggest that although the high frequency component of heart rate variability is mainly under parasympathetic regulation, it may be influenced also by the sympathetic nervous system.

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THE PHARMACOLOGICAL EFFECTS OF ETHANOL, VERAPAMIL AND THEIR COMBINATIONS. M.Otter and A.Schotter

The effects of ethanol and verapamil on cerebral blood flow in rabbits and on motor ability, body temperature, analgesia and behavioral effects in albino rats were investigated. 68 male adult Wistar albino rats of Rappolovo Farm and 10 heterogenous stock of rabbits received one or two dosages of ethanol (1.0 or 0.2 g/kg) alone or in combination with verapamil (Finoptin "Orion") (5.0 or 2.5 mg/kg). It was ascertained that administration of verapamil alone and in combination with ethanol increases in dosage and time dependent manner the local cerebral blood flow of the two structures of the brain measured by means of hydrogen clearance during wakefulness. It is well documented that organic calcium channel antagonists prevent the transmembran flux of extracellular Ca^{++} through ion-selective voltage-sensitive ("slow") channels in vascular smooth muscle cells. In this way verapamil induces vasodilatation in peripheral and cerebral vascular beds. The behavioral tests demonstrated that verapamil exerts several effects which are not entirely attributable to its cerebravascular action. In the experiments in rats verapamil alone did not effect body temperature but potentiated ethanol-induced hypothermia. The combination of both drugs had analgetic effect in "hot-plare" experiment-model. Both verapamil and ethanol impaired motor ability and coordination and this effect was additive. Only in plus-maze tests on anxiety verapamil (5 mg/kg) administrated 15 min before ethanol antagonized the anxiolytic effect of ethanol.

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COMPARISON OF THE ELASTIC PROPERTIES OF THE AORTA OF SPONTANEOUSLY HYPERTENSIVE (SH) AND WISTAR KYOTO RATS (WKY) IN INTACT CONSCIOUS AND ANESTHETIZED ANIMALS.

A.W. van Gorp[#], D.S. van Ingen Schenau[*], H.A.J. Struyker Boudier[*], A.P.G. Hoeks[@], R.S. Reneman[#], J.G.R. De Mey[*].

The elastic properties of the thoracic aorta were investigated in intact conscious 3 month old and anesthetized 3 and 6 month old WKY and SHR. Pulse pressure (ΔP) was measured with an intra-aortic catheter. End-diastolic diameter (D) and diameter changes during the cardiac cycle (ΔD), were measured with a wall tracking system attached to a conventional B-mode imager. From these measurements, compliance (CC) and distensibility (DC) were calculated. Media cross-sectional area (CSA) was measured by morphometry ex vivo and corrected for retraction of the aorta after isolation. In conscious SHR, blood pressure was significantly higher and CC and DC significantly lower than in conscious WKY. There was no difference in D between SHR and WKY. After anesthesia with ketamine/xylazine, diastolic blood pressure (P_{dia}) of 3 and 6 month old SHR was decreased to the level of that in WKY. D was significantly smaller in 3 and 6 month old SHR. At 3 and 6 months of age, CC was significantly lower in SHR than in WKY. These differences in CC were less pronounced than those in conscious SHR and WKY. There was no difference in CSA. The fact that the aorta is less compliant at two different P_{dia} levels in SHR than in WKY indicates that this difference is not caused by a higher pressure alone. The equal CSA and smaller D in SHR, at comparable P_{dia}, indicates remodeling. (supported by NHS and NWO).

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INHIBITION OF BAROREFLEX SENSITIVITY IN RATS AFTER CARDIAC ARREST. Harchenco IB, Tarasova OS, Koshelev VB, Volkov AV.

Chronic experiments on freely moving rats which had a cardiac arrest were done to study the reflectory changes in the heart rate (HR) in response to blood pressure (BP) changes induced by bolus i.v.injections of phenylephrine and nitroprusside. The cardiac arrest was induced by the mechanical occlusion of afferent and efferent vessels for 12 min. In 12 min. the rats were rescuciated by mechanical ventilation and closed-chest cardiac massage. Baroreflex was tested in 1,5 and 3 months after rescucitation. The catheters were implanted in the femoral artery and vein under nembutal (40 mg/kg) one day before the experiment. The test-drugs nitroprusside (5,10,15 mkg/kg) and phenylephrine (1,2,5,10 mkg/kg) were injected into vein's catheter. BP and HR were registered from arterial catheter with computer. Baroreflex was calculated as $\Delta HR/\Delta BP$ ratio. The pressor baroreflex in rescucitated rats in 1,5 months after rescucitation was significantly lower than in the control (1.49±0.16 - experiment; 2.30±0.22 - control; p=0.02), but there were no alterations in the depressor baroreflex. There were no significant differences in pressor and depressor baroreflex 3 months later rescucitation.

The same conclusions were done in the other experiments where the anesthetised (nembutal 40mg/kg) rats in 1,5 months after rescucitation were used. BP rise as response to occlusion of both carotid arteries was tested. As compared with the control BP rising in rescucitated rats was significantly lower (15.8±4.2 mmHg - experiment; 28,5±2.9 - control; p=0.02). The differences disappeared after vagotomy.

Thus, abnormalities of arterial baroreflectory control may be occurred in 1,5 months after cardiac arrest in rats, but no changes were in 3 months.

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THE EFFECT OF K SUPPLEMENTATION OR A POTASSIUM-SPARING AGENT ON BLOOD PRESSURE AND VASCULAR RESPONSES OF SALT-LOADED RATS

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Dietary salt loading increases blood pressure which is accompanied by hypokalemia and enhanced vascular responses. It is in view of this that we investigated the effect of dietary potassium supplement and a potassium sparing agent (spirinolactone) on blood pressure and vascular responses of rats. Eight weeks old male Sprague Dawley rats were fed for 6 weeks on diet containing either 8% NaCl (Salt-loaded rat) or normal rat feed (Control rat). Some of the salt-loaded rats were also given either daily oral administration of spirinolactone or had KCl added to their drinking water. At the end of feeding period the mean arterial pressure (MAP) were recorded directly from the left femoral artery with a Grass polygraph and the serum Na^+ and K^+ concentrations were also measured using flame photometer (Corning 400). The contractile responses of the aortic rings incubated in physiological salt solution which was bubbled with 95% O_2 - 5% CO_2 gas mixture was also measured with an isometric force transducer which was connected to Grass model 7D polygraph recorder. The MAP of salt-loaded rats was higher ($P<0.05$) than that of potassium supplemented rats, Salt-loaded-spirinolactone treated rats or the controls. Salt-loading reduced the serum K^+ concentration and this effect was prevented by potassium supplement or spirinolactone. The increased sensitivity of aortic rings from salt-loaded rats to norepinephrine was lower in the controls, potassium supplemented as well as the spirinolactone treated rats. These results indicate that the salt induced increased in blood pressure and the accompany hypokalemia was attenuated by the administration of KCl or spirinolactone. In addition spirinolactone reduced the sensitivity of vascular smooth muscle to norepinephrine which may partly explain its anti-hypertensive activity.

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ROLE OF THE MEMBRANE SKELETON PROTEINS OF RAT ERYTHROCYTES IN FUNCTIONING OF TRANSPORT ATPases. A.M.Kazennov, M.N.Maslova, Yu.A.Matskevitch, A.D.Shalabodov, O.V.Frolova

Removal of some membrane skeleton proteins (spectrin, actin) from the rat erythrocyte ghosts resulted in a significant decrease of activity of Na,K-ATPase and Ca-ATPase and in a more pronounced diminution of relative phosphatasic activities together with changing of some kinetic parameters of hydrolysis of corresponding substrates. In contrast with intact ghosts the inhibitory effect of increasing concentrations of Mg^{++} on Na,K-ATPase in spectrin-depleted membranes was not revealed and activating effect of the ion on ouabain-sensitive K-phosphatase altered. In addition, the modulating effect ATP and Ca^{++} on ouabain-sensitive phosphatase and Ca-phosphatase practically disappeared. Besides the activating effect of Ca^{++} on these phosphatase activities in the ghosts could be revealed only in the presence of 0.5-1.0 mM ATP. The data obtained show that the function of transport ATPases in unnuclear erythrocyte membranes is related to the membrane skeleton. On the one hand, regulating influence of intracellular ATP and Ca^{++} on the enzymes seems to be realized through the skeleton proteins and, on the other hand, the membrane skeleton proteins could be involved in catalytic cycles of the transport ATPases.

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FUNCTIONAL REENTRY IN THE PREGNANT UTERUS OF THE RAT. Wim J.E.P. Lammers and Kholoud Arafat.

Conduction of individual action potentials ('spikes') was investigated in the isolated rat myometrium at different stages of gestation (day 17 till delivery). Recordings were performed using a multi-electrode array of 15 x 16 electrodes (1 mm inter-electrode distance; 0.3 mm diameter) from the serosal surface of the in-vitro superfused myometrium. Simultaneous recordings of all 240 electrodes was performed from a 2.1 cm^2 area for periods up to 15 minutes.

Spatial analysis of the pattern of propagation revealed marked variations in direction of conduction. In a minority of cases, the propagating impulse was seen to rotate around an area of functional conduction block thereby describing a full circle of propagation with the impulse returning to a previously excited area after a period of 200-500 msec. Further analysis revealed that the impulse then re-excited the area previously activated (= re-entry) and initiated a circus movement. Hereafter, the impulse often continued along the circular pathway for several revolutions. In a few cases, the circle was stable under the electrode array and was terminated by conduction block ahead of the leading wavefront. In most cases, the circulating excitation shifted gradually away from the mapped area until it was no longer visible. We conclude that reentry and circus movement, previously only shown in the heart, can occur in the myometrium with possible pathophysiologic implications.

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GLUTAMATE AGONISTS AND D-ASPARTATE RELEASE FROM HIPPOCAMPAL SLICES FROM DEVELOPING, ADULT AND AGEING MICE. P. Saransaari and S.S. Oja

D-Aspartate is a non-metabolized structural analogue of L-glutamate, the major excitatory transmitter in the brain. In general, it also mimics the behavior of L-glutamate in nervous tissue. In order to shed light on alterations in glutamatergic neurotransmission in the developing and ageing brain, we have now studied the effects of glutamate agonists on the release of preloaded D-[^{3}H]aspartate from hippocampal slices; a preparation also popular in electrophysiological investigations on amino acid transmitters. The release of D-aspartate induced by depolarizing concentrations of potassium ions from hippocampal slices significantly increased during the early postnatal development. This enhancement was further accentuated up to 18 months of age. Of the glutamate receptor agonists only kainate evoked release of D-aspartate from the slices but not the tested 2-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and N-methyl-D-aspartate (NMDA) agonists or antagonists. The kainate effect was discernible in all age groups but more pronounced in adult and old mice than in developing mice. The effect was attenuated by the kainate receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6,7-dinitroquinoxaline-2,3-dione (DNQX). The evoked release of D-aspartate was only partially calcium-dependent, indicating release from both cytosolic and vesicular pools. The results do not indicate any marked qualitative differences in D-aspartate release at different ages but show a marked general enhancement during ageing, probably being of significance with respect to the age-dependent changes in the cognitive functions in which the hippocampus is involved. (Supported by the Medical Research Fund of the Tampere University Hospital and the Medical Research Council of the Academy of Finland)

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THE MECHANISM OF MODULATORY ACTION OF NITROCOMPOUNDS ON CALCIUM ACTIVATED K^+ CHANNELS OF TAENIA COLI SMOOTH MUSCLE CELLS. A.Belevich, A.Zima and M.Shuba.

Nitrocompounds have been previously shown to produce relaxation of visceral smooth muscle (VSM) partly due to membrane hyperpolarization. To elucidate the mechanism of this effect the action of glyceryl trinitrate (GTN) and sodium nitroprusside (SNP) on calcium activated K^+ ($K^+(Ca^{++})$) channels in the membrane of smooth muscle cell isolated from guinea pig taenia coli has been studied using cell-attached path-clamp method. $K^+(Ca^{++})$ channels had a conductance 250±30 pSm, completely blocked by TEA^+ (1mM). 4-aminopiridine lacked to produce any effect on the activity of the channels. Application of GTN (0.1 mM) resulted in the increase of open probability of the channels in 3.4 times and mean open time in 2.2 times. SNP (0.1mM) increased the open probability of $K^+(Ca^{++})$ channels in 3.1 times and enhanced mean open time by factor 2.1. Methylene blue (0.005 mM), guanylate cyclase inhibitor, blocked the effect of both GTN and SNP. 8-Bromoguanosine 3',5'-cyclic monophosphate (1 mM) mimicked the action of nitrocompounds upon $K^+(Ca^{++})$ channels. These results indicate the involvement of $K^+(Ca^{++})$ channels in nitrocompound-produced hyperpolarization and relaxation of VSM. The activation of $K^+(Ca^{++})$ channels by GTN and SNP is supposed to occur through guanylate cyclase pathway.

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MEMBRANE BEHAVIOUR OF HUMAN MUSCLE FIBRES. M.J. Alberink, W. Wallinga, H. Wolters, D.L. Ypey and Th.P. Links.

A theoretical analysis of the electrical behaviour of human muscle fibre membranes is presented with special attention to the effects of defects supposed in hypokalemic periodic paralysis (HOPP), before the genetic defect of the disease was described. The deviations in HOPP are a slightly less negative membrane potential with a normal extracellular potassium level and a positive shift of some tens of mV during paralysis at reduced extracellular potassium level. The simulations were performed with a Hodgkin-Huxley model of the sarcolemmal and T-tubular membrane, describing the transmembrane potential, the capacitive current, the delayed rectifier potassium current, the regenerative sodium current and a leak current. It was studied whether changes in the ionic current components were able to introduce the changes in the membrane potential at HOPP. The addition of a constant sodium leak conductance shifted the original resting membrane potential to a slightly less negative value. A small fraction of non-inactivating sodium channels introduced an extra resting membrane potential less negative than the original one for normal potassium concentration. When, however, this fraction of non-inactivating channels increased with a reduction of the extracellular potassium concentration, the extra less negative membrane potential was only found for HOPP. In the model the removal of the T-tubular compartment did not affect its qualitative behaviour. Whether the genetic defect in the dihydropyridine receptor could be responsible for the changes in the sodium current as in the model has not yet been analyzed. We concluded that HOPP behaviour in the model membrane potential was evoked by changes in the sodium leak conductance and the partial lack of inactivation of the sodium channels for low potassium concentration in the extracellular medium.

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DISTRIBUTION OF FATTY ACIDS BETWEEN MEMBRANE MODEL AND WATER BULK PHASE. V.M.Babenko, I.A.Butovich, S.A.Ohiy.

Polyunsaturated fatty acid (PFA) peroxydation carried out by lipoxygenase is the first step of high potent biological active compounds synthesis. Our recent results allow to suggest that biological membrane play an important role in regulatory mechanism of this reaction. Now we present the data of the last study of PFA distribution between biological membrane model and water phase. For this research a new model of biological membrane based on octadecylsilane modified silica with PFA monolayer adsorbed on it has been proposed. The data obtained have been analysed using equation corresponding to the mechanism of distribution process.

$$[A]\cdot[S]/[A_S]=(K_4-K_2)/(1+K_1/[H^+])+K_2$$

were A and A_S - soluble and membrane associated PFA, respectively; K_1 - dissociation constant of membrane-bound form PFA, K_2 and K_4 - the equilibrium constants of PFA protonated and ionised forms desorption. This equation allows to estimate the individual constants of PFA distribution as well as the concentrations of membrane-bound and soluble PFA. We establish that desorption of PFA at pH<8 is negligible and significantly decreases at pH>10 (up to 50%). If this model contains phosphatidylcholin and PFA in molar ratio 2:1 the desorption of PFA at pH>10 is not more than 8%. The estimated pK_1 value are 9,1±0,1. We suggest that our results can be used for better understanding of physico-chemical properties biological membrane and membrane associated proteins.

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EFFECTS OF DEXAMETHASONE AND POLYPEPTIDE HORMONES ON $(Na^+ + K^+)$-ATPASE IN AMPHIBIAN RENAL DISTAL CELLS (A6) IN CULTURE. B. Lyoussi* and J. Crabbé

The renal amphibian cell line A6 has acquired prominence for studies of transcellular Na^+ transport. These cells, which derive from the distal straight renal tubule of Xenopus laevis, exhibit vectorial Na^+ transport and high transepithelial resistance when grown on appropriate support. We have compared the action exerted on $(Na^+ + K^+)$-ATPase by dexamethasone with that of vasopressine and insulin. $(Na^+ + K^+)$-ATPase activity was determined enzymatically on homogenized A6 cell monolayers, while ouabain binding sites were measured on dispersed A6 cells. After 24 hours of treatment with these agents, 10^{-7}M, Na^+ transport, reflected in short-circuit current increased almost five-fold, from 6.7±0.1 (SE) to 31.7±0.7 $\mu A.cm^{-2}$ in the case of dexamethasone (n=23). This was associated with an increase in $(Na^+ + K^+)$-ATPase activity which went from 5.5±0.3 to 12.6±0.6 μmol P_i/mg protein.h, whereas ouabain binding site density went from 269±29 (n=20) to 459±33 fmoles/10^6cells (n=12). Influence of the steroid on the enzyme remained demonstrable when stimulation of Na^+ transport was prevented by amiloride, 10^{-6} M, or by withdrawing Na^+ on the apical side. By contrast, insulin and vasopressin led to an increase in $(Na^+ + K^+)$-ATPase only when stimulation of Na^+ transport was not interfered with. Thus, dexamethasone acts on $(Na^+ + K^+)$-ATPase in epithelial cells capable of vectorial Na^+ transport directly, whereas insulin and vasopressin apparently do so as a consequence of intracytoplasmic changes (? increased Na^+ activity) which result from enhanced apical Na^+ conductance.

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EXTRACELLULAR PROTONS ACTIVATE AN EMBRYONIC EPITHELIAL CALCIUM-BLOCKABLE MONOVALENT CATION CHANNEL. R. Sabovcik, J. Li, P. Kucera and B. Prod'hom.

A calcium-blockable monovalent cation (CMC) channel is responsible for the large outward rectification of the transmembrane ion current of the ectodermal cells of the gastrulating chick embryo. As some epithelia have a low surface pH, we investigated the possible effect of high extracellular proton concentrations on the CMC channel with the patch-clamp method. Single-channel current recorded in cell-attached configuration in the absence of extracellular calcium decreases with increasing extracellular proton concentrations. The apparent pK_H at -50 mV is 5.7 with a Hill coefficient of 1. Proton binds inside the pore at an apparent electrical distance of about 0.2 from the extracellular side, as indicated by the slight voltage-dependency of the proton block. Spectral analysis of the excess open current noise indicates that the protonation reaction is governed by solvated protons. Extracellular calcium binds to the CMC channel with high affinity, K_{Ca} around 10^{-7} M. At -50 mV pH 7.4 no detectable single-channel current is observed in the presence 100 µM or 1 mM extracellular calcium. However, at pH 4.5 with 100 µM calcium, the inward current through the CMC channel has the same amplitude as the current measured in the absence of calcium. It is frequently interrupted by short closures caused by the calcium block. Increasing the calcium concentration to 1 mM increases the frequency of the short closures. The kinetic analysis of the calcium block reveals that the blocked time is unchanged while the calcium entry rate is decreased 120 times at pH 4.5. pK_{Ca} shifts from 5.7 at pH 7.4 to 3.5 at pH 4.5. In conclusion, these data show that extracellular protons unblock the CMC channel and allow an inward sodium current by reducing the entry rate of calcium without affecting the duration of sojourn of calcium inside the channel.

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The Membrane Potential and Its Ionic Mechanism in Poorly Differentiated Human Nasopharyngeal Carcinoma (CNE-2Z) Cells

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The membrane potentials of poorly differentiated human nasopharyngeal carcinoma (CNE-2Z) cells in culture and the mechanism by which they are established were studied by means of intracellular recording techniques using glass microelectrodes. The results indicated that the membrane potentials of CNE-2Z cells (-27±0.8mV, n=291) were significantly higher than those of the normal nasopharyngeal epithelial cells (-11.7±0.5mV, n=45, $p<0.01$). The coefficient of variation of membrane potentials in CNE-2Z cells was large and the distribution was diffuse. The membrane potential of CNE-2Z cells was inversely proportional to the logarithm of the K^+ concentration in the medium, whereas the membrane potentials did not change significantly when the Na^+, Ca^{2+}, or Cl^- concentration in the medium was increased. This suggests that the outward diffusion of intracellular K^+ through K^+ channels is the main ionic basis that establishes the membrane potentials of CNE-2Z cells.

Key words: Nasopharyngeal neoplasms, Membrane potentials, Ion concentration, Ionic channels

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AN IMPROVED MODEL OF MEMBRANE EXCITATION DYNAMICS.

R.Grebe and R.Vandenhouten

In this paper a model of the electrical properties of giant nerve fibre membranes is presented. Our objectives for this work were 1. to improve the well-known Hodgkin-Huxley (HH) model of membrane excitation by replacing terms of the empirical kinetic description with theoretic concepts while keeping the simplicity of the HH model, and 2. to find a physically plausible mechanism for the inactivation process of the Na+ channels. Similar to the HH model our model consists of a system of four differential equations describing the dynamics of the action potential, the K+ and Na+ conductances, and the inactivation of the Na+ channels, respectively. Unlike the HH model we do not model the inactivation independently of the activation process but instead assume the inactivation rate to be proportional to the actual Na+ current according to some kind of "channel exhaustion" mechanism. This leads to a non-linear term in the equation for the inactivation rate, depending also on the actual activation of the Na+ channels. The model has been used for the simulation of electrical membrane characteristics, in particular the current-voltage and conductance-voltage dependencies, and time series of action potential, K+ and Na+ currents and conductances as well as the inactivation associated with different temporary or constant depolarizations. The simulation results have been compared with experimental data from voltage clamp experiments with giant axons and proved to fit very well although the model is even simpler than the HH model since it renounces voltage dependent relaxation parameters. The voltage dependency of the inactivation kinetics, for example, is now an immediate consequence of the channel exhaustion mechanism. First simulations with varying "channel exhaustivity" suggest that the same model can also be used for describing the excitation dynamics of other types of membranes (like e.g. heart cell membranes).

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A1 ADENOSINE RECEPTORS — MOLECULAR SWITCH TO LATENT *N*-METHYL-D-ASPARTATE RECEPTOR-MEDIATED COUPLING BETWEEN HIPPOCAMPAL NEURONS.

T. Tsintsadze, A. Klishin, N. Lozovaya and O. Krishtal

When performed at increased external [Ca^{2+}] to [Mg^{2+}] ratio (2.5 mM / 0.5 mM), transient block of A1 adenosine receptors in rat hippocampus by 8-Cyclopentyltheophylline (CPT, 100 nM) leads to a dramatic and irreversible change in the excitatory post-synaptic current (EPSC) recorded by *in situ* patch clamp in CA1 pyramidal neurons. The relative contribution of *N*-Methyl-D-aspartate (NMDA) component into EPSC becames more pronounced. New ratio of NMDA to nonNMDA components of the EPSC remaines increased after removal of CPT and no longer depends on the activity of A1 adenosine receptors. When all functional NMDA receptors are previously blocked by irreversible use-dependent blocker, Dizocilpine maleate (MK-801, 100 μM), subsequent application of CPT leads to a partial reappearance of NMDA receptor-mediated current indicating at the recruitment of latent NMDA receptors. Altogether, these findings indicate at the existence of powerful system of latent NMDA receptor-mediated contacts between hippocampal CA3 and CA1 neurons with A1 receptors serving as a switch. The action of this switch depends on the concentrations of external divalents (Ca^{2+} and Mg^{2+}) and in many cases can be impaired by N-type Ca^{2+} channel blocker, ω-Conotoxin GVIA.

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HIGH AND LOW OSMOTIC ELECTROGENIC BICARBONATE TRANSPORT ACROSS CORNEAL ENDOTHELIAL CELLS.

C.G.Wigham and S.A. Hodson.

The objective of this study was to identify the mechanisms that generate electrogenic HCO_3^- transport across the corneal endothelial cell and the contribution of the different components to maintenance of corneal hydration. The effects of amiloride and acetazolamide ($5x10^{-4}M$) on rabbit corneal endothelial cell trans-endothelial short circuit current (s.c.c.), resistance (R_e), net HCO_3^- flux and ability to maintain corneal hydration were measured. Amiloride and acetazolamide reduced s.c.c. by 49 ± 4% and 36 ± 3% respectively, at this concentration R_e was unaffected. A combination of these inhibitors produced a small increase in effect but significantly less than if the inhibitors were acting independently and additively. Acetazolamide reduced net HCO_3^- flux to 42 ± 13 % of the control and no net Na^+ flux was measured across the preparation. Neither amiloride or acetazolamide significantly affected the ability of the endothelium to maintain corneal thickness or reduce the thickness of a pre-swollen preparation. In conclusion, we suggest that more than one, probably two, pathways for electrogenic movement of HCO_3^- across the endothelial cell are operating. One incorporates activity of the Na^+ /H^+ exchanger and cytoplasmic carbonic anhydrase, and its action is non-osmotic. The second pathway is osmotic but its components are not yet identified.

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PATHWAYS FOR SODIUM ENTRY INTO RABBIT CORNEAL ENDOTHELIAL CELLS. H.C.Turner, S.A.Hodson and C.G.Wigham.

The objective of this study was to investigate pathways of Na^+ re-entry into rabbit corneal endothelial cells mounted in vitro. Sodium selective microelecrodes were used to determine the effect of ouabain (1mM) on intracellular sodium concentration in the presence of amiloride ($5x10^{-4}$M) and HCO_3^- free Rabbit Ringer. Steady state $[Na^+]_i$ was 11.9 ± 0.1mM, the addition of ouabain caused $[Na^+]_i$ to increase exponentially, rate constant = 3.2 ± 0.3 $mMmin^{-1}$, from 12.0 ± 1.0 to 35.0 ± 3.1mM during the first 10 minutes of depolarisation. The time taken to reach equilibrium was 22 ± 1.2 mins. Amiloride caused $[Na^+]_i$ to decrease from 11.6 ± 1.2 to 6.9 ± 1.1mM and the ouabain induced Na^+ re-entry rate to decrease to 1.8 ± 0.2 $mMmin^{-1}$, time taken to reach equilibrium was >70 mins. The increase in $[Na^+]_i$ was approximately linear, during the first 10 minutes $[Na^+]_i$ increased from 7.2 ± 1.7 to 18.9 ± 4.3mM. In the absence of HCO_3^- the rate of Na^+ re-entry was also reduced. However, the pattern of $[Na^+]_i$ increase appeared to be biphasic, with an initial phase lasting 10-12 minutes where $[Na^+]_i$ rose rapidly, 2.4 ± 0.3 $mMmin^{-1}$, followed by a slow rise of $[Na^+]_i$, 0.6 ± 0.15 $mMmin^{-1}$. Time taken to reach equilibrium was again >70 mins. Determination of the exact time taken to reach equilibrium was difficult due to the inability to hold impalement for long periods, as the preparation was swelling due to ouabain inhibition. These results suggest that a significant component of Na^+ re-entry takes place via the Na^+-H^+ exchanger and that amiloride is an effective blocker of one component of Na^+ transport into rabbit corneal endothelial cells. A second component of Na^+ re-entry shows bicarbonate dependence although the pathway is yet to be identified.

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FLIP-FLOP OF ANIONIC PHOSPHOLIPIDS AND LONG-CHAIN AMPHIPHILIC SULFONATES IN THE HUMAN ERYTHROCYTE MEMBRANE DEPENDS ON MEMBRANE POTENTIAL

C. Haest, B. Deuticke, R. Ortwein and K. Eidmann

In the framework of our studies on the mechanisms of flip-flop of phospholipids in the erythrocyte membrane, we now studied the effects of membrane potential on the translocation of anionic phospholipids (^{14}C-lysophosphatidylmethanol and fluorescent NBD-labeled phosphatidic acid) and fluorescent long-chain amphiphilic 5-(N-decyl) aminonaphtalene-2-sulfonate from the outer to the inner membrane leaflet and vice versa. Due to the very high membrane-water partition coefficients of these anions (> 10^5), contributions of their potential-dependent partitioning into the membrane can be excluded. Recently, we found (Vondenhof et al, Biochemistry 33(1994)4517, Ortwein et al, BBA 1191(1994)317) that the anionic probes are in part (55-70 %) translocated across the membrane barrier by a flippase mode of operation of the anion exchanger AE1, band 3.

Inside-positive potentials of up to 70 mV due to outward directed Cl^- gradients resulted in an exponential increase of the inward flip rate. The enhancement of flip is suppressed by addition of ionophores that increase the membrane conductance for K^+ or H^+. The membrane potential effect concerns both band-3-mediated and nonmediated flip. Inside negative potentials established by outside-directed K^+ gradients in presence of valinomycin to increase K^+ conductance and after exchange of Cl^- for sulfate to lower anion conductance, decrease inward flip rates. The opposite effects of the positive and negative potentials were obtained for the outward translocations from the inner to the outer membrane leaflet.

The observed potential dependencies indicate that the translocation of the anionic amphiphiles via band 3 is an electrogenic process, whereas the band-3-mediated exchange of hydrophilic anions is electroneutral.

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THE ROLE OF FIBRONECTIN IN HUMAN AND RABBIT TEARS.

S.A. Hodson, N. Al-Tamimi and C.G. Wigham.

Antifibronectin is known to disrupt the physiology of the pre-corneal tear film. The purpose of this investigation was to estimate the concentration of fibronectin in collected tear fluid, resting and stimulated and to localise the fibronectin in the tear film. Fibronectin was assayed using a double sandwich ELISA technique. Localisation of fibronectin in rabbit tear/corneal epithelial interface was determined by electron microscopy of the appropriately conjugated gold particles. Human fibronectin concentrations were 260 ± 61 $ngml^{-1}$, n=38 and <5 $ngml^{-1}$ n=38 resting and stimulated respectively. Fibronectin was not detected (<5 $ngml^{-1}$) in stimulated rabbit tear fluid. Tear fluid total protein concentration in human was 8.14 ± 1.0 $mgml^{-1}$ and 4.6 ± 1.2 $mgml^{-1}$ resting and stimulated respectively. Immunocytochemical investigation of rabbit tear film showed the presence of an extracellular layer of fibronectin closely apposed to the apical membrane of the outer squamous cells of the corneal epithelium. We conclude that an element of fibronectin in human tear film is bound to the epithelial cell apical surface and that this layer might be responsible for the devatating effect on the optical efficacy of the tear film seen when antifibronectin is added. It seems unlikely that the concentration of fibronectin in the collected tear fluid accurately represents the concentration in the tear film. Nevertherless the disproportionate loss of fibronectin when lacrimal secretion is stimulated may suggest that tear fibronectin does not originate in the lachrymal glands.

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FUNCTIONAL PECULIARITIES OF POTASSIUM CHANNELS IN RAT LINES SELECTED FOR THE EXCITABILITY OF THE NERVOUS SYSTEM. A.Vaydo, A.Mokrushin

The aim of the present study was to investigate genetically determined polymorphism by functional characteristics of the potassium channels in four rat lines selected for threshold of the nervous system excitability to electric impulses and differing in some functional properties of the nervous system and behavior.Extracellular field potentials were recorded in surviving tangential slices of the olfactory cortex and Gardos phenomenon was used for estimate of Ca++ dependent K+ -channels in erythrocites membranes. The interstrain differencies were found in: (1) The functional characteristics of K+ -channels connected with GABAb-receptors in olfactory cortex neurons. (2) The functional peculiarities of Ca++ dependent K+ -channels in plasmolemma of erythrocites. These results allow to choose the rat lines being perspective for the gene ingeneering analysis of the structure/function peculiarities of the genes, determining components of different K+ -channels in rat.

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NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR CHANNEL: STRUCTURE AND BLOCKING MECHANISMS. S. Kertser, S. Voitenko, A. Bobrishev, N. Brovtsyna, V. Gmiro, and V. Skok

The dimensions of neuronal nicotinic acetylcholine receptor (AChR) channel were deduced from a correlation between the dimensions of the open channel-blocking molecules and their blocking activities. Both bis-quaternary ammonium compounds, the pentamethonium derivatives, and mono-quaternary ammonium compounds, were used as probes. The blocking activities were estimated from the blocker-induced reduction of the ACh-induced membrane currents recorded from the non-dissociated neurons of rat superior cervical sympathetic ganglion with the whole-cell patch clamp recording method. The open channel block was evidenced by voltage dependence of the blocking effect and by the blocker-induced decrease of the excitatory postsynaptic current (e.p.s.c.) decay time constant. The results obtained suggest that the probability of the blocker interaction with the channel is the highest at the channel cross-profile approximated with a circle of 12.5 Å diameter, and declines at the diameters 11.9, 10.5, 9.1, and 8.4 Å, i.e. with the increasing depth of the funnel-shaped channel. The selectivity filter can be approximated by a rectangle of 5.8 x 8.0 Å.

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MODULATING ACTUION OF PROTEIN A AND PEPTIDOGLYCAN ***STAPHYLOCOCCUS AUREUS*** ON RECULATORY MEHANISMS OF SMOOTH MUSCLE RECEPTOR COMPLEXES. T.L.Davidovskaya, I.B.Phylippov and L.S. Kholodnaya

The aim of the prewsent investigation were studies on the modulating action of protein A (PA) and of peptidoglycan (PG) from ***Staphylococcus aureus*** on signal transmission in Ca^{2+}-polyphosphoinositide messenger system (Ca^{2+}-PPI-system) of smooth muscle. The results obtained so far show that PA and PG inhibit the acetylcholine-induced contractile response of myometrium smooth muscle. Calculations of inhibition constant by the method of Hunter & Downs demonstrate that interaction of these reagents with cholinoreceptors of smooth muscle strips is characteristic for non-competitive inhibition. It is suggested that PA and PG inhibit the key enzymes of the polyphosphoinositide cycle due by lowetring the Ca-mobilizing action of this cascade. PA and PG do not affect the myometrium mechanical response on addition of oxytocin (OT). The same OT action is found in preparations of the swine left coronary artery maybe due to protein-kinase C activation in the Ca^{2+}-PPI-system while contractile response by itself is realized in the Ca^{2+}-independent way. It is established that in smooth muscle PA and PG may alter the propperties of both chemo- and electroexcitable membranes. These reagents enhance the mechanical response of the guinea-pig myometrium for higher concentrations of sodium ions in physiological solution.

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LOW-VOLTAGE ACTIVATED CALCIUM CURRENTS IN THALAMIC RAT BRAIN SLICE. P.Kostyuk, A.Tarasenko and A.Eremin

The patch-clamp technique was employed in thalamic slices to examine the characteristics of low-voltage activated (LVA) Ca currents. Laterodorsal (LD) thalamus nucleus in sagittal slice of rats brain was identified according to the atlas by G.Paxinos and Ch.Watson. To characterize the LVA Ca current *in situ* situation a voltage-clamp study was undertaken since earlier investigations of these channels were performed either in acutely esolated thalamic neurons (ventrobasal complex; Coulter et al, 1989) or in several-week-old cultures of embryonic neurons. Experiments were carried out using 14-day-old rats. LVA Ca currents could be evoked by step depolarization to potential more positive than -75 mV from holding potentials of -95÷-90 mV. They reached maximum at about -55 mV with mean amplitude 765±57 pA (n=16). Inactivation of these currents could be described by an exponential decay with a voltage-dependent time constant of 32±5 ms at -55 mV (holding potential -95 mV). Fractional current (normalized to the maximum current) could be fitted with the following equation: $I/I_{max}=1/\{1+\exp[(V-V_{0.5})/k]\}$. Best fit for averaged fractional steady-state inactivation was obtained with $V_{0.5}$ = -85.6 mV and k=3.1. This restricted the range of potentials (-95÷-75 mV) where LVA Ca channels could effective operate and the time of Ca influx through these.

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SOME FEATURES OF EVOKED BRAIN POTENTIALS AND NEUROPSYCHOLOGIC CHARACTERISTICS OF PATIENT WITH VARIOUS TYPES OF DEMENTIA. P.Kravtsov, S.Sherbakov,A.Snegyr, B.Ivnev

The functional state of cortex structures of the brain in psychially normal subjects (group 1, age range from 64 to 75) and patients with atherosclerotic dementia (group 2) and Alzgeimer's disease (group 3) was studied by the method of auditory and visual evoked brain potentials (EP) as well as by psycholophysiologic testing. A psychophysiologic testing performed during investigation of each group gave statistically significant variations in the parameters of a simple sensomotor reaction (mean time of reaction, the number of erroneous reactions), temping-test (duration of the reaction). The testing results are indicative of lowering dynamics of nerve processes, prolonged time of reaction and low ability to maintain the necessary rhythm of work in patients with atherosclerotic dementia and Alzgeimer's disease. While analyzing EP we found the latent period of P1L1 and P2 components to have no significant variations in all three groups, the amplitude of P3 components in the mode with activation of attention being much greater than that of the same component in the mode without activation of attention (group 1). The principal marker distinguishing EP in groups 1 and 2 is the P3 (P300) component, its latent period much greter than that of P300 in norm. In patients with Alzheimer's disease the P3 component is expressed in significantly against a background of significant by smoothed out components after P2.Comparision of the testing data and EP showed a complete correlation between the degree of disturbances in psychophysiologic testing and EP parameters in groups 2 and 3 especially according to the P300 component in activation of attention.

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CHARACTERIZATION OF NEUROPEPTIDE RECEPTORS IN ASTROCYTES DERIVED FROM RAT CIRCUMVENTRICULAR ORGANS USING CALCIUM MEASUREMENTS. A.R. Müller, E. Gebke, M. Jurzak and R. Gerstberger.

Circumventricular structures (CVOs) of the rat brain, the subfornical organ (SFO) and organum vasculosum laminae terminalis (OVLT), contain neurons and glial cells. The lack of a blood-brain barrier enables the SFO and OVLT to act as receptive structures for circulating hormones and neuropeptides. Angiotensin II (AII), vasopressin (AVP) and endothelins (ET1/ET3), involved in the central control of the extracellular fluid compartment, modify CVO neuronal activity. Information is lacking concerning intracellular signal transduction in CVO neurons, and mainly glial cells as possible transducers between endothelium and neurons. Therefore, primary cell cultures derived from both the OVLT and SFO of one-week old rats pups were used to measure alterations in intracellular calcium ($[Ca^{2+}]_i$) at the single cell level. Astrocytes were subsequently identified by immunocytochemical staining for GFAP as astrocyte-specific marker. After application of the respective neuropeptide, dose-dependent (1-100 nM) transient rises in $[Ca^{2+}]_i$ were recorded from OVLT (AII: 22 (16% responsive), AVP: 13 (38%), ET1/ET3: 42 (17%) and SFO (AII: 22 (5%), AVP: 21 (62%), ET1/ET3: 73 (20%)) astrocytes. The pharmacological characterization of the AVP-receptor subtype revealed a complete, reversible block of the Ca^{2+}-transients in the presence of a V_1-receptor antagonist (OVLT: 5, SFO: 9), whereas the V_2-specific agonist dDAVP could not mimick the AVP-effect (OVLT: 9, SFO: 17). AII-induced Ca^{2+}-transients were reversibly suppressed by the AT_1-specific antagonist DUP 753 (OVLT: 2, SFO: 4), but not the AT_2-specific antagonist PD 123319 (OVLT: 2, SFO: 2). The increase of the $[Ca^{2+}]_i$ by ET1 or ET3 could be reversibely inhibited by the ET_A-antagonist BQ-123 (SFO: 14), but most astrocytes proved to react to ET1 or ET3 exclusively. Not only CVO neurons, but also astrocytes appear to express functional V_1-, AT_1- and ET_A-receptors for the neuropeptides AVP, AII and ET, respectively.

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ANTAGONISM BY TACRINE OF CAFFEINE-INDUCED INHIBITORY EFFECTS. A. Røed

The anticholinesterase drug tacrine may relieve the symptoms of Alzheimer's disease, possibly by prolonging the effect of the neurotransmitter acetylcholine. Caffeine may inhibit neuromuscular transmission and muscular contraction by depleting intracellular stores of Ca^{2+} in the motor nerve terminal and in the muscle cell, respectively. In the present experiments, the interaction between caffeine and tacrine was investigated in the isolated rat phrenic nerve-diaphragm preparation at 37°C in conventional Tyrode solution with pH 7.2. The tension induced by supramaximal twitch (0.1 Hz) indirect or direct stimulation was recorded. Tacrine, 2.5-5.0x10^{-5} M, antagonized the inhibitory effect of caffeine, 10 mM, at the neuromuscular junction during indirect stimulation, and of caffeine, 15 mM, of the muscle cell during direct stimulation. Addition of tacrine after complete caffeine-induced block at the neuromuscular junction also caused a recovery of the twitches, and the contracture tension induced by caffeine was relieved by tacrine. These antagonisms of the caffeine effects were probably not due to cholinesterase inhibition since they were not observed with the anticholinesterase drug neostigmine. However, both drugs caused the typical potentiation during twitch stimulation which is caused by multiple action potentials generated by the prolonged endplate potentials due to the cholinesterase inhibitory effect. Since caffeine inhibits due to a depletion of intracellular stores of Ca^{2+}, a sparing effect of tacrine on these stores, which may supply Ca^{2+} as a cofactor for uptake of choline and/or resynthesis of acetylcholine, may be an additional mechanism of action for the suggested therapeutic effect of tacrine on Alzheimer's disease.

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INFLUENCE OF ACUTE HYPOXIA ON FREE RADICALS IN THE BRAIN TISSUE AND PLASMA OF THE ADULT RATS

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Investigations were performed on adult Wistar rats with acute hypoxia through the ligation of left vertebral artery for ten minutes. We performed a comparative study of nitric oxide (NO) and malonyl dialdehyde (MDA) by measuring GSH, G6-PDH and LDH in samples from the cerebellum, cerebral cortex and blood (control vs.hypoxia). NO was determined indirectly by HPLC function of the citruline content, considering their stoichiometric relationships. MDA, GSH, G6PDH and LDH were measured using the appropriate classic techniques.

NO presented a significant rise in the hypoxic cerebellum from 0.06 nM/g to 0.2 nM/g while no important changes were found in the cortex. This increase of NO was accompanied by intensification of the activity of G6PDH in the cerebellum from 5.26 µM/g/min to 6.50 µM/g/min. In the cortex there was no significant variation of G6PDH ten minutes after hypoxia. MDA formed by lipid peroxidation rised in the blood from 75nM/g Hb to 94 nM/g Hb while GSH decreased both in the neuronal cytosol and in the blood.

Our results bring about new experimental evidence in support of the participation of oxygen-containing free radicals and of NO to the inbalances of metabolism in the brain subjected to acute hypoxia.

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ANXIOLYTIC-LIKE ACTION OF L-NAME, NITRIC OXIDE SYNTHASE INHIBITOR, IN THE ELEVATED PLUS-MAZE. V. Volke, S Kõks, E. Vasar and P.T. Männistö[1]

Nitric oxide (NO) is known as a neural messenger in the central nervous system. The aim of present work was to study the involvement of NO in the regulation of anxiety and to determine its role in the action of anxiogenic and anxiolytic drugs. The elevated plus-maze test of anxiety was used to reveal the action of NO on the rat behavior. NO synthase (NOS) inhibitor N^G-Nitro-L-Arginine Methyl ester (L-NAME) was employed to block the function of NO. The pretreatment of rats with L-NAME (1-20 mg/kg) increased the exploratory activity in reversed U-shape manner. The anxiolytic-like effect of L-NAME was statistically significant at a dose of 10 mg/kg. The similar doses of L-NAME (1-10 mg/kg) tended to reduce the anti-exploratory action of caerulein (5 µg/kg), an unselective agonist of CCK receptors. The anxiogenic-like action of N-Methyl-DL-Aspartate (NMDLA, 30 mg/kg i.p.) was also attenuated by the pretreatment with 10 mg/kg of L-NAME. However, this effect was not statistically significant. Differently from the previous studies the pretreatment of rats with L-NAME did not antagonize the anxiolytic effect of diazepam (2.5 mg/kg) in the plus-maze. Ten mg/kg of L-NAME even augmented the action of diazepam. Consequently, NO appears to act as a neural messenger in the neural networks related to anxiety in the rat.

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A HUMAN NEURON-LIKE CELL LINE FOR THE STUDY OF CALCIUM REGULATION. I. Stevens, R. Nuydens, M. de Jong, F. Cornelissen and H. Geerts

Excitotoxic insults play a major role in many neuropathological conditions. Until now, it was very difficult to monitor the $Ca^{2+}{}_i$ regulation during and after an excitotoxic insult in an *in vitro* human neuronal system.

The human neuroblastoma cell line NT2N [1] can be induced to a postmitotic state by long-term treatment with retinoic acid. We studied the neuronal characteristics of this cell line by morphological and functional criteria.

Immunocytochemical studies revealed expression of specific neuronal markers, such as neuron-specific enolase, neurofilaments and tau proteins, whereas GFAP labelling was negative.

Using Fura-2 as an indicator of free intracellular $Ca^{2+}{}_i$ we studied the $Ca^{2+}{}_i$ response in these cells upon elevation of extracellular K^+, this causes membrane depolarization and a subsequent $Ca^{2+}{}_i$ increase. This suggests that functional voltage-sensitive channels are present. Application of 1 mM glutamate also caused a significant $Ca^{2+}{}_i$ increase [2]. To further dissect the various glutamate receptor subtypes, specific agonists were applied. NMDA, in the presence of glycine and in the absence of Mg^{2+}, led to a significant $Ca^{2+}{}_i$ response.

The data suggest that these cells do possess functional voltage-sensitive Ca^{2+}-channels and some subtypes of glutamate receptors. The relative large availability of the cellular material makes this system an attractive tool to study Ca^{2+}-regulation after excitotoxic insults in a human neuronal cell type.

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ALTERED CA^{2+} HOMEOSTASIS DURING INDUCTION OF APOPTOSIS IN PC12 CELLS. G. Dispersyn*, R. Nuydens, M. de Jong, R. Nuyens, F. Cornelissen, H. Geerts and M. Borgers

Selective neuronal cell death through apoptosis is an important process during normal tissue development, but in some pathological conditions such as chronic neurodegenerative diseases, apoptosis has been documented to occur.

NGF differentiated PC12 cells were used to investigate the effect of nerve growth factor withdrawal on the $Ca^{2+}{}_i$ homeostasis and cell survival (Furukawa et al., 1993).

Upon NGF removal from the medium differentiated cells retract their processes and most cells die within 48 hrs. Cell death occurs through an apoptotic pathway as indicated by morphological assays and specific labeling using ApopTag to detect digoxigenin labelled nucleotide residues which are added by TdT to the 3'-OH termini of DNA strand breaks (Su et al., 1994).

Using Fura-2 as an indicator of free intracellular $Ca^{2+}{}_i$ we compared the $Ca^{2+}{}_i$ homeostasis in differentiated and NGF deprived PC12 cells. Elevation of the extracellular K^+ causes membrane depolarization and subsequent $Ca^{2+}{}_i$ increase. In the NGF deprived cultures the number of viable cells responding to different levels of membrane depolarization was significanty decreased. Using antibodies against ω-conotoxin and fluorescent dihydropyridines, the density of voltage-sensitive channels was studied.

To further evaluate the different intracellular processes intervening in neurotoxicity, we showed that interventions aimed at reducing $Ca^{2+}{}_i$ were able to attenuate neurotoxicity. In addition, olomoucine, an inhibitor of cylin-dependent kinases was also able to reduce neurotoxicity.

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THE RELATIONSHIPS BETWEEN HANDEDNESS, FINGER TAPPING AND SPATIAL MEMORY TASKS. E. Nalçici, M. Çiçek, El Pamuklar and S. Yavuzer

Among 19-21 years old, eighth-one healthy, undergraduate medical school students were randomly choosen as subjects. Their hand preferences were determined by using 13 item questionnaire (L.J. Chapman, 1987). For finger tapping and spatial memory tasks, subjects were required to use a mouse, while they faced a computer screen. The average speed of each hand and dominance score [(right hand-left hand/right hand)x100] were computed after 3 trials. In spatial memory task, subjects were asked to place blocks that were presented previously. After 12 trails, left, right and overall visual field accuracy and latency were measured, and laterality score [(right visual field-left visual field)/(right visual field+left visual field)] was computed for each subject. A significant correlation (r=-.518) between handedness and dominance scores of finger tapping was found. In right handed group (n=66), left hand was slower than right hand, while there wasn't a significant difference between hands of non-right handed group (n=15). Right handed subjects had significantly more left visual advantage, but their performances on spatial memory task were approximately the same with non-right handers. On spatial memory, a negative correlation (r=-.424) between laterality score and total accuracy was observed. This interaction indicated that subjects with high spatial ability had no field advantage. On latency of spatial memory tasks, right handed men (n=35) showed better performance than right handed women (n=31). These results suggest that right handers have more asymmetric brain in comparison to non-right handers, and they have different cognitive strategies.

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LATENT EXITATORY CIRCUITS IN CA1 AREA OF HIPPOCAMPUS, T.Tsintsadze, A.Klishin, N.Lozovaya and O. Krishtal

Inhibition of A_1 adenosine receptors on the background of increased external Ca^{2+}/Mg^{2+} ratio leads to a dramatic and irreversible change in the EPSC recorded by *in situ* patch clamp in CA1 pyramidal neurons. The kinetics of EPSC becomes stimulus-dependent and markedly slows down with the increase in the stimulus strength. The stimulus-dependent fraction of EPSC is carried through NMDA receptor-operated channels, but disappears under either NMDA antagonist, APV, or nonNMDA antagonist, CNQX. This indicates at the polysynaptic nature of acquired stimulus-dependence of the kinetics of EPSC: predominantly nonNMDA receptor-mediated stimulation of CA1 neurons via Schaffer collaterals is followed by the activation of previously silent NMDA receptor-mediated connections between CA1 neurons. These connections become operational on a long-term basis.

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CHANGES IN FEMORAL ARTERIAL BLOOD FLOW AND SYSTEMIC ARTERIAL PRESSURE EVOKED FROM DIFFERENT REGIONS OF MEDULLA OBLONGATA. I.Kukulis, J.Gailans, G.Strazda and I.Taivans.

It is well known that there exist areas in the brain stem which control blood supply of special vascular beds. Our aim was to find in medulla oblongata areas responsible for the control of femoral vascular bed (electromagnetic flow meter) and to clear out how it correlates with generalized cardiovascular control. Experiments were performed on 40 chloralose+urethane anaesthetized and paralyzed cats. In total 1212 points in the medulla were electrically stimulated. Decrease in the FBF accompanied by increase in BP was observed in response to stimulation of 419 points. Such reactions were evoked from the nucleus (nuc.) gigantocellularis, ventral part of nuc. raphe obscurus, dorsal part of nuc. raphe magnus, nuc. centralis medullae, dorsomedial part of the internal division of the lateral reticular nuc., paragigantocellular reticular nuc., and the region which spreads ventrorostrad to ambigual nuc. and encircles the facial nuc. The stimulation of 100 points located mainly in the lateral part of the medulla close to its ventral surface caused elevation of FBF and pressor reaction. The reduction of FBF accompanied by the depressor reaction was elicited from 229 medullary points located mainly in vagal complex and regions dorsal to dorsal accessory nuc. of the inferior olive as well as between lateral reticular nuc. and magnocellular division of the alaminar spinal trigeminal nuc. Increase in FBF accompanied by the depressor reaction was evoked from 98 points located rostrad to olivary complex as well as from small areas in the magnocellular tegmental field ventral to ambigual nuc.

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SUBSTANCE P AND NEUROKININ-A ADMINISTERED AS AN AEROSOL AFFECTS RESPIRATION IN CONSCIOUS GUINEA-PIGS. B. Koch, Å. Edvinsson and L-O. D. Koskinen.

Tachykinins modulates respiration in several ways. However, little information is available on respiratory effects of tachykinins delivered as aerosols. This study was designed to evaluate the inhalation toxicity of substance P (SP) and neurokinin A (NKA) in guinea-pigs when inhaled together with the protease inhibitor thiorphan. Apart from the acute toxicity of the peptides the effects on respiration were investigated. A non-invasive and non-traumatic method evaluating the effects in conscious animals was used. Tidal volume and respiratory rate were recorded on line with headout wholebody plethysmographs.

LC_{50} (15 minutes) for the peptides were 45 and 368 $\mu g/m^3$ for NKA and SP respectively, when inhaled as aerosols and together with thiorphan. Both peptides caused an increase in respiratory rate preceding a decrease in tidal volume. As the exposure proceeded a decrease in both respiratory rate and tidal volume was observed and this continued either until the animals died or to the end of exposure.

The bronchoconstriction caused by the peptides is considered to be mediated primarily by NK-2 receptors to which NKA has higher affinity compared with SP. This is probably the major explanation why NKA is more effective than SP. SP and NKA affects the respiratory regulation by stimulating neuropeptide sensitive vagal afferents in the respiratory epithelium. Furthermore, at least SP, is thought to affect the carotid body chemoreceptor excitation and modulate respiration. The fact that the respiratory rate finally was reduced is probably due to the increasing bronchoconstriction.

Our study clearly indicates that tachykinins modulates respiration when administered as an aerosol and that NKA is more potent than SP.

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PHYSIOLOGICAL RESPONSE TO CHRONIC NOISE EXPOSURE. M.T.M. van Raaij, C.J.G. Dobbe, and M. Oortgiesen.

Chronic noise exposure is a common phenomenon in western societies Besides the development of hearing loss, noise exposure may induce a number of health effects including hypertensia. However, the most common effect is the induction of annoyance, a general feeling of discomfort. Continued exposure may result in a chronic stress condition which renders the organism more vulnerable to novel challenges. The primary goal of this study was to develop a chronic stress protocol to study the effects of chronic noise exposure. Male wistar rats were used throughout the study. The animals were fitted with an indwelling catheter in the vena cava for repeated bloodsampling and were exposed to a chronic intermittent noise protocol for three weeks. This protocol consisted of 180 randomly distributed pulses (1min. duration) of white noise (band: 2-20 kHz) on each day with an intensity of 85 dB or 90 dB. Measurements included plasma catecholamines, ACTH, corticosterone, prolactin and glucose levels. Hormonal levels were significantly effected during the 3 wks noise exposure (especially catecholamines) indicating an "arousal" type of stress condition. In seperate experiments using non-cannulated animals, immunological parameters were measured. Significant changes were observed for splenic NK cell activity, the lymphocyte proliferation response to general mitogens and the phagocytic activity in peripheral blood. The consequences of chronic noise exposure for the vulnerability to novel challenges (e.g. novel acute stress, behavior and immunological status) will be addressed.

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VASOPRESSIN CONTENT IN THE FLUID PERFUSING THE CEREBRAL VENTRICLES AFTER VAGUS NERVE STIMULATION IN RATS. M.Orłowska-Majdak and W.Z.Traczyk

The aim of the present study was to investigate vasopressin (AVP) release into the fluid perfusing the cerebral ventricles. The experiments were carried out on male rats in anaesthesia. The animals had two cannulas introduced into the lateral cerebral ventricles and the third cannula into the cerebellomedullary cistern. Cerebroventricular system was perfused with McIlwain-Rodnight's solution from lateral ventricle to cerebellomedullary cistern and successive 30-min samples were collected. During the collection of the third portion the central ends of both cut vagus nerves were stimulated bipolarly with electric pulses at 60 Hz frequency, 2 msec duration and amplitude up to 8 V, intermittently. Vasopressin concentration in the perfusing fluid was determined by RIA. The highest concentration of AVP we observed in the first portion, because it included the greatest amount of cerebrospinal fluid (CSF). Electrical stimulation of both vagus nerves did not change considerably the release of AVP into the fluid perfusing the cerebral ventricles in rat, although a certain upward tendency could be observed, specially within the first hour after cesation of stimulation. It seems that only AVP raised in circulating blood and not in CSF after vagus nerves stimulation may act on the central nervous structures. AVP can be easily uptaken by CNS structures devoid of blood--brain barrier and in markedly less amounts by structures possessing such a barrier.

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CHRONIC ETHANOL DIFFERENTIALLY CHANGES OPIOID RECEPTOR BINDING AND THE ACTIVITIES OF ENKEPHALIN-DEGRADING ENZYMES IN SEVERAL REGIONS OF THE RAT BRAIN. *M. Rottmann, W.-E. Siems, G. Heder, I. Roske, J. Wolffgramm and M. Melzig*

An important system proposed to be modulated by ethanol and its metabolites is the endogenous opioid system. We have used brain membranes of rats with different and well-defined kinds of ethanol experience in order to study (*i*) the alterations of kinetic parameters for the binding of two highly selective μ- and δ- opioid ligands ([^{3}H]DAMGO and [^{3}H]Ile$^{(5,6)}$-deltorphine II, resp.) and (*ii*) the changes in the activities of enzymes involved in the degradation of enkephalins. Forty-nine male Wistar rats were divided into four groups and maintained on different regimens, each of them characterized by the rats' access to water and ethanol in concentrations of 5%, 10%, and 20% (v/v). The animals of one group (termed as "addicted" rats) were allowed free-choice consumption of any of these four beverages for 20 months; this treatment was restricted to 6 months for a group of "controlled drinkers". "Forced drinkers" were exclusively exposed to ethanolic drinking solution (5%, 10%, and 20% EtOH) for 6 months. These animals were compared to a control group, which received only water. Upon treatment, animals were of about the same age. They were sacrificed and crude brain membranes of eight different brain regions were prepared for receptor binding and enzyme assays. According to our data, the four treatment groups differ significantly in regional K_D and B_{max} values for μ- and δ- opioid receptors as well as in the activities of neutral metalloendopeptidase (NEP) and of angiotensin-converting enzyme (ACE). We conclude that there are characteristic patterns of receptor binding and enzymatic parameters that permit to discriminate certain schemes of alcohol intake.

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EFFECT OF NICOTINE ON AVOIDANCE LEARNING AND MEMORY IN RATS. S. Ayhan, B.Ocakçıoğlu, M. Pasin, A. Elhan, E. Tüccar

Literature on the effects of smoking and nicotine upon learning and memory are contradictory; displaying considerable variation such as improvement, no change and impairment. The inconsistencies may be at least partly due to different types of learning tasks and experimental designs.

The presented research is aimed at the investigation of the effects of different doses (0.2- 0.4 and 0.8 mg/kg) of nicotine on learning and memory and within this scope the two way avoidance learning method was used and the animals were trained by using an automated shuttle box (TKK. Inst.Japan).

The result of experiments showed that avoidance learning of rat was lower in 0.2 mg/kg and higher in 0.8 mg/kg nicotine treated rats when compared with control group. The conditioned avoidance response of nicotine treated animals were found to decrease when they retested after 10 days of the rest period whereas the control rats showed no differences in this test. the re-administration of nicotine completely improved the performance of nicotine pretreated rats.

The present results suggest that nicotine in high dose (0.8 mg/kg) seem to potentiate the acquisition of avoidance learning in rats. On the other hand the pretreatment by high dose of nicotine has been found no-influence on the retention of avoidance response but the re-administration of nicotine induced a complete retrieval.The result of low dose of nicotine (0.2mg/kg) seem to be contradictory. It has been reported that the lower dose but not higher doses of nicotine has a potent antinociceptive action in rats.In our experiments the opposite action of 0.2mg/kg nicotine may be due to the depression of the performance of rats because of the analgesia induced by nicotine.

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PSORALENS — A NEW CLASS OF POTASSIUM CHANNEL BLOCKERS FOR THE SYMPTOMATIC TREATMENT OF MULTIPLE SCLEROSIS. K.H. Bohuslavizki, G. Ditzen, F. Gerst, W. Hänsel, E. Koppenhöfer, A. Reimers and K. Sanmann

Potassium channel blockers like 4-aminopyridine (4-AP) are known to improve the degraded impulse conduction in plaques associated with multiple sclerosis (MS). We found 5-methoxypsoralen (5-MOP), already known from PUVA therapy of psoriasis, to act as a highly selective potassium channel blocker: in potential clamp experiments on intact myelinated axons the potassium currents were blocked in a time dependent fashion leaving the sodium currents unaffected, in particular in the functionally important threshold region. This is a crucidal feature of psoralens depending on the location and nature of certain functional groups. We have found that the methoxy-group in position 8 has nearly no blocking action, but 5-ethoxypsoralen is more effective than 5-MOP, with comparable selectivity. In single-trial tests on MS-patients repeated doses of 5-MOP (0.5 mg/kg·d) administered to 9 patients led within 1 to 2 days to: 1. reduction of spasticity (n=7), 2. reduction of paresis (n=4), 3. reduced need for antispastica (n=6), 4. significantly elevated mood (n=6). All observations were evaluated by means of a standardized disability status score (U.Patzold: Multiple Sklerose, Thieme, Stuttgart, 1985). In other cases salient improvements were found in gait, in bladder and rectal disorders and in potency. Interestingly, the ratio of responders to non-responders could not be enhanced by raising the dosage. Long-term treatment up to two years has not yet revealed any undesired side effects. This seems to be of considerable relevance when comparing the therapeutical potential of 5-MOP to 4-AP.

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A STUDY ON NEUROMUSCULAR EXCITABILITY AT MENOPAUSAL WOMEN. G. Raca, S. Gusti, D. Raca, A. Dunarintiu-Gusti

The women who cross the climacteric period have important hormonal disturbances such as estrogen deficiency and elevated levels of gonadotropins, possible modifications of androgens, cathecolamines, TRH and thyroid hormones. Very frequently, these hormonal changes are accompanied by heightened nervous irritability, augmentation of sympathetic vegetative tonus, a particular psychological status and various somatic manifestations. For women there are many hormonal and nervous factors which influence the neuromuscular excitability. Thus, it is possible that the above factors to modify the neuromuscular excitability at the menopausal women. We have investigated 279 women with certain signs of gonadal hormonal decline, but without having any disease or spasmophylia. These women have constituted two groups - the perimenopausal women and the postmenopausal women. The neuromuscular excitability was determined by the electromiographic method (EMG) activated by ischaemia and hyperventilation; repeated discharges of potentials were considered a positive EMG test. In addition, the plasma levels of calcium and magnesium were determined and the electroencephalogram (EEG) was registered too. Our results are summarized below. For perimenopausal women, the following aspects were found significant: increase of neuromuscular excitability by EMG test ($p<0,02$), low plasma level of magnesium ($p<0,05$), ample and sharp alpha waves ($p<0,001$), ample and grouped theta waves ($p<0,01$). For postmenopausal women, there were found other significant aspects: hypocalcemia ($p<0,001$), low voltage EEG traces ($p<0,001$), low voltage theta waves ($p<0,05$). These results suggest an increase of neuromuscular excitability for perimenopausal women in connection with the endocrine changes, heightened nervous irritability, possible low level of magnesium. This fenomenon is a specific manifestation of menopause or a return of a latent spasmophylia.

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THE ROLE OF INTERACTION BETWEEN 5-HT RECEPTORS AND α2-ADRENOCEPTORS IN THE REGULATION OF SEIZURE SUSCEPTIBILITY IN DBA/2 MICE. T.Semenova, M.Ticku.
The understanding of the mechanisms of audiogenic seizures (AS) susceptibility must ultimately involve the establishment of developmental correlations between AS susceptibility and neurotransmitter traits. The relative importance of various neurotransmitter systems in modulating seizure threshold, especially serotonergic, noradrenergic, GABA-ergic and excitatory amino acid systems was indicated. In this report we have compared the effect of 5-HT$_{1c}$, 5-HT$_3$, 5-HT$_3$ and 5-HT$_4$ receptor antagonists in the regulation of AS in the absence and presence of yohimbine, in younger DBA/2 mice, 20-27 days, the period of maximal susceptibility to AS, and older 30-37 days DBA/2 mice, wich exhibit reduced seizure activity.5-HT$_{1c}$ receptor antagonists (mianserin and cyproheptadine), 5-HT$_3$ receptor antagonist (zacopride) and 5-HT$_4$ receptor antagonist (ICS 205-930) increased the latency of AS and decreased the severity of convulsions in young DBA/2 mice. However, the effect of these antagonists varied in older mice. Ketanserin, 5-HT$_2$ receptor antagonist, was devoid of any activity on AS. Yohimbine, an α2-adrenoceptor antagonist, increased the severity of AS and the anticonvulsant effect of 5-HT receptor subtypes antagonists became more pronounced in the presence of yohimbibe. These observations implicate a role of 5-HT$_{1c}$, 5-HT$_3$, 5-HT$_4$ and α2-adrenoceptors in AS in DBA/2 mice. Furthemore, these results also suggest an interaction between 5-HT receptors and α2 adrenoceptors, and differential development patterns of various 5-HT receptor subtypes in the CNS.

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VISUAL EVOKED POTENTIALS IN SELLAR ZONE COMPRESSIONS. M. Iancau, V. Nestianu, D. Georgescu, M. Bistriceanu.
The data from literature being contradictory, we have studied the alterations of the visual evoked potentials (VEP) at 13 adult patients (7 females and 6 males, aged 23-58), with sellar tumoral pathology (Hypophysis Adenoma) to reveal new aspects of this controversed problem. For stimulation we used pattern reversal with LED, full field 15°, vertical bars 30'; the changing at 1-1.5", at random intervals. Between 100-150 signals were averaged, each representing 500ms of evoked potentials. The latency of the P100 wave has been evaluated, its interocular and inter-emispherical differences, the amplitude of the wave and its interocular differences well as the amplitude of wave and its interocular and inter-emispherical differences as well as the crossed asymmetries, being a well-known fact that the stimulation with LED generates waves of the potentials with a smaller latency as compared with those produced by stimulation with TV pattern. From the tested lot, about 53% showed alterations of the studied parameters. The most accurate parameter in revealing the lesions, proved to be the latency of the P100 wave: 7 presented pathological alterations of latency, 3 being presented monocular. The interocular differences of latency were a slightly altered, only one subject presenting an interocular difference longer than 6 ms. The P100 amplitude is a parameter which presents a more reduced degree of certainly, both by measurement, and by establishing the physiological value of the interemispheric ratio. The VEP alterations were not closely connected with the X-ray aspects of the sellar zone.

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NITRIC OXIDE AND CARDIOVASCULAR CONTROL BY VENTROLATERAL MEDULLA IN CATS. L.N. Shapoval, L.S. pobegailo, T.N. Kovalenko

As ventrolateral medulla (VLM) has been shown to play a crucial role in central cardiovascular control, and endothelial relaxing factor nitric oxide (NO) to be released by nervous cells in the CNS, the objective of our work was to study NO influences the VLM neurons involved in the vascular tone and cardiac activity control. In acute experiments on anaesthetized cats changes in the background and reflector activities in the renal and inferior cardiac nerves together with haemo- and cardiodynamics shifts have been found after unilateral injections of Sodium Nitroprussid, L-arginin, L-NMMA in the neurons in the IVLM and CVLM. NO injections in the IVLM induced attenuation in the descending sympathoexciting influences to the vessels and heart. Location of neurons involved in vascular and cardiac control has been found to be different, and there is functional asymmetry in the VLM influence the ino- and chromotropic function of the heart. NO injections into the CVLM resulted in enhancement of the VLM influences the vessels and heart. On the base of histochemical study we detected fairly large amounts of NO-synthase in the VLM in cats. Data obtained evidence that NO actually may act as central "vasodilator" and be of great importance for the mechanisms of the central cardiovascular control in cats.

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IMMUNE FUNCTIONS AND BEHAVIOR OF RATS SUBJECTED TO CHRONIC IRRADIATION AND NATURAL ANTIOXIDANT DIET. N.Semiletova, E.Novoselova, T.Semenova and N.Medvinskaya.
The ionizing radiation of low doses produces the disfunction of organism, especially in the immune and nervous systems, wich yields the increases in infections and autoimmune diseases, allergies and cancers, the decrease of CNS functional activity. The damage of radiosensitive cells displays itself through changes in the basic parameters of their vital functions. Young male Wistar rats were subjected to chronic low level (12,9 and 3 cGr/day) irradiation and next items were determined: the immune response of T and B lymphocytes to mitogenes in vitro in cell cultures; the lipid composition of plasma-membranes; animals behavior: exploratory activity and sensory attention to the different modality stimuli; the possibility of modification the radiation effects by natural antioxidant ubiquinone Q-9. γ-radiation was shown to produce a wave-like suppression of the lymphocyte mitogenic response and the change in the mitogen-induced responsiveness correlated with splenic cellularity and animals behavior disturbances. We have shown the activation of cholesterol syntesis and the decrease in the membrane fluidity in splenocytes of chronically irradiated rats. The molar ratios of cholesterol to various phospholipid fractions were significantly increased in cells of irradiated rats. Radiation-induced disturbances of the exploratory activity and animals attention as well as suppression of immune functions were partially eliminated by daily ubiquinone Q-9 diet. The data presented here suggest the radioprotective potency of ubiquinones.

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PROBING THE VESTIBULO-OCULAR REFLEX DURING GAZE SACCADES. S. Tabak, J.B.J. Smeets and H. Collewijn

Large shifts of gaze ('gaze saccades') are usually effected by combined, fast rotations of eye and head. On the other hand, between saccades gaze is stabilized against the intrusion of head movements by the vestibulo-ocular reflex (VOR). As the VOR would counter-productive during an intended eye+head saccade, suppression of the VOR during gaze saccades is generally assumed. Despite several experimental approaches, the time course and magnitude of such VOR suppression have remained sketchy because most experimental procedures from which VOR-suppression has been inferred do interfere with the gaze-shift as such. In a new approach, we probed the VOR by superimposing a small (<1 deg), high-frequency (9-15 Hz), oscillation of the head upon active, horizontal gaze-shifts of 40-100 deg. Such horizontal or vertical oscillations were imposed by a helmet, driven by reactive torque, without interfering with voluntary head movements (Tabak and Collewijn, Exp. Brain Res. 102 (1994), 367-378). Eye and head movements were precisely measured with sensor coils in a.c. magnetic fields, and analytical techniques were developed to separate the oscillatory 'probe' from the gaze shift and obtain VOR gain and phase. These involved either the matching and subsequent subtraction of similar saccades with and without oscillation (drawback: low yield) or time-shifting of successive trials to synchronize the oscillations (drawback: slight time-blurring of saccades). The results of both methods were identical and consistent for >10 subjects. Presaccadic VOR-gain values (about 0.9) were reduced by 0.2-0.5 during small and large saccades, respectively, but elevated (about 1.0) in the wake of the saccade. In addition, changes in VOR phase were found: during all saccades the phase of the VOR at the probe frequency shifted by about 20 deg in the leading direction. We conclude that both the horizontal and vertical VOR are partially suppressed during gaze shifts.

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HYPERTONIC VOLUME REGULATION IN BRAIN GLIAL CELLS. I. Mountian and W. Van Driessche

Volume regulation of C6 glioma cells cultured on glass slips was studied by increasing bath osmolality from 300 to 440 mOsm. Cell height was used as an index for cell volume and was measured by an automatic monitoring system. Exposure to hypertonic challenge elicited a typical regulatory volume increase (RVI). The cells immediately shrank and then completely adjusted their volume within 15 min. Mean recovery was 104 ± 2.5 % and mean initial rate of recovery was 3.45 ± 0.03 %.min^{-1}. This process was strictly temperature dependent: at room temperature RVI was completely abolished. Upon returning to isotonic conditions, a post RVI - regulatory volume decrease was clearly observed. The involvement of the $Na^+/K^+/2Cl^-$ cotransporter in RVI was demonstrated by the inhibitory effect of Bumetanide (0.1 mM). In the presence of Amiloride (1 mM), a blocker of the Na^+/H^+ exchanger, the regulatory process was significantly reduced. Volume regulation was also inhibited by removal of Na^+, probably by affecting both the $Na^+/K^+/2Cl^-$ cotransporter and the Na^+/H^+ exchanger. The participation of the Cl^-/HCO_3 exchanger, which is usually coupled with the Na^+/H^+ exchanger, was shown by the diminishing effect of DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid, 0.2 mM) on RVI.

We conclude that C6 glioma cells exhibit RVI in hypertonic conditions. Their ability to adjust cell volume is temperature dependent. It involves the concomitant action of the $Na^+/K^+/2Cl^-$ cotransporter and the Na^+/H^+ and Cl^-/HCO_3 exchangers.

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ASSOCIATED EFFECTS OF THE RESPIRATORY EXERCISES AND ACUPUNCTURE (AP) ON BIOLOGICAL STABILITY IN HUMAN Maria Vrabete, Maria Iancau, M. Tarata and G. Popescu.

The good effects of respiratory exercises on the spiritual quietness explained by the traditional medicine were the model for our research. Aiming to the observation of the physiological pathways activation implied in the mentioning of the organism equilibrium using the two possibilities together: Yoga type breathing and acupuncture, we split the study into two stages: A. The biological performance has been expressed by the EEG, EMG, ECG, and by pH, paO_2 and $paCO_2$, before (t1) and after five minutes breathing (t2); B. The acupuncture needles were inserted in specific points, which are known as antistress points (V_{44}, VS_6). The same procedure as above (A) has been performed just after the needles insertion (t3) and 20 minutes following the insertion (t4). 20 young healthy men, Yoga practitioners, were investigated using the technique above described. The statistical processing of the data allow us to conclude that associating the acupuncture with Yoga type breathing is stimulative for the neuro-humoural pathways which can improve the neuro-motor relaxation and the anabolism. This technique may be very good for the patients in a postagressive stage or for those who need a surgical intervention in the preoperative stage.

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ACTIVITY OF NO-SYNTHASE IN RAT BRAIN: SOME PATHOPHYSIOLOGICAL IMPLICATIONS. M. Yu. Stepanichev, M. V. Onufriev, Yu. V. Zarzhetsky, A. V. Volkov, A. M. Gurvich, and N. V. Gulyaeva.

The enzyme nitric oxide synthase (NOS) synthesizes the messenger molecule nitric oxide (NO), therefore modulation of brain NO-synthase (NOS) is suggested to be of great neurophysiological significance. Effects of different factors on NOS activity in rat brain regions were studied using new approaches. Two methods for quantitative NOS assay were developed: in vitro formation of paramagnetic mononitrotrosyl complex in the reaction of NO with diethyl dithiocarbamate and Fe^{2+} monitored by cryogenic ESR, and fluorometric registration of the rate of NADPH oxidation inhibitable by specific inhibitors of NOS. NOS activity depended on the brain structure studied, on the strain of rats, on their sex and age. Cardiac arrest during 7-15 min (model of post-resuscitation pathology) resulted in the decrease of brain NOS activity; this effect was most pronounced in the cerebellum 1-2 h after resuscitation and depended on individual behavioural characteristics of rats. Treatment of rats with bacterial endotoxins did not change cerebral NOS but did induce NOS activity (presumably of glia in brain regions after the opening of blood-brain barrier by acute epinephrine-induced hypertension. We suggest that new methods of NOS evaluation expand the approaches to elucidation of multiple physiological roles of NO.

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EEG SLEEP ABNORMALITIES OF PAROXYSMAL TACHYCARDIES IN HUMAN EARLY ONTOGENESY. A. Boravova, N. Galkina

Aim of investigation is EEG examination of daytime sleep in infants aged 1 to 3 years who satisfied clinical criteria for cardioarrhythmical form of phychovegetatic syndrom. Infants have genetic liability to phychosomatic disorders. Onset of first paroxysmal episodes of supraventricular tachycardia (paroxysmal, Wolff-Parkinson-White; without cardiac organicity) can be observed between fetal life and 20 months of age. Freqency and severity of repeat attacks were related to season and day time. Before manifestation of paroxysmal tachycardias permanent vegetative disorders and psychovegetative diathesis were diagnosed. Natural daytime sleep EEG (No=48) showed paroxyms such as generalized synchronous short discharge of slow wave activity with different severity during drowsiness, stage 1,2 of NREM sleep (63%); paroxysmal high-voltage, irregular and polymorphic generalized slow wave activity, which long lasting at the moment of passage from NREM to REM sleep (100%). There is increasing synchrony represented as follows frontal-central burst of sharp high-amplitude bilateral theta with frequency 4-5 cps, frontal or frontorolandic high-voltage sleep spindles with slower and faster frequencies, vertex sharp waves, K-complexes, generalized phasic delta wave bursts, fusiform beta activity with high-amplitude. In all cases EEG waking was normal or have mild alterations. Observed abnormal changes of sleep patterns were abrupt increased in children older than 1 years age. Showed enchancement of EEG sleep synchronous processes may reflect the excitability changes of diencephalic-brainstem structure and is one of important risk factor of clinical manifestation of paroxismal tachycardias.

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DIAPHRAGMATIC FATIGUE AND APNOE IN INSPIRATORY RESISTIVE LOAD. N.Aleksandrova and G.Isaev.

At present special attention is addressed to investigation of functional state of the respiratory muscles at airway obstruction. Our goal was to study the mechanisms of diaphragmatic fatigue and apnoe in prolonged resistive inspiratory loads.

Experiments were performed in anaesthetised, spontaneously breathing cats during breathing with air and hypoxic gas mixtures. Electrical activity of diaphragm (EMG) and frenic nerve, transdiaphragmatic pressure and tidal volume were recorded simultaneously. The obtained data evidences that under the breathing hypoxic gas mixture diaphragmatic fatigue developed in moderate resistive load (equal to 70% of maximal load). The loss of diaphragmatic contraction force and decrease in ventilation were related to decreas in diaphragmatic EMG. Phrenic nerve activity remained unaltered. These data suggest a peripharal character of diaphragmatic fatigue related to disorder in neuro-muscular transmission. Combination of hypoxia and heavy resistive load (80% max) produced the respiratory arrest. Transdiaphragmatic pressure decreased gradually just before the respiratory arrest and disorder in contractility process in diaphragmatic fibers was discovered. When air breathing diaphragmatic fatigue developed in heavy loads only and respiratory arrest was not obtained.

Thus disorders in neuro-muscular transmission and contractility process were mechanisms of diaphragmatic fatigue and apnoe in resistive loads. Hypoxia accelerated development of diaphragmatic fatigue and also was one of the reasons of respiratory arrest.

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EXTRACT OUT OF HIRUDO MEDICINALIS POSSESSES CENTRAL EFFECT. Y.N.Khomyakov, I.I.Lyubimov, .V.Phyodorov, V.V.Gusev.

An extract out of the head parts of Hirudo Medicinalis was obtained by method of F.Marcwardt. An activity of the extract out of the head parts of Hirudo Medicinalis was protein was 240-720 AT NIH u/mg. Central effects of the extract were studied in behavioural tests Open Field and Rota Rod with use of white rats (female in puberty, n=10). In 24 hours after intraperitoneumly administration of extract in dose 100 μg/kg in the test Open Field was discoved the reliable decreasing of motor activity and several kinds of vertical and gorizontal explorative activities in comparison with background meanings for this group from 30.90 ± 6.42, 1.80 ± 0.62 and 73.30 ± 6.02 to 12.97± 2.08, 0.10 ± 0.10 and 46.37± 4.73 respectively ($p<0.01$, t-criterion, F=9.48, 46.2) and ($p<0.05$, t-criterion). There were no reliable differences in the groups of rats which received the extract in the dose 1 μg/kg or a solvent by the same way. The extract in the dose 100 μg/kg inhibited processes of acquisition of skills, which bring to increase in working capacity and motor coordination in the test Rota Rod. The reliable effect ($p<0.05$, t-criterion) in comparison with a control group was observed from 2 till 24 hours after the administration of the extract from background meanings 105.00 ± 5.87 to 117.00 ± 12.62 in 20 hours later in the dose 100 μg/kg, from 93.30 ± 7.59 to 167.40 ± 17.24 in the control group. There were no reliable differences between the acquisition of skills in the group of the rats, injected in dose 1 μg/kg and in the control group. These results show extract out of the head parts of Hirudo Medicinalis exerts direct or indirect suppressive action on some structure of the CNS which are responsible for motor and explorative activities.

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NEUROTOXICITY OF AF64A IS MEDIATED BY OXIDATIVE STRESS. N.V.Gulyaeva, M.V.Onufriev, N.A.Lazareva, I.P.Levshina, M.Yu.Stepanichev, and T.J. Walsh

Septohippocampal cholinergic degeneration induced by cholinotoxin AF64A is regarded as a model for Alzheimer's disease. There is indirect evidence that neurotoxicity of AF64A may be mediated by oxidative stress (Walsh et al., 1994). The objective of the present study was to investigate this possibility directly. Parameters of free radical mediated processes (FRMP: level of thiobarbituric acid-reactive material, H_2O_2-induced, luminol-dependent chemiluminescence, superoxide scavenging activity) were measured in selected brain regions of male Wistar rats after intracerebroventricular injection of AF64A (3 nmol/side). Though sham operation caused increase of FRMP in cerebral cortex, hippocampus and the rest of the brain during first 3 days, oxidative stress induced by AF64A was much more pronounced. Increase of antiradical superoxide scavenging activity on day 5 was specific for hippocampus of AF64A-treated rats and was accompanied by the decrease of peroxidation. 4 month after AF64A injection FRMP in brain regions were not different from those in sham-operated controls, however superoxide scavenging activity remained selectively increased in hippocampus, suggesting the compensation of increased free radical generation or of extreme vulnerability of cholinotoxin-treated hippocampus to oxidative stress. In addition, AF64A impaired inter- and intra- regional correlations between different FRMP. We conclude that oxidative stress participates in the mechanism of AF64A-induced neurotoxicity.

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PECULIARITIES OF CELLULAR ACTIVITY AND EEG TOPOGRAMS AT DIFFERENT MOTIVATIONAL-EMOTIONAL STATES. R.G. Kozhedub

Neocortical membrane-synaptic modifications, EEG spatio-temporal organization and synchrony of hypothalamic neuronal impulse activity at different functional states of rabbit and rat were studied by means of analysis of the pyramidal tract responses, of the parameters of neocortical successive EEG topograms and correlation analysis of simultaneous impulse activity of the functionally-identified hypothalamic neurons. During the initial stage of learning, characterized by the dominance of motivational components of behavior the presence of increased excitability of pyramidal cells and the change of fluctuations of the mean level of successive momentary relives of topograms were observed in neocortex. During the late stage of learning, distinguished by the dominance of emotional components of behavior the background of control or lower level of cellular excitability, maximal synaptic efficiency and complication of topogram forms were found. Hypothalamic influences on neocortex favored more strong changes of cellular excitability and global EEG spatial synchronization. Two classes of hypothalamic neurons ("motivational" and "reinforcing") were selected by the character of their activity in dependence on presence in animal of motivation or emotionally positive state after the reinforcement. Specific features of interaction of different classes neurons depended on the predominance of motivational or emotional component in animal's behavior were revealed. The experimental data point to specificity of motivational and emotional hypothalamic influences, realized via "motivational" and "reinforcing" neurons, on the cortical processes at different stages of learning. It is suggested that a mechanism of the action of motivational structure consists in their modulating effects on cellular membrane excitability and global EEG synchronization. The influence of emotiogenic structure consists in their modulating effects on synaptic efficacy and local EEG synchronization.

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THE CHANGES OF FREE RADICALS AFTER THE NOCICEPTIVE STIMULATION. R. Rokyta, V. Holeček, J. Racek, M. Puls, H. Šťastná

As a model of neuropathic pain our own models were used (Rokyta et al. 1985). The both hind limbs were stimulated during five minutes in five consecutive days in the same daily time. In 5th day the animals (laboratory rats of Wistar strain) were anaesthetized and the parts of sensimotor cortex were taken off. The cortical samples were then homogenized, freezed and submitted to the procedure of the evaluation of enzymes and metabolites of lipid peroxidation SOD-superoxidismutase, GSHPx-glutationperoxidase and MDA-malonedialdehyd). Results were evaluated by using ANOVA and Student-t-test methods. After five days stimulation all three compounds were increased in sensorimotor cortex in both hemispheres. After fifteen and thirty days after the stimulation only GSHPx was still increased, SOD and MDA reached their normal values.

It is possible to conclude, that the changes of free oxygen radicals may play a role in the pathogenesis of the pain and could be good indicators of changes in different types of nociceptive stimulation.

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NEW VIEW AT CYTOARCHITECTURE OF AMYGDALA..

L. Kalimullina, A. Karpova, Z. Minibajeva, I. Bajazitov, R. Altinbajev.

Mechanisms of Amygdala's participation in securing such important and complicated processes as adapting behaviour, emotions, memory, stress, visceral and endocrine functions regulations are not exposed as yet. It indicates at the necessity of a further detailed analysis of the peculiarities of its cytoarchitecture in order to work out a rational scheme of investigation of its structural-functional organization. Cyto-architecture of Amygdala was studied at 22 rats (Wistar) in series of sections made in three planes of space and dyed with crezile violet. On the basis of the registration of peculiarities of nuclear-paleocortex interrelations in Amygdala's structure there were marked out anterior, central and posterior divisions. Inside each of the abovementioned divisions, on the basis of image identification theory, there were revealed high-informative sections. They define optimal levels of Amygdala, at which it is recommended to carry out investigations.

Summary. On the basis of cytoarchitectonics criterion and principals of image identification theory an optimal scheme of organization of Amygdala's investigations is proposed.

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LATERAL GENICULATE BODIES REORGANIZATION IN THE BRAIN AFTER DEAFFERENTATION. I. V. IOUCHTCHENCO

The present research is a part of investigations being carried out in the laboratory of prof. N. Lyubimov, deling with he problem of studing the compensation-restoration processes, taken place in central nervous system under different forms of sensory deficit. One year after surgical section of the optical tract the degenerative changes were found in the deafferented lateral geniculate bodies. The changes were species-dependent. In cats, the cytoarchitectonics was preserved, local neuronal losses and layers thinning absent. In night-active apes, with cytoarchitectonic image preserved and focal neuron necrosis absent, the layers more substantially thinned. In apes, with a primate-like lateral geniculate bodies structure pattern, the extended zones of neurons degeneration were found in the layers. The most prominent deafferentation-induced structural lateral geniculate bodies alterations were shown in the central vision projection zones. We can conclude that the central vision after deafferentation are affected more then peripheral one in primates.

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SOMATOSENSORY AFFERENT CONDUCTION ORGANIZATION IN MODIFIRED BRAIN REACTIVITY CONDITIONS. T. V. Orlova, E. V. Damyanovich

Cortical and brain stem somatosensory evoked potentials (SEPs), induced by electrical transcutaneous stimulation of median nerve in the wrist, were recorded and analysed in 12 patients endured an acute stroke with following sensorimotor deficit and also in 16 healthy volunteers practisin the Transcendental Meditation (TM) technology for two years. In the last ones SEPs were registrated before and during the TM. In patients the clinical diagnosis was confirmed by CT scans at acute and restorative stages in all cases. The investigation was performed to estimate somatosensory afferent conduction changes in modifired brain reactivity conditions. All abnormal findings in brain stem and scalp-recorded SEPs in patients depended on lesion location. The SEPs abnormalities in patients with oneside lesion of the forebrain structures were expressed in the amplitude increase of early cortical SEPs components amplitude in intact hemisphere when contralateral median nerve was stimulated. Absence of SEPs or their amplitude significant reduction in both hemispheres after ipsilateral median nerve stimulatin was observed in patients with subtalamic lesions. In meditators SEPs changes during TM were expressed in the amplitude increase of SEPs components without their peak latencies changers. The obtained data can be explaned by involving nonclassic and classic lemniscal pathways in the pathological processe and also by functional changes in the existent simmetrical and asimmetrical inhibitory influences to the relay structures of brain stem.

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PLASTICITY OF AFFERENT PROJECTIONS AS A RESULT OF CONSCIOUSNESS MODIFICATION. E. Istratov, S. Lyubimov

The spreading of the somatosensory cortical afferent projections have been studied in subjects who practiced one of the self-regulation technique - program of Transcendental Meditation (TM). For this purpose method of scalp topographical evaluation of early (up to 100 ms) components of somatosensory evoked potentials (SEP) was used. Topographical results were analysed for two states of consciousness - before and after TM-program. In most (>90%) subjects the distribution of SEP components P30, P45 and N55 after realization of TM-program exhibited tendency to focalization of their registration area: all components obtained over the postcentral gyrus, but their registration area extremly diminished. Based on these data it is possible to conclude that this phenomena is connected with the plasticity of cortical afferent projecitons, resulting from the consciousness modification after TM-program and reflected some kind of 'gating' effect. This last can be provided by the descending inhibitory pathways which passed to the main somatosensory analyser relay nucleus.

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Significance of dynamics of layer transformation of amplitude-time characteristics of thalamo-cortical responses of the rabbits sensorimotor cortex in ontogenesis. Shimko I. A.

The experiments were made on rabbits from 14 days to 2 months old. The high frequency of stimulation of ventroposterolateral (VPL) thalamus nucleus (80 Hr) is assimilated only by the first (PC-1) and the second (PC-2) positive components of heterocomponent thalamo-cortical responses (TCR) sensorimotor cortex (SMC) , which can testify to their pre-sineptical electrogenesis. The arguments in favour of the hypothesis of the generation of invertable PC-2 by the fast-conducting branches of thalamo-cortical fibres, ended at III-IV layers SMC (Bellarge exterior strip), and non-invertable PC-2 - the hyperpolarization centre on inhibition neurons of the afferent entrance, situated in the upper laminae of neocortex, are adduced. The revealed properties of the spatial-temporal characteristics of profile of bioelectric fields of the third negative component (NC-3) TCR are apparently the consequence of the age stages of the development and change of embryonal mechanisms of electrogenesis (the Martinotti embryonal cells, disappearing gradually after 2-3 weeks of post-natal life) for the definitive ones (2 months after birth) in externaly similar negativities.

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THE ACTION OF NEUROPEPIDES ON PERIPHERAL MECHANISMS OF PERCEPTION OF THE SKIN.

L. D. Enin* and G. N. Ponomarenko**

We have studied functional characteristics of skin mechanosensitive afferents by means of mechanical adequate and noxious stimulation under the influence of bradykinin and L-enkephalin.

This study was conducted on male Wistar strain rats, anesthetized by 0.25 $g*kg^{-1}$ hexenal i.p. Mechanical stimuli were applied to the skin of an electrodynamic mechanical stimulator. Firing activity was recorded from single sciatic nerve fibers.

The receptors could be classified as: low-, medium-, and highthreshold units. The injection of bradykinin (10 μg; intracutaneous) promoted an increase of tactile sensitivity in medium- and high-threshold receptors and suppressed impulse activity in low-threshold receptors. However, the bradykinin evoked a tonic discharge in single afferent fibers. The increase of sensitivity threshold in skin medium- and high-threshold receptors and the inhibition of impulse activity in low-threshold receptors under the influence of L-enkephalin (10 μg; intracutaneous) have been determined. Inhibitory effect of L-encephalin on all groups of mechanoreceptors was eliminated by preliminary intraperitoneal or local (intracutaneous) injections of naloxone.

Neuropeptides exert differnt modulate influence on skin peripheral nerve terminals.

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THE FREQUENCY SYNAPTIC STIMULATION PROVOKES THE FORMATION OF THE ELECTRIC PERMEABLE NEURITE-GLIAL CONTACTS. O.S. Sotnikov, I.G.Bretzke

The changes of the neurite-glial (N-G) interaction were investigated under the conditions of frequency pessimal synaptic activation of the cat and frog autonomic ganglions (60, 100 Hz, 10 min) and leech abdominal chain (15 Hz, 10 min). The computer ultrastructural analysis revealed that the significant functional reconstructions of the adjacent neuronal and glial membranes developed in parallel to the decreasing action potential and inhibition of synaptic conducting in the neuropil. Some proteins formed submembrane electron density aggregates and entered into the membranes. The process finished with forming de novo N-G membrane contacts of all known patterns. There were electrical contacts (gap j.) among them. Sometimes multilayer membrane structures with the extremum periods like the normal bilipid membrane (7-8 nm) developed in the new contact places. 5-layer structures were formed by lamella reduction during conjugation of 2 glial and 2 neuronal bilaminar membranes. The process of the new membrane lamella formation occurred in the peripheral part of the membrane aggregate region. Their extremum amplitudes were low. There may be over 10 membrane layers disposed in one newly formed border structure. The data got allow us to suppose that changes of the N-G membrane interactions and membrane molecular destabilization play a considerable role in the inhibition of synaptic conducting.

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CHANGE OF RESPONCE TO THE EXTRACELLULAR APPLICATION OF ACETYLCHOLINE AT THE INTRACELLULAR PERFUSION OF ISOLATED MOLLUSK NEURONES BY BIOGENIC AMINES. Olga P. Yurchenko.

The influence of the extra- and intracellular application of biogenic amines on ion currents induced by acetylcholine on isolated giant neurones of fresh water mollusc *Lymnaea stagnalis* has been analysed with the voltage-clamp and intracellular perfusion methods. Epinephrine and dopamine decreased the response to acetylcholine at the intra- and extracellular application. Intracellular application of 5-hydroxytryptamine (5-HT) produced a significant 30% increase of acetylcholine inward current. Extracellular application of 5-HT to the same cells produced an opposite effect - a 60% decrease of acetylcholine response. All the investigated biogenic amines induced small transmembrane currents with the opposite course at extra- and intracellular application. On the intact brain of *Aplysia depilans* the injection of dopamine in identified neurones also produced changes of acetylcholine responses. Possible existence in the nerve cells of intracellular receptors of biogenic amines that modulate the sensitivity of membrane receptors of neurotransmitters is discussed.

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INTEGRATIVE AND PLASTIC PROPERTIES OF NEURAL STRUCTURES IN THE CAT'S PRESTRIATE CORTEX. Gabibov I.M., Orlov I.V.

The experiments were performed on 20 immobilized cats. During recording session a local anesthetics lidocaine and dicaine were infused into all wound and pressure points. The study of neural receptive fields (RFs) was performed using light bar (2x40 deg. in size). The study of morphological connections was performed using retrograde horseradish peroxidase tracing techniques (50% sol.; 0,2 mkl) in area 21 of intact cat and in two months after callosotomy (Ccsec). It is shown that RFs always comprise the ipsilateral part of visual field, react to any stimulus orientation and consist of 1 to 9 subfields. An analysis of labelled cells were discovered in the medial part of the lateral sulcus (area 17). In area 17 (-7 - -10) were distinguished nine clear groups of labelled cells. The cell numbers in each group varied from 3 to 15. The results of the Ccsec showed that cats do'nt distinguish food during the course of the first week. The RFs consist of 3-4 excitatory subfields and inhibitory zones in RFs become more wide than before surgery. The neurones become selective to stimulus orientation and receive information from the contralateral hemifield of vision only. The study of neural connections showed that in two months after Ccsec the number of labelled cells in the striate cortex was two times more and distribution of these cells is more regular than in intact brain. It is known that after Ccsec or removal of different parts of the cortex in three-four weeks partial recovery of lost neural properties and brain functions begins. As a result of this study it is shown that mechanisms of compensation and plasticity in the brain cortex may provide with reorientation of axon terminations towards prestriate cortex neurons, the main properties of which are integration and full description of spatial information.

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MECHANISMS OF ASCENDING INFLUENCES OF RETICULAR FORMATION OF BRAIN STEM. I.G. Palamarchuk

In experiments at the rabbits and cats under local anesthesia studied influence of transection of brain stem and of following irritation of reticular structures of middle and intermediate brain on evoked potentials /EP/ of neocortex and of single neurons, membrane potential /MP/ of cells of cortex and on thalamo-cortical EP. It is showed mesencephalic transection lead to characteristic structural changes of EP of neocortex: considerable increased amplitude, duration and speed of development of initial electropositive component of EP, depressed negative and following electropositive component of EP. In this time considerable increased /in middle on 17mV, $p<0.001$/ MP of cells of visual cortex, changed the correlation of quantity of neurons which reacted by initial inhibition or excitation on light irritation of eye. At the cats steady characteristic changes of structure of EP marked after of back transection of brain stem and are interpreted by us as result of switching off reticular structures which inhibit the inhibitory mesencephalic structures. Irritation /osmotic, electric, pharmacologic - caffeine, phenamine, adrenaline, noradrenaline, serotonine, acetylcholine, arecoline, proserine, galantamine, nicotine in therapeutic doses/ of rostrale mesencephalic structures or /electric/ nonspecific nuclei of thalamus of preparation cerveau isole of rabbit evoke temporary restoration of structure of EP of neocortex to level of awake. Precollicular transection except possibility of such restoration of EP. Thus by means of reticular formation is conditioned value of MP of cells of cortex and correlation of exciting and inhibiting synaptical influences. Preparation cerveau isole of rabbit after model of EP - new method of indication of reticulotropism of neuropharmacological substances.

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THE FUNCTIONAL ASYMMETRY OF ADRENAL GLANDS INDUCED BY PINEAL PEPTIDES AND OXYTOCIN IN STRESS. M.P. Chernisheva, R.I. Kovalenko, A.V. Shtilik, L.R. Iosifova, I.R. Lutkus, I.A. Novikova.

The pineal indolamines regulate the adrenal functions in stress, but the action of pineal peptides (PP), one of which is oxytocin (OT), is unknown. Male rats (180-200g) had received one i/nas unilateral introduction of OT (0.01ng/ml,0.3mkl) or PP (0.1ng/0.5mkl) with MM 1-4.6 kDa. Control groups had received saline or nothing. The effects had been investigated in the stress--models: sparring (5 sec), food and water deprivation (48h) or open field--test (15 min). Corticosterone (Cst) concentration in plasma (microfluorimetric method), malonic dialdehyde (MDA) (spectrophotometric method) and mass of adrenals in rats with unilateral adrenalectomy and intact ones were determed. The asymmetry of adrenal mass increased ($p< 0.05$) by ipsilateral OT and cotralateral PP in 24 and 72h. The left PP prevented compensatoral hypertrophy of right gland, but right PP decreased left adrenal mass ($p<O.O1$). The asymmetry OT effects in intact rats on corticosteron and MDA had been determed in 48 and/or 72h: right OT increased the hormone level, but left decreased; the most lowering of MDA was found in left adrenal ($p< 0.001$) with no respect to the side OT application. The left PP rised corticosteron and MDA more in right gland than in other adrenal with right PP. These results demonstrate the role of PP and OT in functional asimmetry of adrenals.

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NEURO-HORMONAL MECHANISM OF ADAPTATION TO EXTRAORDINARY INFLUENCES. V.Yelsky, A.Borodin, R.Samsonenko

Mechanisms of adaptation to extraordinary influences were studied in experimental models of adaptation on rats. Radioimmune methods, columnar and thin layer chromatography, fluorescence and spectrometric methods were used to determine a complex indexes of organism state. It was found that changes in substance P, opioid peptides and melatonine release reach a peak during first ten seconds or a minute after the extraordinary influence presents initial phase of the alarm adaptation. Fast-acting system of melatonine, opioids and substance P exerts the activity of hypothalamus - pituitary - adrenal system, and renin-angiotensin-aldosteron system, which provide less intensive but more reliable and long-lasting adaptation. The lack of compensation leads to the disturbance of metabolism, proteolysis, increased activity of lyposomal hydrolysis, accumulation of middle size molecules of ischemic toxins, biogenic amines, products of free radical oxidation, prostaglandines abundance, as well as to lowering of organic acids oxidation and to shifts in pH balance. In favorable conditions the process of unspecific adaptation turns to rising immune resistance, in unfavorable ones it may have a character of immune insufficiency.

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EFFECTS OF INTRACAUDATE DOPAMINE RECEPTOR BLOCKADE ON THE NEURONAL ACTIVITY IN FELINE ENTOPEDUNCLULAR NUCLEUS AND SUBSTANTIA NIGRA. B. Kolomiets, E. Lukhanina

This study is to provide the information on the role of dopamine (DA) receptors in modulation of efferent influences from caudate nucleus (Cn) on the activity of main output systems of basal ganglia. In chronic experiments performed on awake cats the spontaneous activity and responses related to the passive limb movement were recorded from 78 neurons of nucleus entopeduncularis (En) and reactions associated with saccadic eye movements from 86 units located in substantia nigra pars reticulata (SNR). Extracellular recordings were made before and following ipsilateral microinjections of 25 mkg per 5 mkl of DA antagonist haloperidol (Hp) into the head of Cn. Following Hp administration statistically significant increase of the number of neurons with bursting activity was found both in En and SNR (from 16 to 73% and from 34 to 61%, respectively, $p < 0.05$). Excitatory responses of En neurons related to the contralateral passive forelimb movement became more prolonged (1-2 s against 100-300ms before injection) and lost their directional selectivity. Under the same conditions the ratio of excitatory/inhibitory responses associated with saccadic eye movements in SNR neurons increased from 0.04 to 0.4 ($p < 0.05$). The results of present experiments suggest that under conditions of DA deficiency the neuronal activity in basal ganglia output systems become significantly increased that, in turn, leads to enhancement of the hyperpolarization processes in the motor thalamic nuclei resulting in extrapyramidal movement disorders.

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LONG-TERM POTENTIATION IN RAT OLFACTORY CORTEX SLICES IS ACCOMPAINED BY RELEASING NEUROPEPTIDES. A. A. Mokrushin and A.V. Tokarev

The experiments were made on the rat brain olfactory cortex tangential slices. Fluid samples were collected from the donor slice (DS) during four times of the lateral olfactory tract (LOT) tetanization (100Hz, 2-4V, for 30s, with 5-min interval). The samples were used for the perfusion of recipient slices (RSs). The RSs were not tetanized. To determine the changes of the excitability level, focal potentials (FPs) to 0,3Hz test-stimulation of LOT were registered in the pyriform cortex area of the RSs. The fluid samples induced the responses of RSs contrary as compared to DSs reactions. The samples collected from the DS, in which potentiation was developed, induced FPs depression in the RS predominantly. If the tetanization produced depression in the DS, then the potentiation was induced in the RS. Collected from tetanized DSs perfusates were separated into the two fractions according to molecular weights < 50 and > 50 kDa. Fractions > 50 kDa as potentiated as well depressive perfusate produced predominantly the depressive reactions in RSs. The directions of responses of RSs on fractions < 50 kDa were diverse. To establish chemical nature of releasing factors was made treatment of depressive fraction > 50 kDa by immobilized enzyme trypsine. After treatment this perfusate was not able to induce depression reactions in RSs as before treatment.The data obtained evidence that tetanization of DS result in a release of distinct neurochemical factors which are capable to induce potentiation/depression in RSs. In all likelihood these endogenous factors are polypeptides.

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THE LONG-TERM EFFECTS OF EARLY VISUAL DEPRIVATION ON HUMAN ALPHA RHYTHM ONTOGENESIS. T.A.Stroganova, I.N.Posikera.

The focus of the study was the role of early afferential visual input to the brain (pattern vision) in development of a sensory alpha rhythm EEG. Experimental group included infants with bilateral congenital cataracts with visual acuity less than 0,01 before surgery, that completely excluded pattern vision. Eight infants were studied in the "blind" period of deprivation before surgery (age from 5,5 to 11 months); 35 infants (age from 8 to 38 months) in the period of visual functions recovery, after cataracts removal (basically 6-8 months) and correction with optical lenses. Multichannel EEG was recorded in the situation of black homogeneous visual field, thought to be optimal for eliciting alpha rhythm. Developmental trend of alpha rhythm spectral parameters for infants with early visual deprivation comparing to normative group of infants of the first three years of life (30 subjects) was analyzed.

The study revealed that the alpha rhythm generation mechanisms were completely suppressed during "blind" period of deprivation. Pattern vision recovery was followed by immediate appearance of alpha rhythm in EEG. The amplitude of dominant spectral peak in alpha band was significantly decreased and frequency mode was lowered comparing to normative group during short recovery periods (less then 5 months). Prolonging the recovery time (more then 6 months) resulted in the increase of alpha power density (the differences statistically insignificant), however the low dominant frequency in alpha band was observed to remain so throughout the observation period up to three years old. The decreased coherence values in alpha band over the posterior cortical leads differed essentially from the normative group.

The data indicates that early visual experience plays a critical role in the ontogenesis of the rhythmic activity of the human cortex.

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CYTOSKELETAL DISRUPTION REDISTRIBUTES PERINUCLEAR MITOCHONDRIA IN CULTURED NEURONS OF RAT HIPPOCAMPUS.

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Relationship between the cytoskeleton and the arrangement and morphology of mitochondria was examined in new born rat hippocampus cultures using drugs that mostly affect actin microfilaments, or neurofilaments. Spatial distribution, and shapes of mitochondria sampled (homogeneously) in perinuclear cytoplasm were examined via quantitative electron microscopy. The analyses have shown that the disruption of microfilaments causes a dramatic decrease in the number of mitichondria in perinuclear space, without alterations in their shape or size. In contrast, bundling of neurofilaments did not effect the volume occupied by mitochondria in the perinuclear space but was likely to induce the fractionating of the organelles into smaller mitochondria.

These results suggest specific roles of each type of cytoskeletal elements in positioning of mitichondria in cytoplasm, or in maintaining their morphology.

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THE FUNCTIONAL PARTICIPATION OF THE NEURAL CELL ADHESION MOLECULES IN THE GROWTH AND DEVELOPMENT OF NEURONAL AND GLIAL CELLS IN CULTURE.

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Immynocytochemical quantitative study was carried out to show the neural cell adhesion molecules (N-CAM) arrangement on the surface membrane of neurons and glial soma of monolayer cultures of hippocampal neurons from new born rats. N-CAM were labelled with specific antibodies conjugated with colloidal particles on the 5th day and 12th day in vitro. To quantify the labelling, a stochastic geometry approach was used. The experiments with the neurons showed that surface density of labelled N-CAM was found to be ~ 2,5 times higher in growth cone membranes relative to somatic and axonal membranes in 5-DIV neurons. By the 12th DIV, this density decreases in somatic membrane (~18%) and increases in axonal membranes (~60%). The same experiments with the glial cells show that on both 5 and 12 DIV, N-CAM density on the surface of processes is ~ 2 times higher than that in somata: 12-DIV cells showing a lower (~25%) N-CAM surfase density as compared with the 5-DIV cells. This suggests that N-CAM expression in glia surfaces decreases while the cells form contacts, and N-CAM sortind between soma and processes remains stable.

The results revealed regular patterns of N-CAM on the somatic surface and allowed consideration of N-CAM arrangement in a view of adhesion properties.

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PHASE REACTION AS PROPERTY OF THE CATECHOLAMINERGIC SYSTEMS FUNCTIONING AT EXOGENOUS AND ENDOGENOUS INTOXICATIONS. S.A.Kutsenko and S.V.Chepur

Brain and intestinal catecholaminergic systems (CAS) were studied at intoxications of anticholinesterase agents and at disturbance of the detoxication processes in liver at portal hypertension. During complex histochemical investigations phase morphofunctional changes of central and periferal adrenergic structures were observed. There are three period in influence of anticholinesterase substances on brain CAS. In initial period intensivity of the catecholamines (CA) luminance in nuclei didn't change or insignificantly increase. It was coincided with the clinical manifestations of intoxication. Afterwards CA rate decreased below normal value. Restoration of CAS activity corresponded to rehabilitation period of the poisoned animals. Often this changes were revealed in striatum, diligence nucleus and locus coeruleus neurons. Dinamics of CA rate in brain structures didn't correlated with inhibition of acetylcholinesterase activity and the velocity of its recovering these structures. Analogous phase changes CAS activity, closely correlated with portal pressure rate, were observed at portal vein stenosis. The first increase of the CA luminance intensivity in the nerve fibres of the intestinal plexus coincided with initial increase of the portal pressure and finished as the main ways of roundabout bloodflow were distinguished. The second increase of CAS activity was at period of the developing collateral bloodflow when some portal blood hited in common circulation without liver detoxication. In this time portal pressure increase again. Closely correlation of the intensivity of the CA luminance and portal pressure allow to suppose functional connection of these parameters. These data convincely testify that nonspecific change of CAS activity takes part in pathogenesis of the exogenous and endogenous intoxication and become a base to use pharmacological and surgical method of CA rate correction in treatment of toxic pathology.

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MODEL OF PARTICULARY EXPRESSED SPINAL HYPERREFLEXIA AND REFLEX RESPONSES OF SPINAL CORD IN CONDITION OF HYPERREFLEXIA. E.A.Makij, I.A.Krajushkina and A.P.Rogachevsky

It is known that the maximum of white rat spinal cord neurons excitability enhancement is 3-5 days after the nerves cutting. The stable spinal cord reflex arcs excitability enhancement after rat chordotomy is in 5th day and keeps long time. We are supposed that the combination of thesec damages lead to 5 days later excited excitability hotbeds formation in spinal cord which are more intensive than that one after separate damages of nerve or spinal cord. We are studied the evoked monosynaptic discharges of ventral roots (MD VR) in L_5 segment on the cutting nerve side in 5 day after such intervention. It is found that degree of MD VR amplitude enhancement is more significant than that of separate cutting of nerve or spinal cord. We also revealed the discharges with anomalously high amplitude both immediately follows the MD VR and more than twice as much as it. These discharges are registered in 40% experiments approximately. Its enhance in such cases when the amplitude of MD VR have particulary high level. These discharges extremly fit by amplitude and form with responses of ventral roots which have arise by it immediate stimulation. According to data we came to conclusion that: 1) mechanisms of enhancement reflexes after cutting of nerve or spinal cord, have definite differences; 2) reflex discharges can provoke potential action of ventral roots fibres which do not participate in reflex discharge in the condition of pathologically reinforced reflex excitability of spinal cord (efaptic excitation).

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FUNCTIONAL MODULS OF TRACHEABRONCHIAL GANGLIA. A.D.Nozdrachev, A.N.Fedin

Functional modul is consisted of the rhythmical activity generating cells,sensory cells and effector ones which can excite and inhibit muscle activity. One of the neurones that develope rhythmical activity supposedly belongs to spikeless B-cells. It's membrane potential fluctuations are transfered synaptically to the other cell, which generates spikes. These two cells together form rhythmical bursts of discharges. Preganglionar parasympathetic fibers which transfer commands from respiratory center and also fibers from trachea- bronchial receptors are ended on rhythm generator cells. Receptor influences could be excitatory and inhibitory as well. The effector excitatory group activity is maintained by the impulsation from respiratory center and from tonic neurones of ganglion itself. Rhythm generator inhibits the excitatory group of effector neurones via the nicotinic receptors and at the same time activates inhibitory effector neurones. These cells obtain VIP and serotonin receptors. Postganglionic sympathetic nerve fibers transfer the impulsation to the functional moduls neurones through α-adrenorecetors.

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SOME MECHANIMS OF THE HUMAN MENTAL EFFICIENCY. G.M.Chaichenko, L.G.Tomilina, N.B.Filimonova.

In study of 435 the oldest pupils and university students using a battery of psychophysiological parameters (IQ, strength of the excitation process, functional mobility of the nervous processes (FMNP) in stress state and brain efficiency, working memory capacity, strategy of memoring, speed and quality of analytical and logical mentality) determined by means of own original soft ware had been evaluated the psychophysiological rating (PR) in 20-points job scale as an integral index of the cognitive aspects of perception, natural reasoning and problem solving for predict a learning and cognitive abilities. PR had correlated with teaching efficiency (0,36-0,67). The pupil's learning ability first of all depended on IQ and speed of information processing (0,48 - 0,55), in university students the academic performance depended on mainly: in biologists - IQ and speed of analytical thinking (0,57), in journalists- IQ and FMNP (0,37-0,43), in psychologists- the brain efficiency (0,45). The human mental efficiency had been determined by optimal physiological activated level (9,5-10,5 Hz subrange of the EEG alpha-rythm).

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ACID-BASE RESPONSIVE AMMONIAGENESIS IN A GLUCONEOGENIC RENAL EPITHELIAL CELL LINE. Gerhard J.A. Gstraunthaler

In an attempt to study renal ammoniagenesis in tissue culture, we recently characterized the adaptive response of the LLC-PK_1 pig renal epithelial cell line to metabolic acidosis (Am. J. Physiol. 263: C47, 1992). It was shown that LLC-PK_1 cells clearly respond to metabolic acidosis, and that the ammoniagenic pathways reflect the situation in dog kidney, where metabolic flux from glutamate to α-ketoglutarate is primarily performed via transamination by alanine aminotransferase (ALT). In the present study, LLC-PK_1 wildtype cultures were compared with the gluconeogenic substrain LLC-PK_1-$FBPase^+$. First, baseline production rates of NH_3 and L-alanine by the cell strains were correlated with the expression of the corresponding enzyme activities, phosphate-dependent glutaminase (PDG), glutamate dehydrogenase (GDH), and ALT. LLC-PK_1-$FBPase^+$ cells exhibited a higher expression of PDG and GDH activity, respectively, indicative for enhanced oxidative metabolism, compared with the parental (glycolytic) LLC-PK_1 line, which correlated well with increased NH_3 production rates found in the gluconeogenic substrain. Basal alanine production rates were also increased in LLC-PK_1-$FBPase^+$ cultures, which in part is due to the conversion of pyruvate, supplied as gluconeogenic substrate, by LLC-PK_1-$FBPase^+$ cells. Upon exposure of cultures grown to 12-14 days confluence at pH 7.6 to metabolic acidosis (pH 7.0) for up to 72 h, both cell strains clearly adapted with a gradual increase in NH_3 and alanine production. NH_3 accumulated in tissue culture medium, whereas alanine was reutilized by the cell strains at different rates. The ammoniagenic response was much more pronounced in gluconeogenic LLC-PK_1-$FBPase^+$ than in glycolytic LLC-PK_1 wildtype cultures. In addition, LLC-PK_1-$FBPase^+$ cells responded with a doubling in PDG activity expression after 48 h of acidosis, whereas LLC-PK_1 cells lack any adaptive changes in enzyme activity expression. In experiments attempted to increase the state of differentiation of LLC-PK_1-$FBPase^+$ cells and to augment the adaptive response to metabolic acidosis, epithelial cultures were grown on permeable tissue culture inserts. Confluent LLC-PK_1-$FBPase^+$ epithelia generated an apical negative transepithelial potential difference (PD_{te}), in contrast to LLC-PK_1 epithelia, which exhibit an apical positive PD_{te}. Therefore, the altered phenotype of the LLC-PK_1-$FBPase^+$ is pleiotropic. Since LLC-PK_1-$FBPase^+$ cells also clearly respond upon acidosis with an increase in cytosolic PEPCK expression (Am. J. Physiol. 268: C449, 1995), this gluconeogenic cell line represents a valuable in vitro model to study in tissue culture acid-base regulation of renal ammoniagenesis and gluconeogenesis and the interdependence of the two pathways.

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CHROMATIN ORGANIZATION IN RAT KIDNEY CORTEX AND MEDULLA SUBMITTED TO OSMOTIC CHALLENGES. R.Gilles[1], Ph.Compère[2], M.El Goumzili[1], A.Buche[3] and C.Houssier[3].

Kidney medulla cells of mammals have to cope with large changes in environmental osmolarity; a challenge most other mammalian cells cannot easily stand and never have to experience in physiological conditions. In these last cells, application of osmotic shocks lead to important modifications in the organization of the nuclear chromatin. The present paper reports on the changes 1) of cortex and medulla chromatin in situ, in rat kidney slices submitted to osmotic challenges, 2) of medulla chromatin in vitro, in preparation of extracted chromatin submitted to changes in environmental ion concentrations. Our results show that the chromatin of kidney medulla cells 1) does not behave differently from the other mammalian chromatins when submitted in situ or in vitro to osmotic challenges, 2) presents in vitro characteristics similar to the other mammalian chromatins and 3) is protected in vitro, as the other mammalian chromatins, from the disrupting effects of increases in inorganic ion concentration by different so-called compensatory organic osmolytes. The ability of kidney medulla cells to adapt easily to large increases in osmolarity could thus be related rather to a rapid control of the level of such compounds than to some specific, intrinsic molecular adaptations of macromolecules as seen in halophilic archeabacteria for instance

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REGULATION OF RAT KIDNEY Na-,K-ATP-ase ACTIVITY BY ENDOGENOUS INTESTINAL SUBSTANCES

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Substances with molecular weight from 5000 Da to 150 Da were obtained from rat intestinal epithelia, chromatographycally separated and lyophilized. Fractions of these substances with molecular weight 5000 Da and less than 200 Da inhibited ATP-ase from membranes of rat kidney cortex on 25,7% and 34,7% respectively, fractions 400-200 Da activated it on 48,6%. 20-30 mmol/l Na had an influence on activity of all substances - activators and inhibitors both. Sodium ions reduce effect of activators significantly and simultaneously increase effect of inhibitors. It was possible to prevent influence of sodium cations by addition of bivalent cations Ca and Mg in solution. U-V spectroscopic analysis permit us to suppose that all studied substances from rat intestina have nucleoside structure.

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THE DYNAMICS OF SODIUM, POTASSIUM AND WATER TRANSPORT ACROSS THE MAMMARY EPITHELIUM.

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The Dynamic characteristics of ions and water transepitelial transport in mammary alveoli and ducts were investigated. The rate of milk secretion and the intensity of Na, K and water transport into milk were analysed for lactating albino mice using the gravimetric method, flame- and atomic-absorption spectrophotometry, indicator methods founded on mass- beta- and gamma-spectrometry. It was shown that the rate of milk secretion is constant during normal or 3 to 5-fold increases in the time intervals between sucklings; however the Na and K concentrations in milk change step by step. The data suggest that the formation of the milk aqueous phase is a dynamic multistep process. Its base stages occur in alveoli. The initial stage is characterized by high rates of Na, K and water transport to alveolar lumina. Next, the average stage is characterized by low rates of ions and water transport into milk. Then the Na transport into alveolar milk ceases; however, the quantity of K and water entering increases during the third stage. The formation of the milk electrolyte content is finished in the ducts system. The experiments show, that 27—40 % of the Na and 13—14 % of the water contained in milk is transported across alveolar epithelium due to filtration. All the K and a large part of the Na and water enter the milk by transcellular mechanisms. It was shown that near 85—90% of the Na entered in alveolar milk and 25—30 % of the Na moved from the alveolar in the lumina ducts are reabsorbed and exchanged on K by Na-K ATP-ase in the basolateral cellular membrane. This process provides the subsequent intensive K transport in milk through apical cellular membrane down its concentration gradient.

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VOLUME REGULATION BY HYPOSMOTICALLY STRESSED CEREBRAL CORTICAL CELLS: EFFECTS OF -SH GROUP ALKYLATION IN THE PRESENCE OF GABA AND TAURINE.

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Swelling limitation in hypsomotically stressed brain cells is partly mediated by loss of amino acids including GABA and taurine. Recent evidence suggests that in swollen cells this loss (but not basal loss from cells in isosmotic media) is influenced by the availability of -SH groups. The present study examines the effects on steady-state volumes of cells in cortical slices, pre-loaded with GABA or taurine (both lmM), of the -SH alkylating agents N-ethylmaleimide (NEM) and iodoacetate (IA) (both 100 μM) in hyposmotic media (265mosmol/kg). Cell volumes were measured as slice equilibrium non-inulin spaces. The principal findings were as follows:- (a) The small, non-osmometric cell swelling that follows transference of slices from iso- to hyposmotic media was significantly enhanced by IA and NEM in the presence of amino acids: (2) the effects of both agents were abolished when cell swelling was inhibited by addition of 25mM sucrose to media; (3) the -SH reducing agent dithiothreitol (DTT, 100 μM) reversed the swelling effects of IA (partly) and NEM (completely) in media containing GABA but had no effect in the presence of taurine; (4) IA and NEM had no effects on cell volumes in the absence of exogenous amino acids. The results are consistent with the view that moderate cell swelling in hyposmotic media may lead to conformational changes in -SH sites which facilitate volume-regulatory GABA and taurine loss, and further stress the role of amino acids in swelling limitation. However, the diverse effects of DTT suggest that the facilitatory effects of -SH groups on carboxylic and sulphonic amino acid losses may represent discrete processes.

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LACK OF A DIRECT EFFECT OF ENDOTHELIN ON GLOMERULAR ULTRAFILTRATION COEFFICIENT

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Objective: To study the effect of exogenous endothelin-1 (ET-1) on glomerular function of anesthetized dog. **Methods:** Anesthetized beagle dogs, elmag flowmeter, micropuncture of superficial structures. ET-1 at 2.78ng/kg/min intrarenally (i.r.a.). Group 1: "normal dogs", Group 2: dogs infused with a "cocktail" containing alpha- and beta-adrenoceptor, thromboxane, leukotrienes, and angiotensin-II-receptor antagonists i.r.a. and indomethacin i.v. **Results in Group 1:** A decrease in renal blood flow and glomerular filtration rate accompanied by a rise in filtration fraction; an increase in efferent (100%) and afferent (58%) arteriolar resistance: preferential efferent constriction; a decrease in ultrafiltration coefficient (K_f) by 23%. In **Group 2,** vasoconstrictory action of ET-1 was some 30-40% weaker; the increase in resistance was now equally distributed between both arterioles (31% for afferens, 34% for efferens); no change in K_f (9.38 +/- 1.06 prior vs 10.14 +/- 1.12 $ul.min^{-1}.min$ after ET-1, $p > 0.05$). **Conclusions:** ET-1 infused i.r.a. to anesthetized dogs has a vasoconstrictory effect more pronounced on the efferent than on the afferent arteriole. This vasoconstriction is accompanied by a decrease in water, urea and electrolyte excretion rates which seems to be GFR-dependent. Part of this vasoconstrictory action of ET is probably mediated by other substance(s). ET-1 itself has probably no effect on the K_f value.

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ERYTROPOIETIN AS A FACTOR IN INDUCING ANEMIA OF RENAL INSUFFICIENCY. V.Kalaidjieva

The aim of our study was to distinguish the different contribution of erythropoietin (EPO) to renal anemia in acute and chronic renal failure (ARF, CRF). To this end we studied 32 hemodialysed patients (HP) with CRF, divided in two groups according to the duration of renal disease - 0÷5 yr. and 6÷12 yr. We estimated the anemia by measuring the main hematological parameters - plasma EPO level (by RIA), hematocrit (Hct), hemoglobin (Hb), red blood cells count (RBC). Biochemical analysis by standard methods and blood pressure were evaluated as well. An other experimental protocol was made by inducing tubular damage (hystologically evaluated) in Gentamicin (GM) model rats (n = 21). Similar parameters were determined after GM treatment (50mg/kg BW i.p./15d). Some rats were subjected thereafter to hypobaric hypoxia (0.42atm/6h) to test their response to erythropoietic stress. Others were given s.c. rhEPO 50mU/rat/2d. Blood samples were taken on day 0 and 3 after stimulation. The results obtained showed severe anemia in HP - Hb 79.2±20g/l, Hct 0.27±0.06, RBC $2.51\pm0.5.10^{12}$/l vs 140.6±8.4g/l; 0.42±0.04; $4.17\pm0.3.10^{12}$/l in healthy subjects ($n = 15$)($p < 0.001$). EPO levels were found inappropriately low for the degree of anemia but within normal range in all patients 12.34±8.5mU/ml vs 11.80±5.1mU/ml in control group. No changes were observed with age and with the period of dialysis. On the contrary, in acute tubule lesion a lack of EPO response has been seen after hypoxic stimulation 15.3±3.8mU/ml vs 40.4±10.2mU/ml in hypoxic intact animals ($p < 0.01$). Well expressed compensatory reticulocytosis was noted on the third day in GM group - 39‰ ±10,4 vs 2‰±1.3 on day 0 ($p < 0.001$).

The data reveal more marked contribution of reduced EPO production in anemia of acute renal impairment than of chronic disease. Apparently in the latter the capacity of producing EPO may be conserved although at a disturbed set point of oxygen sensing. Involvement of factors other than Epo deficiency can be considered.

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THE RENAL ACTIONS OF ADENOSINE. G.Kövér and Hilda Tost

This study examined the effects of adenosine on the renal function.In ten normal dogs intrarenal adenosine infusion(20 nmol/kg/min) increased the renal blood flow(RBF) from 521±20 ml/min to 582±23 ml/min.The extraction of PAH (E_{PAH}) decreased from 0.85±0.02 to 0.79±0.02, the E_{inulin} from 0.24±0.02 to 0.18±0.02 We conclude that the intrarenal infusion of adenosine modifies the intrarenal redistribution of the blood flow increasing the deep cortical and medullary blood flow.In these experiments the glomerular filtration(GFR) during adenosine infusion calculated from the extraction of the inulin(E_{inulin}) multiplied by the renal plasma flow(RPF) decreased from 79.4±6.4 ml/min to 62.2± 6.6 ml/min and calculated from the $E_{creatinine}$x^{RPF} from 80.3± 6.3 ml/min to 59.3±4.9 ml/min. The E_{PAH}x^{RPF} did not change, it was 241±11 ml/min and 253±13 ml/min, respectively.While the urinary clearances (the clearance calculated by the classic clearance formula; urinary concentration of the substance multiplied by the urine volume and divided by the plasma concentration) in the control periods did not differ from the direct clearances (C_{inulin}=73.3±3 ml/min, $C_{creatinine}$=75±4 ml/min and C_{PAH}=262±15 ml/min) during the adenosine infusion there are considerable differences: the C_{inulin}=40±6 ml/min, the $C_{creatinine}$ =42±6 ml/min and the C_{PAH}=164±22 ml/min. The differences are mathematically significant ($p < 0.01$).During the postinfusion periods the urinary clearances did not differ from the direct clearances.These results show that during adenosine infusion there is a definitive loss of the clearance substances somewhere in the nephron between the glomeruli and the pyelon.These observations suggest that during adenosine infusion there is a back-diffusion of the clearance substances,the permeability of the tubuli changes in the medullary part.The rediffused substances will be retransported into the circulation by the renal lymph flow and that is why they do not appear in the renal venous blood.The rediffusion can explain that the intrarenal adenosine infusion decreases considerably the excretion of the sodium and water in the kidney when there is no or only a small reduction of the glomerular filtration rate.

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EFFECTS OF INTRAPORTAL INFUSIONS OF ARGININE AND GLYCINE ON RENAL FUNCTIONS IN SHEEP FED LOW PROTEIN DIET. M. Szanyiova, S. Faix, A. Cirio* and L. Leng

It is well established that sheep on low protein dietary intake (LP-diet) have a considerable reduced glomerular filtration rate (GFR). This experiment was designed to study the renal response of anaesthetized sheep after 6 weeks of LP-diet to infusions of arginine and glycine to portal vein. Infusion rate for both amino acids was 0.6 mM/ml/min for 90 min. Whole kidney studies showed that arginine significantly elevated urine flow rate (V), GFR and amount of urea excreted ($U_{urea}V$). The plasma urea level (P_{urea}) after arginine was found also higher but without change in fractional urea excretion (FE_{urea}). Sheep infused with glycine showed the significantly increased urinary flow rate only. Free-flow micropuncture of late proximal tubule revealed that the both fluid flow rate and single nephron GFR were increased by arginine without any significant change in tubular fluid to plasma inulin ratio. There were no effects of glycine on studied micropuncture parameters of sheep kidney. In summary, the results of this study show that arginine infusion to the portal system of sheep on low protein diet increases glomerular filtration rate. It is suggested that the synthesis of urea from its precursor arginine in liver and its subsequent delivery to the kidney can be involved in the regulation of dietary induced changes of renal filtration in sheep.

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EFFECTS OF GLUCAGON ON RENAL FUNCTIONS IN SHEEP FED A LOW PROTEIN DIET. S. Faix, L. Leng and R. Boivin*

It is well known that a low protein intake results in a decrease of glomerular filtration rate in sheep. The reduced glucagon plasma level has been suggested to be responsible for this effect of low protein diet. In our protocol the conscious sheep on low protein diet were intravenously infused with glucagon in a rate 100 $ng.kg^{-1}$ body $weight.min^{-1}$. The urine flow rate of sheep decreased in the first 15 minutes from 0.84 ± 0.16 $ml.min^{-1}$ to 0.64 ± 0.23 $ml.min^{-1}$ ($P<0.01$) and it futher dropped as low as 0.48 ± 0.15 $ml.min^{-1}$ ($P<0.01$) after one hour of glucagon infusion. Glomerular filtration rate (GFR) was also reduced upon initiation of glucagon infusion (55.96 ± 7.34 $ml.min^{-1}$) to reach its lowest value (35.95 ± 5.16 $ml.min^{-1}$, $P<0.05$) after 45 minutes of glucagon infusion. It should be stressed out at this place that the same glucagon was checked in rats and it resulted in the known significant increase of both GFR and urine flow rate. The decrease of renal plasma flow of sheep during glucagon infusion was not significant. Glucagon infusion induced also a nonsignificant decrease in the amount of urea excreted. In the first 15 minutes of glucagon infusion the urea clearance decreased from 12.80 ± 2.21 $ml.min^{-1}$ to 8.10 ± 1.53 $ml.min^{-1}$ ($P<0.05$) and remained low throughout the whole infusion time. Conversely, the infusion of hormone did not significantly alter the fractional urea excretion. Presented results do not confirm glucagon as a mediator of adaptation of renal function of sheep to a low protein intake by diet.

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OCCLUSION OF THE ADRENAL VEIN LEADS TO THE DECREASE IN IPSILATERAL RENAL BLOOD FLOW.

P. Abramczyk, J. Przybylski, R. Zięcina, A. Lisiecka, and B. Ciszek

It has been shown that the increased blood flow through the direct adrenalrenal vascular connection (ARVC) results in the development of the arterial hypertension in rat (Abramczyk P,Ciszek B,Papierski K.,Przybylski J.Med. Scien. Res., 1992,20,281). The aim of the present study was to investigate the influence of an acute increase in blood flow through the above rete on the renal blood flow (RBF). The study was performed on 6 male Wistar rats. In the anesthetized animals the laparotomy was done and the probe of ultrasonic flowmeter was placed on the left or right renal vein. Two experiments (in randomized way) were performed. I - the central adrenal vein was clamped. II - tissue between the adrenal gland and the kidney was cut before clamping the central adrenal vein. In the first series, RBF decreased by 25% ±5% SE of the resting values, whereas in the second series neither cutting the ARVC nor subsequent ligation of the adrenal vein have not changed RBF. Short latency of RBF changes due to increase in blood flow through ARVC suggests pivotal role of adrenal cateholamines.

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ALTERED EXPRESSION OF HEAT SHOCK PROTEINS (HSP) IN THE RAT KIDNEY AFTER UNILATERAL NEPHRECTOMY.

W. Neuhofer, E. Müller, A. Ono, K. Thurau, F.X. Beck

In response to reduction of renal mass, the remaining kidney parenchyma, in particular the proximal tubule, hypertrophies. Since it is known that HSPs play important roles during protein synthesis, protein targeting, promotion of correct protein folding and protect against cytotoxic agents, the expression of different HSPs was analysed after unilateral nephrectomy (UNX) or sham operation in male Wistar rats. Using SDS-PAGE and Western blots, three constitutively expressed HSPs (HSP73, HSP25 and the mitochondrial HSP60) and the major stress-inducible HSP (HSP72) were examined in various zones of the kidney (cortex, outer medulla and inner medulla). The HSP72 and HSP25 increased significantly in the outer medulla with peaks at 12-24 h post-UNX, while HSP60 and HSP73 were not elevated. HSP25 exists in one nonphosphorylated (HSP25A) and two phosphorylated (HSP25B,C) isoforms, which are interconverted in a growth-factor dependent way. Thus the HSP25A/B+C ratio was determined by isoelectric focussing.Changes in HSP25 phosphorylation were most prominent in cortex.

The increase in HSP72 and HSP25 after UNX in outer medulla suggests that these HSPs participate in the regulation of growth processes in this kidney zone. Since phosphorylation of HSP25 is reportedly associated with growth-factor-induced cell differentition, the enhanced phosphorylation of HSP25 may be also a component of the adaptive response to UNX.

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EXCRETION OF γ-GLUTAMYL TRANSPEPTIDASE IN SPONTANEOUSLY HYPERTENSIVE RATS AS A MARKER OF TUBULAR INJURY.

J. Pittner, T. Huszár, M. Molnár and L. Rosivall.

There is controversy regarding the value of proximal tubular marker enzymes in the detection of tubular damage. To study the mechanism of excretion of γ-glutamyl transpeptidase (γGT, brush border origin) and β-N-acetyl-D-glucosaminidase (NAG, lysosomal origin), we established a model of accelerated renal injury combining genetic hypertension and streptozotocin-induced diabetes. After administration of streptozotocin, 50 mg/kg bw. iv., to 20 male Wistar-Okamoto spontaneously hypertensive rats (SHR, MAP: 196±17 mmHg), animals were placed in metabolic cages for a 24-hour period once a week for 14 weeks and urine and blood samples were collected. Blood glucose level of the diabetic animals increased 5-fold (44.8±21.7 vs. 9.7±1.9 mmol/l, $p<0.01$), urine volume increased about 5-fold (296.4±192.6 vs. 39.8±21.5 ml/day/kg bw, $p<0.01$) and albumin excretion about 30-fold (12.52±6.17 vs. 0.38±.18 mg/day; $p<0.01$) by the end of week 14, whereas excretion of γGT (Technicon), exhibited a 150-fold increase (29833±14492 vs. 25.6±21.2 mU/day) and excretion of NAG (Boehringer) showed a 2-fold elevation (642.5±209 vs. 270.1±263.6 mU/day, $p<0.01$). The corresponding parameters determined in 20 male SHR animals used as controls were statistically unaltered over the 14-week period. This elevation in γGT, but not in NAG, excretion is well above those usually found in the literature. Proteinuria indicates that γGT does not exclusively originate from the brush border but also from plasma. Assuming the hypothetical situation when γGT is freely filtered through the glomerulus and calculating the maximal possible excretion of the enzyme attributable to filtration, the actually measured excretion still exceeds this value by 90%. Our results indicate 1) that γGT is a good marker of tubular injury in diabetes mellitus, even in the chronic phase, 2) an increased susceptibility of the kidney for diabetes-induced tubular brush border damage in SHR.

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SPONTANEOUS MOTOR ACTIVITY OF HUMAN AND GUINEA-PIG RENAL PELVIS: PATTERNS AND IONIC EFFECTS. E. Neu, M.Ch. Michailov, W. Seidenbusch, U. Welscher, S. Magour, D.G. Weiss

We previously described spontaneous electrical and motor activities of isolated human and guinea-pig (g-pig) ureter and renal pelvis as well as electro-induced modulation of spontaneous phasic contractions (SPC) (*Neu, Michailov et al. *Eur. J. Physiol.* **402**, R48, 1984; *Proc. Int. Un. Physiol. Sci.* Vol. **17**, 529, 1989 and Vol. **18**, 216, 1993/32. IUPS Congr. Glasgow; *Urol. Res.* **8**, 236, 1980). Further observations showed various SPC-patterns of renal pelvis: Regular and uniform contractions in 52.1% of human (n=47) resp. in 62.9% of g-pig (n=205) preparations; irregular and uniform in 23.4 resp. in 20,5%; irregular and non-uniform in 12.8 resp. in 10.2%; burst-like in 4.3 resp. in 1.5%; others in 6.4 resp. in 4.9%. The table demonstrates values of frequency (F) and initial contractile amplitude (A) of SPC [A_o in % of length l of preparations; l_o=9±2 mm for human (n=38) resp. 7±2 mm for g-pig (n=63)] (upper trace) as well as changes of A (in % of initial A_o from single preparations, n=4-5) at different $[KCl]_e$ (1.0x=5.60x10^{-3} M=100%) (middle trace) and $[CaCl_2]_e$ (1.0x=2.16x10^{-3} M=100%) (lower trace) (mean value±SD):

	n	*A_o in %*	*F/min*	*F<3/min*	*(n)*	*F3-6/min*	*(n)*	*F>6/min*	*(n)*
human	38	20.8±11.9	3.9±3.0	1.7±0.6	(20)	4.0±0.9	(10)	8.9±2.2	(8)
g-pig	63	17.1±12.9	5.4±6.2	2.1±0.7	(11)	4.7±0.8	(38)	7.4±1.5	(14)

A in %	*n*	***KCl:** 0.1x*	*0.5x*	*2.0x*	*5.0x*	*10.0x*
human	4	32.2±45.6	67.2±14.4	61.2± 4.4	0±0	0±0
g-pig	5	32.8±44.0	73.8±39.4	113.8±38.6	59.0±58.8	0±0

A in %	*n*	***$CaCl_2$:** 0.1x*	*0.5x*	*1.5x*	*2.0x*	*2.5-3.5x*
human	4	0±0	23.3±33.0	86.6±18.9	75.0±7.1	n.d.
g-pig	5	24.0±14.1	52.5±23.8	102.5±5.5	123.3±11.3	116.8±4.5

The various motor patterns of renal pelvis are obviously the result of differences in complex mechanisms of intra- and intercellular electrical and hormonal regulation of rhythmogenesis as well as in intercellular conduction of excitation whereby K^+ and Ca^{++} and their ratio play an essential role. It is suggested that the motor patterns are correlated to the various electrical patterns (spikes, bursts and burst-plateaus) of g-pig and human detrusor and ureter cells (also in human renal pelvis) which are described as given (*).

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REDUCED LEVELS OF HSP72 IN THE RENAL MEDULLA OF DIURETIC RATS. E.Müller, W.Neuhofer, A.Ohno, K.Thurau, FX.Beck

Heat shock proteins (HSPs) modulate protein folding and targeting in unstressed cells and prevent misfolding and aggregation of proteins in cells exposed to a variety of stress factors (heat, toxic agents, ischaemia). We studied the effect of varying extracellular osmolalities (osmotic stress) on the intrarenal distribution of four different HSPs in the rat kidney in vivo: HSP60, HSP73 and HSP25, three constitutively expressed HSPs only slightly inducible by stress, and HSP72, the major stress-inducible HSP. We studied control rats, animals after 3 weeks diuresis (3% sucrose in drinking water) and animals given normal drinking water for 5 days following this period of diuresis. Urine osmolality fell from 1095 mosmol/kg (control) to 365 mosmol/kg (diuresis) and remained low during the whole period of diuresis. After cessation of diuresis osmolality was 1014 mosmol/kg after 5 days. Western blot analysis with specific antibodies revealed a characteristic distribution pattern for each HSP in control rats. The level of HSP60 was high in the cortex and low in the medulla. HSP73 was distributed homogeneously throughout the whole kidney. Large amounts of HSP25 and HSP72 were present in the medulla but only low levels of HSP25 and almost undetectable amounts of HSP72 were found in the cortex. In diuresis, the amounts of both HSP25 and HSP73 decreased slightly in the whole kidney, while the level of HSP60 did not change at all. In contrast, the amount of HSP72 was markedly reduced (50%) in the medulla.. After cessation of diuresis all HSPs returned to control levels with the exception of HSP60, which again was not influenced. The fact that low osmolality reduces the renal content only of the major stress-inducible HSP72 implies that the high urea and salt concentrations in the medulla of a normally concentrating rat kidney represent a stress situation for kidney cells. Diuresis apparently attenuates the osmotic stress upon medullary cells thus reducing the requirement for high amounts of the cytoprotective HSP72. This hypothesis is strengthened by the observation that a return to normal urinary osmolality again induces HSP synthesis.

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EFFLUX PATHWAYS FOR ORGANIC OSMOLYTES IN RENAL INNER MEDULLARY COLLECTING DUCT CELLS: MULTIPLICITY AND DIVERSITY. B. Ruhfus and R.K.H. Kinne

Renal inner medullary collecting duct (IMCD) cells respond to a hypotonic shock by releasing organic osmolytes such as sorbitol, inositol, glycerophosphorylcholine, taurine, and betaine. The current investigations were aimed to define in isolated cells by flux studies the properties of these efflux pathways and to determine their degree of similarity. Sorbitol efflux was found to be dependent on a rise in intracellular calcium, to be increased by arachidonic acid, slightly inhibited by extracellular ATP and stimulated by SITS, DIDS, 1,9-dideoxyforskolin, niflumic acid, and extracellular cAMP. In contrast, taurine efflux was not calcium dependent, was inhibited by arachidonic acid, regulated by intracellular ATP, inhibited by extracellular ATP and cAMP, and strongly reduced by NPPB, 1,9-dideoxyforskolin, niflumic acid, SITS and DIDS. The same applied to inositol release, however, in general inhibitors seemed to be more effective on inositol efflux than on taurine efflux. In addition, intracellular taurine appeared to modify inositol efflux. These data suggest that in IMCD cells separate efflux routes exist for sorbitol, taurine and inositol. Furthermore the release of organic osmolytes seems to be regulated by different signal transduction pathways and coordinated by "intracellular crosstalk".

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EFFECTS OF LONG-TERM ADMINISTRATION OF VASOPRESSIN ON DEVELOPMENT OF KIDNEY IN GROWING LAMBS. L. Leng, V. Bizub, M. Szanyiova, S. Faix

Effects of three month subcutaneous administration of dDAVP on macromorphology of the kidneys were studied in young growing lambs. Experimental group (n=6) was given 10 ug 1-desamino -D-arginine vasopressin twice daily and control group (n=5) was given saline. At the age of four months the lambs were slaughtered and their kidneys were prepared by conventional histological approach to morphometry. The kidney was completely cut on microtome to sections with 20 um thickness and every 25th section underwent morphometric measurements with digitizing tablet. Computer integrated results showed that the volume of cortex was reduced while the both height and volume of medulla were significantly larger in dDAVP treated lambs. The increment in size of medulla was mainly due to changes of outer medulla dimensions. The surface area of renal pelvis was found to be significantly enlarged by 29 % in dDAVP treated group. The average surface area of renal pelvis in lambs four months old was found to be 5956.76 ± 364.73 mm^2. Presented results suggest that the morphological changes in kidneys of growing lambs caused by increased supply of vasopressin can support the renal recycling of urea and also the concentrating ability of kidneys.

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EVALUATION OF ACTIVE *VERSUS* PASSIVE TRANSPORT OF LITHIUM IN RENAL PROXIMAL CONVOLUTED TUBULES FROM TF/P-RATIOS. A MATHEMATICAL MODEL.

O.Frederiksen, P.P.Leyssac, and N.-H.Holstein-Rathlou.

The question of passive *vs.* active reabsorption of solutes in the renal proximal convoluted tubule may be evaluated from *in vivo* or *in vitro* microperfusion experiments. However, these techniques involve several technical difficulties and potential experimental pitfalls. Thus, the driving forces for transepithelial fluxes may be altered due to the applied techniques, and driving forces may vary with tubular length. A less invasive method would be to evaluate passive *vs.* active reabsorptive mechanisms *in situ* from the tubular fluid to plasma (TF/P) concentration ratios obtained from collections of proximal tubular fluid. We describe a general mathematical model that can be used to evaluate whether the observed TF/P-ratios can be caused by passive transport alone, or whether active transport must be present. Net reabsorption of a solute in a tubule was expressed by combining a mass balance equation with an equation describing the passive transepithelial flux of the solute. The latter was based on the formalism of irreversible, nonequilibrium thermodynamics. This results in an equation relating solute TF/P-ratio profiles to known or measurable parameters: solute permeability (ω), reflection coefficient (σ), and fluid reabsorption and electrical potential difference profiles. We have compared the results of model simulations with our previously obtained data on TF/P-ratios for lithium (Li^+) obtained from pressure controlled tubular fluid collections (Am.J.Physiol. **258**: F1090-F1095 (1990); *ibid* **267**: F86-F93 (1994)). Assuming ω_{Li} and σ_{Li} to be equal to the values for Na^+ the results demonstrate that proximal tubular Li^+ reabsorption cannot be due only to passive transport mechanisms but must involve an active mechanism.

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LACK OF CHLORIDE INHIBITS AFFERENT ARTERIOLAR CONTRACTION. O.Skøtt, B.L. Jensen and P. Ellekvist

The afferent arteriole contains smooth muscle cells and renin-secreting cells. Intracellular chloride is involved in the control of renin secretion, while the role in excitation-contraction coupling in the smooth muscle cells is less known. To investigate this we microdissected, cannulated and perfused rabbit afferent arterioles, and tested the effects of norepinephrine (NE), potassium and angiotensin II (AII) in the presence and absence of chloride in the bath medium. The arterioles equilibrated 30 min after each change in extracellular chloride concentration to assure equilibration with intracellular chloride. The chloride concentration in the perfusate remained at 120 mM in all series. Each concentration of vasoconstrictor was present for 2 min. Results are given as luminal diameters in µm ± SE. Responses to NE were measured consecutively in the same vessels (n=6), while responses to AII (n=6) were measured in separate series because of tachyphylaxis.

Chloride	Control	100 mM K^+	$5*10^{-7}$ M NE	10^{-6} M NE
120 mM	17.8±0.8	2.0±1.0	2.4±1.5	0.8±0.8
0 mM	17.7±0.9	17.7±0.9	11.7±2.5	4.9±1.9
120 mM	17.0±0.9	13.4±2.3	8.7±2.6	0.8±0.8
Chloride	**Control**	**10^{-10} M AII**	**10^{-8} M AII**	**10^{-6} M AII**
120 mM	14.1±0.9	13.4±0.9	2.4±1.6	0.8±0.1
0 mM	19.2±1.5	19.1±1.5	19.1±1.5	17.3±2.4

Removal of chloride left basal diameter unchanged while the sensitivity to NE was markedly and reversibly suppressed, and the responses to potassium and AII were blocked. We conclude that one or more steps in the excitation-contraction coupling are highly sensitive to chloride.

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EFFERENT RENAL NERVE ACTIVITY AND GLOMERULAR FILTRATION RATE DURING HYPOTHERMIA. M.Broman, Ö.Källskog, U.C.Kopp and M.Wolgast. Hypothermia (28°) is associated with decreased renal blood flow (RBF) and glomerular filtration rate (GFR) previously shown to result from change in blood viscosity and an active afferent arteriolar vasoconstriction. The present study performed on Inactin anaesthetized rats to examine whether the increased afferent arteriolar tone was due to increased activation of the sympathetic nervous system. For this purpose the efferent renal nerve activity (ERNA) was measured before, during and after hypothermia. Despite apparent shivering at 28°C (in the same frequency range as the efferent renal nerve signals), ERNA decreased though not significantly during hypothermia from 100% (reference) to 85%, returning to 85 % after rewarming (no shivering at this point). The corresponding values for GFR was 1.15, 0.58 ($p<0.001$) and 1.03 ml/min, respectively, indicative for a decrease in RBF. Mean arterial blood pressure was maintained during hypothermia (110, 113 and 102 ($p<0.05$) mmHg, respectively), whereas heart rate decreased from 362 to 248 beats per min. ($p<0.001$) at 28°C, returning to 349 beats per min. ($p<0.05$) after rewarming. Urine flow was 3.71, 8.49 and 4.02 µl/min, and sodium excretion 219, 650 and 183 nmol/min, respectively.

We conclude that there is no evidence present that an increase in ERNA is responsible for the afferent arteriolar vasoconstriction occurring in the kidney during hypothermia at 28°C. The increase, though not significant, in urine flow and sodium excretion and considering that the activity of shivering will add to the nerve recordings, strongly suggest that ERNA decrease more than shown.

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STIMULATORY ACTION OF PROSTANOIDS ON TRANSCELLULAR Ca^{2+} TRANSPORT IN RABBIT CONNECTING TUBULES. Jürgen van Baal, *Freek J. Zijlstra, ‡Peter H.G.M. Willems, René J.M. Bindels.

The influence of prostanoids on active transcellular Ca^{2+} transport was investigated in immunodissected rabbit kidney connecting tubule cells grown to confluency on permeable supports. Treatment of the monolayer with indomethacin, a specific inhibitor of cyclooxygenase-activity, dose-dependently (EC_{50} = 20 nM) decreased transcellular Ca^{2+} transport. At 0.5 µM indomethacin transport was maximally inhibited from 82 ± 7 to 37 ± 4 nmol/hr/cm^2. This observation suggests that the monolayers normally produce a prostanoid which stimulates transcellular Ca^{2+} transport. In order to investigate this hypothesis, indomethacin-treated monolayers were incubated in medium collected from control cells. Using this protocol, the presence of a stimulatory prostanoid in control medium could indeed be demonstrated. Analysis of apical and basolateral medium following incubation with [^{14}C]arachidonic acid revealed the presence of the full spectrum of radio-labelled prostanoids. Addition of exogenous PGE_2 (0.1 µM) to the basolateral side of the monolayer completely reversed the inhibitory effect of indomethacin on transcellular Ca^{2+} transport. The effect was dose-dependent, with a half maximal and maximal concentration of 7 nM and 100 nM, respectively. A similar dose-relationship was obtained when PGE_2 was added apically. This stimulatory effect was also observed with PGE_1 or PGA_2, but could not be mimicked by PGF_{2a}, PGD_2 or cicaprost, a stable analogue of prostacyclin. In indomethacin-treated cells, the stimulatory effect of the calciotropic hormone PTH was dramatically enhanced (3-fold).

The present study demonstrates that primary cultures of rabbit connecting tubules secrete different classes of prostanoids, at least two of which, namely PGE and PGA, have a stimulatory effect on transcellular Ca^{2+} transport. Moreover, it shows that inhibition of cyclooxygenase unmasks the stimulatory effect of hormones in its full extent.

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ARGININE VASOPRESSIN INCREASES cAMP AND ACTIVE Ca^{2+} REABSORPTION IN RABBIT CONNECTING TUBULES. René J.M. Bindels, Jürgen van Baal, Jaap de Slegte‡, Peter H.G.M. Willems‡.

The effect of arginine vasopressin (AVP) on active transcellular Ca^{2+} transport was studied in immunodissected rabbit kidney connecting tubules cultured to confluency on permeable supports. Addition of AVP to the basolateral side of the monolayer in the effective range of 0.3-10 nM dose-dependently (EC_{50} = 0.7nM) stimulates Ca^{2+} reabsorption from 80 ± 2 to a maximum of 119 ± 5 nmol/hr/cm^2. This stimulatory effect of AVP was paralleled by a dose-dependent (EC_{50} = 2 nM) increase in cellular cAMP production. The selective V_2-agonist dDAVP fully mimicked the stimulatory effect of AVP on both transcellular Ca^{2+} transport and cAMP formation. Neither agonist (10 nM) had an effect when added at the apical side. To determine whether the stimulatory action of AVP and dDAVP on Ca^{2+} transport was mediated by cAMP, monolayers were stimulated with either forskolin (10 μM) or dibutyryl-cAMP (5 mM). Both agents significantly elevated transcellular Ca^{2+} transport by 60 % and 30 %, respectively. Digital imaging microscopy of fura-2 loaded cultured cells showed a rapid sustained increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) following addition of either 10 nM AVP or 10 nM dDAVP to the basolateral compartment. This increase of $[Ca^{2+}]_i$ was mimicked by 30 μM forskolin, suggesting that cAMP is involved in the receptor-triggered increase in $[Ca^{2+}]_i$.

In conclusion, the present study demonstrates that nanomolar concentrations of AVP stimulate transepithelial Ca^{2+} transport via a V_2-receptor. Moreover, the mechanism possibly involved is a stimulated production of cAMP which triggers an increase in $[Ca^{2+}]_i$.

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EFFECTS OF NOREPINEPHRINE AND ANGIOTENSIN II ON CALCIUM IN PERFUSED AND NONPERFUSED RABBIT AFFERENT ARTERIOLES. Mark Kornfeld, Antonio M. Gutierrez, A.E.G. Persson & Max Salomonsson.

Angiotensin II (A II) is a powerful vasoconstrictor, which is supposed to affect L-type Ca^{++}-channels via activation of PKC. Cytosolic calcium concentration $[Ca^{++}]_i$ was measured in smooth muscle cells of rabbit afferent arterioles using an image analysis system and the fluorescent calcium sensitive probe fura-2. Two series of experiments, perfused and nonperfused, were performed.

No significant difference was found between the two series during control conditions. A II (10^{-8}) administration to the bathing chamber caused a monophasic increase in $[Ca^{++}]_i$ (87±11 to 133±21 nM) in the nonperfused and a biphasic respone (73±8 to 148±29, second peak 106±10 nM) in the perfused arterioles. The increase in $[Ca^{++}]_i$ in response to NE administration, on the other hand, was not affected by flow/pressure activation. In control series, the first pulse of A II desensitized the arterioles, i.e. it was not possible to release a second increase in $[Ca^{++}]_i$. This tachyphylaxis could not be reversed by increasing doses of A II (10^{-11} - 10^{-8} M). However, this tachyphylaxis did not develop in the proximal segment when the voltage-sensitive channel blocker Nifedipine (Nif) was applied together with A II in the first administration. Furthermore, administration of Nif together with A II in the bath abolished the second peak in perfused arterioles.

In summary we have shown that during control conditions $[Ca^{++}]_i$ was not affected by perfusion of the arteriole. A II administration caused a biphasic increase in $[Ca^{++}]_i$ indicating an influence of pressure and/or flow. The afferent arteriole showed specific desensitization to repeated administration of A II. Increasing doses of A II could not reverse the tachyphylaxis induced by angiotensin. Nif inhibited development of tachyphylaxis in the proximal segment and abolished the second peak seen with A II administration in the perfused series.

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BLOCKADE OF ENDOTHELIN RECEPTORS ALTERS URODILATIN EFFECTS ON SYSTEMIC AND RENAL CIRCULATION IN RATS

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In the split hydronephrotic kidney endothelin (ET) receptor blockade was previously shown to inhibit the constriction of efferent glomerular arterioles normally observed after natriuretic peptides, like atrial natriuretic factor (ANF) or urodilatin (URO). In the intact kidney of female Wistar rats, anesthetized by Inactin, ET receptors were inhibited using a non-selective antagonist (PD 145065) given as i.v. bolus, 5 mg/kg body weight. The drug decreased mean arterial blood pressure (MAP) from 114±4 to 109±4 mm Hg and renal blood flow (RBF, electromagnetic flowmeter on renal artery) from 6.6 ± 0.3 to 5.8 ± 0.4 ml/min and increased renal vascular resistance (RVR) from 17.7±1.2 to 20.1±2.1 mm Hg·min/ml ($P < 0.01$). Intravenous infusion of URO alone at 0.1 nmol/kg/min decreased MAP by 7 ± 2% ($P < 0.01$), while RBF and RVR remained unchanged. Infusion of URO after PD 145065 pretreatment decreased MAP and RVR by 15±3% and 19±5%, respectively ($P < 0.01$). Heart rate (HR) decreased 14 ± 3% only when URO was given after ET receptor blockade.

Thus, in these experiments a basal level of ET resulted in renal vasodilation in contrast to constriction of systemic vasculature as a whole. Previous blockade of ET receptors enhanced MAP decrease in response to URO and diminished HR, which suggests an effect on cardiac output. URO abolished the increase of renal vascular resistance caused by blockade of ET receptors.

We conclude that endogenous endothelins modify the action of natriuretic peptides on the renal and systemic vasculature. (Supported by DFG)

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DOES BLOCKADE OF Na/H EXCHANGE AFFECT APICAL POTASSIUM CHANNELS IN THE THICK ASCENDING LIMB OF HENLE? R.J. Unwin, S.J. Walter and D.G. Shirley.

When loops of Henle are perfused *in vivo* with a low-Na solution (designed to minimize reabsorption in the pars recta; McKay & Peterson, 1993, Am. J. Physiol. 264, F792), and bumetanide is included in the perfusate (in order to block the Na K2Cl co-transporter in the thick ascending limb of Henle; TALH), net Na reabsorption is almost abolished and net *secretion* of K is consistently seen. This K secretion may be the result of K efflux through apical K channels in the TALH. *In vitro* patch clamp studies have indicated that these channels are pH sensitive (Greger et al, 1991, Kid. Int. 40, suppl 33, S119). On this basis, inhibition of Na/H exchange, which results in intracellular acidification, should reduce the K efflux. To test this, superficial loops of Henle of anaesthetised rats were perfused *in vivo* with the low-Na solution (104mM; isotonicity maintained with mannitol) containing bumetanide (10^{-6}M), with (+) or without (-) the Na/H exchange inhibitor ethyl isopropyl amiloride (EIPA; $2x10^{-4}$M; previously shown to reduce substantially bicarbonate reabsorption in the loop: Capasso et al, 1991, J. Clin. Invest. 88, 430). Reabsorptive rates (means ± SE) of water (J_V), Na (J_{Na}) and K (J_K) are shown in the table.

	Pump rate (nl/min)	n	J_V (nl/min)	J_{Na} (pmol/min)	J_K (pmol/min)
- EIPA	20.7±0.2	19	0.3±0.3	114±44	-16.6±5.2
+EIPA	20.6±0.4	19	-0.2±0.3	11±38	-17.3±2.5

The absence of effect of EIPA on K transport suggests that the K secretion does not occur through apical K channels in the TALH. Alternatively, these channels may be insensitive to pH *in vivo*. *Supported by the Wellcome Trust.*

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POSSIBLE RECIPROCITY: ADP-STIMULATED-Ca^{2+}-ACTIVATED RESPIRATION DURING SUCCINATE OXIDATION IN RATS MITOCHONDRIA IN HYPOXIC HYPOXIA CONDITIONS. I.Shostakovska, M.Doliba, A.Babsky, M.Vatamaniuk, O.Kuka, V.Artym.
Objective of our study was to investigate Ca^{2+}-activated respiration and Ca^{2+}-transport into mitochondria in hypoxic hypoxia conditions. Liver mitochondria were isolated by modified Shneider's method (Kondrashova, 1984). Respiration was registered polarographically. Earlier it was shown, that 1 or 2 days exposing of animals to hypoxic hypoxia (during 4 hours every day; 32mm Hg) lead to decrease of acetylcholine level in rat liver and to activate succinate oxidation in mitochondria. Begining from 7 day of hypoxia treatment the level of acetylcholine in tissue was increasing and it was connected with activation of α-ketoglutarate oxidation (Doliba, 1993). Results we have obtained point, that in initial adaptation days during succinate oxidation observed reliable decrease Ca^{2+}-activated respiration, intensity ions transport into mitochondria and increase of α-ketoglutarate oxidation. On the 7-12 days of adaptation observed increase Ca^{2+} activated respiration during succinate oxidation without reliable influence during oxidation of α-ketoglutarate. We admit that decrease in Ca^{2+}-activated oxidation connected with increasing of ADP-stimulated oxidation. This point of view is similar with Chance's idea that "respiratory activation by Ca^{2+} and oxidative phosphorylation of ADP involved the same energy conserving sites in respiratory chain". Changing in Ca^{2+}-stimulated oxidation during 7-12 days of experiment we explain adaptation processes which take place: prevailing of acetylcholine status of organism, decrease of ADP-stimulated and increase of Ca^{2+}-activated respiration during succinate oxidation.

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CONSENSUS SEQUENCE OF PEPTIDE FRACTIONS' POOL OBTAINED FROM KIDNEY' CORTEX SUBSTANCES AND ITS IMMUNOREGULATIVE INFLUENCE. I.P.Kaidashev
We determined the common consensus aminoacid pool sequnce of peptide extract fractions which were obtained from pig kidneys' cortex substance. Peptides were extracted from tissue by 0,5% solution of trichloracetic acid in the presence of zinc and magnesium ions with following separation of biological material with molecular weight lower 10 kD. Peptide extract was analized by HPLC and sequenced by Edman's degradation. Peptides from kidneys and peptides from MHC molecules class II has similar phisical and chemical characteristics. The common schem of kidneys' peptide organization is dominating residues of Val, Asp, Glu in position 2, Lys in position 3, Asp in position 4 and Arg in 6 position. On the basis of found out motif we determined 7 proteins containing this model: aconitase, cofilin, destrin, pro-TNF-alpha, progastrin, prorelaxin and sorbin. The kidney peptide fractions has ingibiting properties on ConA-induced mice splenocytes proliferation (up to 55%). More hydrophylic fractions are ingibited and more hydrophobic ones are stimulated proliferative response of IL-2-depended cell line (CTLL). This results testify the existence of the cell function organ regulation by peptide molecules which executes proliferation, differentiation and cooperation between immunocytes and special parenchim cells of kidney.

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TO THE MECHANISM OF SEROTONINE INHIBITING INFLUENCE ON ERYTHROPOIETIN BIOSYNTHESIS. N.V.Stepanova, V.I.Philimonov, S.N.Chernova
We had established [1991] that 2-3 multiple increase of serotonine concentration in blood inhibits the erythropoietin (EP) secretion. Taking into account the key part of cyclic nucleotides in EP biosynthesis mechanism [Fisher J.W., 1989], our aim was to study c-AMP and c-GMP concentration in Wistar rats tissue in conditions of hypoxic stimulation of EP production in hyperserotoninemia. Control group rats were injected with physiological solution; the 1-st experimental group was injected with serotonine; 2-nd one - with serotonine in conditions of preliminary serotonine receptors blockade by morphine. After injections all the rats were hypoxically exposed accoding to Schuster S.J.[1987]. C-AMP and c-GMP concentration was determined in the cortical and cerebral renal layers by the radioimmune technique. In the control group c-AMP and c-GMP concentration ratio was 2:1 in both layers. In hyperserotoninemia the response reaction to hypoxia was different: the ratio changes adequately 1:1. In serotonine receptors preliminary blockade hyperserotoninemia hasn't changed the ratio of c-AMP and c-GMP (2:1). Taking into consideration that c-AMP activates A-kinasa - the basic enzyme, stimulating EP synthesis, the ratio violation of c-AMP and c-GMP from 2:1 to 1:1 under the influence of serotonine leads to EP secretion inhibition. Serotonine receptors blockade eliminates this serotonine-effect that proves it's specificity.

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EXPRESSION AND ORGANIZATION OF FOUR DIFFERENT TITIN EPITOPES IN CULTURED HUMAN SKELETAL MUSCLE CELLS. Frank T.L. van der Loop[1], Peter F.M. van der Ven[2], Gert Schaart[1], Guillaume J.J.M. van Eys[1] and Frans C.S. Ramaekers[1].
Titin is one of the first sarcomeric proteins detected in the process of myofibrillogenesis of striated muscle. During embryogenesis this high molecular weight protein is first expressed in a punctate pattern, while during maturation these dots organize into a cross-striated pattern. Cultured human skeletal muscle cells that can be induced to differentiate were used to analyse the dynamic process of titin integration into the sarcomere. For that purpose antibodies against four well-defined titin epitopes, as well as antibodies against desmin, sarcomeric myosin and filamin were applied to study cells in subsequent stages of differentiation. In postmitotic mononuclear myoblasts, the different epitopes in the folded titin molecules were united, displayed as seperate, non-colocalized dots in the cytoplasm of the cells. During elongation and fusion of the cells, the dots move along the stress fiber-like structures to reach their localization at either the Z-line, the A-I junction or the A-band. In maturated, fused myofibers cross-striated patterns of the titin epitopes are observed. The direction of unfolding of the titin molecule (from the amino-terminus towards the carboxy-terminus of the molecule) and the integration of titin in both the Z-line towards and the M-line of the sarcomere can thus be monitored.

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SEGMENT-LENGTH CONTROLLED CONTRACTION OF SKINNED VASCULAR SMOOTH MUSCLE. B.G.V. van Heijst, P. Schiereck and E.L. de Beer.

In principle, the contractile apparatus in vertebrate smooth muscle does not deviate from striated muscle. The sliding filament mechanism, where tension is being developed in the overlap region, operates in the obliquely oriented smooth muscle cells. The amount of overlap, and thus the tension, depends on the functional length of the muscle. From experiments with striated muscle in which isometric contraction is determined as a function of different activation levels, it is known that the length of the contractile units, the sarcomeres, are non-uniformly distributed over the muscle fibre. In smooth muscle it is unknown whether overlap regions are nonhomogeneous distributed over the length of the muscle fibre. Since smooth muscle does not contain sarcomeres of which the length can be easily measured, two markers, attached to the muscle fibre, represent a functional length of the muscle, comparable to the sarcomere in striated muscle. The distance between the two markers thus sets a segment-length and a contraction in which the length of the artificial segment is kept constant results in a truely isometric activation. Aorta's from rabbits were isolated and skinned by means of freeze-drying. Small strips were cut and mounted between two tweezers. Two black cat hairs were glued to the muscle fibre with a distance of approximately 1 mm. A servo-motor unit is used to control either the preparation length or the segment length. Preparation length controlled isometric activation reveals a double exponential course of force development preceded by a delay of approximately 30 seconds. The length of the segment changes during force development to longer segment lengths as well as towards shorters segment length, dependent on the position of the markers and on the shape of the muscle fibre, indicating that activation of smooth muscle results in heterogeneous contraction within the fibre itself.

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EXTRACELLULAR PO_2 REGULATES THE MAXIMUM RATE OF OXYGEN CONSUMPTION OF XENOPUS MUSCLE FIBRES. W.J. van der Laarse, H.P.J. Buschman and M.C. Hogan

Arterial PO_2 and the P_{50} of Xenopus blood are about 70 mmHg and 24 mmHg at 20°C, respectively (Emilio & Shelton, J. Exp. Biol. 60: 565-579, 1974). We have investigated whether these oxygen tensions suffice to reach the maximum rate of oxygen consumption at 20°C (VO_{2max}) attainable by single, isolated muscle fibres dissected from the iliofibularis muscle of Xenopus laevis. A single fibre was mounted in a glass chamber filled with circulating Ringer solution whose PO_2 was monitored polarographically (Elzinga & van der Laarse, J. Physiol. 399: 405-418, 1988). Three types of muscle fibre - 1,2, and 3, two of each - were studied. First, the twitch frequency required to reach VO_{2max} was determined in air-saturated Ringer solution (PO_2=159 mmHg). The twitch frequency, VO_{2max}, and the dimensions of the fibres were similar to previous values (van der Laarse et al., J. Musc. Res. Cell Mot. 10: 221-228, 1989). Then, the PO_2 of the Ringer solution was changed. An increase of PO_2 had no effect on VO_{2max}, but a decrease reduced VO_{2max} to 81±14% (m±S.D., six fibres) at 70 mmHg and to 41±10% at 24 mmHg. These reductions in VO_{2max} with the lowered Ringer solution PO_2 were not changed when the rate at which the Ringer solution circulated in the chamber was reduced to 50%. The results indicate that extracellular PO_2 is a regulator of VO_{2max} attainable in vivo. The results cannot be explained by a simple diffusion model (Hill, Trails and Trials in Physiology, Edward Arnold, p. 217, 1965), which predicts that VO_{2max} of these six fibres should be independent of extracellular PO_2 above 23±8 mmHg.

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ENERGETICS OF MUSCULAR CONTRACTION AND DISSIPATION. J.Mejsnar, F.Maršík and B.Štefl

The rat gracilis, stimulated in vitro to both types of contraction and to an increased dissipation due to simultaneous superfusion and perfusion with medium via its nutrient artery, was used. The evaluation of the entropy balance (for treatment see [1]) yields for the case of: - an isotonic contraction: the Hill equation; - an isometric contraction: tension-time curves resembling experimental data obtained in media without or with external substrates; - a relaxed muscle flow-rate stimulated to energy dissipation: the O_2 consumption-heat relationship [2]. The approach shows, how one assumption of the constant entropy within a muscle during the 3 types of its performance, results in a general unified description of muscle energetics.

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CONTRACTION-INDUCED GLUCOSE TRANSPORT IN MUSCLE AFTER ECCENTRIC CONTRACTIONS: RELATION TO GLUT4 PROTEIN AND mRNA. S. Kristiansen, Aa. Handberg and E.A. Richter.

We studied whether contraction-induced glucose transport in muscle is affected by prior eccentric contractions. The eccentric contractions were induced by stretching electrically stimulated calf muscle. Two days later control (CT), and eccentric (EC) contracted rats had their isolated hindlimbs perfused. After 15 min of isometric contractions the white (GW) and red (GR) gastrocnemius muscle were excised. Western blot showed that EC contractions induced a 51% and 34 % ($p<0.05$) reduction in GLUT4 protein in the GW and GR muscle, respectively, compared with CT muscle. Furthermore, glucose transport during isometric contractions was significantly lower in the EC group in the GW and GR muscle compared with the CT group in spite of similar force production and oxygen uptake in the two groups. Finally, levels of GLUT4 mRNA were quantitated by dot blot technique. In the GW and GR muscle, prior EC contractions decreased the GLUT4 mRNA levels by 63% and 43%, respectively ($p<0.05$), compared with the contralateral CT leg. It is concluded that prior EC contractions decrease contraction-induced increases in glucose transport, GLUT4 protein and encoding mRNA levels. The data strongly suggest that unaccostomed EC contractions decrease contraction-induced glucose transport by a decrease in the GLUT4 protein levels by changes in GLUT4 mRNA synthesis/and or degradation rate

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ADENOSINE STIMULATES GLUCOSE UPTAKE IN CONTRACTING MUSCLE EXPOSED TO β-ADRENERGIC STIMULATION. P.Hespel, S.Van Steenweghen, L.Vergauwen, E.A. Richter.

The role of adenosine in regulating glucose uptake in skeletal muscle exposed to β-adrenergic stimulation was investigated in isolated perfused rat hindquarters. Hindlimbs were perfused with a standard medium containing 6 mM glucose, 1.67 nM isoproterenol and 0 or 100 μU/ml insulin. Selective A_1-adenosine receptor antagonism (A_1RA) was induced by adding 75 μM 8-cyclopentyl-1,3-dipropylxanthine, versus vehicle in the control rats, to the perfusion mix. Glucose uptake by the hindquarter was measured before and after 15 min of isometric contractions induced by supramaximal intermittent electrical stimulation of the Sciatic nerve. In the absence of insulin, glucose uptake rate at rest was similar in the control and A_1RA hindquarters (2.6 ± 0.7 and 3.5 ± 0.7 μmol/g.h, respectively). During electrical stimulation glucose uptake increased in both groups. However, the increase was about 35% smaller ($p < 0.05$) in A_1RA hindlimbs than in controls (+3.5 ± 0.6 vs. +5.3 ± 1.1 μmol/g.h). In the presence of insulin, in contrast, glucose uptake rate at rest was 35% lower ($p < 0.05$) in A_1RA hindquarters than in controls (3.7 ± 0.7 vs. 5.7 ± 0.4 μmol/g.h). During stimulation, however, glucose uptake increased to the same extent in both groups. Thus the difference in resting glucose uptake rate was maintained during contractions (7.8 ± 0.7 in A_1RA vs. 10.9 ± 1.4 μmol/g.h in controls; $p < 0.05$). This study shows adenosine to be very critical to the stimulation of glucose uptake in muscle exposed to β-adrenergic stimulation during contractions.

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ADENOSINE SERVES A GLYCOGEN SPARING ACTION IN OXIDATIVE SKELETAL MUSCLE. L. Vergauwen L., P. Hespel and E.A. Richter

The role of adenosine in regulating glycogenolytic rate in contracting muscle was investigated in isolated rat hindquarters perfused with a standard medium containing no isoproterenol and 0 or 100 μU/ml insulin, or 1.67 nM isoproterenol plus 100 μU/ml insulin. Adenosine receptor (AR) antagonism was induced by caffeine (75 μM) or by a combination of A1- (8-cyclopentyl-1,3-dipropylxantine, 75 μM) and A2-selective (3,7-dimethyl-1-propargylxantine, 75 μM) blockers. Isometric muscle contractions were induced by intermittent tetanic electrical stimulation of the Sciatic nerve. Glycogen concentration was determined in fast glycolytic (FG), fast oxidative (FO) and slow oxidative (SO) muscle fibers before and at the end of the stimulation period. Pre-contraction glycogen concentrations ranged between 33-41, 38-45 and 20-27 μmol/g wet weight in FG, FO and SO muscle fibers, respectively. Glycogen breakdown over the stimulation period was significant in the 3 fiber types, but did not reach the stage of total depletion. With no isoproterenol added to the perfusate, AR antagonism did not affect glycogenolysis during contractions in either fiber type. However, in the presence of isoproterenol and insulin, AR antagonism stimulated net glycogenolysis in FO and SO muscle fibers by approximately 50% ($p < 0.05$) and 55% ($p < 0.05$), respectively. AR antagonism did not affect glycogenolysis in FG fibers during contractions. The data for the first time demonstrate adenosine to inhibit glycogenolysis in oxidative muscle fibers during contractions. This adenosine induced sparing of muscle glycogen requires exposure to β-adrenergic stimulation.

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INFLUENCE OF INORGANIC PHOSPHATE AND pH ON ATPASE ACTIVITY OF THE SARCOPLASMIC RETICULUM IN SKINNED SKELETAL MUSCLE FIBRES OF XENOPUS LAEVIS

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The influence of inorganic phosphate (P_i) and pH on the rate of ATP consumption was determined in mechanically-skinned bundles of myofibrils from the iliofibularis muscle of Xenopus laevis at 5 °C by enzymatic coupling of ATP resynthesis to the oxidation of NADH. The maximal Ca^{2+}-activated ATPase activity and force at 30 mM P_i and at different pH values was determined in the presence and absence of 10 mM 2,3-butanedione monoxime (BDM). The sarcoplasmic reticular (SR) ATPase and actomyosin (AM) ATPase activities were determined by extrapolation of the total ATPase activity to zero force. Addition of 30 mM P_i caused a reduction in the maximum isometric force by 53% and in the total (AM + SR) ATPase activity by 13%. The SR ATPase activity in the absence of added P_i was 32% of the total ATPase activity. It increased slightly whereas the AM ATPase activity decreased (by about 28%) when 30 mM P_i was added. The pH dependence of force and ATPase activity was studied in the range from 6.2 to 7.4. Isometric force showed little dependence on pH: at pH 6.2 it was reduced to 79 ± 5 % of the value at pH 7.1. The total (AM + SR) ATPase activity decreased gradually to 72 ± 6 % at pH 6.2. This reduction was mainly due to a decline in SR ATPase activity: at pH 6.2 the SR ATPase activity was reduced to 39 ± 5 % of its value at pH 7.1, whereas the AM ATPase activity was 90 ± 5 % of the activity found at pH 7.1. These results suggest that the maximum rate of calcium uptake during muscle fatigue is reduced mainly as a result of intracellular acidification.

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EFFECT OF ENDURANCE TRAINING, TENOTOMY AND DENERVATION ON METABOLISM OF PHOSPHOLIPIDS IN DIFFERENT MUSCLE TYPES OF THE RAT

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A relationship between functional status of skeletal muscles and phospholipid (PH) metabolism in myocytes is poorly recognized. In the present study the effect of 4 week endurance training, 4 week tenotomy and 4 week denervation on total PH content, PH composition , incorporation and specific activity of 14-C palmitic acid into different PH fractions in the soleus, red and white gastrocnemius and diaphragm was investigated. PH were separated using thin-layer chromatography into the following fractions: phosphatidylcholine, phosphatidylseryne, phosphatidylinosytol, phosphatidylethanolamine, sphingomyelin, and cardiolipin. It was found that both total PH content and specific activity in skeletal muscles depended on their oxidative capacity.Training resulted in increased total concentration of PH in the red gastrocnemius and diaphragm, reduced specific activity in each muscle studied. Tenotomy did not change total PH concentration in the leg muscles studied. It produced, however, some changes both in PH composition and in their specific activity. Denervation was followed by a reduction in PH content only in the muscle with high oxidative capacity, i.e. in the soleus and the red gastrocnemius. Simultaneously, it produced changes in PH composition and specific activity of different fractions were observed in each muscle. The most striking was decrease in the content of phosphatidylcholine. It is concluded that the functional status of skeletal muscles effects PH metabolism in myocytes.

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Regulation of the excitability of muscle: Sarcolemmal chloride conductance determined by functional ClC-1 mRNA levels and protein kinase C activity

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Blockade of the major chloride channel of skeletal muscle, Clc-1, either by mutations or by drugs, results in hyperexcitability, the hallmark of myotonia. We have studied the effect of allelic mutations and dosage of the myotonia/chloride channel 1 gene, *adr/Clc-1*, in the mouse, by determining conductances in M. sternocostalis fibers and correlating the physiological results to the genotype. The following *adr* alleles were under study: *adr* (insertional mutation); adr^{mto} (stop codon); adr^{K} (missense). Genotypes were determined by PCR diagnosis. In all cases, the G_{Cl}- of homozygous mutants was reduced to ≈ 5% of wildtype, whereas heterozygotes and homozygous wildtype (+/+) littermates showed the same conductance. In heterozygote *adr/+* muscles, the levels mutant and wildtype ClC-1 mRNAs can be determined separately, and functional ClC-1 mRNA was found to be halved. These results imply a regulation at the protein level. A similar observation was made on K^+ conductance which is secondarily affected in myotonia. In +/+ muscle, 2 μM 4-ß-phorboldiacetate reduces G_{total} to 40% of wildtype which would correspond to a reduction of G_{Cl} to 20%. This effect is reversed by 1 μM staurosporin, an inhibitor of protein kinase C. Thus, a fraction of the ClC-1 channels is inactivated by PKC.

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DIFFUSION OF MACROMOLECULES IN SKELETAL MUSCLE CELLS. K.D. Jürgens, S. Papadopoulos and G. Gros

We measured the diffusion coefficients of proteins in intact skeletal muscle cells as a function of protein size. Concentrated solutions of cytochrome c, myoglobin, hemoglobin, catalase, ferritin and earthworm hemoglobin were microinjected into skeletal muscle fibers of the rat. At a wavelength of ~ 410 nm the changes of the absorbance profiles of these iron-containing proteins along the longitudinal fiber axis due to diffusion were recorded. From best fits of the solution of the differential equation describing the diffusional process to the measured curves the diffusion coefficients D_{cell} were obtained. For proteins with a diameter between 3 and 10 nm, injected into rat soleus muscle fibers, D_{cell} was calculated to be about 1/10 of the corresponding diffusion coefficients found in dilute aqueous solution. All D_{cell} values are far lower than the self diffusion coefficients measured in highly concentrated solutions of these proteins. Molecules with a diameter greater than 10 nm exhibit a sharp decrease in intracellular diffusivity, proteins with a diameter of 30 nm do not show significant diffusion at all. These results give evidence that in addition to cytosolic viscosity macromolecular diffusion is strongly influenced by the cell architecture. Myofilaments and its cross bridges, the molecular networks of Z-discs and M-lines as well as the numerous mitochondria are geometrical obstacles which increase the tortuosity of the diffusion path. Differences in the hindrance of protein diffusion between soleus and extensor digitorum longus (edl) muscle of the rat indicate that the diffusivity is lower in muscle fibers with wider Z-discs and higher mitochondrial content. Distances between the myofilaments and the array dimensions of Z-discs and M-lines are in the 10 nm range. From this we conclude that the pore size of the molecular networks seems to determine the limit up to which protein diffusion is possible at all.

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BUMETANIDE SUPPRESSES SKELETAL MUSCLE MEMBRANE POTENTIAL CHANGES INDUCED BY HYPERTONIC MEDIA.

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At certain low medium potassium concentrations (K_o), the membrane potential (V_m) in mouse skeletal muscle fibre (m. lumbricalis) can be either depolarised or hyperpolarised with respect to the normal value (J. Physiol., 1991, 434, 549). This has been attributed to the inward potassium rectifier (IKR). Due to its properties this conductance depends on V_m and K_o, and thus can control the V_m itself. Lowering K_o induces a hyperpolarisation until the reduction in IKR conductance is so large that it induces a depolarisation. At which concentration of K_o this depolarisation occurs depends on the osmolarity of the extracellular medium. In Krebs-Henseleit solutions (290mOsmol) it occurred at $K_o \approx 1$ mM and with higher osmotic solutions at higher K_o values. These osmolarity-induced changes in V_m are relate with symport systems involved in the regulation of cell-volume and intracellular ion-concentrations. We measured the changes in V_m with intracellular micro-electrodes. Mild osmotic shocks were induced by switching the perfusion from control solution (K_o = 5.7 mM) to hyperosmotic solutions (adding Poly-Ethylene Glycol; MW 400). All measured changes of V_m were reversible. Switching from 290 mOsmol to 315 or 340 mOsmol induced depolarisations of respectively $\Delta V_m = 6.9 \pm 0.5$ mV (n = 37) and $\Delta V_m = 10.7 \pm 0.8$ mV (14). Bumetanide induced in control solution a hyperpolarisation (-3.0 ± 0.8 mV, 6) and in both hyperosmotic solutions it made V_m repolarise to values that were comparable to the V_m in control medium plus bumetanide.
The efflux through the IKR and the fluxes through the regulatory symport systems are both energized by the driving forces of Na^+ and K^+ generated by the Na/K-pump. We conclude that the measured interaction occurs by draining on the same driving force.

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MARKERS OF REDOX BALANCE IN THE RAT SKELETAL MUSCLE.

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The nature of the mechanisms underlying the aging process is presently not well understood. Several different investigative strategies and hypotheses have been proposed to identify the constitutional characteristics of organisms, which govern their rate of aging. The development and aging are not two distinc phases of life. Aging is the terminal stage of development and like earlier stages of ontogeny, aging can be characterized by changes in gene expression. Aging is not a genetically programmed phenomenon, but it occurs because of the influence of oxidative stress on genetic programs. The central dogma of free radical theory is that free radicals cause damage to biological molecules and that the accumulation of such damage constituted the aging process. Exogenous antioxidants would prolong life in animals by reducing the level of po-oxidant damage and would thus retard the rate of aging. Oxidative stress causes free radical reactions such as lipid peroxidation, wich have a role in damaging biological structures and cellular functions. Exhaustive exercise was reported to increased oxidative stress in skeletecal muscle. The objetive of this study is the determination of the redox balance. We have used male Wistar rats (3 months old and 27 months old). The animals was physical training and exhausted in Treadmill LETICA LI8706. The experimental protocol was approved of the Faculty of Medicine of the University of Cádiz. Activities of antioxidant enzymes (superoxide dismutases) were measured in tissue homogenates. We have measured of malondialdehyde production by means of thiobarbituric acid assay (TBA test) as an index for determining the extent of peroxidation reactions. Results support the role of oxidative stress in aging.

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A NEW DYNAMOMETER TO MEASURE RAT ACTIVE MUSCLE TORQUE *IN SITU*. M.R. Drost

In chronic experiments, involving e.g. training on a treadmill, it is difficult to evaluate muscle strength without serious surgical intervention, i.e. dissecting free a muscle. The objective of the study was to develop an apparatus in which muscle active torque of rat dorsal or plantar flexors can be measured with a minimal surgical intervention. The experimental procedure is as follows. An anaesthetised rat is laid on its back in the experimental setup. Depending on the protocol the common peroneal nerve or the tibial nerve is exposed and stimulated. The femur is fixed with a hinge fixation at the knee: between femoral and tibial condyles; the foot on a rotational footplate. Care is taken to align the ankle axis with the rotation axis of the dynamometer. The footplate can be rotated with a velocity between 0.01 °/s and 1000 °/s around the anatomical position of the ankle. The starting angle and the stop angle can be chosen between 0.1° and 45° from this anatomical position. The velocity is reached within 10% of the duration of the stroke. Torque is calculated from the current through the linear motor of the dynamometer. Muscle active torque is calculated as the difference between the torques in a movement with muscle stimulation and a movement without a stimulation. As the torques without stimulation are reproducable within 2%, the noise is about 10% during acceleration and 4% during the isokinetic phase.

Conclusion: this apparatus is well capable of measuring rat muscle active torque and mechanical energy delivered of dorsalflexor and plantarflexors during chronic protocols with minimal surgical intervention.

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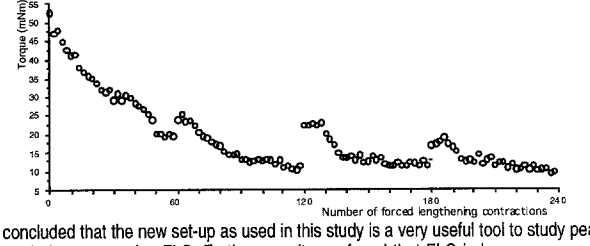
ON-LINE MEASUREMENT OF PEAK TORQUE OF RAT DORSIFLEXOR MUSCLES DURING FORCED LENGTHENING CONTRACTIONS M.K.C. Hesselink, M.R. Drost, H. Kuipers.

Muscle damage induced by eccentric or forced lengthening contractions (FLC) is often accompanied by a decline in muscle force. The objective of this study was to study the decline in muscle peak strength during series of FLC. To be able to do so an experimental set-up was developed, allowing us to measure active peak torque of rat dorsiflexor muscles during isokinetic contractions. Briefly, anaesthetized rats were laid backward in the experimental set-up with the left femur fixed at the lateral and medial epicondyle and the left foot fixed at a rotational, sandal-like footplate. Contraction was induced by electrical stimulation of the exposed common peroneal nerve, dorsiflexor muscles were lengthened by rotation of the footplate (500°/s). Dorsiflexor muscles of 13 male Wistar rats aged 12 weeks were subjected to 4 bouts of 60 contractions (one 300 ms contraction every 3 seconds) separated by 5 minutes recovery. Peak torque of the exercising dorsiflexor muscles was recorded during every single FLC. The results show a very rapid decline in peak torque during the first 60 FLC (52.4 mNm in the first contraction to 19.5 mNm after 60 contractions). Peak torque during the final FLC of the second until the fourth exercise bout was found to stabilize around 10 mNm. Initial peak torque of the third and fourth exercise bout seems to plateau before it falls. During the recovery periods (5 min) initial peak torque was partly restored ($p \leq 0.05$) when compared to the final FLC of the preceding exercise bout and was similar at the beginning of bout 2 and 3.



It is concluded that the new set-up as used in this study is a very useful tool to study peak torque during successive FLC. Furthermore it was found that FLC induce a very rapid decline in muscle active peak torque, this decline is partly reversible during 5 minutes recovery and peak torque never fell below 10 mNm. The mechanism of this rapid decline in peak torque during the first 60 FLC remains to be established.

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Delayed muscle relaxation and aftercontractions in mouse models of neuromuscular diseases

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Spontaneous and induced mutations that lead to neuromuscular disorders in the mouse provide valuable physiological models for human neurodegenerative diseases, e.g. spinal muscular atrophies (SMAs), as well as myotonias and muscular dystrophies. We report here on mouse mutants affected by SMAs and myotonias: SMAs 'progressive motoneuronopathy', phenotype PMN (genotype *pmn/pmn*, Chr 13); muscle deficient, MDF (*mdf/mdf*, Chr 19); CNTF knock-out, CNTF*ko ($Cntf^{o}/Cntf^{o}$, Chr 19); and a new allele of myotonia, ADR*2J (adr^{2J}/adr^{2J}, Chr 6). The ADR*2J myotonia, in which the skeletal muscle is directly affected, shows - like the standard ADR mouse - aftercontractions of > 2 sec. In this case, muscle is directly affected by a mutation that interferes with chloride conductance. In SMAs, in contrast, muscle is indirectly affected by a defect in the CNS. In PMN muscle, the time to peak and half relaxation times were unchanged, whereas in MDF relaxation after single twitches and especially after tetani was prolonged ($T_{\frac{1}{2}}$ rel > 80 ms) although the animal - in contrast to the myotonic mouse - showed no cramps. Twitching was normal in the 160 d old CNTF*KO mouse, but relaxation was prolonged after a tetanus, but to a lesser degree than in the MDF mouse. These physiological results will be related to the histochemistry of affected muscle.

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FUNCTIONAL ROLE OF MYOSIN ATPase SITE "HYDROPHOBIC POCKET". Filenko A.M.,Babiychuk E.B.,Danilova V.M.

Three-dimensional structure of myosin head (S1) has been recently described in the fundamental work by Rayment at al. [Science,1993,261,50]. But the role of different structural unites of S1 is yet unclear. Our investigation treats the problem of denaturative effect of temperature on 50 kDa fragment of myosin S1 in the presence of various nucleosidetriphosphates (NTP). Denaturation changes were evaluated by trypsinolysis extent. It is shown that by protecting action NTPs may be arranged as follows: ATP>CTP>UTP>GTP (1). It is known that by the fluorescence increase caused mainly by tryptophanyls of the 50 kDa fragment various NTPs may be arranged just as in (1); the same is the sequence of lifetime decrease of longliving intermediate complexes M**NDP · P_i. These results suggest that the decrease of the lifetime of M**NDP · P_i complex and fluorescence increase are due to the strength of the NTP binding in the active centre, resulting in the increase of the S1 (mainly fragment 50 kDa) rigidity. Especial importance has the interaction of the NTP nitrous base with the "hydrophobic pocket" of the ATPase centre. Summing up our results we suggest existence of at least two conformational states of the "hydrophobic pocket". One of them is responsible for accumulation of macroergic energy in the structure of the myosin head (stage M**NDP · P_i). Another state takes place at the actin binding when the bond of the nitrous base with the "pocket" abruptly weakens and the macroergic energy is utilized for elementary contraction act.

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SPATIAL STRUCTURE OF THE SKELETAL MUSCLE CELL AND MECHANICS OF THE MUSCLE CONTRACTION.

N.S. Miroshnichenko, P.G. Minchenko .

Muscle contraction occurs when two interdigitating filaments, the thick myosin filaments and the thin actin filaments move past one another. Despite the great efforts undertaken to study this phenomenon the mechanism and the theories of muscular contraction proposed are still rather hypothetical. The most characteristic feature of the existing theories of muscle contraction is the fact that they are actually two-dimensional.Consideration of the spatial arrangement of the contractile elements of the muscle cells is a prerequisite for understanding how they operate. A minimal contractile element (MCE) - such volume of the skeletal muscle cell which sufficiently reflects three-dimensional location of its structural elements - was built. The MCE structure analysis shows that a mutual location of the structures in the MCE makes the simple sliding of the thick over thin filaments very problematical. A new conception of the muscle contraction is being elaborated on the basis of the built model of the skeletal muscle cell. In our model mutual shift of the thick and thin filaments is possible due to the twisting of the myosin filament inside the MCE. The M-line of the sarcomere serves as the border line, the thick filament on both sides of it being twisted in the opposite direction. In our model muscle contraction is realized due to the interdomain rearrangements in molecular motor (myosin molecule head) during power stroke.

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FORCE/LENGTH RELATIONSHIPS IN ACTIVE MUSCLE. S.P.Romanov

In the course of stimulation the special interest is the force changes resulting from muscle (M) length (L) in isometric regime and relationship between M L and force (F) during lifting the different mass loads. In experiments (carried out under general -Nembutal 40 mg/kg - anesthesia) on cats the physiological range of intact M L was measured. The m.soleus and m.gastrocnemius tendons with the part of tuber calcaneum were separated and attached to a tensile load cell for F recording. For isometric regime of M contraction the other end of a load cell is rigidly attached to the scale beam designed to vary the M L within wide limits. In the case that M shortening may lift one or another weight the potentiometric transducer measured the contracted M L. Tibialis nerve was stimulated by pulse train (8-10 impulses with 0.3 ms duration and 3-5 threshold amplitude). Pulse repetition rate lay in the range of 1 to 100 Hz. Between pulse trains M is stretched with 1 mm step from minimal L for which there are not external active F manifestations on the given frequency of stimulation. The results showed that the tetanus contraction reaches its peak at maximal physiological M L. In this range the profiles of isometric F are of the form of S-curve and its incremental slope increases as the pulse repetition rate increases. It has been found that the relation between elasticity and viscosity of the active M reversed under M L changes. Once M was able to lift a load, it continues to shorten if the stimulation is in progress. These findings were used for creating the electronic analogue of M. Ten differential equations describe mechano-chemical coupling and link variables of elasticity and viscosity of serial and parallel compartments and intracellular potential of M fiber with input variables - M L and frequency of stimulation, and the output value - M F as the sum of active and passive F. The same model exhibits various shapes of M contractions with different location of the physiological ranges along the stretch direction on the absolute M L scale. Then contraction types of smooth M, skeletal slow and fast M, and M fibers of a heart are specified by the ratio of the physiological L to absolute normalized (in magnitude of passive F with stretching) L which is evaluated as 0.3-0.5, 0.4-0.7 and 0.8-0.9 for those M.

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THE ROLE OF TEACHING BIOPHYSICS IN PHYSIOLOGY

M.Tuliszka, L.Kubisz, F.Jaroszyk, T.Torlińska,

In our University biophysics and physiology lectures are given jointly as one subject. We present briefly the curriculum of the biophysics part. Biophysics is understood there as an introduction to physiology and its goal is to provide students with a substantial grounding for interpreting cause and effect dependencies in physiological sciences - dependencies, in fact originated by physics as a knowledge about nature. Biophysics understood in this way is a complementary and supporting subject in teaching human physiology. We start with a series of three lectures devoted to environmental factors influencing the human body such as electromagnetic radiation - starting from ionizing radiation through ultraviolet, visual range and infrared, up to low frequency electromagnetic fields, presenting possible applications in medical diagnosis and treatment, and risk factors. The next 4 sessions cover wide-ranging study of classical thermodynamics. Starting from a theoretical background we finish with the mechanisms of the transport and exchange of thermal energy (temperature regulation and metabolism) from one side and solvent and solutes via membranes from the other side. The last part forms specific subjects such as: 1) Biophysical aspects of circulation of blood - covering viscous properties of blood and biophysical aspects of hemodynamics including properties of vascular walls and mechanical work of the heart; 2) Energetics of muscles and muscle rheology - covering the molecular insight into the mechanism of muscle contraction, muscle contraction dynamics and energetics; 3) Biophysical aspects of hearing - including physical properties of sound waves as determinants of physiological perception and its characteristics; 4) Physiological optics - geometrical optics and aspects of common defects of image-forming and their correction.

Each lecture topic is illustrated by a laboratory exercise closely related to problems presented during the current lecture. The stress is put on problems of the importance of quantitative and qualitative description of human judgments and inference by analysis of experimental errors and data processing.

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NEW MEDIA IN HIGHER EDUCATION - EXPERIENCES WITH A MULTIMEDIA DATABASE AUTHORING SYSTEM IN TEACHING PHYSIOLOGY. W. Wiemer, J. Heuser, D. Kaack and M. Schmidtmann

A teaching orientated database information system MILES (Multimediales Informations- und Lehrsystem), the first of its kind in Germany, has been developed at the institution below, and field tested in teaching Physiology to students of medicine (160 p. a.) and biology (40 p. a.). The system is based on a network of personal computers, devised to serve as a common source for all computer and audio-visual teaching materials over the entire curriculum, and to integrate these into lectures, seminars, practicals, and self-study by providing a) database storage for all types of demonstration and working materials - text, video, digitized analogue signals and images, programs, complete teaching units, also from external producers, b) authoring functions for assembling these materials, as required by the above applications, into menus and subsets of the database of varying complexity, c) individual access for students to programs and data including the possibility to compile collections of their own. So far, more than 9000 documents have been stored, including simulations, data processing programs, signal recordings, video and audio modules, about 2500 pictures, a glossary of physiology, and a comprehensive collection of test questions. Experiences hitherto have confirmed the expectations in regard to power, versatility and flexibility of such systems. Teaching results and acceptance by students have been good - but only, if occupation with the system had been integrated into classes, that is, into personal teaching and curricular subject matter; they were distinctly poorer, if materials were offered in by-pass as mere self-education materials. Reference: W. Wiemer et al., MILES. In: Proc. Internat. Conf. on Computer Based Learning in Science, (G. M. Chapman, ed.), Opava 1995, in press.

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A DATA ACQUISITION AND ANALYSIS SYSTEM FOR THE STUDENT OF PHYSIOLOGY. M.Baumann and R.Grebe

Generation, acquisition and interpretation of biomedical data are most important not only in research but as well in the daily life of the physician. That is why every student of medicine has to become familiar with modern data acquisition and the principles of signal analysis. We have designed a special hard-/software device as data acquisition and analysis system which is aimed to draw the students' attention to the data itself instead of needing most of his efforts for to learn how to handle special high complicated measuring devices as oscilloscopes. Our setup replaces plotters, oscilloscopes, and all kind of -meters used by medical students in our laboratories so far.

Six identical data acquisition and analysis systems have been set up designed as follows: 486DX2 at 66MHz, 8 MB RAM, 200 MB harddisk, mouse, no keyboard (!), 15" color monitor, color printer and data acquisition board (A/D-converter). Based on the commercial softwaretool TestPoint (Keithley) running under Microsoft's Windows we have developed our special lesson-adapted and mouse-controlled software which is designed to be controlled by the students with minimal additional help. To achieve this we have done the following: (1) All applications look nearly the same with big data graphs shown on the lower part of the screen and the controlling buttons arranged above. (2) Where possible, buttons have identical names in all applications, which represent their function, like e. g. the name of the exercise to be executed or 'Print'. (3) Buttons are arranged from left to right in the chronological order as they have to be used during the progress of experiments. (4) Only those buttons on the screen can be used, which make sense in the mode of operation. E. g. it is not possible to print unless there are sampled data available. (5) Graph scaling is done semi-automatically which means no auto-scale for 'noise-signal' and not cut off of high signal-amplitudes. (6) Superposed graphs are distinguished by different colors. (7) Demo-data are available from harddisk if an experiment fails. This data can be invoked by the teacher only.

In use since 1993 the system has reduced the variety of hardware needed for teaching so technical problems and costs have decreased remarkable as well. More time is available for to deal with physiological problems instead. In future the system will be enlarged and integrated via a local area network.

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TEACHING PHYSIOLOGY LAB CLASSES WITH MS-DOS COMPUTERS. GC van den Bos, DA Philips, FK Dijkema, AJG Spoelstra

To modernise our physiology lab classes and to increase student participation we have replaced our traditional recording equipment with a personal computer (Stephens and Doherty Am J Physiol, Adv Physiol Educ 8, S23, 1992). Therefore a 486 DX_2/66 PC was supplied with a data acquisition card (PCL-818L; 8 channel, 40Khz/DMA) and was programmed to serve as a universal measurement and recording device. To this end a Windows based software package was developed, in Visual Basic 3.0 and Borland C++ 4.0, which comprises two units: 1. manual for a particular class, illustrated with digitized photographs and slow animations about the subject, to direct the students; this unit also contains help screens to which the student can refer at all times and a variable number of study and practical questions to which answers are provided (scoring of results is optional); 2. the measurement/registration section in the form of a two channel virtual oscilloscope and recorder, coupled via a classroom network to a fast laser printer. Any combination of these units together with the specific information for the particular subject creates a way to interactively modulate all possible practical classes. The software package, virtual instruments included, is fully mouse driven. The input to the PC is through a custom made panel which contains the isolated pre-amps, the rotary switches for sensitivity, off-sets and type of experiment. All transducers are connected to this panel. This part of the system is not computer simulated so that students are also acquainted with the comparable clinical equipment. We have now completed modules to study ECG and heart sounds, pulse wave velocity and systemic compliance, systemic blood pressure, and central venous pressure pulses. Student response to the new approach is favourable and participation has improved.

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Physical model of arterial wave propagation and flow dynamics for student education

T. Arts[1], A.P.G. Hoeks[1], J. Aerts[2]

The arterial circulation has been visualized to elucidate the physical aspects of the arterial flow with special attention to the pulse wave, an arterial stenosis and arteriolar autoregulation. For comparison ultrasonic Doppler flow measurements are performed on the arterial circulation of a student volunteer.

In a physical model the arterial pulse wave has been generated by a pulsatile pump in a system of open channels having the shape of the arterial anatomy. Scaling factors for length, time and pressure are about 0.8, 7.5, and 40, respectively. The arterial pulse wave is so slow (0.6 m/s) that it can be followed visually. At any location in the arterial system pressure can be registered by dynamic measurement of the water level. Flow can be visualized by injecting ink. The ink concentration can also be measured continuously as a function of time.

A simulated arterial stenosis blocks flow partially, causing wave reflections, manifested clinically as arterial pulsations. Flow vortices near the stenosis show up by injection of ink. The decrease of flow can be compensated for by a decrease of the distal peripheral resistance in the model, simulating vasodilatation due to autoregulation.

The arterial flow velocity is recorded in vivo with an ultrasonic Doppler flow meter, applied to a student volunteer. Evaluations are performed on the arterial velocity wave form and the autoregulatory response to reactive hyperemia.

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THE PREPARATION OF RAT LIVER HOMOGENATE: ATTEMPT TO APPROACH CONDITIONS IN VITRO TO CONDITIONS IN VIVO.

Yurij Stephankiv.

It was shown that in intact cells a mitochondries (Mch) are in associative state with endoplasmic reticulum (Shore, Tata, 1972; Katz et al., 1983). The preservation of native structure of these organels in vitro is very important for researching the mechanisms of hormones and neurotransmitters influence on energy processes in Mch (Kondrashova et al., 1994).

We investigated the respiratory and oxidative phosphorylation in rat liver homogenate that was prepared at room temperature with the purpose to avoid the destruction of cytoskeleton proteins (tubulin) and at the conditions of normoxia (the defence of argon, 0.26 l/min). Preliminarily liver was cleansed from blood and extracellular Ca^{2+}. The medium of preparation contained 120 mM KCl, 2 mM K_2CO_3, 1 mM EGTA and 10 mM HEPES (pH 7.2). The medium of incubation contained these substance (except EGTA) and 2 mM KH_2PO_4. Mitochondrial oxygen in homogenate consumption was measured polarographically, with a Clark-type electrode, maintained in a chamber at 28°C. We have found the typical answer of Mch and stimulation of respiratory ADP (200 μM) (succinate 1 mM in the capacity of substrate) in the solution of preparation modified by 1 mM $MgCl_2$, 300 μM alpha-ketoglutarate and tripsine inhibitor 0.26 mg/ml. We have observed these effects of Mch for 60 min in vitro of keeping. At this condition ADP/O and respiratory control reduced by 50%. We suggest that in this conditions it is possible to investigate Mch function in energy answer of cell to the influence of hormones and neurotransmitters.

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MICROCIRCULATORY CHANGES IN RAT MESENTERIC VENULES AS A RESULT OF ISCHEMIA/REPERFUSION (I/R) INJURY.

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The effect of I/R on leukocyte-vessel wall interactions and blood flow was studied in rat mesenteric venules, using intravital video microscopy. Total warm ischemia was induced by temporary ligation of the superior mesenteric artery, after permanent ligation of the inferior mesenteric artery. Number of Rolling Leukocytes per min. (nRL), number of Adherent Leukocytes (nAL; expressed as number stationary for ≥30 seconds per 100 µm length of venule), Red Blood Cell Velocity (RBCV) and Diameter (D) were assessed before as well as 10, 20, 30, 60, 90 and 120 min. following 15, 30 or 60 min. warm ischemia. A sham operated group of rats was included as control. The pre-ischemic values of all parameters were similar in all groups. In the sham group, no significant changes in nRL, nAL, RBCV and D were observed in time (median control values: 58 per min., 1 per 100 µm, 1.2 mm/s and 32 µm). As compared to the sham group RBCV was decreased (1.2, 0.9, 0.9, 0.7 mm/s) at 30, 60, 90 and 120 min. after start of reperfusion ($P<0.05$) in the 15 min. ischemia group. nRL, nAL and D were not significantly different. In the 30 min. ischemia group nRL was increased (130, 135, 128 per min.) at 10, 20 and 30 min. after start of reperfusion ($P<0.05$), nAL was increased (4, 4, 4, 4, 6 and 6 per 100 µm) at 10, 20, 30, 60, 90 and 120 min. after start of reperfusion ($P<0.01$) and RBCV was decreased (1.1, 1.3, 0.9, 0.5, 0.4 mm/s) at 20, 30, 60, 90 and 120 min. after start of reperfusion ($P<0.05$). D was not significantly affected. Rats subjected to 60 min. ischemia all died during reperfusion.

Reperfusion of the mesenteric microcirculation after a short period of total warm ischemia causes a clear and detrimental decrease in blood flow, that is accompanied by a significant increase in leukocyte-vessel wall interactions if the ischemic period lasts longer than 15 min. Reperfusion after 60 min. of ischemia is fatal.

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HYPERGLYCEMIA PER SE DOES NOT AFFECT MICROVASCULAR REACTIVITY IN RAT SPINOTRAPEZIUS MUSCLE. C. Demirci, A.A. van Lambalgen, C.D.A. Stehouwer, GC van den Bos.

To be informed about the effects of local hyperglycemia on microvascular reactivity we measured inside diameter of second (**A2**: 20-40µ) and third order (**A3**: 10-20µ) arterioles in the microcirculation of the spino-trapezius muscle of 6 anesthetized rats (group **G**), using intravital microscopy, before (t=0) and during a 5.5 hrs period of superfusion with 20 mmol/l glucose in tyrode, equilibrated with 95% N_2 and 5% CO_2. We tested the reactivity of the arterioles by adding acetylcholine (Ac: 10^{-5} and 10^{-3}M at the muscle surface) and nitroprusside (NP: 10^{-4}M at the muscle surface) to the superfusate and measured the changes of the inside diameter. During the last hour of the hyperglycemic period we also added N^G-nitro-L-arginine (L-NNA) to the superfusate to see in what way nitric oxide (NO) contributed to the perfusion of the muscle vascular bed. The results of these experiments were compared with those in control rats (group **C**; n=6) in which the same drugs were used but no glucose was added to the superfusate. Diameters were normalized with respect to their baseline value at t=0 (set at 100%). At t=0 $Ac10^{-5}$ and $Ac10^{-3}$ caused an increase in diameter to ~165% and ~180%, and ~195% and ~225% for A2 and A3 respectively; the same values were found after 4.5 hrs of superfusion with or without glucose. Also the effect of L-NNA superfusion was the same in both groups: during L-NNA there was a decrease in diameter to ~70% for both vessel types, $Ac10^{-5}$ increased the diameter to ~125% and ~140% for A2 and A3 respectively (significantly less than without L-NNA) and $Ac10^{-3}$ to ~160% and ~190% for A2 and A3, respectively (not sign. less than without L-NNA). The effect of NP was the same with or without glucose or L-NNA superfusion: increase to ~175% and ~215% for A2 ans A3, respectively. Superfusion of glucose for 5-6 hrs neither affected the NO-dependent and -independent reactivity of these muscle arterioles (10-40µ) nor the contribution of NO to their resting diameter.

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THE EFFECT OF ACETYLCHOLINE ON FINGER NAILFOLD CAPILLARY PRESSURE AND PULSE PRESSURE AMPLITUDE IN HEALTHY VOLUNTEERS. S.J. Morris and A.C. Shore

Nitric oxide (NO) synthase has been demonstrated in endothelial cells of human dermal microvasculature, however the physiological significance of this is unknown. Endothelial cells from a wide variety of sources release NO following stimulation with acetylcholine (ACh), therefore the aim of this study was to investigate the effects of acetylcholine on nailfold capillary pressure and pulse pressure amplitude. 2 fingers on the left hand were studied in 5 healthy volunteers (age 33.8 ± 8.2 years, mean ± S.D., 3 female). Measurements were taken under basal conditions (both fingers) and following each dose of iontophoretically applied 1% ACh (1 finger) or ACh vehicle (1 finger) (7 x 1 mC + 1 x 2 mC, 60 s interval between each dose). The operator was blind to the nature of the solutions. Capillary pressure was measured following direct cannulation at the apex of the capillary loop with glass micropipettes using an electronic resistance feedback servonulling technique. Application of ACh vehicle did not significantly change either capillary pressure or capillary pulse pressure amplitude. ACh significantly increased capillary pressure (from 15.8 ± 2.2 mm Hg basal, to 27.7 ± 3.8 mm Hg, plateau, $P<0.043$, Wilcoxon matched pairs sign rank). Capillary pulse pressure amplitude was also significantly increased following acetylcholine application (from 2.4 ± 2.4 mm Hg basal, to 8.4 ± 2.4 mm Hg plateau, $P<0.043$). The observed increase in capillary pressure following acetylcholine may be explained by a decrease in precapillary resistance or an increase in postcapillary resistance, however the associated increase in capillary pulse pressure amplitude suggests that a reduction in precapillary resistance is the most likely explanation. Supported by The Wellcome Trust

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CAPILLARY DIAMETER CHANGES DURING LOW PERFUSION PRESSURE, REACTIVE HYPEREMIA, AND LOCALLY APPLIED ADENOSINE IN RABBIT SKELETAL MUSCLE. J. Bosman, G.J. Tangelder*, M.G.A. oude Egbrink*, R.S. Reneman*, and D.W. Slaaf.

Capillary (CAP) diameters are usually assumed not to change when CAP transmural pressure is changed during physiological interventions. In bat wing, however, CAP diameters increased by 28% when CAP transmural pressure was increased by only 14 mmHg (Blood Vessels 1989;26:325-334). Aim of the present study was to investigate CAP diameter in skeletal muscle during aorta occlusion and subsequent reactive hyperemia (RH), in the absence or presence of a vasodilator (adenosine; ADO).

Using video intravital microscopy, tenuissimus muscle CAPs of anesthetized rabbits (n=36) were recorded during control (femoral artery pressure: Pfem=83 mmHg), complete aorta occlusion (2 min; Pfem=18 mmHg), and subsequent RH, without or with locally applied 10^{-4}M ADO. Off-line diameters were measured by means of image shearing. Control CAP diameter was 4.4µm (3.2-6.9µm; median and range; n=120). During occlusion diameters decreased to 94% (105- 80%; $p<0.0001$) and during RH diameters increased to 112% (101-132%; $p<0.0001$). ADO induced an increase of control diameter to 126% (109-172%; $p<0.0001$; n=27). Occlusion in the presence of ADO resulted in a diameter reduction to 111% (96-143%; $p<0.0001$). After deflation of the occluder (+ADO) diameters returned to ADO-control levels.

It is likely that the CAP diameter changes during aorta occlusion and RH are passive, i.e., proportional to CAP transmural pressure changes. After addition of ADO, however, CAP diameter during occlusion was larger than during control (- ADO), although in the first situation CAP pressure is likely to be lower than in the latter. The unexpectedly large CAP diameters under ADO may be explained by structural changes of CAP endothelium due to prolonged (at least 20 min) high CAP transmural pressures or by a direct effect of ADO on the CAP wall.

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NORADRENALINE-INDUCED DEPOLARIZATION DETERMINED IN ISOBARIC AND ISOMETRIC RAT SMALL ARTERY PREPARATIONS

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Recently, the reactivity of rat small arteries to noradrenaline (NA) was shown to be higher in isometric than in isobaric vessel preparations. As an explanation, it was suggested, that NA-induced membrane potential changes were in part wall tension dependent, since this would be smaller under isobaric than under isometric conditions (VanBavel E, Mulvany MJ. J Physiol 1994; 477.1: 103-115). Therefore, the effect of NA on the membrane potential of small rat mesenteric arteries was investigated in isometric (n=10) and isobaric (n=12) vessel preparations (see table, data are mean±SEM). The resting membrane potential of the isobaric vessel preparation was significantly less negative, although the wall tension was not different. The membrane potential change induced by the highest NA concentration was 2.6-fold smaller in the isobaric vessel preparation, where wall tension was low, than in the isometric vessel preparation, where wall tension was high.

	Membrane potential (mV)		Wall tension (N/m)	
	isometric	isobaric	isometric	isobaric
resting	- 56.3±1.2	- 49.8±0.8	0.75±0.05	0.81±0.02
10^{-5} M NA	- 33.7±1.2	- 41.2±1.1	3.51±0.30	0.21±0.01
change	+23.0±1.0	+ 9.0±0.8	+2.76±0.26	-0.60±0.02

Thus, differences in wall tension of the isobaric and the isometric vessel preparation cannot explain the difference of the resting membrane potential, but can explain the difference in the membrane potential response induced by NA in the two vessel preparations.

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HYDROSTATIC PRESSURE MEASUREMENT IN DIFFERENT VASCULAR SEGMENTS OF THE STOMACH IN RAT.

J. Peti Peterdi and L. Rosivall

Numerous indirect methods have proved that microcirculatory changes play an important role in the pathogenesis of peptic ulcer. We don't know, however, to which extent does the increase of active or passive resistance of the different vascular segments contribute to the development of the flow reduction. Our goal was to develop a method in which by the use of the micropuncture technique, we can follow the pressure changes in different vascular segments from the arterioles to the venules, in the vascularly intact and innervated stomach of the rat. Rats were aneasthetized by 100 mg/kg Inactin-Byk. Systemic arterial blood pressure was monitored through the femoral artery by a Statham electro-manometer. The stomach was exposed through a midline laparotomy. Temperature of the animal was controlled by a heating pad and that of the stomach by the continuous rinse of thermostat driven saline solution. Vessels of the mucosa were approached from the serosal side. A small window was opened to the mucosa by the careful preparation of the seromuscular layer. Diameter of the vessels of the arterial and venous plexuses was determined in vivo by intravital microscopy by the built in scales of the microscope. At a systemic arterial blood pressure of 100-135 mmHg (n=20) the pressure values were 75-85 mmHg in arteries of 80-90 micron in diameter; 38-42 mmHg in arterioles of 30-40 micron in diameter, and 22-26 mmHg in venules of 30-45 micron in diameter; 16-20 mmHg in veins of 90-120 micron in diameter respectively. Our method is a useful tool for analyzing the microcirculation of the stomach under different physiological and pathological conditions.

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DETERMINATION OF THE MEAN SYSTEMIC FILLING PRESSURE ON THE BASIS OF A SINGLE VENTILATION PROCEDURE.

E.A.den Hartog, J.R.C.Jansen, G.H.Moens, A.Versprille.

Mean systemic filling pressure (P_{sf}) has been defined as the pressure in the systemic circulation if the flow is zero. In the intact circulation P_{sf} was determined based on the linear relationship of venous return (Q'_v) and central venous pressure (P_{cv}): (1) $Q'_v = a - b\ P_{cv}$. P_{cv} was varied by applying inspiratory pause procedures (IPP) at different volumes [1]. P_{cv} and Q'_v were measured during the steady state of the pause. P_{sf} was found from the extrapolation of the linear regression between P_{cv} and Q'_v to zero flow. This method requires 7 IPPs at different volumes with about 5 min intervals and therefore, in total about 45 min to obtain one value of the P_{sf}. The aim of these experiments was to find a faster method to determine P_{sf}. The method that was used was based on Slow Inflation Procedures. Due to the venous capacity, during inflation venous return will decrease more than predicted by the linear relationship, as was observed previously [1], causing an underestimation of P_{sf}. This underestimation would be smaller for slower inflations. In 9 pigs of about 10 kg we studied the effect of the inflation time on the estimation of P_{sf}. The data were compared with those obtained with the IPP-method. Indeed, a linear regression between Q'_v and P_{cv} applied during inflation, produced an underestimation of P_{sf}. An exponential fit through the P_{sf} for different inflation times predicted an inflation time of about 15 s to be on average long enough to yield a reliable estimation of P_{sf} that is not significantly different from the P_{sf}(IPP). The advantage of this method is that the determination of the P_{sf} can be performed much faster than with the method on the basis of IPPs. Such a method could be usefull for conditions of suppressed circulatory control as in anaesthesia and intensive care.

[1] A Versprille, JRC Jansen: Pflug. Arcv. 405: 226-233, 1985.

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A METHOD TO MEASURE BLOOD FLOW DISTRIBUTION IN CONTRACTING SKELETAL MUSCLE UNDER CONTROLLED MECHANICAL CONDITIONS.

C.C. van Donkelaar, M.R. Drost, H.W.M. van Straaten, F.W. Prinzen, J.D. Janssen, A. Huson.

It is known that exercise increases muscular blood flow, while forceful tetanic contractions impede it. Several studies have examined the blood flow distribution between muscles and other organs during exercise, using microspheres. These microspheres stuck in precapillary vessels, thus representing blood flow distribution. However, the influence of a single tetanic contraction on the distribution of blood flow in a skeletal muscle has not been examined before. The objective of the present study was to develop a method for *in vivo* measurement of the regional distribution of blood flow in the rat m. tibialis anterior during a single tetanic contraction, under mechanically well defined conditions. We hypothesised that during contraction, central blood flow decreases more than peripheral blood flow, as a result of a centripetal increase of intramuscular pressure.

During the experiment, the hind limb of an anaesthesized rat was fixed in a rat dynamometer. Both the active moment, generated by electrically stimulated dorsal or plantar flexors of the ankle, and the enforced ankle rotation were measured. Before and during a sustained submaximal contraction of 30 seconds, $2*10^5$ fluorescent, differently labled, 15µm microspheres were injected into the aorta via the a. renalis. After the injection, the rat was perfusion fixated and the m. tibialis anterior was dissected free and embedded in plastic. Using a digitizer, coupled to a fluorescence microscope with filters for different labels, 2D coordinates of the microspheres and of muscle contour points were sampled per section (25µm). A newly developed computer program used these data and the section numbers to visualize 3D muscular blood flow distribution during a sustained contraction.

The first experiments have revealed a random distribution in control muscles, while in contracting muscles regional differences in distribution were seen.

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CAPSAICIN-SENSITIVE NEURAL AFFERENTS IN FEVER PATHOGENESIS. M. Székely, M. Balaskó, A.A. Romanovsky

Cytokines as mediators of exogenous pyrogen action increase the presence/activity of prostaglandin E in the hypothalamus. Their synthesis and/or crossing the blood-brain-barrier takes, however, considerable time.This is not consistent with the remarkably short latency of fever elicited by peripheral administration of the exogenous pyrogens. Other information routes to the hypothalamus (including some neurogenic afferentation) may also be of importance.

In order to destroy a part of non-specific neural afferents cold-adapted female Wistar rats were treated intraperitoneally with low doses of capsaicin (total 25 mg/kg in 6 injections). This treatment causes local damage to abdominal nerve fibers (including vagal afferents) without affecting heat sensitive and nociceptive fibers systemically. Control rats were adapted to cold but had no pretreatment. Two to five weeks after the capsaicin treatment a jugular vein of the rats was cannulated. A further week later 10 µg/kg E.coli endotoxin was injected through the cannula while the animals were kept slightly restrained in a metabolic chamber at the thermoneutral temperature (25°C). Deep body temperature, tail skin temperature, and metabolic rate were recorded continuously, revealing a characteristic biphasic fever in the control group (resulting from co-ordinated changes in effector functions, with an onset of 30, peaks of 55 and 110 min, respectively), while the fever was delayed, greatly attenuated, or completely abolished in the capsaicin treated rats. The basic thermoregulatory reactions were not affected by such capsaicin pretreatment: upon exposure to warm or cold environment these rats responded with increases in heat loss and heat production, respectively, comparable in size to those in controls. These data are in contrast with the effects of systemic large-dose capsaicin desensitization which impaires heat sensitivity and, consequently, has a fever-enhancing effect. The depression of fever in the present experiments suggests that neurogenic information of abdominal origin may play a role in the initiation of fever in response to exogenous or endogenous pyrogens.

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THE ANTIPYRETIC EFFECT OF COLD ACCLIMATION IN THE RABBIT HOLDS ONLY FOR COOL ENVIRONMENT. U.Beckmann and J.Werner

Cold acclimation alters the properties of the temperature control system by morphological changes of the insulation layer, by changes of effector-capacities, or by functional adjustments of control characteristics. Among other physiological states cold acclimation has previously also been shown to reduce a pyrogen-induced fever in the guinea pig (Roth and Zeisberger, 1992), which has been discussed to be the consequence of an antipyretic action of an elevated level of arginine-vasopressin. In this study the influences of cold-adaptive alterations on the regulatory mechanisms during fever (0.5 µg/kg LPS i.v.) at ambient temperatures of 2°C, 22°C, and 30°C were investigated by studies on rabbits acclimated to thermoneutral T_a (8 weeks at T_a=22°C; n=13) and to cold (8 weeks at T_a=2°C; n=10).

Cold acclimation caused an enhancement of fur density and reductions of heat loss via respiratory evaporation and ear blood flow. As an adjustment to the improved insulation, metabolic heat production in the cold was reduced by 10%, but in the afebrile state (saline control; n=10), there was no effect on the threshold temperature for increase in heat production. During fever, nevertheless, the pyrogen-related upward shift of shivering threshold was diminished in the cold acclimated rabbits. This modification of control characteristics caused a significant attenuation of the fever response at lower ambient temperatures, where metabolic heat production acts as the dominant effector for fever generation. The integrated febrile temperature elevation was reduced by about 60% at T_a=2°C and 40% at T_a=22°C as compared to the non-acclimated animals. At T_a=30°C, however, total fever height was enhanced by about 35% after cold acclimation, due to the increased body shell insulation, an adaptive elevation of panting threshold, and a reduced capacity of ear blood flow.

Since central applications of exogenous vasopressin, too, suppress fevers mostly only in cool environments, it seems possible that in the rabbit as well cold acclimation acts antipyretically by an endogenous vasopressinergic mechanism specifically inhibiting the febrile rise of heat production. In a warm environment, however, where fever generation in the rabbit is exclusively depending on the action of heat loss effectors, this mechanism cannot be effective.

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CENTRAL AND PERIPHERAL THERMORECEPTORS AFTER THE LONG-TERM ADAPTATION TO COLD. T.V.Kozyreva, F.K.Pierau

The regulatory parameters of temperature homeostatic system are determined by central and peripheral thermal inputs. One of the important question is what kind of changes in central and peripheral thermosensors could provide some regulatory shifts in temperature thresholds for thermodefence reactions after the long-term adaptation to cold. The experiments on control and cold-adapted (adaptation to cold 4-6 weeks at 5 C) rats were carried out. Hypothalamic thermosensitive neurones were studied in vitro on slices. Skin cold receptors activity was recorded from fibres of n.sapheni in vivo under anaesthesia.

After the adaptation to cold there were some modifications as in central so in peripheral thermal inputs. The portion of neurones which were sensitive in the range of low temperature (35-38 C) reduced while the portion of neurones sensitive in the range of high temperature (38-41 C) increased. In skin cold receptors the maximal dynamic response was decreased and static maxima were shifted by 3-4 C to higher temperatures in cold-adapted animals.

The sensitivity of both central and peripheral inputs reduced at low temperatures but increased at high temperatures. That could provide to cold-adapted organism the increase of thresholds for cooling and the decrease of them for warming.

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SUBJECTIVE SENSATIONS OF A NON-IMMERSED HAND IN RELATION TO TEMPERATURE FLUCTUATIONS DUE TO COLD WATER IMMERSION OF THE OTHER HAND. R. Heus, H.A.M. Daanen, J.A. Kistemaker

When hands are immersed in cold water temperature fluctuations due to cold induced vasodilation (CIVD) occur (Lewis, 1930). Heus and Daanen (1993) found a relation between temperature sensations of immersed hands as compared to these fluctuating hand skin temperatures (HST) and body core temperature. Lewis (1930) showed that during one hand immersion in cold water, the temperature fluctuations of the immersed hand also occur in the non-immersed hand. The present study was designed to study the relation between temperature sensations and HST of the non-immersed hand (NH) at two different thermal states of the body, while the other hand was immersed (IH) in cold water.

Seven male subjects immersed their right hand in a 5°C water-bath; once with a relatively cold body (CB) and once with a relatively warm body (WB). During the experiments core temperature and mean skin temperature were registered. Furthermore, temperatures of the distal phalanxes of all fingers were measured by thermocouples and were averaged to calculate the HST. During the immersion period, subjects were asked after their sensations of comfort (extremely uncomfortable (+8) to comfortable (0)) and temperature (very cold (-10) to very hot (+10)), separately for NH and IH. Also, a general (whole body) comfort rating was given by the subjects.

The results of this study showed that with a CB the HST of the NH is not correlated with the HST of the IH ($r=.04$). The temperature sensation of the NH is significantly correlated with the HST of that hand ($r=.71$). The comfort sensation of the NH under these circumstances has a significant correlation of .35 with the HST of the NH.

With a WB there was also no significant correlation between HST of the NH and the HST of the IH ($r=-.05$). The thermal sensation of the NH under WB-conditions had a significant correlation of .38 with the HST of the NH and the comfort sensation had a significant correlation of .28 with the HST.

These results lead to the conclusion that temperature fluctuations due to CIVD of the immersed hand were not reflected in the non-immersed hand. The second conclusion is that with a relatively cold body differences in temperature of a non-immersed hand were better detected by the subjects. The small fluctuations in skintemperature of the non-immersed hand under warm body conditions have hardly any influence on subjective sensations.

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PHYSICAL PERFORMANCE OF DEHYDRATED AND REHYDRATED SUBJECTS IN COLD ENVIRONMENT. H. Rintamäki, T. Mäkinen, J. Oksa and J. Latvala

The effect of dehydration and subsequent rehydration on different components of physical performance was studied at -20°C. Starting from the evening prior to the tests, on the average 25 years old healthy male subjects (n=7) were dehydrated by water restriction and moderate physical activity. In the first experiment, the effects of dehydration were studied after 60 min exposure to cold. In the second experiment, the effects of dehydration and subsequent rehydration with 5 % sucrose solution were studied 90 min after completion of rehydration, at the end of 60 min cold exposure. In the first experiment dehydration (corresponding 3.4±0.3 % (mean ± SE) loss in body weight) decreased plasma volume by 5.3±2.5 % and cold exposure further decreased plasma volume by 7.8±1.7 % resulting in the total decrease of ca. 13 %. In control subjects the cold induced decrease in plasma volume was 6.9±1.0 %. At submaximal work levels both oxygen uptake and heart rate were higher ($p<0.05$-0.01) in dehydrated subjects while anaerobic threshold was lower: 160±6 W in comparison to 180±5 W in the control subjects. The working time until exhaustion was also shortened from 12.2±0.6 min to 10.6±0.4 min ($p<0.05$). However, dehydration did not affect maximal oxygen uptake and maximal muscle strength. In the second experiment the rehydration (fluid amount corresponding 1.8±0.1 % body weight loss caused by dehydration) decreased oxygen uptake 35.2±2.2 to 28.7±2.7 $ml \cdot kg^{-1} \cdot min^{-1}$ ($p<0.001$) at the end of 10-minute bicycle ergometer exercise (load 125 W). Rehydration also increased systolic blood pressure by 10 mmHg and increased the rate of diuresis. The results suggest lower efficiency, higher physical strain and earlier exhaustion of dehydrated subjects in cold. Although physical performance could be restored by rehydration, a rapid rehydration is not recommended because of increased diuresis and blood pressure.

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THERMOREGULATION AND HEMODYNAMICS IN BROILER CHICKENS DURING DIURNAL CYCLIC TEMPERATURE CHANGES. S. Yahav, D. Luger, I. Plavnik, M. Dotan* and S. Hurwitz

Thermoregulation and hemodynamics were evaluated in broiler chickens which had been acclimated to diurnal cyclic temperature of 12h, 35°C; 12h, 15°C. Ambient temperature (T_a) change was completed within 60 min. Core (T_C) and skin (T_S) temperatures were monitored continuously by implanted thermocouples connected to a data logger. Surface temperature was monitored by Infrared Thermal Imaging Radiometer (Inframetrics model 760), operating within a spectral range of 8-12μ. Plasma volume, pH and pCO_2 in arterial blood, as well as total protein, hemoglobin, hematocrit, osmolality and triiodothyronine (T_3) concentration in venous blood were measured 4-5 hours after temperature change. Average Tc and Ts were lower by 2.2 and 1.6°C respectively at 15°C as compared with 35°C. During decrease in Ta of 0.29°C per min., average surface temperature of the chicken declined at a rate of 0.20°C per min., but the facial temperature declined only at a rate of 0.045°C per min. The increase in Ta was followed by plasma volume expansion from 3.31±0.1 to 4.51±0.24%, associated with an increase in plasma protein concentration, decrease in osmolality and hematocrit, with the development of respiratory alkalosis, but only with a small alteration in circulating plasma T_3. This small change in circulating T_3 suggests only a limited contribution of heat production to thermoregulation during diurnal cyclic temperature changes. The development of respiratory alkalosis and changes in surface temperature indicate the participation of heat dissipation and hemodynamic modulation in chickens exposed to diurnal cyclic temperature changes.

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THE β-ADRENORECEPTOR ANTAGONIST PROPRANOLOL INCREASES BLOOD-VESSELS PERMEABILITY IN HEAT-ACCLIMATED ROCK PIGEONS (COLUMBA LIVIA). Arieli Y., Feinstain N., Raber P. and Marder J.

When exposed to an extremely hot environment (50-60 °C), heat acclimated (HAc) pigeons use cutaneous water evaporation (CWE) as the main cooling mechanism to maintain normal body temperatures (Tb). Moreover, CWE can be induced in HAc pigeons, at room temperature, by administration of propranolol. However, neither heat exposure nor propranalol administration evoked significant CWE in winter acclimated (WAc) pigeons.

We tested the hypohesis that increased CWE in HAc pigeons is a consequence of increased vascular permeability. The distribution of Evans Blue (EB), a tracer of serum albumin was followed electron microscopically. We found that:

a. EB extravasation from the vascular bed into the skin tissue of HAc pigeons, after propranolol administration, was significantly higher comparing to control values, and comparing to WAc pigeons.
b. Propranolol administration to HAc pigeons led to the opening of gaps in the walls of capillaries and venules.
c. Changes in the vascular bed occurred mainly in the first ten minutes after drug administration.

These results show that in HAc but not WAc pigeons, propranolol increases the permeability of small blood vessels walls to water and macromolecules.

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THE ROLE OF ADRENERGIC AND CHOLINERGIC RECEPTORS IN THE CUTANEOUS WATER EVAPORATION MECHANISM IN THE PIGEON (*Columba livia*). Ophir E., Raber P., Arieli Y. and Marder J.

Cutaneous water evaporation (CWE) is known to enable the pigeon, and some other birds that inhabit arid environments, to withstand exposure to high ambient temperatures. In resting heat acclimated (HAc) pigeons, this route is highly efficient and serves as the prime pathway for heat dissipation. The physiological factors involved in the process of CWE in birds are not yet clear and most of the existing data about evaporative cooling mechanisms refer primarily to the respiratory system. In this study we examined the effects of selective and non-selective adrenergic and cholinergic agents on CWE in the HAc pigeon. Intramuscular and subcutaneous injections of propranolol (a non-specific β-adrenergic antagonist) at 24°C Ta resulted in intensive CWE in HAc pigeons. The CWE values that were triggered by propranolol were similar to maximum CWE values induced by exposure to heat in earlier studies. Intramuscular injection of salbutamol (a β_2-adrenergic agonist) drastically diminished CWE induced by either propranolol or exposure to high temperatures, and enhanced panting. Intramuscular injection of ICI-118,551 (a β_2-adrenergic antagonist) also increased CWE. Intramuscular injection of clonidine (an α_2-adrenergic agonist) also elicited CWE, but subcutaneous injection had no effect. Intramuscular injection of atropine (a muscarinic antagonist) resulted in increased CWE. Subcutaneous injection had no effect. Propranolol-induced CWE as well as atropine-induced and heat-induced CWE, but not clonidine-induced CWE, was always associated with visible peripheral vasodilation. The results of this study indicate the important role of α_2- and β-adrenergic and muscarinic receptors in the process of CWE in the HAc pigeon. In accordance with the findings presented here we suggest a hierarchical model for the sympathetic control of CWE in the pigeon.

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MACROMOLECULAR TRANSPORT IN RAT AND GOLDFISH INTESTINE, A COMPARATIVE STUDY. A.J. Kiliaan, P.B. Bijlsma and J.A. Groot

The effects of in vitro application of the acetylcholine analog carbachol and/or forskolin (activator of adenylate cyclase, AC) to intestinal epithelium of goldfish and rat on transepithelial transport of intact horse radish peroxidase (HRP) were compared. Epithelium was stripped from muscular layers and mounted in an Ussing chamber in the presence of TTX to inhibit neuronal activity. Transepithelial transport of the intact protein was calculated from the enzymatic activity of timed samples from the "cold" compartment. Endocytotic uptake of HRP was histochemically semi-quantified by measuring the total diameter of endosomes filled with HRP-product in randomly choosen apical area's of 24 μm^2 of electronmicrographs of the villus cells. Paracellular passage was deduced from filling of tightjunctions with HRP-product. Results showed that, as compared to timed controls, carbachol increased the amount of HRP-product in endosomes and forskolin reduced the amount of HRP-product in endosomes in both species. Carbachol but not forskolin induced an significant increase of the transepithelial flux of intact HRP in rat. The increase was relatively larger than the increase of the flux of mannitol. In the presence of carbachol some lateral intercellular spaces, and focal spots in the tightjunctions in the villi were filled with HRP-product. In contrast, the application of carbachol to intestinal epithelium of the goldfish, did not influence the transepithelial transport while forskolin significantly increased the flux of HRP and the filling of lateral inter-cellular spaces and lateral infoldings with the HRP-product. In the presence of forskolin plus carbachol the filling and the transepithelial flux was not different from control, indicating that carbachol inhibited the effect of forskolin. We propose that carbachol in rats and forskolin in fish can influence the pore-diameter of waterfilled channels in the tight junctions. The presence, *in vivo*, of acetylcholine and AC-stimulating neurotransmitters and hormones in the lamina propria suggest that the regulation of tightjunction-permeability for large, possibly antigenic, molecules from lumen to blood may be of (patho)-physiological importance.

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MODULATION OF CORNEAL ENDOTHELIAL CELL TRANSPORT ACTIVITY. K. Ogbuehi, S.A. Hodson and C.G. Wigham

The objective of this study was to determine the effect of modulation of intracellular cyclic nucleotide activity on corneal endothelial HCO_3^- transport. De-epithelialised rabbit corneas were mounted in modified Ussing type chambers and trans-endothelial short circuit current (s.c.c.) and resistance (R_e) measured. The effects of cyclic nucleotides and their modulators in the presence of inhibitors of traffic proteins was determined. Addition of dibutyryl cAMP and cGMP to the bathing Ringer had no effect on either s.c.c. or R_e. However IBMX ($5x10^{-4}$M), a non-specific phosphodiesterase inhibitor, caused a transient increase in both s.c.c. and R_e that lasted 200 ± 17 min. The maximum stimulation of s.c.c. and R_e was 18 ± 2% and 37 ± 8% respectively (values are mean ± s.e.m., n=3). In an attempt to identify the site of the IBMX induced effect, IBMX was added to preparations previously inhibited with furosemide and amiloride ($5x10^{-4}$M). Furosemide reduced the s.c.c. by 31 ± 5% and had no effect on R_e. Addition of IBMX to the furosemide inhibited preparation increased s.c.c. by 14 ± 4% and R_e by 22 ± 6% (values are mean ± s.e.m., n=3). The effect was not significantly different than that with IBMX alone (p = 0.2, Students' t-test). Amiloride reduced s.c.c. by 49 ± 4% and had no effect on R_e. Addition of IBMX to the amiloride inhibited preparation caused s.c.c. to decrease by 34 ± 6% and R_e to increase by 35 ± 8%. These results suggest that the endothelium is responsive to internally induced modification of cyclic nucleotide activity, that the IBMX effect is not directed at the $Na^+/K^+/2Cl^-$ cotransporter but does involve the the Na^+/H^+ exchanger either directly or indirectly.

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Ca^{2+} ACTIVATES A Cl^- PERMEABILITY IN A MODEL EPITHELIUM, THE FROG SKIN. B.Brodin, K.A.Rytved and R.Nielsen.

$[Ca^{2+}]_i$ is an inhibitor of transepithelial sodium transport. The mechanisms by which $[Ca^{2+}]_i$ exerts its effects on the intact tissue are complex. In this study, we investigated the effect of $[Ca^{2+}]_i$ on basolateral membrane chloride permeability in the isolated frog skin epithelium. $[Ca^{2+}]_i$ was measured using epifluorescence microscopy. Na^+ transport and cell potential were measured in a voltage clamp/microelectrode set-up. Unidirectional Cl^- fluxes and cellular Cl^- were determined in tracer studies, cellular K^+ and Na^+ were measured with atomic absorption photometry. The inhibitor of the ER-Ca^{2+}-ATPase, thapsigargin (TG), causes a small increase in $[Ca^{2+}]_i$ (from 59±9 to 119±16 nM, n=12, $p<0.001$) and a decrease in sodium transport (measured as short circuit current). This is accompanied by a depolarization and a decrease in apical sodium permeability, as previously described (Brodin, Rytved & Nielsen, 1994b). In a situation where nonconductive Cl^- fluxes across the basolateral membrane were blocked with the loop diuretic furosemide (2 mM), TG increased the unidirectional efflux of radiolabelled Cl^- and caused cell shrinkage. The calcium-induced increase in Cl^--permeability could be blocked by the chloride channel inhibitor diphenylamine-2-carboxylate (DPC). Addition of DPC (2 mM), following a $[Ca^{2+}]_i$ -induced depolarisation, caused the cells to hyperpolarize back towards the control value. We conclude that a small, physiological relevant, increase in $[Ca^{2+}]_i$ activates an otherwise quiescent Cl^- permeability in the basolateral membrane. Whether the depolarization observed actually is a result of this increase in Cl^- permeability, or also partly a result of a decrease in K^+-permeability as previously suggested (Brodin, Rytved & Nielsen, 1994a), remains to be established.

Brodin, Rytved & Nielsen, 1994a. Pflügers Arch. **426**:171-173
Brodin, Rytved & Nielsen, 1994b. Acta. Physiol. Scand. **151**(4): 17A

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Ca^{2+} influx induced by intracellular alkalinization in HT_{29} CELLS

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The influence of intracellular pH ($[pH]_i$) changes on the intracellular Ca^{2+} activity ($[Ca^{2+}]_i$) in HT_{29} colon carcinoma cells was examined. $[Ca^{2+}]_i$ and $[pH]_i$ were measured microspectrofluorometrically with Fura-2 and BCECF in independent experiments. Changing the perfusion solution from a phosphate to HCO_3^-/CO_2 buffer led to a concentration dependent acidification of $[pH]_i$. From the basal value of 7.35±0.09 (n=21) $[pH]_i$ decreased by 0.13±0.03 (2% CO_2); 0.29±0.04 (5% CO_2) and 0.39±0,04 (10% CO_2, n=7) units. $[Ca^{2+}]_i$ measured under the same experimental conditions increased slightly and slowly with 5% CO_2 (Δ 8±2 nmol, n=11) and 10% CO_2 (D 15±2 nmol, n=30). $[Ca^{2+}]_i$ changes were similar in Ca^{2+} free solution and were not effected by treatment with thapsigargin. Changing back to the phosphate-buffer after 2 minutes resulted in an alkalinization of $[pH]_i$ by 0.14±0.02 (2% CO_2), 0.27±0.02 (5% CO_2), 0.38±0.03 (10% CO_2, n=7) units above basal level. In parallel $[Ca^{2+}]_i$ increased from 40±16 nmol/l by 20±3 nmol/l (2% CO_2), 65±6 nmol/l (5% CO_2), 143±10 nmol/l (10% CO_2, n=7). The time course of the acidifying $[pH]_i$ changes was not altered by the CO_2 concentrations and was different from that of the $[Ca^{2+}]_i$ changes. The peak $[Ca^{2+}]_i$ changes after HCO_3^-/CO_2 removal were reduced by 90% with a nominal Ca^{2+} free perfusion solution and by 50% with La^{3+} (10^{-5} mol/l). Using NH_3/NH_4^+ we induced $[pH]_i$ changes and $[Ca^{2+}]_i$ transients in HT_{29} cells, too. The peak alkalinization as well as the $[Ca^{2+}]_i$ peak was reached faster (18±2 s and 28±2 s) compared to the HCO_3^-/CO_2 effect. $[Ca^{2+}]_i$ declined to basal levels in the presence of NH_3/NH_4^+ within 200±23 s, whereas $[pH]_i$ still was alkalinized. The $[Ca^{2+}]_i$ peak value was unchanged by Ca^{2+} free perfusion, but the transient was markedly shortened. The $[Ca^{2+}]_i$ transient was interrupted by Ca^{2+} free buffer after the peak had been reached. Removal of NH_3/NH_4^+ led to acidification of $[pH]_i$, whereas $[Ca^{2+}]_i$ did not change significantly. Using NH_3/NH_4^+, propionate, or HCO_3^-/CO_2 for intracellular acidification we could reduce the Ca^{2+} plateau induced by thapsigargin or carbachol by approximately 45% (n=6 for each maneuver). In conclusion: (I) a small increase of $[Ca^{2+}]_i$ was induced by acidic $[pH]_i$ changes (II) an alkaline pH change induced with HCO_3^-/CO_2 led to a $[Ca^{2+}]_i$ transient mostly due to Ca^{2+} influx (III) an alkaline pH change induced by NH_3/NH_4^+ led to a $[Ca^{2+}]_i$ transient due to intracellular Ca^{2+} store release and Ca^{2+} influx.

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ENDOTHELIN INHIBITS Cl REABSORPTION IN THE MEDULLARY THICK ASCENDING LIMB OF HENLE'S LOOP (mTAL), by M. de Jesus Ferreira and C.Bailly.

The recent localisation in the mTAL of ETB receptors and endothelin 1 and 3 synthesis raised the question of their autacoid effect on this segment. Net chloride flux (JCl, pmoles/min), used as the representative parameter of the mTAL reabsorptive function, was determined on isolated microperfused tubules from mouse kidney. Tubules were bathed and perfused with isoosmotic HEPES buffered solutions. In each luminal tubular sample, Cl concentration was determined by microelectrometric titration. On the same tubule, endothelin 1(ET1), 10^{-8} M in the bath, significantly decreased JCl from a control value of 52 ± 2 7.7 to 38.1± 6.1 (N=10), an effect completely blocked in the presence of Ro 46-2005, a specific ETA and ETB antagonist (gift from Hoffmann-Laroche), and not observed in time control tubules (78.7± 12.2 and 75.5 ± 13.0, in two consecutive periods). The ET1 effect on JCl was similar either cellular cAMP content was increased or not by glucagon (25.3 ± 6.9% vs 28.2 ± 3.0%, respectively). Accordingly, 10^{-7} M ET1 did not modify the ADH-induced increase in cAMP content, in microdissected mTALs (20.2 ± 2.5 vs 21.2 ± 5.6 fmoles/4min/mm for ADH and ADH + ET1, respectively). The ET1 effect on JCl was abolished by protein kinase C inhibitors, staurosporine or GF 109203. Moreover, chelating intracellular Ca with BAPTA/AM, 30 min before an experiment, prevented the inhibitory effect of ET1. These results thus indicated that ET1 inhibited the mTAL reabsorptive function, independently of a cAMP decrease, and by a mechanism involving protein kinase C activation and intracellular Ca. This effect might impair the corticopapillary gradient and thus potentiate the ET1-induced inhibition of the ADH action on water reabsorption in the collecting tubule.

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CHLORIDE TRANSPORT IN CULTURED SWEAT GLAND CELLS. G.M. Roomans, A.-C. Mörk, J. Hongpaisan, and A. Ring

Chloride transport in the sweat gland is of interest e.g., in the disease cystic fibrosis, where a defect in the cAMP-regulated chloride channel has been demonstrated. Regulation of chloride transport in cultured sweat gland cells was studied by X-ray microanalysis in the electron microscope, patch clamp technique and fluorescence techniques. Primary cultures of coil cells as well as the immortalized cell line NCL-SG3 were used. For X-ray microanalysis, the cells were generally cultured on very thin plastic films supported by titanium grids. Elemental changes in the coil cells during different stages of isolation and culture could be observed. In primary cultures of coil cells, chloride secretion could be stimulated by carbachol, and in part of the cell population also by cAMP. In addition, purinergic stimulation could be demonstrated. Possibly, part of the coil cells had acquired duct-cell like characteristics during culture. In NCL-SG3 cells which supposedly are of ductal origin, both Ca^{2+} and cAMP-induced chloride secretion could be demonstrated, both by microanalysis and patch-clamp technique. This secretion could be inhibited by chloride channel blockers (9-AC, NPPB, DPC). cAMP-induced chloride secretion was qualitatively independent of the substrate on which the cells were cultured. Although an increase of the intracellular Ca^{2+} concentration by calcium ionophores gave rise to chloride secretion, it could be excluded that the effect of cAMP was due to an increase in intracellular Ca^{2+} concentration. X-ray microanalysis showed that in cells grown on permeable substrates, chloride secretion could be induced by carbachol, but this was not the case in cells grown on impermeable substrates. It is concluded that regulation of chloride secretion in cultured sweat gland cells is complex, and it may not always agree with the situation *in vivo*. X-ray microanalysis and patch clamp technique can be used as complementary techniques to study ion transport at the cellular level.

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INHIBITION OF cAMP INDUCED CL^- SECRETION BY NPY IN HUMAN INTESTINAL EPITHELIUM. H. Bouritius and J.A. Groot.

It has been shown in rat intestine (1) that neuropeptide Y inhibits the forskolin induced short circuit current. Recently a Y_1 receptor has been reported in the undifferentiated human epithelial cell line HT-29 (2). Preincubation with 1 µm NPY for 30 min could completely abolish the forskolin induced cAMP accumulation. This is indirect evidence for the inhibitory action of NPY on Cl^- secretion. We have studied the effect of NPY on forskolin induced Cl^- secretion directly in the secretory cell clone HT-29cl.19A, grown as a confluent monolayer on permeable, translucent Falcon filters and mounted in an Ussing-type experimental chamber. Conventional microelectrode technique was applied to monitor the intracellular potential with respect to the apical side and the transepithelial potential was measured with extracellular electrodes. Transepithelial resistance and the fractional resistance of the apical membrane were calculated from voltage deflections induced by transepithelial current pulses. Application of 3 µM forskolin to the serosal side induced a depolarization of the cell (ΔV_a = 29 ± 3 mV), a reduction of the fractional resistance (ΔfR_a = -0.47 ± 0.06) and concomitantly a serosa positive transepithelial potential change (ΔV_t = 2.3 ± 0.5 mV) and an increase of the short circuit current (ΔI_{sc} = 21.6 ± 3.5 µA/cm²). These changes were nearly sustained. In control experiments the rates of decline of V_t and V_a were -0.02 ± 0.01 mV/min and - 0.10 ± 0.14 mV/min respectively (n=4). Three minutes after forskolin excerted its maximal effect 0.5 µM NPY was added to the serosal bath solution. Immediately, V_a repolarized, fR_a increased and V_t returned to baseline level, which was reached after about 20 minutes. The rate of decline of V_t was -0.10 ± 0.02 mV/min and of V_a -2.08 ± 0.39 mV/min (n=3). We conclude that the human intestinal epithelial cells HT29cl.19A express functional NPY receptors which can directly inhibit cAMP induced Cl^- secretion. Furthermore we found that the intracellular potential change induced by carbachol was strongly reduced after preincubation with NPY.

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2) Mannon, P.J. et al, Am. J. Physiol. 267: G901-G907, 1994.

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FURTHER INCREASE OF INTRACELLULAR CA^{2+} INDUCED BY HYPOTONIC CELL SWELLING IN THE PRESENCE OF THAPSIGARGIN IN HT-29CL.19A HUMAN COLONOCYTES. R.B. Bajnath, A. Borgdorff, W.J. Wadman, H.R. De Jonge and J.A. Groot

Conventional micro-electrode studies with confluent filter grown monolayers of HT-29cl.19A cells in Ussing type chambers showed that hypo-osmotic medium (e.g osmolarity reduced by 20%) in the presence of thapsigargin (TG, the microsomal Ca^{2+}-ATPase inhibitor) [1 µM] induced a further hyperpolarization of the intracellular cell potential (V_a) by 16 ± 5 mV, suggesting a further increase of the basolateral K^+-conductance by cell swelling. This contrasts with the absence of electrophysiological effect of carbachol after TG, indicating a common route for TG and carbachol induced K^+-conductance. To study the involvement of intracellular Ca^{2+} the Ca^{2+} imaging technique was used. We found that hypo-osmotic stress increased $[Ca^{2+}]_i$ from 43 ± 4 nM to 87 ± 7 nM, which was not dependent on the presence of Ca^{2+} in the extracellular solution. Thapsigargin increased $[Ca^{2+}]_i$ by 156 ± 7 nM. Subsequent addition of carbachol did not further increase $[Ca^{2+}]_i$, but in the absence of TG, it increased $[Ca^{2+}]_i$ by 124 ± 7 nM. Application of hypo-osmotic medium after TG plus carbachol induced a further increase of $[Ca^{2+}]_i$ by 85 ± 2 nM, while in micro-electrode studies consecutive application of TG, carbachol and hypo-osmotic medium induced a further hyperpolarization of V_a. The results suggest the involvement of Ca^{2+} in the hypotonicity induced K^+-conductance and a TG-insensitive intracellular Ca^{2+} pool in the hypotonicity induced increase of intracellular Ca^{2+}.

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HYPOXIC INDUCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR GENE EXPRESSION - CLUES ABOUT THE OXYGEN SENSOR. J. Fandrey, S. Frede, and W. Jelkmann

Vascular endothelial growth factor (VEGF) specifically stimulates the proliferation of endothelial cells during angiogenesis. The production of VEGF is greatly enhanced in hypoxic tissue surrounding the central necrosis of solid tumors. Therefore, anti-angiogenesis has been considered as a possible therapeutic approach towards solid tumors. An understanding of the mechanisms of hypoxia-induced expression of the VEGF gene might be helpful in the modulation of angiogenesis. The human hepatoma cell line HepG2 has the ability to sense changes in the pericellular PO_2. Upon hypoxic exposure VEGF mRNA levels rose 7-fold which was quantitated by competitive polymerase chain reaction. Heme proteins of the type b cytochrome family have been proposed to participate in the oxygen sensing process in HepG2 cells (Fandrey et al., Biochem. J. 303: 507-510, 1994). Members of this heme protein family may well sense and influence the redox state of the cell, since among the type b cytochromes are enzymes such as NADPH-oxidase that catalyzes the production of hydrogen peroxide (H_2O_2). We found that HepG2 cells produce H_2O_2 and that its production rate was positively correlated with the pericellular PO_2. We next tested whether H_2O_2, produced under normoxia, might act as an inhibitior of VEGF gene expression. Exogenous H_2O_2 added under hypoxic conditions decreased VEGF mRNA levels. HepG2 cells were then treated with catalase to degrade H_2O_2 and mimic hypoxia at a high pericellular PO_2. Under this condition, catalase increased VEGF mRNA levels. Thus, we hypothesize that H_2O_2 which is abundant under normoxic conditions represses the expression of the VEGF gene. Under hypoxia, however, when H_2O_2 production decreases, full VEGF gene expression is permitted.

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ENDOTHELIUM-DEPENDENT RELAXATION IN HUMAN RESISTANCE ARTERIES: INFLUENCE OF PREGNANCY.

J. Van de Voorde, H. Depypere* and B. Vanheel

Pregnancy is associated with drastic hemodynamic adaptations such as a decrease in peripheral resistance. Vascular tone is importantly determined by endothelium-derived relaxing factors, NO being the most important. This study was designed to investigate whether endothelium-mediated relaxations in human resistance arteries are influenced by pregnancy. Subcutaneous arteries were isolated from abdominal fat tissue. Reactivity of the arteries was studied using a small vessel myograph. Addition of acetylcholine (1 nM - 10 µM) or bradykinin (1 nM - 10 µM) to preparations precontracted with norepinephrine (1 µM) and K^+ (30 mM) elicited concentration-dependent relaxations. These responses were blocked by removal of the endothelium or by addition of the NO-synthase inhibitor nitro-L-arginine (0.1 mM). The relaxations to acetylcholine and bradykinin were compared in vessels from non-pregnant women who underwent a laparotomy (n = 13) and from pregnant women who delivered by cesarean section (n = 8). The relaxation responses were similar in vessels from pregnant and non-pregnant women. Nitro-L-arginine (0.1 mM) had no influence on basal tone and inhibited endothelium-mediated relaxations to a similar extent in vessels from non-pregnant and pregnant women. From these results it is concluded that endothelial NO-synthase activity is not altered by pregnancy in human subcutaneous resistance arteries.

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QUINACRINE INHIBITS ENDOTHELIUM-DEPENDENT HYPERPOLARIZATION IN THE RAT MESENTERIC ARTERY B. Vanheel and J. Van de Voorde.

Several vasodilators, including acetylcholine (ACh), produce an endothelium-dependent hyperpolarization of the membrane of the vascular smooth muscle cells. In the present experiments, the hyperpolarization of smooth muscle cells from the rat main mesenteric artery in response to ACh was investigated. Membrane potential (E_m) was measured with conventional microelectrode techniques in artery strips. Addition of ACh produced an endothelium-dependent hyperpolarization consisting of an initial peak E_m change of 16.4 ± 1.1 mV followed by a partial recovery to an E_m-value significantly more negative than in the absence of the agonist. Ouabain (10^{-5} M) slightly depolarized E_m. In the presence of this inhibitor of the Na/K pump, the ACh-induced hyperpolarization was not significantly affected. Similarly, amiloride (10^{-3} M), an inhibitor of Na/H exhange which substantially contributes to the resting Na influx in vascular smooth muscle cells, had no significant effect on the endothelium-dependent hyperpolarization elicited by ACh. Glibenclamide (10^{-5} M), an inhibitor of ATP-regulated K^+ channels, slightly depolarized resting membrane potential by 1.1 ± 0.4 mV. The inhibitor had no significant effect on ACh-induced hyperpolarization. Iberiotoxin (10^{-7} M), a specific inhibitor of the large conductance Ca-dependent K^+ channel, had no significant effect on the resting E_m. After iberiotoxin pre-exposure, two phases in the peak E_m response to ACh were produced in 3 out of 4 preparations. Nitroglycerin (10^{-4} M) caused a slowly developing, small hyperpolarization (1.8 ± 0.3 mV). The E_m change induced by this NO donor was not influenced by ouabain (10^{-5} M) or amiloride (10^{-3} M) and therefore not mediated by Na/K pump activation. The hyperpolarization in response to ACh was completely and reversibly inhibited by the phospholipase A_2 inhibitor quinacrine (3.10^{-6} M). It is concluded that (1) K_{Ca} channels are likely to mediate at least part of the hyperpolarization in response to ACh and that (2) opening of K^+ channels is inhibited when liberation of arachidonic acid from phospholipids is blocked. The latter finding could mean either that arachidonic acid is a necessary and indispensible precursor in the synthesis or release of the hyperpolarizing factor by the endothelium or that this fatty acid is somehow necessary to allow smooth muscle cell K^+ channel activation by the hyperpolarizing factor.

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ROLE OF ENDOTHELIUM-DERIVED NITRIC OXIDE (EDNO) IN THE REGULATION OF VASCULAR TONE DURING HAEMORRHAGE.

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The problem whether EDNO may function as a true physiological vascular control system with capacity to vary the NO release and, hence, vascular tone in relation to actual circulatory demands was approached by testing its role during graded acute haemorrhage. The study was performed on a cat skeletal muscle preparation using a technique which permitted simultaneous quantitative recordings of vascular resistance (tone) in the whole vascular bed (R_T) and in its consecutive sections, the large-bore arterial resistance ("feeder") vessels (>25 µm; $R_{a,prox}$), the small arterioles (<25 µm; $R_{a,micro}$) and the veins (R_V). Vascular tone was analysed in the absence and presence of local EDNO blockade with L-NAME (10 mg·kg^{-1}, i.a.) under resting conditions and during graded haemorrhage. EDNO blockade at rest (n=9) increased R_T in the sympathectomized vascular bed from 15.4 to 31.9 PRU (+107%; P<0.001) due to selective constriction in the $R_{a,prox}$-section where resistance increased from 8.7 to 24.7 PRU (+183%; P<0.001). $R_{a,micro}$ and R_V were unchanged. A 35% blood loss *per se* constricted all three vascular sections and raised R_T in the steady state by 60% (P<0.001) in the sympathectomized preparation due to humoral catecholamine release. Superimposed EDNO blockade (n=9) increased R_T from 22.4 to 62.2 PRU (+178%; P<0.001) due to selective increase of tone in the $R_{a,prox}$-section from 14.9 to 56.3 PRU (+277%; P< 0.001), whereas $R_{a,micro}$ fell by 26% (myogenic/metabolic compensatory reaction). EDNO blockade thus caused a 2.6-fold larger constrictor response in the $R_{a,prox}$ section during haemorrhage than in the control state (+41.4 vs +16.0 PRU; P<0.001), which indicates a corresponding haemorrhage-induced increase in the EDNO dilator influence (EDNO production) above that at rest. The L-NAME constrictor response was graded in relation to the magnitude of the blood loss (17.5 vs 35 %). The increased EDNO release during haemorrhage seemed independent of the regional sympathetic nerves and more likely was triggered by a humoral factor. Our results indicate that EDNO functions as a specific, and apparently protective, dilator control system for the arterial "feeder" vessels already under resting conditions and much more so during haemorrhage, thereby counterbalancing to a significant extent the simultaneously operating constrictor mechanisms which otherwise would seriously jeopardize nutritive blood flow and flow distribution in the tissue.

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ENDOTHELIUM-DEPENDENT RESPONSES IN HYPERTENSION INDUCED BY LONG-TERM INHIBITION OF NITRIC OXIDE SYNTHESIS. J. Török, A. Holécyová, I. Bernátová, O. Pecháňová.

The aim of this study was to evaluate the influence of 4 week duration of hypertensive state in rats, induced by oral administration of N^G-nitro-L-arginine methyl ester (L-NAME), on the reactivity of arteries representing high- (thoracic aorta, renal artery) and low-pressure circulatory system (pulmonary artery). After 4 weeks of L-NAME treatment (40 mg/kg/d) the increase in systolic blood pressure was 164 ± 3 mm Hg, while total ratio of heart weight to body weight was not significantly changed. Acetylcholine (10^{-9}-10^{-5}M) produced a concentration related relaxation in precontracted arterial rings; the magnitude of relaxation was significantly reduced in rings from hypertensive rats. Indomethacin, a cyclolxygenase inhibitor, did not affect the relaxant responses to acetylcholine in both control and L-NAME-treated rats. Also the endothelium-independent relaxations to sodium nitroprusside (10^{-9}-10^{-6}M) were unaffected. Residual relaxations in arterial rings from hypertensive rats were abolished by additional administration of L-NAME (10^{-5}-10^{-4}M) to incubation medium. On the other hand, pretreatment of rings with L-arginine (10^{-3}M) improved the reduced relaxations. Compared to control rings, contractile sensitivity to noradrenaline was increased in all rings from hypertensive rats. These results support the idea that changes in reactivity of arteries from hypertensive rats are due to reduced production and availability of nitric oxide in both high-and low-pressure circulatory system.

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ENDOTHELIAL FUNCTION - THE FUNCTION OF OXIDATIVE STATUS IN MIDDLE AGED MEN? I.Bukovský, R.Pullmann, M.Lang, M.Országh, J.Hybenová, E.Kosková

Endothelial dysfunction has been experimentally and clinically confirmed as an early event in atherogenesis. In vitro, animal and epidemiological studies suggest that oxidation of lipoproteins plays a very important role in the atherogenesis. Sufficient supply of antioxidants is understood to be one of the most relevant factors related to prevention of the atherogenesis triggering step - lipid oxidation. However, is it possible that parameters of oxidative status can indicate the level of endothelial function? Can the oxidative status be linked with initiating the first changes in blood vessels of middle aged men - the most susceptible segment of industrialized populations? To answer these questions we used high-resolution ultrasound (HRUS) measurements (Sonoline 450, Siemens, Japan) of the diameter of the radial artery to calculate functional (FVDR) and relative vasodilatation reserve (RVDR) and endothelial function index (EFI). Blood samples for lipid profile, beta-carotene (BC), superoxid dismutase (SOD) and malondialdehyde (MDA) analysis were taken within 24 hours of HRUS measurements. Sixty men, 25 - 45 years old, from an area of basically the same level of pollution were examined. We have found significant negative correlations between MDA and RVDR (p=0.001), MDA and FVDR (p=0,001). This relation exists despite no correlation between BC, SOD, MDA levels and smoking. Further analysis shows negative correlation between MDA level and RVDR (p=0.05) as well as FVDR (p=0,032) among smokers. BC alone was not capable of producing protective effect on endothelial function (p>0,05) among smokers.

Despite the lack of correlation between endothelial function and protective activity of BC, analysis suggests strong influence of oxidation on the endothelial function.

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THE ROLE OF PROTEIN KINASE C FOR BARRIER FAILURE OF MICROVASCULAR CORONARY ENDOTHELIAL MONOLAYERS UNDER ENERGY DEPLETION. T. Noll, A. Muhs, H.M. Piper

In a previous study we found that the rise in cytosolic free Ca^{2+}-concentration (Ca_i) triggers barrier failure in microvascular coronary endothelial monolayers under energy depleting conditions. In the same experimental model, we here studied whether Ca^{2+}-dependent activation of protein kinase C (PKC) causes phosphorylation of vinculin, a key protein in anchorage of actin cytoskeleton to plasmamembrane, and whether this effect can be related to increase in macromolecule permeability for albumin during ATP-depletion. It was found: (1) 5 min after addition of 5 mM KCN/5 mM 2-deoxyglucose PKC-activity was augmented (3.7±0.5-fold, mean±S.D., n=5, p<0.05). This was accompanied by an increase of Ca_i (3.1±0.4-fold, mean±S.D., n=5, p<0.05) and albumin permeability (1.8±0.3-fold, mean±S.D., n=5, p<0.05). (2) During the same time of observation, immunofluorescence intensity of vinculin at zonulae adhaerentes and focal adhesion plaques decreased and the ratio of phosphorylated-to-nonphosphorylated vinculin increased (2D-PAGE-Electrophoresis). (3) Increase in albumin permeability, PKC-activation and disappearance of immunofluorescence signal of vinculin could be abolished when intracellular calcium stores had been discharged by a 20-min pre-incubation in presence of 300 nM thapsigargin and extracellular Ca^{2+} was removed.

Conclusion: In energy depleted coronary endothelial monolayers, increase in albumin flux coincides with PKC activation, vinculin phosphorylation and loss of vinculin at cell-cell adhesion sites. These data indicate that under energy depleting conditions phosphorylation of vinculin contributes to endothelial barrier failure.

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RAPID VENTRICULAR PACING IN RABBITS ALTERS THE F-ACTIN CYTOSKELETON IN ENDOCARDIAL ENDOTHELIAL AND SUBENDOTHELIAL CELLS

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Chronic pacing induces heart failure with major alterations in ventricular architecture. The role of cardiac endothelium in tachycardia-induced heart failure is at present unknown. We investigated morphological changes of the *endocardial endothelium* (EE) and subjacent interstitial tissue in rabbits subjected to left ventricular pacing with increasing rate (250-440 bpm). Hemodynamic measurements and gross morphology of the heart confirmed the development of heart failure. Left ventricles of control (n=5) and dilated rabbit hearts (n=6) were processed for light microscopy and *en face confocal scanning laser microscopy*. In control hearts, F-actin staining in EE was usually restricted to the peripheral actin bands. Few cells were present in the subjacent fibro-elastic layer. In the outflow tract and below the mitral valve of dilated hearts, the morphology of EE cells and interstitial tissue cells appeared normal. However, in the apex of dilated hearts, regions of EE cells contained numerous actin filament bundles; peripheral actin bands could not be discerned or were broad and formed by separate actin filament bundles. Moreover, intense staining of nucleoli and cytoplasmic RNA by propidium iodide indicates an increased synthetic activity in EE cells with an altered cytoskeleton. Subjacent to these EE cells, more actin-rich interstitial cells were observed than in controls. The morphological changes in EE and the proliferation of subendothelial cells probably result from the disturbed mechanical stress in the heart wall. The altered cytoskeleton and the increased synthetic activity of EE are probably associated with modified physiological properties, such as changes in permeability and release of EE factors.

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RESTING MEMBRANE CONDUCTANCE OF CULTURED ENDOCARDIAL ENDOTHELIAL CELLS IS DETERMINED BY K^+ AND Cl^- IONS. P.Fransen, M.Demolder, D.Van Bedaf and S.Sys

Release of mediators from endocardial endothelial (EE) cells depends on Ca^{2+}-signalling and, therefore, on the resting membrane potential (V_m) of these cells. Although V_m in EE cells has been reported to be more depolarized than the equilibrium potential for K^+ ions (V_K), the identity of other ions contributing to V_m is still unknown. Single cultured EE cells from the porcine right ventricle were whole-cell voltage clamped and current-voltage (IV) relations were determined in different ionic conditions. In baseline conditions the bathing solution was a normal Tyrode solution, while the patch pipette contained (in mmol/l): 30 KCl, 100 KAsp, 1 $CaCl_2$, 5 $MgCl_2$, 10 HEPES, 10 EGTA, pH 7.2, 37 °C. In these conditions, the main membrane current was the inwardly rectifying K^+-current (I_{Ki}), IV relations were extremely flat at voltages between -60 and +60 mV (current amplitudes less than 2 pA/pF) and the zero-current potential (V_0 = -66.5 ± 8.5 mV, SD, n = 35) was dependent on the extracellular K^+ concentration ($[K^+]_o$). Changing the extracellular Na^+ or Ca^{2+} concentrations had only minor effects on the IV relations, ruling out the involvement of non-selective cation channels to V_m. With 122 mmol/l intracellular Cl^-, V_0 became independent of $[K^+]_o$, but was close to the equilibrium potential of Cl^- ions (V_{Cl}). Intracellular perfusion of the cells with low and subsequently high Cl^-, switched V_0 from values close to V_K (5 mmol/l Cl^-) to values close to V_{Cl} (122 mmol/l Cl^-). Similar transitions of V_0 from V_K to V_{Cl} were observed with changes in extracellular Cl^- from high to low. Results of the present study indicated that the membrane of non-stimulated cultured EE cells from porcine right ventricle was permeable to K^+- and Cl^--ions. We, therefore, suggest that in EE cells both ions affect Ca^{2+}-signalling and the concomitant release of mediators.

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PHOSPHORYLATION OF Na^+-K^+-ATPase IN ENDOTHELIAL CELLS; A $^{133}Cs^+$ NMR STUDY. M.L.H. Gruwel, O. Čulić and J. Schrader.

Recently $^{133}Cs^+$ has been used to study K^+ fluxes in biological tissue and shown to be 100% visible. $^{133}Cs^+$ NMR allows one to study the transmembrane K^+ flux without the use of a shift reagent due to the $^{133}Cs^+$ chemical shift. Putative cAMP/cGMP dependent phosphorylation sites have been identified in the α-subunit of the pump. Therefore we used $^{133}Cs^+$ NMR to study the effects of pump phosphorylation on the Na^+-K^+-ATPase activity in vascular endothelial cells.

Porcine vascular endothelial cells were grown to confluency on microcarrier beads and perfused at 37 ± 0.3°C in a 10 mm NMR tube. For the $^{133}Cs^+$ NMR experiments KCl of the perfusion medium was replaced with CsCl. After switching to Cs^+ medium, 4k data points were accumulated every 10 min. in 64 scans. Up to 24 data poins were collected and fitted to a single exponential in order to obtain the Cs^+ influx at the start of the experiment.

Endothelial cells could repetitively be loaded/unloaded with Cs^+. After complete Cs^+ loading, intra- and extracellular Cs^+ were shifted by 1.36 ± 0.13 ppm. Control experiments showed that the Cs^+ flux ($12 \pm 3 nmol \cdot min^{-1} \cdot (mg\ P)^{-1}$) corresponds to the Na^+ flux as reflected by the the pump stochiometry of Na^+:K^+=3:2. Inhibition of phosphodiesterases by 0.5 mM IBMX reduced the rate of Cs^+ influx by 20%. When perfused with 0.5 mM IBMX + 0.2 mM Br-cAMP, a 60 % reduction of the Cs^+ influx rate resulted. Using 0.5 mM IBMX + 0.2 mM Br-cGMP caused a reduction in Cs^+ influx rate of 35%. These data provide the first in vivo evidence that cAMP/cGMP exerts an apparent effect on endothelial Na^+-K^+-ATPase activity, most likely through phosphorylation of the enzyme.

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Saturated, but not mono-unsaturated, fatty acids induce cell death in neonatal rat ventricular cardiomyocytes. *JE de Vries, THM Roemen, GJ van der Vusse, and M van Bilsen.*

In the present study the effect of saturated and mono-unsaturated long-chain fatty acids on cellular viability of neonatal rat ventricular cardiomyocytes was investigated. To this end, myocytes were cultured in the presence of physiological levels (750 μM) of either palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1), or oleic acid (C18:1), complexed to BSA (150 μM), for up to 48 hours.

Phase-contrast microscopy indicated that cells cultured with C16:1 or C18:1 acid are viable, and form vesicles containing triacylglycerols, as demonstrated by Oil Red O staining. In contrast, the presence of C16:0 or C18:0 resulted in cell death as evidenced by a concomitant release of LDH (140 kDa) and Fatty Acid-Binding Protein (15 kDa) into the medium, starting 12-16 hours following application. Toxic effects were not observed when myocytes were cultured with equimolar amounts of C16:0 and C18:1 (375 μM each). The cells remained viable and developed lipid droplets to the same extent as in myocytes cultured with mono-unsaturated fatty acids only. Apparently, storage of triacylglycerols in lipid droplets, derived from either mono-unsaturated fatty acids only or a mixture of mono-unsaturated and saturated fatty acids, is not harmful to cells.

Possibly, the toxic effects of saturated fatty acids are due to intracellular accumulation of solid depositions of triacylglycerols formed from saturated fatty acids, which may damage the cell. Alternatively, cell death may also be caused by unfavorable changes in cellular membranes when high amounts of saturated fatty acids are incorporated in membrane phospholipids. At present, the mechanism involved in saturated fatty acid-induced cell death remains to be elucidated.

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PALMITATE UPTAKE AND METABOLISM BY CARDIOMYOCYTES FROM ADULT RATS. J.J.F.P. Luiken, G.J. van der Vusse and J.F.C. Glatz.

In cardiomyocytes, oxidation of long chain fatty acids (LCFA) is the major source of ATP production. Since LCFA are poorly soluble in an aqueous environment, cellular proteins, such as cytosolic fatty acid-binding proteins (FABP) are likely to be involved in uptake of LCFA and their subsequent delivery to mitochondria. In order to investigate a possible role for FABP in cardiomyocytes, we intend to compare palmitate metabolism in cardiomyocytes with different contents of FABP. For this purpose, cardiomyocytes from diabetic rats and from rats at different developmental stages will be used, as well as cardiomyocyte cell lines in which the FABP content is manipulated with molecular biological techniques. As a pilot study, we investigated the uptake and metabolism of [1-^{14}C]palmitate in cardiomyocytes from adult rats. Uptake rates were determined after dilution of cell samples in an ice-cold phloretin-containing stop solution. Palmitate oxidation rates were measured as the sum of the production of $^{14}CO_2$ and of acid soluble [^{14}C]metabolites. Palmitate incorporation into cellular lipid pools was analyzed by thin layer chromatography. At a cardiomyocyte density of 2-8 mg wet weight/ml and an albumin concentration of 300 μM, initial palmitate uptake was linear with the amount of cardiomyocytes, linear with time for at least 30 min, and linear with the concentration of palmitate up to 300 μM (palmitate/albumin ratio 1:1). At higher palmitate concentrations, uptake was saturable. We chose a palmitate concentration within the linear range of palmitate uptake as a further object of study. At 90 μM palmitate, the uptake of [1-^{14}C]palmitate was 10 nmol/min per g ww, which is comparable to reported LCFA-uptake rates measured in isolated perfused hearts. The oxidation rate was 5 nmol/min per g ww, with $^{14}CO_2$ production contributing to 55%. Incorporation into cellular lipids was 4 nmol/min per g ww, of which 90% was esterified into triacylglycerols and the remainder into phospholipids. Palmitate uptake and metabolism was not influenced by addition of glucose (5 mM) in the absence or presence of 10 nM insulin. A palmitate/albumin ratio of 0.3:1 with an albumin concentration of 300 μM will be taken as standardized condition to compare palmitate uptake and metabolism in the different cardiomyocyte preparations.

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FATTY ACID-BINDING PROTEIN CONTENT OF HEART AND SKELETAL MUSCLES OF BARNACLE GEESE DURING DEVELOPMENT. M.M.A.L. Pelsers[1], C.M. Bishop[2], P.J. Butler[2], K.W.H. Wodzig[1], G.J. van der Vusse[1] and J.F.C. Glatz[1].

Fatty acid binding protein (FABP) is a small cytoplasmic protein of 15 kD, abundantly present in heart and muscle cells. FABP is the functional equivalent of plasma albumin and serves as an intracellular carrier for fatty acids. As fatty acids are the primary substrate used to provide energy for long-distance flights by birds during migration, a study was initiated to investigate the biochemical development of the cardiac and locomotory muscles of the migratory barnacle goose, *Branta leucopsis*. In addition, the study was designed to investigate the importance of sustained flight experience on locomotor muscle physiology. Tissue samples were collected from birds in the wild (Spitsbergen, Svalbard) and from birds kept in outdoor aviaries (Birmingham, UK). FABP content was measured in cardiac, pectoralis and semimembranosus muscle samples from barnacle geese at 5 and 7 weeks of age and from captive and wild adults. FABP was detected with a Sandwich ELISA using monoclonal antibodies directed against human heart FABP. Western blot analysis showed that these antibodies indentified a single goose muscle protein of 15 kD and results are expressed as the relative human equivalents (μg/g wet weight). During development, the most dramatic increase in muscle mass-specific FABP content was seen in the pectoralis samples. Values increased from 3.8±0.8 μg/g (5 week old goslings) to 68.2±1.6 μg/g (wild adults). Captive adults had significantly lower values of FABP (52.2±5.8 μg/g) than that of wild adults. Heart (52.8±3.8 μg/g) and leg (5.8±0.6 μg/g) muscle FABP showed no significant changes during this developmental period. These data indicate that the leg muscle of the barnacle goose has a low mass-specific capacity to transport fatty acids compared to that of the heart and flight muscles. In addition, FABP content in leg and heart muscles did not change during development, whereas the FABP level expressed in the pectoralis muscle at 5 weeks old goslings was only 6% of that in wild adults. Interestingly, captive adults which had been flight restricted for at least 2 years (and not able to fly for more than a few meters) have a slightly reduced FABP content. These results suggests that development is a more important factor in FABP expression in pectoralis muscle than sustained flight experience.

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ROLE OF PUTATIVE MEMBRANE FATTY ACID TRANSLOCASE (CD36) IN FATTY ACID UPTAKE IN RAT MUSCLES. F.A. Van Nieuwenhoven, C.P.H.J. Verstijnen, N.A. Abumrad, G.J.J.M. Van Eys, G.J. Van Der Vusse and J.F.C. Glatz.

Fatty acids are important substrates for energy-production in heart and oxidative skeletal muscles. Several proteins are proposed to have a role in the uptake of long chain fatty acids (FA) in muscle cells for subsequent oxidation or esterification. Among them is a 88-kDa membrane glycoprotein (FAT) which shows high homology to CD36, and has recently been implicated in the transmembrane transport of FA in adipose cells. Expression of FAT was studied in rat heart during development, in oxidative and glycolytic skeletal muscles and in various cell-types isolated from adult rat heart. FAT-expression was compared to that of heart-type fatty acid-binding protein (H-FABP), which is assumed to participate in the transcytoplasmic transport of FA. We reasoned that if FAT and FABP are involved in uptake and transport of FA, expression of both proteins should correlate with the capacity of the tissues to utilize FA. Furthermore, co-expression of both proteins might indicate related biological functions, e.g. in FA-metabolism. Northern blot analysis showed high expression levels of both FAT and FABP in heart tissue. Expression levels of these proteins in oxidative skeletal muscle tissue were lower and glycolytic skeletal muscle showed the lowest expression levels. In isolated cardiac cells mRNAs of both proteins were detected in cardiomyocytes, but not in endothelial or fibroblast-like cells. Messenger RNA levels of FAT and FABP increased about 5-fold during heart development from the day before birth to day 70. In conclusion, muscle tissues and cell-types with high FA-metabolism show highest expression levels of FAT and FABP. During heart development both proteins show a comparable upregulation of mRNA levels. These results strongly support the hypothesis that FAT and FABP are involved in muscle FA-metabolism.

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EFFECTS OF CGS 9343B ON ISOLATED SKELETAL MUSCLE: DISSOCIATION OF SIGNALING PATHWAYS FOR INSULIN-MEDIATED ACTIVATION OF GLYCOGEN SYNTHASE AND HEXOSE TRANSPORT. P. Shashkin, A. Koshkin, D. Langley, J.-M. Ren, H. Westerblad and A. Katz

The role of calmodulin in insulin-mediated alterations of carbohydrate metabolism in isolated mouse *soleus* muscle was investigated by using the calmodulin antagonist CGS 9343B (CGS). Muscles were incubated in oxygenated Krebs-bicarbonate buffer, frozen and processed for analyses of glycogen synthase activity (GS), hexose transport, glycogen synthesis and metabolites. Insulin (20 mU/ml) activated GS by ≈40% ($P<0.01$) and this increase was not altered in the presence of CGS. Insulin increased the muscle content of glucose 6-P by ≈120% and the incorporation of [^{14}C]glucose into glycogen by ≈800%. However, CGS completely abolished these effects by insulin. Similarly, the insulin-mediated activation of 2-deoxyglucose and 3-O-methylglucose transport into the muscle (by 180% and 100%, respectively) were blocked by CGS. CGS did not affect energy status as judged by the absence of any effect on ATP or phosphocreatine content of the muscle at rest. To check potential sites of CGS action, the muscle was stimulated electrically. CGS had no effect on peak tension of the muscle during electrical stimulation, indicating that the drug did not affect action potentials or sarcoplasmic reticulum (SR) Ca^{2+}-release. On the other hand, relaxation was prolonged in the presence of CGS, which is consistent with the idea that phospholamban mediated activation of SR Ca^{2+} uptake is calmodulin dependent. These data indicate that calmodulin is involved in the insulin mediated activation of glucose transport and glycogen synthesis, but not in the insulin mediated activation of GS.

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EFFECTS OF GLUCOSE AND MANNITOL ON SKELETAL MICROCIRCULATION IN ANESTHETIZED RATS

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AIM : To test the effects of either glucose, or mannitol as osmotic control, or saline, on the diameter of arterioles and venules in the spinotrapezius skeletal muscle of the rat *in vivo*. **METHOD :** Five animals per group were anesthetized with pentobarbital (60 mg/kg, i.p.). After catheterization of the carotid artery and jugular vein, and tracheal intubation, the spinotrapezius muscle was exteriorized and superfused. Microcirculatory fields were visualized by intravital microscopy, and the diameter of arterioles and venules was assessed before and after a 1.3 ml intravenous injection of either glucose or mannitol (1.5 g/kg b.w.) or saline. Three to 6 fields per rat were recorded every 15 minutes and during one hour after the injection, using a video set-up. Arterial blood sampling was performed before, 10 min. after the injection, and at the end of the experiment for glucose and insulin determination. Off-line analysis of the vessel diameter was performed using an image processing system. Mean diameter and amplitude of vasomotion (if any) were computed. **RESULTS** : 1) Glucose, but neither mannitol nor saline, increases the glycaemia (still) significantly 10 minutes after the injection (from 6.8±0.5 mM to 14.1±0.3 mM at 10 minutes) and returns to normal at the end of the experiment. In the same time, insulinemia increases from 52±19 IU to 215± 12 IU at 10 minutes. 2) After short-lasting initial vasodilatation, injection of glucose induces a vasoconstriction (-10 % to -30 %) of the arterioles smaller than 40 µm, and induces a progressive and significant vasomotion.These effects are dealyed and maximal one hour after the injection 3) Injection of mannitol has no effect on the mean diameter of arterioles but induces also vasomotion. 4). No effect is detected with saline or on the venous side. **CONCLUSIONS** : The mixture of high glucose and high insulin induced by the i.v. injection of glucose leads to a delayed activation of the motor activity of small arterioles in the skeletal muscle. This phenomenon is likely related to redistribution of microflow linked with metabolic activity and appears to be partly due to osmotic pressure.

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MUSCLE OXIDATIVE PARAMETERS AND MODERATE PHYSICAL ACTIVITY. M.L. Zonderland, H.A. Keizer, Y.F. de Jong, B.M. Spruijt, J.F.C. Glatz.

Endurance training is known to increase the muscular utilization of fatty acids by increasing the activity of the enzymes of the oxidative metabolism. Cytoplasmic Fatty Acid-Binding Protein (FABP) has been suggested to play a central role in fatty acid metabolism. The objective of our study was the association of moderate physical activity with FABP content and oxidative capacity of heart and skeletal muscles in the rat. Subjects were male Wistar rats, randomly assigned to either standard housing (SH) or enriched environment (EE). SH: standard plexiglass laboratory cage filled with sawdust bedding; EE: large plexiglass cage with sawdust bedding and several objects (tools, climbing racks etc); the arrangement of all objects and position of the food was changed weekly. The quantity and quality of physical activity is enhanced in such environment. Food (standard rat chow) and water were provided ad libitum. After 6 weeks the content of FABP (sandwich-ELISA) and the activities of citrate synthase (CS) and 3-hydroxy-acyl-CoA-dehydrogenase (HAD) were determined in heart, m. soleus and m. extensor digitorum longus (EDL) (see table). All parameters were higher in the skeletal muscles in the EE group (*:p≤0.01, MANOVA). In heart there were no differences.

SH (n=8)	*heart*	*m. soleus*	*m. edl*
FABP (µg/g ww)	912 ± 82.6	214 ± 59.1	80.3 ± 29.8
CS (U/g ww)	87.6 ± 9.6	19.9 ± 3.3	17.3 ± 4.5
HAD (U/g ww)	76.0 ± 7.1	11.4 ± 3.0	8.5 ± 2.7
EE (n=10)		*	*
FABP (µg/g ww)	930 ± 242.6	293 ± 70.3	104 ± 33.1
CS (U/g ww)	85.7 ± 21.5	25.8 ± 6.7	23.1 ± 6.8
HAD (U/g ww)	63.8 ± 28.1	14.5 ± 2.3	8.1 ± 4.0

It is concluded that already moderate physical activity is associated with a significant increase in FABP content and oxidative capacity of rat skeletal muscles (both slow-twitch and fast twitch).

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INTERSTITIAL MUSCLE GLUCOSE AND LACTATE LEVELS DURING DYNAMIC EXERCISE IN HUMANS DETERMINED BY MICRODIALYSIS. D.A. MacLean, B. Saltin and J. Bangsbo.

The purpose of this study was to directly quantitate the interstitial levels of glucose and lactate during dynamic exercise and compare these values to those found in the venous blood draining the muscle. Six male subjects had 3 to 5 microdialysis probes inserted into their vastus lateralis muscle as well as catheters placed into their femoral artery and vein. The subjects rested for 60 min and then performed one legged knee extensor exercise at work intensities ranging from 0 to 50 watts for approximately 8 min. Probe dialysate and arteriovenous blood samples were collected at rest and during each exercise bout. The microdialysis probes were perfused at a rate of 5 ul/min with a Ringer-acetate solution containing 3 mM D-[6- H] glucose and 0.5 mM L-[U-^{14}C] lactate. The blood and dialysate samples were used for glucose and lactate determinations and the relative loss of the isotope (dialysate-perfusate/perfusate) during each exercise bout was used to determine probe recovery and subsequently interstitial glucose and lactate levels. At rest probe recovery was 25.2±4.4 and 28.5±4.2% for glucose and lactate, respectively. At the onset of exercise probe recovery increased markedly ($P<0.05$) and remained elevated throughout the exercise bouts. During submaximal exercise, probe recovery for glucose and lactate increased to 41.4±3.5 and 46±3.4% ($P<0.05$), respectively. At rest the interstitial glucose level (3.19±0.32 mM) was lower ($P<0.05$) than the venous plasma water glucose level (5.31±0.20 mM), however during exercise the interstitial glucose levels increased and were similar to those found in venous plasma water (5.12±0.10 and 5.14±0.07 mM, respectively). In contrast, the resting interstitial lactate level (1.50±0.19 mM) was higher ($P<0.05$) than the venous plasma water lactate level (0.73±0.08 mM) and during exercise interstitial lactate levels remained higher ($P<0.05$) than venous plasma water lactate (3.07±0.36 and 1.66±0.48 mM, respectively). This study is the first to determine probe recovery and quantify interstitial glucose and lactate levels during dynamic exercise in humans and offers a unique opportunity to study transport kinetics of these and other substances.

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IMMUNOLOCALIZATION OF GLUT4 IN RAT GASTROCNEMIUS MUSCLE BY LIGHT MICROSCOPY M.K.C. Hesselink, E. van Breda[1], P. Geurten, H. Kuipers, H.A. Keizer

Transport of glucose across the plasma membrane of skeletal muscle cells is enhanced by insulin as well as by contractile activity. At the cellular level this concomitant increase in glucose uptake is caused by the translocation of intracellular GLUT4 containing vesicles to the plasma membrane. In order to unravel the mechanisms by which GLUT4 is recruited to the plasma membrane it is mandatory to localize the intracellular GLUT4 vesicles. Most studies dealing with GLUT4 distribution are performed by using laborious electron microscopic techniques. Therefore, the goal of the present study was to localize the subcellular distribution of GLUT4 by light microscopy. Transverse cryosections (5 µm) were taken from the mid belly region of gastrocnemius muscle from 12 week old male Wistar rats. The sections were immuno-histochemically labeled using a commercially available monoclonal antibody (purity ≥ 95%) raised in mice against partially pure rat GLUT4. Fresh cryosections were successively rinsed in (1) PBS (10 mM, pH 7.4); (2) 5% normal goat serum in PBS; (3) PBS alone and (4) incubated with 10 µg/ml monoclonal antibody against GLUT4 in PBS with 1% BSA (this step was omitted in the control sections); (5) rinsed in PBS once more and finally (6) incubated with goat anti mouse IgG gold conjugate with an ultra small (ø < 1.0 nm) gold particle in PBS with 1% BSA. To visualize the ultra small gold particles the signal was amplified by silver enhancement. To prevent heat transfer while monitoring the on-going process of enhancement a light microscope with dimmed light conditions was used. Enhancement was judged to be complete when a black stain was visible at a 400 times magnification. Prior to and immediately after this enhancement procedure the slides were washed in distilled water and counterstained where necessary. Finally the specimens were dehydrated in alcohol and mounted in entallan. All previous steps were performed at room temperature in a moisture chamber. Examination of the slides revealed a random distribution of silver stain throughout the intracellular space indicating that in rat gastrocnemius muscle GLUT4 is randomly distributed. This is in agreement with previous findings of other groups using different techniques. Since control sections that were not incubated with the antibody against GLUT4 only showed a very weak background signal, it can be concluded that GLUT4 can be localized at light microscopical level using the technique described in this study.

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REGULATORY EFFECTS OF LINOLEIC ACID DERIVATIVES ON ION TRANSPORT IN CARDIAC SARCOLEMMA AND NEUTROPHIL ACTIVITY. A.Moibenko, G.Cherednichenko, V.Brovkovich, S.Ohiy

It is known that the level of linoleic acid and its derivatives is increased during certain conditions, for example in myocardial ischemia. However, functional significance of these changes was not fully understood. We studied the action of linoleic acid derivatives - 13(S)-hydroperoxide (HPODE) and 13(S)-hydroxide (HODE) on ion transport in highly purified sarcolemmal vesicles from guinea pig hearts and on zymozan-induced chemiluminescence of rat neutrophils. The synthesis of HPODE was performed using immobilized soybean lipoxygenase. HODE was obtained by nonenzymatic reduction of HPODE. We have found that HPODE (0.1-10 µM) increased sarcolemmal Na-Ca exchange activity and exerted digitalis-like action on Na,K-ATPase activity (0.1-100 µM) with IC_{50} of 18 µM. HODE exerted qualitatively similar effects but to a significantly lesser extent. Both compounds increased sarcolemmal ^{45}Ca permeability only at concentrations above 10 µM. HPODE and HODE in a concentration-dependent manner inhibited zymozan-induced chemiluminescence of rat neutrophils. This inhibitory effect was especially obvious in neutrophils obtained from animals after myocardial ischemia-reperfusion. We observed the 80% inhibition of zymozan-induced chemiluminescence in "reperfusion" neutrophils by 50 µM HODE. We suggest that digitalis-like action and inhibition of neutrophil activity may reflect a potential regulatory and even protective action of linoleic acid derivatives in myocardial ischemia and reperfusion.

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MECHANISMS UNDERLYING RENAL AUTOREGULATION IN CONSCIOUS DOGS: ANALYSIS IN THE TIME AND FREQUENCY DOMAIN A. Just, U. Wittmann, H. Ehmke & H. R. Kirchheim

The myogenic response, the tubuloglomerular feedback (TGF), and the renin-angiotensin system (RAS) may participate in renal blood flow autoregulation (AR). In order to determine the involvement of these mechanisms in AR we studied the dynamics of AR in the time and frequency domain in conscious dogs. Time domain: The adaptive changes of renal vascular resistance (RVR) were observed in response to a sudden release of a complete occlusion of the renal artery (60 s). In normal dogs (n=2) RVR was restored to preocclusive levels in *three steps*: *1)* Within the first 5 -10 s RVR rapidly rose from 50 to 70% of control. *2)* After a latency of 50 s RVR further rose (with a transitory overshoot) to 90% of control. *3)* During the subsequent 3-5 min RVR gradually reached the preocclusive level. Interruption of the TGF by furosemide (n=2) abolished the 2nd and enhanced the 1st RVR-response. Converting enzyme inhibition by Ramipril (n=2) slowed the 3rd response. Frequency domain: The transfer function was calculated from spontaneous fluctuations in blood pressure and renal blood flow. The gain of the transfer function indicates autoregulatory efficiency (AE). In normal dogs (n=9), a low AE (high gain) was observed above 0.2 Hz and optimal AE between 0.01 and 0.0006 Hz. The region of increasing AE (0.2 - 0.01 Hz) was characteristically interrupted by a peak of higher gain (lower AE) at 0.025 Hz. An infusion of furosemide (n=5) abolished the peak at 0.025 Hz, but did not change AE below 0.01 Hz. Ramipril (n=6) slightly diminished AE below 0.01 Hz but did not change the peak.

It is concluded, that AR can respond as fast as 0.2 Hz, its AE is optimal below 0.01 Hz and is maintained down to at least 0.0006 Hz. A fast mechanism, which is activated within a few seconds, seems to dominate AR; its fast reaction suggests the myogenic response as the underlying mechanism. A second mechanism, which is activated with a latency of 50 s after a pressure step, has a resonance at 0.025 Hz, and probably reflects the TGF. An intact TGF is not necessary for AR.The RAS may modulate AR below 0.01 Hz.

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DIFFERENTIAL LOCALIZATION OF ENDOTHELIN ET_A AND ET_B RECEPTOR-MEDIATED CONSTRICTION IN RENAL VESSELS

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It has been shown, that endothelin (ET) ET_A as well as ET_B receptors participate in the renal vasoconstriction induced by ET. However, it is not known from functional studies whether ET receptor subtypes are differentially distributed within the renal vasculature. Therefore, we pharmacologically characterized ET receptor subtypes in the renal vasculature of the split hydronephrotic kidney model in anesthetized female Wistar rats. Lumen diameters of arcuate and interlobular arteries and of afferent and efferent arterioles of cortical (C) and juxtamedullary (JM) glomeruli were measured by intravital transillumination microscopy. Glomerular blood flow (GBF) was determined by the dual-slit technique. All drugs were applied into the tissue bath to avoid systemic effects. ET-1 (10^{-9} M, $n = 6$), which has equal affinity to ET_A and ET_B receptors, reduced GBF by $55 \pm 7\%$ (C) and $54 \pm 7\%$ (JM) and constricted preglomerular vessels (6 to 18%) and efferent arterioles (11 to 13%) to a similar degree. The ET_A receptor antagonist BQ-123 (10^{-6} M) and the ET_B receptor antagonists BQ-788 ($2 \cdot 10^{-7}$ M) and IRL 1038 (10^{-6} M) shifted the concentration response curve of GBF for ET-1 to the right (each $n = 6$). BQ-123 significantly antagonized constriction only at preglomerular vessels, whereas BQ-788 and IRL 1038 antagonized constriction at preglomerular vessels and efferent arterioles. The ET_B receptor agonist IRL 1620 (10^{-8} M, $n = 5$) reduced GBF by $52 \pm 13\%$ (C) and $51 \pm 8\%$ (JM) and constricted efferent arterioles (20 to 33%) about two times more than preglomerular vessels (6 to 14%). Vasoconstriction in response to IRL 1620, which has been shown to elicit vasodilation via endothelial release of nitric oxide (NO) in some vascular preparations, was not altered by pretreatment with the NO synthase inhibitor L-NAME (10^{-5} M). Our results suggest, that in renal C and JM vessels of rats preglomerular vasoconstriction is mediated by ET_A and ET_B receptors, while efferent vasoconstriction is predominantly mediated by ET_B receptors. (Supported by DFG)

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INVOLVEMENT OF ENDOTHELIN IN RENAL BLOOD FLOW AUTOREGULATION DURING ACUTE NO BLOCKADE IN THE RAT. P. Fourmanoir and R. Kramp

Acute systemic NO blockade in the rat resets the autoregulatory set-point to a lower renal perfusion pressure (LLA) under our experimental conditions. Involvement of endothelin (ET), a possible mediator of the vasoconstrictor effect of NO blockade, was considered. To address this issue, RBF autoregulation was studied in 17 anesthetized rats using an electromagnetic flow sensor to measure RBF. The autoregulatory set-point was determined by linear regressions. After control (C) measurements, vehicle (V) or 11 µg/min/kg BW of phosphoramidon (P), an endothelin converting enzyme inhibitor, were administered i.v. Thereafter, 10 mg/Kg BW of L-NAME (N) was injected i.v. to block NO synthesis. The hemodynamic changes respectively induced by V, P or N, were as follows :

	MAP (mm Hg)	RBF (ml/min)	LLA (mm Hg)
		Vehicle (n=8)	
C	113 ± 3	8.5 ± 0.6	83 ± 2
V	111 ± 3	8.6 ± 0.5	83 ± 3
N	126 ± 3*	3.6 ± 0.2*	69 ± 2*
		Phosphoramidon (n=9)	
C	119 ± 2	8.4 ± 0.3	85 ± 2
P	117 ± 3	8.7 ± 0.3	88 ± 2*
N	136 ± 2*	4.1 ± 0.3*	84 ± 2*

Mean ± SE. * $P << 0.05$ between periods C-V and V-N, and C-P and P-N : Anova + Bonferroni t-test. n = number of rats.

As shown in the Table, V was without effect but N altered significantly hemodynamics and LLA was reset by ± 15 mm Hg ($P < 0.005$). P only increased slightly LLA ($P < 0.025$) while N again altered significantly hemodynamics but LLA resetting was attenuated greatly. In conclusion, these results suggest that ET may mediate, at least in part, resetting of RBF autoregulation during an acute systemic NO blockade in the rat.

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IMPAIRED EFFECT OF NO SYNTHASE INHIBITION ON TUBULO-GLOMERULAR FEEDBACK IN RATS WITH GENETIC HYPERTENSION. C. Thorup and A. E. G. Persson.

Experiments were conducted on anesthetized male rats from two genetically hypertensive strains (spontaneously hypertensive (SHR) vs. Wistar Kyoto (WKY) and Milan hypertensive (MHS) vs. the Milan normotensive (MNS)), to test the effect of nitric oxide (NO) synthase inhibition. Measurements of changes in proximal tubular stop-flow pressure (Psf) and early proximal flow rate (EPFR) to various loop of Henle perfusion rates (0-40 nl/min) were used to determine TGF characteristics during control and NO inhibited (intratubular infusion of L-NNA at 10^{-3} M) conditions.

During control conditions, SHR showed a significantly higher TGF sensitivity than WKY. L-NNA infusion increased the sensitivity only in WKY and not in SHR. ΔPsf increased with 95 % in WKY, and only with 26 % in SHR. The same pattern was found in MNS vs. MHS. NO inhibition increased the sensitivity in MNS (99 %), but was relatively uneffective in MHS (32 %). The ERFR levels did not differ between any strain during control conditions. L-NNA infusion did not affect EPFR at low tubular perfusion rates. However, at maximal TGF activation (40 nl/min) EPFR were significantly decreased in WKY and MNS but unchanged in SHR and MHS.

In summary, the increased TGF sensitivity seen in normotensive rats after NO blockade is almost totally abolished in SHR and MHS. This is most likely explained by a reduced ability of vessels in these rats to vasodilate. A decreased vasodilation will under normal conditions not be able to counterbalance the TGF mediated constriction of the afferent arteriole, and this might explain the very high TGF sensitivity found in SHR and MHS during development of hypertension and also their inability in the adult state, to compensate for changes in extracellular volume by TGF resetting.

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FUNCTIONAL COUPLING BETWEEN THE BASOLATERAL K^+-CONDUCTANCE AND THE Na^++K^+-ATPase IN RAT CCD.

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In most epithelia K^+ conductances (g_{K^+}) are functionally coupled to the activity of the Na^++K^+-ATPase. We could demonstrate such a coupling also for the g_{K^+} of the basolateral membrane (BM) of principal cells (PC) of rat CCD (Pflügers Archiv 409:81-92,1987). Inhibition of the Na^++K^+-ATPase by ouabain resulted in a decrease in g_{K^+} of the basolateral membrane. In the present study we examined the role of intracellular Na^+ ($[Na^+]_i$) and Ca^{2+} ($[Ca^{2+}]_i$) activities for the functional coupling of the pump-activity and the recently identified K^+ channels in that membrane (Pflügers Archiv 424:470-477,1993). $[Na^+]_i$ and $[Ca^{2+}]_i$ were measured in isolated CCD segments using SBFI and fura-2 as fluorescence indicators.

In 10 PC the transference number for K^+ of the BM was not affected by 10 µM amiloride (AMIL), reduced by 35±9% by 0.5 mM ouabain (OUAB) and again not affected by OUAB in the presence of AMIL. With AMIL $[Na^+]_i$ and $[Ca^{2+}]_i$ decreased by 4.8±0.9 mM (n=18) and by 19±9 nM (n=21), respectively. With OUAB $[Na^+]_i$ and $[Ca^{2+}]_i$ increased by 29.5±7.4 mM (n=8) and by 28±12 nM (n=9), respectively. With both inhibitors present $[Na^+]_i$ increased only by 7.9±2.5 mM (n=7), while $[Ca^{2+}]_i$ did not change significantly (n=13).

Na^+ entry in PC of rat CCD occurs mostly across Na^+ channels sensitive to AMIL. The observed parallel changes in $[Ca^{2+}]_i$ and $[Na^+]_i$ are compatible with the function of the Na^+/Ca^{2+} exchanger present in the BM. The K^+ channels in the BM of rat CCD are inhibited by increases in $[Ca^{2+}]_i$, but independent on cytosolic ATP. These data offer the changes in $[Na^+]_i$ as primary functional link between g_{K^+} of the BM and the Na^++K^+-ATPase of rat CCD. Therefore, consecutive changes in $[Ca^{2+}]_i$ would regulate the activity of the K^+ channels in that membrane.

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EXPRESSION OF THE RAT RENAL $Na^+HCO_3^-$ COTRANSPORTER IN *XENOPUS* OOCYTES: STUDIES WITH mRNA AND cRNA.

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The proximal tubule is the primary site of HCO_3^- reabsorption in the mammalian kidney. Exit of HCO_3^- from proximal tubule cells into the interstitium involves an electrogenic $Na^+HCO_3^-$ cotransporter in the basolateral membrane. Expression cloning strategy initiated by injection of size fractionated mRNA renders this transport phenomenon measurable in *Xenopus* oocytes. In several preparations of mRNA from rat kidney cortex maximum signals related to $Na^+HCO_3^-$ cotransport correspond to identical sucrose gradient fractions of 2 to 3 kb. One of these mRNA preparations was transcribed into cDNA to establish a library capable of coding for membrane transport proteins. Oocytes injected with 40ng of cRNA prepared from several subpools of this cDNA library as well as 40ng of mRNA displayed HCO_3^-- and Na^+-dependent outward currents in a two-electrode voltage clamp device 4 to 5 days after injection. When superfused with 30 mmol/l HCO_3^-, pCO_2 5.33 kPa, pH 7.5, the mean HCO_3^- currents were 333 ± 161 nA (n=6) at -60 mV for mRNA, whereas 139 ± 77 nA (n=5) were measured for the best coding cRNA subpool. Both, the mRNA- and the cRNA-dependent HCO_3^- currents were sensitive to external Na^+: the HCO_3^--dependent current decreased to 137 ± 108 nA (n=6) for the mRNA and to 44 ± 35 nA (n=5) for the cRNA. In both cases water-injected oocytes showed only small HCO_3^-- and Na^+-dependent currents due to the low activity of the oocytes' endogenous $Na^+HCO_3^-$ cotransporter. The smaller signals from cRNA as compared to mRNA are explained by the loss of information density in the transcription processes.

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EXPRESSION OF THE GENE ENCODING COLONIC H,K-ATPase α SUBUNIT IN THE RAT NEPHRON : MODULATION BY POTASSIUM DEPLETION.

S. Marsy, J.- M. Elalouf and A. Doucet.

Functional studies have revealed the presence of two pharmacologicaly distinct H,K-ATPases along the rat nephron : A ouabain-insensitive form present in the collecting duct and a ouabain-sensitive form located in the proximal tubule and thick ascending limb of Henle's loop. H,K-ATPase activity is up-regulated in the collecting duct of K-depleted rats whereas it is down-regulated in other nephron segments. Three distinct isoforms of H,K-ATPase catalytic subunit have been cloned from stomach, distal colon and toad bladder, but the molecular identity of the renal H,K-ATPases has not been established. Therefore, we studied the distribution and level of expression of mRNA encoding the colonic H,K-ATPase α subunit using quantitative RT-PCR on mRNAs extracted from known amounts (1-2 cm length) of nephron segments microdissected from normal (Control) and potassium-depleted (LK-rats) rats. The specific primers chosen for RT-PCR flanked a 390 pb long cDNA region located in the 5' noncoding region (nts 3331-3721). Within this cDNA region, a mutant was generated through deletion of 150 pb between Bsm I and EcoR V sites. Known amounts of the cRNA corresponding to this mutant (usually 500 copies) were added as internal standard to each sample of tubular RNA (0.3 to 2 mm) for RT-PCR. Results are expressed as mRNA copies per millimeter tubular length and are means ± SE of several samples from different animals (n samples, N animals) (ND, not detected) :

	Control	LK-rats
Proximal Convoluted Tubule	ND (6, 4)	ND (4, 4)
Medul. Thick Ascending Limb	ND (9, 6)	ND (4, 4)
Cortical Thick Ascending Limb	430 ± 107 (6, 5)	450 ± 167 (6, 6)
Cortical Collecting Duct	320 ± 65 (7, 6)	9396 ± 2508 (4, 4)
Outer Medul. Collecting Duct	32 ± 17 (7, 6)	14757 ± 2404 (4, 4)

In conclusion, colonic H,K-ATPase α subunit is exclusively expressed in the late nephron where it is up-regulated by K-depletion.

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TAURINE PERMEATION THROUGH VOLUME-ACTIVATED ANION CHANNELS IN RAT INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS IN PRIMARY CULTURE.

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In whole cell recordings on rat IMCD cells (with 140 mmol/l CsCl in bath and pipette) we find an approximately 6-fold increase in membrane conductance within 10 minutes when bath osmolarity is decreased from 600 to 500 mosmol/l (omission of sucrose). This effect is due to the activation of an outwardly rectifying anion channel with voltage-dependent inactivation at voltages above +60 mV and the anion selectivity $SCN^- > I^- > NO_3^- > Br^- > Cl^- > F^-$ > isethionate > gluconate = aspartate = glutamate. The channel is blocked by 10 mmol/l external ATP at positive voltages and requires 2 mmol/l internal ATP for activation. When bath Cl^- is replaced by the organic osmolyte taurine (pH 8.2, 57 mmol/l negatively charged) the current reversal potential changes by +54 mV, from which a $P_{taurine}/P_{Cl}$ of 0.15 can be calculated. With taurine in bath and pipette, the channel exhibits exactly the same sensitivity profile to a variety of anion channel blockers such as NPPB, DIDS, SITS, niflumic acid, and dideoxyforskolin as under symmetrical Cl^- conditions. We conclude that hypotonic stress increases the Cl^- conductance of rat IMCD cells and that this Cl^- conductance mediates taurine efflux.

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FUNCTIONAL CHARACTERISTICS OF THE CLONED RENAL PEPTIDE TRANSPORTER rhaPT EXPRESSED IN *XENOPUS LAEVIS* OOCYTES. H. DANIEL, W.-M. Weber*, M. Boll, M. Herget, M. Wagener, W. Clauss* and U. Wenzel

We have recently cloned the renal high affinity peptide transporter (rhaPT) from a c-DNA library of rabbit kidney cortex. The cDNA (ORF) predicts a protein of 727 amino acids and 12 membrane spanning domains. Injection of the transporter's complementary RNA into *Xenopus laevis* (Xl) oocytes causes a 50-fold increase in peptide uptake compared to water injected control oocytes. Transport is strongly pH dependent with a pH optimum of $\leq$ 6.0 and the transporters V_{max} is directly related to the proton motive force applied. Besides di- and tripeptides rhaPT also mediates uptake of selected peptide mimetics including aminocephalosporin antibiotics and aminopeptidase inhibitors. In order to characterize rhaPT function when expressed in Xl ooyctes we determined its features by a) flux studies with radiolabeled peptides (^{3}H-Gly-L-Gln, ^{3}H-D-Phe-L-Ala) and peptide mimetics (^{3}H-cefadroxil) and b) by two electrode voltage clamp techniques to assess changes in membrane potential and current associated with peptide translocation. Kinetic studies of substrate uptake as a function of [S] and competition experiments revealed that all peptide substrates including negatively charged compounds have similar affinities (< 200 µM) for interaction with the substrate binding site of rhaPT. All substrates caused a dose dependent membrane depolarization and characterstic current-voltage (I-V) relationships. Transport activity of rhaPT expressed in oocytes is sigificantly affected by alterations of membrane potential and the I-V relationships demonstrate that the transporters reversing potential changes as a function of pH.

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MODULATION OF THE KINETICS OF ALBUMIN UPTAKE IN OK-CELLS. M. Gekle and S. Silbernagl

High-affinity endocytotic uptake of albumin in the proximal tubule of the kidney is important to prevent albuminuria. We investigated the modulation of the kinetics (J_{max} and K_m) of endocytotic uptake of FITC-albumin by Ca^{2+}, cAMP and endosomal pH in cells derived from the proximal tubule (opossum kidney cells). Endosomal alkalinization (ΔpH = 0.6) was induced by either 1 µmol/l bafilomycin A_1 or NH_4Cl. Intracellular Ca^{2+} was clamped using the ionophore ionomycin (1 µmol/l). cAMP was added in the membrane-permeable from (dibutyryl-cAMP, 100 µmol/l). J_{max} and K_m of albumin uptake were calculated form the initial uptake rates (15 min) at different substrate concentrations. The parameters under different experimental conditions are listed below:

	K_m (mg/l)	J_{max} (µg/mg)
control	31±5	2.7±0.2
bafilomycin A_1	90±22 *	1.8±0.2 *
NH_4Cl	60±5 *	1.5±0.1 *
Ca^{2+}-free + ionomycin	150±12 *	3.0±0.2
Ca^{2+}-free (extracellular)	169±33 *	2.8±0.4
1 µmol/l Ca^{2+} + ionomycin	29±2	2.9±0.3
cAMP	32±1	1.9±0.1 *
cAMP + Ca^{2+}-free	72±18 *	2.1±0.3 *
cAMP + bafilomycin A_1	75±15 *	1.9±0.2 *

* = $p<0.05$ versus control

These data show that (i) endosomal pH modulates J_{max} and K_m of endocytotic uptake of albumin. Alkalinization reduces both, maximum transport rate and affinity. The underlying mechanisms involve intracellular substrate handling and receptor recycling. (ii) cAMP modulates J_{max} but not K_m, possibly due to enhanced exocytotic vesicle insertion. (iii) Ca^{2+} has no modulatory effect but is a prerequisite for high-affinity endocytosis, whereas the maximum transport rate seems to be Ca^{2+}-independent.

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MEMBRANE CONTACTS AS REACTION OF THE AUTONOMIC NEURONS AT DYSFUNCTION. S.V.Chepur, O.S.Sotnikov, V.V.Malashko, B.A.Gusova

Under the conditions of the different dysfunctions (experimental stenosis of dog portal vein, UV-burns of the rabbit cornea, injection of growth stimulator kormogrisine to pigs and intensive electric activation of autonomic ganglions) new physiologicaly important nonspecific phenomenon was observed. It consisted in ultrastructural changes of the autonomic neuron exitabilitive membrans. As result of the increase of membrane adgesion submembrane aggregates and true membrane contacts between neurons (N-N) and between neurons and glial cells (N-G) were formed. Sometimes contacts creation was accompanied by appearance of the synaptic vesicles. Unusually big quantity of the nonvesical N-N contacts in intestinal nerve apparatus, appearance of the chemical N-N synapses in corneal plexus, nonvesical and vesical N-G contactswhich absolutely absent in intact animals confirm that those structures are newly formation. Gap and tight junction forming finished in several cases by neuritis fusion with construction of syncytial connections. The process of membrane adgesion of neurocyte organells were also activated. There were multiple contacts between mitochondria, aggregation of synaptic vesicles and fusion of cisterns of endoplasmic reticulum and Golgi apparatus. Sometimes these structures formed the contacts with internal surface of plasmalemma in locus of the new cellular junction. For our mind, those processes have common nonspecific mechanisms and are connected with the changes of adgesion property of membrane and cytosolic proteins. Forming of new N-N and N-G connections have to change well regulated interaction of neurons in autonomic plexus and play an important role in pathogenesis of organ dysfunction.

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EFFECT OF SUBSTANCE P AND MET-ENKEPHALIN ON CAT COLONIC SMOOTH MUSCLE ACTIVITY

E. Atanassova and M. Papasova.

Interactions between SP and ME at the smooth muscle of the descending colon were investigated. In vitro experiments on smooth muscle strips cut out in longitudinal or circular direction from cat distal colon were performed. The effects of substance P ($2.5x10^{-8}$ M) (SP) and metenkephalin (10^{-7} M) (ME) on muscle strips contractile activity were compared when administered alone or in combination (SP+ME). SP evoked powerful contractions of the circular muscle strips, 2.29±0.36 g, compared to the spontaneous phasic contractions, 0.65±0.10 g. ME in most cases significantly increased the background activity, from 0.69±0.10 g to 1.18±0.33 g. The two substances applied together produced the most pronounced contractile activity, 3.85±0.43 g. Mean value of the differences between the contraction amplitude of the circular muscle strips after SP+ME and the background amplitude 3.34±0.43 g was significantly different from this after SP - 1.64±0.40 g and after ME - 1.18±0.25 g. The longitudinal muscle strips showed a higher contractile activity, 1.39±0.43 g, compared to that of the circular strips. The increase of this activity was 4.52±0.58 g after SP, 3.25±0.50 g after ME and 6.19±1.19 g after SP+ME. After naloxone the effect of SP decreased significantly in the circular and insignificantly in the longitudinal muscle strips. In some cases the response to SP increased after naloxone. We suggest that ME contributes to the increase of the effect of SP on the contractile acivity of the muscle strips from the descending colon.

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Atherosclerotic Cardiovascular Risk Related to Hypertriglyceridemia in Ovariectomized Alloxan Diabetic Rats. V. Colev, M. Badescu, V. Calatoru, M. Rosca, M. Ciocoiu and C. Bohotin

The assumption that the hypertriglyceridemia plays a pro-atherogenic role in the development of atherosclerosis in diabetes mellitus is of increasing significance.

Triglycerides (TG), total cholesterol (TC), atherosclerotic (Ia), adhesivity (IA) and aggregability (IAg) indices were investigated in the ovariectomized female rats in conditions of alloxan-induced diabetes.

Methods. Young (90-120 g) and adult (180-200 g) Wistar female rats have been divided into four groups: I. Control (C); II. Diabetic by alloxan administration 40 mg/Kgc i.v. in unique dose (Allx);III. Ovariectomized (O); IV. Ovariectomized diabetic (O+Allx).

Results. We have found elevated values of serum triglycerides in Allx and very high levels in O+Allx group. Corresponding with high serum triglycerides levels he have found an elevation of liver triglycerides values in Allx and O+Allx group. In all groups serum levels of total cholesterol were found in the normal range. The greatest values of atherosclerotic, adhesivity and aggregability indices have been found in O+Allx. Histological examination of the vessels reveals platelet adhesion to the endothelium and lesions of coronaries in the O+Allx group.There was no significant difference age related.

Conclusion. Our results suggest that there is a strong relation between hypertriglyceridemia and atherosclerotic cardiovascular risk in the alloxan diabetic female rats in conditions of ovarian hormones deficiency.

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SELECTIVE INPUTS OF NUCLEUS ACCUMBENS ARE INVOLVED IN LEARNING OF TASKS SENSITIVE UNDER PARKINSONISM AND SCHIZOPHRENIA. S.V.Albertin, I.P.Golovacheva

Present research was centered on studying the role of hippocampal and mesolimbic inputs of nucleus accumbens on acquisition of patterned(single and double) alternation in Sprague-Dawley rats performing go-, no-go task with no-symmetrical reinforcement. Neurosurgical sectioning of ventral subicular efferent pathway resulted in 3-4 fold slowing in acquisition of single alternation patterning in comparison with intact and sham-operated animals and failure to learn double alternation of motor responses using both correction and non-correction procedures to skill patterning. This deficit was considerably reduced or eliminated after injection of cholecystokinin agonist CCK-8 only or in combination with dopamine(DA) in medial area of nucleus accumbens, whereas isolated administration of DA was ineffective.

The results obtained as well as our previous data devoted to studying the role of subicular complex in acquisition of latent inhibition learning(Albertin 1992 a,b)evidence that hippocampal-accumbenal pathway is involved in formation of motor learning and memory-related tasks and suggest the potential role of CCK-based drugs under treatment of some neurodegenerative disorders related to glutamate defficiency in humans.

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EFEECT OF CHOLECYSTOKININ ON FOOD INTAKE OF EARLY WEANED PIGLETS. E.Baranyiová and R.L. Hullinger

The effect of cholecystokinin octapeptide (CCK-8) on the consumption of a liquid diet was investigated in 10 piglets from 2 to 23 d after birth. The piglets were weaned on d 1, housed individually in cages and offered a commercial diet (Purina Baby Pig Milk Replacer) for suckling from feeding bottles 9 times a day at 2-h intervals with an 8-h break at night. CCK-8 (Sigma, USA) was administered to piglets (n=5) on d 2, 4, 6, 8, 10, 12, 20 and 23 after birth in single intraperitoneal doses of 4 $\mu g.kg^{-1}$ in saline 5-7 min before the first morning feeding at 6 a.m. Control piglets (n=5) were treated with physiological saline. The relative diet intake per kg live body mass of CCK-treated piglets was reduced to 41, 80, 47, 48, 67, 63, 78 and 71% of that of the controls. When their diet intake was expressed as percentage of whole-day consumption, CCK-treated piglets consumed 5.0, 5.1, 5.4, 6.5, 6.4, 6.6, 9.7 and 8.5% whereas the controls ate 14.0, 6.4, 10.3, 12.0, 10.0, 10.2, 10.1 and 11.4% of their whole-day consumption. Except for d 20, the differences between CCK-treated and control piglets were significant ($P< 0.05$). In addition, on d 2, 4, 10, 12 and 23, the CCK-treated animals ate significantly less ($P< 0.05$) than at their next feeding at 8 a.m. These results provide evidence that CCK-8 exerts a suppressive effect on diet consumption by piglets in the early postnatal period. Moreover, changes were also observed in the quality of consumption, i.e. in their feeding behaviour. Factors involved in food intake regulation in piglets develop in the early postnatal period gradually and show latency before they come into play. However, our data indicate that one of them, CCK, is functional as soon as from d 2 after birth.

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COLONIC SCFA AND ELECTROLYTE ABSORPTION, AND NASAL GLAND DEVELOPMENT, IN RELATION TO NaCl INTAKE IN THE OSTRICH (Struthio camelus). E. Skadhauge, R.P. Prŷs-Jones, D. Swart and C.N. Warüi.

The ostrich is unique among the ratite birds in having a colon more than 10 m long. It functions as a huge fermentation chamber, as in many mammalian herbivores. There is no reflux of urine into the colon, which is separated from coprodeum by a strong sphincter. Concentrations of short chain fatty acids (SCFA's) reach more than 200 mM. This investigation examines the possible regulation of the electrolyte and SCFA absorption in the colon in relation to the NaCl content of the diet. This was studied in semi-domesticated animals prior to commercial slaughtering. The birds were subjected to a high, a medium, and a low NaCl diet. The medium NaCl diet was identical to the NaCl intake, which the birds ingested when drinking natural bore water (BW). For 3 days before slaughter they were offered drinking water containing a water marker, polyethylene glycol 4000. This allowed calculation of relative absorption rates along the length of the gut, as samples of the contents were taken immediately after slaughter at one meter's distance. Plasma and urine samples were also secured. Plasma osmolality: fresh drinking water (FW or BW) intake: 307 $\pm$ 5 mOsm, salt water (1%) (SW) 328 $\pm$ 4 mOsm. Urine osmolality was hypo-osmotic at FW and BW, but 846 $\pm$ 39 mOsm at SW. The heads were dissected, and the weights of the nasal glands measured. Their weight (less than 1 g/gland) was not significantly augmented by NaCl loading. The measurements of colonic SCFA, electrolyte and water marker concentrations indicate a pronounced decrease of colonic NaCl and SCFA absorption induced by NaCl loading, thus possibly compromising energy uptake. BW did not significantly affect colonic absorption.

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IS PARTURITION A STRESSFUL EVENT FOR THE ANIMAL?

E. Hydbring and K. Olsson

Stress and pain during parturition is obvious and women are treated with analgesics during normal labour in contrast to animals. Pain tolerance threshold decreases during fear and increases by motivation and reward. Is parturition a painful and fearful experience for an animal? How much pain shall we allow the animal to endure in these situations? The aim of this study was to evaluate the stress level of goats during labour by measuring haematocrit, blood plasma concentrations of cortisol, β-endorphin, glucose and insulin. We also registered their behaviour. We followed seven Swedish Landrace goats before and during their parturitions. A permanent catheter was inserted into one of the jugular veins before the parturition began. By providing an extension tube to the catheter and fixing the tube to the neck of the goat, blood could be taken without disturbing the animal. Samples were taken regularly before, during and after parturition until the goats had been milked and fed. The intensity of labour pain varied as well as the time; from 10 min to 8 hours. Four of the goats got twins and the other three got one kid. Twin mothers increased their haematocrit during labour but the value had returned to normal when the goats were milked and fed after delivery. Cortisol concentration rose from 101±34 nmol/l to 359±66 nmol/l during parturition and remained high. Plasma concentration of β-endorphin did not change during labour. The blood glucose concentration increased from 3.3±0.8 mmol/l to 10.9±2.4 mmol/l during labour and stayed at this high level during milking and eating. Insulin was non-measurable before and during parturition, but increased when the goats started to eat. Values from goats delivering only one kid corresponded to those of the twin mothers. It is concluded that parturition is a stressful event for the animal. However, we observed no correlation between signs of labour pain and increase of the stress variables.

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PANCREATIC, HEPATIC AND INTESTINAL ARGINASE ACTIVITY IN GALLUS DOMESTICUS. N.Constantin

The evaluation of the arginase activity was carried out in the pancreas, liver, intestinal wall, pancreatic juice, bile and luminal contents of intestine in 49 Rhode Island hens. The aim of these investigations was to identify the presence and the role (metabolic and/or digestive) of arginase in hen. The arginase activity was determined by use of 8 hydroxiquinoleine for microdetermination of arginine (30 min, pH 7,5, 50° C and presence of Ni^{2+}). The results have showed an intensive arginase activity in pancreatic tissue and no arginase activity in pancreatic juice. Arginase activity was high in liver and low in bile and in intestinal wall and luminal contents of small and large intestine. These data indicate pancreatic arginase is a metabolic enzyme with intracellular activity only whereas hepatic and intestinal arginase are metabolic and digestive enzymes with both intracellular and extracellular activity.

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THE PERIPHERAL BLOOD LYMPHOCYTES DEFICIT IN HYPOTHYROID PATIENTS DIAGNOSED AFTER STAY IN THE CHERNOBYL ZONE. N.Khalangot, A.Degonsky, E.Raksha-Slyusareva, G.Latypova, M.Kolomoiskaya

10 patients with primary hypothyroidism (PH) developed 1-4 years after short-time (32±11 days,mean±SE) stay in the Chernobyl zone were examined: free T4, TSH were 3,82±1,33 pmol/l; 26,96±6,26 mU/l respectively. A considerable deficit of total lymphocytes (L) and their populations (LP) as compared to analogous indices of 50 healthy persons was found in peripheral blood of those patients by the method of indirect immunofluorescence using monoclonal antibodies: L (10^9c/l) 1,54±0,22 vs 2,12±0,06($p<0,02$); CD22 0,51±0,08 vs 0,84±0,06($p<0,01$); CD3 0,50±0,11 vs 1,10±0,06; CD4 0,19±0,05 vs 0,58±0,04($p<0,001$); CD8 0,17±0,04 vs 0,35±0,03($p<0,01$). The L and LP levels were also determined in blood of 9 PH patients who hadn't visited the Chernobyl zone and in 10 hypothyroidism-free persons who had visited that zone. A conclusion was drawn that B-lymphocytes deficit is connected mainly with the radiation factor while T-lymphocytes deficit is determined both by the presence of PH and the radiation factor.

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FREE RADICALS INCREASE $[Ca^{2+}]_i$ IN SEVERAL IDENTIFIED NEURONES OF *LYMNAEA* STAGNALIS. H.F.Moghadam, L.L Moroz, W.Winlow Dept. of Physiology, University of Leeds, LS2 9NQ, UK.

Free radicals exist in animal tissues, where they are involved in physiological and pathophysiological phenomena. For example, H_2O_2 is generated at sites of inflammation by leucocytes, but it is an unstable substance and is catabolized by the cells after 8-14 min. H_2O_2 exposure induces a dose-dependent disturbance of intracellular calcium homeostasis, which is independent of the continuing presence of the oxidant and causes a fast rise of intracellular free calcium(1). Using ratio fluorescent microscopy (Magical system) we have measured $[Ca^{2+}]_i$ in cultured, identified, molluscan neurones, which are ideal for studying the intracellular actions of free radicals.

Identified neurones were cultured as previously described(2). Neurones were isolated and cultured for two days, and then incubated in Fura-2 for 2-3 hours to detect free calcium ions. Different concentrations of NO, sodium nitoprusside(SNP), H_2O_2 and t-Butyl hydroperoxide(tBP) were applied as free radical donors.

SNP (10^{-6}-10^{-3} M)increases$[Ca^{2+}]_i$ slightly, but illumination of the medium with white light, increases $[Ca^{2+}]_i$ dramatically due to release of NO. Application of pure NO, H_2O_2 and t-butyl hydroperoxide all riase $[Ca^{2+}]_i$ to varying extents. We have shown that both H_2O_2 and NO inhibit gross Ca^{2+} current in pedal I cluster neurones in *Lymnaea*(3).

In conclusion we have demonstrated that exposure of *Lymnaea* neurones to H_2O_2, t-BP, NO, and SNP causes a rise in free $[Ca^{2+}]_i$ (as measured with Fura-2), probably from internal stores, since these free radicals simultaneously inhibit gross Ca^{2+} currents.

HFM is a Iranian Government scholar.

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THE EFFECT OF ETHYL ACETATE PREEXPOSURE ON THE ADAPTATION IN ANTENNAL CHEMORECEPTORS IN Periplaneta americana (L.).

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In the previous study adaptation of antennal mechano- and chemoreceptors in P. americana was investigated. Continous stimulation at a constant frequency led to a well pronounced adaptation in mechanoreceptors (stimulation by air stream) and in chemoreceptors (air stream + ethyl acetate) in all experimental models. The breaks in stimulation disturbed adaptation, what gives evidence of its existence. In the present experiments we wanted to see in what way one or seven days preexposure to the ethyl acetate evokes change of the pattern of adaptation depending on the time of preexposure. Antennal chemoreceptors were investigated using electroantennographic method (EAG). Two parameters were analized: amplitude in mV and duration of the depolarization of the antenna in s. The EAG was recorded in six successive time sectors every two seconds (t0,t2,t4,t6,t8,t10). The study was performed in 3 experimental models: control, one-day and seven-days preexposure. The results obtained allow to make following conclusions. Adaptation was seen in the form of a fall of the amplitude and time of depolarization both in mechano- and chemoreceptors, but in mechanoreceptors was considerably slower then in chemoreceptors. Preexposition to the ethyl acetate deepened the adaptation in chemoreceptors. Particularly it was seen in the seven-days group (the fall of both parameters of EAG). The breaks (time t4) in stimulation weakened the adaptation in chemoreceptors.

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STRATEGY ASPECTS OF DIVING MAMMALS BIOCHEMICAL ADAPTATIONS. V.Galantsev*, R.Kovalenko*, T.Kamardina*, N.Zavarzina#, V.Perepelitsa*, I.Yanvariova*

Comparative physiological studies of strategy of development of physiological and biochemical adaptations of an organism to environmental conditions and the lack of oxygen in particular are extremely valuable for adaptational medicine. Aquatic and semiaquatic mammals can serve as an appropriate model for such studies. It has been known that the cardiovascular system plays the leading role among adaptational physiological reactions to hypoxia. However, data from our longterm investigations of adaptation of animals to diving shows that biochemical mechanism of using local tissue oxygen as well as one from the blood exists under the same conditions. We are looking for such metabolic tissue source of O_2 by evaluating role of oxygendependent and oxygenconsuming processes (primarily of lipid peroxidation (LP)). Adaptive intensification of LP (detected by the level of dien- and trien-conjugates, Schiff bases and malondialdehyde) was not fond in any organ of mink and musk-rat during apnoe under bradycardia. Nevertheless, increased activity of superoxide dismutase, catalase and peroxidase was detected in variouse organs. This data suggests that O_2, used normally for synthesis of fatty acids' peroxidative compounds in mammals adaptated to diving, is released for maintaining of aerobic bioenergetic mechanism during apnoe. Increasing catalase activity suggests that peroxide hydrogen serves as one of possible sources for O_2.

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ARE THE HIGHER BRAIN CENTRES RESPONSIBLE SIMULTANEOUSLY FOR BOTH BEHAVIORAL INHIBITION AND MEMORY ? Khonicheva N.,Karas A.,Pinkhasov E.

Massive destruction of integrative brain structures and following functional testing was as a general method in this work, concerning memory mechanisms both in vertebrates (Rats, Wistar) and invertebrates (Nereidae, Annelidae). Crucial interval duration between consecutive stimuli (or trials) wich does allow to form association (or behavioral habit) was an universal criterion of the memory.The other independent parameter was an approaching-avoidance characteristic in standart test situation (the analogue of emotional status). After bilateral lesions of amygdalar complex - central part of lymbic system in rats - two correlated phenomena were observed:increasing of approaching reactions in a defensive situation (Vogel-test) and memory defect, which was revealed as impossibility of habit learning during spaced trials(when intertrial intervals was increased up to two hours instead a minute one). The similar parallel phenomena were registrated after supra-oesophagial ganlion ("brain") removal in Annelidae when habituation to repeated stimulus (which is a simple model of memory) was studied. In this case number of defensive responses to external stimulation was considerably lower and habituation was possible only during massed trials (when short intervals were used: 20 s, but not 60 s.). Quite similar correlation (memory disturbance - increasing of approaching reactions) was described for peptides, blocked memory. The idea about mediation of above mentioned central functions by peptides is discussed.

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THE ROLE OF INTENSITIVITI, COUPLING AND POWER OF THE ENERGETIC EXCHANGE IN KEEPING OF RESISTANCE OF THE ORGANISM. M.Tymochko, O.Misakovets and O.Yeliseyeva.

Our investigations, which dealt with physico-chemical processes of the living systema, intensitivity of oxidative reactions, activity of the anabolic exchange under the influence of extremal factors, have proved that resistance of the organism depends on the level of free energy, the latter being kept by high coupling of exchange processes.

It has been found out that effectiveness of the free energy assuming is in direct dependance on the intensitivity and the level of participation of functional structure resources in the energetic exchange and also on their power, the latter being capable to ensure the flow of coupled physico-chemical changes in the formation of the stationary state.

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PHYSIOLOGICAL RESPONSES OF RAINBOW TROUT TO ENVIRONMENTAL STRESS. N.Kazlauskienė, Z.Vosylienė
Various forms of environmental stress can effect the perfomance capacity of a fish impairing the defence mechanisms and predispose them to disease. The aim of the present study was to establish: responses of cardio-respiratory system and hematological parameters of rainbow trout to short-term effect of chemical stressor; to expose fish to handling and to compare the magnitude of the stress responses. Exposure of fish to chemical stressor induced the decrease in gill ventilation frequency and increase in "caughing" rate, changes in heart rate and in its periodic structure (which are induced by the alterations increased activity of n.vagus). As well as there were determined increased hematocrit and blood glucose contents. The data obtained revealed that chemical stress disturbs the activity of cardio-respiratory system impairing fish adaptative abilities. Handling elicited the most sensitive physiological indices of rainbow trout to negligible environmental changes.

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INCREASE OF VASOPRESSIN CONCENTRATION IN THE HYPOPHYSEAL PORTAL BLOOD AFTER SUPERIOR CERVICAL GANGLION STIMULATION IN RAT. S.Lipińska and W.Z.Traczyk.
Our previous experiments (J Physiol Pharmacol 1992, 43, 367) showed an increase in the release of vasopressin into the fluid incubating the posterior pituitary lobe "in situ" after the preganglionic stimulation of the superior cervical ganglion. The aim of the present study was to investigate whether the stimulation of the superior cervical ganglion may influence vasopressin release into the hypophyseal portal blood. In urethane-chloralose anaesthesia the pituitary gland was exposed by transpharyngeal approach in rats. The hypophyseal portal vessels were transsected in the narrowing between the glandular portion of the hypophysis and the infundibulum. The 15-min blood samples from the cut portal vessels were collected before and during electrical stimulation of the superior cervical ganglion (10 V, 20 Hz, 3 ms). Vasopressin content in the plasma was determined by radioimmunoassay. In the control samples the vasopressin content amounted to 3.2 ± 1.03 ng/mL and was about thousand times higher than the concentration of vasopressin in peripheral plasma. Stimulation of the superior cervical ganglion evoked an increase (9.6 fold) of vasopressin release into the blood hypophyseal portal vessels. On the basis of the results obtained, it may be presumed that the sympathetic efferents arising from the superior cervical ganglion participate in the regulation of vasopressin release into the hypophyseal portal blood.

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EFFECT OF THYROID STATUS ON AROMATIC AMINO ACID TRANSPORT IN SINUSOIDAL MEMBRANE VESICLES FROM RAT LIVER. **HELEN F.KEMP & PETER M.TAYLOR**
Transport of thyroid hormones (T_3 and T_4) and aromatic amino acids in erythrocytes and astrocytes shows mutual competitive inhibtion and may occur by the same transport system (system T) [1]. Such interactions may be important in the liver, (which expresses system T activity and is a major site of T_4 action, T_3 synthesis and aromatic amino acid metabolism) especially during altered thyroid status. We have therefore investigated the relationship between the transport of [^{3}H]L-tryptophan and thyroid hormones in sinusoidal membrane vesicles (SMVs) isolated from hypothyroid, hyperthyroid and euthyroid rats. Rats were made hyperthyroid by injection of 60μg/BW L-T_3 on alternate days for 5 doses, controls received vehicle only (0.9% saline). Hypothyroid rats received 0.05% propylthiouracil in drinking water for 28 days. Liver SMVs showed a 14-fold enrichment of plasma membrane marker enzyme, 5'nucleotidase compared to homogenate (irrespective of thyroid status). In euthyroid rats, 1μM [^{3}H]L-Trp transport showed 79, 77, 34, 64, 59% inhibition by 1mM Phe, Trp, Tyr, Leu and BCH respectively and the uptake was also inhibited by 10μM T_3 and 10μM T_4 (by 22 ±15% and 32 ±10%, n=9 livers) indicating a system T transport component. There was no significant change in the uptake of [^{3}H]L-Trp in either of the altered thyroid states as compared to the euthyroids (1.2 ±0.2, 1 ±0.3 and 1 ±0.2 pmol/mg prt/30s, hyperthyroid, hypothyroid and euthyroid respectively), but SMVs from hyperthyroid rats showed a greater susceptibility to inhibition by both T_3 and T_4 (50 ±4 and 52 ±14% inhibition respectively, n=9 livers) compared to those from hypothyroid and euthyroid rats. The data provide preliminary evidence that the extent of interactions between the transport of aromatic amino acids and thyroid hormones in liver membranes is dependent upon thyroid status.
Supported by the BBSRC UK and Dundee University
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THYROLIBERIN (TRH) REGULATION IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) AND MEDIAN EMINENCE (ME) AND IN ISOLATED PANCREATIC ISLETS. V. Štrbák, M. Nikodémová and J. Benický
TRH originally isolated from the hypothalamus is present in various structures possessing many different functions. TRH involved in thyrotropin regulation is synthesized in the PVN (perikarya) and transported to nerve terminals in the ME where it is released into portal blood. In the pancreas TRH was found to be colocalized in the B cells of Langerhans islets together with the insulin, its role here remains obscure. We have compared these systems during static *in vitro* incubation. Rat (SPF Wistar males 300 g) PVN and ME were microdissected and incubated in 95% O_2 + 5% CO_2 atmosphere. Langerhans islets were isolated from pancreases according to Lacy and Kostianovsky. Bacitracin (0.3 mg/ml) substantially increased the TRH detected by RIA in the medium at the end of incubations. The TRH content in pancreatic islets sharply increased during incubation in contrast to that in the hypothalamic structures, where it remained stable. The release of TRH from all structures was stimulated by membrane depolarization induced by 56 mM KCl. D-glucose stimulated the TRH release from the islets but not from the PVN or ME. Cycloheximide added into medium with pancreatic islets inhibited TRH secretion and partially prevented the increase of its content during incubation. **In conclusion: Although preproTRH in hypothalamus and pancreas is encoded by the same gene, TRH content and secretion is regulated differently in different localization thus reflecting various functions.**

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LOCALIZATION OF PREPROENKEPHALIN mRNA-EXPRESSING CELLS IN THE LAMB (EWES AND RAMS) BRAIN WITH IN SITU HYBRIDIZATION. COMPARISON WITH irMET-ENKEPHALIN.

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Among many neuropeptides and neurotransmitters in the brain, the most intensively expressed are the opioid peptides enkephalins. The localization of enkephalins mRNA is prerequisite to investigate their expression and regulation. Therefore, as part of a study dealing with the opioids importance in sheep growth and development, the present experiment was carried out to investigate differences in the synthesis of proenkephalin at the cellular level in several areas of ram and ewe brains. Six-months old Polish Mountain rams (n=5) and ewes (n=6) were used for this study. Brains were removed within 5 min, dissected to obtain the blocks from hypothalamus, hippocampus, striatum, amygdala, nucleus accumbens, cortex, cerebellum, pons, pituitary, pineal and infundibulum. Small fragments of the areas were taken for radioimmunoassay of immunoreactive Met-enkephalin (irMet).The mRNA expression for preproenkephalin was measured by in situ hybridization according to Young et al. (1986). After 28 days of exposure, quantitative analysis of autoradiographic film with an image system show the highest degree of mRNA expressing cells in pituitary followed by hippocampus, striatum, amygdala. Much lower optical density was noticed in hypothalamus and cortex. Unexpectedly, there were clear sex differences in the localization of mRNA. The concentration of irMet varies from 1.90 ± 0.49 in cortex to 35.74 ± 10.40 pmol/g in pineal in rams and from 4.97 ± 0.67 in cortex to 83.30 ± 12.21 pmol/g in infundibulum in ewes. Of considerable interest, the concentration of irMet was higher in ewes than in rams in 7 out of 11 tested brain areas, the exceptions are hypothalamus and pineal. Thus, the synthesis of preproenkephalin appears to be sex dependent and dissociated from the releasing and processing in some brain areas. (Supported by KBN Grant No: 052/P06/95/08).

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CHARACTERIZATION AND PHYSIOLOGICAL REGULATION OF GLUCOCORTICOID RECEPTOR-RELATED TRANSCRIPTS IN THE ANTERIOR PITUITARY GLAND OF THE RAT. H. Morgan and D.A.Carter.

The type II corticosterone receptor is a product of the glucocorticoid receptor (GR) gene, and is transcribed as a 6.5 kb mature mRNA that is widely expressed in mammalian tissues. Previous studies of GR mRNA expression in the neuroendocrine system have used 3' cDNA probes to detect a similar mRNA. The objective of the present study was to identify alternative GR transcripts which may function in the regulation of the stress response. Using a full-length rat cDNA as a probe on Northern blots of poly (A)+ RNA extracted from tissues of adult Sprague Dawley rats, we have detected tissue-specific expression of GR-related RNAs that are smaller than the defined 6.5 kb GR mRNA. In particular, a 2.1 kb RNA is abundantly expressed in the anterior pituitary gland and pineal but not in the liver. Expression of the 2.1 kb RNA is sexually dimorphic being approximately two-fold more abundant in male rats, and we have shown that the sex-difference is reversed following ovariectomy but is unaffected by castration. Adrenalectomy did not alter expression of the novel transcript. In further experiments, we have shown that the 2.1 kb RNA has sequence homology with the 5' end of the GR cDNA. In conclusion, we have demonstrated the presence of novel GR-related transcripts that may function in the regulation of the endocrine stress response, and possibly relate to sex differences in this response.

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GLUCOCORTICOIDS REGULATE CALCIUM CURRENT DENSITY IN GH3 CELLS. M.Sedova, A.Fomina

We studied the influence of hydrocortisone and dexamethasone on low-threshold (LT) and high-threshold (HT) calcium currents and hormone secretion in clonal rat pituitary GH3 cells. The growth hormone secretion (measured by radioimmunoassay method) was increased by 44% after 2 h of incubation in the presence of 1 μM hydrocortisone, while the secretion of prolactin was slightly depressed (10%). The whole-cell and perforated patch clamp techniques were used to assess the regulation of voltage-dependent calcium currents by glucocorticoids. Incubation of GH3 cells in the solution containing 1 μM hormone led to an increase in current densities, that was maximal for both types of currents after 2 h exposure of cells. The elevation of the HT current densities was significantly higher (more than a 4-fold) than that of LT ones (about a 3-fold). Parameters of current inactivation and current-voltage dependence were unaffected. Potentiation of currents was blocked by adding 0.1 mM actinomycine D, suggesting that protein synthesis was required for this effect. The stimulatory action of dexamethasone on HT current was observed under conditions of perforated patch clamp recording (T = 29°C) after 20-30 min from the beginning of hormone application to the bath solution but disappeared with the whole-cell patch technique used. Application of dexamethasone did not influence the basal level of intracellular calcium and calcium transient evoked by high concentration of external potassium. The presented experiments suggest an important role of the regulation of genomic expression of voltage-gated ion channel in glucocorticoids effects on pituitary cells.

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THE PINEALOCYTE AND HYPOTHALAMIC NEURONS NUCLEI VOLUME CHANGES WITH REGARD TO ESTRADIOL, LH, AND CATECHOLAMINE LEVELS IN EWES AFTER OVARIAN STIMULATION.J.Halagan,B.Pastorova,A.Stanikova.

The estimation were carried out on 21 ewes in estrous season. The estrus was synchronized by chlormadinon acetate vaginal tampons.Two groups of ewes (7+7) were treated i.m.with 750 IU or 1,000 IU of PMSG after spongies were withdraw. Blood plasma luteinizing hormone, estradiol and progesterone were monitored by RIA methods. The catecholamines were estimated in tissues by radioenzymatic method. The light and electron microscopy of pineal gland and hypothalamic nuclei as well as karyometric examination of 200 cells were carried out in every specimen. The moderite shift to the left of nuclei volume variation curve of pinealocytes and significant increase of epinephrine values as well as significant increase of estradiol levels were found after stimulation with 1,000 IU of PMSG. The norepinephrine levels remain unchanged after PMSG treatment but an decrease of pineal dopamine content was found after application of PMSG. Positive correlations between levels of endogenous LH and stimulate dose of PMSG as well as multiplication of neurosecretion in the hypothalamus were found in superovulated ewes. Decreasing in the pinealocyte nuclei volume and histological as well as biochemical changes demonsrate an inhibition of the secretory activity of pineal gland after gonadotropine stimulation of ovaries of sheep via estradiol and / or by local catecholamines pathways.

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LETHAL IRRADIATION OF RATS: DAY-TIME EFFECTS ON SERUM MELATONIN, THYROTROPIN AND THYROID HORMONES. E.Ahlersová,M.Kassayová and I.Ahlers

Male Wistar rats adapted to an artificial light-dark (LD) regimen 12:12 h (light switched on at 7 a.m.) were whole-body irradiated with 14,35 Gy of gamma rays from ^{60}Co source and sham-irradiated. The animals were divided into three groups: A-rats irradiated in the night (in darkness) and placed in the 12 h LD regimen, B-rats irradiated in the day-time and placed in the 12 h LD regimen, C-rats irradiated in the night (in darkness) and kept in the constant dark. After real and sham-irradiation the rats starved till the analysis. The rats were quickly sacrificed 6 h to 4 days after exposure and sham-exposure in the dark except the 24 h interval in group B. In the serum were determined: the concentrations of melatonin radioimmunochemically (Charon et al. 1991), thyrotropin (TSH), thyroxine (T4) and 3,5,3´triiodothyronine (T3) radioimmunologically (NIDDK rat TSH-RIA kit from NHP Program, USA, total T4 and T3 RIA kits from IRPAR, Czech Republic). Melatonin levels were increased within days 3-4 after irradiation in the groups A and B. TSH concentration was increased on day 4 postexposure in group C only. Ionizing radiation decreased the levels of T4 and T3 at hours 6 and 72 in group C, in group A at hour 72 postexposure. Different time of day and light regimen during and after irradiation influenced the neuroendocrine reaction to ionizing radiation in serum melatonin, thyrotropin and thyroid hormone response.

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THE EFFECT OF HISTAMINE ON IN VITRO STEROIDS HORMONE SECRETION BY THE PREOVULATORY FOLLICLES OF THE DOMESTIC HEN (GALLUS DOMESTICUS).

H. Paczoska-Eliasiewicz and J. Rząsa

In chicken ovary histamine is present (Schaible and Sturkie, 19900 and significantly changes during follicular maturation (Paczoska-Eliasiewicz and Rząsa, 1993). The aim of the present study was to examine whether histamine affects steroids secretion by the isolated granulosa and theca layers of the preovulatory follicles of the domestic hen. Three largest preovulatory follicles (F_1-F_3; F_1>F_2>F_3) were collected 18 h before F_1 ovulation, granulosa and theca were isolated and separately incubated in the static system for 3 h at 38 °C. Three doses of histamine were used: 1, 10 and 100 ng/ml of Eagle's medium. Progesterone (P_4), estradiol (E_2) and testosterone (T) secreted to medium were determined radioimmunologically. It was observed that histamine statistically decreased secretion of all steroids by the granulosa layer of the examined preovulatory follicles, e.g. for F_1 follicle at the dose of 1 ng/ml: P_4 (25.8 ± 1.7 → 17.4 ± 1.2 ng/mg protein), E_2 (10.2 ± 0.5 → 1.1 ± 0.1pg/mg protein) and T (470 ± 50 → 51 ± 10 pg/mg protein). In the theca layer, histamine significantly decreased P_4 and T secretion but enhanced E_2 secretion, e.g. for F_1 follicle at the dose of 1 ng/ml: P_4 (310 ± 27 → 150 ± 13 pg/mg protein), T (360 ± 41 → 210 ± 17 pg mg protein) and E_2 (9.2 ± 0.8 → 18.3 ± 1.8 pg/mg protein). The results obtained indicate that histamine might be one of the intra-ovarian regulators of steroidogenesis in avian ovary.(Supported by KBN Grant No. 55 3959102).

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SEX HORMONES: THE TOPOGRAPHICAL FEATURES OF ^{3}H-ESTRADIOL-BINDING ACTIVITY IN AMYGDALOID COMPLEX OF BRAIN.

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The level of estradiol binding was determined with method of Bayard et al. (1978) at 52 sexually mature rats. The investigations were carried out on isolated from brain amygdala, excision of which was performed with the original method developed on basis stereometrical characteristics (author certificate 1679246). The results obtained are reflected in Table.

Group animals	Region, fmole per 1 mg DNA ($X \pm S_x$)	
	rostral	caudal
Males	332±55.6	518±11.5*
Total females at all stages of the cycle	564.5±75	823±68*
Females:		
in diestrus	460±46	754.5±35.5*
in proestrus	453±46.5	485±34
in estrus	55±12	514±81*
in metestrus	1861±557	1007±154

* $p<0.01$, significance of differences between region within groups

The numerical data presented in it quite convicingly argue in favor of topographical differences in the level of estradiol binding in the cytosol fraction of neurons by various regions of the Amygdala. These features of the histophysiology of neurons of the Amygdala also are expressed along a rostro-caudal grádient, thus confirming the existence of two poles or centers in the Amygdala in realization of its neuroendocrine potentialities.

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RESPONSES OF HEAT-STRESSED CHICKENS TO REVERSE-TRIIODOTHYRONINE (rT_3) AND ASCORBIC ACID (AA).

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The aim of this study was to examine whether hypometabolic rT_3 facilitates the adaptation of chickens to heat stress (39°C for 72 h). The effects of rT_3 were compared with those of AA known to attenuate stress. 17 day-old chickens were injected (s.c.) with rT_3 (100 µg/day), AA (200 µg/day) or rT_3 + AA for 3 consecutive days. RT_3 or rT_3 + AA increased the mortality of heat stressed chickens (mortality index: dead/n was 5/12 (rT_3) and 7/12 (rT_3 + AA). RT_3 leads to the highest reduction in body weight gain and food consumption. Opposite effects was observed in water consumption. The effect of AA resembled those of rT_3, however, it was less marked. AA retained exogenous rT_3 in the blood plasma of heat stressed chickens; the plasma level of rT_3 in rT_3 + AA treated chickens was 4.2 times higher compared with rT_3 treated chickens. In neutral temperature rT_3 suppressed body temperature but enhanced the hyperthermic effect of heat stress. RT_3 inhibited the rise of plasma adrenaline and increased corticosterone level in hot environment. Hyperglycemic and lipemic effect of heat stress were more manifested in rT_3 treated animals. The effect of rT_3 was more marked in heat stress than in optimal condition. The higher mortality of heat-stressed and rT_3 + AA treated chickens was the result of futher enhancement of the elevated plasma level of exogenous rT_3. Reverse T_3 exert different effect depending on environment temperature. The formation of hypothermia in neutral temperature mirrors its hypometabolic property whereas increased hyperthermia resemble a thyromimetic effect. Decreased plasma adrenaline and enhanced hyperglycemia and lipemia elicited in rT_3 treated birds, support the idea on opposite action of rT_3 compared with T_3.

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PHYSIOLOGIC VARIATIONS OF THE PLATELET COUNT IN NEWBORNS AND MOTHERS ; A.Badarau, E.Nicolescu, M.Artino, R.Cârmaciu, A Iancu.

Abnormalities of the primary hemosthasis during the perinatal period are not un- common.The purpose of this study was to verify if numeric variations of platelets are present in mothers post partum and newborns , and if so, to point out the possible factors of risk arising from physiologic particulars of perinatal period.The blood platelets were counted in 50 newborns and in their mothers immediately after birth, at 72 and 120 hours respectively post partum. Appropriate control values were obtained from 4 - week old infants and non - pregnant women. Maternal platelet count revealed a moderate but statistically significant decrease immediately after birth , reaching the control values after 120 hours .These rezults could be explained by antepartum and intrapartum modifications ; their signification is fully understood only in the context of other changes in primary hemostasis, coagulation and fibrinolysis during and immediately after pregnancy. In the normal newborns at term, the average platelet number was reduced after birth, rising to values comparabile to controls during the next 120 hours; similar data were obtained in new borns with weight deficit problems or APGAR score less than 7.In the premature newborns the platelet count was more reduced at birth and the recovery of the control values was prolonged over 120 hours. The results indicate that prematurity is a risk - factor not to be neglected when primary hemostasis is concerned.

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INSULIN BINDING TO PIG ERYTHROCYTES IN THE NEONATAL PERIOD. H.Antonyak, V.Snitynsky, N.Antonyak

The first step in insulin action is binding to its specific receptor on the plasma membrane. Hormone binding characteristics of mammalian erythrocyte receptors are comparable to that of other insulin-sensitive cells. The present investigation was performed to define the age peculiarities of insulin binding to pig erythroid cells during the neonatal period of ontogenesis. Erythrocytes from newborn, 1- and 10-day piglets were depleted of white cells and platelets by chromatography on mixed α-cellulose and microcrystalline cellulose column. The levels of ^{125}I-insulin binding to red cells were determined by the method of Gambhir et al. (1978), obtained data were analysed by Scatchard plot. We have established that level of insulin binding to erythrocytes was high in the newborn piglets and decreased significantly during the ten-day period after birth. This was conditioned by progressive reduction of receptor number in high affinity site as animals aged. Binding capacity averaged 331±18 (mean±SEM) and 220±11 sites per cell in the newborn and 10-day pigs respectively. The mean affinity constants did not differ significantly during the investigated period. According to our findings the postnatal changes of insulin binding to erythrocytes occur simultaneously with sharp increase of insulin concentration in animal plasma (from 1,2±0,2 μU/ml in newborns to 17,5±3,1 μU/ml in 10-day piglets). The obtained data suggest that insulin may be involved in regulation of its own receptor number in erythrocytes similar to other cell types and provide the evidence of erythroid cell sensitiveness to the regulatory influence of this hormone.

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HORMONAL REGULATION OF ERYTHROCYTE METABOLISM IN PIG DURING THE NEONATAL PERIOD OF ONTOGENESIS. V.Snitynsky, H.Antonyak, V.Danchuk

Blood respiratory function in mammals is known to be conditioned by red cell metabolism level and depends on influence of several hormones. The age peculiarities of thyroid hormone regulatory action on metabolic activity of pig erythroid cell in the neonatal period have not been studied. We have investigated the relations between plasma T_3 levels, intensity of formation of 2,3-diphosphoglycerate (2,3-DPG, modulator of hemoglobin's affinity to oxygen) and activities of glycolysis enzymes - hexokinase (HK), pyruvate kinase (PK), diphosphoglycerate mutase (DPGM) in erythrocytes of newborn, 1-, 5- and 10-day piglets. T_3 levels in animal plasma were measured by radioimmunological assay, 2,3-DPG concentrations - by enzymatic method. We have established that plasma T_3 concentration being low at birth, increased sharply at the first day of postnatal life and remained on the high level in 5- and 10-day piglets. This was accompanied by intensive production of 2,3-DPG in animal erythrocytes during the period of ten days after birth. According to our findings 2,3-DPG accumulation was caused by activation of DPGM and HK and decrease of PK activity levels which occured progressively as newborn piglets aged. Since erythroid cells are known to be susceptible to the thyroid hormone action, we consider that increased formation of T_3 in pig in the period of neonate promotes the alterations in erythrocyte metabolism directed on the decrease of hemoglobin affinity to O_2. This provides the facilitation of oxygen delivery in animal tissues and intensification of oxidative processes in the cells.

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PECULIARITIES OF CONTRACTILE REACTION IN RESPONSE TO ACETYLCHOLINE, EFFECTED TO INTERNAL AND EXTERNAL SURFACE OF THE CHICK AMNION. Nechaeva M.V.

Previously we have reported about our investigaition of possible mechanism underlying regulation of spontaneous rhythmic contractile activity of the chick amnion by two antagonistic neurotransmitters present in the amniotic fluid: serotonin and noradrenalin, which stimulate and inhibit the amnion motor activity, respectively (Turpaev, Nechaeva, 1994). In that study influence of acetylcholine (ACh) (up to 10μM) injected into the amniotic fluid on frequency of spontaneous contractions of the amnion was not found. Yet, it is known, that the chick amnion strip is very sensitive to ACh. Considering the aforesaid and the fact that the chick amnion is a non-innervated smooth muscle devoid of blood vessels having a single layer of epithelial cells on its internal surface, the aim of the present research was to study the contractile reaction of chick amnion to ACh, effected to internal and external surfaces of amnion.

The isolated amnion of the 8-9-day chick embryos was used. Mechanical activity of the amnion was recorded by means of the mechanosensor under isometric condition. The frequency of spontaneous contractions of the amnion at 38^{0}C was equal to 7,2±1/min. ACh, effected both to internal surface of the amnion and external one, was found to induce an increase in the frequency of contractions of the amnion in a concentration-dependent manner. The levels of maximum reaction were similar, however, threshold dose for ACh and K_{50} values were significantly different for two ways of application of ACh. When ACh was effected to internal surface, the values of threshold dose and K_{50} were equal to 1-10μM and 33±12μM, respectively, when Ach was effected to external surface they were 1-10 nM and 13±7 nM respectively. These results indicated significant difference in the sensitivity of external and internal surfaces of the chick amnion to ACh.

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RADIATION INJURY OF THE THYROID GLAND. I.P.Pasteur, N.D.Tronko, V.V.Markov, N.P.Smirnova, E.V.Klochko, Ye.N.Gorban and N.P.Kabatsy

The aim of the present study was to assess the effect of radioactive iodine treatment of the thyroid gland. Experiments were carried out with the Wistar rats, weighing 100-120 g, and newborn pigs. 131-iodine was used in a dose of 5 Gy on the thyroid gland. Thyroids were surgically removed under ether anesthesia and cultured for a further 3 days at 37 C in medium 199 with 10% bovine serum, in the presence or absence of 10 mU/mL thyrotropin (TSH), after which 125-I and carrier iodide were added for 90 minutes. The molecular linkers of lipid peroxidation products with cell proteins (lipid-proteins linkers, LPL) and malondialdehyde (MDA) in the thyroid tissue were determined by proper fluorimetric technique and as its thiobarbituric acid complex, respectively. The medium thyroxine (T4) and triiodothyronine (T3) levels were evaluated by radioimmunoassay. The thyrocytes 125-I uptake was measured by liquid scintillation counting. The results obtained show that radioiodine treatment leads to decrease in medium T4 and T3 levels compared to the control (mean±SD; 12.34±1.38 vs. 21.68±2.13 nmol/L and 0.99±0.17 vs. 1.77±0.23 nmol/L, respectively, $P<0.05$). The LPL coefficient and the MDA content of the irradiated thyroid were higher than those of the unirradiated thyroid (mean±SD; 62.7±8.2 vs. 23.2±2.4 and 2.25±0.45 vs. 1.15±0.15 nmol/mg lipid, respectively, $P<0.05$). Radioiodine statistically significant depressed both basal and TSH-stimulated 125-I uptake by 30,3% and 59,3%, respectively. The data presented above indicate that lipid peroxidation processes and disturbance of transmembrane iodide transport play a pathogenetic role in radiation injury of the thyroid gland exposed to radioiodine in a dose of 5 Gy.

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THE EFFECT OF THYROIDECTOMY AND THYROXINE ADMINISTRATION ON THE RAT UTERUS.

K.O. ADENIYI, K. AMADI, E.J.C. NWANA AND J.A.M. OTUBU

We recently reported that thyroidectomy decreases and chronic thyroxine administration increase the frequency of uterine smooth muscle contraction (Adeniyi et al Pathophysiology 1, 151-154, 1994). The present study is therefore a further extension and an attempt to elucidate the author's earlier work. Rats were divided into four groups, Normal thyroidectomized, thyroidectomized rats treated with thyroxine and thyroxine-treated. The normal and thyroidectomized rats were fed on rat cubes and tap water ad libitum for a period of 45 days while the thyroidectomized rats treated with thyroxine and thyroxine-treated rats were given in addition to rat cubes and tap water, thyroxine (AH Cox and Co. Ltd. Barnstaple England) 6-8mg/100g body weights per day for 45 days. at the end of 45 days rats were killed by cervical dislocation and the uterine horn was removed quickly. Samples were fixed, stained with haemotoxylin and eosin and viewed under the microscope. 150metric contractions were monitored on uterine segments via a force-displacement transducer (Grass FT 03) and recorded on a Grass polygraph (model 7D, grass instrument, quincy MA). The results show that the uteri of the thyroxine-treated rats were markedly shrunken in size. Their endometrial, muscle layers and gland size were all reduced, while the force of contraction of the uterine smooth muscle was potentiated. The thyroidectomized rats had cystically dilated glands which degenerated from columnar to cuboidal epithelium. The overall picture is that of mild cystic endometrial hyperplasia with negative inotropic effect on the myometrium.

It was concluded that thyroid hormones influence the spontaneous contraction of the uterine smooth muscle via their effects on the morphology of the uterus.

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THE MORPHOLOGICAL AND FUNCTIONAL ASPECTS OF THE ENDOCRINE AND EXOCRINE INTERCOMMUNICATIONS UNDER VARIOUS FUNCTIONAL CONDITIONS OF THE PANCREAS.

Our research work is concerned with the study of the dynamics of secretory and incretory processes, as well as with ionic exchange in the pancreas under its normal conditions and in the course of the experimental pancreatitis and diabetes. Investigated were the influences of secretin and cholecystokinin on the dynamics of insulin and glucagon secretion, on the carbohydrate metabolism and the mechanisms of ionic transport in the pancreas under the experimental conditions of pancreatitis and diabetes. Under these conditions were also studied: the morphofunctional parallel of the cellular elements, the capillary circulation in the pancreas and the endocrine-exocrine intercommunications on the structural level. The results of our investigations testify to the existance of intimate functional intercommunications between the endocrine and exocrine apparatus of the pancreas. These intercommunications are realized on different morphological levels with the aid of the nervous and humoral mechanisms of regulation. The nature of these intercommunications lies in the fact that they change under experimental pathology conditions of the pancreas and depend directly on the stage of the pathological processes.

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ACTION OF SOME MODULATORS ON THE THYROID GLAND.

L.V.Gerbilsky, A.P.Rogachevsky and E.M.Staroseletskaya

Because the problem of children thyroid gland pathology will be one of the most important problems of theUkrainian people health, it is needed to study the possibility of directional change of thyroid function and structure by different modulators. That is why we are studied effects of some modulators on the rat thyroid function and structure. It has been studied in vivo and in vitro by means of radiometry method and method of light and electron microscopy.

After administering angiotensin accumulation of radioactive iodine by the thyroid is inhibited. Angiotensin produces the contraction of thyroid arterioles. The cytoplasma of endotheliocytes of perifollicular exchange microvessels becomes dense and luminar surfaces become convoluted. Hydrocortisone, ammonium chloride, pepstatin and contrycal have been studied for their effect on cultured fragments of rat thyroid. The radioactive iodine uptake by thyroid cells is inhibited by hydrocortisone, ammonium chloride and pepstatin. Pepstatin induces formation of vacuoles with nonhydrolysed thyroglobulin in thyrocytes. Heparin inhibites accumulation of radioactive iodine by the thyroid glands. Our findings also show the decrease of degree of rat thyroid intraorgan integrity by the sodium fluoride influence. This influence is shown by the oppressionof functional condition of thyroid gland.

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EFFECT OF DIET ON ADRENAL GLAND IN SAND RAT PSAMMOMYS OBESUS. Y DAHMANI. , F. HADJ BEKKOUCHE. and N. OMARI.

Sand rat is a desertical diurnal rodent that develops an obesity and a non-insulin-dependant diabetic syndrome, when it is submitted to a laboratory chow feeding.
The former works in this animal, have urged us to do a histological and histochemical study of adrenal gland at different stages of the diabetes mellitus.
Our study concerned 15 sand rats divided in two shares:
- a group of 6 animals received a natural feeding based on halophile plants as control group.
- a second group of 9 animals received a laboratory chow feeding and water salted (0,9%).
The laboratory chow feeding, considered as hypercaloric for sand rat, induced an obesity characterized by a weight increase, a normoglycemia and an hyperlipemia. Some among these animals developped diabetic manifestations, with a weight increase (43%), a normoglycemia (53,8 ± 2,6 mg/100ml) an hyperinsulinemia (83%) and an hyperlipemia essentially with the triglyceridemia.
Some among these animals developped diabetic manifestations with a weight increase (53%), an hyperglycemia (260,9 ± 35,7 mg/100ml), an hyperinsulinemia (103%) and an hyperlipemia. These metabolic disorders translate histological and histochemical modifications of the adrenal gland.
This gland presents a general architecture different from that in normal, sand rat, with increase of the zona reticularis and fasciculata . Important alterations are also observed in these diabetic animals medulla comparatively to the control sand rat.
Futhermore, the study of the diabetic sand rat adrenal cortex reveals a Periodic reaction Acid Schiff very positive and an important Soudanophilly essentially in the reticulary zone.
From our study it follows that the metabolic dysfunctions induce histological and histochemical modifications of the obese and diabetic sand rat's adrenal gland.

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BIORHYTHMICITY OF SOME NEUROMOTOR FUNCTIONS IN RELATION WITH OCCUPATIONAL STRESS. I.Anghel, Mihaela Anghel, V.Cojocaru, P.Derevenco, S.Tigan

The aim of the present investigations was to follow up the effects of occupational stress on the circadian variations of certain neuromotor functions. For this purpose we took under study groups of students (n=17), telephone operators(18),controllers at electric power station(6)and operators at an computing center (21). The subjects were investigated in three shifts and the students were studied during the daytime and the night. Reaction time to optic (ORT) and acoustic (ART) stimuli, sensory-motor coordination (SMC) attention (A) and subjective state, were measured. The data obtained show a noctural decrease of the efficacy of the functions, depending on the intensity of the load and the amplitude of the stress and on the functional subsystems prioritarily involved.The finding is of particular importance because all the subjects were submitted to neurosensorial strain,with certain peculiarities. A more marked noctural decrease of A was noted in students, of SMC and of ART in telephone operators, and of ORT in all operators. The probands complained a marked nocturnal discomfort. Vesperal type subjects showed a more rapid adaptation to nocturnal stress. The correlation coefficients between the parameters depended on the degree of functional deterioration in the 3 shifts(0.19-0.71). In sum, the changes in the circadian biorhythmicity of functions represent a maladaptive component to the occupational stress.

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STUDY OF Q-T INTERVAL AND ELECTROMECHANICAL SYSTOLE OF PROFESSIONAL DIVERS DURING SIMULATED DIVING AT DIFFERENT PRESSURE WITH VARIOUS RESPIRATORY MIXTURE. N. Ceamitru, G. Badiu, O. Teren, Luminita Badiu, Nicoleta Ceamitru.

Diving represents a non physiological condition for human being and a very complex stress (high pressure, hyperoxia, temperature, emotional factors, etc.) and might cause several characteristic changes in the cardio-vascular system. This study was done to elucidate the influence of different high pressure (20, 10, 5.5, 3, and 1.5 atm abs) and various respiratory mixtures (nitrox and respectively heliox) on myocardial contractility. A number of 18 male professional divers in a good health condition was included in this study: a helium-oxygen mixture (11 divers) and a nitrogen-oxygen mixture (7 divers) was used in simulated diving. The Q-T interval, electromechanical systole, and heart rate were recorded by non-invasive methods, in according with Weissler method. During diving, as heart rate decreases, the Q-T interval of the electrocardiogram increases. Our preliminary results suggest that the Q-T interval and electromechanical systole are influenced predominantly by pressure and in different mode by the various respiratory mixtures.

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THE INFLUENCE OF HIGH PRESSURE AND VARIOUS RESPIRATORY MIXTURES ON EJECTION FRACTION OF PROFESSIONAL DIVERS. G. Badiu, N. Ceamitru, Luminita Badiu

Hyperbarism represents a non physiological condition for divers and a very complex stress. The purpose of this non invasive investigation was to elucidate the influence of high pressures and various respiratory mixtures on cardiac output and contractility, estimated by means of the ejection fraction. Fifteen healthy professional divers were involved in the simulated saturation diving with nitrox (seven males) and heliox (eight cases) respiratory mixtures and different pressures (1.5 - 20.0 atm abs). The phases of a cardiac cycle and Ej F were determined according to Weissler (1968) and Harris (1974): Ej F = 1.125-1.25(PEP/LVET). The especially high level pressures and probably the various respiratoty gases influence the ejection fraction and left ventricular contractility in different phases of saturation (predive, compression, immediately and after 48 h and decompression) and the myocardium makes special efforts (Starling's type mechanism) in order to maintain its cardiac output.

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ARE ANTHROPOMETRIC AND ECHOCARDIOGRAPHIC PARAMETERS IN ATHLETES ALWAYS CORRELATED? S. Cameli, R. Vannicelli, A. Dello Russo, R. Corsetti, V. Palmieri, A. Gianfelici, E.B. Adamo*, R. Marini*, and P. Zeppilli.

Normalization of data for body surface area (BSA) in commonly utilized in comparing heart and great vessels dimensions athletes of different sports and sedentary people. The aim of present study is to assess if a true correlation between echocardiographic and anthropometric (ANT) parameters really exists in athletes of different sports.

MATERIAL AND METHODS. Left ventricular mass (LVM), by the formula of Deveraux; total cardiac volume (TCV), by a formula which imitates that utilized by Mushoff to calculate heart volume on chest X rays; aortic arch (AA) and inferior vena cava (IVC) size, were measured by two dimensional echocardiogram (ECHO) in: 20 healthy non smokers sedentary subjects (SED) (mean age 25.3 yrs., mean BSA 1.9 m^2); 15 professional cyclists (CYC) (mean age 23.8 yrs., mean BSA 1.8 m^2); 15 professional volleyball players (VP) (mean age 21.7 yrs., mean BSA 2.2 m^2).

STATISTICAL ANALYSIS. Matrix correlation coefficients (r) between heart and vessels dimensions and BSA, height (H) and weight (W), and between the cardiovascular parameters themselves, were calculated in every group: the Pearson's moment product were used to compare data, and 95% confidence intervals were also given. A p value<0.05 was considered statistically significant.

RESULTS. In SED, significant correlations for TCV vs BSA (r = 0.74; p<0.002), W (r = 0.74; p<0.0001) and H (r = 0.44; p<0.04) were found. No correlations were found in CYC significant correlations between TCV vs LVM (r = 0.63; p<0.01) and IVC (r = 0.51; p<0.05), and LVM vs AA (r = 0.62; p<0.01) were found. Finally, no correlations were noticed in VP either between cardiovascular and ANT parameters or cardiovascular parameters among them.

CONCLUSIONS. Correlation between heart and great vessels dimensions and ANT parameters resulted lesser than usually believed. Only in SED, TCV was correlated with BSA, W and less with H, while in CYC a correlation was found only for LVM vs H. In VP, which are very tall and have very large BSA, neither cardiac dimensions nor vessels size were correlated with body dimensions. Normalization of ECHO parameters for BSA, H or W, should not be considered appropriate for every studies comparing dimensional heart and vessels changes induced by training.

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A 6-MINUTE WALKING TEST FOR MEASUREMENT OF MAXIMAL O_2 CONSUMPTION IN MIDDLE-AGED FEMALES. T. Jürimäe, M. Matlep, K. Tammik

The aim of this investigation was to present an optimal duration- (or distance-) walking test in order to measure maximal O_2 consumption (VO_2 max) in middle-aged (40-50 years old) females. In total 20 females were studied (43.5 ± 3.8 yrs, 164.2 ± 5.6 cm, 61.5 ± 10.7 kg, BMI 23.0 ± 2.9). They were moderately physically active. Their body height and mass were measured and the body mass index (BMI) was calculated (kg/m^2). The body fat percentage was measured using the Bodystat-500 (UK) bioelectrical impedance method. A 30 minute walking test at maximal speed was carried out on the indoor track (150 m). The time for covering and the subject's heart rate (HR, Sporttester PE-3000, Kempele, Finland) were fixed at 500 m, 1000 m, 1500 m, 2000 m, 2500 m and 3000 m. Furthermore, the covered distance and the subject's HR were measured with one minute interval starting from the 5th minute of the test. The VO_2 max was measured directly using stepwise increasing loads on a cycle ergometer. The UKK 2 km walk test (P. Oja et al. 1991) index was calculated. Our subjects' mean body fat percentage was 25.2 ± 5.2 ; VO_2 max/kg was 32.8 ± 5.5 $ml \cdot min^{-1} kg^{-1}$ and UKK 2 km walk test's index 109.6 ± 10.2. The correlation coefficient was the highest between VO_2 max/kg and the distance covered within 6-minutes (r = 0.581, VO_2 max/kg = 0.054 (m) - 5.1). There was a close relationship between the distance covered within 6 minutes and the UKK 2 km walk test index (r = 0.843). The results indicate that the 6-minute walking test is appropriate for the measurement of maximal O_2 consumption in middle-aged females.

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RECOVERY AFTER EXERCISE AT 20 °C AND 35 °C IN THE HORSE S. Nyman, A. Jansson, K. Morgan, C. Palmgren-Karlsson, A. Lindholm, J.E. Lindberg and K. Dahlborn

The aim of this study was to evaluate the effects on thermoregulation and fluid balance during the recovery phase after exercise at 35 °C. Like man, the horse dissipates heat mainly by sweating which can result in great fluid losses. Four trained standardbred geldings (3-8 yrs old, mean body weight 476 kg) were used. The horses performed an exercise test at two temperatures, 20 and 35 °C (RH 30-40%) on a treadmill with an incline of 2.5 %. The test consisted of two exercise phases with 2 hours of box rest (at 20 °C in both treatments) in between. Phase 1 lasted for 23.5 min (submaximal intensity) and phase 2 for 26 min (submaximal intensity including a 4 min near maximal run). This exercise program simulated the warming up and race for a trotter racing over a distance of 2600 m. After each exercise phase the horses were showered for 3 min in body warm water before they returned to their stables (at 20 °C in both treatments). The body weight losses caused by sweating and respiratory evaporation were higher after exercise at 35 °C (11.8 ± 1.6 kg) compared to 20 °C (7.7 ± 1.6 kg) as well as the sodium losses in sweat which were 49 ± 8 g compared to 30 ± 4 g. The horses had higher rectal and blood temperatures, and a higher respiratory rate after the exercise program in 35 °C. The rectal temperature was elevated longer after running in 35 °C (after 20 minutes, 40.1± 0.2 °C compared to 39.2± 0.1 °C at 20 °C). Between ten and twenty minutes of recovery after exercise at 35 °C the respiratory rate increased to 101±3 while it had decreased to 44±14 after exercise in 20 °C. This study shows that there was a prolonged recovery phase even after mild climatic heat stress.

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DOES THE DEGREE OF EXCITEMENT AFFECT EXERCISE FATIGUE IN THE HORSE? A. Jansson, S. Nyman, A. Lindholm, J. E. Lindberg, C. Palmgren-Karlsson and K. Dahlborn

It is well known that extreme excitement in a horse during a race leads to an impaired exercise performance. However, the reason for this is unknown. The observations reported here were made in a study consisting of two submaximal treadmill exercise tests (23.5 min) performed at 35 °C (30-40% RH) with a 4 week interval. Four healthy standardbred geldings A, B, C and D were used. Horse A was always bustled during exercise. However, at one of the exercise tests this horse had to be roughly held back in order to prevent it from running faster than the treadmill. At the end of this test horse A had exercised to fatigue and had to be verbally encouraged to keep pace with the treadmill. A mean value, including both exercise tests, was calculated from all horses except horse A which was analysed individually, called AE (excited) and AN (the other time). During exercise blood temperature increased to 41.2±0.2 °C, plasma potassium from 3.8±0.1 to 5.3±0.1 mmol/l and lactate from 0.7±0.1 to 5.6±0.1 mmol/l in B, C and D. The levels of blood temperature and lactate and were markedly elevated during AE (42.7 °C, 9.3 mmol/l) compared to AN (42.2 °C, 7.9 mmol/l) and to the other horses. Plasma potassium was elevated in AE compared to AN (5.4 and 4.9 mmol/l respectively). The increase in blood temperature and plasma potassium indicates that the workload was heavier in AE than in AN. The increase and the delay in plasma lactate appearance observed in AE may be an indication of lowered muscular blood flow, suggestively due to static exercise. The plasma cortisol level was lower during exercise in AE and AN compared to the other horses but increased 25 minutes post exercise AE and AN while it decreased in the other horses. In this study the increase in plasma lactate and potassium is not considered high enough to cause symptoms of fatigue. It is more likely that the body temperature was the limiting factor, since AE had the highest blood temperature recorded.

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ELECTROLYTIC AND BIOCHEMICAL RESPONSE IN ANDALUSIAN HORSES SUBJECTED TO MAXIMAL EXERCISE. Agüera, E.I.; Castejón, F.M.; Santisteban, R.; Escribano, B.M.; Muñoz, A.; Rubio, M.D.

The acid-base equilibrium may be influenced by the concentration of proteins and the presence of other anions such as lactate (Geisser et al., 1994). This led us to analyze the changes caused in the aformentioned blood parameters as a result of subjecting 8 Andalusian horses to a progressively increasing intensity of exercise (4.17; 5.56; 6.94 and 8.33 m/sec.), until exhaustion (9.84 ± 0.31 m/sec). Blood was extracted from the external jugular vein, at rest, on finishing each exercise level and at 2, 4, 6, 8, 10, 20 and 30 minutes of finalizing the whole exercise.

The increase of lactate which occurred in the intense exercise coincided with the other authors consulted (Karlsson, 1986; Wilson et al., 1983; Auvinet and Demonceau, 1992) and as in other previous papers (Agüera, 1994) the anaerobic threshold was not exceeded until the animal was subjected to a speed of 6.94 m/sec (7.95 mmmol/l), with a maximum accumulation (25.11 mmol/l) at 4 min. after finalizing the exercise, with the significant differences ($p<0.001$) being maintained during resting, as from a speed of 6.94 m/sec and throughout the recovery time.

A drop in the pH (7.34) was produced with intense exercise. The difference between the exercise and rest period was significant ($p<0.01$) as from a speed of 8.33 m/sec and these differences were maintained without regaining the initial value. Every acidosis involves a hyperkalaemia as became highly apparent in this paper, when the accumulation of lactate was greater, coinciding with the maximum speed reached by the animal (5.18 mmol/l). As was observed in Andalusian stallions (Rubio et al., 1995), strenuous exercise produced an increase in the Na^+ and K^+ cations and a fall in the Cl^- anion, with an initial value of 101 mmol/l to 94.14 mmol/l at 20 min. of finalizing the exercise. Both the increase in Na^+ and the decrease in Cl^- were seen to be significant, coinciding with the greater concentration of proteins indicating a hemoconcentration caused by sweating.

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COMPARISON BETWEEN FITNESS AND SLOPE OF THE LACTATE vs. VELOCITY CURVE IN ANDALUSIAN HORSES. Muñoz, A.; Castejón, F.M.; Rubio, M.D.; Vivo, R.; Agüera, E.I.; Santisteban, R.

This study was made in order to assess the importance of the slope in the lactate *vs.* velocity during an exercise test of progressively increased intensity, as well as the influence of the intersection with the axis X and Y in the evaluation of fitness and athletic capacity in the Andalusian horse.

Seven 4 and 5 year-old Andalusian horses were subjected to an exercise test. During this test, succesive recordings of heart rate and venous blood samples were obtained in order to determine PCV, blood pH and plasma lactate concentrations.

Statistically significant differences have only been found between the slope of the horses nº 2 and 7, with a lower value in the fittest horse, according to the informations supplied by the jockeys. More significant differences were observed in the X intercept, that fittest horses showed a more removal of the OX point from the coordinate origin in negative direction.

The slope has not shown any significant correlation with the different functional indexes (VLA_2, VLA_4, PLac, maxV, maxPCV, minpH and maxHR). However, strong negative correlations were observed between VLA_2 ($r=-0.977$ ***), VLA_4 ($r=-0.887$ ***), minpH ($r=-0.805$ **) and Y intercept.

According to these results and, although the slope of the lactate *vs.* velocity curve has been proposed in people as a functional index, in the Andalusian horses, this does not seem especially useful, at least in animals subject to the same exercise routine during a defined period of time and finally, which undergo a similar grade of training.

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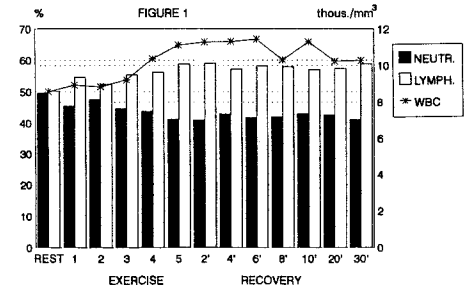
ALTERATIONS PRODUCED IN THE LEUCOGRAM OF ANDALUSIAN HORSES SUBJECTED TO EXERCISE OF PROGRESSIVE INTENSITY. Escribano B; Castejón F; Agüera EI; Muñoz A; Riber C; Rubio MD.

In this study, the alterations caused in the white blood cells of 8 Andalusian horses on being subjected to a maximal effort, after various levels of a progressively increasing intensity of exercise (4.17; 5.56; 6.94; 8.33; 9.84 m/s) were analyzed. All the horses were male and belonged to the Centre of Selection and Training in Jerez de la Frontera (Spain).

The blood was deposited in tubes containing EDTA-3K and was analyzed in a Sysmex (F-800) semiautomatic counter. During resting, the number of leucocytes (Fig. 1) in seen to be similar to that obtained by other authors (Oropesa, 1991; McClay et al., 1992; Weiss et al., 1992). Strenuous exercise caused leucocytosis which manifested itself significantly after the maximal effort. This leucocytosis was maintained during the recovery period, not reaching the resting levels at 30 min. after finishing the exercise, which was in agreement with the authors consulted who reported that from 24 to 48 hours were necessary for recovery. As is shown in Figure 1, leucocytosis is manifested at the expense of lymphocitosis, also significant ($p<0.003$), after the maximum speed and throughout the recovery period. As a result, the N/L relation is inverted and is maintained at under 1, which is indicative of intensity exercises (Rose et al., 1983), during the exercise and the recovery period.



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EVALUATION BY FUNCTIONAL INDEXES OF PHYSICAL POTENTIAL IN ANDALUSIAN HORSES. Muñoz, A.; Santisteban, R.; Rubio, M.D.; Agüera, S.; Escribano, B.M.; Castejón, F.M.

The objective of this research was the evaluation of physical potential in 8 Andalusian horses rangue between 4 and 5 years. Animal performed an exercise test consisting of 2 stages of different intensity. The first stage was of submaximal intensity at 4 speeds progressively increased and the second stage was of maximal intensity. Data on heart rate, plasma lactate concentration, PCV, blood pH and maximum speed were obtained. In relation to these data, 7 functional indexes were considered in the assessment of physical potential: VLA_2, VLA_4, PLac (peak lactate), maxV, maxPCV and minpH.

The information provided by the jockeys about the fittest horse were in agreement with the results of the present paper. This horse showed higher values than the other animals in the two indexed derived from the plasma lactate concentration: VLA_2 and VLA_4. Conversely, lower than the values of the other 7 horses, which may indicate a higher aerobic capacity in the realization of the exercise test. Owing to these lower lactate concentration, a drop in the blood pH value was observed less clearly in this horse, which also showed highest data for maxHR and maxV. Finally, in the horse considered the fittest, narrow variation limits for PCV were observed. The maxPCV was 47.5%, considerately less than the maxPCV of the other horses, which achieved up to 59%.

Due to the great interindividual differences observed in the 7 functional indexes studied and their strong correlation with the subjective estimation of the jockeys, this research may conclude that these indexes are useful as functional markers in the Andalusian horse.

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STUDY OF THE ERYTHROGRAM IN ANDALUSIAN HORSES BEFORE AND AFTER MAXIMAL EFFORT. **Rubio, M.D.; Escribano, B.M.; Agüera, E.I.; Muñoz, A.; Fernández, P.; Castejón, F.M.**

The main aim of this paper was to establish the alterations produced in the red blood cells of Andalusian horses subjected to a progressively increasing intensity of exercise until exhaustion.

An erythrogram (RBC, PCV, Hb, MCV, HCM and CHCM) was made of 8 male Andalusian horses of between 4 and 5 years of age, while resting and after being subjected to speeds of 4.17; 5.56; 6.94; 8.33 and 9.84 ± 0.31 m/sec. and at 2, 4, 6, 8, 10, 20 and 30 min. after finishing the exercise. All the horses were subjected to a training programme (described by Agüera, 1994) habitually used at the Centre of Selection and Training of the Military Stud at Jerez de la Frontera (Spain) to which they belong.

Starting from similar resting values of those obtained in previous studies for stallions of the same breed (Rubio et al., 1994), after the first exercise levels a rise in the erythogram occurred, except for the MCV, which was more significant for Red Cells and Hemoglobin. When the animals were subjected to the third exercise level (6.94 m/sec.), there were PCV (41.9%), which involved a diminution in the MCV during all the stages of the test. However, the initial values (46 fl) were within those reported by Marbach et al., (1978) as being optimal for racing and it was the only parameter of the erythrogram which regained its initial value at 30 min. of finalizing the exercise.

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THE EFFECTS OF ALCOHOL AND EXERCISE ON BLOOD PARAMETERS AND ERYTHROCYTE OSMOTIC FRAGILITY IN RATS. Vila L.; Ferrando, A.; Voces, J.A.; Alvarez, A.I.; Prieto, J.G.

The effects of ethanol and exercise were studied at 4, 8 and 12 weeks in blood obtained via micropunction in the retroorbicular plexus with heparinized microcapilaries

Four groups of animals (Male Wistar rats) were used. Two experimental groups were treated with ethanol and two were used as control. One experimental and one control group were exercised. The ethanol (15%) was added to drinking water for 12 weeks. The rats were exercised along 12 weeks using a treadmill.

The following investigations were carried out: total red cell count (RBC) was determined by chamber method, haemoglobin level (Hb) by the cyanmethaemoglobin method and haematocrit by the micromethod. The mean corpuscular volume (MCV) and corpuscular haemoglobin concentracion (MCHC) were calculated.

The erythrocyte osmotic fragility was determined by the method of Detraglia et al. where X_{50} is the mean erythrocyte fragility and ß is a measure of the breadth of erythrocyte distribution.

Our results of X_{50} does not present statistic differences. The parameter ß present a significant decreased respect to control in all groups estudied.

Elevated serum LDH and CPK are closely related with alcoholic myopathy, LDH and CPK were obtained at the end of each month of treatment. LDH was increased after the first month with the higher values in ethanol and exercised groups. The values decreased in the following months. The highest CPK serum values were obtained in ethanol treated animals without exercise.

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THE DIURNAL VARIATIONS OF FIBRINOLYSIS IN RATS EXPOSED TO " ALIENATION" STRESS. M.F.Artino, R. Cârmaciu, A. Badarau, A. Iancu, E. Huidovici. Previous studies have already revealed the non-specific activation of fibrinolysis by many kinds of stress reactions including exogenous hyperthermia, hypokinesia, reduced gravity acceleration. We are also aware of the circadian rhythm of the fibrinolythic system.The purpose of the present paper is to establish whether the stress-activated plasma fibrinolythic activity (PFA) has any influence on physiological diurnal variations of the PFA.

The experiments were carried out on 300rats transfered in our laboratory from an outside source , therefore " alienated" by a sudden change in their habitat.The PFA was measured as Euglobulin Lysis Time , (E.L.T.) using the von Kaula test . The E.L.T. measured immediately after the animals were brought into their new habitat was reduced, showing minimal values at about 8 30 in the morning .After at least 1 week of adaptation to the new environment, the E.L.T. mean values regained their usually normal level and the normal "pattern" of the diurnal variations, with minimal E.L.T values registered during the afternoon.Our results suggest that this kind of "alienation" stress can alter significantly the biorhythm of the fibrinolythic system in rats ;one must allow for an adaptive period before the normal pattern is regained. Whether or not humans are equally responsive to this kind of stress remains to be seen .

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THE EFFECTS OF INTENSIVE RESISTANCE TRAINING ON THE MYOSIN HEAVY CHAIN ISOFORM EXPRESSION OF HUMAN SKELETAL MUSCLE. J. Jürimäe[1,2] and P.J. Abernethy[1]

The effects of bodybuilding type resistance training on the myosin heavy chain (MHC) isoform content of the triceps brachii muscle were studied in university students (X±SD; 19.5±1.0 years). Fifteen healthy previosly untrained men were assigned to a training (n=11) or a control (n=4) group. After a one week orientation period, the training group exercised 3 times/week for 12 weeks. The training sessions involved the lifting of 70-75% of the subjects' one repetition maximum (1RM) in 4 sets of 8-12 repetitions. The rest period between sets was no longer than 90 seconds. Exercises included triceps pushdown, close grip bench press, triceps kickbacks and biceps curl. Muscle biopsies were taken prior to and following training from the triceps brachii muscle of the non-dominant arm. The percentages of MHC isoforms were analysed with 6% SDS-PAGE electrophoresis from muscle homogenates. In addition, arm circumference and maximum isoinertial strength (1RM) of triceps pushdown were measured. Comparisons between groups and time points were made using a nonparametric Friedman ANOVA for repeated measures. The trained subjects presented a significant ($p<0.05$) decrease in MHC IIb isoforms (39.7±9.2 to 29.2±8.2%), while there was a non-significant increase in MHC IIa (34.0±9.9 to 41.5±10.4%) and MHC I (26.3±7.9 to 29.3±9.6%) proteins. However, no significant changes were found for the control group in the MHC IIb (34.1±6.4 vs 33.3±5.5%), MHC IIa (36.9±7.3 vs 35.5±4.6%) or MHC I (26.6±5.2 vs 27.0±2.6%) isoform composition. There were significant ($p<0.05$) increments in maximum isoinertial strength (41.0±8.3 to 57.0±9.1 kg) and arm circumference (34.9±3.2 to 36.1±3.0 cm) in the training group. No significant changes were monitored in either the maximum isoinertial strength (31.9±7.6 vs 34.1±9.6 kg) or the arm circumference (32.9±3.3 vs 32.8±3.3 cm) for control subjects. These data indicated that 12 weeks of bodybuilding type resistance training increased maximum strength and arm circumference, and decreased MHC IIb isoform content. The MHC isoform data illustrate the plasticity of muscle tissue in response to chronic exercise.

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OPPOSITE EFFECT OF ADAPTATION TO EXERCISE ON Ca-UPTAKE IN SARCOPLASMIC RETICULUM AND ACTIVITY OF ANTIOXIDATIVE PROTECTION ENZYMES IN THE HEART AND SKELETAL MUSCLES. T.G.Sazontova, N.E.Golantsova, F.Z.Meerson and Yu.V.Arkhipenko

Rats were adapted to swimming (daily for 45 days); the duration of the swimming session was increased from 15 to 60 min during the first 2 weeks at 32 ^{0}C. In the heart, the efficiency of the Ca-transport system of sarcoplasmic reticulum (SR) increased with a decrease in the activities of catalase and superoxide dismutase. This was accompanied by an increase in the initial rate of Ca^{2+} transport, lack of inhibition of the Ca-transport system by high concentrations of the cation (compared to control) as well as by a 1.5-fold increase of resistance to induced lipid peroxidation (LPO). In contrast, in skeletal muscles the catalase and superoxide dismutase activities were sharply increased without any rise in the efficiency of the Ca-transport system. There was a decrease in the rate of Ca^{2+} transport, the inhibition of Ca^{2+} transport under LPO was not retarded, while the resistance of the Ca-transport system to high concentrations of this cation decreased 1.7-fold. These findings may be indicative of LPO activation in skeletal muscles (as opposed to the heart) which, in turn, initiates further activation of the antioxidant protection enzymes, however, in a degree insufficient to provide complete protection of the Ca-transport system of SR from free radical damage.

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STUDY OF CONTRACTILE PROPERTIES OF SKELETAL MUSCLE IN THE COSMOS EXPERIMENTS.
M. Rapcsák, V. Oganov and Á. Szöőr

In model experiments we have tested in detail, as to how changes the rat skeletal muscle's contractile characteristics through muscle atrophia. We determinated, that by using these methods the established atrophia is different tonic and tetanical in rat skeletal muscles. The biggest weightloss and contractile strenght decrease was observed in the case of m.soleus consisting I.type fibers, which accordingly the limitation of motion causes primarily the reduction and the weakening of the tonic muscle fibers. On the Cosmos-1514, 1667, 5 and 7 days, on the Cosmos-1887, 2044, 12.5 and 15 days and the Cosmos-1129 18.5 days were in weightless state. From the skeletal muscles of the rats that were participating in the spaceflight, in the case of fiber preparations the significant decrease of produced contractions was observable with ATP-Ca2+ 5, 7, 12, 15 and 18.5 days in flight. This is very much significant by the m.soleus, which is mainly composed of slow fibers. In the other muscles it was smaller degree. The contractile character of the m.soleus changes during short and long spaceflights. After 5 and 7 days of weightlessness there is a decreasing tendency showing in the speed of the contraction of the m.soleus, while after 12.5 days of flight, the speed of the m.soleus does not change, after a long flight there is a small increase in the m.soleus compared to the control. The m.EDL contraction speed did not significant change the 5, 7, 12.5 days in the flown and synchron groups.

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NONUNIFORM NATURE OF STIMULI ACTIVATING MUSCLE ERGORECEPTORS DURING VOLUNTARY STATIC EXERCISE
I.Kukulis, J.Skards, V.Dzerve, A. Paeglitis

Many aspects of the nature of stimuli activating III-IV group muscle afferents and modulating the pattern of sympathetic activation are unclear yet. The aim of this study was to analyse the relationship between metabolic changes in contracting muscle and pressure response during static contraction of forearm with different relative forces. 11 healthy volunteers performed handgrip (H) with 5%, 10%, 15% and 50% of maximal force of voluntary contraction (MVC) till exhaustion. During contraction and recovery period (RP), blood flow (BF), pO_2 and lactate (LA) in arterialised and venous blood from deep forearm veins were determined. Total LA production was calculated from LA release and slow fraction (T 1/2=4,5 min.) of O_2 debt repayment during RP. Mean blood pressure (Pm) was recorded at rest, during H (PmF), and after cessation of H with arrested circulation (PmR) and free BF. Results (mean values ± SE):

	5% MVC	10% MVC	15% MVC	50% MVC
Time, min.	62	41	13	1,8
ΣO_2 debt mM/dm^3	1,7 ±0,21	2,14±0,3	3,48±0,32	6,34±0,52
ΣLA mM/dm^3	0,17±0,02	0,20±0,02	0,39±0,04	1,41±0,21
ΔPmF, %	43±5,1	49±3,3	53±2,9	54±2,3
ΔPmR, %	19±3,1	22±2,9	23±2,1	24±1,3

Time till exhaustion, O_2 debt, total LA production during H with 5% and 10% MVC differ extremely from those after H with 50% MVC. PmF and PmR don't differ significantly. Provided PmR reflects metabolic changes, LA seems not to be the only factor activating III-IV group muscle afferents, some other non-identified factor related with aerobic energetic metabolism could exist.

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PHOSPHOCREATINE DEPLETION AND ACTIVATION OF ANAEROBIC GLYCOLYSIS IN ISCHEMIC AND CONTRACTING FOREARM SKELETAL MUSCLES. D.Matisone, J.Skards, A.Paeglitis, V.Dzerve

The aim of this study was to ascertain the amount of depleted phosphocreatine (PCr) critical for activation of anaerobic glycolysis in forearm muscles during arterial occlusion (AO) of 1-30 min. and during contraction with 10% of maximal force of voluntary contraction (MVC) till exhaustion. Blood flow, arterio-venous difference of O_2 and lactate (LA) during contraction and recovery period and reactive hyperemia were determined. O_2 consumption (VO_2) and LA release were calculated by Fick principle. PCr debt and accumulated LA in muscles were calculated from VO_2 fast and slow fraction during recovery period using stochiometric equations where P/O=3.
It is stated that during ischemia PCr debt in forearm skeletal muscles increases proportionally to the duration of AO, whereas LA release increases proportionally to that in resting muscles only till the 7th min. of AO when PCr debt is 3,6 ±0,22 mmol ATP/dm^3. A further increase of AO evokes an exponential increase of LA release. During static contraction with 10% MVC, a long term (40 min.) steady state between blood flow , VO_2 and LA release is observed. Amount of depleted PCr increases proportionally to the duration of contraction and at the end of contraction when all muscle fibres are exhausted, it reaches 3,25 ±0,29 mmol ATP/ dm^3 corresponding to that observed after 6 min. of AO. Amount of released and accumulated LA during contraction testifies that anaerobic glycolysis is not activated. Our results show that depletion of PCr to definite level at which activation of anaerobic glycolysis doesn't occur, provides a long term aerobic work capacity of muscles, but during a short term ischemia (up to 30 min. AO) PCr acts as an energy store in muscles.

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ENHANCEMENT OF LIPID OXIDATION BY HEART RATE MONITORED EXERCISE. A.Kononoff, K.Manninen, H. Pekkarinen, H.Litmanen, O.Hänninen

Obesity is a major health problem in western societies. Every second middle aged Finn is overweight. Obesity is related to diseases such as high blood pressure, adult onset diabetes, and coronary heart disease. Much of the weight problem is due to diminished physical activity. For those who are mildly or moderately overweight, exercise is recommended for weight control. We have studied both physically trained persons and sedentary subjects on a bicycle ergometer in order to define the optimum level of exertion to oxidize fatty acids. All subjects were on their usual mixed diet. The subjects worked on the bicycle until they reached 80% from their estimated age-related heart rate maximum. Six minute steps were used to ensure a steady state for respiratory quotient (RQ). The RQ, along with oxygen consumption, was used to calculate the proportion of fatty acids used as energy. The caloric transformation used was the thermal equivalent of oxygen for nonprotein respiratory quotient. During mild exercise the RQ reflects a mixture of nutrients used as substrates for energy metabolism. For the non-trained subjects the heart rate where fatty acid utilization seemed to be most effective was lower and the variation in heart rate narrower than for the more fit subjects.

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IMPROVEMENT OF SKELETAL MUSCLE GLUTATHIONE DEPENDENT ANTIOXIDANT STATUS WITH SPRINT TRAINING. M.Atalay, T.Seene, O.Hänninen and C.K.Sen

Endurance training has been shown to enhance the skeletal muscle antioxidant defense. Knowledge is scanty about the effects of sprint training on the antioxidant defense. In the present study, the influence of this regime on rat skeletal muscle antioxidant defense was studied. Male Wistar rats, 16-17 weeks old, underwent sprint training for 6 weeks. Total glutathione (TGSH) level and activities of glutathione peroxidase (GSHPx), glutathione reductase (GRD) and glutathione S-transferase (GST) in different skeletal muscle types were compared in trained and control sedentary animals. Sprint training significantly enhanced TGSH contents of the quadriceps femoris (QF) and the agonist-antagonist gastrocnemius (GS) and extensor digitorum longus (EDL) muscles. Compared with controls, GSHPx activities in GS and EDL were also higher in sprint trained rats. GRD activities increased significantly in EDL. Furthermore GST activity was higher in EDL following sprint training. These results suggest that sprint training increases skeletal muscle glutathione content and activates the glutathione redox cycle enzymes of the agonist-antagonist leg muscles GS and EDL, rich in fast glycolytic fibers.

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BRONCHIAL REACTIVITY TO METACHOLINE AND EXERCISE IN ATHLETES WITH AND WITHOUT EXPOSURE TO COLD AMBIENT TEMPERATURE IN TRAINING

H.Litmanen and H.Pekkarinen

High prevalence of bronchial hyperreactivity and asthma-like symptoms has been shown in cross-country skiers compared to normal controls and this has been linked to training and competing in cold ambient temperature. The purpose of this study was to examine whether athletes not exposed to cold would have lower occurrence of bronchial hyperreactivity and related symptoms compared to skiers. 23 top class cross-country skiers (12 males, 11 females) and 23 Finnish baseball players (13 males, 10 females), nonsmokers and without physician-diagnosed asthma, participated the study. A metacholine challenge test was used to assess non-specific bronchial reactivity. A whole body cold exercise challenge was performed as 10 minute treadmill running in a climatic chamber at -15 °C ambient temperature and flow volume spirometry was used for pulmonary function evaluation. The history of asthma-like symptoms related to exercise and/or cold as well as symptoms related to allergic disorders were collected at an interview. Increased broncial reactivity to metacholine (PC_{20} < 8 mg/ml) was found in 10 skiers and 16 baseball players (p=.07). The prevalence of symptoms did not differ between the groups. Exercise induced bronchoconstriction (EIB) i.e. the fall in FEV1 more than 10% after exercise was found in 4 skiers and 4 baseball players. Of these 7 had PC_{20} < 8 mg/ml (p=.001). No difference was found in exercise-related symptoms between athletes with and without EIB. Hence the occurrence of increased bronchial reactivity to metacholine and exercise and exercise related asthma-like symptoms was similar in athletes with and without training in cold ambient temperature. PC_{20} < 8 mg/ml was highly sensitive but unspecific for identifying athletes with EIB.

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RESPONSES TO MAXIMAL DYNAMIC EXERCISE WITH DIFFERING MUSCLE MASS IN YOUNG AND OLDER MEN. T.Aminoff, J.Smolander, O.Korhonen and V.Louhevaara

The physiological responses to incremental arm crank (1- and 2-arm) and cycle (2-leg) ergometer exercise were examined in 5 younger men (age 25-30 years) and in 6 older men (age 56-59 years). The measurements included anthropometric estimates of muscle mass, heart rate, gas exchange variables, rating of perceived exertion and blood lactate. When the two age groups were compared during 1- and 2-arm exercise, the results showed no significant differences in the maximal cardiorespiratory, subjective, and blood lactate responses. But during 2-leg exercise the younger men showed significantly higher maximal values in work rate, oxygen uptake and heart rate. The maximal oxygen uptake (l/min) (mean ± SE) during the three different exercises (1-arm, 2-arm and 2-leg) were 1.57 ± 0.1, 2.08 ± 0.3 and 3.59 ± 0.2 among the younger men, and 1.61 ± 0.1, 2.08 ± 0.1 and 2.93 ± 0.1 among the older men, respectively. Corresponding heart rates (beats/min) were 148 ± 14, 173 ± 13 and 183 ± 6 among the younger men, and 150 ± 15, 167 ± 9 and 168 ± 9 among the older men, respectively. In conclusion, physically active older men with a well-retained muscle mass had a similar maximal work capacity as younger men in work performed with smaller muscle groups. However, when working with a large muscle group, e.g. 2-leg bicycle exercise, the work capacity was reduced probably because of limits in the cardiorespiratory system.

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RESPONCES TO LONG-TERM STRESSORS IN RAT LINES SELECTED FOR EXCITABILITY OF THE NERVOUS SYSTEM. N.Shiryaeva, A.Vaydo, N.Djuzhikova, N.Lopatina

The phenomena and mechanisms of the results of long-term stress in four rat lines selected for threshold of the nervous system exita bility to electric impulses and differing in some functional properties of the nervous system and behavior were studied. The complex of methods for the estimate of consequences of stressors influences on different organization levels from gene to behavior were used. The stress comprised the random (pr=0.5) pairing of electric shock with light over 15 days.It was shown:(1)Two marginal lines are more sencitive to stress in comparison with middle excitability line.Its reactions have different quality.(2)The results of the stress is long-lasting (to six months).(3)The stress influences on chromosomal aberrations level in bone marrow cells.

Our data suggest: The character of stress reaction depend on exitability of the nervous system.The duration of stress results may be connected with disturbances of chromosome in neurons. It was disscussed the opportunity of examination of last supposition with help molecular genetic methods.

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CHANGES OF MICROTUBULE DENSITY IN CNS FIBRES DURING SLEEP. L.I.Kiyashchenko, E.A. Aristakesian and M.I. Eliava

It is well known that the number of microtubules is regulated in peripheral nerves under functional activation. Our previous investigations of long-term sleep deprivation in rats (water tank method) shown significant decrease of microtubules density in corpus callosum and striatum axons. The aim of our present study is to check the data in the course of stressless short-term sleep deprivation under EEG registration. Sleep was registered in 12 adult male Wistar rats (250-300g) with chronically implanted electrodes. EEG and EMG were polygraphically recorded throughout the 6 h baseline period. Then the total sleep deprivation (TSD) by manual arising under EEG control for 6 h at the same temperature and light conditions was performed in 6 animals (E). Another 6 animals were used as control (C). After the TSD we registered the rebound for 6h. Two weeks after the procedure we repeated the 6h TSD in E group of rats. At the end of the TSD both groups of rats were deeply anesthetized with the overdose of nembutal and perfused transcardially with saline (0.9% NaCl) followed by fixative - 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.4. Then the sections of mediodorsal area of caudate-putamen and medioventral parts of corpus callosum were studied by routine methods of electron microscopy to estimate the density of microtubules. In the myelinated axons of caudate-putamen and corpus callosum of the E group, almost complete disappearance of microubules was found compared to the C group. In nonmyelinated axons significant decrease of microtubules was observed, from 40% to 70%, depending on the fibre size. The microtubules density decrease observed after 6h of TSD may occur at the end of normal waking period. The date may be useful to examine the cellular basis of inhibitory and excitatory processes during sleep.

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ENDURANCE TRAINING AND SURFACE ELECTROMYOGRAM POWER SPECTRUM IN HUMAN MUSCLE. M.A.Pogodin, J.A.Donina

The aim of the investigation was to evaluate the endurance training using spectral electromyographic (EMG) modifications. 9 healthy subjects (5 males and 4 females) aged 18-41 yr volunteered for the experiments. Surface EMG activities were recorded from dexter biceps brachii muscles. EMG signals were analized using a spectrum analyzer (Robotron 01012). In the beginning of static exercises (holding of 7 kg weight) the ratio of power in a low band of EMG frequencies (25-40 Hz) to that in a high one (125-250 Hz), ratio L/H, was approximately 1. Ratio L/H increased up to 14 by the moment when the subject could not hold the weight. Then the weight was taken off. The L/H ratio rose up to 32 in 4-6 s after the release from the weight and by 20-25 s of the release it decreased down to values compared with ones which were observed in the beginning of exercises. The trained subjects exhibited an increase of the L/H ratio in a lesser degree than untrained ones. The results suggest that uninvasive spectral EMG technique may be useful for evaluation of exercise training.

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EMPTYING AND FILLING OF STRAIN GAUGE-BEARING GALLBLADDER (GB). A REAL-TIME ULTRASONOGRAPHIC STUDY IN THE DOG. K. Jonderko and L. Buéno

Aim. The study intended to answer whether and to what extent a strain gauge implanted surgically onto the body of the GB would impair its contraction.

Methods. Patterns meal- and caerulein-induced GB emptying were compared in four dogs equipped with a strain gauge fixed on the GB body and in four unoperated dogs. Caerulein was infused i.v.: (i) at stepwise increasing rates of 0.7 - 2.2 - 7.4 - 22.2 - 66.5 pmol·kg^{-1}h^{-1}, given each for 10 min, and (ii) at a constant rate of 7.4 and 22.2 pmol·kg^{-1}h^{-1}, given each for one hour. The maximum GB dimensions according to X-Y-Z coordinates were measured with a Toshiba SAL32B apparatus and a 5 MHz linear probe. Computation of GB volume was based on the ellipsoid approximation method.

Results. Implantation of a strain gauge onto the GB did not impair either its meal- or caerulein-evoked emptying. In the operated dogs, however, the relative shortening of the long axis of the GB was quicker and more pronounced than the relative reduction of GB diameter. Hence the ratio L:D of the GB length to diameter was significantly greater in the operated compared to unoperated dogs. Contraction of the GB in response to caerulein was linearly related to the logarithm of the dose administered. The corresponding slopes of regression lines did not differ significantly between the two groups of dogs. The dose of caerulein needed to induce GB emptying to 50% of its basal volume amounted to 7.6 pmol·kg^{-1}h^{-1} (95% confidence interval, CI: 6.8 - 8.5) in the unoperated dogs, and to 6.9 pmol·kg^{-1}h^{-1} (95% CI: 6.2 - 7.6) in the dogs equipped with a strain gauge sewn onto their GB. After withdrawal of caerulein infusion (7.4 pmol·kg^{-1}h^{-1}), a delayed return of the diameter, length, and consequently volume of the contracted GB to the basal values was observed in the operated compared to unoperated dogs.

Conclusion. The implantation of a strain gauge onto the GB body does not impair either the meal- or caerulein-induced canine GB emptying. The refilling of the contracted GB appears to be slower in the dogs with a strain gauge fixed onto their GB.

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CALCIUM CHANNELS AND INTESTINAL FLUID SECRETION A. Timar Peregrin, M. Jodal and O. Lundgren

Backgound/aims:The enteric nervous system (ENS) is of importance in explaining the intestinal fluid secretion evoked by several secretagogues amoung them cholera toxin (CT). Several mechanisms involved in the enteric nervous secretory reflex(es) (e.g. action potentials, neurotransmitter release) may be dependent on the flux of calcium across the plasma membrane, which, in turn, may be controlled by voltage gated calcium channels. In our experiments we investigated the importance of plasma membrane Ca-channels for intestinal fluid secretion and tried to determine the site of action of Ca-channel blockers in the nervous reflex arch.

Methods: Two types of studies were performed, in which intestinal net fluid transport, luminal serotonin release (by HPLC with electrochemical detection) and potential difference were measured in anesthetised rats in vivo.(1)The effects on the NFT of A 23187 and cholera toxin, placed in the intestinal lumen, were studied on line with a gravimetric method and various calcium channel blockers were tested on these effects.(2)The effects of nifedipine (L-type channel antagonist) on CT and A23187 calcium ionophore induced 5-HT release, PD changes and secretion were tested.(3)Experiments were performed to test how nifedipine affects the efferent nerves in the reflex arch by using the Ussing chamber method in vitro stimulating the nerves with an electrical field.

Results:A 23187 induced a dose dependent fluid secretion that was abolished by nifedipine (2 mg/kg b.wt. i.v.), hexamethonium (10 mg/kg b.wt. i.v.) or placing lidocaine(1%) on the intestinal serosa. The dose of nifedipine i.v. also abolished the fluid secretion caused by CT. Similarly, the induced release of 5-HT was reduced to the control level and the increased PD was lowered after the administration of nifedipine i.v.. Nifedipine had no significant effect on short circuit changes (SCC) evoked by electrical field stimulation (EFS).

Conclusion:The present study shows that voltage-sensitive calcium channels of a neuronal L-type, which are specially sensitive to dihydropyridine, appear to play an important role in the CT evoked fluid secretion and in the stimulus secretion coupling of 5-HT release from enterochromaffin cells. Moreover, the failiure to reduce the SCC caused by EFS strongly suggest that these kind of L channels (L_n) are located in the afferent part of the reflex arch, on the EC cells and , possibly on the afferent neurones. Our observations above indicate, that the triggered 5-HT release caused by secretagogues, in contrast to the spontaneous 5-HT outflow, is calcium channel dependent.

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EFFECT OF 5-HYDROXYTRYPTAMINE ANTAGONISTS ON CHOLERA TOXIN-INDUCED FLUID HYPERSECRETION IN PORCINE JEJUNUM. M.L. Grøndahl, G.M. Jensen, E. Skadhauge and M.B. Hansen

Background: 5-hydroxytryptamine (5-HT, serotonin) is a mediator in Cholera toxin (CT)-induced hypersecretion in the small intestine. The aim of the study was to determine the effect of the following 5-HT receptor antagonists: ketanserin, granisetron, ondansetron and tropisetron on CT-induced hypersecretion in pig jejunum. Methods: Hypersecretion was induced by CT (1, 5, 10, 20, and 40 µg per 12 cm loop) in ligated jejunal loops. The antagonists were given subcutaneously in the dose of 100 µg x kg^{-1}, since this dose has been reported to give maximal inhibitory effect on 5-HT- and CT-induced hypersecretion in rodents. Furthermore, the effect of intraluminally installed ondansetron was studied. Results: None of the antagonists altered basal absorption or caused fluid hypersecretion. CT caused a dose-dependent electrolyte and fluid hypersecretion. The maximal secretory efficacy was 6.8 ± 0.4 mg accumulated fluid per mg dry loop and was reduced by ondansetron, granisetron and tropisetron by about 40 %, 30 %, and 20 %, respectively, while ketanserin had no effect. Intraluminal ondansetron reduced the effect of CT by about 50 %. Conclusion: Results demonstrate ondansetron to be the most efficient antisecretory agent in CT-induced hypersecretion in pig jejunum. Furthermore, results support species differences in respect to the antagonistic effect of the drugs tested in CT-induced hypersecretion.

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EXPERIMENTAL MODEL OF GASTRIC HYPERMOTILITY

E. Atanassova, D. Nikolovska, E. Itzev, P. Vassilev, A. Noeva, P. Gurkov.

Experiments were performed on dogs with bipolar silver electrodes chronically implanted subserously on the gastric muscle wall. The electrogastromyogram (EGMG) was recorded on an encephalograph (BST 1) at a time constant of 0.3 s. The electrogastrogram (EGG) was simultaneously led off at a time constant of 7 s. non-invasively by cutaneous electrodes fixed on the abdominal wall at the beginning of each experiment. The periods of activity of the MMC were characterised by high-amplitude EGG waves preceeded by bursts of spike potentials with the gastric potentials in the EGMG. The periods of low-amplitude EGG waves corresponding to slow waves in the EGMG characterised periods of quiescence. After recording the background activity the dogs were subjected to another operation: a ligature of the initial part of the duodenum, which allowed for propulsion of the gastric contents. A week after surgery the spike activity was very well pronounced with large groups of high-frequency and high-amplitude spike potentials in the EGMG, preceding high-amplitude waves in the EGG especially after feeding. The mean value of the high-amplitude gastric waves after surgery, 589.75±1.14 µV (n=100) was significantly higher compared to that before the duodenal ligature, 170±0.80 µV (n=50). The mean value of the low-amplitude waves was 67±0.15 µV before and 235.56±0.87 µV after surgery. The increased gastric motility (hypermotility) could be a result of the increased resistance of the initial part of the duodenum, which corresponds to pyloric sphincter stenosis or functional spasm.

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THE ROLE OF INTRACELLULAR CALCIUM STORES IN THE NITRIC OXIDE-INDUCED RELAXATION OF CAT GASTRIC FUNDUS.

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The relaxant effect of two nitric oxide (NO)-releasing agents: sodium nitroprusside (SNP) and 3-morpholino-sydnonimine (SIN-1) on isolated muscle strips from cat gastric fundus was studied. The contractile activity was recorded under isometric conditions using organ baths. Both SNP and SIN-1 completely suppressed the spontaneous tone of fundic strips. Neither tetrodotoxin, nor atropine nor N^{W}-nitro L-arginine (an inhibitor of NO synthesis) significantly changed this effect. 8-Bromoguanosine 3',5'-cyclic monophosphate (8-Br-cGMP) also inhibited the tone but when applied at higher effective doses. SNP and SIN-1 antagonized the contractile effect of acetylcholine and of the specific inhibitors of the sarcoplasmic reticulum (SR) Ca^{2+}-pump, cyclopiazonic acid (CPA) and thapsigargin (TG). In the presence of SNP or SIN-1, CPA and TG exerted only a slight contractile effect. After the complete relaxation induced by SNP or SIN-1, the Ca^{2+}-activated-K^{+}-channel blockers: tetraethylammonium, apamin or charibdotoxin was able to elicit phasic contractions, suggesting that the Ca^{2+} sensitivity of the contractile myofilaments were not affected. Blockade of Ca^{2+}-activated-K^{+}-channels by both apamin and charibdotoxin caused a decrease of the inhibitory effect of SNP, SIN-1 and 8-Br-cGMP on the contractions.

The results suggest that in cat gastric fundus smooth muscles NO-releasing agents caused Ca^{2+} sequestration into the intracellular Ca^{2+} stores from the cell bulk through a protein kinase G-dependent mechanism and activation of SR Ca^{2+}-pump. This process contributes to the activation of a vectorial Ca^{2+} release from the Ca^{2+} stores toward the inner surface of the plasmalemma. Thus, the decreased intracellular free Ca^{2+} concentration is related with an increase of subplasmalemmal Ca^{2+} concentration. This probably results in a cell membrane hyperpolarization due to the activation of K^{+}-channels and whereby to a smooth muscle relaxation.

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INVOLVEMENT OF SEROTONINERGIC SYSTEM IN THE REGULATION OF EXOCRINE PANCREATIC SECRETION IN THE RABBIT. I.-O.Vaasa, S.Teesalu and M.Roosalu.

The effect of serotonin and serotonin receptor agonists and antagonists on the exocrine pancreatic secretion in the rabbit was studied. The rabbits were anesthetized by an i.v. infusion of urethan and the pancreatic duct was cannulated with a polyethylene tube. The intravenous infusion of serotonin in dosis 0.03-0.04 μg/kg/min stimulated the pancreatic juice secretion +198±77%, the protein output +103±85%, the lipase output +140±85% and the amylase output +33±70%. This effect was blocked by nonselective serotonin$_1$(S_1)-receptor antagonist methiothepin but not by selective S_2-receptor antagonist ketanserin and S_3-receptor antagonist ondansetron. Zacopride as a S_3-receptor antagonist and S_4-receptor agonist in dose 10 or 50 μg/kg dose-dependent markedly potentiated the stimulatory effect of serotonin. The fluid secretion was augmented from +198% by stimulation with serotonin administration only to +490% by infusion of zacopride in dosis 50 μg/kg and serotonin in dosis 0.03-0.04 μg/kg/min. Respectively the protein output was increased from +103% to +206% and lipase output from +140% to +220%. The selective S_{1A}-receptor agonist 8-OH-DPAT (2.5-5.0 μg/kg/min) and BAY Q 7821 (30-50 μg/kg/min) had a nonsignificant stimulatory effect on the pancreatic juice flow, lipase and protein output and an inhibitory influence on the amylase output. 8-OH-DPAT induced the twitch of hindlimbs. Our experiments with membran receptor bindings in the rabbit pancreas were without results. We have also no evidence of the presence of serotonergic receptors in the rabbit pancreatic tissue. In conclusion, the serotoninergic system is involved in the regulation of rabbit exocrine pancreatic secretion. This involvement is mediated with different serotonergic receptors, it is possible that with S_{1P}- and S_4-receptors and had a modulatory role in the regulation of exocrine pancreatic secretion.

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ROLE OF NITRIC OXIDE (NO) IN VAGALLY INDUCED PANCREATIC SECRETION J.Bilski, J.Jaworek, S.J.Konturek, W Bielanski

Recent studies have suggested that NO may act as nonadrenergic, noncholinergic neurotransmitter in the pancreas. The aim of this study was to evaluates the role of NO in the nervous control of exocrine and endocrine pancreatic secretion Methods: We compared, in dogs with chronic gastric, esophageal and pancreatic fistula, pancreatic response to vagal stimulation by sham-feeding (SF), i.v. infusion 2-Deoxy-D-Glucose in dose 50 mg/kg-h (2-DG) and insulin (0.1 U/kg-h) or infusion of gastrin releasing peptide (GRP) in dose 0.5 μg/kg-h and urecholine in dose of 50 μg/kg-h (U) with secretion after ordinary feeding (F) and i.v. infusion of 10% glucose. For the *in vitro* experiments rat pancratic slices (containing nerve fibers) and isolated pancreatic acini were prepared. Results: After administration of N^G-nitro-L-arginine (L-NNA), an inhibitor of nitric oxide synthase (2.5 mg/kg + 0.5 mg/kg-h iv) the pancreatic response to SF, F, GRP, U, 2-DG, insulin and i.v. glucose infusion were strongly inhibited. When L-arginine (L-Arg) in dose of 50 mg/kg + 5 mg/kg-h was combined with L-NNA this reduction was attenuated. L-NNA reduced insulin and pancreatic polypeptide release after all stimulants and glucagon release in tests with F, SF and GRP. These effects were partially reversed by addition of L-Arg. Amylase release, both basal and stimulated by U, from pancreatic slices, but not from isolated acini, was diminished by L-NNA (10^{-4}-10^{-3}M) and these inhibitory effects were reversed by addition of L-Arg (10^{-3}M). Conclusion: Endogenous NO participates in physiological control of vagally stimulated exocrine and endocrine pancreatic secretion.

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REGENERATION OF RAT PANCREAS AFTER REPEATED INDUCTION OF PANCREATITIS. A. Dembinski, Z. Warzecha, S.J. Konturek, J. Jaworek, P. Ceranowicz.

Induction of acute pancreatitis with tissue damage and acinar cells loss is followed by recovery. We studied biochemical, histological and functional regeneration of pancreatic tissue after repeated cerulein-induced pancreatitis (CIP). CIP was evoked in rats by s.c. infusion of cerulein (5 μg/kg/h) for 5h. After infusion, rats were divided into three groups. 1st group was infused with caerulein one time, in 2nd group infusion was repeated 10 days later. 3rd group was infused with caerulein 3rd time 10 days after the 2nd infusion. Rats were sacrificed at time sequence of 0, 12, 24, 48, 72 hours and at 5th, 10th, 14th, 21st and 28th day after last infusion. Pancreatic blood flow (PBF) was measured using laser Doppler flowmeter. Plasma and pancreatic amylase, pancreatic weight, RNA and DNA contents, and histological changes were determined. We found that DNA and RNA content, as well, as histological changes in 1st group were showing progress of regeneration after 3 days. Regeneration after CIP was almost completed within 10 days and amylase content in the tissue and plasma amylase level returned to normal values. Repeated infusion of cerulein caused less pronounced destruction of the pancreatic tissue however regeneration occurred progressively later then after 1st or 2nd infusion. Tissue repair after 2nd infusion reached peak at 5th day while after 3rd infusion at 10th day. PBF drooped after first CIP by 50% of control. Repeated CIP caused lower decreases in PBF which was back to control progressively earlier. Conclusion: Repeated CIP leads to a significant delay of tissue regeneration, however, the degree of damage after repeated CIP was reduced when compared to initial value and this effect is correlated with observed preservation of PBF.

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EFFECTS OF PARAQUAT ON CYTOTOXICITY AND GLUCONEOGENESIS IN ISOLATED RAT HEPATOCYTES. M.J. Moreno, J.C. Arenas, A. Berjón and M.P. Fernández-Otero

Paraquat (1, 1'-dimethyl-4, 4'-bipyridylium) is a widely used herbicide. Lipid peroxidation has been proposed as a major molecular mechanism involved in tissue injury induced by paraquat. This process requires the participation of oxygen-derived free radicals, which could attack some biomolecules such as metabolic enzymes and produce changes in metabolism processes. The aim of the present study was to evaluate the toxicity of paraquat as well as its effects on gluconeogenesis from different substrates in isolated rat hepatocytes. These were obtained from the liver of male Wistar rats starved for 48 h by a collagenase perfusion technique and cell viability was measured by the Trypan blue exclusion test. Hepatocytes ($5x10^6$ cells/2.5 ml) were incubated with increasing concentrations of paraquat (0.1-50 mM in distilled water) at 37 °C for 1 h. At the end of the incubation period cytotoxicity was evaluated in terms of leakage of some enzymes such as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and lactate dehydrogenase (LDH). Gluconeogenesis was assessed by the evaluation of glucose produced after the addition to the incubation medium of different substrates (20 mM pyruvate, 10 mM lactate, 20 mM fructose, 20 mM glycerol and 20 mM L-alanine). Glucose production was measured enzimatically after adequate deproteneization and subsequent neutralization. At 1 mM doses or higher, paraquat treatment caused a significant dose-dependent increase in extracellular activity for all enzymes evaluated. With regard to gluconeogenesis studies, a dose-dependent inhibition of glucose production from all different substrates added after paraquat treatment (0.1-10 mM) was observed. Nevertheless, the degree of inhibition observed by the herbicide action was different depending on the specific gluconeogenic substrate added. Thus, when lactate and pyruvate were used as inducers the paraquat inhibitory gluconeogenic effect was observed from 2.5 and 0.1 mM herbicide concentrations respectively. However, a significant decrease in glucose production from L-alanine, fructose and glycerol was only observed with the maximal paraquat concentration used (10 mM). These results seem to suggest that several mechanisms at different levels of the gluconeogenic pathway are involved in the paraquat inhibitory gluconeogenic effect.

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CHANGES OF THE SERIC AND THE SALIVARY IMMUNOGLOBULINS IN PATIENTS WITH ORAL TUMOURS A.Muresan
The location of neoplasm in the facial area varies between 3 and 15% of all tumours.Most oral neoplasms are carcinomae.The biopsy is the examination that indicates the type of tumour,while intravital stain and the cytology may establish an early diagnosis.
The study followed the dynamics of seric immunoglobulins(Ig) and of salivary Ig A at patients with oral tumours.We have taken under study a group of 2o patients,hospitalised at the Maxilo-Facial Clinic,from Cluj-Napoca(6o% males and 4o% females).66% of the patients showed epithelioid carcinoma with horn-like differentiation,16.6%of them malignant lymphoma and 16.6% had osteocondro sarcoma.The values of seric Ig (Ig G,Ig M,Ig A) and salivary Ig A have been measured in all patients taken under study,from unstimulated mixed saliva.All the biological measurements have been determined before the therapy was applied.The results were compared to those of a witness group. The analyse of the results pointed out an insignificant increase of Ig G and Ig M in the serum of patients with oral tumours.A significant increase of seric Ig A has been found in all patients taken under study.The determination of secretive Ig A revealed values that were significantly increased to an average of 13.5U/ml,for all the patients taken under study,comparatively to the average of 4.3 U/ml found in the witness group.The conduct of circulant Ig A was compared to that of secretive Ig A in the same patient. A liniary relation and a positive correlation of seric and salivary Ig A was found between the variation of the two parameters.

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Peptidylglycine Alpha-Amidating Monooxygenase (PAM) in Rat Antrum
J.A. Macro, R. Dimaline and G.J. Dockray

Stimulation of gastric acid by the peptide hormone gastrin, requires a COOH-terminal amide. Amidation is mediated by the multifunctional protein, peptide α-amidating monooxygenase (PAM). We have examined rat antrum for the presence of different forms of PAM. We used the polymerase chain reaction (PCR) with sense and antisense primers corresponding to several regions of rat PAM to characterize transcripts of cDNA derived from rat antrum. Resultant PCR products were cloned into pCRII (Invitrogen) and sequenced. Products were identified that included sequences encoding the peptidylglycine α-hydroxylating monooxygenase (PHM) domain, the peptidyl-α-hydroxyglycine α-amidating lyase (PAL) domain, the protease recognition site between the PHM and PAL domains and the transmembrane domain. A cRNA probe, corresponding to the PHM domain, hybridized to total RNA from rat gastric antrum, revealed a single mRNA species, of around 3.7kb, levels of which did not alter in rats treated with omeprazole (400 $\mu mol.kg^{-1}.day^{-1}$ for 5 days) to inhibit acid secretion. Omeprazole treatment over a period of five days, causes achlorhydria, and leads to a sustained tenfold increase in plasma gastrin and a threefold increase in gastrin mRNA. We have previously shown that the levels of mRNAs of the prohormone convertases, PC1/3 and PC2, thought to play a role in gastrin precursor cleavage, also increase between two and threefold with omeprazole treatment.
Conclusions 1) Transcripts encoding the four major functional domains of the amidating enzyme were identified in rat antrum, compatible with the presence of both membrane bound and soluble forms of PAM. 2) Expression of PAM was not altered in the antrum by omeprazole treatment, indicating that PAM synthesis is unlikely to be the limiting step in the production of amidated gastrin.

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A HISTOCHEMICAL STUDY OF LECTIN-RECEPTORS IN THE SUBMANDIBULAR SALIVARY GLANDS AND PANCREAS
J.V.Makeyeva, A.M.Yashtchenko and M.T.Panasiuk.

With the lectin-peroxidase technique,the glycoconjugates of submandibular salivary glands and pancreas were investigated in 20 male-rats under normal conditions and at hyperthyroidism, caused by injection of a 150mkg/1kg/d l-thyroxin dose within 20 days.T_3 and T_4 levels were determined by radioimmunoassay. Tissue samples were fixed in a 4% neutral formalin dilution,then sectioned into 5-7mkm-thick plates and treated with hematoxylin-eosin and four lectin-types from Arachis hypogaea(1), Sambucus nigra(2),Helix pomatia(3) and Lens culinaris(4) of various carbohydrate specifity. It was revealed, that in the submandibular salivary glands, under normal conditions, lectins (1) and (4) localized in the cytoplasm of granular excretory ducts epitheliocytes, where active biological substances are produced, while (3) - in the cytoplasm of serocytes. In the pancreas under the same conditions, receptors of (2) and (3) were identified in the zymogen granules of pancreatic cells, whereas receptors of (1) and (4) - in the homogeneous area of acinar and some epithelial cells of the duct system. At hyperthyroidism, a mosaic distribution pattern of (2)- and (3)-receptors was observed,both within the submandibular glands and the pancreas. As to the receptors of (1), they were found only in the blood-vessel endotheliocytes and in the cytoplasm of β-insulocytes. Such a redistribution of lectin-receptors testifies to the appearance of disturbances in salivary glands and pancreas functions at exogenous hyperthyroidism in rats.

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THE DETERMINISTIC CHAOS IN CYTOSOLIC CALCIUM OSCILLATIONS IN PANCREATIC ACINAR CELLS AND HEPATOCYTES. I.S.Magura, P.E.Strizhak, T.V.Masyuk, P.N. Schevchuk and A.I.Masyuk
The modified method of the return map reconstruction is applied to the analysis of the intracellular calcium oscillations in the mouse pancreatic acinar cells and in rat hepatosytes. It was shown that within a narrow range of acetylcholine concentrations ($1,4x10^{-7}$-$5x10^{-7}$ M) [Ach] the effect of increasing agonist concentration is to convert the response from chaotic ocsillations, which are characterized by single-peak return map to practically stochastic noise. This data allows to make a decision that the irregularity in the oscillating calcium signals is caused by an appearence of deterministic chaos at low [Ach]. At high [Ach] this irregularity is also connected with stohastic noise. The influence of the another agonist vasopressin (VP) on the calcium signal in hepatic cell was investigated. In this case the irregular part of calcuim oscillations can be characterized as a deterministic chaos for each VP concentrations (0,4; 0,6 and 0,9 mM). Such an analysis is an important tool for the investigation of the chaotic signals which brings a much more information than periodic. Chaotic systems can presumably allow optimal adaptation to fluctuating environmental conditions. It has been argued recently that chaotic signals can be controlled by applying judiciously chosen minute perturbations precisely because chaos is highly sensitive to initial conditions.

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GASTRIC RELATED NEURONS IN THE INSULAR CORTEX OF THE RAT. V.Aleksandrov, V.Bagaev and S.Panteleev.

This study was carried out to investigate the location peculiarities of the insular cortex neurones sending axons to the "gastric" region of dorsal vagal complex and the action the electric stimulation of these ones to motor gastric activity.

HRP solution was injected under visual control into the right or into the left dorsomedial subdivision of the middle portion of nucleus tractus solitarius. Labelled pyramidal neurons were observed in the V layer of the agranular and disgranular insular cortex bilaterally. Their ratio was 3:1 in the contra- and ipsilateral cortex respectively. The main part of labelled ipsi- and contralateral insular neurones (96% and 89%) was located between 0.0 and + 1.05 mm with respect to the joining of anterior commissure. An electrical stimulation of the identified insular cortex area induced both antral tone changes and increasing of an amplitude of antral contractions. The heart rate and breathing rate were unchanged under the same conditions.

The results of present study confirm that insular cortex contains the specific direct output to the bulbar gastric centre.

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HYPOXIA STIMULATES AN APPEARANCE OF NEW ß-CELLS IN INTACT AND STZ-DIABETIC WISTAR RATS. Yu.Kolesnik, A.Abramov

We studed the functional state of neurosecretory nuclei of hypothalamus and islets of Langerhance with different effort of glucose metabolism. The object of experiment was Wistar rats with STZ-diabetes, intact and diabetic rats which due to many days adaptated to hypoxic hypoxia. In STZ-diabetic rats the destruction of ß-cells with decreasing concentration of insulin (I) in them and blood was observed. In acinary part of the pancreas single I-immunoreactive cells were observed. In the paraventicular nuclei of hypothalamus (PVH) hypertrophy and increases of RNA contents in vasopressin (AVP), oxytocin (OT), somatostatin and CRF synthesizing neurons was seen. Hypoxic treatment (HT) of intact rats stimulated synthesis and secretion of I in ß-cells. In acinary part of the pancreas single I-immunopositive cells were observed too. Number of these cells was quite more than one in STZ-diabetic rats. HT of STZ-treated rats inhibited destruction of ß-cells, and increased concentration of I in ß-cells and blood. In diabetic rats HT stimulated appearance of big number of new I-immunopositive cells in acinary pancreas. In the PVH it was notice decreasing of the functional activity of AVP synthesizing neurons, and increasing activity of CRF and OT synthesized neurons. Think that the stabilisation of glucose homeostasis in rats with I-dependent diabetes under the HT is releted with regulatory effects of the hypothalamic neuropeptides on the functional activity of endocrine pancreas, and direct regulatory effects on glucose and fats metabolism.

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GASTRIC SECRETION AT DIFFERENT PATTERNS OF VAGUS NERVE STIMULATION. V.A.Zolotarev, R.P.Hropycheva and S.A.Polenov.

It is clear that not only the total number of nerve impulses but also their bursting pattern may influence the effector responses. The aim of this study was to elucidate the role of the vagal nerve impulse pattern in determinig gastric secretory responses. Methods: In nembutal-anaesthetized male Wistar rats, intragastric perfusion (0.6 ml/min) with saline and continuous monitoring of pH and pCO_2 of the effluent were used for estimation of gastric acid and bicarbonate production. Pepsinogen secretion was estimated in samples of effluent. Left subdiaphramatic vagal trunk was stimulated for 80 min at 6V and 2mc both continuously (10 Hz) and in 1s bursts (50Hz) at 4s intervals, delivering the same total number of impulses in randomized manner. Results: The continuous vagus stimulation produced an enhancement of acid secretion from 0,23±0,06 mM/min (basal level) to 5,26±1,20 mM/min (max.value). Burst stimulation caused a significantly smaller increase in acid production (1,92±0,51 mM/min both in sympathectomized and intact stomach). Bicarbonate production also increased during the continuous stimulation from 0,13±0,02 mM/min to 0,38±0,12 mM/min. Burst stimulation produced less pronounced secretory response especially in the stomach with preserved sympathetic innervation, when no significant changes in bicarbonate production were recorded. Pepsinogen production enhanced to vagus nerve stimulation being not different at either pattern of stimulation used. Conclusions: Continuous rather than burst vagal nerve stimulation is the effective mean of neural control of gastric acid and bicarbonate secretion. (Supported by ISF grant R4Q000).

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COMPERATIVE CHARACTERISTIC OF BIOELECTRICAL IMPEDANCE LINGUAL AND CORPORAL ACUPOINTS TO DECEIT CHANGES GASTRIC SECRETION.

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The purpose of this study was to compare the estimates of bioelectrical impedance analysis (BIA) lingual acupoints (LAP) and corporal acupoints (CAP) in patients with differing secretory function on the background of chronic gastritis (GCH) to define the mechanism of afferent and efferent ways of influencing the visceral functions. The experimental impedancometry (resistance and reactance measurements were made by using a four-terminal bioimpedance analyzer (ICGT-01, REMA, Lviv, Ukraine) on 112 patients with GCH with hypo-, normal, hypersecretory function of the stomack, reference group being of 45 patients. Analysis of the data obtained, BIA, LAP, CAP showed the reduction of impedance module at hyperacid secretion due to the active component ($P<0.01$). Unidirectional changes have been detected in BIA, LAP, CAP at hypo-, and normosecretory function of the stomack depending on the age of the patients and the length of their sickness. Taking into account the combined mechanisms of regulation of LAP, CAP, correlation of BIA data and intensity of secretory process, the method BIA can be applied for the investigition of the first link of the chain of the reflex arc (biological activity acupoints - LAP and CAP - as receptor fields).

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Acute stress opresses the protective function of the mucous barrier of the stomach.

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It was established on the model of acute emotional -painful stress by O.Desiderato (1974) on Wistar rats the development of mucous membrane ulcer of stomach in 100% animals without essential changes PH of the contents of stomach as compared with the control in this conditions in the stomach mucus the level of the markers of glycoproteins-free sialic acids and fucose raised. It's sais about the depolymerization of the main component of mucous barrier of the stomach. The essential role in the weakening of the protective function and in the development stresses ulcers of the mucous membrane of stomach plays the activation of the proteolysis in the stomach tissues, pancras and in the blood serum. The intensification of the proteolytic processes in stress play's more important role in the formation stresses ulcers as compared with the aggressive function of acid formation. The convineing argument of this theis is the authentic decrease of the stoutness of the layer of the stomach mucus in rats, which were exposed to stress, as compared with the control. Thus, the acute stress opresses the barrier function of the stomach mucous membrane, that is the main mechanism of uclerogenesis.

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PECULIARITIES OF NEURO - HUMORAL SUBSTANCES INTERACTION IN THE REGULATION OF OXYGEN PRESSURE (Po2) IN GASTRIC MUCOUS MEMBRANE.

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Numerous mediators and hormones being discharged into the intercellular medium of stomach, the task of the present research was to investigate peculiarities of their interaction on the basis of Po2 registration. Neuro-humoral substances were injected in dose of 100 mkg/kg to the rats narcotized with nambutal. In combined action of acetylcholin (Ach)+ norepinephrine (NE)+ histamine (His) Po2 decreased by 67% , it being based on the dominating effect of NE. Simultaneous action of Ash + serotonin (Ser) + His showed tendency to summerized effect of Ach and Ser, while Po2 decreased. Combined action of Ach+prostaglandine E2 (PGE2) + His at the background of characteristic changes of Po2 dynamics for both Ach and PGE2, is found out to summarize their effects. Received findings are the evidence of various ways of realization of Ach, Ser, PGE2 effect on Po2 regulation in the mucous membrane of stomach.

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NITRIC OXIDE INHIBITS THE CORONARY VASOCONSTRICTION TO ENDOTHELIN-1 IN ANAESTHETIZED GOATS.

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The role of nitric oxide (NO) in the coronary vasoconstriction by endothelin-1 was analyzed in six anaesthetized goats under control conditions and after inhibition of NO synthesis. Endothelin-1 (0.01-0.3 nmol) was intracoronarily injected, and its effects on left circumflex coronary (LCC) artery flow, electromagnetically measured, systemic arterial pressure, heart rate, and left ventricle (LV) systolic pressure and dP/dt were recorded. Inhibition of NO synthesis was performed by i.v. administration of N^G-nitro-L-arginine methyl ester (L-NAME, 42 mg/kg). Under control (C) and after L-NAME, the values for LCC flow, ml/min, were 35±4 (C) vs 32±3 (L-NAME) ($P<0.05$), for mean arterial pressure, mm Hg, 83±4 (C) vs 104±7.5 (L-NAME) ($P<0.05$), for heart rate, beats/min, 77±6 (C) vs 77±5 (L-NAME) ($P>0.05$), for LV systolic pressure, mm Hg, 101±6 (C) vs 121±10 (L-NAME) ($P<0.05$) and for LV dP/dt, mm Hg/sec, 1360±113 (C) vs 1390±108 (L-NAME) ($P>0.05$), respectively. Endothelin-1 reduced LCC flow in a dose-dependent way, which was more pronounced after L-NAME treatment; endothelin-1 did not affect the other variables recorded. The reductions of LCC flow by endothelin-1 under control and L-NAME, respectively, were: 5±2 vs 13±2 % (0.01 nmol) ($P<0.01$), 16±2.5 vs 30±6 % (0.03 nmol) ($P<0.05$), 40±6 vs 64±9 % (0.1 nmol) ($P<0.05$), and 63±5 vs 88±3 % (0.3 nmol) ($P<0.01$). These results indicate: 1) inhibition of NO synthesis reduces basal coronary blood flow, and 2) endothelin-1 produces marked coronary vasoconstriction, which is augmented after inhibition of NO formation. Therefore, under normal conditions NO could produce a basal coronary vasodilator tone and could inhibit the coronary vasoconstriction by endothelin-1.

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DYNAMICS OF CORONARY REGULATION IN RESPONSE TO CHANGES IN HEART RATE IN AWAKE AND ANAESTHETISED PATIENTS WITH CORONARY ARTERY DISEASE.

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In the intraoperative period, during deep surgical anaesthesia, the occurrence of myocardial ischemia is relatively rare compared with the pre and postoperative period. Since vasospasm is suggested to play a role in the aetiology of these ischemic episodes and interferes with the dynamics of coronary flow control, we planned to investigate the effects of anaesthesia on the rate of coronary regulation.

In 9 patients (pts) with coronary artery disease, we studied the rate of adjustment of coronary resistance in response to stepwise changes in heart rate (HR) and subsequently myocardial oxygen consumption (MVO2), before and after induction of anaesthesia. In awake pts, during recording of coronary sinus blood flow (CSBF), HR was increased 25 beats/min (=HR step up) by pacing. 10 min later, CSBF was recorded again and pacing was discontinued (=HR step down). After induction of anaesthesia (fentanyl 75 µg/kg), an identical series of measurements was repeated. The rate of change of the quotient of arterial blood pressure minus right atrial pressure and CSBF, an index of coronary vascular resistance (CVR), was used to asses the rate of coronary regulation, which was then quantified by a t50 value, defined as the time required to establish 50% of the total change of CVR in response to a HR step.

The t50-values (mean ± SD) for the dilating responses to an increase in HR were 5.5 ± 2.3 s in the awake state and 9.5 ± 1.0 s in the anaesthetised state ($p=0.017$). For the constricting responses to a decrease in HR, t50-values were 5.7 ± 2.4 s awake, and 9.1 ± 1.8 s during anaesthesia ($p=0.001$). Baseline values of CVR during anaesthesia did not differ from baseline values awake. Furthermore, anaesthesia had no significant effect on the magnitude of changes in CSBF and MVO2 in response to HR-steps, compared to the awake situation.

General anaesthesia significantly reduced the rate of coronary flow regulation, without changing the steady state regulation. The decelerated coronary response, found in our anaesthetised patients might be involved in the lower incicence of myocardial ischemia during anaesthesia.

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THE GREGG PHENOMENON IS RELATED TO SHEAR STRESS AND DOES NOT DEPEND ON WASH-IN AND WASH-OUT EFFECTS.

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In the isolated heart an increase in coronary perfusion results in an increase in cardiac contractility and oxygen consumption (the Gregg phenomenon). We studied if the Gregg phenomenon was related to shear stress in the coronary vasculature. We induced changes in shear stress in the coronary vessels of the isolated perfused rat heart (n=5) by either changing perfusion flow or increasing the coronary vascular resistance by addition of vasopressin to the perfusion fluid (VP:0.3nM). The hearts were perfused according to Langendorff and contracted isovolumically (LV balloon), at 27°C. Shear stress is proportional to perfusion pressure times vascular diameter. The latter was estimated from resistance assuming Poiseuille's law to hold. In each heart a single linear relationship was found between shear stress and developed left ventricular pressure induced by increases in perfusion flow or increases in coronary vascular resistance. Different flows at similar shear stress lead to similar developed pressure indicating that wash-in and wash-out effects of inotropic factors play a negligible role. We conclude that the Gregg phenomenon is a shear stress related phenomenon.

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SIMULATION OF THE DYNAMIC GREGG EFFECT IN REACTIVE HYPEREMIA.

J. Dankelman and J.A.E. Spaan

The Gregg effect is the pressure dependency of oxygen consumption (MVo_2). This effect should be attributed to capillary pressure (Pc) which depends on arterial pressure and arteriolar vascular tone. Experimentally it is impossible to measure transients in MVo_2 during an occlusion (OC) and reactive hyperemia (RH). Therefore, the oxygen control model was used to predict transients in MVo_2 during 15 s OC and RH taking the Gregg effect into account. The influence of rate of regulation (τ_{tone}) on MVo_2 transients was analysed.

The simulation demonstrates that during OC Pc decreases fast with a rate independent of τ_{tone}, hence, the reduction in MVo_2 due to the Gregg effect is fast as well. Therefore, the Gregg effect reduces the amount of oxygen consumed during OC independent of τ_{tone}. During RH Pc is increased as the result of arteriolar vasodilation inducing an increase in MVo_2 by the Gregg effect. With τ_{tone} < 5 s complete vasodilation occurs during OC. Hence, Pc and MVo_2 increase to high levels during RH. With τ_{tone} > 5 s vasodilation remains lower than maximal and as a result the initial increase in MVo_2 during RH is sensitive to τ_{tone}. The rate of decrease in MVo_2 after peak RH depends on τ_{tone} for high as well as low τ_{tone}. For τ_{tone} > 5 s the smaller initial increase in MVo_2 and the longer duration result in an independency between the total amount of oxygen consumed during RH and τ_{tone}. Hence, the increase in the amount of oxygen consumed during RH is dependent on τ_{tone} for τ_{tone}<5 s but not for τ_{tone}>5 s.

In conclusion, according to this model the Gregg effect influences MVo_2 during OC as well as RH. The amount of oxygen consumed in RH above control is not related to oxygen debt built up in OC as suggested by the often used term oxygen repayment. This extra amount of oxygen can fully be explained by the Gregg effect induced by vasodilation.

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THE EFFECT OF CORONARY VENOUS PRESSURE ON EPICARDIAL LYMPH PRESSURE.

J.W.G.E. VanTeeffelen, D. Merkus, L.J. Bos, I. Vergroesen and J.A.E. Spaan.

We investigated the effect of a transient increase in coronary venous pressure (Pv) on epicardial lymph pressure (Pl). An increased Pv is expected to increase capillary filtration and Pl.

Experiments were performed in anaesthetized open-chest dogs (n=6). The left main coronary artery was cannulated and perfused under controlled pressure. Pl was measured in an epicardial lymph vessel that was cannulated retrogradely. Pv was elevated for 20-100 beats from a control value of about 10 mmHg to approximately 50 mmHg by either inflation of a balloon in the coronary sinus or occlusion of the great cardiac vein. During the first 5 beats Pv increased to ± 65% of the maximal Pv (phase I) and during beat 6-15 to ± 90% (phase II). Subsequently a steady-state was reached (phase III). The Pl response was divided in the same three phases. In each experiment several periods of venous pressure elevation were studied during control and while coronary vascular permeability was increased by intracoronary infusion of histamine (15-75 µg/min).

With normal Pv, mean Pl was 15.7 mmHg (range: 6.0-22.1) during control and significantly increased to 21.2 (10.0-30.4) during the infusion of histamine. Elevation of Pv resulted in a small but significant increase in mean Pl both in control (I-17.4; II-18.6; III-20.0) and during histamine infusion (I-24.6; II-28.2; III-28.4). The rate of Pl response to the increased Pv was similar in each dog both in control and with histamine.

It is concluded that Pl is affected by an increase of Pv. This effect is small and rapid, and not affected by the administration of histamine, suggesting a direct mechanical effect of venous bulging.

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RESPONSE TIMES OF MITOCHONDRIAL OXYGEN CONSUMPTION, TISSUE OXYGENATION AND VENOUS OXYGEN CONCENTRATION DURING STEPS IN HEART RATE IN ISOLATED RABBIT HEART.

B. de Groot, C.J. Zuurbier and J.H.G.M. van Beek

The time courses of mitochondrial O_2 consumption, myoglobin oxygenation, and venous oxygen tension during steps in heart rate were compared in 7 isolated rabbit hearts. The hearts contracted against a water-filled balloon in the left ventricle and were perfused with a constant flow of Tyrode solution at 37 °C. The venous oxygen tension was measured with a fast responding Clark-type electrode. The tissue oxygenation was assessed with a near infrared monitor (RUNMAN, NIM Inc., Philadelphia) using two wavelength bands centered at 760 and 850 nm, whose difference signal mainly reflected changes in myoglobin oxygenation. Steps were made between 120 and 220 beats/min in the electrically paced heart. The mean response time of the venous O_2 tension, defined by Van Beek and Westerhof (*Am.J.Physiol. 260: H613, 1991*) was 13.5 ± 2.3 (SD) s. The similarly defined mean response time of tissue oxygenation was 10.6 ± 1.3 s. Indeed there was a clear change in tissue oxygenation. The calculated mean response time of mitochondrial O_2 consumption was 3.4 ± 1.6 s. Thus the fast change in O_2 consumption was very likely partially supplied from O_2 bound to myoglobin. The consumption of O_2 derived from oxymyoglobin continued for more than ten seconds. That the change in venous oxygen tension was slightly delayed with respect to myoglobin is explained by delay in vascular transport between the cardiac myocytes and the venous oxygen electrode.

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DOES CHRONIC HYPOXIA INCREASE CARDIAC RESISTANCE TO ACUTE HYPOXIA ? M. Pissarek, X. Bigard, P. Mateo, M. Peres, C.-Y. Guezennec, J. Hoerter

To check if chronic hypoxia results in an increased resistance to acute oxygen depletion we measured glycolytic substrate utilisation, energy metabolism and cardiac performance in 3 groups: chronically hypobaric hypoxic pre-treated (28 days, 505 mmHg ≈10 % O_2): H, pair fed: PF and control rats: C. The hearts were perfused at constant flow in isovolumic Langendorff-mode at 37 °C in normoxia, then we stepwise decreased oxygen saturation (75 to 0%). To assess the contribution of oxidative and glycolytic pathways to cardiac function either acetate (10 mM) or glucose (11 mM + insulin 5 mU/ml) were applied as substrate. Cardiac function was assessed by measuring the rate pressure product (RPP= left ventricular systolic pressure x heart rate) and end diastolic pressure (EDP), glycolytic activity by lactate efflux and energetic state by ^{31}P-NMR spectroscopy. During normoxia, contractile function or glycolytic activity of H hearts did not differ from that of the C and PF groups. However, H showed a marked decrease in creatine phosphate (PCr) levels (30%) without change in ATP. Simultaneously, the percentage of Creatine Kinase (CK) BB and MB isoenzymes was enhanced in comparison to C and PF. During anoxia a higher lactate efflux in H than in C and PF (9.7±0.3 vs 7.1±0.3 and 7.2±0.89 µmol/g min, respectively) was associated with lower intracellular pH in glucose perfusion. As pO_2 was reduced, PCr remained lower in H hearts independently from the substrate employed, while ATP decreased similarly in all groups. Despite of this, RPP was diminished similarly in the 3 groups. The rise in EDP was lower in H compared to C only during acetate perfusion. The shift of CK isoenzymes to cytosolic forms and the enhanced glycolysis are potentially favourable under conditions of acute hypoxia. Although this didn't result in an improved systolic function of the H hearts it might contribute to preserve the diastolic performance of these hearts allowing an optimal ventricular filling.

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ANNEXIN V IN THE ADULT RAT HEART. S.W.S. Jans, M. van Bilsen, M. Borgers, Y.F. de Jong and G.J. van der Vusse

The annexins constitute a family of proteins with calcium- and phospholipid-binding properties. In the present study we investigated the prevalence of the different types of annexins in rat cardiac tissue and the cellular and subcellular distribution of annexin V, the most prominent annexin in rat heart tissue, in isolated adult cardiomyocytes and cultered cardiac endothelial and fibroblast-like cells. The presence of annexin I plus II, III, IV, V and VI was positively established with western blot analysis. Western blot analysis and immunohistochemistry with polyclonal antibodies raised against purified rat heart annexin V revealed that annexin V is present in both myocytal and non-myocytal cells. Immunocytochemistry showed that in endothelial and fibroblast-like cells annexin V is localized in the cytoplasm and in cardiac myocytes predominantly in close vicinity of the sarcolemma. This last finding is confirmed by electron microscopy of cardiac myocytes. Northern blot analysis with a rat annexin V DNA probe revealed that all cell types investigated showed expression of annexin V mRNA, with highest levels in the fibroblast-like cells, followed by the endothelial cells and a weak signal was observed in the cardiomyocytes. By means of a sandwich-type enzyme-linked immunosorbent assay (ELISA) annexin V content in adult rat heart, isolated myocytes, cultured endothelial cells and fibroblast-like cells was found to be 0.70, 0.17, 1.63 and 3.84 µg/ml total protein, respectively. The differences in subcellular localization and concentration of annexin V in myocytal and non-myocytal cells suggest that a difference exists in the biological function of annexin V in the various cell types of the rat heart.

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EFFECT OF VARIOUS TYPES OF EXERCISE TRAINING ON 5'-NUCLEOTIDASE AND ADENOSINE DEAMINASE ACTIVITY IN RAT HEART. D.Czarnowski, J.Langfort, B.Wójcik and J.Górski

The metabolic adaptation of the heart to regular exercise depends on a specificity of applied training program. Adenosine has been generally considered to be an important local modulator of physiological processes. It may act as a link between metabolism and blood flow in the heart. A change in adenosine concentration could be produced by a change in the maximum activity of the enzymes which control its metabolism. In the present study an attempt was made to find out whether activities of 5'-nucleotidase (5'NT) and adenosine deaminase (ADA) in left (LV) and right (RV) ventricles of the rat's heart change after endurance and sprint training. Additionally, an influence of a single bout of endurance exercise till exhaustion on activity of these enzymes was investigated. The animals were divided into three groups: sedentary-controls (C), endurance-trained (ET) and sprint-trained (ST). ET-rats were running on the treadmill for 1h daily, 5 days per week for 6 weeks. The running speed was gradually increased during the first 4 weeks and then it was maintained at 28 m·min^{-1}, 0° inclination in the final week of training. ST-rats ran for 10s separate bouts with 50s intervals between them, 5 days per week for 6 weeks. The speed, number of exercise bouts and inclination of the treadmill were gradually increased up to 58 m·min^{-1}, 30 repetitions per day and 15° of treadmill inclination which were attained in the 4th week of training and maintained for its final period. Half of each animal group performed a single bout of endurance exercise to exhaustion with an intensity of 30m·min^{-1} 48h after completing the training session. It was shown that both types of training increased 5'NT, but did not change ADA activity in either ventricles. Acute exercise till exhaustion did not affect 5'NT activity in C and ST, but decreased its activity in ET-group. ADA activity after exhaustive exercise increased in C only in LV, in ET in both ventricles, but decreased in ST-animals. It is concluded that physical training affects cardiac adenosine metabolism and the type of training may have an influence on purine nucleotides metabolism in the heart during exhaustive exercise.

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MULTIPLE PATHWAYS OF CONVERSION OF ANGIOTENSIN I IN THE HEART. L.S. Mihailescu, F.L. Abel and V. Nestianu

The heart possesses its own renin - angiotensin system, which produces renin, angiotensinogen and converting enzyme. Certain experimental results show a complete abolition of the myocardial and coronary actions of angiotensin I after converting enzyme inhibition, while others show only a reduction. We performed 18 experiments in adult cats, anesthetized with sodium pentobarbital (30 mg/kg). The hearts were isolated and perfused, according to the Langendorff technique, at a constant perfusion pressure of 75 mm Hg. The Krebs - Henseleit buffer (37^{0}C, pH=7.4, pO_2=500-600 mm Hg) was used as perfusate; the left ventricular pressure and the aortic flow were recorded. Angiotensin I (10^{-12} M to 10^{-7} M) produced a dose dependent increase in : heart rate by 6.9 - 23 %, left ventricular pressure by 1.4 - 12 %, +dP/dt by 2.5 - 23 %, -dP/dt by 8 - 20.5 % and coronary flow by 12.9 - 34 %. In four experiments, pretreatment of the isolated hearts with enalaprilate (10^{-7} M) significantly reduced the cardiac effects of angiotensin I, by around 50 %. In other four experiments, a synthetic angiotensin II AT_1 receptor blocker (losartan) was infused after the highest concentration of angiotensin I used (10^{-7}M). Losartan (10^{-7}M) completely reverted the cardiac actions of the decapeptide. Our results show that in isolated hearts angiotensin I exerts its effects after conversion into angiotensin II and this conversion is realized by multiple enzymatic pathways.

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STIMULATION OF CARDIOMYOCYTE GLUCOSE TRANSPORT BY SEROTONIN OCCURS THROUGH A MONOAMINE-OXIDASE DEPENDENT PATHWAY. Y.Fischer, J.Thomas, J.Kamp, E.Jüngling, C.Carpéné†, G.D. Holman‡ and Helmut Kammermeier.

We have previously reported that serotonin (5-hydroxytryptamine, 5-HT) causes a slow (30-90 min) increase in glucose transport of isolated rat cardiomyocytes, and that this effect is not mediated by a classical 5-HT receptor (J Mol Cell Cardiol **24** suppl.V, S22). We have now further explored the mechanism underlying this action of 5-HT, as well as the intracellular signal involved. By using the photoaffinity label ^{3}H-2-*N*-[4(1-azi-2,2,2-trifluoroethyl)benzoyl]-1,3-bis-(D-mannos-4-yloxy)propyl-2-amine, we found that 5-HT induces an increase in the amount of the glucose transporters GLUT1 and GLUT4 in the plasma membrane (1.8- and 1.5-fold, respectively, vs. control). Next, the involvement of 5-HT metabolism was examined. The monoamine oxidase inhibitors clorgyline (1 µM, MAO-A selective), and tranylcypromine (1 µM, non-selective) completely suppressed the effect of 5-HT on glucose transport, whereas the control and insulin-stimulated rates of glucose transport were unaffected. In contrast, the MAO-B selective inhibitor L-deprenyl (1 µM) was ineffective. Addition of catalase or glutathione diminished the 5-HT-dependent stimulation by 50%; these two factors are known to favour the degradation of hydrogen peroxide (which can be formed during the deamination of amines by monoamine oxidases). Glutathione also depressed the stimulatory action of exogenously added hydrogen peroxide (200 µM) by 30%. Furthermore, in cells treated with 5-HT, a time-dependent accumulation of 5-hydroxy-1*H*-indole-3-acetic acid (a product of 5-HT metabolism via monoamine oxidases) was observed, which paralleled the changes in glucose transport.

In conclusion, the stimulation of glucose transport by 5-HT in cardiomyocytes is likely to occur through the degradation of 5-HT by a monoamine oxidase (type A) and concomitant formation of hydrogen peroxide, which eventually leads to the recruitment of glucose transporters GLUT1 and GLUT4 to the plasma membrane (supported by the Deutsche Forschungsgemeinschaft).

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IMPACT OF ACUTE HYPERTENSION IN THE CORONARY CIRCULATION ON MYOCARDIAL FUNCTION AND METABOLISM. A.I. Khomazjuk, A.P. Nescheret, I.V. Shepelenko, I.V. Gonchar and N.V.Okhrimenko

To assess the effects of increased coronary artery perfusion pressure (CAPP) the experiments on 32 dogs were performed without opening the chest under chloralose anaesthesia. Catheterization and programmed extracorporal autoperfusion of left circumflex coronary artery (CA) and continuous drainage of coronary sinus (CS), catheterization of heart and vessels, recording of cardiodynamics and CAPP, blood oxygen saturation and determination of glucose, FFA, lactate, pO_2, pCO_2, pH in arterial and coronary venous blood were performed. The increase of CAPP from 13.3 to 20.0 and 26.6 kPa was induced either by augmentation of perfusion volume or by intracoronary injection of arginine-vasopressin (AV; 0.1-1.0 IU). The hyperperfusion and coronary hypertension caused marked elevation of heart contractility only in hypoperfused state but not detectable changes of myocardial contractility,metabolism and O_2 consumption in normally perfused hearts.Injection of AV produced rise of CAPP (+4.8±0.78 kPa) and left ventricular dP/dt (+135.0±10.5 kPa/s), increase of myocardial glucose uptake from 0.4 ±0.11 to 0.6 ±0.08 mmol/l and lactate, FFA and glycerol release into CS (-0.6±0.19; -0.4±0.10; -0.2±0.05 mmol/l, resp.). The reaction was followed by reflectory counterregulatory decrease of heart contractility, peripheral vessels dilatation and blood pressure reduction. All components of the reaction but CA hypertension were induced by activation of endogenous catecholamines by AV and were blocked by propranolol (2.0 mg/kg, i.v.). It is suggested that acute coronary hypertension does not produce detectable independent changes of adequately perfused heart and does not elicit counterregulatory reflexes.

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EFFECT OF HISTAMINE SUPERFUSION FOLLOWING MAST CELL STABILIZATION ON LEUKOCYTE ROLLING IN MESENTERIC VENULES OF THREE DIFFERENT STRAINS OF RATS. S. Tromp, M. oude Egbrink, W. Engels, D. Slaaf, R. Reneman, G. Tangelder

Histamine causes an increase in leukocyte rolling in mesenteric venules of Sprague-Dawley rats pretreated with the mast cell stabilizer cromoglycate (Kubes et al., J. Immunol. 1994;152:3570). In this pilot study we investigated this phenomenon in three different rat strains: Sprague-Dawley (SD; n=2), Lewis (LE; n=4) and Wistar (WI; n=3). All rats were anesthetized with pentobarbital (24 mg/kg/hr) and received cromoglycate (5 mg/kg i.v.) before surgical preparation of the mesentery. After a stabilization period of 30 minutes, 10^{-5}M histamine, 10^{-4}M histamine and Tyrode's solution were superfused successively during 25-30 minutes each.

The vessel numbers studied in the SD, LE and WI were 7 (median diameter: 24 µm), 7 (25 µm) and 12 (26 µm), respectively. The basic levels of leukocyte rolling per minute were 9, 21 and 29; these values were significantly different from each other (p=0.02; Kruskall Wallis test). The normalized level of leukocyte rolling in the SD venules showed a gradual increase till 1.44 with 10^{-5}M histamine and 1.67 with Tyrode (p=0.06; Wilcoxon test). Levels in the LE and WI groups showed no significant change during histamine or Tyrode superfusion. No changes were observed in venular diameter, reduced velocity or heart rate. Mean arterial pressure remained unchanged during superfusion of 10^{-5}M histamine, but decreased by on the average 9% with 10^{-4}M histamine. Systemic leukocyte counts decreased during an experiment, but returned to normal values at the end; the percentage of polymorphonuclear cells increased in all groups by 10% to 46% (median values).

In summary, superfusing the mesentery with histamine after mast cell stabilization induced an increase in leukocyte rolling in SD rats, but not in LE and WI rats. This suggests that in these three strains of rats differences may exist in presence of mesenteric mast cells, mast cell constituents and/or distribution of histamine receptors.

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ROLE OF ENDOGENOUS NITRIC OXIDE IN THROMBOEMBOLISM IN RABBIT MESENTERIC ARTERIOLES AND VENULES. M.A.W. Broeders, M.G.A. oude Egbrink, D.W. Slaaf, R.S. Reneman and G.J. Tangelder.

The antithrombotic role of nitric oxide (NO) in vivo was investigated in rabbit mesenteric arterioles and venules (diameter: 20-38 µm) using intravital video microscopy. Thromboembolism was induced by wall puncture with a glass micropipet (tip: ≈6 µm). The involvement of NO was investigated by superfusing the mesentery of 9 anesthetized rabbits with the NO Synthase (NOS)-inhibitor Nω-nitro-L-arginine (L-NA; 0.1 mM). In another group (n=11) the vehicle (Tyrode's solution) was used. Wall puncture induced the formation of a thrombus that stopped bleeding and from which emboli were shed. L-NA did influence neither bleeding time (overall median: 4.0 s) nor thrombus height (overall median: 65% of local vessel diameter). In venules L-NA significantly ($p<0.005$) increased the median number of emboli produced per vessel from 2 to 11, as well as the duration of embolization (from 50 to 511 s). In arterioles, L-NA had no significant influence on the number of emboli produced. In both vessel types no influence was found on the rate of embolization (overall median: 6.5 s per embolus). L-NA had no significant effect on the number of leukocytes adhering to the venular wall (control median: 7/100µm; L-NA median: 4/100 µm). The number of rolling leukocytes was lower in the L-NA than in the control group (control: 25/min.; L-NA: 15/min.), although this difference was not significant (p=0.09). The effects induced by L-NA could not be explained by fluid dynamic factors. The finding that NOS-inhibition has a significant effect in rabbit mesenteric venules, but not in arterioles, indicates that endogenous NO plays a more important role in thromboembolism in venules. A possible role for substances released by rolling leukocytes in the interactions between platelets and the venular vessel wall cannot be excluded. *Supported by the Dutch Heart Foundation, grant # 92.339*

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EFFECTS OF ISOMETRIC EXERCICE ON UTERINE AND OMBILICAL ARTERY BLOOD FLOW VELOCITY WAVEFORMS IN NORMAL PREGNANCY. A. W. Kedra, N. Ciraru-Vigneron, P. Bonnin, C. Bazzi-Grossin, E. Savin, O. Bailliart, J.P. Martineaud.

OBJECTIVE: Our study was designed to evaluate the effects of isometric exercise of upper extremity (handgrip - HG) on uteroplacental circulation in late normal pregnancy. METHOD: Thirty three healthy women with normal singleton pregnancy between 22 to 40 weeks gestation (mean 30.6 weeks) were explored by Doppler ultrasound duplex using a 3.5 MHz pulsed Doppler system in combination with a real-time linear array scanner. The maternal heart rate (MHR), arterial blood pressure (BP) and uterine artery flow velocity waveforms (FVW) were recorded at rest and at the end of 3 min HG at 30-40% of maximal force in 22 patients; the fetal heart rate (FHR) and ombilical FVW were recorded in 11 subjects. The uterine and ombilical artery resistance index (UARI, OARI) were calculated as a ratio of (systolic -- diastolic)/systolic velocities. RESULTS: Mean BP increased during HG (mean BP +/- SD was 75.2 +/- 9.9 to 92.9 +/-14.1 mmHg; $p<0.01$) but there was no significant variation of MHR (89.4 +/- 12.6 to 91.6 +/- 15.1 beats/min; NS), FHR (148.3 +/- 9.2 to 147.7 +/- 7.8 beats/min; NS), UARI (.43 +/- .07 to .43 +/- .08; NS) and OARI (.61 +/- .06 to .63 +/- .08; NS). CONCLUSION: In advanced normal pregnancy isometric exercice increases the mean arterial blood pressure but does not influence the uteroplacental circulation.

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ANALYSIS OF INTRAVASAL AND TRANSMURAL DYNAMICS OF PERFUSION BY COMPUTER AIDED PERFUSOGRAPHY.

J.Hektor, H.Heidtmann, H.Horstkott, R.Grebe

Adequate perfusion is most important for normal function of all tissues. The efficiency of the microvascular system highly depends on the flow of volume between intravascular space and interstitium. That's why it is not enough to measure the transcapillary bloodflow but the transmural flow has to be taken into account as well. The aim of this project is to develop a model based procedure to quantify intravasal and transmural volume flow.

In this study the rat mesenterium has been used as an example of an easy to observe microcircular region. In this method the fluorochrome Na-fluoresceine is used because it freely diffuses with the body fluid between vascular and interstitial space. The fluorescence is stimulated by a strong light source with an according bandpass-filter and is recorded on video tape by a CCD-camera equipped with a low-pass filter. Later it is digitized by a transputersystem and evaluated on high performance computers.

A bolus of the dye is injected into an artery and the appearance of the fluorescence is observed by microscope (25x). A parametric image (perfusogram) with a color chart of appearance times is calculated, which resembles the dispersion of circulation through visualization of the velocity of inflow as a dynamic portrait. Three stages of perfusion are distinguished: 1. the primary intravasal flow (arteriol-capillar-venul), 2. the prolonged diffuse interstitial appearance of fluorescence, 3. the final outflow via the venul system.

In addition a model of the microcirculation has been developed by which the whole process can be simulated and which gives the basis to calculate the intravasal and the interstitial volume flow from the experimental data.

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IS OXYGEN DELIVERY TO SKELETAL MUSCLE TIGHTLY REGULATED? Toth, A., M. Pal, R.D. Shonat, M.E. Tischler and P.C. Johnson

Changes in oxidative metabolism of the parenchimal cells are believed to play a significant role in regulation of local blood flow and ultimately oxygen delivery. Better understanding of this role however was handicapped for a long time by a paucity of methods to assess in vivo metabolic state in small enough tissue volumes. A special computer controlled intravital microscope has been developed in our lab with spatial and temporal resolution needed for evaluating changes in metabolic state of the tissue on the microcirculatory level while also allowing for simultaneous determination of flow parameters.

To evaluate the possible dependence of the microcirculatory flow regulation on changes in oxidative metabolism rat spino-trapezius and cat sartorius muscle preparations were used. Changes in tissue NADH fluorescence level and RBC velocities were determined during and following different perturbations in blood flow (ischemia, functional hyperemia and sympathetic stimulation).

Our results show, that in resting or moderately excercising skeletal muscle oxygen delivery is high enough to avoid anaerobic shifts in the oxidative metabolism. We also found a substantial spatial heterogeneity in oxygen reserves. This heterogeneity however was dependent on local flow conditions and independent of location of the tissue site in proximity of the arterioles.

The results led to the conclusion that there is a significant surplus of oxygen present in the muscle and the first level of microcirculatory flow regulation is probably not casually linked to shifts in oxidative metabolism. A ~50% decrease in RBC velocity lasting for ~30 seconds and microvascular oxygen tensions <5 mmHg are needed to induce anaerobic shifts in oxidative metabolism which has been suggested to activate the classical metabolic feed back mechanisms.

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STANDING LEADS TO RAPID AND PRONOUNCED PLASMA VOLUME DECLINE, A RESPONSE ENHANCED AT RAISED ENVIRONMENTAL TEMPERATURE. POSSIBLE ROLE IN ORTHOSTATIC FAINTING REACTIONS. P. Lindgren and J. Lundvall.

Quiet standing leads to reduction of plasma volume (PV) by filtration in dependent regions but studies have indicated that this process is relatively slow and apparently without more significant haemodynamic importance unless standing is markedly prolonged. However, the rate and magnitude of the PV reduction might have been seriously underestimated in the past as indicated by recent studies from our laboratory. The present study, in fact, strongly suggest that standing still leads to pronounced PV decline within very short time and, further, that the response is markedly exaggerated during heat stress. Male volunteers ($n=6$; 78.3 ± 3 kg; 182 ± 4 cm) were exposed to quiet standing under comfortably warm conditions (room 23-25°C) and, with short-term exposure, also under heat stress (room 35-40°C). Determination of control PV prior to standing and of haemoconcentration changes in response to experimental intervention provided measures of PV changes. Under comfortable conditions PV decreased by $11.6\pm0.3\%$ or by 442 ± 19 ml after 5 min standing. The reduction was $16.6\pm0.7\%$ (633 ± 36 m) and $17.5\pm0.9\%$ (667 ± 39 ml) after 10 and 20 min of standing still. Quiet standing thus was associated with very rapid and large PV loss completed virtually within 10 min. The response was further enhanced during heat stress when 5 min standing evoked a $15.0\pm0.5\%$ or 580 ± 29 ml PV reduction. **Comments.** Many studies have been devoted to the problem of PV changes on standing, but almost exclusively with the use of prolonged 20-60 min experimental intervention. Normally, however, in every-day life, prolonged standing still is unusual whereas short quiet standing instead is common. The demonstrated very rapid and large PV reduction (11.5% or ≈450 ml after only 5 min standing under comfortably warm conditions) then seems a potentially very important component in the circulatory challenge in the upright posture in normal life. It is suggested that PV reduction rather than the early terminated blood pooling might be the process that ultimately may lead to circulatory failure. The observation that the PV decline was significantly enhanced during heat stress, i.e., under conditions when orthostatic, fainting reactions are known to be common, supports this contention.

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THE EFFECT OF OVARIAN STEROIDS ON ELASTIC PROPERTIES OF FEMALE LARGE ARTERIES DURING THE NORMAL MENSTRUAL CYCLE. C. Willekes, H.A. Keizer, H.J. Hoogland, A.P.G. Hoeks, R.S. Reneman. CARIM, University of Limburg, The Netherlands.

Introduction: In previous studies the elastic properties of the common carotid artery were found to differ between men and women. In these studies, however, the phase of the menstrual cycle or the use of oral contraceptives were not taken into consideration. It was the aim of the present study to investigate the effect of different ovarian hormone levels during the menstrual cycle on arterial wall properties of female large arteries.

Material and methods: The study was performed on the elastic right common carotid artery and the muscular right femoral artery of normotensive female volunteers in the age range of 18-35 y (n=12). The end-diastolic diameter (d), and the absolute (Δd) and relative diameter changes (Δd/d) during the cardiac cycle were assessed with the use of an ultrasonic wall tracking device (Hoeks et al, Ultrasound Med Biol, 16: 121-128; 1990). Arterial pulse pressure was calculated from automatic brachial artery cuff blood pressure measurements. These variables were used to calculate cross-sectional arterial distensibility (DC) and compliance (CC). Plasma estrogen and progesteron levels were measured with a radioimmunoassay.

Results: DC and CC of the carotid and femoral arteries did not change significantly during the menstrual cycle despite significant changes in ovarian hormone levels (Wilcoxon and Friedman statistical analyses). On the contrary, it was noted that bladder filling (needed for abdominal follicle detection) influenced arterial wall properties of the muscular femoral artery. Δd, Δd/d, DC and CC decreased significantly in case of a full bladder.

Conclusions: From the data of a small group of healthy female volunteers we conclude that the menstrual cycle does not influence the arterial wall elasticity of female large arteries. By chance we found bladder filling to reduce the elastic properties of the femoral artery, a reduction which cannot be explained by changes in blood pressure.

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NORADRENALINE FACILITATES MYOGENIC RESPONSIVENESS BY PRESSURE-DEPENDENT DEPOLARIZATION IN CANNULATED RAT SMALL ARTERIES. J.P.M.Wesselman*, R.Schubert, E.VanBavel, H.Nilsson, M.J.Mulvany

Myogenic responsiveness (MR), the ability of blood vessels to constrict with increased transmural pressure, is present in vessels with basal tone, but can be augmented by e.g. noradrenaline (NA). We tested how NA affected the relation between pressure and smooth muscle membrane potential (Em). Rat mesenteric small arteries were cannulated using micro-pipettes. The outer diameter (D) was monitored with a video camera. Em was measured using micro-electrodes. Pressure was raised stepwise between 10 and 120 mmHg, either without drugs or with 10 μM NA. D is normalized to D at 120 mmHg and full dilation. Without drugs pressure elevation increased both D and Em. 10 μM NA at low pressure caused both constriction and depolarization, as well as vasomotion and oscillations in Em. Pressure elevation did not change D, but evoked extra depolarization (see table, data are means$\pm$SEM, n= 6-41 vessels, 'min' and 'max' refer to minima and maxima of NA-induced oscillations, *: $p<0.05$ by t-test).

P, mmHg	diameter, %			Em, mV		
	PSS	NAmin	NAmax	PSS	NAmin	NAmax
10	65.4±0.5	45.6±0.8	47.3±0.8	-56.0±0.7	-56.7±2.3	-40.0±2.4
22	71.8±0.6*	46.0±0.7	47.6±0.6	-55.3±1.2	-49.6±1.9*	-40.1±1.9
44	86.3±0.6*	46.6±0.8	48.4±0.9	-54.1±1.3	-45.3±1.4*	-37.9±1.7
80	93.2±1.1*	50.3±2.8	52.0±2.9	-51.4±1.3*	-42.7±1.6*	-37.7±2.7
120	92.4±1.7*	49.3±1.8	50.7±2.2	-47.3±1.8*	-39.0±1.5*	-34.8±1.9

Thus the depolarization upon pressure elevation is enhanced by 10 μM NA. We conclude that the potentiation of MR is, at least in part, due to pressure-dependent depolarization.

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THE LEVEL OF LEUKOCYTE ROLLING DECREASES WITH INCREASING AGE IN CONSCIOUS RATS. G.H.G.W. Janssen, G.J. Tangelder, M.G.A. oude Egbrink, R.S. Reneman.

Leukocyte rolling (LR) is the first step in the interaction of leukocytes with the vessel wall, preceeding adhesion and diapedesis. Rolling leukocytes move distinctly slower than the free flowing red blood cells. In the present study, we investigated whether the level of leukocyte rolling in skin venules of conscious Lewis rats changes with increasing age.

Six male Lewis rats were trained to sit quietly in a restrainer unit, with a hindpaw stretched and fixed on a plateau using plastic clay. Intravital videomicroscopy with epi-illumination was used to determine the number of rolling leukocytes in skin venules in the nailfold of a toe. Two venules per animal were studied at different ages (see table).

Level of leukocyte rolling in skin venules of conscious rats.

AGE	10-11	12-14	20-23	33-35	39-40
WEIGHT	280-340 (295)	300-350 (310)	350-400 (360)	390-440 (410)	420-500 (430)
LR	9-23 (15)	5-21 (13)	7-21 (11)	4-17 (8)	2-14 (7)
AGE: weeks; WEIGHT: g; LR; cells/minute. Ranges and (median).					

LR negatively and significantly correlated with age (r_s = -0.44; $p<0.001$; Spearman rank correlation). No changes were observed in rolling velocity and in hemodynamic parameters like red blood cell velocity, vessel diameter and reduced velocity. This finding suggests that at increasing ages the initial interaction of leukocytes with the venular endothelium decreases in conscious rats. This cannot be explained by changes in hemodynamics.

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THE EFFECT OF THE PARTICULATE NATURE OF BLOOD ON TISSUE OXYGENATION AS PREDICTED BY A NUMERICAL MODEL. C. Bos, L. Hoofd, T. Oostendorp, B. Oeseburg

Since the beginning of this century, modelling of tissue oxygenation has been part of the research on oxygen transport in tissue. Modelling of tissue oxygenation started with the classical model of Krogh (1919). For simplicity, blood was considered to be homogeneous regarding oxygen transport. In the last decade, models have been developed that reflect the particulate nature of blood. These models describe at most a few capillaries or arterioles. Meanwhile mathematical models have been developed describing large volumes of tissue with up to a hundred capillaries. It was shown that large volumes of tissue should be modelled to investigate tissue oxygenation distributions. However, on such a large scale computational limitations prohibit the modelling of blood as a heterogeneous oxygen carrier. To overcome this problem, the effect of the particulate nature of blood can be introduced into these models as an additional oxygen partial pressure drop needed to extract the oxygen out of the capillary into the tissue. This drop will be referred to as the Extraction Pressure (EP). In this study we use a numerical model which describes a small part of a capillary with adjacent tissue, comparable to the volume supplied by a single red blood cell (RBC). In the previous analytical models the RBCs where considered as point-like sources. From the analytical models it was derived that the EP depended on the velocity of the RBCs and the spacing of the RBCs. These results are confirmed by the current numerical model.

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A COMPREHENSIVE MODEL OF MUSCLE TISSUE OXYGENATION. L. Hoofd, C. Bos

Calculation of oxygen distribution in tissue is a difficult and still not resolved problem. Important stages in the distribution process occur on micrometer scale, while a correct conception of tissue oxygenation is only possible on a much larger scale of about 100 μm. This fact alone already makes the problem virtually inaccessible for computer calculations - it would imply calculation of complicated and intertwined processes on a scale of at least several hundred steps in each of the three dimensions involved. But the problem is much too complicated for analytical solutions too. The only possibility seems to be a combined solution of approximate analytical descriptions and numerical treatment of the remaining parts. Such an approach is possible for a tissue where capillaries run in parallel. Muscle tissue is a good candidate. First, The micrometer scale problems of O_2 release from erythrocytes in the capillary blood are isolated and solved in terms of an extraction pressure (EP). Then, the whole-scale tissue problem is solved assuming average values for capillary O_2. Finally, this capillary O_2 is coupled to the erythrocytic O_2 via EP. This leads to a description of tissue oxygen status for a tissue part containing many capillaries, each with many red blood cells. The model can account for a different situation for each capillary. Tissue pO_2 histograms are quite heterogeneous, as found with O_2 electrode measurements. It was remarkable, that small-scale flow redistributions had almost no effect on such tissue pO_2 histograms.

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EFFECTS OF PEEP ON SYSTEMIC VASCULAR COMPLIANCE IN INTACT PIGS. B.Lambermont, O.Detry, A.Fossion, P.Gérard, T.Pochet, J.O.Defraigne, V.D'Orio.

Objective of the study.

Increased intrathoracic pressure (PEEP) has been largely demonstrated to decrease both cardiac output and aortic blood pressure, secondary to impediment in venous return. Alternatively, little attention has been focused on the mechanisms by which thoracic blood inflow is reduced by PEEP. We tested the hypothesis that potential changes in systemic vascular compliance could play a substantial role in this phenomenon.

Methods.

Systemic vascular compliance (SVC) was therefore studied in six anesthetized pigs submitted to increasing PEEP from 0 to 20 cmH_2O. SVC was referred to as the slope of the linear relationship obtained from the simultaneous changes in filling pressure and blood volume measured in the inferior vena cava during artificial and temporary interruption of caval blood flow.

Results.

Application of PEEP was associated with a significant reduction of cardiac index by 49%, and a fall in aortic pressure of 22%. Surprisingly, heart rate and systemic vascular resistance remained unchanged. Concomitantly SVC was increased from 18.3 ± 4 to 21± 9 ml/mmHg (17% increase) during the period of PEEP application.

Conclusions.

We conclude that cardiac performance is reduced consequently to application of PEEP, and circulatory adaptation does not occur. This apparent hemodynamic deterioration may be due in part to enhanced systemic vascular compliance.

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MYOGENIC REACTION OF ARTERIAL VESSELS AND ISOVOLUMIC AUTOREGULATION OF BLOOD FLOW DURING DECREASED PERFUSION PRESSURE IN RESTING FOREARM SKELETAL MUSCLES. D.Matisone, J.Skards, V.Dzerve

The task of this investigation was to ascertain whether the myogenic reaction of arterial vessels to transmural pressure (Ptransm.) changes exists and if so, whether this reaction is associated with isovolumic autoregulation of blood flow (I) in the forearm skeletal muscles during decreased perfusion pressure (Pperf.). Pperf. was decreased by raising the arm above the heart level. Forearm muscle I and pO_2 in arterialised and venous blood from deep forearm vein were determined and O_2 uptake was calculated. Compliance (Ca) of forearm arterial vessels was calculated from volume pulse amplitude and pulse pressure, haemodynamic resistance (R) of precapillary vessels - from data of regional Pperf. and I. It is stated that after reduction of Pperf. in the forearm, Ca of arterial vessels rapidly (in 2-3 s) increases by 72 ±4% and remains unchanged during reduced Pperf. Skeletal muscle I always decreases and R correspondingly increases, but afterwards two alternative patterns are observed. One, where I and R remain unchanged and the other, where after 20-30 s I begins to increase gradually untill it returns to nearly basal level and R correspondingly decreases. Such isovolumic autoregulation of I is observed in cases when basal value of I (before reduction of Pperf.) is below 1,4 ml/100g min. and basal O_2 uptake can't be provided by increase in O_2 extraction during reduced Pperf. Final value of pO_2 (20 ±2 mm Hg) in venous blood determines critical O_2 uptake in skeletal muscles. Our results show that myogenic reaction of arterial vessels and isovolumic autoregulation of I during decreased Pperf. are two different phenomena which realise in different parts of skeletal muscle arterial tree.

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THE CROSS-SECTIONAL AREA VERSUS PRESSURE RELATION OF THE PULMONARY ARTERY OF PIGS IN VIVO AT SPONTANEOUS AND FIXED HEART RATES. *L. Kornet, J.R.C. Jansen, F.C.A.M. te Nijenhuis, G.J. Langewouters and A. Versprille.*

Objective of the study: The pseudo-static compliance of the pulmonary artery, i.e. the relationship between cross-sectional area (CSA) and pressure was determined.

Methods: The study was done in vivo in eight pigs. An adapted conductance method was used to determine CSA. The pseudo-static compliance was calculated from changes in average CSA and changes in the mean arterial pressure ranging from approximately 30 down to 5 mmHg. Two series of measurements were analyzed, one at a fixed (paced) and one at spontaneous heart rate.

Results and discussion: Pseudo-statical compliance depended on pressure. This dependence on pressure, could be attributed to 1) the different contribution of collagen and elastin in the vessel wall to stretch at different pressure levels and 2) to a stretch dependent smooth muscle tone. An increase in heart rate (pacemaker) caused a constriction of the pulmonary artery. Our results in combination with literature indicated that pseudo-static compliance does not depend on heart rate itself but that pseudo-static compliance will be overestimated if measured at an increasing heart rate.

Conclusions: The pseudo-static compliance of the pulmonary artery depends on pressure and should be determined at a fixed heart rate.

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NITRIC OXIDE INHIBITS THE IN VITRO RENAL VASOCONSTRICTION TO VASOPRESSIN. G. Diéguez, A.L. García-Villalón, L. Monge, N. Fernández and B. Gómez.

The isometric contraction to vasopressin was studied in isolated, 2 mm long, segments from branches of the rabbit renal artery mounted in an organ bath. In 25 arterial segments, arginine-vasopressin (10^{-10} - 10^{-7} M) produced concentration-dependent contraction and the mean EC_{50} value was $3.0x10^{-9}$ M and the mean maximal contraction was 276±78 mg. Endothelium removal (8 segments) increased the maximal response by 153% ($P<0.05$) and shifted the concentration-response curve to the right 1.5 times ($P<0.05$), and the inhibitor of nitric oxide synthesis N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) (7 segments) increased the maximal contraction by 204% ($P<0.001$) without modifying the EC_{50} values, to vasopressin. Both the specific V_1 antagonist $d(CH_2)_5Tyr(Me)AVP$ ($3x10^{-9}$ - $3x10^{-8}$ M) (15 segments) and the V_1-V_2 antagonist $desGly\text{-}d(CH_2)_5\text{-}D\text{-}Tyr(E)ValAVP$ (10^{-7} - 10^{-6} M) (16 segments) shifted to the right in a parallel way the concentration-response curve to vasopressin, but the V_1 antagonist ($pA_2=9.43$) was more potent than the V_1-V_2 antagonist ($pA_2=8.45$) ($P<0.0001$). The specific V_2 agonist desmopressin (10^{-10} - 10^{-7} M) (8 segments) did not produce any effect in renal arteries. These results suggest that vasopressin produces renal vasoconstriction by stimulation of V_1 receptors, and this vasoconstriction is inhibited by endothelial nitric oxide. (Supported by CICYT and FIS).

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SKIN BLOOD FLOW AND FINGER BLOOD PRESSURE VARIABILITY. K.Jagomägi, R.Raamat

Physiological meaning of the synchronized fluctuation in the circulatory system is still uncertain. The aim of this investigation was to study the inter-relationship between spontaneous fluctuations in finger-tip skin blood flow and mean finger blood pressure. We recorded finger-tip skin blood flow (thermal clearance method), mean finger beat-to-beat blood pressure (differential oscillometric method) and respiratory movements at rest in 10 healthy volunteers. Thermal sensor frequency response allows recording of skin blood flow changes with period ≥5 seconds. Spectral analysis by fast Fourier transform revealed two main rhythmic oscillations in beat-to-beat finger blood pressure variability: high frequency component synchronous with respiration and low frequency component synchronous with vasomotor waves. Partial correlations of skin blood flow, mean finger blood pressure and respiratory spectra showed high individual variability, which partly could be explained by different degree of cold induced vasoconstriction (finger temperature varied from 23.2 to 32.6° C). Correlation coefficients between blood pressure and skin blood flow spectra were higher (r=.51-.95) than between blood pressure and respiration (r=.17-.39). The spontaneous fluctuations in skin blood flow have a significant impact on the finger blood pressure. Simultaneous recording of finger skin blood flow as an indicator of vasoconstriction would greatly improve the reliability and accuracy of finger arterial pressure measurement.

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COMPARISON OF VOLUME CLAMP AND DIFFERENTIAL OSCILLOMETRIC METHOD FOR NON-INVASIVE BLOOD PRESSURE MEASUREMENT ON FINGERS. J.Talts, E.Hendrikson, E.Länsimies

Monitoring of finger arterial pressure is an important development in clinical autonomic nervous regulation research. We compared beat-to-beat values of mean non-invasive finger blood pressure recorded from adjacent fingers of the same hand by volume clamp and differential oscillometric method. The first one was implied by commercially available apparatus (Finapres, Ohmeda, USA), and second one by UT9201 device. Finapres records the full pressure wave, UT9201 gives the beat-to-beat value of mean pressure. Seven healthy volunteers (aged 25 to 53 years) were studied at rest and during deep breathing with a fixed rate of 6 breath/min. We compared 100 pairs of values during rest and all values during 4 cycles of deep breathing (DB). The mean differences (Fina - UT) at rest and during DB were -2.8 ± 4.3 mm Hg ($p>0.05$) and -2.8±3.9 mm Hg ($p>0.05$) respectively. The expiratory/inspiratory ratio measured using Finapres was 1.18±0.11 and using UT9201 1.15±0.07 (difference 0.03±0.06, $p>0.05$). The correlation coefficients for the whole material during DB were 0.92, 0.94 and 0.92 for the mean, maximal and minimal values respectively. In our comparative study special attention was paid on proper application of finger cuffs. Finapres appeared to be more than UT9201 sensitive to finger cuff malapplication. No systematic significant differences between the two methods were found. We find that both non-invasive techniques - the volume clamp method (Finapres) and differential oscillometric method (UT9201 device) - gave similar mean finger blood pressure changes and both devices are of valuable use in cardiovascular research.

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CUTANEOUS VASODILATION INDUCED BY TRANSCUTANEOUS NERVOUS STIMULATION. R.Orasan

Cutaneous circulation can be enhanced by transcutaneous nervous stimulation (TNS) to the patients with leg ulcer. The vasodilation has been testfied by recording the following data before and after stimulation:

- the partial pressure of O_2 which increased approximately 3 times in the patients
- the skin blood flow which doubled as compared to the pre-stimulatory levels
- the skin temperature which raised to 1.5°C after a latency span of time of approximately 30 minutes.

The above modifications suggest the release of neuro-humoral substances during the stimulation, responsible for the vasodilation and the fast healing. Three physiological mechanisms seem to be mainly involved :

- the decrease of the sympathetic vaso-motor tonus
- the release of humoral vasodilator mediators
- the axon reflex.

The patients with leg ulcer to whom this method has been applied for a month with a daily rate of 30 minutes showed spectacular healing of the ulcers as compared to the reference group.

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A MATHEMATICAL MODEL FOR BLOOD MICRO-CIRCULATION

by DANIEL PASCA[1] and GALINA CAMENSCHI[2]

The problem of modelling of blood flow has been approached by several authors so far, each of them proposing one model or another for the modelling of fluid flow (blood), as well as for the modelling of blood vessel walls.

These paper aim is to propose a model, that we consider adequate for modelling blood micro-circulation. Blood is modelled as a Newtonian viscose incompressible fluid, and the blood vessels as a thin pipe, axi-symmetrical to the thin wall, considered as a linear elastic membrane. In this paper we consider the influence of variable viscosity on the main characteristics of fluid motion (pressure and the components of the velocity of the fluid), and we obtain the analytical solutions using the asymptotic analysis. The classical Hagen-Poisseuille model is obtained as a first asymptotic approximation model of our proposed model.

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NEURAL CONTROL OF GASTRIC FUNCTIONS IN RATS: ROLE OF AUTONOMIC NERVE DISCHARGE PATTERN.

S.A.Polenov, M.V.Lensman, V.A.Zolotarev, V.S.Demjanenko, R.P.Hropycheva and V.F.Shalenkov.

In nembutal-anaesthetized Wistar rats, we compared the effects of regular continuous and burst stimulation of regional autonomic nerves on gastric microvasculature, secretion and motility. In vivo TV-microscopic studies on submucosal microvasculature revealed more powerful and better sustained arteriolar contractile responses to stimulation of the left splanchnic nerve in 1s bursts at 4s intervals at 5-40 Hz compared with continuous stimulation at 1-8 Hz, delivering the same total number of impulses. In contrast, submucosal venules responded equally to stimulation in bursts and continuously, reflecting differences in neuroeffector organization of arterioles and venules. Arteriolar contractile responses to stimulation in high-frequency bursts persisted partly after adrenoreceptor blockade suggesting possible involvement of nonadrenergic cotransmitter release. In the perfused sympathectomized stomach model, continuous vagal stimulation for 80 min at 10 Hz produced much more pronounced increase in acid and becarbonate secretion than stimulation in 1s bursts at 4s intervals at 50 Hz, with no differences in pepsinogen secretion. In pylorus and oesophagus ligated rats, regular continuous stimulation of vagal efferents at 1-16 Hz evoked an increase in intragastric pressure whereas high-frequency burst stimulation (20-40 Hz for 1s at 4s intervals) invariably resulted in gastric relaxation. This seems to be the first evidence of vagally induced gastric relaxation in animals without a preliminary atropinization. We conclude that the rat gastric functions may be effectively controlled merely by changing the pattern of autonomic nerve impulse activity. (Supported by ISF grant R4Q000).

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ULTRASTRUCTURE AND FUNCTIONING OF CARDIAC LYMPHATIC CAPILLARIES.

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We performed postmortem ultrastructural examination of miocardium in 43 patients (age group 39 - 85 years) which died from acute myocardial infraction (37) or some other diseases: pulmonary embolism (2), aortic aneurysm rupture (2), sudden cardiac death (2). Tissue samples were taken by transthorax express-necropsy immediately after the patients' death in clinic.

Three types of lymphatic capillaries could be distinguished in the myocardium according to their diameter and wall structure : terminal lymphatic capillaries (TLC), moderate lymphatic capillaries (MLC) and large lymphatic capillaries (LLC). TLC had clearly visible discontinuous basal lamina which did not include pericytes. TLC were found close to the cardiomyocytes. MLC had anchoring filaments which could partly prevent the collapse of these microvessels. Pericytes sometimes appeared as a component of MLC wall. LLC demonstrated close relationship with neighboring cardiomyocytes by means of elastic legaments and couplings which could transmit cardiomuscular movements upon LLC maintaining their vasomotion and lymph circulation. Couplings included anchoring filaments which were braided into the subendothelial reticulum formed by collagen and reticular microfibers which appeared around the LLC.

From this data we conclude that different parts of myocardial lymphatic capillaries show typical structural features and functional accomodations for the maintenance of vasomotion and lymph circulation.

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ROLE OF THE NA^+/K^+ PUMP IN PROLIFERATIVE RESPONSES OF VASCULAR SMOOTH MUSCLE CELLS TO MITOGENS.

S.R. Sampson and S. Ganis-Shen

Proliferation of vascular smooth muscle cells (VSMC) is believed to be important in the production of atherosclerotic lesions in blood vessels, but the signals involved in the mitogenic response of VSMC are as yet unclear. We have investigated the role of the Na^+/K^+ pump in mediation of the proliferative responses VSMC to mitogenic stimulation. Studies were done on A7r5 VSMC made quiescent by incubation in serum-free DMEM for 48 hr. We measured [^{3}H]-thymidine uptake, Na^+/K^+ pump activity, and c-*fos* protein expression by conventional methods. Insulin and phorbol esters caused a large increase in [^{3}H]-thymidine uptake measured during 18 hr. This was associated with increases in cell numbers. This effect was significantly reduced by ouabain given up to 2-4 hr after the mitogen. When given longer than 2 to 4 hr after the mitogen, ouabain failed to reduce the proliferative response. Angiotensin had no clear effect on [^{3}H]-thymidine after 24 hr but caused an increase after 48 hr, an effect also reduced by ouabain. Insulin caused Na^+/K^+ Pump activity to increase within 5-15 min, and this was blocked by amiloride, which inhibits Na^+/H^+ exchange. Phorbol esters caused Na^+/K^+ Pump to increase after 30-60 min, but this effect was not altered by treatment with amiloride. All mitogens tested induced c-*fos* protein within 30-60 min, and this effect was not influenced by treatment with ouabain. We conclude that changes in Na^+ and K^+ ion fluxes play a key role in responses of VSMC to mitogenic stimulation. (Supported by the Otto Meyerhoff Center, Ben and Effie Raber Research Fund, and the Harvett-Aviv Neuroscience Research Fund.)

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SMOOTHELIN, A MARKER FOR HIGHLY DIFFERENTIATED VASCULAR SMOOTH MUSCLE CELLS.

Frank T.L. van der Loop[1], Giulio Gabbiani[2], Gert Schaart[1], Frans Ramaekers[1] and Guillaume van Eys[1].

Smoothelin is cytoskeleton associated constituent in smooth muscle cells. This 59 kDa protein is expressed in differentiated, contractile smooth muscle cells (SMC) in a broad range of species and tissues. Smoothelin was not detected in myofibroblasts, myo-epithelial cells, skeletal or cardiac muscle. It was also absent in established smooth muscle cell lines. In this study the presence of smoothelin in normal and proliferative vascular SMC was investigated. Smoothelin is found in aorta SMC, that express either vimentin or desmine next to α-smooth muscle actin. Confocal scanning laser microscopy of aorta SMC indicated that smoothelin is organized as filaments organized but does not colocalize with desmin, vimentin or α-smooth muscle actin filaments. In a number of SMC of the T. media of muscular arteries, smoothelin was more abundant than desmin. In some of the atherosclerotic plaques groups of smoothelin positive cells were detected, mostly at the luminal side of the plaque. This indicates that there are fully differentiated cells of smooth muscle origin present in these plaques.

Smoothelin appears to be a marker for both vascular and non-vascular contractile smooth muscle cells.

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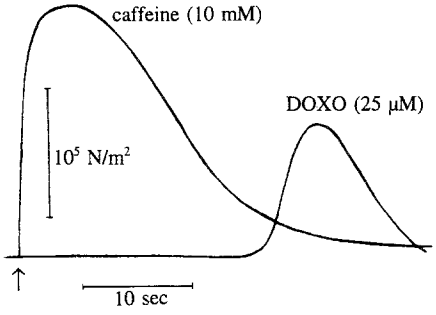
DOXORUBICIN AFFECTS ACTIN-MYOSIN INTERACTION AND CALCIUM RELEASE FUNCTION OF SARCOPLASMIC RETICULUM.

A.E. Bottone, B.G.V. van Heijst, P. Schiereck, E.E. Voest, E.L. de Beer.

Doxorubicin (DOXO) is an important chemotherapeutic agent. Its use is limited by cardiotoxicity, related to the cumulative dose applied. The aim of this investigation is to study the direct effect of DOXO on the actin-myosin interaction as well its effect on the sarcoplasmic reticulum (SR).

Rabbit psoas fibres were either skinned by freeze-drying, thereby disrupting the SR membrane, or by glycerol, leaving the SR functionally intact. Isometric tension of single fibres was measured with a force transducer (SI, Heidelberg, Germany). SR of the fibres was loaded by immersing the fibre in Ca^{2+}-uptake solution (pCa 6.4) for 10 min. In freeze-dried fibres, DOXO (1, 5, 10 and 20 μM) increased maximal tension depending on both incubation time and sarcomere length.



The figure shows two tension transients evoked by caffeine or DOXO in the same fibre. The arrow indicates the moment of immersion. In Ca^{2+}-loaded glycerol-skinned fibres, caffeine (10 mM) immediately evokes a tension transient, while DOXO (25 μM) releases the SR after a delay.

This delay of about 20 sec is reduced after subsequent loading and releasing with DOXO. These results implicate that DOXO affects both cross-bridge interaction and the SR.

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EXPRESSION OF GAP JUNCTION PROTEINS IN HUMAN UMBILICAL CORD ARTERIAL SMOOTH MUSCLE CELLS.

R.S. de Boer, H.V.M. van Rijen, M.J.A. van Kempen and H.J. Jongsma

Gap junctions establish direct cytoplasmatic contact between cells. They consist of proteins called connexins of which several isoforms are known. In the vascular wall gap junctions are thought to play a role in maintaining homeostasis and coordination of vasoconstriction. In order to obtain an in vitro model system for vascular smooth muscle cells (SMC) we used the explant method to culture SMC from the human umbilical cord artery (HUCA) and compared the connexin43 (Cx43) expression in situ and in vitro using immunohistochemical techniques. The HUCA has a well defined morphology. It consists of an intima with endothelial cells (EC) and a lamina elastica bordered on a media with longitudinal and circular layers of SMC. The artery is localised in the so-called Wharton's jelly which is comparable to the adventitia and contains mainly connective tissue with fibroblasts. Antibodies against smooth muscle actin (SMA) and desmin were used as markers for SMC. Three distinct layers of SMC were found in the HUCA media. Two, longitudinally orientated layers flanking one circularly orientated layer. All layers were SMA positive whereas desmin showed only distinct staining in the two outer layers of the media. Cultured SMC were all SMA positive whereas desmin positive cells were only seen sporadicaly in primary cultures. With antibodies against Cx43 a clear staining was found in HUCA sections, especially in the outermost longitudinal layer of the media and in the EC of the intima. In cultured SMC, Cx43 showed a more intense staining with increasing passage number, whereas SMA staining became fainter. We conclude that initial cultures of SMC are comparable to SMC in the HUCA while SMC with higher passage number are not because they change into a different phenotype.

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NONGENOMIC EFFECTS OF SYNTHETIC STEROID SEX HORMONES ON THE VASCULAR SMOOTH MUSCLE CELLS.

J.Aivars, A.Uljanov, G.Praulite

The aim of current investigation was to proof the possibility of rapid nongenomic effects of synthetic steroid sex hormones on the reactivity and sensitivity of vascular muscle cells. We used the miography of isolated strips of rabbit aorta in the standart solution and synthetic steriods Noretisterone (NT) and Ethynyl-oestradiole (EO) in the concentracion of $3{,}3.10^{-6}$ to $3{,}3.10^{-4}$ M/l. Rapid and reversible effects of NT and EO were revealed. Synthetic steroids provoke the increase of both the basal tonus and the amplitude of Norepinephrine (NE)- induced contractions, increase the Hill's coefficient of NE - "dose-response"curve and shift the curve to left. Those also increase the tonic component of NE - contraction for 35,2±2,5%, decrease the amplitude of the contractions in Ca-free solution for 18,1±4,2%, decrease and elongate the contractions elicitd by potassium. Both NT and EO do not influence the contractions elicited by Angiotensine. The similar series of experiments was perfomed on the isolated aorta of a rat.The changes of the parameters of both NE - contractions in Ca-free medium or contractions induced by potassium were similar in both series. In conclusion, the obtained results confirm hypothesis that the steroid sex hormones like aldosterone can produce not only delayed genomic effects but also rapid non-genomic effects on the membran functions of vascular smooth muscle cells.

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REDUCED CONTRACTILITY IN MESENTERIC RESISTANCE ARTERIES FOLLOWING MYOCARDIAL INFARCTION IN THE RAT. M.J.J.M.F. Willemsen, F.R.M. Stassen, G.E. Fazzi, J.G.R. De Mey.

Following myocardial infarction, blood pressure is reasonably maintained through elevation of peripheral vascular resistance resulting from the stimulation of various neurohumoral mechanisms. We evaluated whether alterations of peripheral resistance arteries develop under these conditions. In rats, myocardial infarction (MI) was induced by permanent ligation of the left coronary artery. Mesenteric resistance-sized arteries (MrA) were isolated after 3 and 5 weeks. After sympathectomy and removal of the endothelium, their reactivity (myograph experiments) and structure (morphometry on cross sections) were compared to those of sham operated animals. Following MI, the optimal diameter and the media cross sectional area of the MrA were not modified. Also the sensitivity of the vessels for the contractile effects of noradrenaline, phenylephrine (PHE), serotonine and vasopressin were not altered. Maximal contractile responses to these agonists and those to high potassium (K+) were, however, significantly reduced at 3 and 5 weeks post-MI. The calcium-sensitivity of the vessels was evaluated after removal of extracellular calcium and depletion of intracellular stores. In the presence of high K+ and of both high K+ and PHE no alteration was observed. Calcium-sensitivity in the presence of PHE was, however, drastically reduced. This was more pronounced at 5 than at 3 weeks post-MI. Thus, in the rat a progressive reduction of the contractile reactivity of peripheral resistance arteries develops after MI despite maintenance of vascular wall structure. This and the observed reduction of the calcium-sensitivity suggests alterations of the excitation-contraction coupling.

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COMPARATIVE EFFECTS OF α - TRINOSITOL ADMINISTERED EXTRA- AND INTRACELLULARLY (BY USING LIPOSOMES) UPON RAT AORTA RINGS. D.D. Brănişteanu

Many groups have investigated the inositol phosphate - calcium signalling system. Although the involvement of inositol trisphosphate formation is well documented in many functional processes such as muscle contraction, metabolism, cell secretion and differentiation, there are still some areas less understood. The effects of α - trinositol, a D - myo - inositol [1, 2, 6] trisphosphate derivative, were studied upon desendothelised rat aorta rings. The organ bath conatined Krebs - Henseleit solution (pH 7.4), kept at 37°C and aerated continuously with 95% O_2 + 5%CO_2. α - trinositol was applied extracellularly as well as intracellularly (by using liposomes as drug carriers). Upon extracellular administration, the drug reduced the level of contraction induced by 40 mM K^+ or by phenylephrine (10^{-5} M). No effects were observed upon relaxed preparations. Liposomes containing α - trinositol induced a dose dependent contraction of the preparations under resting tension with a threshold of 10^{-5} M in the aqueous phase. These contractions were heparin - insensitive but were significantly blocked by either D - 600 (10^{-5} M) (a L - type Ca^{2+} channel) or in Ca^{2+} - free medium. Our data suggest that α - trinositol has a plasmalemmal mechanism of action which could involve Ca^{2+} influx from the extracellular space.

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EFFECTS OF ADENOSINE DEAMINASE - LOADED LIPOSOMES UPON CONTRACTIONS IN THE ISOLATED AORTA INDUCED BY K^+, PHENYLEPHRINE OR BY LIPOSOMES CONTAINING D - MYO - INOSITOL 1, 4, 5 TRISPHOSPHATE. S.M. Slătineanu, E. Brăiloiu and D.D. Brănişteanu

This work examines the effects of adenosine deaminase (ADA) - loaded liposomes (L_{ADA}), administered either as pretreatment or during the contraction plateau in rat aorta rings stimulated with: high K^+ (40 mM), phenylephrine (PHE 10^{-5} M), or D - myo - inositol 1,4,5 trisphosphate - loaded liposomes [$L_{Ins\ (1,4,5)P3}$]. The experiments were performed on rat thoracic aorta denuded by endothelium. The cut rings were mounted between hooks in the organ bath,containing Krebs - Henseleit solution at 37°C and aerated continuously with 95% O_2 + 5% CO_2. Administration of L_{ADA} upon the relaxed preparations did not change the resting tension level. Excepting the $L_{Ins(1,4,5)P3}$ - induced contraction, L_{ADA} application resulted in relaxing effects which were dependent upon the contractile agent used and the moment of its administration. These effects were similar to those of the Ca^{2+} - channel blocker D 600 (10^{-6} M). Under identical experimental conditions, L_{ADA} were able to either increase or decrease the effects of D 600 upon the high K^+ - and PHE - induced contraction. In its turn, D 600 showed a similar influence upon the effects of L_{ADA}.

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THE EFFECTS OF pH ON THE RAT PULMONARY ARTERY. J.Ramsey, D.Curran, C.Austin, S.Wray.

Changes of extracellular pH (pH_o) have been known for many years to alter vascular tone. In some vascular tissues at least this is due to large and rapid changes in intracellular pH (pH_i) (Austin & Wray, 1993). Hypoxia, which is thought to produce a decrease in pH_i, has been shown to constrict pulmonary vessels, whilst relaxing or having no effect on systemic vessels (Yuan *et al.*, 1990). The reason for this is unknown but may reflect different functional responses of pulmonary vessels to pH. We have therefore investigated the effects of pH_i and pH_o on the pulmonary artery.

pH_i and tension were monitored simultaneously in small strips of isolated rat pulmonary artery (1st branch), loaded with the pH-sensitive dye carboxy Snarf. Tissues were constantly perfused with a HEPES buffered Krebs solution at 37°C, pH 7.4. pH_o was altered by the addition of HCl or NaOH and pH_i by the addition of sodium butyrate or trimethylamine (40 mM). All experiments were carried out in tissues pre-contracted with KCl (40 mM) which was isosmotically substituted for NaCl.

A decrease in pH_o caused a relaxation of all tissues studied, while an increase caused a contraction. When pH_i was measured simultaneously, it appeared that changes in pH_o caused large (approximately 50%) and rapid (approximately 5 minutes) changes in pH_i which were closely associated with the changes in tension. Increases or decreases in pH_i alone seemed to cause a contraction or relaxation of tissues respectively.

It appears therefore that decreases in both pH_o and pH_i cause relaxation of pulmonary artery while increases in pH cause contraction. This is similar to the effects previously reported in other vascular tissues.

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INFLUENCE OF HYPODYNAMICS STRESS ON CONTRACTILITY OF SMOOTH MUSCLES OF THORACIC AORTA, MORPHOLOGY OF MYOCARDIUM, AND AORTA AND CHANGES OF ELECTROCARDIOGRAMS M. Kušleikaitė, S. Stonkus, D. Reingardienė, K. Daukša, R. Gailys.

Hypodynamic stress was induced in rabbits by placing them in tight metal hutches (according to Fiodorov B. M., 1991). Twenty five rabbits of 2.5-3 kg were distributed into three groups: a control group of 8 rabbits which had no intervention was kept in ordinary vivarium conditions, group 2 consisting of 8 rabbits was kept immobilised for 1 day and night, and other 9 rabbits were kept immobilized for 28 days and nights. Contraction force (provoked by adrenaline and acetylcholine 10-5M) of thoracic aorta smooth muscles preparations from rabbits was registred every 3 minutes (in 3, 6, 9, 12 and 15 min) by micromechanographic metod in isometric regime and expressed in percent. Electrocardiograms (ECG) were recorded in three standart derivations. The obtained results show that after 28 days of hypodynamics, thoracic aorta contractility force of smooth muscles effected by adrenaline and acetylcholine significantly ($p<0.05$) increased as compared with the data of the control animal group. After 1 day of hypodynamics these data changes were not significant. Sladge phenomenon, disintegration of myofibrils and their setting down in flakes, swelled endothelium of aorta, twisted elastic fibre, some of them fragmentary, were obtained only after 28 days of hypodynamics. The obtained results of ECG show that ischemic ST segment changes were more pronounced after 1 day of hypodynamics stress.

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FISH DIET AND OIL, DHA-CONTAINING OIL AND PLASMA LIPID AND COAGULATION FACTOR LEVELS. O. Hänninen, J. Ågren and G. Hornstra

The study was carried out to clarify the effects of fish diet, fish oil and DHA-containing oil on fasting and postprandial lipid levels and on coagulation factors. Subjects were healthy male students (n=59). Fish meals were provided at workdays to subjects in the fish diet group for 14 weeks (the amount of meals actually eaten was 4.3±0.5 per week). This provided 0.38±0.04 g EPA and 0.67±0.09 g DHA per day. Fish oil group ate 4 g fish oil per day, and it provided about 1.3 g EPA and 0.9 g DHA. DHA-group ate 4 g per day oil which contained about 42 % DHA. One group served as controls. Oral fat-loading tests were made before and after the dietary period. Both fasting plasma triglyceride levels and postprandial triglyceride response were reduced in all test groups. In the fish diet group, plasma triglycerides decreased from 1.36±0.47 to 1.16±0.40 mmol/l. In fish oil and DHA-oil groups they decreased from 1.21±0.35 to 0.89±0.13 and from 1.17±0.38 to 0.97±0.21 mmol/l, respectively. Most of these changes occurred in VLDL-triglycerides. The increase of total plasma triglycerides (mmol/l x h) after fat load was 4.6±1.4 before and 3.3±2.1 after the dietary period in the fish diet group. In the fish oil group these values were 4.3±2.4 and 3.0±1.2, and in DHA-oil group 3.7±2.0 and 3.1±1.0. The increase in chylomicron triglyceride concentration followed the same pattern. Total cholesterol levels were not changed but the HDL2/HDL3-cholesterol ratio increased in all test groups. The levels of tissue factor pathway inhibitor, prothrombin fragment 1+2, modified antithrombin III or fibrinogen were not changed. Factor X level was decreased by fish diet. These results show that fasting and postprandial triglyceride levels can be decreased with moderate intakes of long-chain n-3 fatty acids either from a fish diet or fish oil.

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PARTICIPATION OF ENDOTHELIN IN ACTIVE MYOGENIC REACTIONS OF THE VASCULAR SMOOTH MUSCLES. M.N. Tkachenko and V.F. Sagach.

The purpose of the study was to elucidate the possible involvement of endothelin in the modulation of length-tension dependence of the vascular smooth muscles.Graded distension of the intact strips of the rat portal vein resulted in the rise of the amplitude of phasic contractions. Increase of the distension (force vs. initial level) to 10 mN was accompanied by the maximum increment of the amplitude of vascular smooth muscle contraction with subsequent fall at 12 mN. Mechanical deendothelization of the strips significantly decreased the volume of active myogenic length-tension reactions. The amplitude of contraction was maximum at 4-5 mN. Similar effect was produced by phosphoramidon (3 x 10-6M), which inhibits endothelin-converting enzyme. At the same time, addition of endothelin-1 (10-9M) to the perfusate increased both the initial force of contractions of the vascular smooth muscles and the volume of increment of contraction of strips at the increase of their length. The change of the length of vascular preparation led to a more steep rise of the curve of phasic contraction amplitude - the force of graded distension. Thus, the perfusion with endothelin resulted in a lesser distension needed to be applied to the smooth muscle to produce more significant contraction.The data presented suggest the involvement of endothelin (released by the vascular endothelium at distension of the vascular wall) in the development of active myogenic reactions of the vascular smooth muscles at their distension.

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ENDOTHELIUM-DEPENDENT FLOW-INDUCED RELAXATION OF BOVINE MESENTERIC LYMPH VESSELS. Lobov G., Orlov R., Pankova M.

Contractile activity of smooth muscle cells was investigated on isolated perfused segments of bovine mesenteric lymphatics, consisting of two lymphangions. Both lymphangions had rythmic contractility when on input and output of preparation the transmural pressure was 5 cm water. The spontaneous activity of both lymphangions inhibited at increase transmural pressure on input to 10 cm water. In same experiments by pretreatment with 10^{-5} M methylene blue or remove of endothelium of the whole preparation restored the spontaneous activity of smooth muscle cells in both lymphangions at increase of pressure on input, but spontaneous activity of lymphangions not was restored by pretreatment with 10^{-5} M aspirin. The removal of endothelium in distal lymphangion resulted to the spontaneous activity in the lymphangion without endothelium and this activity was absent in the proximal lymphangion. If the endothelium was removed in proximal lymphangion and it was intact in distal lymphangion the increase of pressure on input resulted to the ingibition of spontaneous activity in both lymphangions, but this activity of both lymphangions was restored in solution with 10^{-5} M methylene blue. These results suggest than constant flow of solution in lymphatics may produce endothelium-dependent relaxation smooth muscle cells through stimulating synthesis of guanosine 3',5' cyclic monophosphate.

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PHARMACOLOGICAL EFFECT OF LIPOSTABIL UPON LIPID LEVEL IN CULTURE OF CELLS FROM A MAN'S AORTA WITH ATHEROSCLEROTIC ALTERATIONS AND UPON AGGREGATION ABILITY OF THROMBOCYTES. H. Kurdanov, L. Batyrbekova, E. Popov.

In the present work we determined the lipostabil ability to decrease the cholesterol (CH) concentration in cultured aorta plaques of a man in vitro. Lipostabil in concentrations beginning from 250 mkg/ml positively decreases the CH content for 35-40% in cells taken out of atherosclerotic plaques. While increasing the preparation dosage up to 500-1000 mkg/ml, the further growth of CH decreasing action was not determined. In concentration of 250 mkg/ml lipostabil inhibits the ADPh-induced aggregation of thrombocytes (0.5 mkM ADPh) for 55.2 ± 3.1%. If lipostabil concentration is more than 2500 mkg/ml, the aggregation is inhibited completely. Using of PAPh-10 nM and collagen - 0.2 mg/ml as aggregation inducers showed that lipostabil (250 mkg/ml) has the most expressed effect upon PAPh-induced aggregation, depressing it for 85.1 ± 2.6%. The received data indicate that lipostabil in certain concentrations is able to decrease HC in primary organ culture of a man's aorta, taken out of atherosclerotic affections, which is connected with its influence upon HC going out of cell, and also it inhibits the aggregation ability of thrombocytes.

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CORRELATION MEASURES FOR ACTIVATION PATTERNS DURING ATRIAL FIBRILLATION. B.P.T. Hoekstra, C.G.H. Diks, M.A. Allessie and J. DeGoede

The spatiotemporal complexity of atrial activation is partly characterized by estimating the spatial extension of coherent myocardial regions. Conventional linear correlation measures can be applied, e.g. the spatial correlation function. Also, nonlinear measures derived from information theory may be used such as generalized redundancies. We studied the right atrial activation pattern determined from high-density mapping during electrically induced fully developed atrial fibrillation in a patient undergoing open-chest surgery. We used the linear spatial correlation function and the redundancy $I(r,\epsilon) = \ln[C_{xy}(\epsilon)/C_x(\epsilon)C_y(\epsilon)]$, in which r is the spatial separation between electrodes positioned at x and y and $C(\epsilon)$ the Grassberger-Procaccia correlation integral estimated at a resolution ϵ in reconstructed phase space. Correlation measures (fig.) were estimated choosing a row of unipolar electrodes (interelectrode distance 2.25 mm).

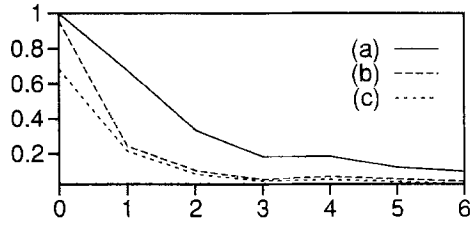

Fig. Spatial correlation function (a) and Redundancy at resolution 0.05 (b) and 0.1 (c) in a.u. against electrode separation. Correlation lengths l_c from fits with an exponential function are 4.2 (a), 1.7 (b), 2.0 (c) mm.

The example suggests that the correlation lengths l_c estimated by the two methods are clearly different. The precise physiological interpretation of this difference remains to be investigated.

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BASAL FIBRINOLYTIC ACTIVITY AND FOLLOWING DYNAMIC SHORT-TERM EXERCISE IN HEALTHY SUBJECTS AND IN PATIENTS WITH CORONARY HEART DISEASE. F.Gligor, R. Rinea, N.Giurgea, V.Lupu, V.Ossian, C.Vlad, D. Predescu, D.Zdrenghea

The authors aimed to find wheter short term exercise at ergonomic bycicle, besides the sympathetic activation, can induce significant changes in the fibrinolytic activity of the coronary patients already presenting EKG changes at low exercise thresholds.

Two groups of subjects were studied: 10 healthy subjects aged 21±3 years and 18 patients with coronary heart disease(CHD)aged 54±7 years. The following determinations were performed in all cases before and after the exercise test: LTDC (lysis time of the diluted clot formed by coagulation with thrombin of the blood sample diluted 10 times with acetate buffer), TRAS (time of residual serum activation), fibrinogenemia, lipemia, cholesterolemia, hematocrit, glycemia, Quick and Howel times, EKG, systolic and diastolic blood pressure at various exercise levels. TLCD did not show significant changes in controls versus the basal levels. But in patients with CHD and positive exercise test the values were significantly decreased (261.42±7,97 as against 223.66±15.22; ($p < 0.05$). The rise was very significant ($p < 0.001$) for blood pressure and arterial heart rate levels ($p < 0.001$). In conclusion, even short-time exercise in patients with CHD produced a marked activation of the fibrinolytic activity which was beneficial for the patient and which could counteract the effect of the circulating catecholamines of the increase of blood viscerity (increased hematocrit), that could determine the occurence of a thrombotic coronary event.

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THE STUDY OF THE CARDIAC CONTRACTILITY AT THE OBESE AGED PEOPLE.-St. IONESCU

The studies were performed on 9I cases,among wich28 with normal weight and 63 overweight,of both sexes and ages between 40 and 76.There were measurings of arterial pressure,of the electrocardiogram,phonocardiogram and carotidogram simultaneous recordings;we used as indicators: the heart rate, the preejection period,the preejection/ejection ratio,the maximal oxygen consumption(VO_2max.).The overweight group included 44 men and I9 women, the majority of them with light obesity(+I5-25%) and only 6 cases with severe obesity(+40-50%).65% of overweight patients also presented anothers risc factors such as: high blood pressure, sedentarism.The results:-The heart rate in overweight patients in repose shown light, insignificant increases.Major increases are observed in obese patients who performed a physical effort. The preejection period shown increases in obese persons,from 83ms. to II0ms.(and to I26ms. in 50% of cases),similar for both sexes;but according to age the increases are higher in men 55-65 of age and women of 76.The ejection period was not importantly modified in overweight women,but it decreased from 293ms. to 260ms. in overweight men at the age of 55-65.The prejection/ejection ratio increases from a normal value of 0,34 to 0,44 in obese persons and to 0,50 in 67% of overweight men.The increasements are higher in aged patients.It was stated a VO_2max. increase,especially in obese women suggesting a higher cost of cardiac activity and a reduced efficienty of the cardiac contractility.The values of the diastolic and men arterial pressure are increased in obese women and indicate an increase of the resistance in the systemic circulation.

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THE FIFTH DETERMINANT OF CARDIAC PERFORMANCE.R.Cârmaciu
Although it is generally accepted that intraventricular conduction defects alter the heart's functioning as a pump,until the present day most Cardiologists have failed to recognize the pattern of ventricular activation as a primer determinant of cardiac performance (along with preload,afterload,inotropic state and heart rate). From a theoretical point of view there are some favourable consequences of physiological activation of the ventricles: The secvential activation provides the heart muscle with a "peristaltic-like" wave of contraction,a sort of synchronism of systole and an optimal manifestation of the "idioventricular kick" phenomenon. In our work we describe a representative case which demonstrate undoubtfuly our assumptions. A patient,aged 58,suffering with SSS,having a pacemaker type VVI and submitted to cardiac catheterization,for IHD,showed a systematised arrhythmia,consisting of 3-4 heartbeats in sinus rhythm,alternating with 3-4 heartbeats in pacemaker rhythm;in both circumstances the cardiac frequency was about 50-65 bpm. We measured the following systolic and diastolic parameters of the left ventricular performance,during heart catheterization :EDP,ESP, Mean and Systolic Aortic Pressure,+dP/dt and -dP/dt.STI. Cut-clear results were obtained.EDP varied between 5 and 8 mmHg in both sinus and pacemaker rhythms. However,the ventricular and aortic systolic pressure were 140+/-5mmHg during sinus rhythm,and respectively 115+/-4mmHg during artificial rhythm ($p<0.001$). The peak +dP/dt was 1250+/-27mmHg in sinus rhythm,and only 720+/- 22mmHg in pacemaker rhythm ($p<0.01$).The -dP/dt varied nesemnificatively about 2000 +/-45 mmHg. The PEP was 120+/-8 ms in sinus rhythm and respectively 170+/-9 ms in pacemaker rhythm,and LVET was 356+/-15 ms in sinus rhythm and only 258 +/-12 ms in artificial rhythm. Another feature of ecg proved that we were not faced with a "pacemaker syndrome".

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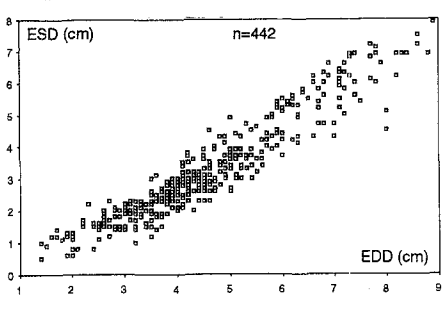
VENTRICULAR SYSTOLIC & DIASTOLIC DIAMETERS IN DOGS ARE RELATED OVER THE FULL PATHOPHYSIOLOGIC RANGE. A. Roos, G. ter Haar, S. Kocsis, G. Voorhout, A.A. Stokhof and P.L.M. Kerkhof*.

Aim of the study is to investigate whether ventricular dimensions can uniquely be characterised independent of underlying cardiopathology. Cardiac output is known to relate to body mass, and ventricular output dependence on preload has often been studied in the form of a Starling curve. To assess ventricular diameter interrelationships we analysed echocardiograms of the left ventricle measured in 442 dogs. These animals were referred to our animal clinic because of cardiac problems, including e.g. mitral regurgitation and dilated cardiomyopathy. Body mass ranged from 0.5 to 80 kgs, and age from several months to 14 years. We applied linear regression analysis, yielding

$$ESD = 0.917\ EDD - 0.92 \text{ with } n = 442 \text{ and } r = 0.93$$

where end-systolic (ESD) and end-diastolic diameter (EDD) are expressed as cm (ranges 0.4 to 8 cm, and 0.6 to 9 cm, respectively). The slope of the regression line is significant ($p < 10^{-6}$). This implies the presence of certain volumetric regulation principle(s) which still have to be delineated. Since fractional shortening (FS) depends on the interplay between ESD and EDD, FS obviously relates to ESD. Moreover, this equation permits the pressure-volume area to be expressed as an explicit function of ESD.

We conclude that the regression equation found offers a useful starting point for further studies on volume regulation and for calculations on indices for ventricular performance, particularly on FS and myocardial oxygen consumption.



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Optimal left ventricular fibre orientation for homogeneous sarcomere length during ejection in the normal heart

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During the ejection phase of the cardiac cycle, left ventricular muscle fibres shorten while generating force. It was hypothesized that fibres are oriented in the wall such that the amount of shortening is the same for all fibres. We evaluated this hypothesis for the equatorial region of the left ventricle.

In a finite element model of left ventricular wall mechanics fibre orientation was quantified by a helix angle which varied linearly from the inner to the outer wall. Fibre length was characterized by sarcomere length, set at 1.95 µm everywhere in the passive state of 0 transmural pressure. For a cavity pressure of 15 kPa, considered representative for ejection, inhomogeneity in mechanical loading was expressed by the variance of the sarcomere length. The variance was minimized by adapting the transmural course of fibre angle. First, only the slope was optimized and in a second optimization this was done for both slope and intercept.

Optimal helix fibre angles were 69.6° endocardially, 0° at the middle of the wall and -69.6° epicardially for the first optimization and 78.2°, 20.7° and, -36.7° respectively for the second. Sarcomere length changed from 1.95 to 1.975±0.012 and 1.981 ±0.004 µm (mean±SD) respectively. Conclusion: After optimization calculated helix fibre angles were in the physiological range. Describing the transmural course of fibre angle with slope and intercept significantly improved homogeneity in mechanical load.

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BAROREFLEX SENSITIVITY DURING ATRIAL NATRIURETIC PEPTIDE INFUSION IN WISTAR RATS. D.Mikchov, R.Girchev, P.Markova, B. Piryova

Conscious freely moving (n=10) as well as 35 mg/kg b.w. Nembutal anesthetized male Wistar rats (n=20) were used. 40 ng/min per kg b.w. Atrial Natriuretic Peptide was infused through jugular vein catheter in the course of 80 min. Systolic artery pressure (SAP), diastolic artery pressure (DAP), mean artery pressure (MAP) and heart rate were measured using Statham transduser connected to femoral artery catheter by Biopac MP100WS data acquisition system. Computer *on line* record and continued analysis after data collection with the help of AcqKnowledge software was made. Baroreceptor-heart-reflex sensitivity was assesed before and during Atrial Natriuretic Peptide infusion by evaluating the reaction of the inter-beat interval (IBI) in response to either blood pressure rise (i.v. Phenylephrine Hydrochloride 5-10 µg/kg b.w.) or drop (i.v. Sodium Nitroprusside 5-10 µg/kg b.w.) and the slope of the linear regression function defined baroreceptor-heart-reflex sensitivity (ms/mm Hg). Atrial Natriuretic Peptide infusion per se did not change arterial pressure and heart rate. The infusion of Atrial Natriuretic Peptide did not change the baroreceptor-heart-reflex sensitivity responding to blood pressure rise and drop in Nembutal anesthetized rats. IBI/MAP slope in conscious rats remained unchanged during Atrial Natriuretic Peptide infusion as compared to control period (0.92 ± 0.21 versus 1.28 ± 0.28, $p > 0.05$). In conclusion the baroreflex sensitivity is not changed during Atrial Natriuretic Peptide infusion in anesthetized as well as in conscious male Wistar rats.

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EFFECT OF INTRACEREBROVENTRICULAR (ICV) ATRIAL NATRIURETIC PEPTIDE (ANP) IMMUNONEUTRALIZATION ON THE FLUID-ELECTROLYTE HOMEOSTASIS IN CONSCIOUS UNRESTRAINED WISTAR RATS, N. Belova and B. Piryova

To clarify central ANP participation in fluid-electrolyte balance 20 male Wistar rats with cannulated third cerebral ventricle were put on high (H-Na) or low (L-Na) sodium regimens. The conscious freely moving in the metabolism cages rats were subjected to the following separate ICV treatments: 1) Controls; 2) Angiotensin II (AII); 3) ANP antiserum (ABANP); 4) AII 3h following ABANP. Fluid intake and urine flow rate (V) were registered for 6 hours periods following the treatments, and electrolyte excretions ($U_{Na}V$, $U_{Cl}V$, $U_{K}V$ and $U_{Osm}V$) were evaluated per 100g b.m. and per minute. Major results: AII was a potent stimulus for fluid intake and for V and $U_{Na}V$, especially in the H-Na rats that were drinking 1%NaCl solution. Central ANP immunoneutralization per se produced a significant increase in V and $U_{Na}V$. This effect was also predominantly expressed in the H-Na group. In the H-Na rats V rose from 3.4±0.4 to 16.5±5.5µl/100g/min ($p<0.0003$) and $U_{Na}V$ increased from 1.4±0.2 to 2.9±1.1µmol/100g/min ($p<0.05$). In the L-Na rats the respective changes for V were from 1.6±0.1 to 3.0±0.04µl/100g/min ($p<0.0002$) while $U_{Na}V$ increase was insignificant. In the ABANP+AII group an increase in fluid intake , V and $U_{Na}V$ was observed. The changes were most prominent in the H-Na rats again. These results confirm our previous data for putative diuretic and natriuretic central ANP immunoneutralization effects per se and in combination with central application of 0.6M NaCl. In both protocols the effect of central ANP immunoneutralization is especially pronounced on the background of pre-stimulated natriuresis.

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REPRODUCTIVE PERIOD AFFECTS WATER INTAKE IN HEAT-STRESSED DEHYDRATED GOATS. K. Olsson, M. Josäter-Hermelin, J. Hossaini-Hilali, K. Cvek, E. Hydbring and K. Dahlborn

Water intake following dehydration was studied in acutely heat-stressed pregnant (N=5), lactating (N=4), and nonpregnant, nonlactating (N=5) Swedish domestic goats to investigate if reproductive period affected drinking. The goats were water deprived from 09.00 h until 15.05 h the next day, when they got free access to water at the temperature of 35±1°C. They were fed morning and afternoon. On the second day, ambient temperature was increased from 20°C to 38-39.5°C for 5.15 h to accelerate water losses with minimal disturbance of food intake. Respiratory rate increased most during pregnancy and lactation causing greater water losses than during the nonpregnant, nonlactating period. To some extent this was counteracted by a greater increase in rectal temperature during these reproductive periods. Cutaneous moisture loss was small in all periods both during dehydration and rehydration. During dehydration plasma Na and vasopressin concentration increased most in pregnant and lactating goats. Pregnant goats lost 2.2 kg of their body weight. They drank 3.5 l immediately followed by 2.5 l more during afternoon eating. Lactating goats lost 4.9 kg and drank 6.3 l at once, and 3.9 l more during feeding. Nonpregnant, nonlactating goats lost 1.7 kg and drank 2.6 l followed by 0.6 l. The large water consumption in pregnant and lactating goats caused marked hyponatraemia and plasma vasopressin concentation fell below measurable levels, but in nonpregnant, nonlactating goats these values only returned to control levels. Urine flow and renal free water clearance increased in all periods, but with long-lasting water diuresis only during pregnancy. The results imply that the thirst center had become less sensitive to inhibitory signals from the oropharyngeal tract and to hyponatraemia in pregnant and lactating goats during rehydration.

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EFFECTS OF ENDOTHELIN-1 ON GLOMERULAR FILTRATION RATE AND SODIUM EXCRETION IN CONSCIOUS DOGS.

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The effects of endothelin-1 (ET-1) at three doses (40, 400 and 4000 fmol·kg^{-1}·min^{-1}) on renal and hemodynamic parameters were investigated in 6 slightly water deprived conscious dogs. After a 30 min control period ET-1 was infused continuously for 120 min. Only one dose was investigated each day. The losses of urinary sodium were replaced during the experiment. Infusion of 40 fmol·kg^{-1}·min^{-1} increased sodium excretion (9±2 to 48±21 µmol·min^{-1}) and osmolar clearance (Cosm) (0.60±0.05 to 0.95±0.17 ml·min^{-1}). Urine flow increased (0.15±0.01 to 0.35±0.16 ml·min^{-1}) while glomerular filtration rate (GFR), potassium excretion, free water clearance (CH_2O), heart rate (HR) and mean arterial pressure (MAP) remained unchanged. Infusion of 400 fmol·kg^{-1}·min^{-1} also increased sodium excretion and Cosm (6±2 to 55±18 µmol·min^{-1} and 0.65±0.06 to 1.05±0.20 ml·min^{-1}, respectively). Urine flow increased (0.16±0.01 to 0.53±0.14 ml·min^{-1}) and GFR, potassium excretion, CH_2O, HR and MAP remained unchanged. At 40 and 400 fmol·kg^{-1}·min^{-1} the natriuretic effects peaked after termination of ET-1 infusion. Infusion of 4000 fmol·kg^{-1}·min^{-1} increased MAP (104±4 to 138±4 mmHg) and decreased HR (71±4 to 46±3 beats·min^{-1}). GFR and the excretion rate of sodium decreased (33±2 to 12±3 ml·min^{-1} and 8.3±1.1 to 1.2±0.2 µmol·min^{-1}, respectively). Also potassium excretion and Cosm decreased (16±2 to 8±2 µmol·min^{-1} and 0.7±0.1 to 0.2±0.1 ml·min^{-1}, respectively) while CH_2O increased (-0.50±0.07 to -0.02±0.01 ml·min^{-1}). After the infusion had stopped urine flow increased (0.17±0.02 to 0.53±0.11 ml·min^{-1}). It is concluded that low doses of ET-1 increase sodium excretion without affecting GFR, HR and MAP and high doses of ET-1 decrease sodium excretion, GFR and HR while increasing MAP. It seems likely that low doses of ET-1 inhibit tubular Na^+-reabsorption without any measurable effects on GFR.

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ON SOME ELECTROLYTIC AND WATER METABOLISM MODIFICATIONS IN RATS SUSCEPTIBLE TO AUDIOGENIC CONVULSIONS. M. Uluitu, R. Chis, Al. Badescu.

Following our research in the relation between mechanisms of blood transportation of sodium and cerebral excitability, we studied rats susceptible to audiogenic convulsions and rats with normal response to acoustic stimulation, in order to determine: 1. The degree of Na interaction with blood serum proteins; 2. Free motor behaviour; oriented behaviour, motivated behaviour; 3. Influence of variable Na intake on renal elimination of Na, K and water. Results showed cerebral excitability is associated with several modifications; 1) Absence of Na interaction with blood serum proteins in normal animals; 2) There is significant hypermotricity in the animals susceptible to audiogenic seizure; 3) Hyperexcitable animals consume significantly more NaCl solution, which they freely choose in a motivational manner, than normo-excitable animals; 4) Increased, motivated consumption of NaCl is associated with increased renal elimination of Na in animals susceptible to audiogenic seizure. Increased renal elimination of Na is accompanied by increased water elimination, therefore with protection of renal concentration mechanisms; 5) The NA/K ratio indicating the function of the corticosuprarenal is greater in animals with cerebral hyperexcitability, also involving the mineralcorticoid hormones, similarly to body Na loading; 6) Our data suggest that normal cerebral excitability is compatible with existence of Na transport mechanisms in interaction with blood proteins.

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POSSIBLE PHYSIOLOGICAL MECHANISMS OF LIGHT NEGATIVE HYDROAIRIONS ACTION IN CELLS AND ORGANISM. I.Stavrovska, A.Babsky and I.Shostakovska

The physiological and therapeutic influence of light negative hydroairions (HI) obtained by spraying water due the balloelectric effect was investigated. We found out pronounced normalization influence of HI on respiration and oxidative phosphorylation in rat liver mitochondria (MCH) altered by stress due to immobilization or injection of physiological dose of adrenaline. Adaptation to such influence on MCH level is connected with oxidation Krebs cycle substrate -succinate- in active metabolic state (ADP addition or active uptake of calcium ions in MCH). The respiration by HI during 20 min changed some physical-chemical indices of blood, namely decreases: the reaction of settling erythrocyte, protein coefficient of blood (relation of albumine and globuline) that limited viscosity of blood, calcium and glucose concentration, increases the concentration of hemoglobin. The HI effects are probably mediated by the changes of properties of biologic solutions such as water and blood. The flow of HI blown over the water surface increases its calorific capacity and decreases surface tension that is due to cooperative formation of structures in water. We suppose that lungs besides gas and water exchange have regulation function of electrical charges of colloidal and cell elements in blood. Thus, lungs electroexchange is the primary step of HI action whereas electrochemic interactions among colloidal systems of blood and tissues (tissues electroexchange) are secondary step.

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INVESTIGATION OF CELL MEDIATED IMMUNE DEFICIENCY IN RATS WITH PROTEIN ENERGY MALNUTRITION. N.Esen, E.Bahçeci

In developing countries, Protein Energy Malnutrition (PEM) is even an important cause for morbidity and mortality. The reason for morbidity and mortality in PEM is infections that are seen due to cell mediated immune deficiency. In previous studie, it was observed that lymphoid tissues especially thymus became atrofied and the proportion of sheep-erythrocyte-rosette forming cells was decreased. In addition it is reported that there is dysfunction of the complement system. Nevertheless the mechanism of immune deficiency in malnutrition is not actually explained. We think that the low proportion of the rosette forming cells and the dysfunction of lymphocytes may be caused by CD2 receptors that works inproperly. In this study, 20 wistar-albino rats were fed with a diet containing 5.5 % protein for three weeks. On the other hand, control group was fed with normal diet containing 24 % protein. At the end of three weeks there was a 18 % weight loss and serum total protein was decreased significantly in the protein deficient group. Lymphocyte subpopulations including CD3+, CD19+, CD4+, CD8+, CD2+ cells were investigated with immunoflourescance technique. IgG, IgM, IgA levels were measured with radialimmunodiffusion plaques. The proportion of CD3 + cells was not changed while CD19+ and CD8+ cells were increased significantly with a significant decrease in CD4+ and CD2+ cell proportions. There was a correlation (r= 0.52) between the decrease both in IgG and IgM levels, and CD4 as well as CD4/ CD8 proportions. We think that PEM causes a decrease in number and/ or function of lymphocyte subpopulations. As a result cell activation and antibody production occure inproperly.

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IMPORTANCE OF PORE/CELL SIZE RELATIONSHIP IN DETERMINING ERYTHROCYTE DEFORMABILITY BY CELL TRANSIT ANALYZER. A. Temiz, O. K. Başkurt, F. Kandemir.

In the cell transit analyzer (CTA), time course of the passage of individual RBC through a special filter is monitored by a computerized system. The widely used filter has 30 pores of 5μm diameter and 15μm length. CTA with this pore-size filters has been used in studies on human blood with satisfactory reproducibility and sensitivity. However, the data on other mammalian blood is very limited. Most mammals used in laboratory studies have smaller RBC that can pass through 5μm pores with lower resistance indicated by shorter transit times (TT) under the same pressure gradient. The aim of this study is to test the influence of pore/RBC size relationship on reproducibility and sensitivity of CTA parameters. Coefficient of variation (CV) for repeated measurements and the sensitivity to the mechanical alterations induced by heat treatment (HT) (48^{o}C 30 min.) and glutaraldehyde (GA) treatment (0.005%, 30 min., at 20^{o}C) were determined with human and rat blood, using 5 and 3μm pore-size filters. Mean cell volumes for human and rat RBC were 89.71±0.74 fL and 54.50±0.50 fL respectively. CV of TT determined using 5μm filters for human RBC was 3.07% while it was 4.83% for rat RBC. TT increased 17.72±0.02% and 15.32±0.03% for human and rat RBC respectively after HT. GA incubations resulted in 19.40±0.04% and 31.91±0.03% increments in human and rat RBC. Human RBC did not pass through 3μm filters under 3 cmH_2O pressure gradient. CV determined using 3μm filters for rat RBC was 6.27%. TT increased 60.58±16.63 % after HT and 64.10±14.07% after GA treatment. In conclusion, rat RBC TT had slightly higher CV with both 3 and 5μm filters. 5μm filters can be used for RBC deformability measurements of rat and human with similar sensitivity. The sensitivity for the changes in rat RBC mechanical properties can be increased significantly by using 3μm filters.

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A STUDY OF INTRACELLULAR FREE CALCIUM IN AN IN VITRO MODEL OF APOPTOSIS IN AVIAN GRANULOSA CELL SHEETS. L.Leybaert and K. D'Herde

It is known that calcium ions play a role in the triggering of apoptosis. Experimental work on in vitro models indicate that an increase of the intracellular free calcium concentration ($[Ca^{2+}]_i$) probably initiates the pathway to apoptosis. In the present study we have used an in vitro model of apoptosis in avian granulosa cell sheets to study apoptosis related changes of $[Ca^{2+}]_i$. The in vitro model of apoptosis consists of culturing granulosa cell sheets, isolated from quail, under serum-free conditions. These granulosa cell sheets are at one side delineated by the basement membrane and at the other side by the vitelline membrane; the normal cellular organisation is thus more or less preserved in this preparation, in contrast to dissociated granulosa cell preparations. Granulosa cell sheets kept for 24 h in serum-free culture did not show any evidence for apoptosis. However, following 96 h serum-free culture an important fraction of the cells showed the hallmarks of apoptosis as evidenced by acridine orange, electron microscopy and in situ-end labeling. We have used the calcium-sensitive fluorescent indicator fura-2 to study the resting level of $[Ca^{2+}]_i$ in this granulosa cell preparation. $[Ca^{2+}]_i$ was measured in a sub-area of the preparations, encompassing approximately 100 cells. The resting $[Ca^{2+}]_i$ averaged 457 ± 148 nM (n=10) following 24 h serum-free culture and 83 ± 17 nM (n=6) following 96 h in culture.

The experiments suggest that, in this in vitro model, apoptosis is associated with a lowering of $[Ca^{2+}]_i$. This conclusion is in line with recent observations that demonstrate that apoptosis can be induced with calcium chelators. It furthermore supports the hypothesis that a disregulation of the $[Ca^{2+}]_i$ homeostasis sets the way to apoptosis.

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STUDIES ON GAP JUNCTIONAL COMMUNICATION BETWEEN TERM PREGNANT HUMAN MYOMETRIAL CELLS BEFORE LABOR. H.N.Çîray, B-E.Persson, G.M.Roomans, T.Bäckström, and U.Ulmsten

Regulation of gap junctional communication between the smooth muscle cells of human myometrium is of importance as initiation of labor to terminate pregnancy is achieved by increased frequency of low-resistance channels provided by the gap junctions. Control of gap junctional regulation will allow better understanding of the mechanisms of labor, and thereby of disorders of labor which might arise from junction malfunction. To understand the mechanisms that regulate the gating of the channels, we have employed the dye-coupling technique, and injected the fluorescent probes lucifer yellow (LY) and carboxyfluorescein (CF) into myometrial strips from women who were in term pregnancy but not in labor. We have found that human myometrial cells are dye-coupled before labor, and coupling was more extensive when CF was the injection probe (injections resulting in coupling: 75% compared to LY: 15%). With both probes quantification of coupling was poor, and therefore we studied electrical coupling between the cells by passing a constant current (1nA) through the injection pipette. This gives the input resistance of the cells which mainly varies with the quantity and gating of the gap junctional channels. We have used the putative gap junctional uncoupling agent octanol to inhibit gating between the cells. Octanol increased the input resistance of the cells compared to the untreated group (23.9±10.7 MΩ in the untreated and 32.6±10.3 MΩ in the octanol treated group). As progesterone has been shown to increase the tonus and the frequency of contractions in human myometrial strips we have also studied the effects of progesterone on the input resistance in the non-labor tissues. The input resistance after progesterone treatment did not differ (22.1±6.0 MΩ) from the control group, and application of octanol to the progesterone treated group increased the input resistance (41.6±7.6 MΩ) indicating that progesterone does not act via gap junctions.

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COMPERATIVE EXAMINATIONS ON THE BASIS OF NK AND K CELL ACTIVITY CHANGES OF HEALTHY BLOOD-DONORS AND PREGNANTS

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Objective : On blood-donors in reproductive age, we examined the possibility of the change exerted on the cellular immune reaction caused by genetic differences, taking into consideration the cell systems NK and K, compared with the pregnants in the first trimester.
Study design : 200 voluntary blood-donors (100 men, 100 women) as well as 100 pregnants in the first trimester took part in our examinations. For the study we used approx. 4 ml of blood drawn from the cubital vein and placed in plastic test tubes containing heparin. We began analysing the blood samples within 1 hour. We examined the NK cell activity in relation to target cells originating from the K-562 myeloid leukemia cell line. We examined the antibody-dependent cellular cytotoxicity (ADCC) of the K cells in relation to "O" Rh+ (D) red blood cells. We used the cytotoxic enzyme kinetics model to determine the maximum NK and K cell activity. We used the BMDP Mann-Whitney, Kruskal-Wallis trials in our mathematical analysis.
Results : We have ascertained that no significant difference can be shown amongst these three groups examining either the cell system NK or K.
Conclusions : As to the two above elements of the cellular immune reaction, there is no difference between the sexes in the reproductive age.

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EFFECT OF PROTEIN C INHIBITOR ON *IN VITRO* FERTILIZATION. X.L. Zheng, M. Zechmeister-Machhart, P. Uhrin, P. Hufnagl, M. Geiger, B.R. Binder

Protein C inhibitor (PCI) is a relatively non-specific serine protease inhibitor which has been originally described in plasma as an inhibitor of activated protein C and other coagulation factors. PCI is also synthesized throughout the male reproductive tract and is present in high concentrations in seminal pasma (Laurell et al., J. Clin. Invest. 1992). We have shown recently that PCI inhibits the sperm protease acrosin and that endogenous PCI is immunocytochemically localized to disrupted acrosomal membranes, while intact sperms were negative for PCI-antigen (Zheng et al., Am. J. Physiol. 1994). Under physiological conditions acrosin is activated and released in the female reproductive tract in the immediate vicinity of the ovum and is thought to be involved in the fertilization process. In the present study we therefore analyzed PCI-synthesis in the female reproductive tract and the effect of purified PCI on *in vitro* fertilization. Northern blotting of mRNA isolated from mouse ovaries revealed that PCI is also synthesized in the female reproductive tract. When zona-intact mouse eggs were exposed to purified human PCI (5µg/ml) during *in vitro* fertilization by mouse epididymal sperms, the number of sperms bound to the zona pellucida (1.4±0.3 sperms/egg, mean ±SE) and the percentage of fertilized eggs (29.6±7.8%) were significantly lower ($p<0.01$) than in control experiments (3.2±0.8 sperms/egg; 46.6± 8.3% fertilized eggs). In zona-free eggs PCI (5µg/ml) reduced sperm-egg binding by 32% and *in vitro* fertilization by 52%. Heat inactivated PCI had neither an effect on sperm-egg binding nor on *in vitro* fertilization. These data suggest that PCI, present in the female reproductive tract might modulate the ferilization process possibly by a mechanism involving the inhibition of sperm acrosin.

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CHOLECYSTOKININ IN THE REGULATION OF EMOTIONAL BEHAVIOR. E.Vasar, S.Kõks, V.Volke, V.Võikar, A.Lang, A.Kask and P.T.Männistö

The recent evidence suggests the role of neuropeptide cholecystokinin (CCK) in the regulation of fear and anxiety both in laboratory animals and in man. The aim of present study was to characterize further the involvement of CCK in anxiety in rats. The elevated plus-maze model of anxiety was employed in rats. In one part of the study the intraventricular cannula was implanted into the third ventricle under the chloral hydrate anesthesia (350 mg/kg). Caerulein (5 µg/kg), an unselective agonist of CCK receptors, induced the anxiogenic-like action in the plus-maze.The pretreatment of rats with L-365,260 (10 µg/kg), an antagonist of CCK_B (brain subtype) receptors, blocked this action of CCK agonist. Devazepide (10 µg/kg), an antagonist of CCK_A (peripheral subtype) receptors, tended to augment the effect of caerulein. The acute administration of CCK antagonists did not affect the behavior of rats. However, after the repeated treatment (12 days, twice daily) the situation apparently changed. L-365,260 (50 µg/kg) and devazepide (50 µg/kg) both inhibited the exploratory behavior of rats. The action of L-365,260 was accompanied with a strange alteration of behavior. Namely, after the placing into the central platform animals had difficulties to enter into the enclosed arm and due to that the exploratory activity of these rats was markedly reduced. The administration of devazepide (250 ng) into the third ventricle, differently from L-365,260 (250 ng), induced the anxiogenic-like action on the behavior of rats. Consequently, the results of present study are in favour of different role of CCK_B and CCK_A receptors in the regulation of emotional behavior. CCK_A receptors seems to mediate the anxiolytic action, whereas CCK_B receptors are related to the anxiogenic effect in the rat.

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THE PHAGOCYTIC ACTIVITY OF RATS WITH STEREOTAXIC DISTRUCTIONS IN THE MAIN ZONES OF AMYGDALA. M. Dorofteiu, R.Orăsan, C.Marina, M.Zirbo, A.Vaida, A.Deyyoub

The amygdala is involved in some of the most complex functions of the brain including emotion and memory. A lot of the limbic system efferences run towards the hypothalamus which takes part in the coordination of energetic metabolism and phagocytic activity. The investigation were carried out on 58 adult Wistar albino rats, divided in 4 groups with billaterally distructions in: the corticomedial part, basolateral part and central part of the amygdala, and the last group which received diazepam before and after the billaterally electrocoagulation in the basolateral part. After the experiment, the animals were killed and the position of the lesions was checked by microscopically examining brain serial sections. Three days before and on the 3rd and 7th day after the intervention, blood was taken from the retroorbital sinus, in order to determine glucose,proteins and lipids, as well as the phagocytic activity.In the fourth group, the investigations were performed,two hours after the administration of diazepam(0.5 mg/kg) by intragastric tube in normals animals and on the same rats, 2 weeks after the billateral damage in the basolateral part of amygdala.The distructions in the three main parts of amygdala decrease significantly the phagocytic activity, the ratio of NBT positive white cells and induce a remarcable rise of the lipids level in the blood. In the animals with lesions in the corticomedial part of amygdala an augmentation of the blood glucose level was noticed. Diazepam declined the phagocytic activity and the ratio of NBT positiv leukocytes.

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METHOD FOR MEASUREMENT OF CHANGES OF HUMAN LIMBS PERIMETERS. Ü. Kristjuhan

In occupations where a worker is not engaged in heavy manual labour, precise measurement (to within 0.2 mm) of the perimeters of upper and lower limbs and their parts, fingers and toes, may be useful. It enables to clear up the changes of volumes of soft tissues, extracellular and intracellular fluids, which depend on the working position, physical activity, peripheral fatigue processes, microclimate, clothing etc. As the scientific literature did not offer practical methods for small changes of limb perimeters (from 0.5 to 2.0%) we have developed a perimeter meter which consist of a measuring tape from metal or plastic and loads at the end of the tape, for guaranteeing constant pressure between the tape and skin. In recent years we have used plastic hyaline tapes with a round section plane and a diameter from 0.2 to 0.3 mm. These are convenient in research and not expensive. One circle of the tape was measured using a calibrated ruler (to within 0.1 mm). The measurements were carried out in the same horizontal position of the limb. Our results during 12 years showed that the method was one of the best for the measurement of physical changes due to workload in industry and services and for finding practical solutions of work rationalization.

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ENVIRONMENTAL AND PATHOLOGICAL FACTORS INFLUENCING SALT AND SWEET PREFERENCE IN THE UNITED ARAB EMIRATES. A. Al-Hashimi[1], T.Y. El-Sharkawy[2] and N. R. Banna[2].

A study of the effects of different variables (urbanization, age, gender, hypertension and diabetes) on salt and sweet preference in samples of United Arab Emirate nationals was conducted. Hedonic preference was measured by ad libitum salting or sweetening of food by the subjects (Ss). Ss were asked to ad NaCl to unsalted tomato juice, or sugar to unsweetened 2% lemon solutions, to their level of preference. Towards the end of the experiment, the resulting mixtures were analyzed, respectively, for their chloride content gravimetrically after ashing, and for their sugar content polarimetrically.

In the case of salt preference no significant differences (t-test) were observed between urban men and women, between 2 age groups (above or below 35 years) or between hypertensive and normal Ss. However, urban men showed a significantly higher preference for salt than rural men, and rural women showed a highly significant preference for salt compared to rural men, probably due to the women's excessive and dark-colored clothes in a hot environment. Furthermore, there was a highly significant increase in salt preference in diabetic (non-IDDM) patients, but no difference in hypertensive patients. In the case of sugar preference, no significant differences were observed between urban and rural Ss or between men and women in the two environments. However, older (>35y), diabetic and hypertensive Ss showed a significantly lower sugar preference than young, nondiabetic and normotensive Ss, respectively.

In conclusion, the major trends observed were an enhancement in sugar preference in the younger age group that is increasingly being exposed to a modernized way of life, a clear and physiologically-driven preference for salt in bedouin women, and a decrease in sugar preference amongst hypertensives and diabetics.

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THE PHYSIOLOGY AND THE ONTOLOGY OF THE HUMAN BEHAVIOUR Vasile Astărăstoae, Sebastian Slătineanu, Călin Scripcaru

The authors consider that according to the present - day conceptions, the elaboration of a hierachical system of behaviour in man depends on the integration and adjustment levels, namely genetic, humoural, neurologic, psychic and social.

The authors have investigated each of these levels. For this purpose they have analysed the results of 25 studies (including their owns) on 2097 pairs of monozygotes twins, 974 dizygotes twins and 75 separately brought up monozygotes, a group of 14 children resulted from incestuous relations, 91 gonosomic mutations cases and 1029 socially assisted minors followed during 5 years.

It results therefore that there is a hierarchic and integrated behavioural system structured under the influence of genetic and ambient factors. The interactions of genetic and environmental factors does not support each other, but are so much interpenetrated that their non - dis - sociability becomes a postulate. So, behaviour can be equated with a genetic - mesological character of quasi - continuous variation. Heredity could express itself in the existence of a subjacent tendency (with a continuous variable), but the phenotypical distribution of individuals is discontinuous (on either side of the average), depending on the conjugated effect of different factors.

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ON THE TREATMENT OF DEPRESSIVE DISTURBANCES IN PHARMACOLOGICALLY KINDLED RATS. A.Shandra, L.Godlevsky, P.Chuyev and R.Vastyanov

In the course of kindling development induced via repeated subthreshold picrotoxin or pentylenetetrazol injections to male Wistar rats the appearance of seizures including the repeated ones were observed. The progressive decreasing of locomotor activity, explorative, consumptive behaviour as well as the reduction of the paradoxical sleep was observed in interictal period. The "neuroleptic catalepsy" components become prevalent in the posture and locomotor disturbances in the course of kindling. The investigations of systemic administration of naloxone and yohimbine (alpha-2-adrenergic receptors antagonist) were performed in fully kindled rats. It was established that i.p. naloxone (2-5 mg/kg) engendered the restoration of locomotor and exploratory activity thus lessening depressive disturbances. Naloxone also reduced the seizure threshold. It was established also established that yohimbine normalized the locomotor activity and caused the appearance of opiate - dependent components in postseizure depressive syndrome. Yohimbine also heightened the seizure threshold. Gained data permit to conclude that pharmacological kindling may be considered as the model of depressive state development. which could be of certain significance for the development of questions of pharmacological treatment of depression and related disturbances.

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QUANTITATIVE ESTIMATION OF HUMAN OLFACTORY REACTIONS. M.R.Gzhegotsky

Solution of some applied problems of physiology is connected with the experimental definition and probability estimation of olfacty (lim I) imparted by xenobiotics to water, food, etc. Volunteers of 12-15 in each group were shown 10 odorous substances water solutions in different concentrations (C, mg/l). The obtained results were compared by revealing the dependencies of dose-effect (A) and dose-response (B) relation-ships. According to the Weber-Fechner Law A was approximited to $I = a \cdot \lg C + b$ straight line where I is smell intensity from 0 to 5 grades; a and b - coefficients. The coefficients were defined applying the least square method. Considered lim I in the capacity of concentration C-50 corresponding to I = 1. For its probability estimation the mutual conformity of space for I and C was taken into account. In the case of B probit method was used and lim I was estimated as usually in a state of CE-50 proceeding from the frequency of individual estimation I = 1 when testing C concentrations in the narrower value diapason. When C-50 and CE-50 were defined in the independent experiments we received accidental estimations CE-50 $\gtrless$ C-50. When proceeding from the same experiment it is always CE-50 < C-50 and "correct" (accepted) estimation of lim I is the subject of agreement. Variant A provides the estimation stability and probability of simultaneous definition of smell sense practical bound for I = 2 and of necessary (for instance in the extremal situations) for I = 3. These advantages allow to recommend A variant for examining category problems solutions.

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CUMULATIVE AND ADAPTIVE EFFECTS OF EXOGENOUS TAURINE. V. I. Fedorenko and B. M. Shtabsky

Taurine was introduced daily into the stomach of Wistar male-rats in the doses of 3000 mg/kg (1), 600 mg/kg (2), 120 mg/kg (3), 15 mg/kg(4), 3 mg/kg(5), 3 mg/kg(6) for a 30 days. The activities of erythrocyte"s total, Mg^{2+} - and Na^{+} - K^{+} - ATP-ases, liver Ca^{2+} - ATP -ase, cerebral cortex and liver cholinesterase (ChE) and monoamineoxidase (MAO), calcium and urea contens in blood plasma, and behavioral reactions were determined on the 10th, 20th and 30th days. Liver and testis morphology and spermatogenesis functional indicators were investigated after finishing experiment. On the 20th day of experiment some animals that accepted taurine in 4-6 doses were introduced to taurine (7500 mg/kg) or trichlorfon (190mg/kg) as a load; the activities of ATP-ases and ChE and liver morphology were investigated. We established the dose-dependent inhibition of erythrocyte ATP-ases and activation of liver ATP-ase. In diapason from 1 to 4 doses we also revealed brain ChE and motive reactions inhibition, liver MAO activity and calcium level increase in blood plasma. 1-3 doses have brought to body mass decrease and to hepatocyte dystrophy with focal necrosis; 1 and 2 - to death of some rats and all test shifts. Reactions to both loads were considerably weaker than that in intact animals, but occured in inverse ratio from 4 to 6 doses. In pharmacology and dietology in should be taken into account the ratio of cumulation and adaptation phenomena under the influence of taurine different doses. Toxic and physiologic effects are appeared in 1-3 and 4-6 doses diapasons correspondingly.

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TYPE A BEHAVIOR PATTERN: HETEROGENEITY PSYCHOLOGICAL DETERMINANTS. A NEW APPROACH TO DIAGNOSTICS. L. SEVERYANOVA, A. ZAVYALOV, V. PLOTNIKOV, D. PLOTNIKOV.

A complex psychological, psychophysiological and neurophysiological investigation was completed on medical university students with type A behavior according to Jenkin"s questionnaire. Our investigation has shown, that generalized people with different psychological characteristics have type A behavior pattern. Exterior similar signs of behavior were used as the basis for generalization. Four main types of accentuated profiles of type A behavior persons were described as follows: 1)hyperthymergastic-active type (its signs are: stable elevated mood, mental and psychomotor activation, thirst for activity), 2)cyclothymic type (alternation of hyperthymic-active and dysthymic states, i.e. bipolar affective accentuation), 3)emotional type (elevation of intensity and appearance of emotions, hyperdrawing into activity and events), 4) mixed type (combination of mono- and bipolar affective accentuation and signs of emotional type). It was established that socio-environmental factors play essential role in the formation of type A behavior. Type A behavior persons differ in individual parameters (character and temperament) subjected to the action of the environment. These parameters inconsiderably differed in physiological indices of activation (electroencephalograms, electrocardiograms, heart rate frequency, blood pressure and etc).

A new test for differential value of psychophysiological profiles of type A behavior persons was worked out.

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THE INVESTIGATION OF POLYPEPTIDE GROWTH FACTORS IN BLASTODERM AND YOLK OF EARLY CHICK EMBRYOS.L.Solohub, S.Kusen, H.Antonyak, I.Pashkovska, N.Antonyak
We have isolated from blastoderm and yolk of chick embryos at gastrula stage two trypsin-sensitive factors capable of stimulating Swiss 3T3 fibroblast anchorage-dependent proliferation. Isolation was performed by acid-ethanol extraction, gel filtration on P-Biogel, HPLC and Heparin-Sepharose chromatography. One of the factors with molecular weight of 6 kDa positively reacts with anti-insulin antibodies. It also binds specifically to a 350 kDa membrane protein of rat liver cells whose molecular weight coincides with that of the insulin receptor. Hence, it follows that the first isolated factor is insulin. The second factor has a molecular weight about 30 kDa and binds to a 180 kDa receptor of rat liver plasma membranes. After heating for 10 min at 70°C its mitogenic activity decreases by 20%, and at 90°C by 80%.
It was found that ^{125}I-labeled embryonic factor, insulin, EGF and TGF-β bind to different plasma membrane receptor proteins during incubation. Nevertheless, the receptor binding activity of 30 kDa embryonic growth factor increases significantly in the presence of both insulin and epidermal growth factor. TGF-β appears to have no effect on the intensity of embryonic factor-receptor complex formation. Two protein fractions with receptor-binding activity were found after chromatography of this embryonic factor on Heparin-Sepharose column (eluted at 1,2 M and 2,0 M NaCl). Our data show that the 30 kDa factor belongs to basic heparin-binding growth factor family and probably is involved in regulation of angiogenesis and myogenesis during early embryonic development.

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ATRIAL FIBRILLATION IN THE GOAT: A MODEL FOR DEDIFFERENTIATION OF CARDIOMYOCYTES. J. Ausma, M. Wijffels, F. Ramaekers, M. Allessie, M. Borgers.
Chronic atrial fibrillation (AF) was induced in chronically instrumed goats by electric pacing. After 2-3 months of sustained AF the goats were sacrificed and atrial myocardium was studied by light- and electron microscopy. The large majority of the cardiomyocytes showed marked changes in their cellular substructures: loss of myofibrils, accumulation of glycogen, mitochondrial shape changes and reduction in mitochondrial size, fragmentation of sarcoplasmic reticulum and dispersion of nuclear chromatin. With immunocytochemical procedures the expression and organisation patterns of contractile and cytoskeletal proteins such as titin, cardiotin and α-smooth muscle actin were assessed in atria altered as such and compared to normal atria. The occurence of titin in a punctated pattern and the diffuse or even complete absence of cardiotin in chronic AF cardiomyocytes are indicative for an embryonic phenotype of these cells. The re-expression of a-smooth muscle actin, a protein that is absent in adult cardiomyocytes in AF cells support this hypothesis. Cardiomyocytes with similar structural changes were shown in the left ventricle of the chronic hibernating myocardium and those seen during embryonic development. Therefor these changes are considered to be part of a dedifferentiation process. This model of chronic AF represents an animal model in which cellular adaptation occurs in a way comparable to chronic hibernating myocardium and, in view of the uniformity of the cell change, lends itself well to study the cascade of events leading to dedifferentiation.

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RELATIONSHIP OF THE CONTRACTILE FORCE OF ONE BEAT TO THE MEMBRANE POTENTIAL AND OUTWARD CURRENT OF THE PRECEDING BEAT IN ISOLATED FERRET MYOCARDIUM.
P.Arlock, B.Wohlfart, and M.I.M. Noble.
The contractile force was studied in 13 ferret papillary muscles subjected to voltage clamp depolarisations, using the single sucrose gap method. Prolongation of a single test depolarisation within a train produced potentiation of the following contraction. We studied the effect of varied duration and membrane potential of the test depolarisation upon the potentiated force of the following beat. The relationship between the peak contractile force of the following potentiated beat and the systolic membrane potential of the test depolaraisation revealed an equilibrium of -20mV to -15mV; this was manifest after a 100msec period of no effect. More positive potentials caused enhancement of force of the following beat; more negatice potentials caused suppression of force of the following beat. These results were interpreted by assuming that force of a beat was an index of calcium entry on the previous depolarisation. We postulated that such calcium entry, if carried by an electrogenic mechanism, would be revealed as a membrane current developing after 100msec. In all experiments, membrane current at these times was outward. When the duration of the test depolarisation was progressively prolonged, outward current just prior to repolarisation progressively increased. Force of the following beat was significantly correlated with this current in a curvilinear manner. When the duration of the test depolarisation was held constant, outward current was varied by variation in membrane potential. Force of the following beat was proportional to both the test clamp membrane potential and current just prior to repolarisation. These results are compatible with the hypothesis that potentiation of force following a prolonged depolarisation is derived from calcium entry into myocardial cells by reversed sodium-calcium exchange.

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IS MEMBRANE STRETCH THE SIGNAL THAT TRIGGERS A SWELLING-INDUCED CHLORIDE CONDUCTANCE IN CHICK HEART CELLS? J. Zhang and M. Lieberman.
The volume of cultured heart cells was increased by four perturbations to identify the signal that triggers the swelling-activated chloride conductance. Swelling of single chick heart cells was induced by 1) reduction of external osmolarity; 2) elevation of intracellular osmolarity; 3) isosmotic urea uptake; and 4) positive pressure injection. Changes in cell volume and whole-cell currents were recorded simultaneously and a comparison among differently activated whole-cell currents was made in terms of time course, reversal potential (E_{rev}), whole-cell conductance, and response to a number of channel blockers. Although the time course of cell swelling varied between the experimental maneuvers, the resultant whole-cell current displayed nearly identical current-voltage relationships: outward rectification and a reversal potential near the calculated chloride equilibrium potential (E_{Cl}). The induced currents were inhibited comparably by the Cl^- channel blockers, diphenylamine-2-carboxylate (DPC) and 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), and were almost completely suppressed by gadolinium. Activation of the Cl^- conductance by hyposmotic swelling was largely reversed when cell volume was reduced by applying negative pressure through the whole-cell patch pipette. In cardiac myocytes, the observed relationship between the degree of cell volume increase and current activation indicates that the Cl^- selective conductance was activated by a swelling-induced membrane stretch.
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SYNCHRONIZATION OF RABBIT SINOATRIAL CELLS STUDIED WITH COUPLING CLAMP AND MODEL CLAMP. R.Wilders, E.E.Verheijck, R.W.Joyner, A.C.G. van Ginneken, and H.J.Jongsma

We have investigated the effects of gap junctional conductance on the synchronization of sinoatrial node cells. The synchronization of two single isolated rabbit sinoatrial node cells was studied using a computer-controlled version of the "coupling clamp" technique introduced by Tan and Joyner (*Circ Res* 67:1071–1081, 1990). With this digital coupling clamp technique, the membrane potentials of two single isolated cells not in physical contact with each other are recorded using patch-clamp amplifiers in the current clamp mode. These cells can be electrically coupled at any desired value of intercellular conductance by means of a computer-controlled circuit that continuously supplies time-varying currents to each cell with a sign and magnitude that would have been present if the cells would have been physically coupled. This allows the rapid independent measurement of the intrinsic cellular properties and then the analysis of the effects of a wide range of intercellular conductance values on the electrical behavior of the coupled cells. As an expansion of the digital coupling clamp system, we have developed the "model clamp" system, in which one of the isolated cells has been replaced with its mathematical model counterpart, running real-time on a fast computer and communicating bidirectionally with the real cell through a fast data acquisition board. In a model clamp experiment, individual membrane currents of the model cell can be "recorded," and intrinsic cellular properties can be changed. We have used the single cell model developed by Wilders, Jongsma, and van Ginneken (*Biophys J* 60:1202–1216, 1991). In both coupling clamp and model clamp experiments we consistently found that an intercellular conductance of 0.05–0.5 nS was sufficient for frequency entrainment, and that waveform entrainment occurred at conductance values ≥10 nS. Recalling that the conductance of a single cardiac gap junction channel is ~0.07 nS, we conclude that very few gap junctional channels are required for synchronous firing of sinoatrial node cells.

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THE RELEVANCE OF THE DIASTOLIC THRESHOLD FOR POST REPOLARIZATION REFRACTORINESS. H. Leerssen, M. Vos, K. den Dulk, J. van der Zande and H. Wellens.

Post Repolarization Refractoriness (PRR) is the continuation of the Ventricular Effective Refractory Period (VERP) after completion of repolarization, expressed by Action Potential Duration (APD). PRR (increase VERP/APD) has been described in ischemic tissue and at short Pacing Cycle Lengths (PCLs) after class I drugs, such as Procainamide (P). Since the Diastolic Threshold (DT) has never been quantified in relation to PRR we tested whether DT increase is responsible for the paradoxical lengthening of VERP, resulting in PRR. To cause PRR, DT increase at short PCLs after P, should approach or even exceed the margin of twice DT during Control (C) at the longer PCL. Therefore, DT and VERP/APD were determined at different PCLs in 15 dogs with chronic (≥2wks) AV-block during C and after P (20mg/kg/5min bolus plus 5mg/min infusion) with a MAP catheter in the right ventricle, using 0.1mA resolution (group I, n=11) and 0.01mA resolution (group II, n=4). Data expressed as mean±sd.

	VERP/APD		DT [mA]	
	PCL>300ms	PCL≤300ms	PCL>300ms	PCL≤300ms
C	0.86±0.07	0.84±0.08	0.05±0.01	0.07±0.02*
P	#0.92±0.06	#1.04±0.15	#0.07±0.01	#0.16±0.08*

In group I an increase in DT was found in 2 dogs during C and in 8 after P at the shortest PCL tested. In group II (table), VERP/APD and DT increased after P (#, $p<0.05$). Furthermore DT increased for PCL≤300ms during C (*, $p<0.05$). DT even tripled for the combination PCL≤300ms and P (*, $p<0.05$). As a consequence capture was lost in 3 dogs at the shortest PCLs tested. Conclusion: the increase in DT using short PCLs combined with P leads to lengthening of the VERP and as a consequence to PRR.

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CHANGE IN ESTIMATED CIRCUIT SIZE DURING SPONTANEOUS TERMINATION OF ATRIAL FIBRILLATION. F. Mast, F.J. Chorro, M.C.E.F. Wijffels, R. Dorland and M.A. Allessie

Termination of atrial fibrillation (AF) is thought to depend on the chances that the present multiple reentering wavelets will fuse and die out. The aim of this study was to examine whether spontaneous termination of AF was associated with an increase in estimated circuit size (ECS) of the multiple reentrant circuits, defined as the product of AF interval and conduction velocity. AF was continuously reinduced in conscious goats (n=7) by burst pacing. The goats were chronically instrumented with epicardial quadrupoles, located at the left and right atrial free wall, and the right and left atrial appendages. While during control electrically induced AF usually terminated within a few seconds, after a few days of AF the paroxysms of AF lasted for several minutes. In the this study we analyzed electrograms recorded from 4 quadrupoles during the last 12 seconds before spontaneous termination of AF. Local activation times were determined from the steepest negative deflection of the unipolar electrograms. Conduction velocity (CV) was calculated from the mean conduction vector of all consecutive activations in the two triangles making up a quadrupole (interelectrode distance: 6 mm). Ten to 15 beats prior to spontaneous termination the fibrillation interval increased progressively from 113±2 to 166±3 ms ($p<0.0001$). Twelve seconds before termination the average CV was 64±7 cm/s. During the last 3 beats conduction had sped up to 101±6, 103±7, and 97±6 cm/s ($p<0.01$). As a result the average ECS increased from 7 to 16 cm. These observations suggest that spontaneous termination of paroxysmal AF is primarily caused by a progressive slowing of the rate of fibrillation. Together with the concomitant higher conduction velocity this leads to a considerable increase in the size of the reentrant AF waves. An increase in atrial circuit size up to 16 cm will favor the chance of fusion of individual AF waves, and thereby increase the probability of spontaneous termination of AF.

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HEART RATE VARIABILITY AND SENSITIVITY TO PROPRANOLOL ARE REDUCED IN TRANSGENIC MICE OVEREXPRESSING B1 ADRENERGIC RECEPTORS IN ATRIA.

Pascale Mansier[1], Claire Médigue[2], Jean Clairambault[2], Brigitte Bertin[3], Bernard Swynghedauw[1].

To appreciate the role of the myocardial phenotype in the genesis of Heart Rate Variability (HRV), we have designed transgenic mice (T) overexpressing human β1 AdrenoReceptors, β1AR, in atria. The Heart Rate, HR, was continuously recorded on a computer in control mice (C) and T using telemetry. (i) T survive normally without any arrhythmias. (ii) The HRV, as assessed by Wigner-Ville spectral analysis, was nearly abolished in T as compared to C, with a Low Frequency, LF, peak (0.8 Hz) amplitude in T (17,407 ± 41,63 ms^2/Hz) which was half that of C (35,723 ± 11,700 ms^2/Hz, $p<0.05$). (iii) Injection of propranolol induced a comparable slowering of HR in both groups. Nevertheless, propranolol doubles LF amplitude in C (to 65,875 ± 26,893 ms^2/Hz, $p<0.05$) whereas no major change was evidenced in T (to 19,095 ± 3,991 ms^2/Hz, p ns). (iv) Non linear dynamical analysis using Recurrence Maps confirmed the low variability observed in T, especially in presence of Propranolol. To conclude, increasing the number of β1AR in atria in the absence of underlying disease abolishes HRV but did not induce arrhythmias, nor it shortens lifespan. Therefore a lonely loss of HRV is not per se a risk factor. In addition, it has a paradoxal effect on the sensitivity to β-antagonist which is strongly attenuated.

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PATTERN ANALYSIS OF HEART RATE FLUCTUATIONS

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Heart rate variability is the expression of physiological irregularities in heart beats. A simple quantitative measure of heart rate variability is the mean of the modulus of successive R-R interval differences (|ΔRRI), the so-called beat to beat variability. Analysis of the inherent time structure of heart rate fluctuations by computation of power spectra shows two pronounced rhythms in short range time scale, the 0.25 Hz and the 0.1 Hz rhythmicity. Both methods give no information about the specific pattern of these fluctuations. The aim of our study is a description of the dynamic pattern of heart rate fluctuations and a better understanding of the mechanisms generating these patterns.

To describe the dynamic pattern of successive R-R interval differences ($\Delta RR = RR_{n+1} - RR_n$) two approaches of different sophistication were applied.

1.) As a simple measure of asymmetry between increasing and decreasing R-R intervals we define the asymmetry-index $A_{RR} = (\#_+ - \#_-)/(\#_+ + \#_-)$. Here $\#_+$ means the number of R-R intervals where the difference ΔRR is larger than a minimum acceptable difference Δ, and $\#_-$ is the number of R-R intervals where the difference ΔRR is smaller than -Δ. Note that a symmetric distribution of R-R interval differences leads to an index zero.

2.) A more detailed characterization of the dynamic pattern of R-R interval sequences can be achieved by reducing the information content of ΔRR sequences to symbol sequences e.g. -1, 0, and 1 describing decreasing, unchanged, and increasing R-R intervals, respectively. Special attention is given to the frequency distribution of short symbol sequences.

Data from 12 male volunteers (25±1 yrs., 179±2 cm, 74±2 kg) showed interindividual differences regarding the asymmetry variable A_{RR} as well as regarding the frequency distribution of 5-symbol sequences. These results were not correlated with mean RR and mean |ΔRRI.

Comparing empirical time series with simulated ones drastically restricts the plausible range of parameter values of the model and helps to understand the mechanisms being responsible for specific patterns of successive R-R intervals. In conclusion, pattern analysis of heart rate fluctuations seems to be a tool for understanding the cardiac effects of autonomous interaction and coordination.

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EXERCISE INTENSITY OF LONG DISTANCE MARCHING IN ELDERLY PEOPLE. G. de Wild, M. Peeters, W. Hoefnagels, B. Oeseburg and R. Binkhorst.

The aim of this study was to examine the relative exercise intensity ($\dot{V}O_{2MARCH}/\dot{V}O_{2MAX}$) of long distance marching in elderly participants in the 1993 Nijmegen Four Days March (30 km/day). During the march self selected speed (v) was measured and $\dot{V}O_{2MARCH}$ was estimated using the linear relation of $\dot{V}O_2$ with v^2 as assessed in 18 of the subjects in the laboratory. $\dot{V}O_{2MAX}$ was measured using incremental bicycle ergometry. 91 men aged 76.7 ± 4.6 years (mean ± sd) and 49 women aged 72.8 ± 3.6 years were included. Self selected v was 5.00 ± 0.57 km/hr in men and 4.95 ± 0.47 km/hr in women (p = ns), comparable to self selected v of younger subjects. $\dot{V}O_{2MARCH}$ was 10% higher ($p < 0.0001$) and $\dot{V}O_{2MAX}$ was 7% lower ($p = 0.02$) in women compared to men. $\dot{V}O_2/\dot{V}O_{2MAX}$ was 52 ± 10% in men and 63 ± 9% in women ($p < 0.0001$). The sex difference in $\dot{V}O_{2MARCH}$ was in accordance with the reported relation of energy expenditure during walking with the inverse of leg length[2]: leg length and step length were lower in women compared to men, women took more steps compared to men at a certain speed while $\dot{V}O_2$/step was not different between men and women. The sex difference in relative exercise intensity was caused by the higher $\dot{V}O_{2MARCH}$ and lower $\dot{V}O_{2MAX}$ in the women compared to men. Most subjects in this study, in contrast to studies on younger subjects, met established criteria for maintaining and improving fitness ($\dot{V}O_2 > 50\%\ \dot{V}O_{2MAX}$) and reducing the risk for cardiovascular diseases ($\dot{V}O_2$ = 40-70% $\dot{V}O_{2MAX}$) during long distance marching. Supported by the Dutch Heart Foundation, grant no. 43.007.

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EFFECTS OF WORK LOAD AND BODY TEMPERATURE ON VENTILATORY RATE, HEART RATE, AND PREFERRED PEDAL RATE DURING CYCLE ERGOMETRY. Horst Olschewski, Frank Leweke, and Kurt Brück. Zentrum für Innere Medizin, Justus Liebig University Giessen, D-35392 Giessen, Germany

According to the most customary exercise protocols core temperature rises in parallel with work load (WL) and experimental time (TIME). Physiological variables, however, may be related to each of these factors. In order to investigate effects of WL independent of TIME and body temperature (TEMP) we employed four moderate work loads in 4-min steps between 35 and 65% VO_{2peak} in randomized order. In order to investigate independent effects of TEMP the same work protocol was performed both after resting in comfortable ambient temperature (CONT) and after a double cold exposure (precooling test, PRET) where core temperature (T_c) and the temperature set point are decreased by approximately 0.6 and 0.3 °C, respectively. Eight male subjects (24 ± 1.9 yr, VO_{2peak} 4.9 ± 0.5 l/min) worked on a cycle ergometer in a climatic chamber. Heart rate (HR) and breathing frequency (BF) but not preferred pedal rate (PR) were positively correlated to (T_c), the slopes amounting to 17 and 3.75 $min^{-1}/°C$ for HR and BF, respectively. The regression appeared linear over the whole temperature range and the regression lines were not shifted by precooling. PR was increased by TIME, but PRET-CONT differences of PR and T_c were inversely correlated ($r = -0.50$, $p<0.01$). The effects of WL were highly significant on HR, VO_2 and rating of perceived exertion (RPE) but not on BF, PR and sweat rate. The relation of RPE to HR was shifted by precooling. In conclusion, during moderate cycle exercise, heart rate and breathing frequency are linearly correlated to core temperature, whereas pedal rate is increased by a thermoregulatory response to cold obscuring an opposed temperature effect.

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SKELETAL AND CARDIAC TROPONIN I AFTER ECCENTRIC-BIASED AND CONCENTRIC-BIASED EXERCISE. A. Koller, S. Sorichter, J. Mair, W. Gebert, D. Rama, C. Calzolari, E. Artner, and B. Puschendorf

This study examined the effects of eccentric-biased (20 min of downhill running, 16% incline, 70% of maximum heart rate, 4 mmol lactate, N=13) and concentric-biased (20 min of level running, 0% incline, 70% of maximum heart rate, 4 mmol lactate, N=5) exercise on muscle damage. Serum concentrations of total (TnI) and cardiac (cTnI) troponin I were measured before exercise, immediately postexercise, 2h, 6h, 24h, 48h, 72h, 144h, and 216h postexercise by 2 independent immunoenzymometric assays (IEMA).

TnI increased rapidly after exercise with peak values at 6h after finishing. Peak values were significantly higher in downhill vs. level runners (mean: 30.6 vs. 6.4 ug/L; P=0.003, t-Test).The time courses, however, were identical (ANOVA, P=0.122). cTnI could not be detected in any sample taken, thus excluding a troponin I release from the heart.

The profile of circulating TnI values is suggested as a new marker of muscle fiber damage during exercise. Our results provide evidence that the high muscle force associated with the eccentric contraction or the length change occurring during the eccentric contraction causes a rapid degradation of skeletal troponin I.

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EFFECTS OF A SINUS NODE INHIBITOR, S-16257 ON THE MAXIMAL AEROBIC EXERCISE PARAMETERS F. Carré*, J. Beillot*, J. Dassonville*, H. Alberini**, T. Denolle**, C. Weber*** and G. Lerebours***

The detrimental effects of classical bradycardic agents concerning maximal aerobic exercise performance are well-described. S 16257 is a new specific inhibitor of channel i_f and a double-blind randomised placebo (P) controlled study was performed to evaluate its effects on the classical maximal exercise parameters. Single increasing intravenous (IV) bolus doses (1, 2, 4, 8, 16, 24 mg) were injected to 60 males healthy volunteers who performed 3 maximal graded exercise on ergocycle : the day before (1), 1 hour (2) and 8 hours (3) after the drug injection.

Results :

Dose (n)		Heart rate max. (bpm)			VO_2 (l.min-1)			Ventilatory threshold (% VO_2 max)		
		1	2	3	1	2	3	1	2	3
P	m	192	189	190	3.92	3.84	3.80	72.6	72.3	74.2
(12)	± sd	7.4	6.2	8.9	.75	.76	.72	8.0	8.5	7.1
8 mg	m	194	164**	176*	4.02	3.86	3.86	80.0	74.6	75.9
(8)	± sd	6.6	4.8	7.5	.61	.62	.57	9.0	7.8	5.1
16 mg	m	193	150**	167**	4.12	3.91	4.05	70.0	67.5	71.9
(8)	± sd	6.4	9.6	6.9	.50	.59	.61	5.6	5.5	4.7
24 mg	m	190	144**	164**	4.03	3.79	3.88	69.3	73.1	73.5
(7)	± sd	5.7	6.8	7.7	.42	.53	.47	8.3	12.9	6.3

$p< .05$;**$p< .01$ versus placebo. No significant effect was observed at 1, 2 and 4 mg. Exclusively maximal heart rate and rate pressure product were significantly reduced for the three highest dose levels. These effects had disappeared 24 h after dosing (control exercise testing).

Conclusion : it appears that the IV injection of S-16257, a new specific blocker of the sinus node ionic channel carrying i_f is accompanied with a deeply, dose-dependent, expected and exclusive decrease in maximal heart rate. No other detrimental effects on classical maximal exercise parameters were observed. The present results suggest that this drug is devoid of negative inotropic effect.

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EXHAUSTIVE RUNNING AND SUBSEQUENT BED REST FOLLOWED BY LIGHT RUNNING: EFFECTS ON SERUM CREATINE KINASE ACTIVITY. E.Havas, J. Komulainen and V.Vihko.

Effects of exhaustive running (EX) and subsequent bed rest followed by an additional light running (LR) exercise on the appearance of creatine kinase (CK) in serum were studied in 10 well trained endurance athletes. Subjects ran a 18 km cross-country race in 87±4 min, the intensity was respective to anaerobic threshold. After the race the subjects were divided into bed rest (BR, n=5) and control (C, n=5) groups. The bed rest started within 20 minutes after the run and lasted for 23 h while the control group maintained their normal daily routines. 23 h after the EX all subjects jogged for 45 min. Blood samples were taken before and at several time points after the exercises and analysed for albumin (Alb) concentration and CK activity. Alb increased by 20 % ($p<0.01$) during the EX in both groups. 7 h after the EX the values of BR's were back to normal level but those of C's were still 10 % higher than before exercise. CK (corrected by Alb) was increased by twofold after the EX and continued to increase in similar way in both groups reaching values of 342 % in BR and 258 % in C at 7 h after exercise. Thereafter CK decreased to 254 % in BR ($p<0.05$) but increased to 304 % in C ($p<0.05$) at 23 h after EX. The LR on the next day caused an additional increase by 40 %-units ($p<0.01$) in CK at 1 h after LR in C and at 3 h after LR in BR, respectively.

The bed rest had no effects on CK during the first 7 h after exercise. During the next 16 h the bed rest caused a significant decrease in CK whereas controls exhibited a respective increase. Similar responses to LR in BR and C groups on the next day suggest, however, that no marked accumulation of CK in the interstitial fluid had occurred. These findings suggests that in well trained athletes the lymphatic transport of CK molecules from muscle interstitium into the circulation after exercise was maintained by other mechanisms than the muscular activity. This effect, however, may disappear during the next 16 hours.

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EFFECT OF ORAL CREATINE SUPPLEMENTATION ON MUSCLE PHOSPHOCREATINE AND PERFORMANCE DURING HIGH-INTENSITY EXERCISE K. Vandenberghe, Van Hecke P., Van Leemputte M., Gillis N., Vanstapel F. and P. Hespel

Creatine is transported in the muscle cell via a Na^+-dependent specific carrier which, via activation of the Na^+K^+-pump may be stimulated by adrenaline. The aim of this study, therefor, was to examine the effects of oral creatine ingestion in combination with adrenergic stimulation (caffeine and physical training) on muscle phosphocreatine (PCr) and on the capacity to perform streneous intermittent exercise. Healthy male volunteers (N=9) participated in a double-blind study during which they received during 6 consecutive days and in random order either placebo (P), or creatine (0.5g/kg.day)(C), or creatine (0.5g/kg.day) plus caffeine (5mg/kg.day) (CC). The treatment periods were separated by a wash-out period of 3 weeks. On days 1 to 5, the subjects participated in a 1 hour training session with the dominant leg only. Before and at the end of each experimental period NMR spectroscopy of the M. Gastrocnemius was performed and torque production of the M. Quadriceps was evaluated during knee-extensions on an isokinetic dynamometer. Three bouts of 30 dynamic maximal voluntary contractions were performed, with a rest interval of 1 min. P did affect neither muscle [PCr] nor muscle torque production. In the control leg, compared with P, [PCr] was increased by 8% both after C and after CC ($p<0.05$). Muscle torque production was increased ($p<0.05$) by 17, 15 and 5% by C over the 3 bouts of exercise, resp., but not by CC. Physical training did not alter the effects of C and CC neither on muscle [PCr] or performance. The data show oral creatine supplementation to elevate muscle PCr level and to improve performance during intens intermittent exercise. These effects are not facilitated by training. The ergogenic effect of oral creatine intake is abolished by caffeine.

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THE EFFECT OF RUNNING AND CYCLING ON DUODENAL MOTILITY AND OESOPHAGEAL pH WITH A CARBOHYDRATE DRINK OR WATER AS SUPPLEMENT. H.P.F. Peters, J.W.C. Wiersma, L.M.A. Akkermans, E. Bol, W.L. Mosterd

To gain more insight into the mechanisms of gastro-intestinal symptoms during exercise, we have examined the effects of different modes of exercise and different drinks on duodenal motility and oesophageal pH. Eight male triathletes performed two tests (50 min periods of running, cycling and running at 70-75% $\dot{V}O_{2max}$), either with a 7.5% carbohydrate drink (C) or water (W) (3.0 ml/kg bodyweight every 15 min). Motility and pH were measured for 18 hrs with an ambulant manometry/pH system at night and during exercise. During exercise, the number of phases-III per hr (cyclic short-lasting migrating contractions with maximal frequency and high amplitude during the fasting state) was lower ($P<0.05$) with W (mean±SD: 0.1±0.1) than with C (0.4±0.3), and were lower during exercise than at night (0.7±0.2). Phases-III were shorter during exercise with C than at night (3.3±0.7 vs 6.9±0.6 min; $P<0.05$). With W only one subject showed a phase-III, which was also shorter (3.8 min) than at night. With C more phases-III per hr were measured during cycling than during running (0.9±0.6 vs 0.2±0.4, $P<0.05$). Gastro-oesophageal reflux (defined as % of time pH<4) during exercise was higher with C than with W (running: 37.0±8.5 vs 8.7±10.2; cycling: 41.3±27.6 vs 0.2±0.5; and running: 39.6±29.6 vs 2.7±3.9; $P<0.05$). With C reflux occurred more often during running than at rest. Cycling with W, however, resulted in less reflux than at rest. We conclude that exercise results in a disordered duodenal motility, both in the fasting (water) and fed (carbohydrates) state, e.g., phase-III occurrence in the fed state. Moreover, exercise elevates the % of time oesophageal pH is lower than 4. This is related to the type of fluid supplementation, but not to the mode of exercise. These changes might explain the induction of gastro-intestinal symptoms during exercise.

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RISK FACTORS FOR CORONARY HEART DISEASE IN FORMER ATHLETES. E.Pihl, T.Kaasik and T.Jürimäe

The aim of the study was to examine risk factors for coronary heart disease(CHD) in former men and women athletes retired from the active sport 10-15 years ago. The study population consisted of 147 women and 123 men, aged 40-50 years. They divided into 4 groups: 1)physically active exathletes (AA), continuingly exercising at recreational level; 2)physically passive exathletes (PA) having sedentary lifestyle; 3)recreational exercisers (RE) and 4)nonexercisers (NE). Body composition of the subjects was measured by impedance method, physical working capacity (PWC_{170}) was estimated by a cycle ergometer test, serum total cholesterol (CHOL), high density lipoprotein cholesterol (HDL-C), triacylglycerols (TG) and glucose were measured. The results of the study showed that AA had significantly lower fat percentage than PA and NE. PWC_{170} was the highest in AA but there were no significant differences for PWC_{170} between AA and RE in men. No differences were disclosed for blood biochemical indices between any of the women groups. Among the men, AA had significantly lower TG level than all the other groups and higher HDL-C level and HDL/CHOL ratio than PA. In conclusion, the impact of physical activity was more obvious in men than in women. Former top-level athletes who have had no exercises during 10-15 years had the same risk for CHD than subjects who had never exercised.

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CHARACTERIZATION OF THE ENDOGENOUS Ca^{2+} INACTIVATED Cl^- CHANNEL (CaIC) IN *XENOPUS* OOCYTES. W.-M. Weber, K. Liebold, F.W. Reifarth, W. Clauss

Removal of external divalent cations activates large currents through Cl^- channels in the plasma membrane of defolliculated *Xenopus* oocytes. These currents can be inhibited specifically by several Cl^- channel blockers such as flufenamic acid, niflumic acid, and DPC. However, SITS showed no inhibitory potency and even further activated the currents. The reversal potentials measured in dependence of the external chloride concentration exhibit a shift from -17 mV with 25 mM external Cl^- to 23 mV with 125 mM Cl^- in the bath solution. This linear relationship yields a slope of 58 mV per decade as predicted by the constant field equation. Only small amounts of external Ca^{2+} inactivate this CaIC (K_i for Ca^{2+}: 20 µM) while intracellular Ca^{2+} concentrations show absolutely no effect on the CaIC. Single channel analysis of the CaIC shows a slope conductance of about 80 pS and at least 4 different substates.

Increasing the intracellular cAMP concentration via micro injection or incubation with permeant analogues of cAMP has a stimulating effect on the CaIC while cGMP remained without effect. Cholera toxin and pertussis toxin also increase the activity of the CaIC. Activation of protein kinase C via phorbol ester induces further stimulation of the CaIC. GTP-γ-S strongly influences Ca^{2+} channels which are dependent on intracellular Ca^{2+} but shows no influence on the CaIC. Preincubation of the oocytes with Cytochalasin D, which is known to disrupt actin filaments, reduces the currents mediated by the CaIC indicating an influence of the cytoskeleton on the CaIC. Taken together, all these data give evidence for the fact that the so-called leak current after Ca^{2+} removal in *Xenopus* oocytes is indeed a Cl^- channel.

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A NEW RED CELL BICARBONATE CHANNEL. W.F.Widdas and G.F.Baker

When human red cells are first inserted into low ionic strength media they undergo a triphasic volume change. The initial shrinkage (Phase A) and the rapid reflation (Phase B) have been shown to depend on both the red cell carbonic anhydrase and the band 3 anion exchanger. The swelling of Phase B is probably caused by a rapid production of HCO_3^- from CO_2 by the red cell carbonic anhydrase in an alkaline environment (Widdas *et al* 1995), and the re-entry of Cl^- in exchange using the band 3 anion exchanger. The final slower shrinkage (Phase C), is roughly equal in amplitude to the swelling in Phase B, and needs the prior swelling of Phase B which is due to the re-synthesis of bicarbonate. Thus, Phase C is deduced to involve the osmotic loss of red cell bicarbonate but this loss is independent of the anion exchanger. The swelling of Phase B, and the shrinkage of Phase C are both markedly accelerated by the four lower monohydric alcohols. This strongly suggests a common dependence which is postulated to be that of carbonic anhydrase. Therefore, Phase C is considered to involve the loss of bicarbonate ions on channels made up of dimers of carbonic anhydrase molecules. The biophysical properties of such unique transporters have been derived and simulated on a computer. Since only CO_2 crosses through the membrane lipid phase the transport pathway is more substrate specific than that of other membrane transporters. However, it has complex effects on the pH of those cells which have this transporter in their membranes and also contain cytosolic carbonic anhydrase.

Reference: Widdas, W.F., Baker, G.F. & Baker, P. (1995) *Cytobios* (In the press).

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ANNEXIN V INHIBITS A CALCIUM SENSITIVE CHLORIDE CURRENT

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Annexin V, a member of a family of more than a dozen proteins exhibiting Ca^{2+} dependent binding to phospholipids, has been examined for electrophysiological response when injected into oocytes of Xenopus laevis. Oocytes, either untreated or pre-injected with mRNA for the metabotropic glutamate R1a receptor were injected with 20 nl 100 mM KCl solution with varied concentrations of annexin V isolated from Ehrlich ascites tumor cells. Electrofysiological measurements were performed 2 hours or more after injection. Untreated oocytes were permeabilized to Ca^{2+} by incubation in a Ringers solution containing 1 µM A23187. Injection of 20 nl of annexin V solution (1.4 mg/ml) giving an intracellular concentration of about 1.6 µM completely inhibits the native Ca^{2+} sensitive chloride conductance when triggered directly by an external Ca-pulse using permeabilized oocytes, or through the second messenger system by application of quisqualate to oocytes with glutamate receptors expressed. To mimic the Ca^{2+} binding effect of annexin comparable amounts of calmodulin (CaM) was injected, but CaM showed no effect on the calcium sensitive chloride conductance. SDS-PAGE and Western blot analysis of oocyte cytoplasm showed only trace amounts of annexin V in untreated cells compared to the annexin V injected oocytes. Conclusion: Annexin V is a potent blocker of the calcium-sensitive chloride conductance found in oocytes of Xenopus laevis. The block is due to an interaction between the chloride channel and annexin V, and is not a consequence of the calcium buffering capacity of annexin, since injection of CaM which binds calcium in the same concentration range as annexin V has no effect. It is not known whether the block is due to a direct interaction between annexin V and the channel or is due to annexin binding to neighboring phospholipids. Annexin V thus resembles annexin IV which has been shown to block a Ca^{2+} sensitive chloride conductance in colonic T84 cells (Kaetzel & al. 1994, J. Biol. Chem. 269, 5297-5302).

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FLIP-FLOP OF LONG-CHAIN AMPHIPHILIC ANIONS VIA THE ERYTHROCYTE MEMBRANE ANION EXCHANGER

B. Deuticke, CWM. Haest, R. Fischer, R. Ortwein

The study of membrane carrier transport, historically devoted to hydrophilic non-electrolytes and ions, has recently been extended to the translocation of lipophilic and amphiphilic substrates, largely present in living systems in association to binding proteins or membranes. Such substrates probably approach their translocation site on a carrier or pump molecule not via the aqueous phase but by lateral movement in the *cis* leaflet of the membrane lipid bilayer, followed by translocation to the *trans* leaflet and release from the carrier by lateral diffusion. Translocases mediating such active or passive flip-flop processes have been demonstrated for phospholipids, were claimed for fatty acids and may also comprise carriers mediating the extrusion of hydrophobic cytotoxic drugs from malignant cells.

We have now shown that the anion exchanger of the erythrocyte membrane (Band 3, AE1) not only accepts a wide variety of hydrophilic anions but also acts as a flippase mediating e.g. flip and flop of long-chain N-alkyl (C10-C14) derivatives of 5-amino-naphthalenesulfonate. These fluorescent anions partition almost exclusively ($P>10^5$) into the membrane and are translocated at half times of 6-22 min at 37°C, as determinded by following the time-dependent decrease of extractability of the probe from the outer membrane lipid layer by serum albumin. About 65% of the transport occur via AE1, the remainder is simple diffusion via the lipid domain. The flip-mode of AE1 shares with the exchange-mode sensitivity to non-covalent and covalent inhibitors, competition with other small anions and pH-dependency. It differs from the exchange-mode in being stimulated by alcohols and papain treatment and being affected by surface potential gradients. Our results demonstrate that flippases and classical transporters may not require basically different molecular organisation and intermediates between the two can exist.

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AMINO ACIDS INVOLVED IN PROTEIN-LIGAND INTERACTIONS OF THE Na^+/D-GLUCOSE COTRANSPORTER.

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The relationship between the structure and the binding sites for ligands of Na^+-dependent glucose transporters (SGLT) is virtually unknown. Cloning of several isoforms and homologs has permitted a molecular comparison to identify potential residues involved in ligand binding. There are five well conserved charged residues in the putative membrane domain: D176, E225, D273, K321, R427. In site-directed mutagenesis we replaced these residues with alanine. The expression of the mutant proteins in *Xenopus* oocytes was followed by Western blots, cytochemical and freeze-fracture analysis and the function was determined by electrophysiological and radioactive tracer experiments. D176A introduced only subtle changes in steady-state kinetics of αMDG transport, but affected the stability and conformational transition of the unloaded protein. Elimination of the negative charge in position 225 did not alter cotransport kinetics: this residue is probably not located within the membrane domain. D273A showed reduced affinity for αMDG (α-methyl-D-glucopyranoside) and reduced transport activity by ~80%, suggesting that D273 is of some functinal significance. R427 plays a critical role in protein folding or insertion into the membrane since immuno-fluorescent cytochemical and freeze fracture analyses localized the R427A mutant close to, but not in the oocyte plasma membrane. It causes defective insertion of the protein into the plasma membrane. K321A dramatically decreased the affinity to Na^+-ions and substrate ($K_{0.5}^{Na}$~70 mM, $K_{0.5}^{\alpha MDG}$~40 mM) compared with wild-type ($K_{0.5}^{Na}$~10 mM, $K_{0.5}^{\alpha MDG}$~0.2 mM). The inhibitory constant for phlorizin increased from 10 μM to 700 μM. We conclude that K321 is involved in Na^+-binding. This strategy appears to be useful in the indentification of residues critical for cotransporter function.

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A membrane K channel attached to fusion protein imaged by AFM.

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Molecular biological techniques enable the production of purified, complex proteins. Atomic force microscopy (AFM) has the potential to produce high resolution images of such molecules. In 1993 a renal plasma membrane K+ channel, ROMK1, cloned, expressed (1) and subsequently investigated (2). Recently, a fusion protein (GSH-S-transferase (GST)+ ROMK1=ROMK1-GST) has been used to produce a polyclonal antibody for immunocyto-chemical localisation of ROMK1 (3). We used the (AFM) to produce images of ROMK1-GST in aqueous conditions. The fusion protein was dissolved in phosphate buffered saline and spread on freshly cleaved mica coated with cethylpyridinium chloride to neutralize the electrical charges (4). Nanomolar concentrations were used to attach about 20 molecules per μm^2 of mica surface. Scanning was performed under fluid with a newly designed AFM (BioScope, Digital Instruments) that allows a lateral and a height resolution in the range of 5 and 0.5 nm, respectively. We could image a homogeneous population of homodimeric fusion particles (ROMK1-GST=GST-ROMK1) firmly attached to mica. The volume of a single fusion protein was calculated treating the 3D image as a sphere's segment. The individual volume of such a protein averaged 118±5.7 nm^3 (S.E.,n=18). From this value the molecular weight of the fusion protein could be calculated according to the equation: $Vpr=M \bullet (V_1+d \bullet V_2)/N$. In this equation M is the molecular weight, N is Avogadro's number, V_1 and V2 are the partial specific volumes of the fusion protein (0.74 cm3/g) and of water, respectively. d is the extent of protein hydration (0.4 mol/mol). The calculated value was 65 kDa. It matches the known molecular weight indicating that we imaged a single molecule. In some images we found two homodimers joining each other. We conclude that native membrane proteins can be imaged in fluid by AFM and thus could be accessible to functional studies at the single molecular level.

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FUNCTIONAL RECONSTRUCTION OF THE VOLTAGE-DEPENDENT SODIUM CHANNEL WITH S4-45 AND P PEPTIDES.

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The voltage-dependent sodium channel supports action potentials initiation and propagation in excitable membranes. Since its primary sequence was made available, a number of topological models and expression experiments with mutated cDNA, highlight S4 and P segments in each of the four homologous domains as responsible for voltage-dependence and ion selectivity, respectively. Dissecting these functions with synthetic segments reconstituted into planar lipid bilayers is an interesting alternative approach, also allowing to assay their secondary structure. The addition of the short connecting loop S45 between the transmembrane helices S4 (the voltage-sensor with arginines every three residues) and S5.(highly hydrophobic) to the previously studied S4 does not impair the intrinsic voltage-sensitivity but interestingly reduces the single-channel conductance (to ca. 10 pS) and improves sodium selectivity. Thus, the implication of S45 in the permeation pathway of the open channel is supported. Circular Dichroism demonstrates a Helix→β-sheet transition for this segment when the polarity increases. The helical content of the whole S4-45 fragments is reduced by 10% in domain II, as compared to domain IV, due to the presence of a proline. As for synthetic peptides mimicking the P-region (between S5 and S6), their secondary structure is relatively rich in β-sheet both in a large concentration range of organic solvents and in lipid vesicles. In planar lipid bilayers, unit conductance ranges from 6 to 20 pS (in 0.5 M KCl) and P_{Na}/P_K=0.7-3 according to which domain (presenting either neutral, negatively- or positively-charged residues in the presumed β-bend) is assayed. Thus, each of the P segments contribute differently to the presumed selectivity filter. Taken together, these results suggest that if S45 and P regions are in series in the lumen of the open channel, an appreciable selectivity, albeit still lower than in the *in situ* channel, can be achieved with the peptide reconstitution.

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COMPARISON OF ADULT AND INFANT RESPIRATORY CHEMOREFLEXES. N.Calder, B.Waites, S.J.Wong and M.Hanson

The respiratory chemoreflex response to alternate breaths of Fio_2 0.21 and 0.16 develops in size in the neonate (Calder et al. Ped Res 35(3):321-324, 1994), but whether it increases further beyond infancy is unknown. We used the technique used previously in infants to measure the response in 14 awake seated adults breathing through the nose. Respiration was measured by inductance plethysmography and calibrated using a pneumotachometer. A mass spectrometer sampled end-expiratory gases. A computer measured tidal volume (V_T), inspiratory (T_I) and expiratory time (T_E), calculated frequency (f), V_T/T_I, T_I/T_{TOT} and V_T/f for each breath, and switched inspired gas between two lines on a breath-by-breath basis at the start of each expiration. Gas was delivered to the subject at 25lmin^{-1} via a nose mask. Control runs alternated between Fio_2 0.21 and 0.21, and test runs between Fio_2 0.21 and 0.16; 3 of each were performed in each subject.

9 of the 14 adults showed a significant chemoreflex respiratory response. Their responses were not significantly greater than infant responses (Mann-Whitney U test P>0.05).

Table 1. Median (range) chemoreflex responses (mean % breath-by-breath alternation) in infants and adults.

	V_{ti}	T_I	T_E	f	V_{ti}/T_I	V_{ti}/f
INF	2.5	0.8	3.0	1.6	1.6	0.8
	-2.8,8.9	-4.3,4.9	-2.5,7.0	-3.9,4.8	-0.1,5.4	-0.8,6.6
AD	1.1	0.3	0.8	1.71	1.6	2.0
	-1.1,9.7	-1.2,13.9	-0.7,6.7	-0.8,8.2	-0.3,4.2	-1.1,4.5

Our results suggest that the magnitude of the chemoreflex respiratory response to an alternating hypoxic stimulus is no greater in adults than in infants, however further studies in the adult during quiet sleep are needed to confirm this finding.

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BILATERAL LESIONS IN THE RED NUCLEUS ABOLISH THE BIPHASIC RESPIRATORY RESPONSE BUT NOT THE BIPHASIC CARDIOVASCULAR RESPONSE TO HYPOXIA IN DECEREBRATE NEONATAL RABBITS. B. A. Waites, G.L. Ackland, R. Noble & M.A.Hanson.

During the perinatal period, hypoxaemia evokes cardiovascular responses but respiratory responses are inhibited. Previously we showed that a descending inhibitory mechanism involving the red nucleus (RN) reduces the reflex respiratory response to hypoxia in decerebrate neonatal rabbits (Ackland et al., *J. Physiol.*,**483**, 89P, 1995). We have now used RN lesioning techniques to investigate whether cardiovascular responses are also affected by this descending mechanism.

Rabbits (27±1 days old; n=6) were anaesthetized (2% halothane) and artificially ventilated. After pre-collicular decerebration and bilateral vagotomy, anaesthesia was discontinued and rabbits were paralysed. RO (right phrenic nerve; expressed as a % of control), heart rate and arterial blood pressure (MAP) were measured. Rectal temperature was maintained (38.5-39°C). The response to 8 min of isocapnic hypoxia (PaO_2 ca.28mmHg) was measured before and after placement of electrolytic lesions in the RN bilaterally. Sites of lesions were confirmed histologically.

Before lesioning both RO and MAP increased from control values to a peak after 2-3 min (mean±SEM; 152.7±20.8% & 111.0±7.8mmHg respectively) followed by decreases to a nadir (75.9±11.7% & 85.8±6.8mmHg) by min 7. After lesioning RO increased and remained elevated throughout the hypoxic period (peak=139.3+10% & nadir=130+9%). However, the changes in MAP remained biphasic (peak=108+7.2mmHg & nadir=80.6+8.3mmHg). Heart rate was not affected by lesioning and as the animals were vagotomized, it did not change in hypoxia. Therefore although descending inhibitory mechanisms, involving the RN, reduce RO they do not affect cardiovascular responses during hypoxia in neonatal decerebrate rabbits. The mechanism by which these responses are controlled independently needs further investigation.

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HEMODYNAMIC CHANGES IN AWAKE EARLY PREGNANT RATS. B.F.M. Slangen, I.C.M. Out, C.M. Verkeste, L.L.H. Peeters.

Background: The mechanism of hemodynamic adaptation in early pregnancy is still unknown. To identify the onset of the hemodynamic changes and their interrelationship, we determined longitudinally in conscious, chronically instrumented rats the following variables: cardiac output (CO; EMF probe ascending aorta), mean arterial pressure (MAP; femoral artery catheter), hematocrit (Hct) and cardiac contractility (ECG wires s.c.). Pregnant rats (P) (n=9) were studied daily from day 4 till day 12 and in late pregnancy (term=23 days), along with nonpregnant controls (n=9).

Results: In the P rats, Hct began to decrease by day 6 to reach a value of 9 % below the initial level, by day 8. This fall in Hct was paralleled by a similar rise in CO, due to a selective rise in SV. By day 10 cardiac contractile performance had increased in the P group, as indicated by the fall in the PEP/LVET ratio and the rise in aortic flow acceleration, peak flow and stroke work. Changes in MAP were small and inconsistent.

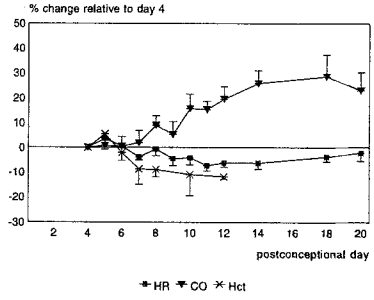
By day 18, CO in the P group had further increased to ± 40 % above the value in the NP group, due to both an increase in HR and SV.

Conclusion:

Hemodynamic changes can already be identified on day 7 of rat pregnancy, 1 day after implantation.

Speculation:

In early pregnancy systemic vascular relaxation leads to a secondary rise in plasma volume, CO and cardiac contractility.



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NEUROPATHOLOGIC AND HEMODYNAMIC CONSEQUENCES OF TOTAL UMBILICAL CORD OCCLUSION IN MIDGESTATION FETAL SHEEP. H. Keunen[1],C.E. Blanco[2], J.L.H. van Reempts[3] and T.H.M. Hasaart[1].

Poor neurological outcome of the neonate is thought to be related to prenatal intrauterine asphyxia. We describe neuronal outcome following asphyxia induced by total umbilical cord occlusion.

Methods: Thirty-two midgestation (85-90 days) fetal sheep were instrumented with a reversible inflatable umbilical cord occluder and catheters in both femoral arteries. Following a three-day recovery period, occlusion was performed during 0 min (sham, n=9), 10 min (n=11), 15 min (n=8) and 20 min (n=4). Fetal arterial blood pressure (MAP) and heart rate were monitored continuously. Fetal acid-base balance was determined before, during and after occlusion. Three days after the experiment the fetal brain was perfused *in vivo* by intracardial infusion of Karnovsky fixative. Toluidin blue sections (2μm) of parietal cortex, hippocampus and cerebellum were scored for neuronal damage (criteria: dark neurons, cell coagulation and/or nuclear shrinkage).

Results: All fetuses survived. Histological evaluation did not show neuronal damage in any group.

GROUP:	sham	10 min	15 min	20 min
pH	7.34 ± 0.01	6.92 ± 0.01[*)]	6.82 ± 0.06[#)]	6.71 ± 0.07[$)]
▲ % MAP	0	-21.77 ± 3.77[*)]	-45.04 ± 1.48[#)]	-40.99 ± 9.44[$)]
base excess	-1.0 ± 0.8	-12.5 ± 1.9[*)]	-17.1 ± 1.5[#)]	-20.9 ± 2.1[#)]

Values are mean ± SEM, $p<0.05$ compared to sham [*)], 10 min [#)], 15 min [$)] (Mann-Whitney-U-test).

Conclusion: Three days after severe asphyxia, induced by total cord occlusion up to 20 min, no neuronal damage was observed in midgestation fetal sheep.

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VENOUS OXYGEN SATURATION (SvO_2) AND CHANGE IN LACTATE DURING PROGRESSIVE HYPOXIA IN 10 DAY OLD PIGLETS. M. van der Hoeven, W. Maertzdorf and C. Blanco

Background: Venous oxygen saturation (SvO_2) reflects the residual oxygen after tissue oxygen extraction and could be used as a critical indicator of oxygen sufficiency at the tissue level. We studied SvO_2 and arterial lactate during progressive hypoxia to assess the relation between SvO_2 and tissue hypoxia.

Subjects: Eight 10 day old anesthetized ventilated piglets.

Intervention: We induced progressive hypoxia by giving different oxygen/nitrogen mixtures. SaO_2 and SvO_2 were measured continuously by a fiberoptic catheter (Oximetrix, Abbott Lab.) in the aorta and pulmonary artery. Aorta flow (Qt), arterial and venous bloodgases and lactate were measured.

Results: Basal values before hypoxia were 98.5 ± 3.4 % and 59.6 ± 8.1 % for SaO_2 and SvO_2. During hypoxia SaO_2 and SvO_2 were gradually reduced to 21.7 ± 8.6 % and 2.4 ± 2.9 %. Qt remained unchanged. A value of SvO_2 of < 15 % was frequently associated with an increase in arterial lactate concentration > 1 mmol/L. Positive Predictive Value: 88 %

Conclusion: SvO_2 decreases during hypoxia reflecting an increased oxygen extraction to avoid tissue hypoxia. When SvO_2 was < 15 %, arterial lactate increased and tissue hypoxia developed.

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CHORIOALLANTOIC ARTERY BLOOD FLOW OF THE CHICK EMBRYO FROM STAGE 34 TO 43. MCG van Golde, TL Mulder, H van Straaten and CE Blanco

Little is known about the allantoic blood flow adaptations during advancing gestational age of the chick embryo.

It is reported that the chorioallantoic artery blood flow represents about 50% of the combined cardiac output in the 17-19 days old chick embryo, this is comparable to the placenta in mammalians. The baseline blood flow profiles of the chorioallantoic artery and the heart rate and changes with 5 minutes anoxia (100% N_2) were measured in 100 chick embryos from stage 34 (day 9)* until stage 43 (day 17) with a transonic flow-probe (VB-series 0.5 mm). The eggs were opened and placed in a small plexiglass box with continuous flow of a N_2/O_2 mixture (5 l/min). The chorioallantoic artery was localized near the fetal abdomen and placed in the lumen of a transonic flow-probe. Heart rate was derived from the blood flow signal.

Results

			normoxia(21%O_2)		anoxia(100%N_2)	
stage	n	weight (g)	flow (ml/min)	heart rate (bpm)	flow (ml/min)	heart rate (bpm)
34-36	30	2.2	0.41	202	0.16	144
37-40	40	6.4	1.22	246	0.55	150
41-43	30	13.3	3.04	280	1.08	136

After anoxia there was a overshoot only in blood flow during the reperfusion, which lasted for about 6 minutes. In summary, chorioallantoic artery blood flow and the heart rate increases with advancing gestational age of the chick embryo. The chemoreflex seems to be elictable since early in fetal life. The chicken embryo could be an useful and attractive model for perinatal research.

*Hamburger, V. and Hamilton, H.L. J. Morphology, 1951: 49-98

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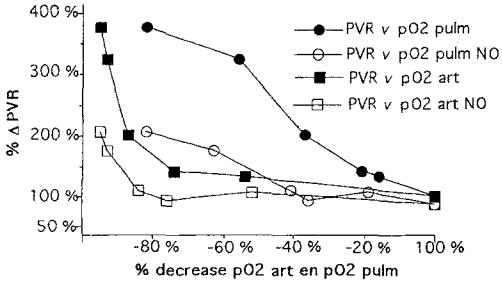
INHALED NITRIC OXIDE (NO) PRIMING PARTIALLY PREVENTS PULMONARY VASOCONSTRICTION IN ANAESTHETIZED NEWBORN PIGLETS. Th.M. Hoorntje, G. Geskes, C.E. Blanco.

Introduction: Hypoxia induces pulmonary hypertension by inhibiting nitric oxide synthetase (NOS) and decreasing NO production. We wondered whether this could be prevented by providing inhaled NO (80 ppm) before and during the hypoxia challenge.

Materials and Methods: Eight newborn piglets (11-18 days, 3.2-5.2 kg) were instrumented under anaesthesia with catheters in the femoral artery and vein, transonic flowprobes were placed around the main pulmonary artery and ascending aorta, a fiberoptic catheter was inserted in the pulmonary artery distal from the flow probe for continuous measurement of mixed venous saturation and pulmonary artery pressure. Another catheter was placed in the left atrium. The piglets were mechanical ventilated with a gasmixture of N_2/O_2. We started ventilating with a FiO_2 of 1.0 and gradually decreased the FiO_2 to 0.1. After a recovery period priming with NO at 80 ppm was started and the same protocol was repeated. Percent change of pulmonary resistence was plotted against the percent change of mixed venous pO_2 and arterial pO_2 with and without the inhalation of NO.



Conclusion Priming with NO partially prevents hypoxic vasoconstriction.

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THE EFFECT OF N-ω-NITRO-L-ARGININE (NLA) TREATMENT ON POST HYPOXIC-ISCHEMIC BRAIN INJURY. CA Dorrepaal, M Shadid, P Steendijk, ET van der Velde, JH Meinesz, M van de Bor, J Baan, F van Bel.

Background: Post hypoxic-ischemic (HI) brain injury is characterized by an initial cerebral hyperperfusion, followed by cerebral hypoperfusion, decreased cerebral O_2-consumption ($CMRO_2$) and decreased electrocortical brain activity (ECBA). Excessive nitric oxide (NO) production upon reoxygenation and reperfusion may play an important role in this post-HI reperfusion injury (e.g. NO-mediated glutamate neurotoxicity).

Subjects: 18 newborn lambs, subjected to severe HI

Interventions: Changes from pre-HI values were measured for brain blood flow (carotid flow [ml/min]; Qcar), (relative) $CMRO_2$ and ECBA [μV] at 15, 60, 120 and 180 min after HI. Immediately after completion of HI, 6 received a placebo (CONT), 6 a low dose NLA (10mg/kg/iv; NLA-10), and 6 a high dose NLA (40 mg/kg/iv; NLA-40).

Results: In contrast to NLA-10 and NLA-40, CONT showed a clear initial hyperperfusion. From 1h after HI, cerebral perfusion was about 80% of the original cerebral perfusion in all 3 groups. In contrast to NLA-10 and NLA-40, $CMRO_2$ was significantly decreased after HI in CONT. ECBA decreased in all lambs, but only recovered to pre-HI-values in NLA-10 at 180 min after HI. Convulsions (ECBA) were detected in 4/6 CONT, 0/6 NLA-10 and 2/6 NLA-40. Brain to body weight ratio was 15.4 ± 1.8 (CONT), 12.5 ± 2.6 (NLA-10) and 11.3 ± 2.2 in NLA-40 (p=0.0086), suggesting less cerebral edema in NLA-treated lambs.

Conclusion: Preservation of $CMRO_2$, recovery of ECBA, and the absence of convulsive activity in NLA-10, suggests that a low, rather than a high dose NLA may reduce post-HI-brain injury.

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ALTERED HEMODYNAMICS IN CHICK EMBRYOS AFTER TREATMENT WITH ALL-TRANS RETINOIC ACID AND AFTER CLIPPING THE VITELLINE VEIN. MLA.Broekhuizen, B.Hogers, HGA.Bouman, RE.Poelmann, AC.Gittenberger-de Groot, JW.Wladimiroff

Cardiovascular performance was studied in stage 34 white Leghorn chick embryos (day 8 of a 21 day incubation) treated with all-trans retinoic acid (RA), vitelline vein (VV) clipped, and control embryos. We simultaneously measured dorsal aortic flow velocities with a 20 MHz pulsed Doppler velocity meter and vitelline artery blood pressures with a servo-null system. Following wave form recordings all embryos were examined histologically. Embryos treated with RA and VV clipped embryos showed malformed hearts. After RA treatment the majority of the hearts showed a spectrum of a rightward shift of the aorta with and without ventricular septal defect (VSD). The rightward positioned aorta was still connected to the left ventricle in caes without a VSD and were classified as having no septation abnormalities. In the presence of a VSD, in combination with the rightward positioned aorta, this anomaly was diagnosed as double outlet right ventricle (DORV). In VV clipped embryos outflow tract anomalies (e.g. DORV), ventricular septal defects, and heart defects in combination with abnormal great arteries were observed.The hemodynamic data were correlated with the morphology and statistical comparison was performed between control and experimental values. Peak systolic and mean velocities, peak systolic and mean blood flows, peak acceleration and stroke volume were reduced in RA treated embryos ($p<0.01$). However, peak systolic and mean velocities, peak systolic and mean blood flows, and stroke volume were elevated in VV clipped embryos ($p<0.01$). Heart rate was reduced in both experimental groups. Pressure readings were not statistically significant. The hemodynamic changes observed in RA treated embryos suggest a decrease in cardiac contraction force. We propose that these changes are mainly due to myocardial dysfunction. The elevated hemodynamic parameters in VV clipped embryos suggest the presence of a compensation mechanism.

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BLOOD VELOCITY WAVE FORM OF THE INTERNAL CAROTID ARTERY (BVWF-ICA) AS A LEFT VENTRICLE (LV) FUNCTION INDICATOR.

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Background: Serial echographic estimation of LV-funtion in unstable (preterm) neonates is important but requires time and may induce apnoe. Experimental studies showed relation between BVWF-ICA and myocardial contractility. We studied this relation in the newborn.

Patients: 70 babies: GA>34wks: no asphyxia(A)/asphyxia(B), GA<34wks: no RDS(C)/RDS(D).

Measurements: On day 1, 2, 3, 5-7, 14 the time between Q(ECG) and peak velocity, acceleration time and time between Q(ECG) and ejection onset (preejection period; PEP-ICA) of BVWF-ICA and echocardiographically determined LV-output, fractional shortening, mean circumfer. fiber shorting, LVPEP, LVET, LVPEP/LVET-RATIO were measured simultaneusly.

Results: Although significant correlations were found between all BVWF-ICA features and LV-funtion indicators, the strongest was: PEP-ICA/LVPEP and PEP-ICA/RATIO(table)

		total n=70	A n=26	B n=15	C n=13	D n=16
PEP-ICA/ LVPEP	r	0.66	0.65	0.69	0.59	0.48
	p	<0.001	<0.001	<0.001	<0.001	<0.001
PEP-ICA/ RATIO	r	0.55	0.55	0.53	0.51	0.48
	p	<0.001	<0.001	<0.001	<0.001	<0.001

Conclusion: PEP-ICA could be used to investigate changes of LV-function even in the most unstable neonate.

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BIOMECHANICS OF INTRA- AND EXTRACRANIAL ARTERIES FROM CADAVERS WITH BRAIN ANEURYSM. M. Tóth, G.L. Nádasy, T. Kerényi, I. Nyáry, E. Monos

OBJECTIVE: Multiplex appearance of brain aneurysms suggests that these local lesions may represent a systemic vascular pathology. To clarify this hypothesis three-dimensional stress-strain analysis was made on non-aneurysmatic intra- (arteria cerebri anterior) and extracranial (a. radialis, a. dorsalis pedis) vessels from 10 aneurysmatic (age: 43-77) and 17 control (age: 26-85) human cadavers. METHODS: An in vitro quasi-static large-deformation mechanical test was applyed. Cilindrical vessel segments of 2-3 cm length dissected from cadavers were cannulated on both ends and mounted in a tissue bath containing oxygenated nKR solution. The intraluminal pressure (IP) was changed slowly between 0-200-0 mmHg, while external diameter and axial extending force were continuously recorded. The geometrical and elastic parameters were computed using a special-purpose software developed by us. Histological examinations were also made with light microscopy. RESULTS: In the aneurismatic group the wall thickness of arteria cerebri anterior was significantly larger (e.g. 0.0137 ± 0.0021 vs. 0.0095 ± 0.0004 cm at 100 mmHg IP) and internal radius of arteria dorsalis pedis was smaller (e.g. 0.0973 ± 0.0105 vs. 0.1369 ± 0.0176 cm at 100 mmHg IP), than in the corresponding control arteries. There were no significant differences in the other geometrical (external radius, mean radius/wall thickness) and elastic parameters (incremental elastic modulus, distensibility, characteristic impedance). Most of the aneurysmatic cadavers had history of hypertension, but the degree of physiosclerosis found by histological examinations did not correlate with the age of the subjects. CONCLUSIONS: These biomechanical and histological findings suggest that brain aneurysm is not only a local vascular disease, but may be coupled with systemic biomechanical and morphological alterations of arteries. (Supported by grants ETT 291-93 and OTKA 1113-91/94)

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CEREBRAL ARTERY RESPONSE TO ABRUPT DECREASE OF BLOOD PRESSURE IN MAN. J. Siegelová, B. Fišer, E. Savin, Ph. Bonnin, J.P. Martineaud

The aim of the paper was to study the time course of autoregulatory reaction after the abrupt decrease of blood pressure (BP). The suprasystolic pressure in occluding cuffs placed on both thighs ceased circulation completely in man in the supine position. The rapid decrease in occluding pressure (after 5 minutes, from 180 to 60 mmHg) elicites decrease in peripheral resistance, and thus decrease in BP. BP was measured noninvasively (Peňáz, Finapres, Ohmeda), middle cerebral artery velocity and temporal artery velocity were determined using transcranial Doppler velocimeter, common artery blood flow was measured by using a range gated Doppler velocimeter. Ten healthy subjects were examined. The decrease in blood pressure of 9% caused the decrease in flow velocity in a. cerebri media to 80% and in a. temporalis to 70%. Five second later, BP remained constant, but the velocity in a. cerebri media increased to 95% and in a. temporalis to 80%. Differences between both arteries are significant (Wilcoxon, $p<0.05$). The values of flow in a. carotis commun. are similar to a. cerebri media. The noninvasive method of determination of autoregulatory reaction of a. cerebri media can be used in clinical examinations.

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CHANGING CEREBRAL CIRCULATION BEFORE VASOVAGAL SYNCOPE ASSESSED WITH NIRS

WNJM Colier, RA Binkhorst and B Oeseburg

Vasovagal syncope or common fainting is usually described as a sudden and transient loss of consciousness which resolves spontaneously. Cardio-circulatory changes are well described during and before syncope. About changes in the cerebral circulation however only little is known. In this study near infrared spectroscopy (NIRS) was used to assess the cerebral circulation during 80° head-up (HU) tilt. As a stimulus for vasovagal syncope 500 mL of blood was drawn from each of 10 healthy subjects. Cerebral oxygenation of the right frontal lobe was measured with NIRS. This technique measures concentration changes in oxy- and deoxyhemoglobin (O_2Hb and HHb). Oxygenation Index (OI) was defined as the difference between O_2Hb and HHb. Blood pressure, heart rate and cardiac output were monitored with a finger plethysmographic device (Finapres). The protocol was divided into 2 stages, each consisting of a 15 min stabilisation period in supine (SUP) position, 15 min in HU position and 10 min again in SUP position. Between stage 1 and 2 blood was drawn from the subject at Red Cross Blood Bank. Haemoglobin concentration (ctHb) was measured before and 30 min after withdrawal of blood. No compensatory hemodilution was observed. During HU in the 2nd stage 6 subjects fainted (F), 4 did not (NF). A significant difference ($p=0.02$) between F and NF was found in the observation that prior to fainting the OI of F showed a steady decrease (-1.4±0.5 µM/min) compared to NF (-0.18±0.16 µM/min). This indicates that the onset of (pre)syncope is preceded by a mismatch of oxygen demand and oxygen supply in the cerebrum. Using NIRS enabled us to monitor this mismatch and to predict the onset of a syncope *before* clear signs in cardio-circulatory variables were visible.

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THE ROLE OF L-ARGININE NITRIC OXIDE SYSTEM IN VISUAL ACTIVATION ELICITED CORTICAL BLOOD FLOW RESPONSE.

A. G. B. Kovách, J. Marczis, Z. Lohinai, M. Reivich.

The constitutive expression of endothelial nitric oxide (NO) synthase (ecNOS) is essential in the physiological regulation of cerebral vascular tone in a regionally heterogeneous manner. 7-nitro-indazole (7-NI) inhibits neural constitutive NO synthase (ncNOS) without increasing MAP in the mouse. In the cat 7-NI decreased regional cerebral blood flow (rCBF), as well as cortical NO content and ncNOS catalytic activity. In vitro vascular reactivity and ecNOS catalytic activity are not affected by 7-NI. Present studies were designed to investigate the role of ec- and nsNOS in CBF changes elicited by functional activation (FA). The experiments were performed in chloralose urethane anesthetized cats (n=11). Local CBF was measured by the laser Doppler (LDF) method, and the cortical NO concentration (NO cc.) by Diamond General microelectrodes. Visual stimulation significantly increased both LDF to 131± 5.9% and NO cc. to 141±7.3% of baseline ($p=0.009$ and 0.008, respectively). In 6 cats both the ecNOS and the ncNOS were inhibited by 30 mg/kg N^G-nitro-L-arginine (NOLA) iv. The LDF decreased in 20 min to 54.5±7.4 % and the NO cc. to 48.7±6.9% of baseline ($p=0.015$ and 0.011, respectively). NOLA attenuated the FA elicited LDF and NO cc. responses to 5.6±4.3% and 4.7±4.5, respectively. L-arginine (50 mg/kg iv) after 25 min restored the baseline and activation elicited blood flow and NO cc. responses which have been inhibited by NOLA. In 5 cats only the ncNOS activity was inhibited by administering 7-NI (30 mg/kg ip). LDF decreased after 7-NI to 67 ± 5.3 % and NOcc. to 37.5±7.9% of baseline by 20 min. The FA elicited LDF and NO cc. increase was attenuated by 7-NI (6.7±.5.5% and 4.9± 6.8% respectively). In conclusion: neural tissue generated NO may play a role both in the maintenance of baseline CBF and also in FA elicited CBF metabolism coupling.

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CYCLIC GMP INDEPENDENT EFFECTS MEDIATE SPECIFIC ATTENUATION OF NORADRENERGIC VASOCONSTRICTION BY NITRIC OXIDE IN PIGS.

J. Zanzinger, J. Czachurski and H. Seller

We studied hemodynamic effects of nitric oxide (NO) during experimental increases of vascular tone by vasoconstrictor agonists in anesthetized pigs after vagotomy and ganglionic blockade. Either noradrenaline (NA), angiotensin II, (AII), arginine vasopressin (AVP) or endothelin-1 (ET-1) was infused intravenously to produce increases in mean arterial blood pressure (MAP) of about 50 mmHg. Bolus administration of the NO-donors sodium nitroprusside (SNP, 50µg/kg) or S-nitroso-N-acetylpenicillamine. (SNAP, 25 µg/kg) evoked arterial dilator responses that were significantly greater during infusion of NA when compared to AVP and AII or control conditions. Inhibition of cGMP formation by methylene blue (MB, 10-50 mg/kg), unmasked similarly heterogenous dilator effects of NO on the venous side but had no significant effects on arterial dilator responses to NO-donors. Additional blockade of calcium dependent K^+-channels by charybdotoxin (CTX, 2 µg/kg iv) caused blood pressure increases and reduced the dilator effect of NO-donors while subsequent inhibition of NO-synthesis by NG-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg) resulted in similar vasoconstriction as observed after MB or control. However, the differences between the dilator responses to NO-donors after vasoconstriction by infusion of the agonists were significantly smaller after CTX. These results suggest that NO differentially attenuates vascular tone dependent on the active constrictor principle. The effects are cGMP-independent and possibly related to direct smooth muscle hyperpolarization by NO in vivo.

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EXPERIMENTAL NO-DEFICIENT HYPERTENSION: METABOLIC CHANGES IN RATS.

I. Bernátová and O. Pecháňová

In the study, an involvement of NO in the modulation of proteosynthesis was investigated after 4 weeks of oral administration of nitro-L-arginine methyl ester (L-NAME). L-NAME in both 20 and 40 mg/kg/day doses increased significantly systolic blood pressure by 34% and 30% respectively, while the heart rate decreased by 15% and 20% respectively as compared with controls. L-NAME in 20mg/kg/day increased total RNA and DNA content by 10% and 214% in myocardium, by 155% and 56% in aorta, by 47% and 400% in brain, by 8% and 31% in liver and by 13% and 29% in kidney respectively. The dose 40 mg/kg/day increased total RNA and DNA content by 15% and 238% in myocardium, by 254% and 200% in aorta, by 85% and 350% in brain, by 12% and 79% in liver and by 36% and 92% in kidney respectively. The [^{14}C]leucine incorporation into the protein of above tissues showed significant increase in proteosynthesis of myocardium (by 100%), aorta (by 40%), brain (by 38 %) and liver (by 166 %) after the administration of L-NAME in 20mg/kg/day. However, the dose 40 mg/kg/day increased significantly [^{14}C]leucine incorporation also into the protein of kidney (by 117%). Despite of no significant change in heart/ /body weight ratio, the results suggest significant role of NO in nucleic acids and protein metabolism of investigated tissues.

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CAROTID BIOMECHANICS AND BAROREFLEX SENSITIVITY IN HEALTHY HUMANS. I. Bonyhay, G. Jokkel, and M. Kollai

Cardiac vagal activity (CVA), which has gained renewed interest because of its cardioprotective action, exhibits considerable variability among individuals. As arterial baroreceptors provide the dominant excitatory input to cardiac vagal motoneurones, interindividual variability in CVA is largely accounted for by variations in baroreflex sensitivity (BRS). The source of BRS variability might be in any element of the reflex arch. The aim of this study was to investigate whether individual differences in static and dynamic characteristics of carotid elastic behaviour contributed to the variability of arterial baroreflex function. Studies were performed on young, healthy volunteers (n=17, age:18-26 yrs.). R-R intervals were monitored from continuous ECG, brachial artery pressure was measured by sphygmomanometry, and finger blood pressure was monitored by the Penaz method. BRS was determined as the change of R-R interval in response to increases of systolic arterial pressure induced by iv.phenylephrine (22 ± 6.5 ms $mmHg^{-1}$). After locating the common carotid artery with "2D echo scan", a phase-locked "echo-tracking" device was used to monitor vessel wall movement. The maximum (diastolo-systolic) change in vessel diameter (Δd), end-diastolic diameter (d) and pulse pressure (ΔP) were used to calculate the distensibility coefficient [$DC = (2 \times \Delta d/d)/\Delta P$] ($5.4 \pm 1.1$) x 10^{-3} $mmHg^{-1}$. As dynamic parameters the expansion time ("t") (mean± SD: 114 ± 15 ms), the mean velocity of carotid artery expansion ($v = \Delta d/t$; 6.1 ± 0.4 mm s^{-1}), and the maximum rate of change in diameter (11.1 ± 2.8 mm s^{-1}) were determined. Multiple regression analysis revealed a significant and close relationship between BRS and carotid distensibility ($R = 0.77$; $p < 0.001$), but excluded dynamic parameters as predictors of BRS. Our results suggest that in young, healthy individuals BRS is proportional to the static elastic properties of the carotid artery and independent of the dynamic parameters of wall movement.

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PLASMA NORADRENALINE AND BAROREFLEX SYMPATHETIC ACTIVATION IN HUMANS: OVERFLOW OF TRANSMITTER TO PLASMA FLOW-LIMITED AND NOT CAPABLE TO REFLECT THE SYMPATHETIC DISCHARGE. J. Lundvall and H. Edfeldt.

In humans measurement of the plasma concentration of noradrenaline (NA) has attracted great interest as a biochemical test of alterations in the body's sympathetic outflow and of the more or less non-uniform alterations in the sympathetic discharge to specific organs or tissues. The widely accepted concept that the NA variable that provide the best measure of regional sympathetic discharge is the rate of which released NA spills into the venous drainage of the organ was tested. The LBNP-technique, with application of low 15 to high and barely tolerated 85 mm Hg negative pressure, was used to elicit graded, mild to pronounced sympathetic baroreflex activation. Forearm blood flow and regional NA concentration changes in the venous effluent were followed. Graded LBNP caused (1) graded neurogenic vasoconstriction with pronounced steady state flow decline (75 ± 4%) and resistance increase ($344\% \pm 33$) at high LBNP; (2) graded increases in venous NA nicely correlated to the resistance response and with quite conspicuous > five-fold rise at high LBNP from 1.05 ± 0.07 to 5.8 ± 0.79 nmol lit^{-1}; (3) clear-cut increase in the overflow of NE from muscle to blood. This increase, however, showed poor correlation to the magnitude of LBNP ($\approx 100\%$ rise at low, moderate, as well as high LBNP) and to the induced vascular resistance change. Specific analysis of data at high LBNP ('standardized, integrated sympathetic activation') showed a direct relation ($p < 0.001$) between regional NA overflow and regional plasma flow, indicating that overflow of NA was flow-limited. This seemed confirmed by the finding that forearm exercise superimposed on high LBNP caused 10 to 15-fold increases in both regional forearm blood (plasma) flow and in regional NA overflow. **Comments.** It is concluded that LBNP is capable to evoke very efficient, graded baroreflex sympathetic activation nicely reflected in graded and pronounced increases of the concentration of NA in forearm venous plasma. However, data strongly indicated a flow-limitation of the quantitative overflow of NA from tissue to blood. The latter finding seem to refute the widely accepted concept that the NA overflow provides a measure of regional sympathetic discharge.

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A TRANSIENT ROLE OF THE KIDNEY IN THE MAINTENANCE OF HYPERTENSION

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Objective: Since we have seen recently that "hypertension travels with kidney", the long-term role of the kidney in the maintenance of hypertension was studied. **Methods:** Prague Hypertensive Rat (PHR), a model derived from the Wistar rat, in which a normotensive parallel (the Prague Normotensive Rat, PNR) was bred from the same parent pair (so that organs can be transplanted between both parallels without signs of rejection), was used. **Results:** Transplantation of a kidney from PHR to a bilaterally nephrectomized (BNX) PNR led to an increase in systolic blood pressure (SBP) in the recipient for 10 weeks after grafting. Similarly, a decrease in SBP was seen in BNX PHR for the same period of time after grafting a kidney from PNR. If, however, the SBP was measured over a longer period of time since grafting a kidney from PHR to BNX PNR, the elevated SBP slowly drops being less than 130 mmHg in the fourth month and thereafter. If BNX PHR receives a kidney from PNR, the decrease in SBP is permanent, amounting to 126.3 +/- 12.7 mmHg one year after transplantation. In **conclusion,** the presence of a kidney from PHR is necessary for the development but is not sufficient for the maintenance of hypertension.

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HIGH FREQUENCY COMPONENT OF HEART RATE VARIABILITY REFLECTS THE MAGNITUDE OF PARASYMPATHETIC MODULATION RATHER THAN PARASYMPATHETIC "TONE". A. Hedman and M. Hakumäki

The objective of this study was to evaluate the effect of modulation in cardiac parasympathetic input on the high frequency component of heart rate variability. We developed an animal model, in which other neural outflows to the heart were eliminated but pure vagal effects could be produced by electrical stimulation. We stimulated the right vagus nerve with three different stimulation patterns in anaesthetized, vagotomized and spinal anaesthetized dogs. We kept the mean stimulation frequency constant; controlled the amplitude of modulation in programmed stimulation patterns and analyzed the resulting heart rate variability by power spectral analysis. Constant frequency vagal stimulation increased cardiac interval but did not change markedly heart rate variability. There was a slight increase, from 11 ± 2 ms^2 to 27 ± 11 ms^2, in high frequency component. However, when the instantaneous stimulation frequency was modulated at 0.20 Hz frequency, we could produce a clear variation in heart rate with 91 ± 9 % of the variation corresponding to the frequency of the modulation. The high frequency component was 12932 ± 7701 ms^2 . When the magnitude of modulation, i.e. the difference between minimum and maximum instantaneous frequency, in stimulation increased, the high frequency component increased to 32711 ± 17943 ms^2. In conclusion, the high frequency component of heart rate variability reflects the magnitude of fluctuation in the cardiac parasympathetic input rather than parasympathetic "tone".

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MECHANISMS OF CALCIUM SIGNALLING IN DIFFERENT TYPES OF MAMMALIAN NEURONS. P.G.Kostyuk, A.N.Verkhratsky, A.V.Shmigol, A.N.Tarasenko

Calcium signals (transient elevations of intracellular free Ca^{2+} concentration) are the most important components of the activity of excitable cells. Their characteristics are determined by a complex system which include activation of a whole spectrum of Ca^{2+} permeable channels, cytosolic Ca^{2+} buffering, Ca^{2+} uptake and release by intracellular stores and Ca^{2+} extrusion from the cell. The relative role of these mechanisms is different in different cells, determining their specific functional properties. We have studied them in a large variety of rat and mice neurons (first and second order sensory, cerebellar, hippocampal, hypothalamic, neocortical) at different stages of ontogenetic development. Substantial differencies were found in the distribution of low-voltage and high-voltage activated (LVA and HVA) Ca^{2+} channels. LVA channels in most neurons were highly expressed during the early postnatal period and disappeared with different velocity during maturation; however, in hypothalamic neurons they continuosly remained dominant. The majority of studied neurons expressed Ca^{2+}- induced Ca^{2+} release from internal stores (CICR); the only exception were small size primary sensory cells. However, the characteristics of CICR varied substantially in different neurons: it was readily available in large primary sensory neurons due to rapid spontaneous refillement of intracellular stores, but less effective in central neurons, depending on preceeding charging of these stores. The IP_3 - induced Ca^{2+} release (IICR) was less prominent in the studied neurons, depending in some cases again on their developmental stage. Dramatic changes in the effectiveness of both plasmalemmal Ca^{2+} transport and intracellular Ca^{2+} storage were observed during neuronal aging. On the background of substantially elevated background level of free Ca^{2+}, the amplitude of calcium signals became decreased and their recovery time constant considerably prolonged due to changes in the effectiveness of both Ca^{2+} storage and Ca^{2+} extrusion processes.

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CHANGES OF INTRACELLULAR FREE CALCIUM FOLLOWING MECHANICAL INJURY IN A SPINAL CORD SLICE PREPARATION. L.Leybaert and A.de Hemptinne

Intracellular calcium ions are, in addition to free radicals, an important mediator of tissue destruction following traumatic injury to the spinal cord. In vivo measurements of calcium in the interstitial space and in the tissue suggest the occurrence of a post-traumatic shift of calcium from the extracellular to the intracellular compartment at the injury site. No information is, however, available on the post-traumatic changes of calcium in the intracellular compartment, where the ion exerts its crucial messenger function. We developed an in vitro model of local traumatic spinal injury, using a spinal cord slice preparation, allowing to investigate injury-related changes of intracellular free calcium. The injury consisted of the impact of a small needle; the intracellular free calcium concentration ($[Ca^{2+}]_i$) was measured with fura-2.

Application of the injury at different places within the gray matter caused a transient and reproducible increase of $[Ca^{2+}]_i$. This injury-induced $[Ca^{2+}]_i$ increase was largely, but not completely, suppressed under zero extracellular calcium conditions. It was also largely depressed in the presence of high extracellular potassium and in the absence of extracellular sodium. The injury-induced $[Ca^{2+}]_i$ increase was modestly depressed by the calcium channel blocker nifedipin, by the calcium release channel blocker dantrolene and by the gap junction blockers halothane and octanol. The calcium channel blocker flunarizine, the NMDA-receptor channel blocker MK-801 and the endoplasmic reticulum calcium-ATPase blocker thapsigargin had no effect.

The experiments suggest that the injury-induced $[Ca^{2+}]_i$ increase is mediated by calcium influx, partly via L-type calcium channels, but also by the mobilisation of intracellular calcium. They furthermore give evidence that sodium influx and intercellular communication via gap junctions contribute to the injury-induced $[Ca^{2+}]_i$ response.

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BRAIN INTRACELLULAR CALCIUM MEASURED WITH INDO-1 FLUORESCENCE DURING ISCHEMIA: A MULTIPARAMETRIC STUDY L. Ligeti*&, A. Mayevsky$, Z. Ruttner*&, M. Reivich^, A.G.B. Kovach^&, and A.C. McLaughlin*

Introduction: Increased intracellular calcium levels have been suggested as a primary cause of hypoxic or ischemic cell damage. The aim of this study was to corralate extracellular and cytoplasmic Ca^{2+} concentration measured *in vivo*, in a model of ischemia in the cat.

Methods: Indo-1 fluorescence was measured above the suprasylvan gyrus. Extracellular calcium and potassium levels, NADH fluorescence, ECoG and relative cerebral blood flow (laser Doppler) were measured using a "multiprobe" assembly. Ischemia was induced by cardiac arrest. $\Delta F400$ and $\Delta F506$ were calculated from the fluorescence signals at 400 nm (calcium-bound form of Indo-1) and 506 nm (free form of Indo-1) by subtracting the autofluorescence observed at these wavelengths before Indo-1 AM was loaded. Changes in $\Delta F400$ and $\Delta F506$ were corrected for hemodynamic artifacts (using the saline flush approach) and NADH fluorescence changes. The ratio $\Delta F400/\Delta F506$ was used to monitor intracellular calcium levels.

Results: Typical changes in extracellular potassium, relative CBF, NADH fluorescence, and ECoG were observed during ischemia. Significant decreases in extracellular calcium were observed after 4 minutes, but no significant changes in $\Delta F400/\Delta F506$ were observed, even after 8 minutes of ischemia.

Comments: In this model of ischemia, the $\Delta F400/\Delta F506$ ratio of Indo-1 fluorescence did not provide a reliable *in vivo* monitor of changes in intracellular calcium levels, even under conditions that would be expected to give large increases in intracellular calcium, i.e., 8 minutes of ischemia. The reasons for this lack of significant fluorescence change need to be investigated further.

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VOLTAGE DEPENDENT K^+ AND Na^+ CURRENTS OF FRESHLY ISOLATED HIPPOCAMPAL NEURONS DURING GRADED AND PROLONGED HYPERTONIC EXPOSURE. R. Huang and G.G. Somjen

Brief exposure to strongly hypertonic solutions suppress voltage dependent and agonist-induced ion currents of hippocampal neurons[1,2] but the concentration dependence and adaptation to prolonged exposure have not been explored. We now report two sets of trials: (1) Exposure to various degrees of increased osmolarity (π_o) for 3 minutes, separated by 5 min recovery periods; no channel blocking drugs were used and Na^+ and K^+ currents were measured on the same trace. (2) Na^+ or K^+ current was isolated by pharmacological means and hypertonic condition was maintained for 30 min, followed by 30 min recovery. Cells were isolated by Kay and Wong's technique[3]; whole-cell ion currents were evoked by voltage steps in V-clamp. π_o was raised by adding mannitol to otherwise normal bathing solution. Depression of maximally activated currents was measured from leak-corrected traces; rundown was corrected by linear interpolation. The effect varied widely among cells, but Na^+ currents were invariably more depressed than K^+ currents in the same cell. Increase of π_o by 25 mosm/kg depressed Na^+ currents in most cells, but had no effect on K^+ currents. In 3 min, +50 mosm/kg depressed mean total (mixed) outward (K^+) currents by 31%, and Na^+ current by 44%; +100 mosm/kg depressed K^+ current by 43% and Na^+ current by 50%. During prolonged exposure depression deepened and reached maximum in 10-15 min, coincident with maximal shrinkage of the cells observed by microscope. Patch-clamped cells recovered some of their volume but ion currents showed no obvious recovery during 30 min elevation of π_o. Following return to normal π_o, ion current amplitude sometimes overshot control amplitude, even when cell volume did not fully recover. We conclude that cells are capable of some volume regulation even when open to the patch pipette; but ion current depression is not directly linked to volume change.

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HYPOXIA INDUCIBLE EXPRESSION OF THE ERYTHROPOIETIN GENE AND LOCALIZATION OF SPECIFIC ERYTHROPOIETIN BINDING SITES IN DEFINED AREAS OF THE MOUSE BRAIN

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Erythropoietin (EPO), the major regulator of erythropoiesis, is induced in an oxygen-dependent manner. The main site of EPO production switches during development from fetal liver to adult kidney. So far, EPO has been reported to act mainly on erythroid precursor cells through binding to its receptor. Now, we have detected mRNA encoding both EPO and EPO-receptor (EPO-R) in mouse brain by reverse transcription PCR (RT-PCR). Exposure to 0.1% carbon monoxide resulted in a 20-fold increase of EPO mRNA in brain. In addition, incubation of primary astrocytes isolated from neonatal murine brain at 1% oxygen led to marked elevated levels of EPO mRNA. Binding studies on mouse brain sections revealed defined binding sites for radioiodinated EPO at distinct areas. The specificity of EPO binding was assessed by homologous competition with an excess of unlabeled EPO and by using two monoclonal antibodies against human EPO, which are known to be inhibitory and non-inhibitory, respectively, for binding of EPO to EPO-R. Major EPO binding areas were observed in the hippocampus, capsula interna, cortex and midbrain areas. To investigate whether EPO gene expression occurs also in primate brain, we analyzed human and monkey (*macaca mulatta*) brain by RT-PCR. We detected EPO mRNA in biopses from human hippocampus, amygdala and temporal cortex and in all tested brain areas of monkey: cortex, cerebellum, hippocampus, hypothalamus, and nucleus caudatus. EPO gene expression was hypoxia-inducible in monkey brain. Finally, we showed that EPO-R mRNA is also present in the brain of man and monkey. Functional expression of the EPO-R and hypoxic upregulation of EPO suggest a new, yet to define role of EPO in the brain.

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Acetylcholine enhances optokinetic modulation of floccular Purkinje cells. H.S. Tan and J. van der Steen

Over recent years, behavioural studies have pointed out the possibility of a modulatory role for acetylcholine in the cerebellum. Microinjections of carbachol in the cerebellar flocculus enhance the optokinetic responses elicited by a rotating drum. The present study is a search for electrophysiological confirmation of this observation. For this purpose, a total of 63 Purkinje cells (P-cells) in the rabbit cerebellar flocculus were identified as being sensitive to optokinetic stimulation around a vertical, or a horizontally axis oriented at 135° azimuth.

Iontophoretic application of acetylcholine (-90 nA) enhanced the depth of optokinetically induced simple spike modulation in 39/63 P-cells. On average, the modulation depth was increased 2-fold, whereby the effect on VA P-cells tended to be stronger than on HA cells. The basal firing rate usually changed during the iontophoresis, but the direction in which it did varied. Current injection through a saline barrel, which was used as a current control, never affected modulation depth of our Purkinje cells.

Conclusion: acetylcholine seems to function as a positive neuromodulator in the cerebellum at the level of the Purkinje cell. This modulatory effect of acetylcholine probably underlies the previously reported enhancement of the optokinetic reflex by microinjection of carbachol in the flocculus.

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SPECIFIC UP-REGULATION OF NMDA COMPONENT OF HIPPOCAMPAL EPSC IN ISCHEMIA. T.Tsintsadze, N. Lozovaya, A.Klishin and O.Krishtal

Electrophysiological recording were made from the hippocampal slices exposed to *in vitro* ischemic conditions in which the superfused medium was hypoxic and deprived of glucose. The excitatory postsynaptic current (EPSC) recorded from CA1 pyramidal neurons were initially depressed. Reoxigenation with normal solution for 2 hours after brief (10 min) ischemia led to a dramatic and irreversible change in the EPSC. Kinetics acquired stimulus-dependence and markedly slowest down with the increase in the stimulus strength. The stimulus-dependent fraction of EPSC is carried through NMDA receptor-operated channels and disappears under NMDA antagonist. When slices were exposed to a severe ischemic conditions for 40 min initially inhibited EPSC transiently increased and after few minutes disappeared. This transient increase was accompanied with predominant enhancement of NMDA component and was prevented by the antagonist of NMDA receptors, APV, and antagonist of metabotropic receptors RS-α-Methyl-4-carboxyphenylglycine.

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ROLE OF SIGMA RECEPTORS IN REGULATION OF BEHAVIOUR: INVOLVEMENT OF DOPAMINERGIC SYSTEM. A.Lang, E.Vasar and A.Soosaar

Although little is known about the functional role of sigma receptors, several lines of evidence suggests that these sites might be implicated in the control of behaviour. The widespread distribution of sigma receptors in the limbic and extrapyramidal structures seems to reflect their role in the control of emotions and motor functions. The aim of present study was to reveal the role of sigma receptors in the behavioural effects induced by the stimulation of dopamine and by the blockade of NMDA-gated channels. Several selective sigma antagonists (rimcazole, BMY 14802, cinuperone and remoxipride) were employed for that purpose. All studied sigma antagonists antagonized MK-801, a non-competitive antagonist of NMDA, induced hypermotility in mice. Apomorphine-induced climbing and yawning, amphetamine-induced hypermotility were also blocked by these drugs. Only remoxipride, but not the other sigma antagonists, reversed apomorphine-induced stereotyped behaviour and aggressiveness. However, no correlation was found between the affinity of sigma antagonists at sigma receptors and their behavioural effects. By contrast, the behavioural activity of these compounds correlated with their affinity at dopamine D_2 receptors. Nevertheless, it seems possible that sigma receptors are modulating the activity of dopaminergic systems, especially those regulating the motor functions. The interaction with dopaminergic system is probably a major target in the action of drugs interacting with sigma receptors.

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CALCIUM SIGNALLING IN GRANULE NEURONES OF THE MOUSE CEREBELLAR SLICES. N. Voitenko, S. Kirischuk, A.Kulik and A. Verkhratsky

We investigated the mechanisms of $[Ca^{2+}]_i$ homeostasis in fura-2/AM loaded granule neurones on acutely isolated cerebellar slices prepared from mice of two different age groups, namely 6 - 8 (P6) and 25 - 30 (P30) days old. The depolarization-triggered $[Ca^{2+}]_i$ elevation in these neurones were mediated via both LVA (T) and several types of HVA (N and L) calcium channels as revealed by using specific channel blockers. Caffeine (30 - 40 mM) induced the release of Ca^{2+} from Ca^{2+} -induced Ca^{2+} -release (CICR)-related ER Ca^{2+} stores in neurones from both P6 and P30 animals. Glutamate and its analogues (quisqualate and kainate) triggered $[Ca^{2+}]_i$ elevation via several distinct routes, including Ca^{2+} entry through voltage-dependent Ca^{2+} channels and ionotropic glutamate receptors (GluRs) as well as by initiating $InsP_3$-induced Ca^{2+} release (IICR). The latter has been observed only in neurones from P6 mice. Quisqualate was the preferential agonist for metabotropic GluR-mediated IICR, whereas another metabotropic GluR agonist trans-ACPD failed to trigger IICR, suggesting the expression of mGluR1 and/or mGluR5 isoforms in mouse cerebellar granule neurones. The expression of both CICR and IICR mechanisms undergoes developmental regulation: in cerebellar granule neurones in early ontogenesis IICR is more prominent, while during maturation CICR mechanism became principal.

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ACTIVITY OF NITRIC OXIDE SYNTHASE IN BRAIN REGIONS OF WAG/Rij RATS: EFFECTS OF SEX AND ACOUSTIC STRESS N.V. Gulyaeva, M.V. Onufriev, M. Yu. Stepanichev, G.D Kuznetsova, and E.L.J.M. Van Luijtelaar

It is suggested that the inbred strain of rats WAG/Rij (showing trains of 7-10 Hz spike-wave discharges in the cortical EEG and associated behavioural manifestations) is a useful model for human non-convulsive absence epilepsy. The objective of this study was to investigate the activity of nitric oxide synthase (NOS, evaluated as the rate of N(G)-nitro-L-arginine inhibitable NADPH oxidation) and the generation of active oxygen species (GAO, using luminol-dependent chemiluminescence) in selected brain regions of WAG/Rij rats as well as the response of these parameters to acute acoustic stress. Basal NOS activity and GAO appeared to be slightly higher in females than in males. The regional distribution of both NOS and GAO differed from that in Wistar rats (e.g. in WAG/Rij rats maximal NOS and GAO was not characteristic for cerebellum as it is in Wistar rats). Acoustic stress induced electrophysiological and behavioural phenomena, including catalepsy. NOS activity in hippocampus and GAO in most regions depended on the sex, and NOS activity in thalamic region - on stress (ANOVA). In cerebral cortex of male and female rats stress induced decrease of NOS activity; in females NOS activity decreased in hippocampus and increased in thalamic region. Basal and stress-induced differences of NOS and GAO in male and female rats may be related to differences in their stress response (e.g. less prominent stress-induced calalepsy in females).

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SYSTEMATIC VARIATIONS IN METABOLIC PROPERTIES WITHIN SINGLE-TYPE MUSCLE FIBRE POPULATIONS. D. Kernell and A. Lind

Young adult female rats were used for investigating the patterns of co-variation between histochemically determined properties of muscle fibres in extensor digitorum longus (EDL), tibialis anterior (TA), peroneus longus (PerL) and gastrocnemius medialis (GM). Based on their reactions in staining for myofibrillar ATPase (mATPase), the fibres were categorized into types I, IIA, IIBd and IIBm. Within each muscle, fibres were sampled from two separate regions, one comparatively rich in (presumedly slow) type I fibres ('red' region) and one (almost) lacking such fibres ('white' region). The staining intensity (optical density) was measured for succinic dehydrogenase (SDH), alpha-glycerophosphate dehydrogenase (GPD) and fat. Systematic co-variations in these parameters were frequently found between fibres all belonging to a given mATPase-category and sampling-region (particularly common for IIBd fibres; typically significant negative correlations SDH vs. GPD, significant positive correlations SDH vs. fat). For SDH and GPD, but not for fat, there were also systematic differences between single-type fibres of 'red' and 'white' regions ('red', as compared to 'white': high SDH, low GPD). The results underline that (a) metabolic properties co-vary in a largely continuous and systematic manner across fibre populations of single muscles, and (b) the manner in which various histochemical (and, presumably, physiological) fibre properties are combined (i.e. the fibre 'profiles') differs systematically between different regions of the same muscle.

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VARIABILITY OF FAST FATIGUABLE MUSCLE UNIT PROPERTIES C.J. de Ruiter, A. de Haan and A.J. Sargeant.

Rat medial gastrocnemius (MG) muscle is compartmentalized: branches of the motor nerve innervate discrete parts of the muscle differing in fibre type composition. Type IIB fibres in the most proximal (prox) compartment are smaller and display higher succinate dehydrogenase staining intensities than the IIB fibres of the most distal (dist) compartment. It was hypothetized that the prox IIB fibres belong to smaller fast fatiguable (FF) muscle units (MUs) with higher resistance to fatigue compared to the dist IIB fibres. The properties of single FF MUs were studied by splitting ventral roots of anaesthetized rats (250-310 gr). The MG was prepared free and distally connected to a force transducer. Nerves of other muscles and the primary nerve branches innervating intermediate MG muscle parts were cut. An isolated single MU therefore belonged either to the most prox or the most dist MG compartment: as distinguished by EMG. From five glycogen depleted prox and dist MUs, fibre type (myosinATPase), innervation ratio (IR) and area (IA) were determined. It was confirmed that the FF MUs contained only pure type IIB fibres (no IIX). There were no significant (α = 0.05) differences in contraction times, relaxation times, sag properties and the 50/200Hz force ratio. However, the prox MUs (n=11) had their optimum for force (200Hz) production at lower muscle lengths than the dist (n=10) and produced less force (means±sd): 142±25 versus 229±86 mN. Twitch tetanus ratio was lower for the prox (0.25± 0.05) MUs than for the dist (0.31±0.03). The IRs were not different: 216±22 (prox) and 174±105 (dist). The prox fibres were smaller 1642±191 µm2 than the dist (2808±562) and the prox IAs (mm2) were smaller (3.4± 0.5) than the dist (10.0±4.0). During the first 1.5 min of a Burke fatigue test, the prox MUs tended to fatigue less than the distal. However, thereafter force in the prox MUs continued to decline in contrast to the dist MUs, which slightly recovered. Therefore, contrary to our expectations, the prox fatigue index (FI=force 2 min after peak force/peak force) was lower (0.23±0.07) than the dist (0.31± 0.08). Since during the second part of the test, force output was almost reduced to twitch level, the lower prox FI could be related to the relative small prox twitches and/or differences in susceptibility to electrical fatigue between prox and dist FF MUs. Clearly, categorisation of muscle units based upon a standard fatigue test can be misleading if their location in the muscle is disregarded.

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RECRUITMENT OF TYPE IIB FIBRES IN RAT MEDIAL GASTROCNEMIUS DURING TREADMILL RUNNING IS RELATED TO THE MUSCLE COMPARTMENTS. P.E.M.H. Habets, C.J. de Ruiter, A. de Haan, A.J. Sargeant.
Rat medial gastrocnemius (MG) is a compartmentalized muscle: different branches of the motor nerve innervate discrete regions of the muscle.The most proximal (prox) and distal (dist) IIB fibres are innervated by the most prox and dist branches of the motor nerve, respectively. Prox IIB fibres are smaller and show higher succinate dehydrogenase staining intensities. It was hypothetized that these differences are associated with functional differences during in vivo motor behaviour. Rats were subjected to different running protocols (matched for total work output) on a motor-driven treadmill (20° incline). After familiarization with running for 10 days, rats were randomly divided into 3 groups (n=6): control, continuous running (CR) at 16.3 m/min for 18.38 min, and intermittent sprinting (IS; five 30sec sprints at 40.7 m/min; 2.5 min at 16.3 m/min in between). After the exercise rats were anaesthetized and MG muscles were excised and frozen. Sections were stained for mATPase and glycogen (PAS). After IS relatively sharp boundary lines between depleted and non-depleted muscle areas were seen. These lines matched the compartmental boundaries which were identified by De Ruiter *et al.* (1995). Glycogen levels in prox IIB fibres decreased with (%; mean ± sd) 14±11 (CR) and 28±19 (IS) ($p<0.05$). No glycogen depletion in dist IIB fibres was seen after CR and only in 10 % of the dist IIB fibres glycogen was decreased after IS. Pilot experiments showed that a decrease in glycogen in almost all dist IIB fibres was achieved only after maximal galloping (no incline) or trotting (incline 20°) at 47.1 m/min. After maximal intermittent in situ stimulation (n=6) the distal IIB fibres demonstrated a higher maximal rate of glycogenolysis. Glycogen decreased with 61±4% in the dist IIB versus 48±6% in the prox IIB fibres. Thus, the differences in glycogen breakdown between prox and dist IIB fibres after running were opposite to those expected if both populations were recruited to the same extent. Therefore, it was concluded that the prox IIB fibres were recruited at lower intensities of exercise than the dist IIB fibres. Furthermore, recruitment was organized in relation to the innervation of muscle compartments.

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RECOVERY OF SLOW-TWITCH CHARACTERISTICS IN RAT SOLEUS MUSCLE FOLLOWING 3 WEEKS OF HINDLIMB SUSPENSION.C. Huchet-Cadiou, V. Bonnet and C. Léoty
The purpose of the study was to analyse the recovery of the slow-twitch characteristics of rat soleus muscle following 3 weeks of hindlimb suspension and to see wether the changes were fully reversible. Twitches and K^+-contracture (146 K^+ mM) were analysed on intact fibers isolated from rat soleus muscles at 1, 2, 3, 5 and 8 weeks of recovery from 3 weeks of tail suspension. The effects of cyclopiazonic acid which is a specific sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor were also investigated. After 3 weeks of suspension the soleus muscle weight was decreased by 38%. At 8 weeks of recovery the soleus muscle weight was found similar to the control. Following hindlimb suspension the characteristics of the twitch and of the K^+ contractures became similar to those found in EDL muscle. At 8 weeks of recovery the twitch amplitude was still reduced by 38 % (n=6) while at 3 weeeks its time to peak and its time constant of relaxation were similar to control. At 3 weeks of recovery the kinetics of 146 K^+ were identical to normal K^+ contractures. The amplitude of the K^+ contracture that increased from 5.5 ± 1.2 to 26.5 ± 4.6 mN/mm^2 (n=7) during 3 weeks of recovery remains smaller than control 40.5 ± 5.7 even at 8 weeks 23.6 ± 3.6 mN/mm^2 (n=7). The sensitivity of the twitch and the K^+ contractures to 10 µM cyclopiazonic acid became progressively identical to those found in normal soleus fibers after 8 weeks of recovery. These results indicate that 3 to 8 weeks return to terrest gravity reversed the effect of 3 weeks suspension. However it appears that the recovery of the contractile parameters occured with different time courses and that some modifications due to suspension in rat soleus muscle were still present at 8 weeks.

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ALTERNATIVE CONDITIONING PROTOCOL TO TRANSFORM SKELETAL MUSCLE FOR CARDIOMYOPLASTY. J.H.L. Davidse, F.H. van der Veen, H.G. Kaulbach, J.W. Habets, T. van der Nagel, H.J.J. Wellens
Chronic electrical stimulation has been shown to transform fast fatigue muscle fibres into fatigue resistant fibres and this phenomenon has been used in dynamic cardiomyoplasty to apply skeletal muscle for cardiac assist. The present study examined if partial fibre type transformation is an alternative possibility to maintain adequate force and fatigue resistance and to prevent the muscle from atrophy and lipomatosis when stimulating the muscle in dynamic cardiomyoplasty. Goats (n=7) had two intramuscular electrodes in the proximal part of the Latissimus Dorsi (LD) muscle and a pulse generator implanted (Telectronics Pacing Systems, MYOSTIM 7220). A stimulation protocol with an average of 6 contractions per minute was used during a 3 months training period. Stimulation started on 1 pulse, while every 2 weeks 1 pulse was added to finally 6 pulses per burst. Mean peak force of the LD muscle did not significantly differ after the training period (5.8 ± 3.4 compared to 3.5 ± 1.7 N). This is in contrast to findings of clinically used conditioning protocols which demonstrate a reduction in peak force. Histological analyses showed great inter and intra fascicular heterogeneity of the percentage type I fibres (range 40-90%). In conclusion: an intensity reduced conditioning protocol for dynamic cardiomyoplasty maintains a forceful muscle, which is accompanied by a large variation in muscle fibre type distribution. Clinical relevancy of this protocol should be studied in animals after a cardiomyoplasty procedure.

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CHANGES IN ELECTROLYTE CONTENT OF MUSCLE DURING REPETITIVE SUBMAXIMAL ISOMETRIC EXERCISE. E.Verburg, N.K.Vøllestad, O.M.Sejersted
Exercise is accompanied by electrolyte shifts in the working muscle. K^+ is lost to the circulation (Vøllestad et al., 1994) and in rat EDL muscle Ca^{2+} and Na^+ content is increased after 0.5 to 4 hours of continuous stimulation (Everts et al. 1993). It is not known whether such calcium shifts are seen in fatigued human muscle. We have investigated whether repetitive submaximal isometric exercise at 30%MVC, carried out until exhaustion, leads to changes in total muscle K^+, Na^+, Ca^{2+} and Mg^{2+}. Two-legged isometric contractions of the quadriceps muscle were held for 6s with 4s rest between. Biopsies were taken from the vastus lateralis at rest, repeatedly during exercise and at exhaustion (mean 58min, sd 33) from 11 male subjects. Biopsies were analyzed for total ion and water content. Maximal force fell linearly with exercise time reaching about 55% of pre-exercise control. Ca^{2+} and Na^+ in muscle biopsies increased significantly (repeated measurements one-way anova, $p=0.0092$ and 0.025) from 3.07 ± 0.098 to 3.39 ± 0.13 mmol Ca^{2+}/kg dw (10.4%) and from 116 ± 17 to 143 ± 17 mmol Na^+/kg dw (23%), resting and during exercise respectively. This increase seems to occur early in exercise after which the ion content remains stable. The content of Mg^{2+} and K^+ did not change significantly. However, femoral blood-samples showed an early large increase inV-A difference for K^+. These data indicate a K^+ loss from the muscle of about 3% of its total content. The biopsy data show the same tendency, but precision of the method for K^+ analysis does not allow detection of such small changes. During exercise muscle water content tended to increase, but at most by 3%. Assuming that the extra water entering the muscle has the same ion concentration as blood plasma, the increase in water content can explain at most half of the observed increase in muscle Ca^{2+} and Na^+ content. We conclude that during the first minutes of repetitive submaximal isometric exercise total muscle content of Ca^{2+} and Na^+ increase and K^+ decreases.
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SKELETAL MUSCLE WORKING CAPACITY IN ISCHEMIA IN MAN. P.Ozolins, L.Plakane

In experiments with anaesthetised cats it was shown that the decrease of skeletal muscle working capacity after blockade of blood supply is not an uniform process, but is interrupted by a transient significant increase of contraction force (Ozolins et. al. 1994). The purpose of this work was to test the appearance of the increase of force in ischemia also in man. In 18 healthy men and vomen the thenar muscles (m.adductor pollicis and m.flexor pollicis brevis) were stimulated by brief trains (0,33 s) of electrical impulses (0,1 ms duration, 100 Hz) every 2 s. 56±8 s after arrest of muscle blood supply by applying 200 mm Hg pressure in a cuff placed on the upper arm, a transient increase of muscle contraction force by 56±11% for 38±6 s was demonstrated in 13 of the persons. When 30 s complete tetanic contraction was performed one min after arrest of blood flow in the arm, the muscle working capacity, estimated by fatigue index, was significantly increased to 82±3% compared with 69±3% in control conditions. Physiological mechanism(s) transiently increasing muscle working capacity in ischemic conditions are active also in man.

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CONTRACTILE AND METABOLIC RESPONSES TO PROLONGED DYNAMIC EXERCISE IN MAN. K. Sahlin, K. Hirakoba, J. Seger, K. Söderlund and M. Tonkonogi.

The contractile properties of the quadriceps muscle were measured in 7 healthy male subjects before, during and after prolonged cycling to exhaustion (work rate 75 % of estimated VO2max; exercise duration 85 ± 9 min ($\bar{x}$ ± SEM). Maximal voluntary isometric force (MVC) decreased already after 5 min of exercise to 91 ± 4 % of the pre-exercise value ($P<0.05$) and decreased further to 82 ± 6 and 66 ± 5 % after 40 min cycling and at exhaustion, respectively. Reversal of MVC occurred in different phases where T½ of the initial rapid phase was about 2 min. MVC was 80 ± 2 % of the pre-exercise value after 30 min and was not affected by superimposed electrical stimulation. Maximal voluntary concentric (CON) and eccentric force (ECC) decreased to 74 and 80 % of initial values at exhaustion ($P<0.05$). In contrast to high-intensity exercise where there is a pronounced slowing of relaxation, the kinetics of isometric contraction were not affected by the prolonged cycling. Muscle metabolites were measured in another group of subjects before and after cycling to exhaustion (n = 8; work rate 75 % of VO2max; duration 76 ± 7 min). Muscle glycogen decreased to 34 % and PCr decreased to 37 % of the pre-exercise value. PCr was rapidly repleted during the recovery period and was higher than the pre-exercise value after 5 min (105 %; $P<0.05$). A slight increase (n.s.) in muscle lactate was observed at the end of exercise but due to the breakdown of PCr estimated muscle pH was slightly increased. Plasma hypoxanthine (Hx) increased during exercise and subjects with large increases in Hx also exhibited pronounced catabolism of ATP to IMP. It is concluded that prolonged exhaustive cycling results in reduced force generating capacity during isometric, concentric and eccentric conditions. The changes in muscle contractile properties may in part be related to metabolic factors (e.g. availability of high-energy phosphates) but the slow phase of force restoration, despite complete reversal of PCr indicate independence from muscle energetics.

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ERECTOR SPINAE MUSCLE FATIGUE IN SYMMETRIC AND TWISTED TRUNK POSTURES. J.H. van Dieën, A.F. Housheer, M. Steultjens and H.H.E. Oude Vrielink

The coordination of trunk muscles in asymmetric postures and hence their activity and resulting fatigue development are little understood. The external oblique muscle and asymmetric activity of the erector spinae muscles have been proposed to be the main actuators for torsion. The former would imply symmetrical activity and fatigue development of the back muscles, while their endurance at a given relative extension torque will be affected due to the opposing torque caused by the abdominal muscles. If asymmetric activity of the erector spinae muscles is used to stabilize the twisted posture, endurance will not be affected.

Coordination and fatigue development in the back muscles was studied in 12 volunteers. Subjects performed 3 s isometric trunk extensions in 4 postures (0, 15, 30, and 45 degrees torsion). On 2 separate days the 0 and 30 degrees condition were executed till the limit of endurance. EMG was sampled continuously from the main tracts of the erector spinae muscle and of the external oblique muscles. Maximum force and EMG-amplitude were the dependent variables for the short contractions. The endurance time and the slopes of the EMG median and mean power frequency were studied in the endurance contractions.

Per 15 degrees of rotation a 10% drop in the MVC occurred, while asymmetric activity of the back muscles increased, concurring with little abdominal activity. Endurance at 40% MVC did not differ between the 0 and 30 degrees condition (139 ± 64 s vs. 144 ± 76 s, respectively). The slope estimates of the spectral EMG parameters indicated selective fatigue of the contralateral iliocostalis muscle in the 30 degrees condition.

The results show that a coordinative strategy avoiding cocontraction is chosen, which minimizes effects on endurance and on spinal load.

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DOES FIRING RATE DETERMINE FLUCTUATIONS OF SPECTRAL PARAMETERS OF A SURFACE ELECTROMYOGRAM? H.H.E. Oude Vrielink, J.H. van Dieën, C.T.M. Baten, H.J. Hamberg, A.F. Housheer

Surface electromyography (SEMG) is often applied to study non-invasively physiological processes in muscles during activity. One of the parameters indicating muscle fatigue, the mean power frequency (MPF) of the SEMG, is reported to decrease in time. This decrease, however, is not always obvious, due to considerable variation in the MPF-estimates. The variation might relate to the reported synchronous firing of motor units (Broman *et al.*, Brain Res. 337, 311-319, 1985). In the present study, the characteristics of this variation were investigated, and the question was raised whether firing rate fluctuations are dominating them.

Five healthy males performed a 40% of their maximum isometric trunk extension until endurance. Force development and SEMG of the multifidus l. muscle were sampled continuously (100Hz and 1024 Hz, respectively). Variation of the MPF of the SEMG signals were analyzed for subsequent and partially overlapping epochs of 2s. In parallel these epochs were analyzed for the average firing rate (AFR; Baten *et al.*, Abstract book 10th congress of ISEK, June 21-24, 1994, Charleston, USA, p. 136-137). Fluctuations in force, MPF and AFR were analyzed for their median frequency.

MPF and AFR showed regular fluctuations (range of their median frequencies: 0.13-0.25Hz and 0.13-0.19Hz, respectively) of considerable amplitude (estimated peak-peak value: 6-16% and 20-56% of the mean, respectively) during full contraction length (endurance: 104-126s). Scatter-plots showed no relation between both, nor with force variation.

In conclusion, fluctuations in MPF are such that inappropriate sampling strategies might result in masking of fatigue effects. Though fluctuations in MPF and AFR appear to occur around the same frequency band, no deterministic relation was found.

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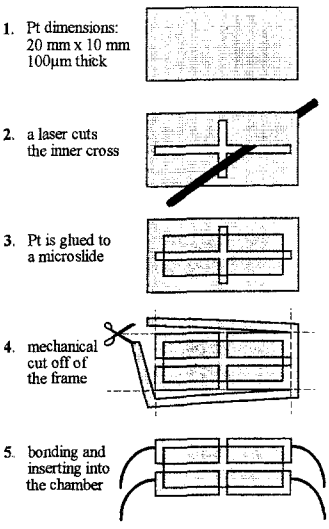
A MICROCHAMBER FOR ELECTRICAL MANIPULATION OF CELLS UNDER OPTICAL CONTROL. M.Baumann and R.Grebe

The influence of electric fields on erythrocytes and other cells has been investigated in numerous experiments. Most often there is a lack of direct optical information about the influence of the manipulations. We have developed a simple and cheap method to construct microchambers which allow the continuos observation of biological cells while manipulating them simultaneously by any electric field.



The electrodes are made of platinum (Pt) because of its chemical resistance (1.). A laser is used to cut a cross into a piece of Pt as shown in (2.), leaving small bridges on each side to keep the distance of exactly 100 µm. Then this formation is glued onto a glass microslide while leaving the bridges free (3.). After cutting mechanically the frame off an arrangement of four electrodes is obtained as shown in (4.). Finally copper wires are bonded to each of the resting electrodes and the formation is inserted into a chamber made of plastic (5.). These steps produce a four electode microchamber which can be loaded with the cell suspension. A coverslip is set on top of the microchamber to form a capillary space, which can be used in every orientation. To keep the removable coverslip in place we use a vacuum which is generated in a separate small channel surrounding the whole chamber. At the moment such a cheap chamber is used both to elongate erythrocytes with high frequency electric fields and to porate the same cells in pulsatory electric fields.

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COUNTERCURRENT VASCULAR NETWORKS: BLOOD FLOW AND HEAT TRANSFER. J. Werner and H. Brinck

A three-dimensional vascular model was constructed which computes the spatial variations in the arterial, venous and tissue temperatures. It was developed by considering closely spaced, countercurrent pairs of thermally significant vessels. The model was applied to the cross-sectional area of a human extremity. It has an idealized three layer organization of the vasculature of the peripheral circulation: the core contains the countercurrent central artery and vein and the surrounding tissue. These vessels are the starting points of a countercurrent arterio-venous network in the muscle layer. The countercurrent pairs of arteries and veins are constantly branching. The muscle layer is surrounded by the skin layer in which the blood is supplied by isolated larger riser vessels. The three-dimensional vascular model presented here is unique in describing the vascular system explicitly in terms of the physical details. The pre-arteriole and post-venule vessels of the muscle layer are incorporated into the model. The arterial blood temperature is not constant along the length of the countercurrent network. The deviation from the arterial blood temperature at the beginning of the vascular tree increases when the perfusion rate is small. The temperture equilibrium occurs with passage of the blood through the vascular tree in the muscle tissue near the skin surface. At the end, the blood is equilibrated with the surrounding tissue. Temperature profiles are presented for various conditions. Blood temperature profiles and profiles describing heat flow across the vessel walls are calculated. Assumptions inherent in bio-heat formulations of Pennes (1948) and Weinbaum and Jiji (1985) for the convective heat transfer are questioned. When the perfusion rate is high and, when at the same time, there is a high metabolic rate there is a heat flow into the artery and a minor heat flow out of the vein. This is obviously a countercurrent effect. The vascular model presented here treats the effect of blood perfusion on a vessel-by-vessel basis and predicts the temperature in and near individual blood vessels. However, detailed information about vascular geometry is rarely available. Even when it is, the resulting computational task is formidable. Such a model is useful only in modelling small volumes of tissue. Whole body models, however, should be constructed without the physical details of the vascular system.

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THE ORGANISM IN EXTREME SITUATIONS: PREDICTIONS BY SIMULATION OF HEAT AND COLD. J. Werner, X. Xu and A. Mehrle

Since the early reports of Stolwijk (1966, 1970), Wissler (1961, 1964) and Werner (1975, 1977), various models of human temperature regulation based on the cylindrical representation of the body components have been developed. Whereas more complex three-dimensional models that take into account the true geometry of the body (Werner and Buse 1988; Werner et al. 1989) are computationally demanding and require sophisticated computers, cylinder models are now generally available for use on personal computers (Werner and Webb 1993). This contribution outlines the basis of a six-cylinder model, named THERMOSIM, which has been written in Microsoft^R-FORTRAN and is available for use with WINDOWS^R. The controller equations evolved from an earlier three-dimensional model (Werner et al. 1989). The overall model characteristics can be summarized as: ease of application via WINDOWS-shell, 6 cylindrical body compartments, one-dimensional computation where temperature is a function of the radial coordinate, different parameters for the 4 compartment layers of core, muscle, fat and skin, and air layer included beneath the clothing layer. The model was validated against very diverse data sets involving transient exposures to hot and cool conditions, and nude exposures to cold air. Additional testing was done against data of exercising subjects under changing ambient temperatures and of subjects in sleeping bag trials. Reasonable values were obtained in most cases. An important point made was the fact that experimental data are sometimes less reliable and often more variable than the errors in prediction. The THERMOSIM model has proved to be a viable tool for predictive purposes under diverse conditions: heat, cold, and exercise. Problems of reliability do not seem to be greater than that found with experimental results. Although the application of the model is simple, use of the model beyond the tested range should be accompanied by thorough physiological knowledge and experience. For example, the limits of thermoregulation depend primarily on the minimum or maximum values of the effector mechanisms which yet have to be carefully and individually worked out. The present min/max input data have to be regarded as modelling constructs.

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MECHANICAL INTERACTION BETWEEN SKELETAL MUSCLE CONTRACTION AND PERFUSION: A MODEL STUDY . W.J. Vankan, J.M. Huyghe, M.R. Drost, J.D. Janssen and A. Huson

In skeletal muscles interstitial pressure rises and blood perfusion decreases during contraction. Although the pressure rise is suspected to be responsible for this flow reduction, the exact mechanism of this interaction is not known. In this study we focus on the mechanical aspects involved in this interaction, leaving vasoregulatory aspects out of consideration. Because pressure distribution, deformation and perfusion are expected to be non-homogeneous, we developed a three-dimensional finite element model of a contracting, blood perfused skeletal muscle. The model geometry was based on rat m. gastrocnemius medialis. Perfusion, modelled by conductivity of the muscle tissue for blood flow, was prescribed as homogeneous and isotropic for arterial-, capillary- and venous blood compartments. The model predicted realistic vascular blood pressures (circa 100, 90, 20, and 10 $mmHg$ for arterial, arteriolar, capillary and venous blood, respectively) and regional capillary perfusion (circa 20 $ml/(100g\ min)$) in resting muscle. During maximal tetanic contraction the interstitial pressure increased non-homogeneously with a maximum of circa $150mmHg$ (Fig. 1), venous pressure developed up to circa 50 $mmHg$ and regional capillary perfusion halved.

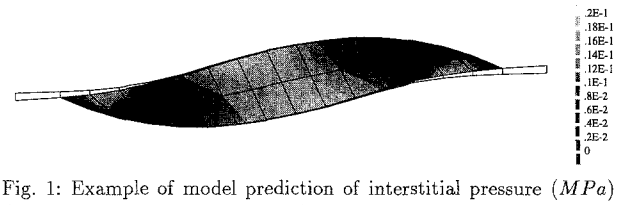

Fig. 1: Example of model prediction of interstitial pressure (MPa) during maximum tetanic contraction.

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A MATHEMATICAL MODEL OF O_2 TRANSPORT IN ERYTHROCYTES. Yu. Ya. Kislyakov

A model is suggested which describes oxygen diffusion in the erythrocyte and in capillary plasma as well as red blood cell reaction kinetics. It was used for the calculation of the dynamic O_2 distribution in the erythrocyte and in the plasma inside the cylindrical capillary. In this model, the velocity of red blood cell O_2 desaturation depends on the gradient of the O_2 partial pressures during oxyhaemoglobin deoxygenation and the O_2 dissolved in erythrocyte plasma. The transport of O_2 in erythrocyte and capillary plasma is described by the diffusion equation. O_2 partial pressure on the surface of the capillary has an average tissue value of 20 torr. A numerical method is used to solve the system of differential equations describing the O_2 partial pressure (pO_2) fields of a single erythrocyte surrounded by plasma in the 3-dimensional space of the capillary. The model has been applied to investigation of the velocity red blood cell O_2 desaturation during its movement along a capillary from the arterial to the venous end. Simulation shows that the average pO_2 in erythrocyte plasma decreases by 67 torr (from 94 to 27 torr) during its motion in the capillary. At the same time, the pO_2 of the haemoglobin deoxygenation decreases by 46 torr. This means that the erythrocyte has a considerable O_2 stock at the venous end of the capillary. Therefore, haemoglobin deoxygenation conditions in capillaries and for O_2 tissue supply can remain constant in different extreme and pathological situations. This model was used for the analysis of O_2 transport conditions in a microcirculation system under different physiological situations.

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CLOSED-LOOP CONTROL OF LEFT VENTRICULAR FILLING PRESSURE. S.A.A.P. Hoeksel and J.J. Schreuder

Nitroglycerin is often used in left ventricular failure to reduce left ventricular filling pressure and to prevent pulmonary edema. We evaluated the feasibility of a closed-loop nitroglycerin delivery system in an operating room environment. As input of the closed-loop system we used mean pulmonary artery pressure as an indicator of left ventricular filling pressure. The system validated the quality of the pulmonary pressure signal by comparing each pressure beat with a moving average model of the previous pressure beats. The pulmonary pressure values were corrected for the respiration artifact by fitting them in a least-square sense on the first harmonic of the ventilator signal. The closed-loop system consisted of a proportional-integral regulator which updated the infusion rate of an infusion pump every 5 seconds. A supervisory program was used to adapt or overrule the proportional-integral regulator when necessary. The pressure validation algorithm was evaluated in 20 patients who were scheduled for cardiac surgery. The algorithm did not falsely label data from invalid pressure signals as valid data. The average correction made for the respiration artifact on the pulmonary pressure values was $\pm$ 1.3 (SD 0.4) mmHg. The mean pulmonary artery pressure calculated by the algorithm provided a stable enough input signal for the closed-loop system. Preliminary results using the closed-loop system in a clinical setting indicated that the closed-loop system was fast enough and provided stable control without the need to manually overrule the controller's actions.

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INTRINSIC BIOMOLECULAR PHOTONIC EMISSIONS AND NEUROPHOTONIC EMISSION SPECTROSCOPY-BASED TOMOGRAPHY

S. M. H. Bukhari^ and Z. H. Shah

Objective: Aim of the study was anunderstanding of quantum physical phenomena inherent in the normal and malignant living cell macromolecules and their effects on cell physiology and pathology.

Methods used: The study began with the STEM Microscopy and microelectrode analysis of cultured and chemically processed rat neurons followed by their X-Ray Crystallography to determine the molecular structures and biophysical interactions of both benign and malignant cell proteins. The second phase initiated with the selection of seven human subjects, including three with malignant brain tumours, who underwent a detailed neurological study with the help of a specially-designed medical analysis system, which revealed the presence of well-defined electromagnetic emissions from both the healthy and malignant lesion cells with distinguishable characteristics as frequency, intensity and resonance, detectable by a specialized magneto-optical instrumentation. Later, observations of seventeen cases with suspected brain tumours were taken on with the system using the devised method and thirteen of these were diagnosed tumour-positive. A subsequent MRI tomography verified the results. In the third and last phase, these tumour patients were detained and made to go through an integrated chemotherapy & Quantized Photodynamic (QPD) therapy strategy using drug-coupled irradiation with state-quantized non-ionizing radiations. After a constant planned therapy for just a month, eleven out of fifteen patients showed visible signs of progress with reduction in the tumour pathoanatomical size and associated symptoms.

Conclusions: Combined results of the three phases of research led to the inferrence of the theory of intrinsic biomolecular photonic emissions from the living cells intrinsically-related to physiological and pathological conditions, on the basis of which a new non-radiological method for the diagnosis and localization of tumours was defined, called "Neurophotonic Emission Spectroscopy-based Tomography (NEST)". Besides, the devised technique of Quantized Photodynamic (QPD) therapy, integrated with suitable chemotherapy, came forth as a promising new way for the non-ionizing effective treatment of drug-resistant malignant tumours.

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MATHEMATICAL MODELING OF THE ARTERIAL BARORECEPTOR AND HIS RESETTING PROPERTY

V. Nestianu, I. Iancu and E. Iancu . The purpose of this work was to develop a mathematical model of the arterial baroreceptor and to study his behavior during steady state and transitory state. We used the experimental data published in literature for carotid sinus baroreflex in anesthetized and vagotomized dogs. For the carotid sinus baroreceptor model without resetting, the block diagram showed three output components. One component is proportional to blood pressure and two components are specifics for low-pass filter. One of them is blood pressure derivative proportional. We developed the baroreceptor resetting model as a transfer function $H(s)=k\omega^2/(s^2+2\zeta\omega s+\omega^2)$ which introduced a supplementary negative feed-back and works like a follower system. For the carotid sinus baroreceptor model with resetting property, the block diagram showed the output components presented in the model without resetting and two components proportional to second and third derivative of blood pressure.To validate the baroreceptor model we evaluated the differences between native and modeling baroreceptor responses to the same stimulus. The rezults indicated similar behavior for native and modeling baroreceptor . We also developed the model of carotid sinus baroreflex as an autonomous nonlinear system, including the two models of the baroreceptor: with and without resetting. Through simulation we proved the role of resetting in stabilizing blood pressure . Moreover we concluded that baroreceptor with resetting property works like a controller in the control systems.

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ANALYSIS OF TRACER DISPERSION AND DELAY IN SAMPLING LINES.
Jurg L. Jaggi, Joel H. Greenberg, Martin Reivich, Abraham Noordergraaf.
Quantitation of cerebral blood flow (CBF) with diffusable radio-labeled compounds requires a measurement of the arterial tracer concentration function. For that purpose, blood is commonly withdrawn with a pump through a small bore catheter of radius R, and at some distance L from the subject the activity in the line is counted with a fixed detector system of diameter d. Although this method allows for automated and continuous sampling, care has to be taken to account for ***delay*** and ***dispersion*** of the measured arterial time course.
We analyzed the flow distribution of a tracer through a thin tube. A perpendicularly arranged detector distant to the sampling site monitors the tracer activity passing through the tube as follows, $\mathbf{V} = \pi R^2 d\,(1 - 4\mu L / GR^2 t)$ **= const (1 -** τ **/ t)**, which represents the arterial blood isotope concentration over time. Interestingly, the response to a step change is not, as commonly assumed, an exponential but rather a simple ***hyperbolic function*** with the dispersion constant τ. Poiseuille's law describes the volume rate of a fluid in a tube as a function of pressure gradient, G; radius, R; and viscosity, μ. Substituting the gradient in Poiseuille's law into the equation above, an explicit expression of the dispersion constant is obtained, $\tau =$ **V / 2Q**, with V being the volume of the sampling tube and Q the volume rate of flow. As seen in the hyperbolic function, τ is also defined as the minimal delay of tracer between the sampling site and detector system. As a consequence, delay and dispersion caused by the sampling line are described with only ***one*** (and not two) variables.
To test if this reduction in parameters improves the reliability of the fitting procedure, we incorporated this new concept into the operational equation of the positron emission tomography CBF analysis. Data from six normal controls were analyzed in two ways: First with the 3 parameter model, solving for cerebral blood flow, dispersion and delay separately, and secondly, with the new 2 parameter model which solves for flow and τ. Results show that when the number of fitting parameters is reduced, smaller variances of flow and dispersion are obtained with only a minimal increase in the sum of squares.
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A MATHEMATICAL MODEL OF SUBCORTICAL ISCHEMIA. M.W Roos and G.O. Sperber
Subcortical infarctions cause stroke and dementia in humans. We have developed an experimental model useful for studying the process of subcortical infarction in rabbit brain. Small vessel occlusion is obtained by injecting plastic beads into the heart. In short-term experiments, ischemic foci can be seen all over the brain and in long-term experiments infarctions, mainly located in subcortical structures, develop. The distribution of ^{14}C-2-deoxyglucose (2-DG) was obtained through autoradiography and agreed well with simulations, with suitably chosen parameters.

Simulations: Three equations of the form: $\frac{dC}{dt} = D\left[\frac{d^2C}{(dr)^2} + 2\frac{dC}{r\,dr}\right] - Q$

describe diffusion and consumption of 2-DG, oxygen, and glucose in a spherical volume of tissue where **C** is the substrate concentration, **D** the diffusion coefficient, **t** the time, **r** the radius and **Q** the consumption. Further equations obeying a modified Michaelis-Menten kinetics specify **Q**. The system was solved numerically. The results are compatible with the view that in the ischemic center the glucose and 2-DG concentrations are close to zero which in turn causes a central decrease in 2-DG accumulation. Hopefully this theoretical model together with animal experiments can bring new information about the pathophysiology of subcortical infarctions in humans.

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THE INFORMATION VALUATION OF ADAPTIVE CHANGES OF A HUMAN THERMOREGULATION SYSTEM. Yu. Gorgo, A. Drobacha.
The conditions and adaptive changes in healthy humans thermoregulation system were analysed by local application of cold of a certain duration. The reduction of time of skin temperature recovery was used as a cold adaption criterion after local dosed cooling. For this index was measure of organization of system (R) on works H. von Foerster. They were evaluated R at creation of stereotype constant to definite on force and duration to local cools. Person were training to perception of weak cools, thus control were strong cooling effects, and vice versa. More intensive on force of effect cool causes and more difficult the organized reactions of thermoregulation system. At control strong cooling effects R=0.5, and at control weak R=0.47. The system became more determined by applying more heavy cooling a so less able to learn. For device to conditions of environment system must lower by its determination. We observed, that creation of adaptive reactions to colds will be better at action of weak cool. And training of organism to strong cools did not generate devices to weak. Value R at weak cooling, caused after training strong cooling loads, grew with 0.47 to 0.51. The adaptation to weak loads was accompanied by reduction R with 0.50 to 0.46, that testified about adapting fixtures of thermoregulation system. The effect of carry of adaptation to weak irritations on strong, but not vice versa, is display of specific role of weak irritation of any nature in process of adaptation.

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A PHYSIOLOGICAL MAN-MACHINE-SYSTEM FOR THE PREVENTION OF HEAT STROKE. M. Hexamer and J. Werner
Heat stress reduces human comfort and performance. It may lead to collapse, due to a temporary breakdown of central arterial blood pressure or to a letally high core temperature due to heat stroke. The latter is a serious threat for humans working in high environmental temperatures and/or high humidity, as they produce internal heat which might not be transferred adequately to the environment. For compensation of extreme heat stress, expecially for minors, fire-workers and astronauts, it is recommended to wear cooling garments. This is essentially an underwear which is provided with a network of flexible tubes through which cooling liquid is pumped. In the past, temperature and flow of cooling liquid is chosen according to the expected climatic and working conditions, or it is controlled by the subject according to the comfort sensations. This yields generally unsatisfactory results, especially on account of extreme oscillations of the man-machine control loop i.e. temperature of the cooling liquid changes oscillatorily in a broad range. We have investigated systematically the possibilities to control the cooling circulation automatically according to the physiological status of the subject. By this, a technical control loop which is programmed by physiological signals is superimposed on physiological temperature regulation which eventually might not cope with the thermal stress. As controller input various physiological signals and combinations of them were tested: oxygen consumption, heart rate, body temperature, mean skin temperature and sweat production. The final result is a man-machine-system controlling mean skin temperature by proportional plus integral control. However, a second physiological input for an adaptive adjustment of the set-point according to the work-load turned out to be necessary. We chose heart rate as a quick signal correlated with the strain induced by muscular exercise. This system meets equally the requirements of the physiological status (low load error) and of a reasonably comfortable sensation.

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DIFFERENTIAL EFFECTS OF EXTRACELLULAR ADP AND ATP ON INTRACELLULAR CALCIUM OF DIFFERENTIATING EMBRYONIC RED BLOOD CELLS, Leech E. and Baumann R.

Extracellular adenine nucleotides are paracrine effectors for a variety of cellular systems and some celltypes such as endothelial cells, release ATP under physiological conditions.
Embryonic erythropoiesis begins in blood islands of the yolc sac, where erythroid cells develop in intimate contact with endothelial cells. We have investigated the effect of ADP and ATP on red cell calcium of the first erythroid cell generation (primitive embryonic RBC) harvested from 2 to 6 day old chick embryos. During this time the cells mature from proerythroblasts (day 2) to mature postmitotic RBC (day 6): ATP and ADP cause different signals: ADP produces a transient calcium spike (Δ Ca_i max: 130 nM) followed by a plateau, whereas ATP produces only a small increase (< 40 nM) to a plateau. Pretreatment of cells with PMA inhibited both ADP and ATP dependent calcium increase. Similarly pretreatment of cells with ATP inhibited the ADP dependent calcium spike. ATP dependent inhibition was released when cells first treated with suramin, which is known to inhibit ATP binding to P2y receptors, whereas suramin had no influence on the ADP induced calcium spike.
The ADP dependent calcium spike was only observed in proliferating but not mature RBC, whereas the ATP signal was observed in both. Treatment of cells with thapsigargin abolished the ADP and ATP response. The data suggest that immature embryonic RBC have two different nucleotide receptors addressing different intracellular calcium pools, of which one responding to extracellular ADP is a marker of proliferative capacity of the erythroid cell. The results also suggest that extracellular adenine nucleotides may serve as physiological effectors during embryonic erythropoiesis.

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NEW APPROACH TO ELECTROCARDIOGRAM ANALYSIS FOR RECOGNITION OF PATIENTS PREDISPOSED TO SUDDEN DEATH
A. Parkhomenko, A. Andrushchenko, M. Perepelitsya.

OBJECTIVE OF THE STUDY
Diagnostic of patients prone to sudden cardiac death after acute myocardial infarction (AMI) remains the essential problem in cardiology. One of possible approaches to alleviate the problem consists of an analysis of electrocardiograms (ECG). Our purpose is to develop a method to detect the mentioned patients by an analysis of one-lead ECG.
METHODS USED
Here, such an approach based on a nonlinear forecasting technique for time series is applied. First, we embed an ECG signal in a specially chosen multidimensional space. Characteristics of embedding are estimated directly from the signal. Second, a coefficient to evaluate a quality of forecasting is computed. ECG of 20 patients after AMI divided into two equal-size groups were analyzed by this technique. The first and second groups are characterized by the presence and absence of heart arrest in the follow-up period, respectively.
RESULTS
The proposed technique was an efficient tool to discriminate the groups. A separating rule (between the groups) with 100% sensitivity and 100% specificity was derived.
CONCLUSIONS
The nonlinear forecasting technique for a time series analysis is perspective for the mentioned purpose. A larger quantity of patients is needed to implicate this approach in clinical practice.

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CONTROLLED CHAOS AND FRACTAL STRUCTURES IN BRAIN ACTIVITY DYNAMICS. A.Mogilevsky, L.Derzhiruk, A.Panchokha, Ya.Verbny

The main goal of our study is to indicate the fractal characteristics of nonlinear processes in dynamics of brain and neuronal activities during controlled experiments provided: a)the positive reinforcement of the certain EEG-patterns due to electrostimulation of hypothalamic emotion-positive zones, b)the sensor stimulation associated with EEG-patterns and c)the associative intracellular stimulation of the single neurons. The controlled self-stimulation was found to occur by means of the common complex dynamic system formed in brain; the above system organizes cognitive processes directed to satisfy the certain motivation. In this case the dynamic system develops the strategy resulting in occurence of reinforcing stimulation, for which the brain generates in EEG the highly periodic process permitting the realization of this strategy. The control may be realized too by the formation of a great number of different models with the increased number of attractors. The increase of the information content of the stimulus would result in the development of the absolutely different character of the modelling process able to realize other cognitive-orientative strategy of the incomparable greater or smaller internal freedom. The construction of such structure determines the effective chaos control even by weak deviant signals. These peculiarities are reflected in characteristics of controlled chaos dynamics on the single neurons level during the formation of plastic reconstructions of their activities under different modes of intracellular electrostimulation.

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ON-LINE ANALYSIS OF CONTINUOUS PATCH-CLAMP DATA.
A. Nesterov and V. Serebryakov
The computer methods for the analysis of continuous patch-clamp single channel data developed so far allow to obtain output information from the experiment only upon its completion and after all data are collected by the computer. The software developed in our laboratory allows to manage single channel patch-clamp data on-line. During an experiment the data are continuously analyzed by a computer. We use an IMB 486 DX2-66 personal computer. The data are acquired with a sampling rate of up to 50 micro-seconds per point. To detect the single channel events we use thresholds for the closed and the open states of an ion channel. To distinguish these states we set for each state the limits of the current amplitude. An ion channel is considered to be in one of the possible states if the value of the current amplitude is within amplitude limits, pre-setted for a given state. Amplitude intervals and their location may vary because of a base line drift or because of ion channel amplitude changes. A special algorithm is used for the correction of the location of these intervals. Thus a large amount of raw data is transformed into the so-called event array on-line. The event array contains a list of ion channel states registered throughout an experiment with the corresponding life times for each state. The event array is used to produce values of 1) the open state probability, 2) the probability of activation, 3) the mean times of open and closed states for a single ion channel averaged in an interval of user-defined length. These parameters are visualized graphically, so we can follow their evolution throughout an experiment. The program saves the raw data along with the information obtained in the process of the on-line analysis on the hard disk. To reanalyze pieces of data that has been processed incorrectly on-line because of abrupt base-line shifts, sudden occurrences of multiple open states etc., our program can be used also for the off-line analysis of the raw data.

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PHYSIOLOGICAL STRAIN OF FIRE FIGHTERS IN A JOB-RELATED TRAINING DRILL. R. Ilmarinen and K. Koivistoinen.

Cardiac and heat strain was studied in a job-related test drill developed in the Finnish Emergency Services College for fire-fighting and rescue work in extreme conditions. The subjects were 42 healthy male fire-fighting students aged 19-26 years and with $\dot{V}O_2max$ ranging from 42.6 to 70.5 ml/kg·min. The test drill, conducted at air temperature of 5-25°C and performed with own speed, lasted 50 to 70 min and consisted of nine common fire-fighting tasks. The drill was done with a self-contained breathing apparatus and permeable fire-protective clothing (total weight of 28 kg) and it was divided into three consecutive work sessions with a 10-min rest between each session for drinking and changing the air container. HR was continuously recorded and rectal temperature (Tre) was registered at the beginning and at the end of each sessions. Sweat production was determined from the changes in body weight, corrected for fluid intake and accounting for the amount of sweat absorbed into the clothing. Tre increased steadily over the duration of the drill and exceeded 39 °C (±0.3 °C) at the end of the drill on average. One subject could not complete the drill because of exhaustion and five subjects were actively cooled at the end of the test (Tre 39.5 °C). The range of the individual mean HR was 150-170 bpm with a corresponding near maximal cardiac strain of 75-96 %HRmax. HR of the seven subjects exceeded 200 bpm and the highest recorded value was 206 bpm. The average sweat production was 1.9 l (range 0.5-3.5 l) and respectively, water deficit 2.5 % (range 0.9-4.2 %). The results indicate that there is a risk for exhaustion and heat related disorders in fire-fighting test drills even in thermoneutral conditions. In actual fire-fighting work under high thermal radiation and wearing a waterproof fire-protective clothing (obligatory in many countries), the risk of heat disorders, even of lethal heat stroke, increases. The present results emphasize the need for heat tolerance test in selection of fire-fighting students for occupational training and the need for regular evaluation of the fire fighters' ability to work in heat.

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PHYSICAL PERFORMANCE IN TWO CONSECUTIVE EXPOSURES TO HEAVY DYNAMIC WORK AND HOT ENVIRONMENT WITH FIRE-PROTECTIVE CLOTHING AND EQUIPMENT. V. Louhevaara, R. Ilmarinen, B. Griefahn and C. Künemund

Physical performance was studied in two consecutive tests simulating demanding fire-fighting and rescue operations. Both tests included the same exposure to heavy dynamic work (treadmill walk, speed: 4.5 km/h, angle: 2°, VO_2: 25 ml/min/kg), to hot dry environment (T_a: 50 °C, P: 1000 W/m^2, RH: 20 %, v_a: < 0.3 m/s), and to the use of fire-protective clothing and self-contained breathing apparatus (total weight: 25.9 kg, I_{cl}: 1.85 clo). The subjects were 12 male fire fighters aged 26-46 years. The range of their weight, height and VO_2max was 69-101 kg, 174-187 cm, and 33.4-73.3 ml/min/kg, respectively. The consecutive tests (15 + 15 min) were repeated with four combinations of the length of recovery and T_a during recovery between the tests (15/0, 30/0, 15/20, and 30 min/20 °C). The time interval between the test occasions was at least one day. The criteria for the interruption of the tests was the exceeding of 90 % HRmax. Three subjects passed all tests without interruptions. Seven second tests were interrupted when the preceding recovery time was short (15 min) and warm (20 °C). The mean performance time was 13.2 min. For the tests combinations of 15/0, 30/0, and 30 min/20 °C the corresponding values were 5/13.6, 4/14.5, and 5 interruptions/14.1 min, respectively. The length and T_a of the recovery period between the tests did not affect significantly the number of interruptions in the second test but the difference was significant ($p<0.001$) in endurance times. The present type of consecutive multi-exposure was too heavy for about a half of the subjects who were experienced fire fighters with an average or good VO_2max. In actual work situations, repeated exposures encompassing heavy dynamic work, hot environment and the use of protective clothing and equipment should be avoided or given sufficient time for recovery.

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MUSCULAR EXERCISE IN THE COLD : NITRIC OXIDE PRODUCTION IN EXHALED AIR. A. Therminarias, M.F. Oddou-Chirpaz, A. Favre-Juvin, J. Eterradossi, M. Delaire and F. Grimbert.

Performing muscular exercice in the cold is known to favour the occurrence of bronchoconstriction. Among the factors influencing airway reactivity, nitric oxide (NO) formed within the respiratory system could be involved in relaxation of vascular and airway smooth muscle, and consequently could modulate airway reactivity. The aim of this study was to investigate the pattern of NO measured in exhaled air during exercise performed in a cold environment. Well endurance trained subjects were tested in a climatic chamber, the room temperature being controlled either at 25°C or -12°C. The protocol consisted of exercise increasing in intensity in 30 W increments every 3 min until exhaustion. Minute ventilation, oxygen uptake, NO and carbon dioxide productions were continuously measured. At 25°C, the NO production increased as a function of exercice intensity up to 80-90% of maximal oxygen uptake. At this level, the expired amount of NO reached approximately 5 times the rest value. At -12°C as compared to 25°C, minute ventilation, and oxygen uptake did not significantly changed. On the other hand, the expired amount of NO diminished considerably.

Apparently, exposure to cold influences exhaled NO produced during incremental exercice. Since NO has been suggested to modulate airway reactivity, the possibility exists that a decrease in NO production is one of the factors favouring the occurence of bronchoconstriction during exposure to cold.

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THERMOREGULATION IN NEWBORN LAMBS

M E Symonds and L Clarke

Cold exposure to the extra-uterine environment is an important stimulus to metabolism in the newborn. This study aims to determine if modest changes in ambient temperature at birth influence thermoregulation.

Six sets of twin lambs were all born normally at term. One lamb was immediately placed in the warm (30°C;WD) and its twin in to a cool (15°C;CD) ambient temperature. Lambs were not allowed to feed. Colonic temperature (Tc) was recorded continuously between 2-6 hours of age and O_2 consumption (VO_2) was measured during non-rapid eye movement sleep between 5-6 hours of life.

Between 2 and 5 hours of life Tc gradually declined in all lambs, a response that was greater in CD than WD lambs (CD 0.67 ± 0.15; WD 0.38 ± 0.08°C). However, by 6 hours of life Tc remained 0.67°C higher in the CD group (CD 39.14 ± 0.33; WD 38.47 ± 0.27°C). At this stage VO_2 was 70% higher in CD lambs (CD 21.4 ± 2.3; WD 12.3 ± 1.3 ml/min per kg body weight). Irrespective of delivery temperature Tc was positively correlated with VO_2 ($Tc = 37.43 + 0.082\ VO_2$, $P = 0.015$).

In conclusion, delivery and maintenance in a cool environment can improve the newborn lambs ability to thermoregulate when not allowed access to its mother or feed.

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THE INSULIN - RECEPTOR INTERACTION IN ADIPOSE TISSUE OF HYPOTHERMIC RATS.T.Torlińska, P.Maćkowiak, L. Nogowski and J. Kożlik.

Glucose intolerance and suppresion of insulin release have been observed in patients undergoing hypothermic cardiac surgery and in animals subjected to experimental hypothermia.The aim of the study was to compare the number and affinity of insulin receptors in adipose tissue of both normothermic and hypothermic rats. Plasma membranes from epididymal adipose tissue were prepared and purified according to Havrankowa and binding assay was performed using / ^{125} I / - iodoinsulin . The kinetic parameters of the hormone - receptor interaction were analysed by the method of Scatchard using the LIGAND - Pc.v.3.1. computer program of Munson and Rodbard. Maximum specific insulin binding to plasma membranes in adipose tissue was decreased in the hypothermic rats but half-maximum displacement of tracer insulin was similar in the normothermic and hypothermic group suggesting that reduced receptor numbers rather than reduced affinity accounted for the difference.The changes in the number of insulin receptors took place despite the decrease in insulin serum concentration which seems to be in apparent conflict with the theory of "down an up" regulation. On the other hand insulinopenia observed in hypothermia may be beneficial for organism by favouring lipolysis in response to the need for energy substrates.

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COLD AND FEVER

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Fever has a great influence on thermal preference of an animal. The majority of mammals seek higher ambient temperatures following pyrogen injections. In these animals temperature of the body increases. However, because of their small size mice injected with pathogenic organisms in cold ambient temperature show a decrease of body temperature. The aim of this study was a comparison of changes in thermopreferendum in mice injected with pyrogen and preexposed or nonpreexposed to cold. Thermoregulatory behavior was assessed in these experiments by monitoring the thermopreferendum of adult mice in a thermal gradient by a computer system. Mice nonpreexposed to cold or preexposed to cold (30 min) received i.p. pyrogen / 50μg/kg / or saline and were put in the thermal gradient. Such short cold preexposure exerted stronger influence on thermal preferendum in mice receiving saline (mice selected higher ambient temperature) than in mice receiving pyrogen. Starting from 30 min after the injections during 6 hours of experiment in mice injected with pyrogen selected ambient temperatures were within much narrower range (2^{o}C) than in saline injected mice (5^{o}C). Mice nonpreexposed to cold and injected with pyrogen preferred higher ambient temperature than mice receiving saline. The differences were significant at 60, 120 and 210 min after injections. On the other hand, the difference between chosen ambient temperatures in mice preexposed to cold and receiving saline vs mice preexposed to cold and receiving pyrogen were not significant.

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THERMOGENESIS OF BAT IN HIBERNATORS AND NONHIBERNATORS

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Hypersensitivity to calorigenic actions of noradrenaline /NA/ following chronic cold exposure has been well documented in various mammals. In our earlier experiments golden hamsters receiving noradrenaline died from heat stroke. The animals were NA injected at a room temperature and the only way to prevent the heat stroke was the cold exposure. The aim of the present experiments was a comparison of the efficacy of brown adipose tissue (BAT) thermogenesis in hibernators and nonhibernators. Because it is generally recognized that behavioral adjustments constitute an integral part of physiological temperature regulation, thermal efficacy (BAT) in hibernators and nonhibernators was determinated by monitoring their thermopreferendum in a thermal gradient by a computer system. The selected ambient temperature was dependent upon thermal danger i. e. it was inversely related to body temperature. The experiments were done on 6 golden hamsters and 4 *Muscardinus avellanarius* (both of animals are hibernators) and on 6 wild and 6 dwarf mice (both of them are nonhibernators). In hibernators higher efficacy of brown adipose tissue was inferred from their selected ambient temperature (14^{o}C) which was markedly lower than in nonhibernators (20^{o}C). In experiments carried out on nonhibernators we were not able to show any influence of body mass on BAT efficacy. In hibernators group, however, *Muscardinus avellanarius* members being smaller hibernators chose lower ambient temperature than golden hamsters. It must be stressed that behavioural method of investigating the BAT efficacy is safe and noninvasive.

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EFFECTS OF INTRAVENOUS FLUID INFUSION ON CIRCULATORY FUNCTION IN DEEP (25°C) HYPOTHERMIA. T.Lauri

This study determines the effects of an acute fluid infusion on the canine circulatory system.Cardiac catheterization was performed on 18 anesthetized beagle dogs.11 of them cooled and received 40ml/kg dextran intravenously at a body temperature of 25°C while the control group of seven dogs received same amount of dextran without cooling at a body temperature of 37°C.Circulatory function was controlled for every one degree during cooling and rewarming.In deep hypothermia the effects of fluid infusions were minimal.Heart rate,cardiac output and mean aortic pressure increased only slightly after infusion in hypothermia when compared to normothermia.Mean left ventricular filling pressure and left ventricular end-diastolic pressure increased significantly as signs of cardiac decompensation as also did in normothermia.Decrease of systemic vascular resistance in hypothermia was small and cardiac contractility did not increase.During rewarming heart rate and cardiac output increased significantly up to 29°C while mean left ventricular filling presure and left ventricular end-diastolic pressure stayed at higher level and systemic vascular resistance decreased.Every parameter recovered to normal during rewarming.According to this study the fluid infusion in deep hypothermia at first fills existing physiological hypovolemia and blood volume increases during early phase of rewarming.Clinically this may lead to overloading and cardiac decompensation.

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EFFECTS OF COLD AND EXERCISE ON OXYGEN EXTRACTION IN HELMETED GUINEA FOWLS. H. Johannesen and C. Bech

Both low ambient temperature (T_a) and exercise increases oxygen uptake (Vo_2) in birds. The supply of O_2 by the ventilatory system involves three variables: respiratory frequency, tidal volume and oxygen extraction (Eo_2), i.e. the percentage of oxygen extracted from the inspired air. In some species, Eo_2 increases during cold exposure. In other species, the increase in Vo_2 at low T_a is accompanied by an equivalent increase in total ventilation. There have been fewer studies of Eo_2 in exercising birds. From data obtained during running, flying and swimming it can be seen that Eo_2 is constant or decreases compared to that in resting birds. However, an increase in body temperature (T_b) during exercise affects ventilation and hence Eo_2. Studies on exercise energetics in the Helmeted Guinea fowl have shown that T_b is constant or slightly reduced during treadmill running at 4.5 km h^{-1} at 25°C. This species is therefore suitable for comparing the effects of cold and exercise on Eo_2. In the present study, Vo_2, T_b and partial pressures of oxygen (Po_2) in inspired air and in abdominal and interclavicular air sac gas were measured in post absorbtive birds at two experimental conditions: resting in darkness at day at thermoneutral (35°C) or low (5 and -15°C) T_a; running on a treadmill for 15 min at 2.5 or 4.5 km h^{-1} at thermoneutral (35°C) or low (25 and 5°C) T_a. Values of Po_2 were converted to fractional O_2 contents. Eo_2 was calculated from the fractional O_2 contents of inspired air and interclavicular air sac gas. The results show that Po_2 in the abdominal air sac gas increased, while Po_2 in the interclavicular air sac gas decreased in resting birds at low T_a. Therefore, values of Eo_2 were higher during cold exposure than at thermoneutrality. In exercising birds, Po_2 in both air sacs increased, and Eo_2 decreased at both running speeds at all T_a's, even though T_b did not increase at low T_a. These changes in air sac gas composition indicate hyperventilation of the lung. However, the increase in Po_2 in both air sacs was greatest at 35°C. It can be concluded that in the Helmeted Guinea fowl, Eo_2 increases with increasing Vo_2 induced by cold exposure, but decreases with increasing Vo_2 due to exercise. Furthermore, an increased Po_2 in both air sacs during exercise under isothermic conditions indicates that factors other than hyperthermia contribute to the exercise hyperventilation in this species.

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Elevation of prostacyclin synthesis preceds the increase in PGE_2 production in mouse hypothalamus during the febrile response L. Abramovich, V. Fraifeld and J. Kaplanski

Prostaglandin E_2 (PGE_2) is generally considered to be the major central mediator of various pyrogens, acting directly on thermoregulatory center in hypothalamus. However, the possible involvement of several other prostanoids has been also suggested. The aim of the present study was to examine the effect of E. coli LPS on hypothalamic PGI_2 and PGE_2 production in mice. Male ICR mice (30-40 g) were injected ip with 0.1 ml saline containing 50 μg of E. coli LPS. Following 2, 4, 6 and 24 h post-injection, mice were decapitated, their hypothalami were excised and incubated. PGE_2 and 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2), content in incubation medium were determined by radioimmunoassay. Two hours post-injection, it was found a significant decrease in rectal temperature (- 2 °C, $p < 0.05$), which returned to the control level at about 6 h and remained unaltered up to 24 h. LPS-induced elevation in hypothalamic PGI_2 production in hypothermic phase of body temperature response (117±12 vs. 72±6, 103±5 vs. 74±9, 124±13 vs. 81±7 pg/hr per mg tissue, $p < 0.05$; 2, 4, 6 h after LPS administration, respectively). In contrast to PGI_2, the increase in hypothalamic PGE_2 production was found only in the late phase (145±18 vs. 77±11 pg/mg tissue/hr, $p < 0.05$; 24 h after LPS injection). In conclusion: (i) LPS caused a considerable increase in PGI_2 and PGE_2 by mouse hypothalamus and this increase occured in time-dependent manner; (ii) the elevation in PGE_2 production was preceded by that of PGI_2.

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INTERACTION OF PROPRIOCEPTORY AND THERMORECEPTORY SENSORY INPUTS IN PROGRAMMING POSTURAL AND THERMOREGULATORY ACTIVITY OF HUMAN MOTOR SYSTEM A.Yu.Meigal, and Yu.V.Lupandin

Thermoregulatory muscle activity (TMA) is very similar with the postural activity, some kinds of movements and enhanced physiological and pathological tremors by the motor units activity and biomechanical characteristics (Meigal et al, 1993, Pozos et al, 1992). The hypothesis is that temperature might influence postural activity and postural tonic reflexes, because both TMA and posture utilize the same types of motor units and this leads to competition betwee them. We investigated by ME3000p EMG-device (MEGA Electronics Ltd, Kuopio, Finland) the influence of tonic neck (TNR) and labirynthine (TLR) reflexes on TMA distribution in the ipsi(DM_{i1}) and contralateral deltoid muscles (DM_{c1}) provoked by the lateral head bendings during sitting, standing and tip-toe positions at +5°C and +22°C. The lateral head bends evoked 49.02±23.03% increase ($p<0.01$) of TMA intensity in DM_{c1} without significant effect on the DM_{i1}. TMA significantly increased when changing position from sitting to standing on tip-toes ($p<0.01$). Postural reflexes did not manifest themselves at room temperatures. Postactivation effects (PAE), that is aftercontraction tonus, was tested in the brachial biceps muscles EMG at room (+22°C), cold (+5°C) conditions and at sauna (+65-85°C). PAE increased twofold in cooling conditions ($p<0.01$) and it decreased almost twofold at sauna ($p<0.01$) in comparison to room conditions. The data obtained evidence that TMA and posture widely interact, though thermoregulatory and motor sense of this interaction is still obscure.

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THE GENESIS OF THE HYPOXIA UNDER HYPOTHERMIA CONDITIONS. P. Beloshitsky, A. Pischalenko

The difficulties of the studing the problems of the genesis of hypoxia under hypothermia are that hypothermia reduce the oxygen tissue consumption and, at the same time, leads to a couple of changes in oxygen transport and utilization. That is why the evaluation of the correspondence the O_2 transport to O_2 consumption is possible only after analysis of the all the influencing factors. It was shown, that in hypothermed rats during the prolonged nonmedicamental hypothermia (20 hours, 22°C) there is a decreasing of the O_2 consumption, arterial and interventricular pressure, bulk flow velocity, an increasing the processes of free radical oxydation, the rate of discoordination of working all the organism systems and ultrastructural disturbances, hyperkaliemia, metabolic acidosis. At the same time, there are no changes in O_2 tension in tissues as well as in the activity of oxydative enzymes. It was discovered, however, that under prolonged hypothermia and adequate O_2 delivery the rats show the signs of hystotoxic hypoxia, coupled with circulatory hypoxia during terminal stage.

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MATHEMATICAL MODELS OF RESPIRATORY SYSTEMS AND CIRCULATION OF THE BLOOD SYSTEMS AS WELL AS THE ESTIMATION OF ORGANISM'S RESERVES AND OF THE RELIABILITY OF SYSTEM'S FUNCTION. N.I. Aralowa, P.V. Beloshitskii and Yu.N. Onopchuk

The main function of systems both of respiration and of circulation of the blood, i.e. the adequate and timely delivery of oxygen to the metabolic tissues as well as the removal of the produced carbonic acid from organism, is studied. The process both of respiration and of circulation of the blood is presented in the form of controlled dynamic system; as the main active executive mechanisms of regulation there are considered the respiratory muscles, the heart muscles and the smooth muscles of vascular system. It is adopted that the aim of regulation is to put a dynamic system in the state of the comparative balance; and the optimal regulative reactions are considered those providing the compromise agreement for all conflict situations which arise between the different tissue regions fighting for oxygen. Analysis of mathematical model, both qualitative and numerical, in modelling of various conditions of vital functions of organism, allows to estimate its reserves, to give an appreciation of the extent of arising hypoxia and hypercapny, to prognose the degree of tension of regulative mechanisms in regime of supporting the equally-balanced state, to form the reliability criteria of functioning both of the respiratory system and of the circulation of the blood system, to study the system's adaptive possibilities of organism.

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MUSCLE WORK EFFICIENCY IN PHYSICAL TRAINED AND COLD ADAPTED SUBJECTS. V. Divert, E. Tkachenko.

Muscle work efficiency depends on a lot of exogenous and endogenous factors. The most important factors are work load and environmental temperature. The optimal load is mostly determined by muscle energetics and can indicate it changes after adaptation.
The influence of different work loads on the body energy cost was studied in physical trained and cold adapted subjects. Physical training was carried out in warm or cold environment. The changes of work efficiency and optimal work load after physical training and cold adaptation are different. Maximum of work efficiency rises after the physical training in warm, but decreases after the adaptation to cold. Physical training in cold doesn't change it. The optimal work load is lowered after the adaptation to cold and rises after training in cold or in warm.
True oxygen can be used as the index for respiratory system efficiency. At exercise the maximum value of true oxygen lowers after cold adaptation and rises after training in warm. The exercise load for true oxygen maximum were the same as for the whole body efficiency maximum in both cold adapted and physical trained in warm subjects. Physical training in cold lowers the optimal efficiency of respiratory system and the optimal work load in comparison with training in warm.
The shifts of optimal exercise load at various environmental temperature accompanied with different energetic processes. From one hand, energy cost of work decreases due to increase of cardio-respiratory system efficiency. On the other hand, energy cost of work increases due to activation of lipid metabolism. Analysing these processes allows to determine the optimal external temperature for any work load.

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SEASONAL PECULIARITIES OF T_3 LEVEL INCREASE INDUCED BY ULTRA-VIOLET IRRADIATION IN VITRO OF HUMAN BLOOD. DOLOMATOV S.I.
It has been shown that ultra-violet irradiation (wave length 254 nm) in vitro of men`s blood in age of 20-35 years old by means of mercurydischarge lamp with irradiation at blood surfase 15,5 Wt/m2 leads to dose-dependent increase of triiodothyronine (T3) concentration. Dynamics of level increase of T3 and absolute values of its growth has seasonal dependense: in the winter period after 2 min. exposure to UV quality of T3 increases reliably ($P<0.001$) in exposed blood plasma ($n=16$) from 2.43 ± 0.22 nmol/l ($\bar{x}\pm S\bar{x}$) to 5.05 ± 0.54 nmol/l in comparison with non-irradiated blood; in the summer period under analogous UV-exposure T3 content increases from 1.41 ± 0.06 to 1.68 ± 0.08 nmol/l ($P<0.05$ $n=22$). After UV-exposure of blood at 20 min. T3 levels in plasma were: in the summer series 3.90 ± 0.17 nmol/l,in the winter series 10.61 nmol/l ($P<0.001$). There was no reliable change of thyroxine (T4) concentration at the whole range of applied doses. It is supposed thet T3 level increase under UV-irradiation is attributed to output of deposited form of hormone from erythrocytes or as a result of modification of hormone-receptor interaction, since maximum sorption of thyrozine is closed to irradiation spectrum of an applied source, or as a result of deep rebuilding of erythrocyte membrane. Correlation analysis of obtained data hasn`t reveal any negative correlation T4-T3 before irradiation and at the whole rande of applied UV-doses. Absence of such correlation validates indirectly that induced increase of T3 is the result of deposited form`s output rather than the activation of conversion of T4 to T3.

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THERMAL RESPONSES OF YOUNG CHILDREN (AGE 6 MONTHS-4.5YEARS) DURING HEAT EXPOSURE. K. Tsuzuki-Hayakawa.
This study was conducted to investigate the thermoregulation of young children in comparison with that of adults. Twenty five children (ages 6 months-4.5 years), with only three being three or above, and sixteen adults first rested in a thermoneutral room (25°C or 28°C, 50% Rh, 0.2 m/s). They were then exposed to a hot room (35°C, 70% Rh, 0.3 m/s) next door for 30 minutes, and then returned to the thermo-neutral room where they stayed for an additional 30 minutes. The Rectal temperature(Tre), skin temperatures at 7 points, heart rate (HR), total sweat rate(TSR), local sweat rate and the NA+concentration of the sweat were measured. There was no significant difference in Tre between the children and their mothers in the rest phase. However, the Tre of the children elevated as soon as they entered the hot room and was significantly higher than that in the control, and that of the mothers during heat exposure. Moreover the relationship between the rise of Tre and age for the young children was significantly observed during the heat exposure. Mean skin temperature (Tsk), forehead, abdomen and instep skin temperatures were significantly higher in the children during both the thermoneutral and heat exposure. TSR was significantly higher and Na+ concentrations in the sweat on the back and upperarm were significantly lower for the children during the heat exposure. They had a greater body surface area-to-mass ratio than the mothers by 64%, which indicates they had the advantages of thermal regulation. However, the sweating and skin temperature responses of the children were not enough to prevent the rise in body temperature. These results suggest that the young children had a disadvantage in being heated up easily due to their smaller body sizes and there may be maturation-related differences in thermoregulation during the heat exposure between young children and mothers.

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INTRACELLULAR CALCIUM AND ADH STIMULATED SODIUM TRANSPORT IN FROG SKIN. H.F. Bjerregaard, M.S. Nielsen and B. Brodin.

The isolated frog skin is an often used model for studying Na^+ transport and its regulation in tight epithelia, such as the urinary bladder and the distal part of the kidney. Stimulation of active Na^+ transport by antidiuretic hormone (ADH) in frog skin is mediated by the intracellular messenger cAMP. However, evidence for involvement of free intracellular calcium ($[Ca^{2+}]$) has been reported (1). We have previously shown that the intracellular calcium antagonist TMB-8 inhibit ADH stimulated Na^+ transport (2) and cAMP production (3). In this study the involvement of $[Ca^{2+}]$ was further investigated. High extracellular Ca^{2+} (10 mM) attenuated the inhibitory effect of TMB-8 (200 µM) on ADH (50 nM) stimulated cAMP production with 107 ± 11 % (n=12, P<0.005). Furthermore, it was shown that TMB-8 did not affect neither the basal nor the stimulated adenylate cyclase activity in isolated cell membranes from frog skin epithelia. These data indicate that the effect of TMB-8 could be caused by a reduced release of $[Ca^{2+}]$ from the intracellular stores due to TMB-8 blocking of Ca^{2+} channels. This hypothesis was supported by measurement of $[Ca^{2+}]$ with fura-2, showing that $[Ca^{2+}]$ within 1 to 2 min decline from 81 ± 14 nM to 69 ± 11 nM (n=10, P<0.02) and that ADH in this situation had no effect on $[Ca^{2+}]$. Other experiments showed that TMB-8, after addition of 10 mM extracellular Ca^{2+}, was not able to decrease the baseline $[Ca^{2+}]$ or inhibit the increase in $[Ca^{2+}]$ induced by extracellular calcium. In conclusion, this study demonstrate that an ADH induced increase in $[Ca^{2+}]$ could be necessary for optimal activity of adenylate cyclase in frog skin epithelia cells.

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SIGNAL TRANSDUCTION IN HUMAN BREAST CANCER CELLS: IN VITRO PROGRESSION FROM ER+ NON-INVASIVE INTO ER⁻-INVASIVE/METASTATIC CELLS. Anwar A. Hakim

Uncoordinated P53 Phosphorylation/Dephosphorylation systems modulate breast cancer suppression or promotion (Hakim FASEB Experimental Biology A724, 1995). The objective of the present study was to examine signal transduction in human breast cancer ER⁻ cells MDA-MB-231, and ER+ cells NCF-7 and T47D, which were maintained in an estrogen E, PDGF, TGF, & FGF supplemented and free media (Hakim Diagnostics & Clinical Testing 27: 30-37, 1989 Surg. Oncology 40: 21-31, 1989). Cellular Phospholipid (PL) levels correlated directly with phosphorylated protein tyrosine kinase (PTK) & inversely with protein tyrosine kinase phosphatase (PTKP). Progression of ER+HBCC into ER-aggressive HBCC is accompanied with 25 folds increase in PL levels. GDP/GTP exchange rate increased simultaneously with the PL. While C-erbB2-185P was undetectable in normal human breast epithelial cells (NHBEC) & in MCF-7. 185P was several times amplified and over expressed in metastatic/invasive breast tumor cells. Human unresponsive ER⁻ & PR⁻negative breast cancers differ from ER+positive cells in their proliferative and invasive rates in vivo and in vitro. ER+ positive cells acquire multiple characteristics of ER⁻ negative during acquisition of resistance to stepwise-increasing concentrations of Adriamycin or during prolonged withdrawal from estrogen. Treatment of HBCC with progestins results in a suppression of PR-expression. The decline in receptor protein is accompanied with a parallel decrease in the steady state of PRmRNA & a decrease in the level of PR-gene transcription. In contrast to the effect of estrogen on ER-expression. Progestin have no effect on PRmRNA half-life but mediate a decrease in PR protein half life. Thus PR expression is transcriptional & post-translational events modulated by signal transduction. The proto-oncogenes induced shortly after mitogenic treatment of cells include C-fos, C-myc, C-myb & C-Jun. These are induced by estrogen & progesterone in breast cancer. Tamoxyfen down regulates C-mycexpression causing tumor expression in patients tumors. Estrogens & growth factors activate nuclear-oncogenes by transducing signals through phosphorylation reactions.

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EFFECTS OF NA⁺ CHANNEL BLOCKERS ON PRIMARY CULTURED HUMAN NASAL CYSTIC FIBROSIS EPITHELIUM. U. Blank[1], W. Clauss[1], B. Eistert[2], A. Fryen[2], H. Glanz[2], W.-M. Weber[1]

Cystic fibrosis airway epithelia are characterized by a defect in cAMP-dependent chloride secretion through the cystic fibrosis transmembrane conductance regulator (CFTR) and an increased rate of sodium absorption through amiloride-sensitive apical Na^+ channels. The enhanced Na^+ absorption in cystic fibrosis airway epithelia under basal conditions seems to be a major reason for the defects in CF, as following dehydration of the mucus and impaired mucociliary clearance leads in turn to chronic infections of the airways. Human airway epithelial cells were disaggregated from surgical specimens removed for nasal reconstruction. We established a primary culture of human nasal epithelia which reached confluency on an area of 1.5 cm^2 on a permeable substrate. These high resistance preparations were mounted in an Ussing chamber to quantify the transport characteristics of sodium ions. Primary cultured human cystic fibrosis nasal epithelium showed large transepithelial short circuit current (I_{sc}). The complete short circuit current was mediated by Na^+ conductances and could be blocked entirely by apical application of amiloride with a K_i of 2.73 µM. Benzamil, an analogue of amiloride, also blocked the I_{sc} completely and reversibly, however, with a K_i of 14 nM it has a much higher affinity than amiloride. Benzamil is known to block Na^+ absorption better than amiloride in many epithelia, but to our knowledge it never was testet in cystic fibrosis epithelia. Our results might therefore indicate possible facilities for the symptomatic therapy of cystic fibrosis.

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SODIUM AND CHLORIDE TRANSPORT IN THE COLON OF VOLES. I. Choshniak. and R. Mualem.

The present study was designed to characterize sodium and chloride transport mechanisms in the colon of the vole (*Microtus socialis*)- one of the smallest mammalian herbivores. *In vitro* short circuit techniques were employed to measure potential difference (PD), short circuit current (Isc), tissue conductivity (G) and unidirectional Na^+ and Cl^- fluxes across the colon of the vole. In the proximal and distal colon, net Na^+ flux was not significantly different from that of Cl^-, and both were higher than the measured Isc. Amiloride (1 mM) resulted in a decrease in net Na^+ and Cl^- fluxes, whereas no effect on the electrical parameters was recorded. Amiloride (0.1 mM) had no effect on any of the tissue parameters. Employing Cl^- or Na^+ free solutions, significantly reduced net Na^+ and Cl^- fluxes as well as tissue PD. Mucosal furosemide reduced Na^+ and Cl^- in the proximal but not in the distal colon. Following the mucosal addition of SITS, both Na^+ and Cl^- fluxes were significantly reduced in the distal colon only. When HCO_3^- free solutions were used Na^+ and Cl^- fluxes, Isc PD and G were significantly reduced in the proximal colon but not in its distal part. The results suggest that parallel exchangers for Na/H and Cl/HCO_3 underlay the colonic electroneutral transport mechanism for Na^+ and Cl^-. In addition, a Na-Cl cotransporter is present in the proximal colon only.

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ABSORPTION OF ^{14}C-MANNITOL AND ^{51}Cr-EDTA IN CAT SMALL INTESTINE *IN VIVO*. SOLVENT DRAG EFFECTS OF GLUCOSE TRANSPORT AND OF THE COUNTERCURRENT MULTIPLIER. P. B. Bijlsma, B.-M. Fihn, A. Sjöqvist, J. A. Groot, J. A. J. M. Taminiau, M. Jodal.

The lumen to tissue transport of ^{14}C-mannitol and ^{51}Cr-EDTA was studied in *in vivo* perfused jejunum of anaesthetised cats using different isotonic (310 mOsmol/kg) perfusion solutions. In each cat (n=8), four 10 cm long segments of jejunum (with intact blood supply) were perfused with about 20 ml of Krebs-Henseleit solutions at 37 °C containing trace amounts of the probes. The four perfusion solutions contained resp. Krebs + 30 mM glucose (KG), Krebs + 30 mM mannitol (KM), Na^+-free $Choline^+$- Krebs + 30 mM glucose (CG), and Na^+-free $Choline^+$- Krebs + 30 mM mannitol (CM). The transport of water was monitored and the clearance of the probes was expressed in µl (min x g intestine)$^{-1}$. Values are mean ± s.e.m.

Perfusion solution	CM	CG	KM	KG
Water absorption	-0.45 ± 1.05	1.60 ± 0.53	6.86 ± 1.08	12.92 ± 1.42
^{14}C-mannitol clearance	1.11 ± 0.32	1.28 ± 0.40	2.01 ± 0.55	2.66 ± 0.75
^{51}Cr-EDTA clearance	0.41 ± 0.14	0.39 ± 0.07	0.45 ± 0.12	0.39 ± 0.10
Cr-EDTA/mann. ratio	0.58 ± 0.17	0.53 ± 0.13	0.33 ± 0.09	0.24 ± 0.07
Glucose clearance		18.75 ± 2.15		27.50 ± 2.84

There was a significant positive correlation between water absorption and ^{14}C-mannitol clearance from the different perfusates ($r = 0.99$; $p < 0.01$). This correlation was absent for ^{51}Cr-EDTA clearance ($r = 0.03$; $p < 0.95$). Hence there was a significant negative correlation between water absorption and ^{51}Cr-EDTA/^{14}C- mannitol clearance ratio's ($r = 0.98$; $p < 0.02$). These results indicate a prominent effect of solvent drag on mannitol absorption, through pores which exclude Cr-EDTA. We conclude that carrier-mediated glucose transport only partly contributes to the observed water absorption, and that the effect of glucose on mannitol absorption is secondary to an increased influx of water and not due to a change in epithelial permeability. Hallbäck *et al* (Acta Physiol. Scand. 1979; 107: 89-96) have shown that the difference in water absorption from the used solutions in cat small intestine is caused by the magnitude of villus tissue hyperosmolality, in turn depending on the efficiency of the vascular counter current multiplier. This indicates a causal relation between the efficiency of this mechanism and mannitol absorption *in vivo*. As a similar counter current mechanism is present in humans (Hallbäck, Gastroenterol.1978; 74: 683-690), we propose that this relation is essential for the interpretation of clinical intestinal permeability tests with this probe.

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HANDLING OF NEUTRAL AND CHARGED SUBSTRATES BY THE INTESTINAL PEPTIDE TRANSPORTER PepT1 EXPRESSED IN OOCYTES. M. Boll, W.-M. Weber*, M. Herget, M. Wagener, I. Gebert, U. Wenzel, W. Clauss* and H. Daniel

The intestinal peptide transporter PepT1 is responsible for absorption of dietary di- and tripeptides. PepT1 has recently been cloned from a rabbit small intestinal cDNA library. Based on 20 proteinogenic amino acids, 400 dipeptides and 8000 tripeptides could serve as substrates from which a number of peptides carries net positive or negative charge at physiological pH. PepT1 translocates neutral substrates by electrogenic proton coupled cotransport. The question of how this unique transport system translocates anionic and cationic substrates has not been investigated. We studied the handling of charged peptides in *Xenopus laevis* (*X.l.*) oocytes expressing PepT1. Peptide transport characteristics were assessed a) by two electrode voltage clamp techniques and b) by determining peptide mediated changes in pH_{in} in oocytes containing the pH sensitive fluoresecence indicator carboxy-SNARF1. Injection of the transporter´s complementary RNA into Xl oocytes results in a 30-fold increase of uptake of the dipeptide ^{3}H-Gly-L-Gln. Transport of neutral peptides is strongly pH dependent with a pH_{out} optimum of 6.5 and the transporter´s V_{max} is directly related to the transmembrane ΔpH. Peptide uptake regardless of their net charge causes a reversible membrane depolarization and decline in pH_{in}. However, the I-V relationships show distinct differences between substrates. Whereas at pH_{out} 6.5 transport is dependent on membrane potential (pd), the pd dependence of neutral but not anionic substrates is lost at $pH_{out} \leq 5.5$. Recordings of pH_{in} and current suggest that transport of the anionic species occurs with a higher H^+ flux-coupling ratio than uptake of neutral and cationic substrates.

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DOES THE MDR-1 GENE PRODUCT PREVENT INFLUX OF HYDROPHOBIC DRUGS INTO EPITHELIAL CELLS. H. DANIEL, E. Korasiak and H. Bartels

The MDR-1 gene product (p-gp) is expressed in epithelial cells such as the renal cell line MDCK and acts as an ATP-dependent transporter for xenobiotics including the anticancer agent daunomycin (DM). The presence of p-gp in the apical membrane of MDCK cells grown as monolayers results in a threefold higher basal to apical (BA) than apical to basal (AB) DM flux. The AB fluxes are as high as mannitol fluxes and appear to occur by the paracellular route. Net secretion (BA-AB) of 2.5 µM DM into the apical medium is saturable (K_m 7.6 ± 2.0 µM) and completely blocked by 10 µM cyclosporin A (CSA) and 100 µM verapamil. Since MDCK cells always accumulate more DM when presented to the basal side than when applied from the apical side, it was speculated that p-gp may prevent entry of hydrophobic drugs into the cell by directly extruding them from the cell membrane. We have determined unidirectional influx and efflux of ^{3}H-DM in control MDCK cells (C-cells) grown on Transwell filter supports. As a tool for assessing the role of p-gp in transmembrane fluxes, MDCK cells were in addition cultured in the presence of 100 nM DM to increase expression of p-glycoprotein (treated cells: T-cells). In T-cells BA flux of DM is enhanced threefold (V_{max}) compared to C-cells without changes in the AB fluxes. Although the verapamil sensitive efflux of DM from the T-cells into the apical medium is increased significantly, unidirectional influx is not different between C-cells and T-cells. In both cell types influx across the apical membrane exceeds basal influx 2-fold but apical entry is neither verapamil nor CSA-sensitive. We conclude, that the side specific accumulation of DM in MDCK cells does not result from a restricted apical entry but from local intracellular concentration differences.

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NUTRIENT INTERACTIONS WITH P-GLYCOPROTEIN IN THE APICAL MEMBRANE OF CACO-2 AND MDCK CELLS. E. Korasiak, H. Korte and H. Daniel

The p-glycoprotein (p-gp) belongs to the ABC transporter family and acts as an ATP-dependent export pump for xenobiotics. Its overexpression in cancer cells is asscociated with pleiotropic resistance against a variety of anticancer agents. Although p-gp is found in normal epithelial cells its role and its potential substrates in these cells are not yet defined. We have characterized p-gp mediated transport of ^{3}H daunomycin (^{3}H-DM) in monolayers of intestinal Caco-2 and renal MDCK cells and investigated the interaction of selected nutrients with p-gp transport activity. Cells were grown on porous filter supports and the presence of an intact monolayer was assessed by determining TEER. Both cell types show similar net transepithelial secretion of ^{3}H-DM with basal to apical (BA) fluxes exceeding the apical to basal (AB) fluxes two- to threefold. Net secretion into the luminal compartment showing saturation kinetics is completely blocked by the reversing agents cyclosporin A (CSA) and verapamil. Since p-gp prefers hydrophobic substrates we determined the transepithelial fluxes of 2.5 µM ^{3}H-DM in the absence and the presence of lipophilic nutrients (25µM) in 2.5% (v/v) DMSO. From the fat soluble vitamins only retinol and menadion (K_3) were found to inhibit ^{3}H-DM fluxes significantly. Vitamin K_3 caused a 40 ± 5 % inhibition of net ^{3}H-DM secretion and consequently increased cellular daunomycin accumulation by 42%. Free cholesterol also inhibited net ^{3}H-DM secretion by 20 ± 3%. In addition thiamine as the only cationic water soluble vitamin reduced p-gp mediated transport significantly. Although none of the nutrients was found to be as efficient as the classical drugs in altering p-gp activity, our studies suggest that nutrients interact with the epithelial p-glycoprotein.

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pH_{in}-RECORDINGS IN CACO-2 CELLS AS A TOOL FOR CHARACTERIZING PROTON COUPLED PEPTIDE TRANSPORT.

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Intestinal absorption of dietary di- and tripeptides as well as of a variety of ß-lactam antibiotics is mediated by an electrogenic proton coupled cotransport system in the apical membrane of epithelial cells. As small intestinal enterocytes, the human colon carcinoma cell line Caco-2 expresses a peptide/H^+ cotransporter with similar or identical characteristics. We have used intracellular pH recordings in Caco-2 cells to assess the substrate specificity and characteristics of proton coupled influx mediated by the peptide transporter. Polarized Caco-2 cells (TEER $\geq$ 300 Ω/cm^2) were loaded with 5 µM of the lipophilic acetoxymethylester of BCECF. Single cell intracellular pH was determined by video imaging based on the intensity of emission of BCECF at 530 nm after excitation of the fluorescent dye at the isosbestic point at 439 nm and the pH-sensitive wavelenght at 495 nm, respectively. Since peptide uptake requires an inwardly directed proton gradient, pH_{in} changes to substrate addition were determined at apical pH_{out} of 7.4, 6.5, 6.0 or 5,5 while keeping the basal medium at pH 7.4. Zwitterionic peptides and ß-lactam antibiotics cause a substrate specific and dose dependent decrease of pH_{in} when pH_{out} is decreased from 7.4 to 6.0. Below pH_{out} 6.0 the pH_{in} changes are less pronounced. Initial acidification (ΔpH/min) correlates significantly with influx of the corresponding radiolabeled substrates indicating that pH_{in} changes in response to substrate addition are solely the consequence of peptide-induced proton flow. In contrast to zwitterionic substrates, anionic peptides and ß-lactams cause pH_{in} changes only if buffer pH_{out} is $\leq$ 5.5. Peptides carrying net positive charge at pH 5.5 to 7.4 do not affect intracellular pH although they significantly inhibit the influx of neutral peptides into the Caco-2 cells.

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PGE_2 INHIBITS ADH INDUCED Na^+ TRANSPORT AND cAMP PRODUCTION IN FROG SKIN.

K.A. Rytved, H. Andersen and R. Nielsen.

Introduction: Prostaglandin E_2 (PGE_2) and antidiuretic hormone (ADH) both stimulate the absorption of water and electrolytes across tight epithelia. The activation is due to a stimulation of adenylate cyclase that increases cellular cAMP. The increase in cAMP stimulates the absorption of Na^+ by increasing the sodium permeability of the apical membrane. In toad urinary bladder and cortical collecting tubule, PGE_2 is able to antagonize the ADH dependent water absorption. In the cortical collecting tubule PGE_2 also inhibits ADH dependent sodium transport. The interaction between ADH and PGE_2 have not been demonstrated previously in the frog skin. These experiments were therefore dedicated to investigate the interactions between AVT (amphibian ADH) and PGE_2.

Methods: Na^+ transport was measured as short circuit current (I_{sc}), cellular potential (V_{sc}) was measured by conventional microelectrodes and cAMP contents was determined by a radio immuno assay.

Results: AVT stimulated the I_{sc} in the upper and lower dorsal, abdominal and thoracic areas of the frog skin, but subsequent addition of PGE_2 inhibited the AVT dependent sodium transport only in the abdomen. Pieces of abdomen was therefore selected for further investigation. AVT stimulated the I_{sc} and depolarized the cells indicating an increased Na^+ permeability of the apical membrane. Subsequent addition of PGE_2 inhibited the I_{sc} and hyperpolarized the cells indicating a decrease in the Na^+ permeability of the apical membrane. Measurements of cAMP production in isolated epithelia revealed that PGE_2 significantly inhibited AVT dependent cAMP production in theophylline treated epithelia.

Conclusion: PGE_2 inhibits the AVT dependent Na^+ transport, by decreasing cAMP contents of the epithelia, thereby lowering apical Na^+ permeability. The inhibition of AVT dependent Na^+ transport in strongly regionalised and only takes place in the abdomen of the skin.

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EFFECT OF Ca^{2+} AND cAMP ON SECRETION FROM EXOCRINE GLANDS OF FROG SKIN.

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The aim of this study was to investigate the relative effects of stimulation by Ca^{2+} and cAMP agonists on Cl^- secretion from the exocrine glands of the frog skin (*Rana esculenta*). We have measured Cl^- net flux by the use of unidirectional tracer fluxes, and $[Ca^{2+}]_i$ with the epifluorescence technique. Addition of carbachol (20 µM) resulted in an increase in the netflux of Cl^- from 0.57 neq cm^{-2} min^{-1} to 4.53 neq cm^{-2} min^{-1}. This Cl^- secretion fell to the prestimulatory level within the first hour of stimulation. The effect of carbachol on $[Ca^{2+}]_i$ was an increase from 102 nM to 270 nM (n=10). The effect on Cl^- netflux and $[Ca^{2+}]_i$ could be abolished by the addition of the muscarinic antagonist atropine (4 µM). Activation with the cAMP agonist PGE_2 (2 µM) gave an increase in Cl^- flux from 0.72 neq cm^{-2} min^{-1} to 8.55 neq cm^{-2} min^{-1}, a level of stimulation which could be sustained. The $[Ca^{2+}]_i$ after stimulation by PGE_2 were not statistically different from the prestimulatory value (P=0.20, n=4).

From the data presented we conclude that activation by carbachol via $[Ca^{2+}]_i$ lead to secretion of relatively short duration compared to stimulation by the cAMP agonist PGE_2, which causes sustained secretion. This is consistent with the fact that stimulation via $[Ca^{2+}]_i$ in the living frog is obtained by pulsed releases of acetylcholine from the periglandular nerves, whereas the cellular cAMP concentration can be raised by both nerve transmitters and by hormones circulating in the frog.

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THE ROLE OF K^+ CHANNELS IN cAMP MEDIATED Cl^- SECRETION IN DISTAL COLON.

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Cl^- secretion in rat and rabbit distal colon proceeds mostly in mid and base crypt cells. We have shown recently that one of the first steps in the up-regulation of Cl^- secretion by cAMP is the increase in the Cl^- conductance of the luminal membrane. The uptake of Cl^- occurs via the loop diuretic sensitive $Na^+2Cl^-K^+$ co-transporter system. The recycling of K^+ across the basolateral membrane occurs via K^+ channels. These K^+ channels are activated by cAMP. We have shown recently that blockage of these channels by the chromanol 293B inhibits Cl^- secretion almost completely, indicating that the basolateral K^+ conductance is required to drive luminal Cl^- secretion. We have also shown that 293B does not affect the K^+ conductance under control conditions. We have further investigated the mechanism of the cAMP-induced up-regulation of the basolateral K^+ conductance in patch-clamp studies in rat colonic crypt base cells. In a first series of experiments (n=12), it was shown that a specific type of channel appears in the basolateral membrane of cell-attached patches when the crypts were stimulated by forskolin. These channels have a conductance of 10 to 20 pS, and their reversal potential is indicative of a K^+ channel. Once activated, these channels were blocked reversibly by 293B (0.1 mmol/l). The inhibition occurred after a delay of approximately 1 to 2 minutes. In a second type of studies, whole-cell patch-clamp experiments were performed, and it was shown that forskolin enhanced whole-cell conductance (G_m) from 10 ± 1 to 18 ± 2 (n=29) nS and that it depolarized the voltage (V_m) from -86 ± 1 to -35 ± 1 mV (n=63). 293B (10 µ mol/l) had no effect in control cells on V_m or G_m (n=7). However, in the presence of forskolin, it depolarized V_m significantly from -42 ± 1 to -33 ± 1 mV (n=22), and it reduced G_m from 14 ± 1 to 10 ± 1 nS. In a third series of experiments (n=7), the forskolin activated K^+ channels were studied in excised inside/out patches. They were highly selective for K^+ over Na^+ and had a conductance of 10 to 20 nS. Upon excision, they showed a run-down in open channel probability. They could be reactivated by adding ATP (1 mmol/l) and catalytic subunits of the proteinkinase A (100 kU/l) to the bath solution (n=5). Once activated in excised patches, the open probability could be reduced by high concentrations of 293B. In conclusion, the present data indicate that cAMP enhances the open probability of a K^+ channel in the basolateral membrane. This K^+ channel is of small conductance type, and it is blocked reversibly by the chromanol 293B. Supported by DFG Gr 480/11.

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ION TRANSPORT AND STRUCTURAL PROPERTIES OF MITOCHONDRIA-RICH CELLS IN TOAD SKIN EPITHELIUM.

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The mitochondria-rich (MR) cell of the amphibian skin epithelium is specialized for uptake of Cl^- against steep concentration gradients and for secretion of H^+. In the present study we have investigated the interrelationship between transport mechanisms for Cl^- and H^+ in the apical membranes of MR cells. Toad skin bathed in Cl^--free mucosal medium were capable of acidifying this solution corresponding to a proton flux (J_H) of 0.83 neq/cm^2/min. In lightly TRIS-buffered toad Ringer (111 mM Cl^-, no CO_2) the apparent J_H was reduced to approximately 0.09 neq/cm^2/min. When CO_2 was removed from the serosal bathing solution proton secretion was practically abolished (J_H decreased from 1.1 to 0.025 neq/cm^2/min). The estimates of the active chloride flux *in vitro* (J_{Cl}=1.2-2 nmol/cm^2/min) corresponds to that obtained *in vivo* (J_{Cl}=0.6-0.9 nmol/cm^2/min (Jørgensen et al., Acta Physiol.Scand. 30:178-190, 1954)). When live animals were exposed to Cl^--free media (distilled water or 100 mM $NaNO_3$) for two days or more the density of MRC is increased, and light-microscopy and keratin immunocytochemistry revealed numerous MR cells characterized by deeply located cell somas and with extensively prolonged neck regions stretching all the way to the apical surface of the stratum granulosum. The recruitment of MR cells was accompanied by a near-proportional increase in passive Cl^- conductance but not with any increase in proton secretion. The passive Cl^- conductance can be activated by increased intracellular [cAMP], by increased mucosal [Cl^-], and by apical membrane depolarization. MR cell could be isolated by a combined collagenase/trypsin treatment and the apical cell membranes patched with patch-clamp micro-pipettes. In cell-attached patches depolarization-activated apical Cl^- channels with unit-conductances of 7-10, 20-40 and 250-300 pS were detected. Supported by DNSRC grants 11-0083 & 11-0971.

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GENETIC INFLUENCE ON THE AGING PROCESSES IN HUMAN BRAIN

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The alteration of the information and energetic processes in the brain with aging were studied in healthy subjects and in the persons genetically predisposed to the Alzheimer's disease (AD). Visual evoked potentials (VEP) and DC-potentials were examined in the groups of healthy persons and the relatives of AD patients of different age (20-80 years). In healthy persons the alterations of information processes appear in 5 decade of life and they are characterised by the delay of VEP components latencies. The intensity of energetic processes progressively decreases beginning from 3 decade of life. In the AD patients relatives the premature delay of VEP components latencies is observed connected with high level of brain energetic expenses. It is supposed that the decrease of energetic supply can cause the alteration of information processes. The genetic factor may affect the velocity of brain aging.

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INTERHEMISPHERIC ASYMMETRY AND NEUROIMMUNE MODULATION IN NORMAL AGING AND ALZHEIMER DISEASE

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In normal aging and dementia of Alzheimer's type (DAT) there is high correlation between interhemispheric differences of amplitude and latency parameters of visual evoked potential (VEP) and immunological characteristics of peripheral blood. Both in normal and pathological aging the people with different functional interhemispheric asymmetry (on base of electrophysiological criteria) have different intensity of neuroimmune interaction. Functional interhemispheric asymmetry (determining using DC-potentials and interhemisperic difference of latency of VEP P3 component) correlates significantly with FGA-induced activity of T-cells. In patients with DAT functional activity of T- and B-cells is decreased especially when the latency of VEP P3 components is shorter in right hemisphere than in left one.

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IDENTIFICATION OF THE Na-Ca EXCHANGE CURRENT IN SECRETORY CELL MEMBRANE

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In order to identify Na-Ca antiport providing lead out Ca from secretory cells, we for the first time by the method of whole-cell voltage clamp and intracellular perfusion have registered the Na-Ca exchange current in the secretory cells membrane of the salivary gland in Chironomus larvae. As a result of the hyperpolarization (from -20 mV) in the presence of physiological Na and Ca gradients arises a slow inward current through membrane of dialysed secretory cells of this gland. The half-time of the current increase due to the membrane hyperpolarization from -20 up to -60 mV is 734 mc. During the time of membrane hyperpolarization the increasing of current amplitude are not found. The current amplitude and direction are determined by the motive force of the Na ion transport ($\Delta E_{Na} = E_m - E_{Na}$). The current amplitude dependence on ΔE_{Na} is described by a multiplicative function. The inward current coresponds to the negative value of the potential fixed ΔE_{Na} and the outward current to the positive value. This current is non-channel because ΔE_{Na} sign change is not followed by the change either in the current direction or in the current amplitud dependence on ΔE_{Na}. Thus, given results testifies that this current was a reflection of the electrogenic nature of Na-Ca exchange.

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DECREASE IN PROSTACYCLIN SENSITIVITY AFTER ALFA-2 ADRENERGIC STIMULATION IN ISOLATED RAT ARTERIOLES. NTP Bakker; N Westerhof; P Sipkema.

We studied the possible interaction between the α2 component of norepinephrine (NE) and the endothelium-derived relaxing substances nitric oxide and prostacyclin (PGI_2) in isolated first order arterioles of the cremaster muscle of Wistar rats.

Vessel segments (avg diameter 232 μm) were mounted in a pressure myograph at 75 mm Hg distending pressure without flow. In the first group (n=4) responses in outer diameter to acetylcholine (ACh, 0.1 μM) and arachidonic acid (AA, 1 μM) were recorded during spontaneous tone (Sp) and after adding NE (0.1 μM) supplemented with prazosin (0.1 μM) and propanolol (5 μM) to block α1 and β receptors. Responses to ACh (% of maximum) were not affected by α2 stimulation with NE: 74 vs 81%. In contast, dilation to AA was abolished: 66 vs -5%. In the second group (n=11) responses to Iloprost (PGI_2 analogue) were recorded during Sp, and α2 (NE or clonidine, Clo) or α1 (phenylephrine, PE) stimulation superimposed. Responses to Iloprost were impaired after α2 stimulation with NE and Clo (see table). In contrast, α1 stimulation did not affect responses to Iloprost.

Iloprost	Sp (n=11)	NE (n=7)	Clo (n=5)	PE (n=3)
10^{-8}	65 ± 10	27 ± 12*	3 ± 3*	61 ± 23
$2*10^{-8}$	85 ± 6	35 ± 11*	21 ± 7*	77 ± 20
$5*10^{-8}$	94 ± 4	76 ± 9	63 ± 14*	98 ± 2

Data expressed as % of maximum dilation ± SE; * significant p≤ 0.05.

It is concluded that the sensitivity to prostacyclin is impaired specifically by α2 adrenergic stimulation in these arterioles.

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INVOLVEMENT OF α_1 AND α_2 ADRENOCEPTOR IN MODULATING VASCULAR TONE VIA ENDOTHELIUM IN GUINEA-PIG THORASIC AORTA. A.Khoshbaten, Norozian, and A.M.Sharifi

Male guinea pigs weighing 400-500 gram were anaesthetised by sodium pentobarbital (i.p). Thorasic aorta was dissected free from surrounding tissue and were cut into rings 5mm long. The rings were suspended at 37°C locke solution under their optimal resting force using two intraluminal parallel wires, one of which was connected to a force transducer. The locke solution was gassed with 95% O2: 5% Co2. Removal of endothelial layer were done either by mechanical rubbing or adding Nw-Nitro-L-arginin (NLA) into the bath.

Epinephrine and phenylephrine induced dose-dependent vasocorstriction. These responses were abolished by phentolamine and prazosin respectively. Clonidine induce relaxation *per se*. Adding prazosin augmented this response but with adding yohimbin relaxation changed to contraction. Removal of endothelial layer virtually reduced the vasodilator effect of clonidine.

These results demonstrate that thorasic aorta of guinea pig contain both α_1 and α_2 adrenoceptors on both smooth muscles and endothelium. It seems α_2 receptors on endothelium are mainly responsible for mediating relaxation.

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CHRONIC HEAD-UP TILTING RESULTS IN CAPILLARY RAREFACTION OF RAT HIND LIMB MUSCLES. E. Monos, G. Dörnyei, and O. Hudlicka+

In earlier experiments we found that long-term head-up tilting induced an immediate steady elevation of the sapheno-femoral venous pressure with a concomittant increase in the passive vein caliber and the venous myogenic reactivity (Am.J.Physiol. 256: H1185-H1191, 1989). This study was aimed at elucidating the role of gravitation induced hemodynamic load in remodelling of the muscle microcirculation. Adult rats were kept tilted head-up at 45° in specially designed tube-like restricting cages for three weeks. Capillary density (CD), capillary/muscle fibre ratio (C/F), fibre density (FD), as well as weights of extremity muscles - tibialis anterior (TA), extensor digitorum longus (EDL), and soleus (SOL) - were compared with those from animals of similar movement restriction but at horizontal position, and from freely moving control animals. Movement restriction caused loss of weight (atrophy) in fast muscles (TA, EDL) and gain of weight in the slow postural SOL of the tilted animals. Neither of these changes were due to alterations in the water content. Capillary supply (CD, C/F) was not affected by movement restriction but was significantly decreased, particularly in the oxidative core region of TA and in SOL of tilted rats (C/F: 1.78±0.11 vs. 2.11±0.05, and 2.06±0.08 vs. 2.29±0.10, respectively). It is supposed that this capillary rarefaction may be the result of a venoarterial reflex causing cessation of flow through some capillaries with their consequent closure and disappearance. (Supported by OTKA 1113-91/94, CO 194/B, and ETT 191/93 grants.)

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THE GLYCOSAMINOGLYCANS LEVEL IN THE THORACIC AORTA OF RABBIT WITH VASOPRESSIN HYPERTENSION AND EXPERIMENTAL HYPERCHOLESTEROLEMIA. J.Drobnik, R.Dąbrowski, A. Szczepanowska, L.Giernat[1]

Atherosclerosis is related to alterations of both pattern and metabolizm of glycosaminoglycans (GAG). The aim of this study was to investigate the effect of two different atherogenic stimuli (vasopressin hypertension and hypercholesterolemia) on GAG fractions in the internal and external part of the thoracic aorta. Forty New Zealand rabbits were divided into four groups. The animals of the first group were injected intravenously with arginine vasopressin in the dose of one U/kg daily over 25 days to induce hypertension. The systolic blood pressure (BP) was elevated from BP_1= 110 mmHg (on the begining of the experiment) to BP_2= 166 mmHg (on the end of the experiment). The rabbits of the second group received food containing 0,25% cholesterol (BP_1=112 mmHg, BP_2=121 mmHg). Animals from 3rd group were injected intravenously with 0,9% NaCl in the dose of 0,1ml/kg; (BP_1 =108,3 mmHg, BP_2=110,3 mmHg). Intact rabbits served as controls (BP_1=108,5 mmHg; BP_2=113,7 mmHg). Measurements of the systolic blood pressure were performed by using noninvasive method. Diet with cholesterol increased both total (up to 617,2mg%) and LDL (up to 378,3mg%) cholesterol level in the blood comparing with the other groups (measured by enzymatic method). After 14 weeks of the experiment rabbits were sacrificed and thoracic aorta was dissected into internal and external parts (controlled under microscope). GAG fractions in each part of the aorta were separated according to the method of Antonopoulos and estimated as uronic acids using the carbasole method of Bitter and Muir. In both internal and external part of the thoracic aortal wall, an elevated level of the total GAG was found in vasopressin treated rabbits ($p<0,05$). Cholesterol diet, however, increased the total GAG content in the internal part of the aorta ($p<0,05$). Augmented heparan sulphate (HS), chondroitin-4-sulphate (CH-4-S) and heparin (H) level has been also shown in the internal part of the thoracic aorta in rabbits received cholesterol ($p<0,05$). On the other hand, in vasopressin treated rabbits, increased content of hialuronic acid (HA), HS, CH-4-S was found in the internal part of the aorta. In the external part, augmentation of chondroitin-6-sulphate (CH-6-S), dermatan sulphate (DS) and H level was observed ($p<0,05$). The results indicate that both atherogenic stimuli vasopressin hypertension and hypercholesterolemia increased GAG content in the thoracic part of the aortal wall. In cholesterol mantained rabbits, the changes localized only in the internal part of the thoracic aorta and concern only HS, CH-4-S and H. The effect of vasopressin hypertension was visible in both internal (HA, CH-4-S, HS) and external part (CH-6-S, DS, H) of thoracic aorta.

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EFFECT OF DEOXYCORTICOSTERONE ACETATE ON BLOOD PRESSURE AND UPTAKE OF LOW DENSITY LIPOPROTEIN BY AORTA OF NORMOTENSIVE RATS

R. Medina[1], L.E. Cardona-Sanclemente[1], G.V.R. Born[1] and M.J. Brown[2]

We have shown that the uptake of low-density lipoprotein (LDL) by arterial walls is accelerated by adrenaline in anaesthetized rabbits, and in conscious , unrestrained normotensive rats. Angiotensin II increases the uptake of LDL and fibrinogen by aortic wall of normotensive rats, while increasing diastolic blood pressure without affecting heart rate. The uptake of LDL or fibrinogen was not increased in spontaneously hypertensive rats in spite of their high blood pressure. Simultaneous administration of Deoxycorticosterone acetate (DOCA) s.c. with 0.9% NaCl drinking water significantly increases blood pressure within 3 weeks. The purpose of this study was to determine the effect of DOCA (25 mg/kg biweekly s.c. for 4 weeks) on LDL uptake in conscious, unrestrained normotensive rats. Blood pressures were measured via intracarotid cannulae. LDL labeled with ^{125}I-tyramine cellobiose was injected i.v. 24 hours before sacrifice and the radioactivity of the aortas was determined. DOCA-salt (n=6) significantly increased blood pressure compared to control (n=6) and vehicle (n=6) groups (151±9 vs 125±5 and 116±3 respectively). DOCA-salt also significantly increased the uptake of labeled LDL by aorta compared to control and vehicle groups (3.6±0.2 vs 2.7±0.1 and 2.7±0.2 ng protein/mg dry weight respectively). Thus, in normotensive rats DOCA-salt increases both blood pressure and LDL uptake, so that this effect is associated with yet another pressor mechanism.

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PHARMACO-PHYSIOLOGICAL PROPERTIES OF HUMAN SPERMATIC AND OVARIAN VEINS.

M.Ch. Michailov, E. Neu, H. Meißner, U. Welscher

We observed species- and organ-specific differences in pharmaco-physiological properties of vascular and non-vascular preparations (*Urol. int.* **38**, 234, 1983; *Canad. J. Physiol. Pharm.* **72**, 164, 1994). In this context we examined applicability of human *v. spermatica* (Vsp) and *v. ovarica* (Vov) (first report in 1978: *6th Int. Biophys. Congr.*, Kyoto, Abstracts, p. 157) as test preparations for the evaluation of the relevance of results from experiments with animal tissue for humans. Motor activity of isolated preparations was recorded isotonically in Krebs-Henseleit solution at 37°C (method: *Strahlenther.* **165**, 860, 1989). Over 50% of Vsp (n=10) and Vov (n=15) showed spontaneous rhythmic contractions (SC) of 5-10% of the initial length with a frequency of 0.5-1.5/min. Acetylcholine (1nM-1 μM) had no effect. Adrenalin (0.01-1 μM), 5-hydroxytryptamine (0.1-1 μM), histamine (1 μM) and prostaglandin $F_{2\alpha}$ (0.3-300 nM) induced a concentration-dependent tonic contraction and an increase of SC as well as of contraction amplitudes (up to 40%) following electrical stimulation (CES). CES with 10 and 100 Hz, 0.1-0.3 ms pulse and 3 s series durations were blocked by tetrodotoxin (0.3-15 μM) and phentolamine (0.1-1 μM), but atropine (up to 1 μM) had no influence, indicating that the reaction was of α-adrenergic neurogenic origin. CES at 10 Hz, 40 ms, 3 s were not influenced by these drugs. Db-cAMP (10 mM) decreased (up to 70%), db-cGMP (1 mM) increased (up to 30%) CES. $NaNO_2$ (1.5 μM) and papaverine (10 μM) induced a strong relaxation and a decrease of CES (and SC). Indomethacine (10 μM) induced a decrease of CES. Short time (5-30 s) cooling (37 to 25, 15, and 5°C) induced a biphasic reaction in Vov: a fast contraction was followed by a large slow tonic contraction after rewarming (from 15 and 5 to 37°C). Vsp reacted only with a single contraction to cooling; at 25°C the adrenalin contraction of Vsp was potentiated. In Vsp 50 kV X-rays (at 5 to 30 Gy, 30 Gy/min) induced immediate contractions, whereas in the highly radiosensitive Vov doses of 0.1 to 5 Gy (at 2.5 Gy/min) were sufficient to induce a contractile response and an increase of SC. Reactions of human *a. ovarica* and *vasa uterinae* to hormones, CES, temperature, and X-rays were similar to those of Vov. We suggest that human spermatic and ovarian veins and other urogenital vessels (surgical material) can be used as test preparations in experimental medicine for the purpose of physiological, pharmacological, radiological and toxicological investigations.

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EFFECTS OF CHRONIC LOW DOSE ANGIOTENSIN II INFUSION ON VASCULAR REACTIVITY IN RATS.

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Angiotensin II (AII) plays a key role in hypertension and congestive heart failure. AII has previously been shown to acutely facilitate sympathetic neurotransmission and to potentiate vascular responses to noradrenaline (NA). However, long term effects of elevated AII levels haven't been studied extensively. We studied vascular reactivity and structure of mesenteric resistance arteries (MrA) in rats that had been infused with 250 ng/kg/min AII by s.c. implanted osmotic minipumps (ANG). After 2 weeks mean arterial pressure was significantly enhanced: 155±6 vs 121±5 mmHg p=.001). MrA were isolated, mounted in a myograph and set at their individual optimal diameter. In sympathectomised MrA, dose response curves (DRC) for NA were constructed, while in MrA with intact nerve endings neuroeffector mechanisms were studied with electrical field stimulation (EFS). Direct potentiating effects of AII were studied by adding AII (30nM) to the organ bath preceding the DRC for NA or EFS. All experiments were performed in the continuous presence of propranolol, indomethacin and L-NMMA. At the end of the experiments, MrA were fixed and processed for histological examination. In the ANG-group, maximal contractile responses to NA and EFS were increased by 36 and 21%. Vasoconstrictor responses to 30 nM AII were not altered. Adding AII to the organ bath increased sensitivity for NA or EFS only at low concentrations/frequenties with no changes in maximal responses. Cross sectional area, media thickness and wall/lumen ratio were increased by 38, 36 and 31% resp.. This indicates that increased maximal contractile responses resulted from an increase in vascular wall mass, since maximal active wall stress was not altered. Thus, chronic elevation of circulating levels of AII resulted in hyperreactivity but not hypersensitivity of resistance arteries. The hyperreactivity seems to be entirely due to wall hyperthrophy.

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MEMBRANE POTENTIAL RESPONSES TO ENDOTHELIUM-DEPENDENT AND -INDEPENDENT VASODILATORS IN RAT AORTA AND MESENTERIC ARTERIES.

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The membrane potential (E_m) of vascular smooth muscle cells importantly determines the tone of the vessel. In the present experiments, the resting E_m and its response to the endothelium-dependent vasodilator acetylcholine (ACh) was compared in the aorta and the main and smaller (first or second branch) mesenteric artery (MMA and SMA, respectively) isolated from rats. E_m was measured with conventional microelectrode techniques in strips of the aorta or MMA, and in ring segments of SMA. In aorta and MMA, resting E_m was -51.9 ± 0.6 and -53.1 ± 0.6 mV respectively. In SMA, resting E_m was more negative (-59.7 ± 0.6 mV) and a slow hyperpolarization with time (hours) was observed. Values obtained in the 10th microelectrode impalement in the same preparation averaged -65.0 ± 3.6 mV. In aorta, ACh produced an endothelium-dependent hyperpolarization consisting of an initial peak E_m change of 12.5 ± 0.8 or 8.8 ± 1.4 mV (10^{-5} and 10^{-6} M respectively), followed by a partial recovery to an E_m-value significantly more negative than in the absence of the agonist. In MMA, 10^{-6} M ACh produced larger hyperpolarization (16.4 ± 1.1 mV), while in SMA E_m changes were comparable to those in aorta (-11.9 ± 1.0 and -6.9 ± 0.8 mV with 10^{-5} and 10^{-6} M ACh respectively). In some SMA, a small depolarization was observed during the maintained phase of the ACh response. Glibenclamide (GLI, 10^{-5} M), an inhibitor of ATP-regulated K^+ channels, had no significant effect on the resting E_m in aorta. It slightly depolarized MMA (1.1 ± 0.4 mV), while depolarization was significantly larger in SMA (6.6 ± 0.5 mV). GLI had no significant effect on ACh-induced hyperpolarization in aorta and MMA. In SMA, the response to ACh was increased after (depolarization with) GLI. In all preparations, nitroglycerin (10^{-5} M) caused a small hyperpolarization of E_m. In SMA, this response was more transient and sometimes followed by a small maintained depolarization. It is concluded that (1) K_{ATP} channels play a role in maintaining resting E_m at a relatively hyperpolarized level in SMA but not in MMA and aorta and (2) in all preparations, ACh induced hyperpolarization consists of an initial peak E_m change followed by a more slowly developing second component. In SMA this latter component can sometimes be a maintained depolarization, similar to caused by exogenous NO in these preparations.

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EFFECTS OF NO-SYNTHASE INHIBITION ON THE MICRO-CIRCULATORY EFFECTS OF TRH. L.-O. D. Koskinen and M. Koch

Several vasodilators elicit their effects by an endothelium dependent release of nitric oxide (NO). The peptide TRH affects the regional blood flows in a complex pattern. We have studied the effects of L-NAME on the microcirculation and the interaction with the effects of TRH. Anaesthetised male Sprague-Dawley rats were artificially ventilated. Blood pressure (MAP), arterial blood gases and pH were monitored. Regional blood flows were measured by microspheres. After a control blood flow measurement L-NAME (30 mg/kg) was given i.v. in order to inhibit the NO-production. Blood flow was measured 25 minutes later. Thereafter 0.3(L) or 3 mg/kg (H) TRH was given i.v.. Five minutes later the third blood flow measurement was performed. Control animals received only TRH. Blood gases and pH were normal during the experiments. TRH(L) elicited a 19±8% ($p<0.05$) increase in total cerebral blood flow (CBF_{tot}) and TRH(H) produced a vasodilation by 98±28% ($p<0.02$). The corresponding effects in the hemispheres (CBF_{hsf}) were 18±7% ($p<0.05$) and 103±30% ($p<0.02$) respectively. L-NAME induced an increase in MAP and a decrease in CBF_{tot} by 35±6% ($p<0.001$). TRH(H) normalised the CBF_{tot} but did not elevate the blood flow over the control level. CBF_{hsf} was affected in a similar way. No significant cerebrovascular effect of TRH(L) was observed after L-NAME treatment. The vascular resistance in the heart decreased from 5.05±0.80 VRU to 4.31±1.22 by TRH(L) and to 2.74±0.45 ($p<0.005$) by TRH(H). L-NAME increased the vascular resistance in the myocardium from 4.81±0.73 VRU to 8.05±1.25 VRU ($p<0.005$) and TRH(H) normalised this effect (5.01±0.51 VRU). L-NAME produced vasoconstriction in several peripheral tissues and TRH had a complex pattern of effects on this vasoconstriction. The results indicate involvement of NO in the regulation of regional blood flows. The effect of the neuropeptide TRH is affected in a complex pattern suggesting several underlying mechanisms.

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LEUKOCYTE-MEDIATED ENDOTHELIAL CELL DYSFUNCTION. V. Smieško, F. Kristek and A. Holécyová

Surgical isolation of blood vessels in vivo results in trauma of the vessel wall. This, probably, alter neutrophil-endothelial interaction which is supposed to be a cause of endothelial cell dysfunction. To test the hypothesis, in 8 dogs anesthetized with sodium thiopental, two different endothelium -dependent dilatory responses were studied 3 and 7 hours after the surgery of the femoral artery (FA). FA diameter was monitored by the inductive transducer and FA blood flow was monitored by the electromagnetic flowmeter. As dilatory stimuli, an increase in blood flow through the FA (by opening of arteriovenous shunt) and a bolus i.a. injection of acetylcholine were used. The results indicate no change in the acetylcholine-induced dilation between the two time intervals. However, significant decrease in flow-induced dilation to 42.3 ± 14.1 % was observed 7 hours after the surgery as compared with dilation after 3 hours. In both time intervals samples of the FA were taken for transmission electron microscopy. Micrographs showed an increase in neutrophil number in subendothelial space: 3 hours after the surgery there were none or some of them, after 7 hours neutrophils created even clusters or continual layers, thus push away endothelium from smooth muscle layer. The data support the hypothesis that neutrophil accumulation in subendothelial space in response to surgery may induce endothelial cell dysfunction, manifested by the observed dissociation of flow- and acetylcholine-induced vasodilation.

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Ca^{2+}-DEPENDENT POTASSIUM AND NONSELECTIVE CATION CHANNELS ACTIVATED BY BRADYKININ ON CORONARY ARTERY ENDOTHELIAL CELLS. A. Baron, M. Frieden, F. Chabaud & J.-L. Bény.

Bradykinin (BK) induces endothelium-dependent relaxation of pig coronary artery. We have previously shown that endothelial cell activation by BK resulted in a transient hyperpolarization approaching the K^+ equilibrium potential. Using the patch-clamp technique, we studied the ionic channels activated by BK on cultured endothelial cells to further understand electrophysiological events underlying cellular activation. In "cell-attached" mode, BK (94 nM) activates two types of Ca^{2+}-dependent channels : a large conductance K^+ channel (285 ± 12 pS, high sym. K^+) whose open state probability is increased by membrane depolarization, and a lower conductance inwardly rectifying nonselective cation channel (43.7 ± 0.8 pS , high sym. K^+). Divalent cations such as Ca^{2+} can flow through this cation channel, with nearly the same permeability than monovalent cations ($P_K : P_{Na} : P_{Ca} = 1 : 1 : 0.7$). The cation channel openings duration is more than doubled in presence of BK. This effect is delayed compared to the increase in the channel open state probability and rapidly lost in "inside-out" configuration. Caffeine, releasing Ca^{2+} from endoplasmic reticulum, also activates the cation channel but more transiently than BK and without any effect on the channel mean open time. Both K^+ and cation channels are activated by a rise in intracellular Ca^{2+} in which both Ca^{2+} release from endoplasmic reticulum and Ca^{2+} entry are thought to be involved. On primary cultured pig coronary endothelial cells, BK induces a rapid rise in cytosolic Ca^{2+} level, measured with Fura-2, generally maintained during BK application. If the role of Ca^{2+} in activation of NO-synthase and release of relaxing mediators is well documented, the role of endothelial cells hyperpolarization is quite unclear. If the involvement of the K^+ current in endothelial cell hyperpolarization seems obvious, the role of the cation current is more difficult to assess. Ca^{2+} entry via cation channels could contribute to activate Ca^{2+}-dependent K^+ channels, but the effect of membrane potential variations on K^+ channels activity, as well as the depolarizing effect of cation entry must also be taken into account to further understand the involvement of such channels in electrophysiological events induced by BK on endothelial cells.

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IONIC CURRENTS ACTIVATED BY PROSTAGLANDINS E IN SMOOTH MUSCLE. S.Zakharenko and V.Serebryakov

The effects of prostaglandins E (PGE) on ionic currents, membrane potential (E_m) and intracellular free calcium concentration ($[Ca^{2+}]_{in}$) in rat aortic smooth muscle cells (SMC) were investigated using patch-clamp technique (whole-cell configuration), microelectrode method and fura-2 spectrofluorimetry. PGE_1 and PGE_2 (100 nM) increased the amplitude of voltage-dependent outward currents at positive potentials and induced inward currents at negative potentials in freshly isolated SMC. Outward currents activated by PGEs were blocked with external Ba^{2+} and internal Cs^+. PGE_1 and PGE_2 induced depolarization of the SMC membrane in rat aortic strips (see the table below):

SOLUTION	E_{mr}, mV	$\frac{Em(PGE_1)*100-100^*}{E_{mr}}$	$\frac{Em(PGE_2)*100-100^*}{E_{mr}}$
normal	-46±3	16.5±1.5 %	21.7±1.6 %
Ca-free	-47±3	18.5±1.3 %	21.3±1.9 %
Ca,Na-free	-56±2	10.7±1.3 %**	12.5±1.5 %**

* E_{mr} - resting E_m; $E_m(PGE)$ - PGE-induced E_m

** $p<0.05$ vs. PGE effect in the normal solution

Niflumic acid (100 μM), a blocker of chloride permeability, decreased PGE-induced depolarization by 60 % in the normal physiological solution and eliminated PGE-induced depolarization in the Ca,Na-free solution. PGE_1 and PGE_2 increased $[Ca^{2+}]_{in}$ in aortic strips and single aortic SMC in the normal and Ca^{2+}-free external solutions. These results suggest that PGEs enhance the K^+ outward current presumably through Ca^{2+}-dependent K^+ channels. The PGE-induced inward current and consequent depolarization of the SMC membrane assumed to be mediated through Ca^{2+}-dependent Cl^- and non-specific cationic channels.

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EFFECT OF REDUCED GLUTATHIONE ON LONG-TERM PRESERVATION OF ENDOTHELIAL FUNCTION IN ISOLATED CORONARY ARTERIES. E.Kevelaitis*, N.C.B.Nyborg, Ph. Menasché

The aim of this study was to test the direct effect of reduced glutathione (GSH) on vascular smooth muscle reactivity and endothelial function of isolated coronary arteries (CA) subjected to long-term cold storage. Artery segments (length 2 mm) were taken from proximal (diameter 552 ± 37 μm) and distal (diameter 281 ± 17 μm) part of left anterior descending CA of rats. Ring preparations were mounted and stretched on the isometric wire myograph to their optimal lumen diameter (90% L_{100}). Serotonine (10^{-9} - 10^{-4} M) and phenylephrine (10^{-8} - 10^{-4}) were used to assess vascular smooth muscle contractile activity. Endothelium-independent and -dependent relaxation to isoprenaline (10^{-9} - 10^{-5} M) and acetylcholine (10^{-8} - 10^{-4} M), respectively, were tested using precontraction of CA with $PGF_{2\alpha}$ (10^{-5} M). After control measurements, the segments of CA were stored at +4°C for 15 hours either in saline (group1), or in a new heart preservation solution, Celsior®, (group 2), or in Celsior® solution supplemented with 3 mM of GSH (group 3). Following 15 hour of cold storage, there were no significant differences in maximal contractile response and endothelium-independent relaxation in all groups, as compared with control values. The endothelium-dependent relaxation was reduced in both proximal and distal segments of CA stored in GSH-free Celsior® solution (group 2). However, addition of GSH reversed this impairment and completely preserved endothelial function of isolated CA after cold storage. In conclusion, cold storage preserves the ability of the vascular smooth muscle to contract and relax. GSH improves preservation of endothelial function of CA after an extended period of cold storage in Celsior® solution used during heart transplantation procedure as a storage medium and perfusion fluid.

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THE EFFECT OF ADAPTATION TO EXERCISE ON ENDOTHELIUM-DEPENDENT RESPONSES AND NITRIC OXIDE PRODUCTION IN THE RAT. E.B.Manukhina, A.V.Lapshin, F.Z.Meerson, V.D.Mikoyan, L.N.Kubrina, and A.F.Vanin

Adaptation to exercise (AE) is known to play an important role in prevention and treatment of cardiovascular disorders (Meerson, Pshennikova, 1988). Both at the systemic and at the local level AE reduces sympathetic tone, attenuates pressor responses, and suppresses vascular sensitivity to adrenergic agents (Wiegman et al., 1981; Jennings et al., 1984). However the contribution of endothelial nitric oxide (NO) to adaptive protection of the organism, remains controversial (Endo et al., 1994; Gilligan et al., 1994). The aim of the present study was to investigate the effect of AE on endothelium- mediated constrictor and dilatory responses of isolated rat aorta and on NO production in the organism. Wistar male rats were adapted to exercise by swimming for 1 hour daily, for 36 days. The tension of isolated rat aortic rings was measured by an isometric force transducer. The NO content was assayed by electron paramagnetic resonance in the heart, liver, intestine, kidneys and spleen. After AE, the contractile response of aorta to norepinephrine (NE) was decreased as compared to the control in the presence [ED_{50}(x10^{-8} M) 2.76±0.20 vs. 3.88±0.44 respectively, $p < 0.05$] but not in absence of the endothelium [ED_{50}(x10^{-8} M) 1.71 E+-16 vs. 1.58±0.12 respectively]. The endothelium-dependent relaxation of NE-preconstricted aortic rings induced by acetylcholine was greater by 27% ($p < 0.05$) in the aorta from adapted rats than in control. In addition, AE significantly potentiated the NO production in all the organs studied by 3-10 times. We suggest that AE may prevent excessive vasoconstriction characteristic of many cardiovascular diseases by enhancing the modulating effect of endothelium on vascular tone.

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INFLUENCE OF PERIVESSEL ENDOTHELIZATION ON VASCULAR WALL FUNCTION

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The endothelial denudation and myointimal hyperplasia are the causes of early and late complications, when autogenous vein is using for popliteal or tibial bypass operations. The modern reseach showed the leading role of endothelial cells (EC) in vessels pathology. In this reseach we studied the influence of perivessel endothelzation (PE) on smooth muscle tone after vascular wall deendothelization. The experiments were carried out on 3 cm-long segment of human saphenous vien, harvested at the proxymal part. Endothelial function was assessed in an organ bath where the studied vessel was submaximally contracted with a predetermined dose of an agonist (noradrenaline) and then exposed in en endothelial dependent relaxant (acetylcholine). This experiment was repeated with an endothelial independent relaxant (sodium nitroprusside). The acetylcholine reaction on vascular wall after denudation was absent and restored its function with autogenous culture of EC aplicated perivessel. This reaction of vasodilatation was inhibited by methylene blue and oxyhemoglobin. Methylene blue also prevented relaxation, caused by sodium nitroprusside. Thus, we can presume, that the PE of EC, which release endothelial factors of vessel relaxation, takes part in vascular wall vasomotion reactions.

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ELECTROPHYSIOLOGICAL MODIFICATIONS INDUCED BY MYOCARDIAL STRETCH IN DIFFERENT ANIMAL SPECIES. M.Sabău, Gh.Bărbat, D.Dobreanu, Adriana Habor, S.Rață

1.Objective: To determine how myocardial stretch modulates the electrophysiological properties of the heart by the mechanoelectrical feedback in isolated preparations in different animal species. **2.Methods:** Frog ventricular strips and papillary muscles obtained from rats, guinea pigs, hamsters and rabbits (n=10) stimulated at a rate of 12/min were used. Resting and action potentials were recorded using standard microelecrode technique, simultaneously with isometric mechanograms at Lo and Lmax. The stretch at the Lmax was obtained gradually. **3.Results:** In all cases a small reduction of membrane potentials was observed with no significant modifications of the amplitude of action potentials. Their duration is slightly decreased, except the guinea pig where an increase of the duration was noticed. In 8% of all cases pacemaker activity appeared after stretch, the incidence of such activity being not influenced by high Ca^{2+} concentrations /5 mM/. In whole in situ frog, rabbit or dog hearts the acute volume or pressure overloading can induce occasionally arrhythmia or normalization of a preexisting one. The role of mechanoelectrical feedback in the initiation of such arrhythmias is possible, their incidence rising as the amount of stretch increases. The ionic channels activated by stretch or tension increase within the sarcolema might explain mechanoelectrical feedback effects. The intracellular Ca^{2+} concentration may be influenced by length variations via Ca^{2+} influx or Na^{+}-Ca^{2+} exchange, participating in the initiation of stretch induced arrhythmias. **4.Conclusions:** In all species studied it is difficult to explain or to predict the apparition of an arrhythmia, which depends on the complex conditions and the amount of stretch in a certain case.

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THE VARIATION OF THE ACTION POTENTIAL DURATION OF THE MYOCARDIAL FIBER UNDER LINEAR VARIABLE FREQUENCY STIMULATION - C. Stanciu, Madalina Stanciu

Previous studies showed the action potential duration (APD) of the ventricular myocardial fiber depends on the stimulation frequency (SF). This relation was demonstrated only by using constant frequencies. There are also known a lot of biochemical and structural changes of the myocardial fiber at a stimulus application. When the frequency is constant, only the first stimulus can modify the initial state, the followings coming in a different state (recovering state after the first stimulus), but the same for all the secondary stimuli. The analysis of these effects obtained when each stimulus acts in a different state of myocardial fiber is more reliable for understanding the relation between APD and SF. Our study examines this relation using an original experimental model. The frequency varies constantly, so each stimulus is in fact like a "first stimulus" for a certain SF and it is applied after a free interval which is progressively shortened. The results show that APD is not in a linear dependency on SF, but is almost constant even for relative high frequencies. We have also noticed some sporadic action potentials which are longer than the others, despite the linear increase of the SF. Our data are in opposition with the hypothesis of changes in gating-channels mechanisms which could influence the relation APD - SF, and also with that of exclusive origin in interstitial fluid of Ca++ ions implied in phase 2 of the action potential. It is proposed another hypothesis which is able to explain even the Ca++ channels blockers action.

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SEVERE ARRHYTHMIAS IN CHRONIC RESPIRATORY FAILURE AT SEA LEVEL OVER 1,000 M - PRELIMINARY RESULTS

Š. Kujaník, M. Sninčák, M. Mayer, J. Vokáľ, E. Zubajová, F. Vlasatá.

In a multicenter study (at the sea level 1150- 1350 m) the occurence of severe arrhythmias [ventricular tachycardia (VTACH), run of ventricular extrasystoles (VEBR), pairs of extrasystoles (VEBP), and bigeminy (BIG)] was investigated by Holter ECG monitoring during 24 hours in 43 men over 50 years with respiratory failure (RF) and in the control group (CG) of 9 cardiopulmonary healthy men. Except of single supraventricular or ventricular extrasystoles (ES) other arrhythmias were not present in CG. In RF the summary occurence of severe arrhythmias was the following: ventricular fibrillation was not present, VTACH 20 times ($P<0.001$ compared to CG), VEBR 34 times ($P<0.001$), VEBP 255 times ($P<0.001$), BIG 54 times ($P<0.001$). Our results show that cardiac arrhythmias are not rare in RF in higher decennia at the middle high altitude. Except of numerous single supraventricular and ventricular ES also severe arrhythmias including R on T are present, most frequently VEBP. It is assumed that the cause of higher occurence of those arrhythmias is the increased adrenergic activation of the heart elicited by hypoxia (decrease in the partial pressure of oxygen in the inspired air by more than 20 mmHg). A stay of patients with RF in the middle high altitude may be dangerous under some conditions.

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THE VENTRICULAR FIBRILLATION THRESHOLD AND HEART RATE AT THE VENTILATORY CHANGES IN RATS. CHRONOPHYSIOLOGICAL STUDY.

P.Švorc, I.Podlubný, Š.Kujaník, I.Bračoková, F.Vlasatá.

The circadian rhythms of the ventricular fibrillation threshold (VFT) and the heart rate (HR) were investigated in female WISTAR rats (adaptation to daily light - dark cycle 12:12 hours with dark period from 6.00 p.m to 6.00 a.m.; pentobarbital anaesthesia 40 mg/kg i.p.; open chest experiments) under conditions of the ventilatory change from the initial normoventilation (NV) to the subsequent hypoventilation (HV) (1.exp.group; $n = 6$) and on the contrary (2.exp. group; $n = 4$). During NV (1.exp. group) VFT and HR showed the circadian rhythms with the higher values in dark (VFT - 2.26 dark vs. 1.75 mA light; HR - 353 dark vs. 340 beats/min. light). In this time, the values of VFT were the smallest during NV after HV (2.exp. group) (1.82 light vs. 1.08 mA dark) and the circadian rhythm had the opposite course. HR was not changed (348 light vs. 346 beats/min. dark). HV significantly decreased ($\alpha = 0.001$) VFT and HR and the circadian course of VFT showed the double-peak character with the first smaller one between 15^{00} - 18^{00} h. and the second higher one between 24^{00}- 3^{00} h. in the both groups. The values of VFT were indepedent on HR and the circadian rhythms of HR were not corresponding with rhythms of VFT in the both groups. It is concluded, that HV decreases VFT and HR during the whole 24 - hour period, where circadian course of VFT is changed and acquires the double - peak character in female WISTAR rats. The circadian rhythm of VFT acquires the reverse course after the return from hypo- to normooxic conditions. VFT is probably independent on changes of HR during both types of ventilation.

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PROPERTIES OF GAP JUNCTION CHANNELS IN ADULT RABBIT ATRIUM AND VENTRICLE; S. Verheule, M.M.P. Hermans, B.R. Kwak and H.J. Jongsma.

Electrical coupling between heart cells is mediated by gap junction channels. These channels are made up of two hexamers of connexin (Cx) proteins, with each cell contributing one hexamer to form a complete channel. The mammalian Cx-gene family has at least 12 members. Gap junction channels consisting of different Cx's have different electrical properties (single channel conductance, open probability and voltage dependence) and have differential sensitivity to second messenger modulation. In the heart, local conduction properties vary as a result of differences in tissue organisation and possibly, differences in Cx expression.

Myocytes were isolated from either the atria or the ventricles of the hearts of adult rabbits by enzymatic dissociation. We used the double whole cell voltage clamp method on cell pairs to study the electrical properties of gap junctions in these tissues. A continuous transjunctional potential difference was applied and junctional current was measured to determine junctional conductance. Macroscopic conductance did not differ significantly between ventricular and atrial cell pairs (mean±s.d.: 46.2±28.1 and 53.6±9.2 nS, respectively). The junctional conductance could be reduced with the uncouplers heptanol or halothane. Just before total uncoupling, openings and closing of single gap junction channels could be resolved. Amplitude histograms of these transitions from ventricular cell pairs showed a single population with a mean conductance of around 100 pS (CsCl as charge carrier), irrespective of transjunctional voltage. However, amplitude histograms from atrial cell pairs show a major peak at 200 pS at a driving force of 25 mV. With increasing transjunctional voltage the incidence of this conductance was dimished, but another population with a conductance of 100 pS could be resolved. When a steplike increase in transjunctional potential difference was applied, the larger channel opened and closed preferentially at the beginning of the step. The behavior of the larger channel observed between atrial cells is in agreement with the properties which have been described for Cx40. The smaller atrial channel and the ventricular channel correspond with the less voltage dependent Cx43 channel.

In order to confirm this hypothesis, we used the polymerase chain reaction technique to detect Cx40 and Cx43 mRNA in myocytes isolated from either the ventricles or the atria. Both Cx40 and Cx43 mRNA's were present in atrial myocytes, but only Cx43 mRNA could be detected in ventricular myocytes. The observed difference in connexin expression might be implicated in differential modulation of ventricular and atrial conduction.

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THE ROLE OF HISTIDINES ON pH SENSITIVITY OF CONNEXINS 43 AND 45. M.M.P. Hermans, P. Kortekaas, H.J. Jongsma, and M.B. Rook

Protonation of histidine (H) residues in the cytoplasmic loop of gap junctional proteins (connexins) has been proposed to play an important role in pH regulation of junctional permeability in heart and other tissues. The major cardiac connexin is connexin43 (Cx43). Its cytoplasmic loop contains three H-residues on positions 95, 126 and 142. Cx45, another connexin reported to be expressed at low levels in working myocardium, has four H-residues at positions 97, 104, 124 and 162. To study the role of the number and the position of histidines on pH gating, H126 or H142 in Cx43 cDNA were substituted by glutamine (Q) via site-directed mutagenesis. Wild-type Cx43 and mutant (Cx43-H126Q and Cx43-H142Q) cDNA's were stably transfected into coupling-deficient SkHep1 cells, which express low levels of endogenous Cx45. Junctional conductance (g_j) between cell pairs was measured using the dual voltage clamp technique. pH_i was manipulated with the NH_3/NH_4 pH-clamp technique (Grinstein et al., *Am.J.Physiol.* 267: C1152, 1994). Under normal conditions (pH_i=7.0), g_j in cell pairs transfected with either wild-type or mutant Cx43 cDNA's was substantially higher (up to 50 nS) than in parental cells (up to 3 nS). At a pH_i of 6.3, g_j in cells expressing wild-type Cx43 gap junctions was reduced by 55% of control values, in Cx43-H126Q cells this reduction was only 25%, while in Cx43-H142Q cells g_j dropped to virtually zero. In untransfected cells (Cx45) g_j was also reduced to zero. At pH_i 5.8, all cell types became completely uncoupled (g_j=0). Any pH-induced uncoupling always was reversible upon returning to pH_i 7.0. At normal pH_i, g_j could be reversibly blocked by exposure to halothane in all cell types and at g_j levels approaching zero, single gap junction channel events could be recorded. Single channel conductances of Cx43 wild-type and mutant channels were similar: ~ 40 pS. Cx45 channel conductance in untransfected cells was ~20 pS. Single channel events could also be measured in all cell types prior to complete uncoupling caused by reduction of pH_i. Their conductance was not markedly different from the values found at normal pH_i. From our data we conclude that: 1) Cx45 is far more pH sensitive than Cx43. 2) The position rather than the total number of histidine residues in the cytoplasmic loop determines the pH sensitivity of Cx43. 3) pH-induced uncoupling results in a reduction of gap junction channel open probability but not in channel conductance.

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MEASUREMENT OF THE ATRIAL REFRACTORY PERIOD AND THE WIDTH OF THE EXCITABLE GAP DURING SUSTAINED ATRIAL FIBRILLATION IN THE GOAT. *M.J.P. Killian, F. Mast, M.C.E.F. Wijffels and M.A. Allessie*

In healthy goats, instrumented with atrial mapping electrodes, electrically induced atrial fibrillation (AF) lasts for only several seconds. We found in most of these goats that sustained AF (>24h) will develop when AF is continuously electrically reinduced. It appeared that in chronically fibrillating goats the fibrillatory process could be regionally entrained by rapid pacing, indicating the presence of an excitable gap. The objective of this study was to measure the refractory period (RP) and estimate the width of the excitable gap during sustained AF. A bipolar stimulator-amplifier (Medtronic SP3111) was used to monitor both spontaneous and stimulated local atrial activity at the stimulation site. Electrode polarization caused by stimulation was reduced by feeding back a controlled voltage function. Spontaneous atrial activations at the stimulation site were sensed by detection of peaks in the bipolar electrogram. These senses were used to schedule premature stimuli. Peak detection was set critically to avoid false senses. After 10-20 senses a 4 times threshold premature stimulus was given. The local atrial RP was defined as the shortest coupling interval evoking a premature response in at least 2 out of 4 observations. On line criteria used for determination of capture: 1. absence of spontaneous AF wave within the expected AF interval following the premature stimulus; 2. electrograms obtained at and close to the stimulation site show a response within 10-20 ms after the stimulus; 3. at the stimulation site there is mean ratio above 1 of: [interval between capture response and return AF wave] / [interval between previous AF wave and capture response]. Procedures were validated off line by examination of atrial activation maps. A radial activation pattern around the stimulation site was taken as evidence for capture. We have found RPs as short as 57 ms, corresponding to excitable gaps of up to 40 % of the AF interval.

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DISRUPTION OF CARDIOMYOCYTE COHESION IN CHRONIC FIBRILLATING ATRIA OF THE GOAT. J. Ausma, M. Wijffels, F. Ramaekers, M. Allessie, M. Borgers.

In chronically instrumented goats chronic atrial fibrillation (AF) was induced for 2-3 months by electrical pacing. The large majority of atrial cardiomyocytes showed marked ultrastructural changes. The main characteristics were the replacement of myofibrils by glycogen, shape changes of mitochondria, fragmentation of the sarcoplasmic reticulum and dispersion of the nuclear chromatin. The changes were not degenerative in nature but were interpreted as phenotypic adaptations to the embryonic/fetal state. This "dedifferentiation" hypothesis was supported by immunocytochemical localalization of early development markers. The number of cells and the degree of cellular dedifferentiation appeared to correlate, at least in part, with a simultaneous increase in atrial pressure. This led us suppose that next to contractile unloading (fibrillation), stretch of the cardiomyocytes (elevated pressure) might at the base of the observed adaptative structural changes. To further substantiate this hypothesis we investigated the intercellular cohesion of the cardiomyocytes. With immunofluorescence we saw that the anchoring of the intermediate filament protein desmin to the desmosomes was detached. Other desmosomal proteins such as desmoplakin and desmoglein were still present. However, these desmosomal proteins appear clustered together as large dots at the border of the cardiomyocytes. Many of these cells had lost contacts with neighbouring cells. At present it is not clear whether the loss of intracellular contacts is causely related or merely the consequence of the "dedifferentiation" events.

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ACTION OF TEDISAMIL ON RABBIT SA-NODE

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Tedisamil® is a well known class III antiaritmic agent that prolongs refractory period by prolongation of the action potential duration by a combined blockade of the transient outward and delayed rectifying potassium currents (1). In addition the drug has a remarkable bradycardic effect. We undertook the present study to clarify the mechanism of the depressant action on the activity of the SA node. Experiments were done in the SA node of the isolated rabbit right atrium and in isolated nodal cells. In the atria the electrical activity of nodal fibres was recorded using classical microelectrodes; in the isolated nodal cells voltage clamp was applied through both whole cell and perforated patch methods. Tedisamil was added in concentrations of 5 and 10 μM. In isolated atria Tedisamil increased cycle length respectively with 16% (N=5, SD ±12) and 26% (N=5, SD ±6). The main cause was an increase of the duration of the action potential with about 50% in both primary and secondary pacemaker fibres at 5 μM. Also diastolic depolarization rate was significantly depressed, in primary pacemakers more (65% in the paced preparation, N=23, SEM ± 7) than in secondary pacemakers (21%, N=43, SEM ±6). In most cells also the maximal upstroke velocity was depressed significantly.

Voltage clamp on isolated nodal cells demonstrated conclusively a reduction of the total outward current that was activated upon depolarizing voltage steps and a strong reduction of the tail currents after stepping back to the holding potential. This makes a strong depression of the delayed rectifier potassium current very likely. In most cells also a depression of the high threshold calcium current ($I_{Ca,L}$) was found, but spontaneous run down of this current could not be excluded sufficiently. No effect was observed on I_f. We conclude that the bradycardic action of Tedisamil is primarily caused by a reduc-tion of I_K, with a possible contribution of a depression of $I_{Ca,L}$.

1. Dukes ID, Morad M. The mode of action of Tedisamil on voltage dependent K+ channels. Cardiovasc Drugs Ther 1992;6:321-327.

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CHARACTERISTICS OF UNIPOLAR ATRIAL ELECTROGRAMS DURING ELECTRICALLY INDUCED ATRIAL FIBRILLATION IN MAN. KTS Konings, CHJH Kirchhof, JRLM Smeets, HJJ Wellens, OC Penn, MA Allessie.

Atrial fibrillation (AF) was induced by rapid pacing in 25 WPW patients undergoing open-chest surgery to interrupt their accessory pathway(s). The right atrial free wall (RAFW) was mapped with a regular array of 244 unipolar electrodes (interelectrode distance 2.25 mm). All potentials in the unipolar electrograms during 4 seconds of AF were classified either as: Singles, Short Doubles (2 negative deflections <10 ms apart), Long Doubles (2 deflections 10-50 ms apart), and Fragmented (multiple deflections). On the basis of the activation patterns 3 types of AF were defined. In type I, single wavelets propagate fast and almost uniformly across the RAFW. In type II, 1 or 2 wavelets conduct irregularly, and in type III activation of the RAFW is highly fragmented by 3 or more wavelets. Singles (77±12%) were almost exclusively associated with fast and uniform conduction (positive predictive value (PPV) =0.96). Short doubles (7±3%) were frequently found in areas where two wavefronts collided (PPV=0.33) whereas long doubles (10±7%) were associated with arcs of functional conduction block (PPV=0.84). Fragmented potentials (6±4%) were recorded both at pivoting points around an arc of block and in areas of very slow conduction (<7.5 cm/s) (PPV=0.87). Going from type I to type III AF the incidence of long doubles (4±2%, 12±3% and 18±7% respectively) as well as fragmented potentials (2±2%, 6±3% and 10±4%) increased ($p<0.05$). Specific areas with a high incidence (>5%) of double or fragmented potentials were not found. *Conclusions*: The morphology of unipolar electrograms during AF reflects specific conduction disturbances which may be used to distinguish dysfunctional areas and different types of AF in man.

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EFFECTIVENESS OF CIBENZOLINE VERSUS FLECAINIDE IN TERMINATING SUSTAINED ATRIAL FIBRILLATION IN GOATS.

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Recently we developed a new model of Sustained Atrial Fibrillation (SAF) in chronically instrumented conscious goats. While in normal goats electrically induced AF usually terminates spontaneously within less than 5 seconds, continuous maintenance of AF resulted in a progressive prolongation of AF episodes and sustained AF (>24h) within 1-3 weeks. In the present study we determined the effectiveness and safety of two class Ic drugs, Flecainide (F) (n=4) and Cibenzoline (C) (n=9) on the fibrillation interval (AFCL) and termination of SAF. To evaluate the effects on the AV-node and the ventricle the RR-interval and width of the QRS-complex were measured. Both drugs were given as a continuous infusion (0.1-0.2 mg/kg/min) until one of the following end points was reached: 1) Sinus rhythm, 2) widening of the QRS-complex of >70-100% and 3) Occurrence of multiple ventricular extra-systoles or tachycardia. **Results**: F prolonged the AFCL from 92±5 to 139±25 ms (52%) and C from 95±10 to 175±33 ms (85%) ($p<0.08$). The RR-interval during SAF was not affected by both drugs. While both drugs increased the width of the QRS-complex to a similar degree from 38±4 to 69±9 (F) and from 40±8 to 71±21 ms (C), C was more effective in terminating SAF. C restored SR in 8 out of 9 cases (89%), whereas F was only effective in 1 out of 4 cases (25%). When, for both drugs the effects on atrial fibrillation was compared with the class Ic effects on the ventricle (AFCL/QRS ratio) C had a more specific effect on AF than F. The AFCL/QRS ratio of C was 1.12 compared to 0.67 of F. **Conclusions**: At concentrations causing a similar increase in QRS-width two class Ic drugs, Cibenzoline and Flecainide, had different effects on AF. C was clearly more effective than F both in prolonging AFCL and converting AF to SR.

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INFLUENCE OF BDM ON ISOMETRIC ATP CONSUMPTION AND FORCE IN SKINNED CARDIAC AND SKELETAL MUSCLE

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The influence of 2,3-butanedione monoxime (BDM) on isometric force and the rate of ATP consumption was determined in Triton X-100 skinned cardiac trabeculae from the rat at 20 °C and in single fast-twitch fibres from the iliofibularis muscle of Xenopus laevis at 5 °C. ATP consumption was determined by enzymatic coupling of ATP resynthesis to the oxidation of NADH. Both in skeletal and in cardiac muscle, BDM caused a reduction in isometric force and ATP consumption. Isometric force was half-maximal at 12 mM BDM in trabeculae and at 2 mM BDM in Xenopus fibres. In cardiac trabeculae the rate of ATP consumption was depressed less than isometric force whereas in skeletal muscle fibres ATP consumption and force varied in proportion. At 100 mM BDM force in cardiac trabeculae was reduced to 3.0 ± 0.5 % and ATP consumption was depressed to 29 ± 2 % of the control values in the absence of BDM. These results indicate tension cost (ATP consumption/isometric force) is independent of BDM in skeletal muscle fibres but increases markedly at high BDM concentrations in cardiac muscle. BDM influenced the dynamic properties of the cardiac trabeculae. Stiffness derived from the amplitude of the force change to a shortening of 1% of the initial length (L_o) varied at different BDM concentration in proportion with isometric force. The rate of force recovery after small (1% L_o) shortening and after unloaded shortening (10% L_o within 2 ms) was reduced significantly at [BDM] ≥ 20 mM. Using a two-state model for the crossbridge cycle in which isometric force and stiffness are proportional to the number of attached crossbridges, these results indicate that the (apparent) rate of crossbridge attachment is reduced by BDM in both skeletal as well as in cardiac muscle. In addition, to explain the increase in tension cost in cardiac muscle, the apparent detachment rate should increase in cardiac muscle.

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CONCERNING THE INTERNAL RECIRCULATION OF CONTRACTILE ACTIVATOR IN ISOLATED CARDIAC MUSCLE.

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Papillary muscles were subjected to trains of stimuli or sqaure wave voltage clamp depolarisations. The introduction of extrasystoles or of single prolonged depolarisations produced potentiation of the following contractions. The decay of force from the first to the second potentiated beats was used as a method of assessing the recirculated fraction (RF) of contractile activator from one excitation to the next. Force decayed faster when studied under voltage clamp stimulation (when inotropic state was very high) than with normal action potential activation (normal contractility). When, over a very wide range of inotropic state (including both stimulation protocols), the second potentiated beat was plotted against the first, a sigmoid curve was obtained. The initial slope was steep, followed by a less steep phase and a final steep phase as the saturation level was reached. We attempted to alter sodium-calcium exchange by changing membrane potential. Reduction in diastolic membrane potential resulted in a loss of a fixed amount of force on the second potentiated beat which was not proportional to the force of the first potentiated beat. i.e. there was no change in RF. This result was interpreted to mean that a more favourable diastolic membrane potential (more negative) for calcium extrusion caused a loss of a fixed amount of calcium rather than an amount proportional to calcium release. Reduction of the systolic membrane potential from +20mV to 0mV caused an inconsistent fall in RF plus a fall in contractile force of the second potentiated beat not proportional to force of the preceding beat. The fraction of contractile activator recirculated from one beat to another appears to depend on inotropic state, and is compatible with the concept of competing mechanisms for the sequestration of calcium released intracellularly upon depolarisation.

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CA^{2+}-HOMEOSTASIS IN ISOLATED RAT CARDIOMYOCYTES IS NOT AFFECTED 24 HOURS AFTER IN-VIVO HEAT STRESS.

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It has been recently shown by us that heat stress pretreatment negatively affects the normoxic *ex vivo* left ventricular pump performance when the extracellular calcium concentration ($[Ca^{2+}]_e$) is low or when the heart must perform against a relatively high afterload. These findings indicated that heat-shocked hearts are less sensitive to Ca^{2+}. Therefore, Ca^{2+} homeostasis was investigated in more detail using rod-shaped cardiomyocytes isolated from adult rats exposed to hyperthermia or anesthesia (Control) 24 hours earlier. Ca^{2+}-overload was induced using veratridine (0-100 µg/ml) or high $[Ca^{2+}]_e$ (up to 20 mM) in quiescent or paced cells. The number of Ca^{2+}-overloaded cells upon exposure to these stimuli was not different in both groups of cells. In another set of experiments, intracellular calcium-concentration ($[Ca^{2+}]_i$) in quiescent cells was determined using Fura-2 fluorescence. The same technique was applied to study changes in myofilament Ca^{2+}-sensitivity (assessed by calcium/force transients; stimulation frequency 0.5 Hz) in cells with $[Ca^{2+}]_e$ up to 10 mM. No differences were observed in the $[Ca^{2+}]_e$-dependent $[Ca^{2+}]_i$ between control and heat-shocked cells. In addition, the Ca^{2+}-sensitivity in heat-shocked cells was not attenuated at the various $[Ca^{2+}]_e$ tested. These results suggest that Ca^{2+}-sensitivity investigated under these experimental conditions, is not changed in heat-shocked cells.

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CARDIOTIN, A STRUCTURAL COMPONENT IN THE MYOCARDIUM, RELATED TO THE SARCOPLASMIC RETICULUM. G.Schaart, G.van Eys, L.Moens* and F.Ramaekers

Cardiotin, a structural component of the cardiovascular system, is expressed in cardiomyocytes of several species. It is not found in smooth muscle tissue, or any other type of mesenchymal, epithelial or neural tissue. Cardiotin seems to be organized longitudinally between the myofibrils, perpendicularly to the desmin striations.

Immunohistochemical studies in heart tissues of different species of different age, showed that the expression of cardiotin in the myocardium is age-related. During embryonic development no immunoreactive cardiotin is detected, while in 3 months old monkey heart cardiotin expression can be found. In chronic hibernating myocardium, the cardiotin distribution is affected and may disappear completely. The subcellular localization and the age-related expression of cardiotin suggest a possible link with the sarcoplasmic reticulum. Immunoblotting experiments have shown that cardiotin is a high molecular weight protein. In absence of ß-mercaptoethanol a 300 kDa is found. This 300 kDa protein can be reduced or degraded by enzymatic digestion into smaller fragments of 100, 60 and 30 kDa. Microsequence analyses of the 100 kDa and the 60 kDa fragment, showed for both a aminoterminal-residue of 14 amino acids, which gives 100% indentity with human skeletal muscle α-actinin on position 11-24. The amino-terminus of the 30 kDa fragment appeared to contain a 20 amino acid sequence with no homology to known sequences.

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EFFECTS OF CYCLOPIAZONIC ACID ON TWITCH AND CAFFEINE CONTRACTURE OF ADULT AND NEWBORN FERRET CARDIAC FIBERS. V. Bonnet and C. Léoty

The aim was to evaluate the participation of sarcoplasmic reticulum (SR) in intracellular Ca regulation in adult and newborn ferret heart. Cyclopiazonic acid (CPA) was used to inhibit selectively the SR Ca-ATPase. On adult and newborn (5-6 days old) ventricular trabecules, the twitch obtained by electrical stimulation (0.1 Hz) and the contracture due to 10 mM caffeine were investigated in the presence and absence of 20 µM CPA. After 5 min-treatment of CPA, the amplitude of the twitch was decreased in adult and newborn preparations by 74 ± 6 % and 70 ± 4 %, respectively. The time to peak was significantly increased in adult (control: 455 ± 70 ms, CPA: 722 ± 90 ms) and in newborn fibers (control: 352 ± 27 ms, CPA: 522 ± 14 ms). The time constant of relaxation was also significantly increased in adult (control: 525 ± 63 ms, CPA: 639 ± 83 ms) and in newborn fibers (control: 400 ± 30 ms, CPA: 551 ± 50 ms). In adult fibers, the characteristics of caffeine contracture were not affected by CPA. In newborn fibers, the amplitude of caffeine contracture was significantly decreased by 40 ± 15 % while the kinetics were not changed. Concerning caffeine contracture, similar results have been found in free-Na solution which blocks Na-Ca exchange. In conclusion, the reduction of SR Ca content due to CPA was not sufficient to alter the caffeine contracture amplitude except on newborn fibers in which the SR amount is smaller. The reduced effect of CPA on the caffeine contracture in free-Na solution suggest that the relaxation was not dependent on SR and Na-Ca exchange but related to an additional Ca transport system at the sarcolemmal level.

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MONENSIN REVERSES POSITIVE FORCE-FREQUENCY RELATIONSHIP AND REST-INDUCED DECAY OF CONTRACTION IN CARDIAC MUSCLE. K. Mubagwa, Wei Lin and W. Flameng

Effects of the Na^+ ionophore monensin on contraction were studied to test the hypothesis that species-related differences in cardiac force-frequency relationship (FFR) are determined by differences in intracellular sodium concentration ($[Na^+]_i$). Isolated rabbit and rat papillary muscles were superfused with Tyrode solution (Ca_o = 1.8 mM) at 30 °C and were paced with regular stimuli at various frequencies (0.02-1 Hz) or with paired pulses. In rabbit muscle, isometric force amplitude increased, while in rat muscle force decreased with frequency. Paired pulse pacing-induced potentiation of contraction (PPP) was marked at all frequencies in rabbit muscle, but was absent at low frequencies in rat muscle. In rabbit muscle monensin (15 µM) increased force only at low frequencies (at 0.02 Hz: from 3.1 ± 0.57 to 8.0 ± 0.62 mN/mm^2, mean±SEM, n = 5; at 1 Hz: change from 6.6 ± 0.77 to 4.5 ± 0.57 mN/mm^2), hence reversing FFR from positive to negative, and decreased PPP at low frequencies. Monensin added during rest in the absence of depolarisation-induced Ca^{2+} influx reversed rest-induced decay of force (after 75-min rest: 6.8 ± 0.79 vs. 1.4 ± 0.16 mN/mm^2, in monensin vs. in control, respectively). The decay of PPP upon stopping paired pacing was slower and related to time in rabbit muscle, while it was related to the number of beats in rat muscle. Recirculation of intracellular calcium as measured from the decay of PPP was low but increased at high-frequency pacing in rabbit muscle (at 0.2 Hz: 0.1 ± 0.06; at 1 Hz: 0.8 ± 0.20), while it was large and frequency-independent in rat muscle (at 1 Hz: 1.1 ± 0.26; at 0.2 Hz: 0.8 ± 0.12). The results suggest 1) that FFR depends on $[Na^+]_i$, 2) that in the presence of high $[Na^+]_i$, cellular and sarcoplasmic reticular Ca^{2+} load are increased during diastole possibly via reverse-mode Na^+/Ca^{2+} exchange.

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TRANSIENT INHIBITION OF L-TYPE CA^{2+} CURRENT DURING SPONTANEOUS CA^{2+} RELEASE FROM THE SARCOPLASMIC RETICULUM. K.R.Sipido, G.Callewaert, J.Vereecke, and E.Carmeliet

Spontaneous Ca^{2+} release from the sarcoplasmic reticulum in cardiac cells induces $[Ca^{2+}]_i$-dependent, transient changes in membrane potential. We investigated whether $[Ca^{2+}]_i$-dependent changes in I_{Ca} contribute to these transient membrane currents. Enzymatically isolated guinea-pig ventricular myocytes were studied under whole-cell voltage clamp, and $[Ca^{2+}]_i$ was monitored with K_5fura-2 (70 μM, included in the pipette solution). With impermeant monovalent cations in the internal and external solutions, the Na^+ current, K^+ currents, non-specific cation currents, and Na/Ca exchange current were eliminated. Ca^{2+} overload of the cell induced spontaneous Ca^{2+} release from the sarcoplasmic reticulum. These Ca^{2+} oscillations were accompanied by an outward shift of the membrane current at potentials between -40 mV and +60 mV. A similar outward current shift could also be observed during caffeine-induced Ca^{2+} release from the sarcoplasmic reticulum, in the same potential range. This current shift was not sensitive to Cl^- substitutions. If the Ca^{2+} current was suppressed with verapamil, the current shift was also depressed. These observations indicate that the outward current shift results from $[Ca^{2+}]_i$-dependent transient inhibition of the inward Ca^{2+} current. We conclude that during spontaneous Ca^{2+} release from the sarcoplasmic reticulum, transient inhibition of I_{Ca} can contribute to Ca^{2+}-dependent changes in membrane potential.

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EFFECTS OF STREPTOZOTOCIN (STZ)-INDUCED DIABETES ON NEUROGENIC INFLAMMATION OF GINGIVOMUCOSAL (GM) TISSUE IN RAT. P. Benedek, A. Györfi, Á. Fazekas, L. Rosivall

It has been suggested that, the unmyelinated small diameter afferent nociteptive C-fibers are impaired in diabetes mellitus. We have recently demonstrated that the fibers are the prerequisite for neurogenic inflammation induced by mechanical or chemical irritations. These experiments were designed to characterize the neurogenic inflammatory responses of GM tissue in the early phase of experimentally induced diabetes mellitus in rat. Effect of dental ligature on the GM vascular permeability was studied in control rats and in rats pretreated with STZ at days 7 and 14 following STZ administration. In separate groups of control and STZ diabetic rats studies were also performed to investigate the effect of local capsaicin application on GM vascular permeability on day 14. Vascular permeability was assessed by means of Evans blue extravasation. The ligature placed around the mandibular left first molar caused a significant increase in vascular permeability of GM tissue on the ipsilateral side both on days 7 and 14 after the ligation in control rats. In STZ diabetic rats on day 7, there was a significant elevation of Evans blue extravasation in the tissue tested on the left ligature side, too. However, on day 14 the ligation failed to produce any changes in Evans blue extravasation on the ipsilateral side, i.e. no difference in GM vascular permeability could be recorded between the two sides in STZ diabetic rats. Topical capsaicin administration produced significant Evans blue extravasation in GM tissue of control rats as compared to that observed in diabetic rats on day 14 after STZ treatment. Electron- and light-microscopic studies demonstrated fiber degeneration of the C neurons and less inflammatory cells in STZ-induced diabetes in the GM tissue. These findings appear to indicate that the inflammatory responses induced by mechanical (dental ligature) and/or chemical irritants (topical application of capsaicin) in the GM tissue are altered in STZ diabetic rats and this alteration is due to the diabetes-induced damage to the C fibers.

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DOES THE CIRCULATING FREE LIPOPROTEIN-LIPASE MOLECULE CONTROL THE RECEPTOR MEDIATED UPTAKE OF VLDL-REMNANTS BY THE LIVER? L. Szollár, L. Tornóci, K. Rischák, I. Karádi and L. Romics

It has recently been shown that the lipoprotein lipase molecule increases (40-50 times) the speed of apo E mediated uptake of chylomicron remnants through the Lipoprotein Related Protein (LRP/alpha-2M) receptor HepG2 cells. Reanalysing our previous lipoprotein kinetics data, we have shown that the free lipoprotein lipase entering the circulation after heparin administration increases the clearance of VLDL-remnants considerably both in healthy subjects and in individuals suffering from primary hypertriglyceridaemia. After inhibiting the action of heparin by protamine-sulfate, the VLDL-TG concentration increases, and this increase is proprotional to the lipoprotein lipase activity. The heparin induced clearance is a result of two factors: the hydrolytic activity of the enzyme (to a lesser degree) and the uptake through LRP (to a greater degree). The increase of VLDL-TG after inhibition is a result partly of production and mainly of release from the LRP receptor. This phenomenon plays a role in the catabolism of VLDL remnants, too. A defect in this process may play a role in the pathogenesis of certain cases of hyperlipidemias.

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EFFECT OF INGESTION OF BRANCHED-CHAIN AMINO ACIDS ON PERFORMANCE IN McARDLE'S DISEASE. AJM Wagenmakers, JH Coakley and RHT Edwards.

Exercise and oral ingestion of branched-chain amino acids (BCAA) both increase BCAA oxidation in muscle. In the BCAA aminotransferase reaction α-ketoglutarate is used as amino group acceptor. The alanine aminotransferase reaction (pyruvate + glutamate --> alanine + α-ketoglutarate) subsequently functions in healthy subjects to regenerate α-ketoglutarate, so that the ambient level of tricarboxylic acid (TCA)-cycle intermediates can be maintained. Patients with McArdle's disease lack glycogen phosphorylase and cannot increase muscle pyruvate during exercise, a prerequisite for this mechanism to be effective. Here we investigate the effect of BCAA ingestion prior to exercise on exercise performance in two patients with McArdle's disease. Both patients participated in two exercise tests with 20 watt increments every 20 min until a maximum of 80 watt. The tests were performed on two consecutive days. One of the tests was performed in the postabsorptive state and the other 30 min after oral ingestion of BCAA (10 gram each of leucine, isoleucine and valine in an aqueous solution). One of the patients was stopped in both tests after 120 min of cycling, the last 60 min at 80 watt. The other patient was exhausted after 90 min in the control test (while cycling at 80 watt) and after 55 min in the BCAA test (while cycling at 60 watt). Plasma ammonia concentration, perceived exertion (Borg scale) and heart rate were higher after BCAA ingestion in both patients at all work rates. We conclude that BCAA ingestion prior to exercise without access to muscle glycogen has a negative effect on performance as would be predicted from the supposed role of the alanine aminotransferase reaction in the maintenance of the ambient level of TCA-cycle intermediates during exercise.

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DEAMINATION OF AMINO ACIDS IN MUSCLE IN McARDLE'S DISEASE. AJM Wagenmakers, D Halliday, JH Coakley and RHT Edwards.

Exercise increases the oxidation of branched-chain amino acids (BCAA) in muscle. The amino group, thereby, is first transferred to α-ketoglutarate in the BCAA aminotransferase reaction and then to pyruvate, leading to alanine production and regeneration of α-ketoglutarate, in the alanine aminotransferase reaction. Patients with McArdle's disease lack glycogen phosphorylase and cannot increase muscle pyruvate during exercise, a prerequisite for this mechanism to be effective. As plasma ammonia increased to very high concentrations during exercise in these patients, we here investigated whether the amino group of the BCAA was deaminated instead of being released as alanine. The leg exchange of alanine and ammonia was measured in three patients with McArdle's disease during incremental exercise (product of femoral arteriovenous differences and leg blood flow) and compared with published data on healthy subjects. Muscle ATP concentrations were measured at rest and at exhaustion. L-[^{15}N]leucine was injected intravenously and the ^{15}N-enrichment of the released ammonia measured by isotope ratio mass spectrometry. Alanine release at exhaustion was 26% of that in healthy subjects. Ammonia release was 11-fold higher. Net ATP breakdown accounted for less than 50% of the ammonia release. The femoral venous plasma ammonia ^{15}N-enrichment was between 0.3 and 1.0% of the arterial ^{15}N-leucine enrichment. We conclude that exercise without access to muscle glycogen leads to: 1. a reduced alanine release from muscle; 2. excessive ammonia release and 3. deamination of BCAA instead of release of the aminogroup as alanine.

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SIGNALLING IN BONE: ELECTRIC AND DYE COUPLING BETWEEN CALVARIAL CELLS IN VITRO. K. Schirrmacher, D. Nonhoff and D. Bingmann

Widespread reactions of the bone to local stimuli indicate that this tissue is equipped with an efficient signalling system in which the signals may consist of fluctuations of membrane potentials or intracellular second messenger concentrations of bone cells. Such signals might be transferred to neighbouring cells via gap junctions (gj) which then would be key elements in signal transduction. For a better understanding of cell-cell communication in bone properties of gj between osteoblast-like cells (OB) derived from calvarial fragments of new-born rats were analysed in vitro in more detail. Gj channels were found to be composed mainly of connexin 43 and in less amounts of connexin 45. The electrophysiological studies on gj in extended cells revealed that (i) electric- and dye coupling was abolished by octanol. (ii) The electric coupling strength did not vary with shifts of the membrane potential between -60 and -10 mV. (iii) Changes of extracellular H^+ and Ca^{2+} concentrations had no effects on electric and dye coupling. (iv) Elevations of intracellular Ca^{2+} concentration achieved by the injection of Ca^{2+} via a patch pipette did not affect the coupling in extended cells, while in the double whole cell configuration a decoupling was observed. (v) Parathyroid hormone and calcitonin had opposite effects on the coupling strength. (vi) Phenytoin and carbamazepine known to cause osteomalacia in chronically treated patients weakened the coupling strength.

The present findings are in line with the assumption that signalling in bone strongly depends on the function of gj channels.

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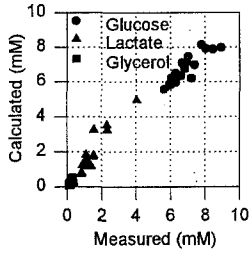
MICRODIALYSIS IN ADIPOSE TISSUE, CAN IT BE USED QUANTITATIVELY? B. Stallknecht, J. Madsen, H. Galbo and J. Bülow.

To examine adipose tissue in humans in vivo is difficult because humans do not have a vein selectively draining adipose tissue which is easy to cannulate. Microdialysis is a technique by which you can determine interstitiel concentrations of different metabolites in e.g. subcutaneous adipose tissue in humans. The **objective** of the present study was to investigate, if adipose tissue venous plasma concentrations can be predicted from adipose tissue interstitiel concentrations determined by microdialysis. **Method**: In 6 anaesthetized dogs the inguinal fat pad was isolated so that it was drained by 1 vein and feeded by 1 artery. Four microdialysis probes were placed in the tissue. In the basal state and during and after epinephrine or adenosine infusion microdialysis fluid and venous blood were collected. Concentrations of glucose, lactate and glycerol were determined in microdialysate and plasma. Venous plasma concentrations were also calculated from microdialysate concentrations (C_i) using "Fick's law of diffusion for a thin membrane": $C_{v\ calc} = (C_a - C_i) \cdot e^{PS/Q} + C_i$. **Results**: Calculated and measured plasma concentrations of glucose, lactate and glycerol showed linear correlations with correlation coefficients of 0.90 ($p<0.05$), 0.93 ($p<0.05$) and 0.65 ($p<0.05$), respectively. Calculated and measured concentrations differed for non of the metabolites ($p>0.05$). **Conclusion**: Adipose tissue venous plasma concentrations of glucose, lactate and glycerol can be calculated from interstitiel concentrations determined by microdialysis. Consequently, microdialysis can be used quantitatively in adipose tissue.



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ONE-STEP PURIFICATION OF RAT HEART FATTY ACID-BINDING PROTEIN EXPRESSED IN E.COLI. F.G. Schaap, G.J. van der Vusse and J.F.C. Glatz

Fatty acid-binding proteins (FABPs), 14-15 kDa proteins present in the cytoplasm of several tissues, belong to the family of lipid-binding proteins which also comprises retinoid- and bile acid-binding proteins. These proteins apparently function as intracellular carriers for their hydrophobic ligands. Likewise, heart-type FABP (HFABP) is believed to transport fatty acids in cardiac cells from the sarcolemma to metabolic sites, e.g. mitochondria. As we plan to use reconstituted lipid vesicles to examine this putative function, large quantities of pure protein are required. For this, we expressed rat HFABP cDNA in E. coli. Rat heart RNA was reverse transcribed and amplified by PCR using rat HFABP-specific primers. The resulting cDNA was ligated into the pET3a expression vector and transformed to E. coli strain BL21(λDE3)pLysS. Upon addition of IPTG, these cells produced large amounts of rat HFABP. The recombinant protein was purified to homogeneity in a one-step procedure by anion-exchange chromatography with a linear gradient of 0-100 mM NaCl in 10 mM imidazole (pH 7.0). Fractions were screened for HFABP with ELISA and tested for fatty acid-binding activity using the Lipidex assay. SDS-PAGE revealed no impurities in FABP-containing fractions. Two isoforms were detected in IEF with isoelectric points of 4.9 and 5.2, presumably representing the protein with (about 10%) and without (about 90%) *N*-formyl-methionine at its *N*-terminus. In this study we obtained high-level expression of rat HFABP in E.coli; the recombinant protein made up about 25% of total cytosolic protein. This high yield (about 45 mg rat HFABP per litre culture) enables us to perform studies aimed at elucidating the physiological role of rat HFABP.

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GUANIDINOSUCCINIC ACID, AN ENDOGENOUS METABOLITE ACCUMULATING IN UREMIC ENCEPHALOPATHY, IS A N-METHYL-D-ASPARTATE RECEPTOR AGONIST IN THE CA1 REGION OF RAT HIPPOCAMPAL SLICES. M. Diltoer, Ph. Lebrun, M.W. Chowdhury, M. De Ridder, S. Engelborghs, E. Williams, R. D'Hooge, P.P. De Deyn, F. Colin and J. Manil.

Guanidinosuccinic acid (GSA), an endogenous protein metabolite accumulating in renal insufficiency, ressembling L-aspartic acid and N-methyl-D-aspartate (NMDA), may play a role in the pathophysiology of uremic encephalopathy (memory loss and convulsions). We studied the effects of tonic applications (40') of GSA (from 31.25 µM to 1 mM) on the field potentials of the pyramidal cells of the CA1 region of rat hippocampal slices after electrical stimulation of the Shaffer collateral pathway at a frequency of 1/min. We found that GSA (from 62.5 to 125 µM) induced a dose dependent long-term potentiation (GSA-LTP) and occluded the tetanic LTP considered to be an electrical correlate of memory at the cellular level. At concentrations of 250 and 500 µM, spontaneous activity and depression occur followed by the developpement of a GSA-LTP after washout. The application of 1 mM GSA did not allow any recuperation of the response and was toxic for most slices. All those effects were dose dependently antagonized by the NMDA-receptor antagonists D-AP5, MK801 and high extracellular Mg2+. The effects of GSA were amplified by 10 µM glycine and inhibited by the glycine-site antagonist 7 Cl-kynurenic acid (50 µM). These results support the hypothesis that GSA is a NMDA agonist inducing a GSA-LTP facilitating convulsions and memory dysfunctionin uremic encephalopathy.

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THE INFLUENCE OF AGING AND DIET ON THE RATE OF PROTEIN SYNTHESIS IN RATS. K.L. Pisarchuk

Peculiarities of intensity of protein synthesis (PS) were measured in both fast and slowly renewing tissues (liver, skeletal muscle) by means of continuous constant infusion of L-[14C]leucine and further preparatory amino acids analysis on Hitachi Analyzer. The study was conducted on male Wistar rats aged 5 and 24 mo raised during the suckling period (up to 21 days) in litters of 8-10 pups (control) and 2 pups/dam (experiment). During one week before the infusion they received the rations with low (2.5%), normal (15%) and high (60%) protein content. It has been shown that differences in the early postnatal feeding have influenced the PS neither in liver nor skeletal muscle. Analysis of age peculiarities of PS showed that under the given experimental conditions its level in both liver and skeletal muscle remained unchanged in the control animals. Thus, the relative specific radioactivity in the liver was 66.4±6.7 in young and 95.7±23.3% in old rats (P>0.05); in skeletal muscle - 4.1 ± 0.6 and 3.9±0.5%/day (P>0.05), respectively. In the experimental group there was a significant rise of the PS level in the liver of young (59.0±5.7) and old (9.4±13.4%) rats (P<0.05), while in the skeletal muscle no difference was found: 4.4±0.5 and 4.7±0,8 %/day (P>0.05), respectively. A tendency towards the PS activation in tissues has been observed with lowering the protein content in the diet. It can be concluded that with aging the above tissues retain a considerable adaptive ability in the regulation of biosynthetic processes.

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TURNOVER OF NITROGEN METABOLITES IN SHEEP. J. Varady, L. Leng*, J. Fejes*, S. Faix* and M. Szanyiova*

The effect of intraruminal and combined intraruminal and intracaecal nitrogenous nutrition on the parameters of urea turnover, pool and clearance were investigated in sheep with the fistulas of the rumen at synthetic and semisynthetic diets. At combined nutrition, a half dose of nitrogen was administered by infusion into the caecum during feeding. The animals have been gradually adapted to intake of synthetic and semisynthetic diets. The lowest values of clearance, metabolic pool and turnover for urea were found at synthetic diet. At semisynthetic diet with oral nitrogen intake those parameters were higher. However, they significantly increased at intracaecal nitrogen nutrition, especially in clearance, total and exogenous urea turnover. Secretion of endogenous urea into the digestive tract (endogenous turnover) was without significant differences at both diets and it was independent on the way of nitrogenous nutrition. The increased absorption of ammonia from caecum with both higher urea synthesis and renal excretion was found during intracaecal nitrogen nutrition. In the next series of experiment the secretion and excretion processes of endogenous urea, uric acid and creatinine were investigated quantitatively. It was found that urea penetrating the rumen wall was mostly hydrolyzed to ammonia and partly entered the rumen content as a whole molecule. The passage of endogenous urea into the isolated rumen pouch reached the value of 100 $umol.h^{-1}$, while the values of uric acid and creatinine were only 0.52 and 0.41 $umol.h^{-1}$, respectively. The secretion rates of these metabolites into the milk were in a similar proportions. Fractional excretion (FE) of creatinine by the kidney was found to be 141.5% and for uric acid 63.3%. The FE for urea was 75.6%. Presented results confirm the important role of the recycling of nitrogen substances in ruminants.

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METABOLIC DISTURBANCES &
EFFECT OF ZINC ADMINISTRATION IN DIABETIC RATS

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Objective of the study: We have observed the blood modification in proteins, glucose, LDH, and G-6-P-ase simultaneously with weight variations and urine glucose levels on young Wistar rats with alloxanic (Allx) diabetes.

Method used: The animals were divided into four groups, each comprising 10 young male Wistar rats. Rats in group I received a regular diet; those in group II received Zn sulphate: 70 mg/kg/day, six weeks; those in group III received alloxan (Allx): 40 mg/kg, in unique dose i.v. and those in group IV received Allx and Zn (before and after Allx) in the same dose. The animals are not submitted to a treatment or/and hypoglycemiant regimen.

Results & conclusions: Zn administration decrease blood glucose and urine glucose levels to the diabetic rats. Blood glucose concentration was 20.9 mmol/L at alloxanic rats, 6.075 mmol/L in normal rats and 6.35 mmol/L in Zn+ Allx rats; that prove a increase of existing insulin activity and a relative insulinlike effect of Zn. The diabetic group showed a significant reduction of plasmatic proteins (9,06 ± 0,2 g/dl) compared to the normal (N) group (10,04 ± 0,14 g/dl) while Allx+Zn presented values nearing those of the N group. Also the average weight of the diabetic rats which were administered Zn (116 g) is nearing that of the N animals (148 g). The positive effect of Zn on protein metabolism and implicitly on body growth is caused by metal enzymes as Zn is a component of several DNA and RNA-polymerases and by the STH synthesis increase. The significant increase of LDH plasmatic activity with 148% in diabetic animals compared to the N group proves alteration of hepatocyte membrane (the protein synthesis site) caused by the oxygen free radicals result in allx diabetes. Administration of Zn to the diabetic group improves LDH values but does not bring them to normal. Increased values of G-6-P-ase in the Allx group confirms membrane injuries (G-6-P-ase is the marker enzyme for microsomal membrane). Administration of Zn salts generates cellular protection in diabetic animals, where the G-6-P-ase value is nearing that of the witness group.

The present data suggest a favorable effect of zinc in ameliorating metabolic disorders in the evolution of the aloxanic diabetes.

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AMBIVALENT IMPACTS OF GLIBENCLAMIDE ON BETA-CELLS IN VITRO. A.V.Timofeev, T.I.Danilova, L.Yu.JULIOVA, and M.I.Balabolkin

In attempt to elucidate the reasons for secondary failure of sulfonylurea therapy in non-insulin-dependent diabetic(NIDD) patients we investigated an effects of glibenclamide (GB) in primary cultures of human fetal pancreatic cell clusters (HFPC). HFPC from 24-wk cadaveric fetuses were incubated for 24 hrs in serum-containing medium with 0.1 or 1.0 ng/ml GB. HFPC were then cultured for 72 hrs without GB and fixed in paraformaldehyde. Sections of HFPC were immunostained for insulin (INS). In presence of GB, INS release from HFPC (determined by RIA) increased 1.5 - 5-fold; an effect was dose-dependent. Elevated INS accumulation in the medium persisted over 72 hrs after GB removal. After GB treatment (1 ng/ml), proportion of INS^+ cells in HFPC increased to 8.5 $\pm$ 2.3 % vs. 2.2 $\pm$ 1.2 % in untreated HFPC; $p < 0.01$. To explore whether an increase in number of INS^+ cells after GB treatment was due to proliferation and/or differentiation of beta-cells (BC) we studied effects of GB in hamster HIT T-15 cell cultures (containing both mature BC and BC precursors). Cells at confluency were shifted to quiescence by 96-hr incubation in serum-free medium with ^{14}C-dThd. GB was added to the medium for the final 24 hrs of the step-down phase. Cell proliferation was stimulated by addition of serum-containing medium with ^3H-dThd. At various time intervals after stimulation cells were either lyzed for estimation of $^3H/^{14}C$-ratio or stained for INS. GB at doses 1 and 10 ng/ml caused 2.5- and 5.7-fold increase in number of INS^+ cells at 36 hr after stimulation but did not promote entrance of cells into S-phase. At 50 or 100 ng/ml GB suppressed INS accumulation over the step-down period by 45 or 82 %, caused 1.8-fold decrement in number of INS^+ cells and delayed the onset of DNA replication by 4 - 6 hrs. Thus, GB at low doses facilitates BC maturation, while at high doses GB suppresses BC function and maturation in vitro. We speculate that the long-term, high-dose GB therapy of NIDD patients may cause functional and reproductive deterioration of BC.

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AUTO/PARACRINE GROWTH REGULATORS IN HUMAN FETAL ISLET CELL CONDITIONED MEDIUM. A.V.Timofeev, A.V.Labzin, T.I.Danilova, L.Yu.JULIOVA, and A.A.Olschanskaya

Beneficial impacts of human fetal pancreatic cell (HFPC) transplantation in insulin-dependent diabetic (IDD) patients can not be explained merely by replacement of the host islet cells (IC) because an amount of grafted HFPC is in most of cases insufficient to maintain glucose homeostasis. Long-term effects of HFPC transplantation (stabilization of the course of IDD, retardation of IDD complications) may be due in part to induction of growth and/or maturation of the host residual IC or IC precursors by factors produced by grafted HFPC. In searching for these factors we investigated morphogeneic properties of culture media conditioned by HFPC. Precultured HFPC clusters from 16 - 26-wk cadaveric fetuses were incubated for 48 hrs in serum-free RPMI-1640 meduim (glucose conc. 11 mmol/l) supplemented with HSA, vitamines, and nicotinamide. Conditioned media (CM) were concentrated 100 - 200-fold (Amicon PM10), desalted (G25 column), and lyophilized. CM from non-pancreatic cell cultures were used for control. Effects of CM were studied in monolayers of hamster IC tumor cells (HIT T-15). Combined autoradiography and immunocytochemistry enabled us to demonstrate that HFPC CM preparations (1 - 10 mcg of protein per 1 ml of culture medium) markedly increased the number of INS^+ cells and of INS^+ cells labelled with ^3H-dThd. Proliferation- and differentiation-promoting activities of HFPC CM were completely abolished by repeated freezing-thawing of CM, heating CM for 30 min at 60° C, or by protease digestion. Treatment of CM with antiserum against IGF-1 (a well-known promoter of IC renewal) did not block the effects of CM. We suggest that HFPC in vitro produce peptide regulators of IC morphogenesis, which may be distinct from IGF family.

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CHANGES IN CALCIUM SIGNALLING IN PRIMARY NOCICEPTIVE NEURONS OF DIABETIC MICE. E.P.Kostyuk & A.V.Shmigol.

Pathological changes in the functioning of the nervous system are regular complications of chronic diabetes mellitus, potentiation of the transmission ot nociceptive signals beeing most common. A possible reason for such changes might be pronounced alteration of cellular calcium homeostasis during diabetes which has been already detected in several tissues. Therefore the aim of the present study was to analyse possible changes in calcium signalling in primary nociceptive neurones in experimental conditions (mice with streptozotocin-induced or genetic diabetes). $[Ca^{2+}]_i$ transients were measured using Indo-1 based microfluometry in isolated dorsal root ganglion (DRG) neurons of different size taken from mice with STZ-induced diabetes or db/db line mice (with spontaneously occuring illness). It has been found that in control mice DRG neurons of different size handle depolarization-induced $[Ca^{2+}]_i$ transients in a different way. In large (exteroceptive) neurons these transients are rapid due mainly to fast uptake of Ca^{2+} ions from the cytosol into caffeine-sensitive intra- cellular stores. In small (nociceptive) ones such uptake is negligeable, and signal termination is due mainly to plasmalemmal extrusion of Ca^{2+} ions. In diabetic mice the duration of $[Ca^{2+}]_i$ transients became substantially prolonged just in small neurons, without detectable changes in large ones. The changes were more prominent during STZ-induced diabetes comparing with the spontaneous one. We suggest that the prolongation of calcium signals only in small sensory neurons is related to high dependence of calcium homeostasis here on extrusion of Ca^{2+} ions by plasmalemmal Ca pump, which according to biochemical data is substantially depressed during insulin-dependent diabetes. The described changes might be the basis for potentiation of nociceptive signal transmission during diabetic neuropathiae.

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COMPARISON OF WOUND HEALING IN NONDIABETIC AND DIABETIC RABBITS BY USING LOW VOLTAGE ELECTRICAL STIMULATION.S.Hajizadeh, A.Khoshbaten, M.Khaksari, A.Asgari, A.M.Sharifi

In this study we examined the effect of low voltage electrical Stimulation on wound healing in nondiabetic (ND) and diabetic rabbits.White adult rabbits weighing between 1.7 - 2.3Kg were used in this study. Diabets was induced by injection of Alloxan (170 mg / Kg , iV) . Animals were anesthetised by sodium pentobarbital (40mg / Kg , iv) and fullthickness incision wounds were made on both sides of vertebral column . Animals were electrically stimuluted via surface electrodes 24 hours after surgery for tow hour periods twice a day. In this study 1- percentage of healing on 4th,8th and 10th days after surgery for ND rabbits were significantly increased from 17.4% , 33.5% and 54.8 % for control to 36.42% , 75.1% and 94% for stimulated rabbits respectively . 2- Percentage of healing on 4th, 8th and 12th days after surgery for diabetic animals were 12.5% , 16.3 % and 51.2% for control that were increased to 26.15%, 33.1 % and 69.3% for experimental group respectively. 3 - Duration of healing for ND and diabetic control groups were 14.9±0.58 and 16.5 ± 0.42 days that significantly decreased to 10.37 ± 0.7 and 13.55 ± 0.44 days respectively($p < 0.01$) . These results showed that wound healing was delayed with diabetes and also that electrical stimulation could accelerate wound healing process in either diabetic or non-diabetic animals .

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EFFECT OF FAT EMULSIONS AND ALPHA-TOCOPHEROL ON LIPOPEROXIDATION STATUS *IN VITRO* AND *IN VIVO*.

Z. Červinková, P. Živný, V. Palička, J. Vávrová

Recent papers document that parenteral administration of fat emulsions leads to enhanced concentration of malondialdehyde (MDA) in sera. It is also known that fat emulsions for intravenous use are liable to peroxidation during their storing in spite of adding vitamin E. Because saturated fatty acids are more resistant to peroxidation stimuli we examined the influence of long chain triglyceride (LCT) and medium chain triglyceride (MCT) oils on the degree of lipoperoxidation *in vitro* and *in vivo*.

Methods:

a) *in vitro* study. To 500 ml of anticlotting full blood following substances were added: saline as a control group, LCT emulsion enriched with alpha-tocopherol (AT), Intralipid 10 % (LCT), Lipofundin 10 % (LCT/MCT), Intralipid 10 % + Ephynal (dl-AT-acetate).

b) *in vivo* study. We used 2/3 hepatectomy (PH) as a lipoperoxidation stimulus in adult male rats. Saline, Lipofundin 10 % or Lipofundin 10 % enriched with AT were administered intravenously by infusion started always immediately after PH lasting 6 h. Plasma concentration of AT, triglycerides and MDA were measured in both studies, in *in vivo* study at 6 and 24 h intervals after PH also MDA concentrations in lung and liver homogenates.

Results and conclusions: Significantly lower concentrations of MDA were found in blood which was supplemented *in vitro* with Lipofundin ($p<0.001$) resp. Intralipid + Ephynal ($p<0.001$). On the other hand we did not find any changes of plasma MDA concentration in hepatectomized rats treated with Lipofundin enriched with AT. Because we found significantly lower MDA concentration in lung homogenates obtained from hepatectomized rats treated with Lipofundin enriched with AT in comparison to control group treated with saline there is a question if plasma MDA concentration really reflects the degree of lipoperoxidation in tissues. To answer this question requires further experiments.

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EFFECT OF OVARIAN STEROIDS ON INCORPORATION OF ^{14}C-PALMITIC ACID INTO DIFFERENT LIPID FRACTIONS OF RAT UTERUS.

J.Świątecka, J.Górski

Ovarian steroids, estradiol and progesterone, are important regulators of uterine metabolism. However, a relationship between effects of estrogen and progesterone on uterine lipid metabolism is poorly recognized and not clear defined. The aim of the present study was to examine effects of *in vivo* 17-βestradiol(E) , and progesterone(P) (in E-primed animals) treatment on incorporation of [1-^{14}C]palmitic acid (PA) into different lipids fractions,on content(CPL) and specific activity of phospholipid fraction (SA PL) of rat uterus. The animals were virgin female Wistar rats, weighing 230-260gm at autopsy. For the study of the effects of exogenous hormones all rats were ovariectomized (ovx) and 25-28 days later divided into three groups, treated as described below: Group C-untreated , Group E received a single s.c.E injection 50mg/100g bw, Group E+P-received a single s.c. P injection 2mg/100g bw (72h after the E- priming).Uteri horns were excised 24h after the last injection, under thiopental-anaesthesia, with care to remove all adhering fatty connective tissue. Horns were oppened by longitudinaly section and incubated either 5 or 30 min at 37°C in a 3% albumin bath medium, bubbled with 100% O_2, containing Hepes buffer, fatty acids (183,6±5,2mEq/l) and 10μCi PA (specific activity 57.00 mCi / mmol)/35ml. After incubation, uterine segments were washed three times with fresh bath medium without[1-^{14}C]PA at 2°C,frozen in liquid N_2 and pulverized.Lipids were extracted and separated using thin-layer chromatography into the following fractions:phospholipids(PL), mono-(MG),di-(DG) and triacylglycerols(TG),free fatty acids(FFA),cholesterol(Ch) and cholestyresters (ChE).Most of the label was found in PL and TG. It was shown that E increased PL content, and P reduced that effect. SA PL in both E- and E+P-group, was lower than in C-. It was significantly higher after E than after P treatment. E increased incorporation of ^{14}C-PA into MG, DG and TG and this effect was also inhibited by P.It is concluded that ovarian steroids effect markedly lipid metabolism in the rat uterus.

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THE PLASMA-BORNE UNESTERIFIED LONG-CHAIN FATTY ACIDS ENTER THE RAT LIVER NUCLEI.

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The nucleus contain different complex lipids that play an important role in its function. This study was aimed to investigate whether extracellular unesterified long-chain fatty acids (FFA) enter the nucleus. Two experiments have been carried out on Wistar rats. In one wxperiment ^{14} C- palmitic acid complexed to serum was administered intravenously in a group of 10 male and in a group of 10 female rats. The liver samples were taken in 5 and 30 min after administration of the label. The nuclei were isolated. Lipids were extracted from the nuclei and from a portion of the whole liver tissue. The FFA fraction was isolated using thin-layer chromatography and its radioactivity was counted. It has been found that the nuclei contained considerable percentage of the total ^{14} C- palmitic acid found in the whole liver in both sexes. However, the number of counts both in the liver and the nuclei obtained from females was nearly two times higher than in the males. In the second experiment the confocal laser scanning microscopy was used to evaluate FFA transportinto the nucleus. The hepatocytes were isolated and incubated in the presence of 12-NDB-stearate (a fluorescent derivative of stearic acid) (complexed to albumin). Four different 12-NDB-stearate/albumin ratios were used . There was a gradual accumulation of the fluorescence both in the cytosol and in the nucleus. Fluorescence intensity increased faster in the cytosol than in the nucleus. The initial uptake rates of the fluorescent stearate in the cytosol and the nucleus increased linearly with elevation in the extracellular concentration of the acid. It is concluded that the extracellular FFA enter the nuclei of the rat liver. The accumulation of FFA in the nucleus increases with increased concentration in the cytoplasm.

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THE EFFECT OF MELATONIN ON METABOLIC CHANGES INDUCED IN FEMALE RATS BY CONTINUOUS IRRADIATION AND DIMETHYLBENZANTHRACENE.

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The experiment represents an initial step in the project analyzing the mammary carcinogenesis induced by continuous irradiation in female Wistar rats. Selected parameters of lipid and carbohydrate metabolism were analyzed by conventional methods. Young /50 day old/ virgin female rats were continuously irradiated by daily dose of 100 mGy of gamma rays up to 15 days. 7,12- dimethylbenz/a/anthracene /DMBA/ in a low dose /5 mg per rat/ was administered by gavage to a part of irradiated and non-irradiated animals between postnatal days 55-60. Another part of animals drunk melatonin /MEL/ in the tap water at the concentration 20 μg per ml. Two, 30 and 100 days after irradiation end the analyses were realized. DMBA administration increased the serum glucose and lipid concentration, increased the content of liver and thymus lipids and elevated the liver glycogen content, especially in non-irradiated rats. In contrary, DMBA decreased the concentration of myocardial glycogen and of bone marrow triacylglycerols /TG/. Exogenous MEL modified some DMBA-induced changes: the increase in liver TG and cholesterol and the decrease in myocardial glycogen and bone marrow TG were inhibited. The beneficial metabolic effects of exogenous MEL could contribute to the expected role of pineal gland in rats, subjected to continuous irradiation and DMBA administration.

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SERUM LIPOPROTEIN PARAMETERS IN RELATION TO OVERWEIGHT AND LIFESTYLE IN SCHOOLCHILDREN. K.Aasvee, M.Saava and K.Neilinn-Lilienberg

Cardiovascular risk factors were studied in a representative group of 13-15 year old schoolchildren (n=290,124 boys and 166 girls) of Tallinn. To elucidate overweight, body mass index (BMI) and sum of triceps and subscapular scinfolds(T+S)were calculated. BMI of child's mother (BMIm) and father (BMIf) were determined by a questionnaire, filled in by parents. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglycerides(TG) were measured enzymatically, apolipoproteins A-I and B (apo A-I and B) by immunophoresis. Out-of-school physical training per week in hours (sport) and sleeping hours per night (sleep) were registered by interview. The linear correlation coefficients (r) were as follows:

	BMI	T+S	BMIm	BMIf	Sport	Sleep
TC	0.06	0.10*	0.00	0.20**	0.01	0.08
HDL-C	-0.14**	-0.15**	-0.06	-0.05	0.12**	0.09
TG	0.14*	0.19***	-0.10	0.17*	-0.10*	0.01
Apo A-I	-0.07	-0.05	0.09	-0.05	0.03	-0.06
Apo B	0.15**	0.12*	-0.03	0.21**	-0.02	-0.12*

* P< 0.05 ** P< 0.01 *** P< 0.001

Cardiovascular risk in children, according to lipoprotein parameters, was potentiated by their overweight and a short resting time at night, positive correlations were found with fathers' BMI as well. Physical exercises revealed favourable effect on blood lipids, but not on apo A-I or B level.

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EXPRESSION OF GLUT4 IN SKELETAL MUSCLE MICROVASCULAR ENDOTHELIUM H.A. Keizer, M.K.C. Hesselink, H. Kuipers.

Glucose transport across the plasma membrane of insulin sensitive tissues such as skeletal muscle is facilitated by an intracellular glucose transporter (GLUT4). Whether this is also true for endothelial cells in those tissues is a matter of debate. Immunocytochemical studies using a monoclonal antibody against GLUT4 (1F8) have localized GLUT4 in capillary endothelial cells from insulin-sensitive tissues (heart and skeletal muscle, white and brown adipose tissue) [2]. However, these results could not be reproduced by others using the same monoclonal antibody (1F8) and 2 different polyclonal antibodies (anti-IRGT$_{12}$ and anti-IRGT$_{19}$). In endothelial cells no reaction was observed using any of the polyclonal antibodies. Only an occasional reaction with 1F8 was reported and considered to be a spurious reaction of 1F8 [1]. To shed more light upon this discussion we performed immuno-gold silver staining with another commercially available monoclonal antibody against partially pure rat GLUT4 (21/71 Biogenesis, UK). Briefly, cryosections of gastrocnemius muscle of male Wistar rats were incubated with the primary monoclonal antibody against GLUT4 (21/71), subsequently a secondary gold (ø<1 nm) conjugated antibody was used, the ultra small gold particles were visualized at light microscopical level by silver enhancement. We observed that the walls of capillaries in rat gastrocnemius muscle show a positive reaction, whereas background staining (where incubation with the antibody against GLUT4 was omitted) was negative. This indicates the presence of GLUT4 in endothelial cells of skeletal muscle capillaries and confirms the results of Vilaró et al. [2]. Therefore, we conclude that the previously reported positive reaction of 1F8 in endothelial cells of insulin-sensitive tissues is not solely a spurious reaction [1].

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THE INFLUENCE OF SHORT FASTING ON METABOLIC REACTION TO STRESS IN WISTAR RATS. B. Beszczyńska, E. Wasilewska, E. Święcka, Z. Bargiel

The aim of the present study was to estimate whether short fasting can influence reaction to stress in rats exposed to acute immobilization at room temperature (IM), cold (-5°C, C) or cold and immobilization simultaneously (CIM). In fed and 24h fasted rats the blood glucose and lactate levels and the content of glycogen in liver and muscle were compared. Fasting alone significantly decreased the content of glycogen in liver and muscle and blood glucose level, but increased the blood lactate concentration. In fasted rats hepatic glycogen significantly decreased in all stress conditions, whereas in fed ones the decline of liver glycogen was observed only after IM. In fed rats both IM and CIM significantly decreased muscle glycogen level, but after C no change was found. In fasted rats decline in muscle glycogen was observed after C, IM, and CIM and the deepest drop was observed after CIM. Increase in blood glucose level was noted in fasted rats after IM and in fed ones after CIM, whereas in the former CIM significantly decreased blood glucose level. Neither C nor IM and CIM changed blood lactate level in fasted rats. However in fed ones increase in concentration of this metabolite after IM was observed. It seems, that the short fasted rats respond to stress in a different manner than the fed ones, probably because of their different initial metabolic state.

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THE EFFECT OF ARG^8-VASOPRESSIN ON THE ARACHIDONATE CASCADE OF RAT PLATELETS. Z. Czank, B. Kis, Zs. Mezei, Á. Gecse, G. Telegdy.

The salt-water balance, the renin-angiotensin-aldosteron system and vasoactive peptides play important role in the regulation of blood pressure. The vascular tone might also be modified by different arachidonate metabolites, synthesized in platelets and/or in endothelial cells. It is known, that vasopressin is a vasoconstrictor and induce platelet aggregation, but the mechanism of these actions has not yet been clarified. The aim of present experiments was to investigate the role of Arg^8-vasopressin (AVP) on the arachidonate cascade of rat platelets. Washed thrombocytes were isolated from male rats of Wistar strain. Platelets were preincubated with AVP (10^{-12}-10^{-8} mol/L) in Parker medium 199 (pH 7.4) at 37°C, for 5 minutes. The enzyme reaction was started with the introduction of 1-^{14}C-arachidonic acid (AA) into the incubation mixture. The incubation was carried out at 37°C, for 10 min and then the AA metabolites were extracted with ethyl-acetate at acidic pH. The ^{14}C-eicosanoids were separated by overpressure thin-layer chromatography, and quantitatively determined with liquid scintillation. AVP (10^{-8} mol/L) did not significantly modify thromboxane synthesis, while the formation of $PGF_{2\alpha}$ was reduced, and that of 12-hydroxiheptadecatrienoic acid was stimulated. The lipoxygenase pathway was inhibited by AVP (10^{-11}-10^{-9} mol/L) and at the same time the ratio of lipoxygenase to cyclooxygenase metabolites was significantly reduced. Summarising these experiments, we may conclude that the vasoconstrictor effect of Arg^8-vasopressin is not mediated by the alteration in platelet eicosanoid synthesis. On the other hand, the reduction of platelet lipoxygenase products may play a role in the vasopressin induced platelet aggregation. **This work was supported by the grants from OTKA (T-6084, 2683, T-3 1354) and by the Ministry of Social and Welfare of Hungary (T-11 549/93) and FEFA (1008/1).**

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EICOSANOID SYNTHESIS OF PLATELETS ISOLATED FROM BLOOD GROUP "A" PATIENTS. B. Kis, Sz. Lénárt, Zs. Mezei, Á. Gecse, G. Telegdy.

It is well known that platelets play role in the pathogenesis of diseases, but the exact mechanism of action has not yet been clearly defined. The aim of the present *in vitro* experiments was to investigate the arachidonate (AA) cascade of isolated platelets of human blood group "A" - most frequent blood group antigen in human population - and detect any corellation between the synthesis of eicosanoids and the platelet count. Washed thrombocytes were isolated from healthy 20-40 year old men not taking any drug for at least 1 week before the experiment. Platelets (10^7 to 10^9 cell/ml) were preincubated in Parker medium 199 (pH 7.4) at 37°C, for 5 minutes. The enzyme reaction was started with the introduction of 1-^{14}C-arachidonic acid into the incubation mixture for 10 min, then the AA metabolites were extracted with ethyl-acetate at acidic pH. The ^{14}C-eicosanoids were separated by overpressure thin-layer chromatography, and quantitatively determined with liquid scintillation. The arachidonate cascade was most active when the platelet count (10^8-$5x10^8$cell/ml) was around the physiologic range of human plasma. The synthesis of cyclooxygenase (CO) products and TxB_2, as well as the ratio of vasoconstrictor to vasodilator CO metabolites were positively correlated, with the in vitro applied platelet count while the ratio of lipoxygenase (LO) to CO products was inversely correlated with the platelet count in the incubation media. The synthesis of 12-hydroxiheptadecatrienoic acid (12-HHT) was most effective when 10^8 platelet/ml was used. Lower or higher cell concentrations resulted in reduced synthesis of 12-HHT. Conclusion: isolated human platelets are useful tool to investigate human cellular arachidonate cascade. The arachidonic acid metabolism of human platelets is dependent on the cell number. The arachidonate cascade of thrombocytes may play role in the pathomechanism of diseases that are accompanied by alterations in the platelet count.

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THE ARACHIDONATE CASCADE OF ISOLATED HUMAN PLATELETS WITH DIFFERENT BLOOD GROUPS. Zs. Mezei, Sz. Lénárt, B. Kis, Á. Gecse, G. Telegdy.

It is suggested that certain diseases might be associated with the ABO blood group antigens. Platelets express surface blood group antigens like erythrocytes. The aim of these *in vitro* experiments was to examine the arachidonic acid (AA) metabolism of human platelets isolated from persons with different blood groups and to compare the results to that of the rat ones. Washed thrombocytes were isolated from untreated, healthy 20-40 years old men and from male Wistar rats. Platelets were preincubated in Parker medium 199 (pH 7.4) at 37°C, for 5 minutes. The enzyme reaction was started with the introduction of 1-^{14}C-arachidonic acid (AA) into the incubation mixture containing 10^8 platelet/ml. The incubation was carried out at 37°C, for 10 min and then the AA metabolites were extracted with ethyl-acetate at acidic pH. The ^{14}C-eicosanoids were separated by overpressure thin-layer chromatography and quantitatively determined with liquid scintillation. Blood group dependency was observed in the activity of lipoxygenase and cyclooxygenase pathways of human platelets. The platelets isolated from blood group "O" synthetised significantly ($p < 0,05$) more lipoxygenase (LO) and less cyclooxygenase (CO) product than platelets of blood group "B" patient. This was mainly due to reduced synthesis of TxB_2 in platelets with blood group "O". There was no significant difference in the synthesis of 12-hydroxiheptadecatrienoic acid (12-HHT) in the platelets of patients with different blood groups. Human platelets (10^8 cells/ml) synthesized less LO metabolite and more TxB_2 and 12-HHT than rat platelets. Our results suggest that the arachidonate cascade of rat platelets can not be used as a model for human ones. The work was supported by grants: OTKA (T-6084, 2683, T-3 1354), the Ministry of Social and Welfare of Hungary (T-11 549/93) and FEFA (1008/1).

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CATECHOLAMINE TURNOVER IN SOME ORGANS OF COLD ACCLIMATED RATS AFTER SURGICALLY INDUCED DEFICIENCIES IN THE SYMPATHETIC NERVOUS SYSTEM. R. Bertin, F. De Marco and R. Portet.

Are rats deprived of adrenal medulla or with sympathectomy of interscapular brown adipose tissue (IBAT) capable to live in a cold environment?

Surgical interventions were performed on 3 week old male Sprague Dawley rats, born and reared at 16°C or 28°C: sympathectomy of the IBAT, by cutting the five nerve fibre bundles that enter each of the two pads of this brown fat deposit; adrenal demedullation, by the technique of autograft (the adrenals were removed after severing the nerve and vascular connexions, then they were put exactly at the same place). The measures were made 2 months later after the regeneration of the adrenal cortex.

The levels and turnover rates of norepinephrine (NE) being known to be reliable indicators of sympathetic nervous system activity (very important in cold acclimation) were studied by measuring these two parameters in IBAT, heart, and adrenals. The levels and turnover rates of epinephrine (E) in adrenals were also determined.

In the 28°C rats, after sympathectomy of the IBAT, there were substantial decreases in the IBAT NE basal level and turnover rate but there were no changes in the heart or the adrenals; in these rats the bilateral adrenomedullectomy did not induce changes in the catecholamine turnover.

In the 16°C rats, after sympathectomy there were decreases in the basal level and turnover rate of the IBAT NE and significant increases in level and turnover rate of NE in the heart and of E in the adrenals. Bilateral adrenal demedullation led to increases in NE basal level and turnover rate in the IBAT and the heart in the cold acclimated rats

In rats born and reared at an environmental temperature lower than thermoneutrality compensatory increased catecholamine levels and turnover rates persist in some organs two months after a partial sympathectomy of BAT or bilateral adrenal demedullation.

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RESPIRATION RATES OF HEPATOCYTES FROM HYPERPHAGIC RATS. L. Lionetti, S. Iossa, M.P. Mollica, M.D. Brand* and G. Liverini.

We have previously shown that rats fed an energy dense diet become hyperphagic, but fail to exhibit excess weight gain through a facultative increase in energy expenditure. A contribution to the increased energy expenditure could be given by the liver, since it consumes about 20% of the total oxygen used by the organism. We therefore measured respiration rates of isolated hepatocytes from hyperphagic rats. The results show a significant increase in oxygen consumption, both in the presence and in the absence of added substrates. To identify which block of reactions is responsible for the increased respiration rate, we applied top-down elasticity analysis both to hepatocytes and to isolated mitochondria. In hepatocytes from hyperphagic rats there were significant kinetic effects on substrate-oxidation pathway, while there was no effect on the kinetic of proton leak plus ATP turnover. In isolated mitochondria we found a decrease in substrate oxidation pathway with NAD-linked substrate, while no variation was found with FAD-linked substrate. These results are consistent with our working hypothesis that the increased respiration rates of hepatocytes from hyperphagic rats essentially involve flavin-linked oxidations. Increased flavin-linked oxidation might cause a decrease in hepatic metabolic efficiency which could contribute to the increased energy expenditure of hyperphagic rats.

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INTRAOSSEOUS PRESSURE AND TRABECULAR BONE MECHANORECEPTOR SENSITIVITY ON VARIOUS LEVELS OF LOWER EXTREMITY IN PRIMARY OSTEOARTHRITIS OF THE HIP. A.Mertens, M.Vitola and A.Čūriškis

An elevated intraosseous pressure is one of the possibly pathogenetic factors in primary osteoarthritis (OA). The degree of pain in OA is generally connected with the intraosseous pressure rise. The measurements were performed in iliac crest, head of femur, proximal metaepiphysis of tibia and calcaneus on the side of OA. Ninety females participated in the study of the age from 44 to 77. A total of 182 trials are reported in this paper. The intraosseous pressure was measured with patients in horizontal position by connecting a bone cannula to a pressure transducer. Isotonic sodium chloride solution with controlled pressure and volume was injected intraosteally in order to increase the intraosseous pressure. The intraosseous pressure increased in the direction from iliac crest to calcaneus. In iliac crest and head of femur the intraosseous pressure was significantly lower than in calcaneus ($P<0,05$). Iliac crest was 14,2±4,5 mm Hg, head of femur 24,3±3,8 mm Hg, calcaneus 32,1±2,8 mm Hg. The bone mechanoreceptor sensitivity in distal parts of the lower extremity was significantly higher than in proximal parts. The bone mechanoreceptor sensitivity rose gradually from iliac crest to calcaneus. During Valsalva manoeuvre the intraosseous pressure increased only in iliac crest and head of femur. Arterial occlusion probe for 3 minutes demonstrated that active hyperemia response was connected with intraosseous pressure increase both in tibia and calcaneus. These results indicate that trabecular bone intraosseous pressure in various levels of lower extremity is modified by local factors.

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THE INFLUENCE OF THE TREATMENT WITH THIOCTIC ACID UPON NERVE CONDUCTANCE VELOCITY IN DIABET RATS. #B.Nickel, A.Shandra, L.Godlevsky and V.Zaporozhan

The prophylactic activity of TOA(±) against experimental diabet neuropathy was shown earlier. It was also determined that the activity of TOA(+) was in charge for the observed beneficial effects. The aim of present work was to investigate effects of the treatment design with TOA (±), (+) and (-) on the motor nerve conductance velocity (NCV) declining in streptozotocin (STZ)- administered rats. Diabet was induced in rats via i.v. STZ (50 mg/kg). In rats with severe hyperglycemia (>300 mcg/dcl) which suffered from the diabet during two weeks and had substantially reduced NCV the treatment with TOA(±),(+) and (-) (30 mg/kg daily and twice/day) during two and four weeks was performed. The determination of NCV at the end of treatment revealed that there was not restoration of declined NCV to that level which was observed in age and weight-matched control animals but further declining of NCV was significantly reduced. This effect as well as the reduction of hyperglycemia was registered only in animals treated with TOA(±) and(+). Discontinuation of the treatment was followed by declining of NCV which was more pronounced in animals treated during two weeks and to a less extent - in four-weeks treated rats. Therapeutic effect was also more pronounced in twice/ day treated rats in comparison with one/day administration of drugs. Obtained data showed that TOA(±) possessed therapeutic activity under conditions of well pronounced diabet polyneuropathy. TOA(+) mediate therapeutic efficacy of TOA(±) and caused more pronounced action in a course of prolonged and frequent administrations.

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THYMIC FUNCTION AND ACTIVITY OF NK CELLS IN GENETICALLY DETERMINED DIABETES MELLITUS IN BB RATS. V.F.Chebotarev, G.A.Zubkova, T.F. Zacharchenko

Previously we showed a considerable decrease (8-10times, $P<0.01$) of thymic serum factor (TSF) level assessed according to J.-F.Bach (1975) in experimental (streptozotocin induced) and clinical type 1 diabetes mellitus. At the initial stage of the disease there is a significant increase of NK cells activity (assayed by the method of M.P.Rykova). The aim of the pressent research was to study these indices in BB which rats are known to be a classical model of hereditary insulin-dependent diabetes mellitus, before and after the first sighs of hyperglycemia have arisen. A significant decrease of TSF level (3.5+0.21 vs 5.3+0.19 in controls) and increase of NK cells activity (39.0 +1.5 vs 15.0+4.0 in controls) were noted in animals, homozygous in the gene of diabetes as compared to heterozygous ones, on the 80th day of life, i.e. before hyperglycemia occurs, which becomes detectable on the 100-120th day of life. After the first signs of the disease have emerged the studied indices decrease by 15-20%. Thus, it was shown that inhibition of thymic function and stimulation of the NK cells activity precede hyperglycemia and, therefore, are not caused by the changes in insulin level. An attempt has been made to attribute the results obtained to the changes in the immunological status effected by the autoimmune process which is the background for type 1 dibetes mellitus. The role of NK cells in etiology and pathogenesis of insulin-dependent dibetes mellitus in suggested.

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COMPORISON EFFECTS OF CALMODULIN ANTAGONIST AND STEROID DRUGS ON BURN EDEMA.

KHAKSARI M.,KHOSHBATEN A.,HADYIZADEH S.,RAHIMI M.

Previous expriments have revealed that calmodulin antagonists are effective drugs in treatment of some of skin burns.In our previous expriments we investigated the effect of these drugs on burn edema formation,and we found that trifluoperazine (TFP) is effective drug.

The present study was designed to comparise the betamethasone ,clobetasol and TFP effects with other.Experimental burns wee produced on the shaved back of rats.Two series of standard $4cm^2$ burns was inflicted using 2-sec contact with a metal rod heated to 100°c.

Vascular permeability changes were assessed by calculation of water content and by extravasation of Evans blue , drugs were injected immediatly after induction of burns.These studies have shown that TFP and betametazone caused signifacant reduction ($p<0.05$) of Evans blue dye extravation and therefore reduce leakage of albumine in burned skin . However TFP was more effective than betamethasone. Also water content in burned skin was siginficantly,reduced by betametasone. Certainly ,a discrepancy was observed between albumine leakage and acumulation of fluid. These data suggest that oxygenation products of arachidonic acid and ca++- calmodulin pathway may be important in albumine leakage and that factors other than colloid osmotic pressure are resposible for edema formation.

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THE ROLE OF DISTURBANCES OF MEMBRAINE FUNCTIOS IN THE DEVELOPMENT OF LENS OPACITIES. I. Metelitsina, S. Kolomiychuk and N. Leus

There is no single theory, which explains the mechanisms of development of ageing lens opacities because of numerous factors, promoting their origins and progression. Terefore we have undertaken an attempt to determine regularities of cataractogenic alterations in lens. With this purpose we have eleborated a model of ligt cataract similar by clinical signs to senile cataract. In conditions of chronic irradiation of animals by polychromic ligt of high intensity inhibition of enzymes γ-glutamyltranspeptidase and Na,K-ATPase and also diminution of Vitamin E level and peroxide resistance of erythrocytes in blood was revealed in early stage of development of experimental cataract and in some cases in preclinical stage.By modelling of ligt cataract in the conditions of injection of dimethylsulfooxide was determined increase a number of precataractogenic alterations 2 times and increase of cases of primary cataract on 46%, and also more marked diminution of erythrocytes membrane stability and activity of γ-glutamyltranspeptidase in blood plasma, Na,K-ATPase and thiaminpyrophosphatase in liver and lens. Correlation between functional state of biomembranes metabolic status of organism and sensibility of animals lens to the common action of polychromic light and small doses of ionizing radiation was determined Therefore disturbances of membrane structures of whole organism and lens are important mechanism in the development of lens opacities out of dependence the factors, which are caused these opacities.

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TO THE QUESTION OF DIABETIC MICRO- AND MACRO ANGIOPATHIAS OF THE PULMONARY CIRCULATION

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Gemodynamics of pulmonary circulation of ill people with diabetes mellitus is studied little because of complication and jeopardy of catheterization of heart of these ill people. By means of invasion method with the help of taking cardiography, phonocardiography, jugular flebography, ventricle cardiac right cardiography with the carrying out of calculation of pressure in pulmonary artery according to the formula Burstein in L.I.Levina's modification (1976), 34 ill people were studied. Among them 21 people had IDDM, 13 ill people had IIDM. There were 25 men and 9 women. 26 ill people had decompensation of II degree, 8 people had II-III degree. Age range of ill people was 14-60. The research was conducted in dynamics for ill people before 40 in the condition of decompensation and compensation. Material was processed statistically. As the research shows, ill people with diabetes after 40 in the condition of decompensation have the bigger period of isometric enfeeblement of ventricle cardiac right, which characterizes the altitude of pressure in pulmonary artery, than ill people before 40. In the condition of compensation ill people before 40 have the smaller period and accordingly pressure in pulmonary artery, which at the same duration of disease and absence of pulmonary diseases proves that microangiopathias in the system of pulmonary artery are present.

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CHERNOBIL'S SYNDROME. A. Beloshitsky, H. Kurdanov, A. Pischalenko, A. Krasuk, A. Ivanov

The study of the different aspects of the problem of functional reliability of the organism under extreme radioactive conditions for Ukraine population is considerably important. On the first step of our study we put attention on the similarity of the High Altitude Disease and Chernobil's syndrom. It was discovered that the Chernobil's syndrom is the result of external and internal radioactive influence and include vegetovascular distony, discirculatory encephalopathy, respiratory allergy, hemodynamic and microcirculation disturbances. It is of paramount importance the polyfunctional disturbances in the system of O_2 delivery and utilization, that leads to hypoxic states. The complex treatment of the patients with Chernobil's syndrom on High Altitude (2100m, Elbrus region) included step by step adaptation, terrencures, vitamin and antioxydant drags, were lead to stable remission and recovery. To evaluate the oxybiotic processes of O_2 transport and utilization we developed the special Kibernetic model for dynamic gas transport studing.

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The mechanism of Human adaptation

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The action of new ecologikal factors on human body produces the chanses of cell structure and functions of different organs.We studied the action of small doses of toxic substances (endotoxins,xenobiotics) in experiments on Wistar rats on blood leucocytes, mast cells and lung macrofags. In experiments the stracture of cells was damased,the cell metabolism was distroyed, the activity of dehydrogenases, phosphotases and oxydate enzymes decreases. The vacuolization of cytoplazm was observed.

The pesticede - hexachlorcyclohcxan was given orally in non - toxic doses during 6 mouths. It stimulated metabolism, function and cell resistance during first two months: the organism adaptation takes place.

The continueing administration of pesticede decreases the adaptive abilites of an organism.The administration of non-toxic doses of pesticede - hexochlorcyclohexan produces the adaption of the organism cell systems.

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AIR DISTRIBUTION, FLOW-VOLUME CURVE AND ALVEOLAR GAS COMPOSITION IN DIFFERENT BODY POSITIONS. P.-H.Kingisepp

The effect of the supine in comparison with the upright position on the lung function was studied in 58 human volunteers. The forced expiratory flow-volume curve was registered by the spiroanalyzer Pneumoscreen II (E.Jaeger, Germany), and alveolar gas composition was recorded by a mass spectrometer (MX 6202) in 15 men (mean age 19.8±0.4 years). All data are represented as mean values±standard errors. Gas distribution was measured by the single-breath N_2 technique (P.K.Morgan, Ltd., England) in 43 persons (24 females and 19 males, mean age 20.1±0.8 and 19.2±0.7 years respectively). The differences between the data collected in the sitting and supine positions were evaluated by the paired t-test.

Compared to the upright position in the case of the supine posture, diminished forced expiratory flow rates were observed, which confirms the previous studies and suggests increased airflow resistance. We found that alveolar CO_2 was higher in recumbency (6.01±0.07 %) than in upright position (5.69±0.08 %) and the alveolar N_2 slope (phase III) was steeper and the closing volume was higher in females in the supine than in the sitting position (0.83±0.06 and 0.99±0.05 % per liter; 0.195±0.02 and 0.276±0.026 l respectively).

Conclusion. The decreased forced expiratory flow-volume curve parameters in the supine position suggest increased respiratory resistance. Changes in body position also influence the distribution of the inspired air and ventilation-perfusion relationships.

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DYNAMIC SPIROMETRY IN CHILDREN: SEX DIFFERENCES. J.Kivastik

A study was performed to find out whether there are sex differences in the performance of lungs in children.

We made a cross sectional study of 1305 children (625 boys) aged 5.0 to 18.5 years. We measured standing height and weight, and the spiroanalyser Pneumoscreen II, *Jaeger* was used to record forced vital capacity (FVC), peak expiratory flow (PEF) and forced expiratory flow when 50% and 75% of FVC had been exhaled (FEF_{50} and FEF_{75}). The maximum envelope of at least three similar flow-volume loops was analyzed. To examine the expiratory flow relative to lung volume, we divided PEF, FEF_{50} and FEF_{75} for each subject by his/her FVC. The results were grouped in 5 cm blocks so that the first group represented children of standing height 115.0 . . . 119.9 cm inclusive, and so on. Differences between the same height groups in boys and girls were assessed for statistical significance using the Student's unpaired t-test. FVC was higher for boys in all groups although the difference was not significant in the height range 150.0 . . . 164.9 cm. Before controlling for lung size, PEF tended to be higher in boys, except the height range 150.0 . . . 164.9 cm. After 130.0 cm, female FEF_{50} and FEF_{75} were higher, although the performance was significantly better only between heights of 150.0 . . . 164.9 cm. Volume-adjusted FEF_{50} and FEF_{75} in girls were significantly higher in all groups after 130.0 cm. Volume-adjusted PEF was also higher in girls, but the differences were significant only in the height range 145.0 . . . 165.0 cm.

In conclusion, when lungs of the same size are considered, sex differences are found. Girls have superior airflow per unit lung volume, the higher female performance is particularly noticeable in the later flow measures (FEF_{50} and FEF_{75}). This finding confirms the suggestion that girls have shorter but wider airways than boys.

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IN VITRO MEASUREMENTS OF FLOW WITHIN BRONCHIAL BIFURCATIONS. A.Tippe; S.Häußermann.

Convective transportation and deposition of aerosol particles within the bronchial tree are controlled by a complex air flow. Due to the capabilities of modern computers, calculations of this bifurcating three-dimensional flow field, e.g. by the FIDAP software, are considerably improved. Nevertheless, the results should be controlled by experiments. We, therefore, developed a method to obtain hollow-casts of bronchial tree bifurcations consisting of high transparency, elastic walls (n_D= 1.41). Applying a modified Pulsed-Laser-Light-Sheet method (M.Kratzer, J.Kinder,Biomed.Technik: 20,11-12,1975) streamlines of a fluid flow (n_D= 1.47) are visualized and the 3D-velocity fields at different Reynold-numbers are quantitatively measured using the OPTIMAS software for image analysis. A comparison of stationary flow fields within hollow-casts and glassmodel bifurcations will be presented by means of a short video-film. Secondary flow regions which appear in the glassmodels at relative small Re-numbers (Re≈50) are not observed within the hollow-casts.

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DIAMETER-BASED ANALYSIS OF THE BRANCHING GEOMETRY OF FOUR MAMMALIAN BRONCHIAL TREES

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A diameter-based classification technique, which we have previously used to analyse human bronchial geometry (Phillips et al., Respir. Physiol. 98:193-217, 1994a) is applied also to the partial measurements of the dimensions of the conducting airways of four mammals, made by Raabe et al. (Tracheobronchial Geometry: Human, Dog, Rat, Hamster, 1976). The local branching patterns are characterised by means of three parameters, which reflect the asymmetry and the degree of expansion at each bifurcation, and the ratio of the length of each branch to its diameter. The mean values of these parameters as functions of diameter, calculated from the raw morphometric data, are shown for the four species. A statistical reconstruction technique is then used to estimate from the incomplete measurements of Raabe and coworkers some geometrical properties of the whole system of conducting airways. These include the distribution of the total airway volume between branches of different diameters and the mean and variance of the total lengths of different pathways through the bronchial tree. Using a simple flow model, we demonstrate how these geometrical characteristics are manifest in the distribution of velocity within the bronchial tree, and the variation of "time of flight" from trachea to respiratory region. Our results show significant differences between the branching structures, and thereby the velocity distributions, in all four species. In particular, the human airways branch much less asymmetrically and branches of a given diameter are typically much longer than in the other species. These differences in branching geometry between species have implications for the extrapolation of gas/particulate transport data obtained from animal experiments to humans.

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PERIPHERAL AND CENTRAL VENTILATORY DOPAMINERGIC CONTROL IN THE RAT. D. Lagneaux and J. Lecomte

In anesthetized Wistar rats, iv injection of dopamine (DA) provoked a transient inhibition of the ventilation (max -37 ± 18 % at 8 s, -8 ± 4 % at 50 s for 85 μg/kg). As DA does not cross the blood-brain barrier (BBB) and as this inhibition was suppressed by domperidone (DO, 1 mg/kg), a peripheral D2-receptor blocker, exogenous DA clearly inhibits the peripheral glomic chemoreceptors afferences. To explore the role of an eventual central dopaminergic system in the ventilatory control, D2 agonist bromocriptine (BR) which penetrates BBB has been used. BR first iv injection induced a delayed and prolonged hypoventilation (-22 ± 4 % at 35 s, -9 ± 6 % at 5 min for 100 μg/kg). Following injections were without ventilatory effects indicating tachyphylaxis. Furthermore, when DA was injected 5 to 20 min after BR, DA ventilatory inhibition was reduced (-12*± 3 % at 8 s, -4 ± 5 % at 20 s). This suggests a prolonged BR fixation on its receptors. After DO peripheral blockade, even the first BR injection failed to modify the ventilation. But, after peripheral and central blockade by haloperidol (HA, 1 mg/kg), BR first ventilatory response became biphasic. The initial hypoventilation was reduced (- 9*± 2 %) and a delayed hyperventilation developed (5* ± 0.6 % at 5 min). These differences between antagonistic activities of DO and HA indicate that D2 dopaminergic mechanisms play a role in ventilatory control at central and peripheral levels. Both are essentially inhibitory, but their simultaneous blockade by HA also evidenced a secondary central BR excitatory effect. It could be explained by a stimulation of extra D2-receptors mechanisms or by an activation of non-dopamigergic process.

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SIMULATED COUGH AND EXPULSION EFFECT OF HIGH FREQUENCY JET VENTILATION IN RABBITS. *K. Javorka, Ľ. Šutarík, M. Petrášková, A. Drgová, P. Račay*

The aim of the study was to determine effectiveness of the expulsion effect of the high frequency jet ventilation (HFJV) itself and in combination with simulated high frequency modulated cough.

In acute experiments in 26 anaesthetized, artificially ventilated (HFJV) rabbits, the dye (Evans blue - 10 mg) in plasma (1 ml/kg) was injected by means of the HFJV inpulsion effect into the lungs. During the next 60 minutes the first subgroup of the rabbits were ventilated by the neutral regime (ti=0.5), the second subgroup by the expulsion regime (ti=0.7) and the third by the expulsion regime combined with an simulated high frequency (300 x min^{-1}) modulated cough. During the experiment, the parameters of the mechanics of breathing, ventilatory parameters, airways and blood pressures were continuously recorded. After the experiment, the lungs were withdrawn and the amount of the dye remainder was evaluated.

The biggest percentage of the eliminated material was in third subgroup with the expulsion effect combined with simulated cough (40.7 ± 3.2 %), in subgroup with expulsion effect it was 24.4 ± 3.5 % and with the neutral regime 13.7 ± 2.8 %. Concomitant changes in parameters of the mechanics of breathing, ventilation pressures, blood gases and in indexes of oxygenation correlated well with the percentage of the elimination.

The results corroborated the previous findings (Brychta et al., Europ J. Anaesth. 1985, 2, 2 and Javorka et al., Europ. Resp. J. 1993, Suppl. 17, 445) that the expulsion regime of the HFJV is effective method for cleaning the bronchoalveolar compartments and that this method can be amplified by the use of the high frequency modulated artificial cough.

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VARIABILITY OF LUNG FUNCTION WITH AGE IN NON-SMOKING BLACK SOUTHERN AFRICANS

M. H. Shamssain.

We studied 1566 black Southern African adults from Transkei aged 20-60 years. The subjects were non-smokers. Forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and forced expiratory flow between 25% and 75% of FVC (FMF) were studied. We applied the American Thoracic Society (ATS) 1987 criteria for selection of the best pulmonary function test. Logarithms of lung function parameters were estimated. Logarithms of lung function was regressed against age and logarithm of height separately for each sex. Results are presented in the table. The present study illustrates that there is a veriability of lung function with age and that the lower limits of normal lung function should be replaced by 5th or 10th percentiles.

MALES:

FVC (l) = 0.049HEIGHT - 0.031AGE - 1.684
FEV1 (l) = 0.025HEIGHT - 0.029AGE - 0.061
FMF (l/s) = 0.022HEIGHT - 0.051AGE + 1.341

FEMALES:

FVC (l) = 0.035HEIGHT - 0.024AGE - 1.712
FEV1 (l) = 0.023HEIGHT - 0.024AGE - 0.473
FMF (l/s) = 0.015HEIGHT - 0.036AGE +1.464

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DIFFERENT RESPIRATORY EFFECTS OF BARORECEPTOR A- AND C-FIBRES. R. Staak, S. Staak, K. Scheufler and H. Opitz

In rabbits, the aortic nerve is containing afferent baroreceptor A- and C-fibres, but no chemoreceptor fibres. Therefore, we used rabbits to study the different effects of electrical stimulation of baroreceptor A- and C-fibres with respect to respiration.

In 18 anaesthetized spontaneously breathing rabbits the central end of the cut left aortic nerve was stimulated electrically and the compound action potentials were recorded. Respiratory flow was measured by pneumotachography. The inspiratory and expiratory times and the duration of late-expiratory zero flow were computerized.

Selective stimulation of baroreceptor A-fibres (0.02ms, 80Hz, 2-4V) induced a slight decrease in breathing frequency and a prolongation of the late-expiratory zero flow. The peak respiratory flow increased significantly.

In contrast, simultaneous stimulation of baroreceptor A- and C-fibres (2ms, 10 and 20Hz, 10V) markedly increased breathing frequency, due to a decrease in expiration time. Both the negative respiratory flow and the late -expiratory zero flow phases were shortened. The amplitude of respiratory flow was found to be significantly reduced.

Both types of stimulation produced a fall in arterial blood pressure.

It is concluded that the two types of baroreceptor afferent nerve fibres elicit different respiratory effects.

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MODULATION OF BRONCHIAL RESPONSIVENESS BY LOCAL INFLAMMATION. EFFECTS OF CALCIUM CHANNELS BLOCKERS. *Fr. Schneider, C. Bunu, G. Tanasie, I. Siska, V. Păunescu, D. Vancea, V.M. Tudorache*

Bronchial hyperreactivity and local inflammation are characteristic features of airway hyperresponsiveness. We studied the isometric contraction on human bronchial spiral strips fixed into organ-bath. We obtained the dose-response curves to Histamine and Acetylcholine and we followed the relaxing effect of Nifedipine (Tab I). To examine the relation between ventilometric parameters changes and local inflammation, we investigated 16 asthmatic patients (AP) in comparison with 6 healthy subjects. AP were nonsmokers, clinically stable, without corticotherapy and had no evidence of chest infection. Ventilatory function tests and airway resistance measurements were performed in basic conditions (Raw1) and 30 min after a single sublingual administration of 20 mg Nifedipine (Raw2) using a computerized Jaeger pneumotachometer. In AP, cytologic examinations - total cells (TC) and leukocyte populations - of bronchoalveolar lavage (BAL) and blood were done after cyto-centrifugation and staining with May-Grunvald-Giemsa. Specific binding of monoclonal antibodies for CD3(T cells), CD4(T helper), CD8(T cytotoxic), CD19(B cells), CD16/52 (NK), HLA-DR and CD11c (integrin family) was analyzed by direct immunofluorescence using a Becton-Dickinson flow-cytometer (Tab II). We can conclude that "in vivo" airway responsiveness to calcium channels blockers is less potent than "in vitro" due to local inflammatory process and shedding of epithelial cells (EC) into the bronchial lumen. Bal cellular infiltrate is characterized by the presence of activated eosinophils (E) and Th CD4+. The expression of CD11c on BAL and blood E is correlated with the preferential E recruitment to the asthmatic airway.

Drugs	Contractile dose			10^{-4} niffedipine effect on 100% induced contraction					
	minimal	50 %	100 %	5 min	10 min	15 min	20 min	25 min	30 min
Hist.	$8x10^{-5}$M	10^{-4}M	10^{-3}M	-28%	-50%	-100%			
Acet..	10^{-5}M	$4x10^{-5}$M	10^{-3}M	-	-	-	-22%	-46%	-70%

Tab.II

BAL									Raw kPa/(l/s)			
TC	E	N	CD4	CD8	HLA	CD11c	NK	EC/TC	PATIENTS		CONTROL	
10^{-3}/ml	%	%	%	%	DR%	%	%		Raw1	Raw2	Raw1	Raw2
150	6	5	2.8	6	3.7	10	.8	.7	.756	.491	.386	.338
±70	±4	±3	±1	±2	±1	±5	±.6	±1	±.23	±.10	±.31	±.12
									p<0.02		p>0.05	

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CONTRIBUTION OF Na^+- GLUCOSE COTRANSPORT TO LIQUID ABSORPTION FROM THE PLEURAL SPACE. L.Zocchi, E.Agostoni and A.Raffaini

The relationship between the net rate of liquid absorption from the pleural space and the volume of liquid injected (0.5; 1; 2 ml) was determined in anesthetized rabbits during hydrothoraces with phloridzin 10^{-3} M. The relationship was parallel to the control one, and displaced downwards by 0.09 ml/h ($P < 0.01$): this suggests the occurrence of a Na^+ - glucose cotransport on the luminal side of the pleural mesothelium, operating also under physiological conditions. Since we have previously shown the occurrence in the pleural mesothelium of a Na^+/H^+ - Cl^-/HCO_3^- double exchange (Agostoni and Zocchi, Respir. Physiol. 92: 101-113, 1993), we also made 2 ml hydrothoraces with SITS $1.5 \cdot 10^{-4}$ M and with phloridzin plus SITS. In the hydrothoraces with phloridzin plus SITS the decrease in net rate of liquid absorption was 0.18 ml/h, i. e. similar to the sum of those produced by SITS (0.09 ml/h) and by phloridzin (0.10 ml/h) alone. Finally, we performed 0.5; 1; 2 ml hydrothoraces with phloridzin plus amiloride $2 \cdot 10^{-3}$ M. The relationship between the net rate of liquid absorption and the volume of liquid injected was displaced downwards by 0.17 ml/h ($P < 0.01$), i. e. to an extent similar to that previously found with amiloride alone. This likely occurs because amiloride at this concentration (besides inhibiting the double exchange) may inhibit also the Na^+ - glucose cotransport (Cook et al., Am. J. Physiol. 253: C199-C204, 1987) and/or the Na^+/K^+ pump (Soltoff and Mandel, Science 120: 957-959, 1983).

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α-TRINOSITOL ABOLISHES THE INCREASED NEGATIVITY OF INTERSTITIAL FLUID PRESSURE (P_{if}) IN RAT TRACHEA AFTER ALLERGEN CHALLENGE. K. Woie, E. Westerberg*) and R.K. Reed.

Acute airway inflammation induced by mast cell degranulation or vagal nerve stimulation induces increased negativity of P_{if} concomitant with edema formation. A negative P_{if} will greatly enhance the capillary fluid filtration and edema formation. Since airway inflammation is important in the pathophysiology of asthma, the effect on P_{if} of allergen challenge to sensitized rats was studied. The anti-inflammatory effect of 6.25 mg hydrocortisone or 10 mg α-trinositol (D-*myo*-inositol-1,2,6-tri-phosphate (Perstorp Pharma, Sweden)) was also studied. 28 rats were sensitized with i.p. injection of 2 mg egg albumin in 1 ml Complete Freund's adjuvant given on day 1 and 5. Experiments were performed on day 21-26, with i.v. injection of the same solution. P_{if} was measured in rat trachea, after circulatory arrest had been induced in pentobarbital anesthesia, with sharpened glass micropipettes connected to a servocontrolled counterpressure system. Circulatory arrest is induced to prevent edema formation which would cause underestimation of a potentially increased negativity of P_{if}. Allergen challenge to sensitized rats decreased P_{if} from -1.27 ± 0.4 (n=7) (SD) mmHg to -5.76 ± 0.5 mmHg (n=6) ($p < 0.01$), but had no effect in non-sensitized rats. α-Trinositol, given either prior to or after allergen challenge, abolished the increased negativity in P_{if} ($p > 0.05$ compared to control and $p < 0.05$ compared to allergen alone), while hydrocortisone had no effect. Airway resistance was calculated from maximum inspiratory pressure using a constant volume respirator and increased significantly in sensitized rats after allergen challenge (from 2.69 ± 0.61 to 5.88 ± 1.74 breaths*mmHg/ml) ($p < 0.05$), but did not change in control rats. Pretreatment with α-trinositol had no effect on airway resistance. Thus, increased negativity of P_{if} contribute to the edema formation in this acute asthmatic reaction. α-Trinositol, but not hydrocortisone, abolished the increased negativity of P_{if}.

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OXYGEN AVAILABILITY IN MUSCLE TISSUE OF RATS AT HIGH AMBIENT PRESSURE. J.A. Donina, G.V. Troshihin, A.S. Ivanov.

The nature of the influence the density of inhaled gas mixture on oxygen metabolism in organism remains unclear. For this purpose we investigated the oxygen availability (aO_2) in femoral muscle and rectal temperature ($T^\circ r$) in unanestetised rats exposed at 40 ATA in helium-oxygen (He-O_2) mixture (N_2-O_2). A constant partial pressure of O_2 (0.21 ATA) was maintained in all gas mixtures and under all pressures. The temperature N_2-O_2 mixtures was 26°C, but in He-O_2 was 28°C or 30°C. The results indicated, that under conditions at 6 ATA when the density of N_2-O_2 mixture corresponds to that of He-O_2 mixture at 40 ATA, changes in aO_2 in muscle and in $T^\circ r$ were similar to those under control conditions. Increasing the pressure N_2-O_2 mixture to 20 ATA resulted in substantial decrease of aO_2 in muscle while $T^\circ r$ did not change significantly. This decrement of aO_2 in muscle cold be due to the increased density or to nitrogen narcosis. The results suggests that the decrease of aO_2 in muscle tissue resulting from exposure at 40 ATA in He-O_2 mixtures is not related to the increase of the density inhaled gas, but is a result the cooling effect of helium. When a higher temperature (30°C) is used the heat balance in rats is not disturbed and the aO_2 in muscle remain normal. Thus a five-fold increasing the density of inspired mixture (40 ATA He-O_2 and 6 ATA N_2-O_2) did not appear to be an important determinant of oxygen metabolism in muscle.

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THE CONTROL OF BREATHING UNDER CONDITIONS OF ALTERED DENSITY OF THE GAS MILIEU DURING EXERCISE.
M.O.SEGIZBAEVA AND G.G.ISAEV

In 10 young healthy subjects at resting the substitution of air by a helium-oxygen ($He-O_2$) or elegas-oxygen (SF_6-O_2) normoxic mixture didn't induced significant changes of minute ventilation (V_E), tidal value (V_T), the duration of the each respiratory cycle (T_I, T_T), breathing frequency (f), alveolar P_{CO2} (P_{ACO2}) in normobaric conditions. In the moderate constant-load exercise (approximately 50 % from maximal work capacity) the inhalation of $He-O_2$ reduced V_T and increased f, but the inhalation of SF_6-O_2 increased V_T and reduced f from the first respiratory cycle after substitution of air by $He-O_2$ or SF_6-O_2. No significant differences were observed in pulmonary ventilation and alveolar P_{CO2} during air, $He-O_2$ and SF_6-O_2 inhalation in the exercise. Both at resting and in exercise the substitution of air by $He-O_2$ significantly reduced the central inspiratory drive, the work of breathing and electrical activity of inspiratory parasternal muscles, whereas the inhalation of SF_6-O_2 led to obvious effects: the effort for even the least maximal inspiratory flow increased in the same lung ventilation as when breathing air and in constant chemoreceptor stimulus. The central inspiratory activity and EMG parasternal muscles increased nearly three-fold. The compensatory responses of the respiratory system seem to appear on the basis of afferents from the lungs and respiratory muscles mechanoreceptors as well as on account of the segmentary level reflexes and the properties of a muscular fibre itself.

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THE ROLE OF SENSORY COMPONENTS IN RESPONSES OF HUMAN RESPIRATORY SYSTEM TO INCREASING LOADS
G G.Isaev and M.O.Segizbaeva

In 13 healthy male subjects performing an increasing work on veloergometer in conditions of resistive respiratory loads, the moments of sensation of respiratory difficulties and refusal from the work were recorded. The volume-time parameters of respiratory cycle (pneumotachography) were registered in the course of the experiment, bioelectric activity of inspiratory muscles, initial inspiratory activity P100, esophageal pressure was registered, respiratory work and the value of non-elestic resistance in lungs was calculated, electrocardiogramme was registered and the minute volume of blood circulation was defined by the method of return breathing. The analysis of the respiratory system functionas revealed that the respiratory discomfort occurred with increasing of the alveolar Pco2 up to a certain value and that the refusal occurred on a certain level of inspiratory activity. The subjects believed that the moment of refusal coincided with the complete exhaustion of the respiratory system reserves. Their lung ventilation, however, as well as inspiratory effort were far from reaching the maximal values obtained in special tests. It was established that the most important factor restricting the tolerance of exercise in added resistance to breathing was the limitation of functional possibilities of respiratory muscles. The initial signs of their fatigue were defined. It is suggested that the increased afferentation from respiratory muscles in the sourse of sensation of dyspnea and makes man to stop work thus preventing about extreme tension in the activity of respiratory muscles The situation gets worser by the haemodynamic disorders in lung because of the increase in extracardial pressure in thorax

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CHANGES IN POTENTIAL DIFFERENCE OF ISOLATED TRACHEAL WALL AFTER COUGH RECEPTOR STIMULATION. T. Tyrakowski, I. Wojciechowska, B. Banach, A. Mosciboda

We have studied if the gentle mechanical stimulation of cough receptors of isolated rabbit tracheal wall can influence the transepithelial potential difference (PD) by means of liberated neuropeptides. The study was performed in a modified Ussing apparatus and stimuli were jet rinsing or touching with a loop of polyethylene tubing. By applying different inhibitors of ion transport and blockers of receptors, evidence was produced that Cl^- and Na^+ transport processes were involved in an augmentation of PD after stimulation of cough receptors, and the probable mediators of the reaction were belonging to the NANC system. We put forward the hypothesis that similar reactions in vivo could be responsible for production of respiratory tract epithelial lining fluid during and after cough reflex.

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CYTOCHROME B558 OF NADPH OXIDASE IS NOT ESSENTIAL FOR HYPOXIC INDUCTION OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR AND ALDOLASE GENES.

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Many cell types respond to hypoxia with increased transcription of certain genes, e.g. those coding for erythropoietin, vascular endothelial growth factor (VEGF) and glycolytic enzymes like lactate dehydrogenase A or aldolase. The heme protein cytochrome b558 of the NADPH oxidase complex such as the one found in leukocytes has been proposed as a key component of the oxygen sensing mechanism. Cytochrome b558 is a heterodimer of the p22phox and gp91phox polypeptides and is essential for superoxide generation via NADPH oxidase in phagocytes and B-lymphocytes. Mutations of the p22phox or gp91phox genes are the molecular basis of cytochrome b558-negative Chronic Granulomatous Disease, where superoxide generation via NADPH oxidase is deficient in phagocytes, B-lymphocytes and B cell lines derived from the patients.
Two normal B cell lines expressing cytochrome b558 showed increased levels of mRNA for VEGF and aldolase after 16 or 65 h of hypoxia (1% oxygen), compared with normoxia (20% oxygen). Two p22phox- and two gp91phox-deficient Chronic Granulomatous Disease B cell lines were tested under identical conditions; all four showed unimpaired hypoxia-induced upregulation of VEGF and aldolase mRNA compared with the normal B cell lines.
Thus, cytochrome b558 of the NADPH oxidase complex appears not to be essential for hypoxia-induced activation of the VEGF and aldolase genes in human B lymphocytes and can be excluded as a candidate for a universal oxygen sensor.

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ARE DOPAMINE AND CARBACHOL INVOLVED IN RECEPTOR MEDIATED DEGRADATION OF PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE IN THE NORMOXIC CAT CAROTID BODY.

R.P. Strosznajder and M. Pokorski

Dopamine (DA) and acetylocholine are neurotransmitters in the carotid body (CB) chemoreceptor cells but their action remains still highly controversial. It is known from our previous studies that the signal transduction mechanism of the CB is connected with enzymatic breakdown of phosphatidylinositol-4,5-bisphosphate (PIP_2) to inositol-1,4,5-trisphosphate (IP_3) and diacylloglicerol (DAG). In this study we wished to examine the effects of DA and carbachol, a muscarinic agonist, on PIP_2-phospholipase C (PLC) activity. CBs were dissected from normoxic (20 min; $PaO_2 \approx 90$ mmHg) cats under otherwise normal arterial blood gas and acid base status. CBs were preincubated in the Krebs-Ringer buffer pH 7.4 in the presence of 100 μM DA together with 10 μM pargyline or 1 mM carbachol for 6 min at 37°C in a shaking water bath. Subsequently, CBs were homogenized. The homogenates were then used as the source of PLC for the determination of this enzyme activity acting on the exogenous substrate [^{3}H]PIP_2. The activity of PLC was determined from the level of IP_3 radioactivity. The results showed that both 100 μM DA and 1 mM carbachol stimulated PIP_2 degradation by about 20-30%. The effect was augmented in the presence of 100μM GTPγS, a nonhydrolizable analog of GTP. We conclude that dopamine and carbachol may have modulatory effect on the signal transduction pathway in the CB through IP_3 and DAG liberation.

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DEMONSTRATION OF NMDA RECEPTORS IN CATFISH ELECTRORECEPTOR ORGANS.

G.N. Andrianov, F. Bretschneider and R.C. Peters

Electroreceptor organs of fish are considered to be part of the acoustico-lateral system. L-glutamate appears to be one of the most probable neurotransmitter at the afferent synapse of catfish electroreceptor organs, and its action is mediated by distinct kainate and AMPA subsynaptic receptors. NMDA has no effect, even at 5.0 mM. With the aid of electrophysiological approach, we studied whether NMDA receptors are present and functional at this level. Recording of action potentials from primary afferents was performed in isolated flaps of skin of the catfish *Ictalurus* while the serosal surface of the skin was constantly perfused with normal and test solutions. Our results show that addition of 5 mM NMDA to the Mg^{2+}- free solution induced a pronounced and enduring increase in the firing rate and a sustained level of resting activity. Under the condition of electrical stimulation of the sense organs (frequency 9 Hz, current density 70 nA/cm^2) application of NMDA produced a clear-cut frequency increase, even in the normal solution. Specific NMDA antagonists such as ketamine, APV, and 7-chloro-kynurenic acid (7ClKyn) in concentrations of 0.1-1 mM inhibited both the resting discharge and the electrically evoked activity. The 7ClKyn also antagonized the excitatory effects of NMDA in Mg^{2+} - free solutions. The glycine agonist D-serine reversed the inhibitory action of 7ClKyn. Glycine (0.01 mM) potentiated the NMDA responses in a Mg^{2+} - free solution. In conclusion, our results suggest the presense of NMDA receptors at the afferent nerve fibre synapse of electroreceptor cells.

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OFFSET CURRENTS IN CATFISH ELECTRORECEPTORS MEASURED WITH THE VIBRATING PROBE.

F. Bretschneider, R. Créton, M.R. Dohmen, P.S. Heijmen and J.P. Versluis.

After it had been found that fresh-water media lacking calcium inhibited the functioning of electroreceptors (Roth A, 1971: Z. vergl. Physiol. 75, 303–323), we found that this malfunctioning could be both mimicked (by a lumen-outward current) and corrected (by a lumen-inward current). This apparent equivalence of ion composition of the medium and direct current stimulation led us to postulate the hypothesis that receptor cells and normal epithelial cells differ in their response to the composition of the fresh-water environment, resulting in an outward offset current. In addition, the normal receptor functioning had been suggested to depend on a steady, inward bias current (Zakon HH, 1986: in Electroreception., Bullock, TH & Heiligenberg W, eds., Wiley, New York). Unfortunately, the concomitant voltage differences are too small to be measured with conventional microelectrode techniques. Here, we present the results of applying the so-called vibrating-probe technique (Jaffe L & Nucitelli R, 1974: J. Cell Biol. 63, 614–628) to measure the mentioned currents around catfish electroreceptors. This technique employs a vibrating metal microelectrode together with a lock-in amplifier (synchronous detection). We found that the offset current, expected in the calcium-free water, does indeed occur, and that the order of magnitude is approximately correct. In the normal medium, however, the average bias current is outward. Since we found currents of both polarities, it is more likely to assume that each receptor organ has an incidental equilibrium current of random polarity, dependent on local homeostatic factors.

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FUNCTIONAL AND MOLECULAR CHARACTERISATION OF THE LUMINAL Ca^{2+} BINDING SITE OF THE INOSITOL TRISPHOSPHATE RECEPTOR.

I. Sienaert, L. Missiaen, J.B. Parys, H. De Smedt and R. Casteels.

The inositol trisphosphate receptor ($InsP_3R$) is regulated by a variety of mechanisms including luminal Ca^{2+} content. The regulation of the $InsP_3R$ by luminal Ca^{2+} was studied by measuring unidirectional $^{45}Ca^{2+}$ efflux from saponin-permeabilized A7r5 smooth muscle cell monolayers and by measuring interaction of $^{45}Ca^{2+}$ with different fusion proteins expressing parts of the luminal loop of the $InsP_3R$. Ca^{2+}-sensitive dyes can be loaded into the intracellular stores to monitor luminal Ca^{2+}. Since these dyes can potentially interfere with $InsP_3$-induced Ca^{2+} release (IICR) and its regulation by luminal Ca^{2+}, we first studied if those dyes modify IICR. Our results demonstrate that $InsP_3$ (0.5 μM) was less effective in emptying stores of permeabilized cells that were loaded with 40 μM of the low affinity Ca^{2+} dye Furaptra/AM than control A7r5 cells or A7r5 cells loaded with 2 μM of the high affinity Ca^{2+} dye Fura-2/AM. The inhibitory effect of Furaptra/AM on IICR confirms that luminal Ca^{2+} is an important regulator of the $InsP_3R$. In order to understand how luminal Ca^{2+} regulates the $InsP_3R$ at the molecular level we expressed the luminal loop of the $InsP_3R$ (aa 2461-2568) as GST fusion protein. Overlay experiments revealed that this region bound Ca^{2+} and ruthenium red. Ca^{2+} binding was subsequently mapped to a non-conserved acidic subregion of the luminal loop (aa 2463-2528). Ca^{2+} affinity and specificity were studied in $^{45}Ca^{2+}$-overlay experiments. Simulation of the data demonstrated a Hill coefficient of 1.34 and a K_D value of 0.19 μM. Divalent cations were tested as potential competitors of the $^{45}Ca^{2+}$ binding site. These cations competed with the $^{45}Ca^{2+}$ binding site in the following order $Ca^{2+} > Co^{2+} > Sr^{2+} > Ba^{2+} >> Mn^{2+}, Ni^{2+} >> Mg^{2+}$. We were unable to detect $^{45}Ca^{2+}$ binding to the conserved hydrophobic subregion (aa 2529-2568). Further experiments will be needed to determine whether the described Ca^{2+} binding site has a regulatory role or plays a role in the channel function.

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MECHANISMS OF X-RAY-INDUCED VASOMOTOR REACTIONS: EFFECT OF FACTORS INFLUENCING $[Ca^{++}]_i$. U. Welscher, M.Ch. Michailov, E. Neu, E. Hochhäuser, L. Schachinger, N. Willich, D.G. Weiss

X-Ray-induced motor reactions of various vascular preparations of human (surgical material) and animal origin have been reported previously: [Michailov et al & Neu et al, in: *Intraoperative Radiation Therapy (IORT)* (eds Willich, Schildberg, Krämling), pp 27-39, Blaue Eule, Essen, Germany (1993); Welscher et al: *Arch Pharmacol* **346**, R27 (1992) & *Hepato-Gastroenterol* **41**, 16 (1994)]. A summary of new and earlier results is given in order to elucidate action mechanisms participating in these X-ray-induced acute reactions: **(1)** Vascular preparations of human: *vasa renales, ovaricae, uterinae, et v. saphena* and animal: *aorta, v. cava, vasa pulmonales, mesentericae, et renales* [chicken, frog, guinea-pig (gp), pigeon, rabbit, rat] react to X-irradiation (50 kV, 5-5.000 cGy at 125-3.000 cGy/min) with immediate (latency: 1-20 sec) reversible, semi- or irreversible fast (phasic) and slow (tonic) contractile responses. Species and organ specific radiosensitivities vary widely. **(2)** Activation of receptor-coupled, G-protein-dependent transmembrane signal transduction by hormones [effectiveness (0.01-30 nM): noradrenalin > adrenalin > prostaglandins ($F_{2\alpha} > E_2$) > serotonine (≥ 1 μM)], application of phospholipases (PLA_2, PLC, 1-100 mU/ml) and of certain anesthetics (bupivacaine, fentanyl) sensitized significantly, whereas calcium antagonists, cyclooxygenase and phosphodiesterase inhibitors, as well as db-cAMP inhibited these X-ray contractions. **(3)** An increase in extracellular [$CaCl_2$] (1.25 to 2.50 mM) and in [LiCl] (IP_3 phosphatase inhibitor, 1-30 mM), a decrease in $[H^+]$ (35.5 to 25.1 nM, i.e. pH 7.45-7.60) and in [$MgCl_2$] (1.2 mM by 80-100%) also had a strong radiosensitizing effect. **(4)** The factors given in **(3)** augmented X-ray effects also in non-vascular smooth muscle, such as *colon* (chicken); *fundus ventriculi* (rat); *vas deferens* (gp); *vesica urinaria* (human, gp, rat). Considering the above results we suggest that in radiotoxicity, besides known processes such as formation of oxygen radicals, lipid peroxidation, and membrane perturbation, also participate mediators of transmembrane signalling, especially those of the phosphatidyl-inositol-4,5-biphosphate and arachidonate cascade may be effective in X-ray-induced increases in $[Ca^{++}]_i$ that at toxic levels lead to excessive activation of calcium-dependent processes and subsequent ATP-consumption, and further to apoptosis. Due to the concerted effects of various endogenous first and second messengers these DNA-damage independent X-ray reaction mechanisms are probably effective also *in vivo* and in other cell types. Taking into account the above suggestions data obtained by experimental X-irradiation of cells (threshold doses, radiotoxicity, etc.) under classical tissue culture conditions (monotype, monolayer, no addition of hormones) may have to be reconsidered.

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MICROVILLI CONTRIBUTE TO THE SENSITIVITY AND THE SHAPE OF THE FREQUENCY CHARACTERISTICS IN AMPULLARY ELECTRORECEPTOR ORGANS P.S. Heijmen, M.A.H. Braks, A. Boele and R.C. Peters

The receptor cells of the ampullary electroreceptor organs of Ictalurus nebulosus bear microvilli on the apical membrane. Whereas microvilli in mechanoreceptive hair cells have a transduction function, the function of these membrane specializations in electroreceptor cells is not fully understood. In order to investigate the role of apical microvilli, the ampullary organs were apically exposed to the microfilament disruptive agent cytochalasin B and D and to hyperosmotic sugar solutions.

Electrophysiological measurements demontrated that cytochalasin treatment resulted in a strongly reduced sensitivity, a slightly reduced spontaneous activity, and altered shapes of the frequency curves. Electron microscopical observations showed that cytochalasin treatment induced disorganization of the apical microvilli. Calculations with an electrical equivalent circuit of an receptor cell indicated that the reduced sensitivity and the altered shapes of the frequency curves might be due to a reduced apical surface area in combination with extra closed or less conductive ion channels. Hyperosmotic treatment also resulted in a reduced sensitivity and altered shapes of the frequency curves. Calculations indicated, however, that in this case a reduction in apical surface area only can account for the observed effects.

We conclude that intact apical microvilli are necessary for proper functioning of ampullary electroreceptor organs. Both experiments indicate that, although only the basal membrane is thought to be involved in stimulus transduction, also the apical membrane contributes to the sensitivity and the shape of the frequency curves.

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ARE THE MECHANO-ELECTRICAL TRANSDUCTION CHANNELS IN HAIR CELLS OF THE FROG SACCULUS CA CHANNELS ? F.Jørgensen and A.B.A. Kroese.

The mechano-electrical transduction by the hair cells depends on a minimal Ca^{2+} concentration of the apical solution. The role of Ca^{2+} in the transduction process is not known. The transduction channels do not discriminate between monovalent cations where-as the relative permeability to Ca^{2+} is debated.

The extracellular receptor currents from hair cells evoked by step displacements of the otolithic membrane of the isolated saccular macula of *Rana esculenta* were recorded under transepithelial voltage clamp conditions. The basolateral membranes of the hair cells were depolarized with solutions of high K^+ concentration and the electrical properties of the macula are then expected to be regulated by the apical membranes.

High K^+ concentration in the basolateral solution (62 mM) caused a shift in the non-linear receptor current-voltage relation along the voltage axis with an increase in the slope of this relation. The transepithelial voltage, at which the receptor current was zero, V_{rev}, was reduced -51 mV $\pm$ 10 mV; (n=32) compared to the normal preparation. These observations suggest that the resting membrane potential of the hair cells was depolarized and that the fractional resistance of the apical membrane was increased.

The effects of different concentrations of Ca^{2+} in the apical solu-tion on on Δv_{rev} and on the receptor current-voltage relation were examined. Fitting a modified constant field equation to ΔV_{rev} gave an estimate of P_{Ca}/P_K of the transduction channels of about 200. Also, fitting constant field current equations to the receptor current-voltage relation produced values of P_{Ca}/P_K in this range.

The influence of divalent cations on V_{rev} showed that $P_{Ca} > P_{Ba} = P_{Sr}$, whereas Mn^{2+} inhibited the receptor current without a significant change in V_{rev}. The receptor current was inhibited by amiloride (IC_{50} 3.2 μM $\pm$ 3.2 μM) and nifedipine (IC_{50} 1.9 μM $\pm$ 0.6 μM).

These results indicate that the mechano-electrical transduction channels of the frog saccular hair cells are highly selective to Ca^2.

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ARACHIDONIC ACID IS FUNCTIONING AS A SECOND MESSENGER IN ACTIVATING THE CA^{2+} ENTRY PROCESS ON H_1-HISTAMINE RECEPTOR STIMULATION IN DDT_1 MF-2 CELLS. L. van der Zee, A. Nelemans* and A. den Hertog*.

This study was carried out to identify the cellular component activating the histamine stimulated Ca^{2+} entry process in DDT_1 MF-2 smooth muscle cells. Stimulation of H_1-histamine receptors results in a rise in intracellular Ca^{2+} concentration caused by Ca^{2+} release from inositol-phosphate sensitive Ca^{2+} stores and Ca^{2+} entry from the extracellular space and is accompanied by a transient Ca^{2+} activated outward K^+ current. This histamine evoked K^+ current was still observed after preventing inositol phosphate-induced Ca^{2+} mobilization by intracellularly applied heparin. The current was activated by Ca^{2+} entry from the extracellular space since it was abolished in the presence of the Ca^{2+} channel blocker La^{3+} or under Ca^{2+}-free conditions. Histamine activated Ca^{2+} entry was also observed in the presence of intracellularly applied Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, depleting their respective Ca^{2+} stores and pre-activating the inositol phosphate-regulated Ca^{2+} entry process. Thus the ability of histamine to activate Ca^{2+} entry independently of Ca^{2+} mobilization and the formation of inositol phosphates suggests that another component is involved to initiate the Ca^{2+} entry process. It was observed that stimulation of H_1-histamine receptors resulted in a pronounced release of arachidonic acid (AA) in DDT_1 MF-2 cells. Exogenously applied AA induced a concentration-dependent increase in internal Ca^{2+} concentration due to activation of Ca^{2+} entry from the extracellular space. Inhibition of the lipoxygenase or cyclo-oxygenase pathway did not affect this AA induced Ca^{2+} entry. The histamine-induced change in internal Ca^{2+} concentration and the concomitant K^+ current were decreased in the presence of AA and caused by Ca^{2+} mobilization from internal stores. Blocking this Ca^{2+} release by heparin, in the presence of AA, resulted in abolition of the histamine induced Ca^{2+} regulated K^+ current. These observations show that AA, released on H_1-histamine receptor stimulation in DDT_1 MF-2 cells, is functioning as a second messenger to activate plasmamembrane Ca^{2+} channels promoting Ca^{2+} entry from the extracellular space.

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THE EFFECTS OF CLONIDINE ON TRACHEAL SMOOTH MUSCLE CONTRACTION. Gh. Petrescu, C. Brailoiu, N. Costuleanu and M. Costuleanu

Recent investigations have clearly demonstrated that centrally acting drugs like clonidine induce their antihypertensive effects primarily by high affinity binding at the imidazoline receptors. These imidazoline binding sites are distinct from adrenergic and histaminergic receptors and are widely distributed. In order to assess the existence of imidazoline sites in airways, we tested the effects of clonidine on isolated rat tracheal rings. The experiments were performed on tracheal smooth muscle preparations, in organ bath, at 37°C , in Krebs - Henseleit buffer and continuously bubbled with 95% O_2 +5% CO_2. The control contractions were induced by K^+ 40 mM and carbachol 10^{-5} M, respectively. In the plateau of these contractions, clonidine was administered in a cumulative way, in the presence of yohimbine 10^{-5} M, in concentrations between 10^{-10} M and 10^{-3} M. Clonidine relaxed either depolarization - and agonist - induced contraction with an aproximatively ED_{50} of 5 x 10^{-5} M and 6 x 10^{-4} M, respectively. These results show the existence of some type of imidazoline receptors on rat trachea, which need further characterization.

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SUBSTANCE P: ROLE OF MEMBRANE LIPID MATRIX IN THE INTERACTION WITH CELL. B.R.Mogilevich, V.K.Rybalchenko

The well-known conception of ligand-receptor interaction postulates the necessity of the direct binding of substance P (SP) with receptors for the purposeful action on the cell. But there are some biological effects of SP which does not explaine this theory. We investigated of the SP surface active properties and its ability to modify lipid membranes. It was shown that SP had its own surface activity. Lipid membranes imitating target cell plasma membrane initiated and increased SP adsorption from the solution. SP molecules were not only adsorbed on the artificial membrane surface. They are also incorporated into lipid matrix and formed potential-dependent ion channels in the BLM. These results suggest that interaction of SP with membrane developes in two stages. At the first stage (electrostatic interactions) SP is accumulated close by acceptor sites on the membrane-associated proteins. This process could explain the effects of extremely low dose of peptides. On the second stage hydrophobic interaction and implantation of SP molecules into lipid matrix proceeds. SP molecules assume only one possible conformation in contrast to set of conformers in solution. At the same time matrix physico-chemical characteristics are changed and modulate activity of the membrane-bound proteins. Possibly in lipid environment of membrane SP might also interact with transmembrane localised proteins and display non-specific effects.

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HEMODYNAMICS OF HYPERTROPHY AND FAILURE: IMPORTANCE OF REFLECTIONS. N.Westerhof and M.F.O'Rourke

In the young adult wave reflections return mainly in diastole, paying no contribution to the cardiac load while augmenting *diastolic* pressure and so aiding coronary perfusion. With aging the conduit vessels stiffen. The result is increased pulse pressure due to a direct stiffening effect of the conduit arteries and due to the early return of wave reflection as a result of the increase in wave speed. The result is increased systolic wave augmentation posing a larger load on the heart. Increased aortic systolic pressure is associated with increased systolic ventricular pressure which may lead to ventricular hypertrophy. Hypertrophy may lead to failure.

Wave reflection adds to pressure but subtracts from flow. In youth with the diastolic arrival of reflected waves pressure is augmented. In hypertrophy, however, the ventricle functions as a flow source. This means that the reflections returning in *systole* mainly affect the pressure but not the flow. However, in failure, when contractility of the cardiac muscle is low and when the ventricle is dilated, the heart approaches a pressure source. This implies that reflections returning in *systole* affect the flow rather than the pressure. Since reflected flow waves subtract the measured flow wave is diminished by the reflection. This means that cardiac output is reduced by the reflections.

It is therefore suggested that in cardiac failure reflections should be diminished and delayed so that they arrive at the heart in the *diastolic* phase.

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THE EFFECTS OF CHRONIC VENTRICULAR PACING ON LOCAL MYOCARDIAL BLOOD FLOW AND WALL THICKNESS. M.F.M. van Oosterhout, F.W. Prinzen,T. Arts, R.S. Reneman.

Previous acute canine experiments indicated that asynchronous electrical activation reduces mechanical load and blood flow in early activated regions and increases them in late activated regions. We investigated the structural adaptation and the distribution of blood flow in the left ventricular (LV) wall during chronic asynchronous activation, as induced by ventricular pacing. Under general anaesthesia 5 dogs received a pace-maker, with the stimulus electrode implanted 1 cm below the base of the LV free wall (LVFW). After full recovery the dogs were paced at physiological heart rate (A-V sequential, A-V interval 25 ms). On 2D-echocardiograms the cross-section of the LV wall at the level of the stimulation electrode was divided into 6 sectors, from which mean local wall thickness was measured. Myocardial blood flow was determined in these sectors using fluorescently labeled microspheres: during sinus rhythm (SR) and ventricular pacing (VP), both during the implantation and the termination procedure. After 6 months of VP, wall thickness of the early activated LVFW decreased by 9.1±6.5% and thickness of the late activated septum (S) increased by 29.8±10.8%, compared with baseline. The ratio of LVFW and S (LVFW/S) thickness decreased from 1.14±0.13 to 0.93±0.06. The LVFW/S blood flow ratio was calculated as an index of blood flow distribution. This ratio was 0.81±0.22 during SR and decreased to 0.62±0.10 after 15 min of VP. After 6 months of continuous VP the LVFW/S ratio had returned to baseline values (0.80±0.22) and increased to 1.19±0.22 15 min after return to SR. We conclude that 1) myocardial structural adaptation to mechanical overload occurs locally and that 2) local wall mass adapts so that myocardial blood flow per unit weight returns to initial (pre-pacing) values.

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ROLE OF p42-MAP-KINASE IN MYOCARDIAL HYPERTROPHY INDUCED BY CATECHOLAMINES. H.M. Piper, K.-D. Schlüter, M. Schäfer, A. Simm, W. Hoppe

Catecholamines can induce cardiac hypertrophy by α- or ß-adrenoceptor stimulation. In spite of different initial signalling pathways, cellular hypertrophy is characterized in both cases by increased protein and RNA contents. It has recently been suggested, that both signalling pathways converge at the level of MAP-Kinase activation by the tyrosine kinase MAP-kinase kinase, leading to a hypertrophic response of the heart. To study this hypothesis MAP-kinase activation in isolated cardiomyocytes from adult rats was determined. It was found that α-adrenoceptor stimulation by phenylephrine (PE, 10 μM) of cultured cardiomyocytes activates p42 MAP-kinase. Activation was demonstrated on Western-blots by use of an anti-phosphotyrosine antibody and a monoclonal antibody against p42 MAP-kinase. Stimulation of ß-adrenoceptors with isoprenaline (1 μM), however, did not activate p42 MAP-kinase.To investigate, whether p42 MAP-kinase activation plays a role in PE induced cardiac hypertrophy, isolated cardiomyocytes were treated with PE or PE plus the tyrosine kinase inhibitor Genistein (Gen, 100 μM). PE increased the protein mass of cardiomyocytes within 24 hrs from 46.04 ± 3.18 to 52.95 ± 2.30 (PE alone) and 53.83 ± 3.23 (PE+Gen) μg protein/μg DNA (n=12, $p<0.05$). Similar results were obtained for PE-induced RNA increase. PE induction leads also to significant increase in the specific activity of the fetal type isoform of creatine kinase, CK-BB, from 0.26 ± 0.05 U/mg protein (Control) to 0.65± 0.06 U/mg protein (n=4, $p<0.05$). This effect of PE, however, was significantly reduced in presence of Gen to 0.50 ± 0.09 U/mg protein (n=4, $p<0.05$). In conclusion these results indicate, that activation of p42-MAP-kinase by tyrosine phosphorylation is not a common signal in induction of cardiac hypertrophy by α- or ß-adrenoceptor stimulation. It seems required for induction of fetal type proteins in α-adrenoceptor mediated hypertrophy.

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BIVENTRICULAR HYPERTROPHY INCREASES THE ARRHYTHMOGENIC EFFECTS OF D-SOTALOL IN DOGS.

SC Verduyn, MA Vos, J van der Zande, SHMA de Groot, HJJ Wellens.

We have reported that in dogs with complete chronic AV-block (AVB, 9 weeks), d-sotalol (*dS*) results, with an incidence of 50%, in early afterdepolarization (EAD) dependent Torsade de Pointes arrhythmias (TdP). The by chronic AVB induced volume overload causes biventricular hypertrophy (BVH): left ventricle/body weight (LV/BW) increases from 4.3±0.9 to 5.8±0.9 g/kg and the RV/BW from 1.4±0.4 to 2.6±0.9 g/kg (<0.05). BVH is associated with prolongation of action potential duration (APD) and sudden death. The role of BVH in the genesis of TdP is not known. In anesthetized dogs, the effect of *dS* was therefore tested twice: at the moment of AVB and after 5 wks. Cycle length (CL) was kept constant in both experiments. LV and RV APD, presence of EADs and dispersion (ΔAPD=LV-RV APD) and incidence of TdP were measured.

Results: At baseline (CL=1720±215ms), both APDs significantly increased after chronic AVB (*, table). These values increased significantly after *dS* (<0.05). *dS* did not induce TdP at 0 wks, but resulted in 4/5 TdP after 5 wks. This was associated by a more pronounced increase in ΔAPD and more EADs at 5 vs 0 wks (Table).

	0 wks	5 wks	0 wks *dS*	5 wks *dS*
LV APD	315±35	390±70*	355±35	495±80*
RV APD	270±15	315±45*	300±20	380±60*
ΔAPD	45±40	75±35	55±30	110±45*
EAD	0%	10%	40%	80%
TdP	0/5	1/5	0/5	4/5

Conclusions: BVH increases the (arrhythmogenic sensitivity) to dS resulting in a higher incidence of TdP. This study also again emphasizes the role of ΔAPD and EADs in the genesis of TdP.

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ANALYSIS OF ELEMENTS IN DAMAGED MYOCARDIAL TISSUE USING THE PROTON MICROPROBE. B.A.W. Verhoef, P.M. Frederik, P.H.H. Bomans, G.J. van der Vusse, M.J.A. de Voigt

Particle Induced X-ray Emission (PIXE) was used to measure intracellular concentrations of Na, Mg, K, Ca and Fe in normal and diseased myocardium. With a 3*3 μm^2 proton microbeam spatial distributions of the above mentioned elements were measured in areas of about 50*50 μm^2. Scanning Transmission Ion Microscopy (STIM) was used to localize individual cells within freeze-dried cryosections of rat heart. Intracellular concentrations were determined in hearts subjected to normal perfusion, ischemia and reperfusion. Besides, hearts severly damaged by Ca-paradox experiments were analyzed. Substantial changes were found in reperfused hearts, where locally elevated Ca levels (from control levels of about 100 up to 500-3000 mg/kg dry weight) and decreased K levels (from control levels of about 13,000 down to 6000 mg/kg dry weight) were observed. Similar, even more pronounced changes were found for hearts subjected to Ca-paradox (Ca levels increasing to at least 2000 mg/kg dry weight for all hearts). Next to these changes in K and Ca, the Ca-paradox induced a steep increase in intracellular Na concentrations and a dramatic efflux of Mg and Fe from the intracellular compartment. It can be concluded that the proton microprobe is a versatile tool to measure alterations in elemental concentrations in damaged myocardium, which can be used as an indicator of the degree of injury inflicted upon the heart.

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THE EFFECT OF A NEW NITRIC-OXIDE DONOR, GEA 3162 ON THE MECHANICAL PERFORMANCE OF THE ISCHEMIC LANGENDORFF-PERFUSED HEART. M. Szekeres*, T. Metsa-Ketela**

The effect of a new nitric-oxide (NO) donor agent, a mesoionic 3-aryl substituted oxatriazole-5-imine derivative, GEA 3162 was studied on the constant flow-perfused rat heart. The perfusion was kept on the rate of 16 ml/min. Ischemia was performed on 0.8 ml/min low flow for 30 minutes and was followed by 40-min. reperfusion period. Experiments were divided into two groups: a control (n=10) and a GEA 3162-treated group (n=10). The infusion was started before the ischemia till the end of the experiment by the rate of 1% of the actual coronary flow at the dose of 10 uM entering the heart. The perfusion pressure (PP) was measured continuously. A balloon was inserted into the left ventricle in order to measure the left ventricular pressure (LVP). LVP, heart rate (HR) and contractility (dP/dt) was detected at certain times according to a computer program as the indicators of cardiac performance. In the control group there was a continuous increase in PP during reperfusion by 85% in average indicating an endothelial damage that was verified by bradykinin-test. In the treated group PP decreased by 62% in av. after the administration of GEA 3162, and the increase in reperfusion was 46% in av. that indicates an improved coronary function compared to controls. The preischemic values did not differ significantly in the two groups. In both groups at the early reperfusion the performance of the heart reached the preishemic values, but after 20 minutes there was a significant fall in LVP to 70% of initial value in the controls, but in the NO-treated group it remained stable. The dP/dt values returned to normal by the end of experiments in both groups. The HR was 140 bpm in av. during the high flow and it temporarily fell down by 10% in av. in the middle of reperfusion in both groups. According to the above results GEA 3162 seems to have a protective effect on the ischemic perfused heart that is mostly seen in the preserved cardiac function.

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INTRACARDIAC DEGRADATION OF URIC ACID AS A MARKER OF OXIDATIVE STRESS DURING CORONARY REPERFUSION IN MAN. B. F. Becker, G. Münch*) and G. Richardt*)

Uric acid has been proposed to act as a physiological antioxidant in man, and disappearance of urate from blood during pulmonary passage has been ascribed to oxidative degradation in the course of radical scavenging (B.F. Becker, Free Rad Biol Med 14:615;1993). However, in the non-failing human heart there was rather a net formation of urate, coronary sinus (CS) blood containing about 14μM more urate than arterial blood. In the present study, 9 patients with myocardial infarction undergoing coronary reopening with PTCA catheters consented to having arterial and coronary sinus blood sampled before, immediately after and 5-10 min after reopening. Blood was analyzed for purines by HPLC after cold-stop and deproteinization with $HClO_4$. Reperfusion was graded by the TIMI score. **Results**: Pre-interventional arterial blood contained 233±23μM urate (mean ±SEM), the CS value was virtually identical (230±24μM). Of the 9 patients, 6 revealed good reperfusion (TIMI flow 3), but 1 apparently had some spontaneous reflow befor the intervention (TIMI flow 2). For the 5 acutely reperfused subjects, CS urate after 5-10 min was 57±21μM lower than the arterial value ($P<0.05$), which did not essentially change. The fall in CS urate was less marked immediately after reopening (-21±19μM). The 4 other subjects displayed little coronary arterio-venous difference in urate(-0.5±11.4μM). In contrast to urate, the CS levels of adenosine, inosine and hypoxanthine were slightly but uniformly elevated as compared to the respective arterial values. **Conclusions**: The observed fall in urate blood levels during coronary passage in acutely reperfused hearts may be ascribed to direct chemical degradation, in accordance with the presumed generation of oxygen free radicals in this setting. Thus, uric acid acts as a physiological antioxidant in the human heart during a condition of high oxidative stress.

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SUSCEPTIBILITY OF CARDIOMYOCYTES FOR HYPERCONTRACTURE IS INCREASED BY ANOXIA.

Y.V.Ladilov and H.M.Piper

Reperfusion of ischemic myocardium can elicit hypercontracture of the myofibrils and consecutive cell necrosis. It was investigated if the susceptibility of cardiomyocytes for hypercontracture is increased after simulated ischemia. Isolated ventricular cardiomyocytes of adult rats were anoxically superfused with Tyrode's solution (pH_o 6.4) for 70 min and than reoxygenated. During anoxia cells developed cytosolic Ca^{2+} overload (10^{-5} M; fura-2) and acidosis (pH_i 6.5; BCECF). Continuation of acidotic extracellular pH during first 10 min of reoxygenation prevented spontaneous hypercontracture and allowed recovery of cytosolic Ca^{2+}. Susceptibility for hypercontracture of anoxic/reoxygenated cardiomyocytes was compared with control cells. For this purpose the cells were loaded with calcium at normoxic conditions by high-frequency electrical stimulation at elevated extracellular Ca^{2+}. The analysis of the relationship between the cytosolic Ca^{2+} concentration and the extent of hypercontracture revealed that anoxic/reoxygenated cells developed irreversible hypercontracture at lower cytosolic Ca^{2+} (0.46 $\pm$ 0.06 μM) than control cells (0.85 $\pm$ 0.05 μM, $p<0.01$).

Conclusion: Anoxia causes an increased susceptibility of the cardiomyocytes for hypercontracture. After reoxygenation, therefore, hypercontracture may develop at Ca^{2+} concentrations which are not normally harmful.

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STIMULATORS AND INHIBITORS OF INSULIN SECRETION INDUCE CHANGES OF MEMBRANE POTENTIAL IN INS1-CELLS

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The aim of this study was to characterize the effects of modulators of insulin secretion on membrane potential of INS-1 cells. In contrast to other commonly used insulin secreting cell lines such as RIN- and Hit-cells, INS-1 cells respond to glucose at physiological concentrations. The resting membrane potential of the cells at 0.5 mmol/l of glucose was - 80 ± 0.9 mV (n=42). Using the cell attached patch clamp configuration membrane depolarisation of 3 ± 1 mV (n=7); of 9 ± 1 mV (n=10); of 12 ± 5 mV (n=3) and of 24 ± 1 mV (n=20) was induced by 2.8, 5.6, 8.3 and 16.7 mmol/l of glucose, respectively. Superimposed action potentials (AP's) were observed sparsely at 5.6 mmol/l glucose, frequently at 16.7 mmol/l glucose, but were absent at 0.5 and 2.8 mmol/l glucose. In three cells, slow wave membrane potential oscillations with a burst phase of AP's of 13 s and a silent phase of 25 s have been observed at 3 mmol/l of glucose similar to those described for normal β-cells. Forskolin (5 and 10 µmol/l) had no effect on membrane potential nor did it increase the frequency of action potentials induced by glucose. Adrenaline (1 and 0.1 µmol/l), a potent inhibitor of insulin secretion, blocked AP's and hyperpolarized the plasma membrane by 23.5 ± 2.3 mV, n=4. Similar effects have been observed with somatostatin (1 µmol/l) and galanin (0.1 µmol/l). Insulin secretion was measured by radioimmunoassay. Glucose (16.7 mmol/l) stimulated insulin release 2.3-fold over 30 min (from 0.87 ± 0.12 (n=24) to 2.96 ± 0.41 (n=16) % of cellular insulin content). Forskolin (10 µmol/l) potentiated the glucose effect 6-fold (to 18.76 ± 2.23 % of content (n=8)). Adrenaline inhibited stimulated secretion in a dose dependent manner with a half maximal effect at 0.03 µmol/l. Somatostatin and galanin were less potent inhibitors of insulin secretion. The results demonstrate INS-1 cells as a suitable model to study regulation of insulin secretion by physiological modulators such as glucose and adrenaline.

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LPS- AND PROSTAGLANDIN-INDUCED ACTH SECRETION: THE INVOLVEMENT OF HYPOTHALAMIC HISTAMINERGIC NEURONS. M.Anthonisen, D.Akman, U.Knigge, A.Kjær, H.Jørgensen, J.Warberg.

Lipopolysaccharid (LPS) endotoxin immunochallenge stress is a potent stimulator of ACTH secretion. However the exact mechanisms by which LPS stimulates ACTH is not fully understood, but is in part mediated via interleukins and prostaglandins (PG). In addition, the hypothalamic neurotransmitter histamine (HA), which is an important ACTH secretagogue, may be involved in mediation of LPS-induced ACTH secretion.

We investigated 1) the importance of hypothalamic histaminergic neurons in LPS- and PG-induced ACTH secretion, and 2) the importance of PG's for HA-induced ACTH secretion.

1) LPS stimulated the release of ACTH dose-dependently and increased the hypothalamic concentration of the HA metabolite telemethylHA significantly and that of HA slightly, indicating an increased turnover of neuronal HA. Pretreatment with the HA synthesis inhibitor α-fluoro-methyl-histidin (α-FMH) administered intracerebroventricularly (i.c.v.) or intraperitonealy (i.p.) inhibited the ACTH response to LPS about 60%, whereas i.p. administration of the H_3-receptor agonist R(α)methylHA, which inhibits the HA synthesis and release, decreased the response about 50%. Pretreatment with the H_1-receptor antagonist mepyramin (MEP) i.c.v. inhibited the hormone response to LPS about 50%, while pretreatment with equimolar doses of the H_2-receptor antagonist cimetidin (CIM) i.c.v. had no effect on the LPS-induced release of ACTH. PGE_1 stimulated the release of ACTH 4 fold. Pretreatment with the HA synthesis inhibitor α-FMH i.c.v. or the H_1-receptor antagonist MEP i.c.v. reduced this effect, while pretreatment with the H_2-receptor antagonist CIM i.c.v. had no effect on the LPS-induced ACTH release.

2) Pretreatment with PG synthsis inhibitor indomethacin did not affect HA-induced ACTH release.

We conclude that 1) Hypothalamic histaminergic neurons are involved in the mediation of the ACTH response to LPS - and PG stimulation via activation of postsynaptic central H_1-receptors, and 2) PG-induced ACTH release is, at least in part, mediated by HA.

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TESTICULAR STEROIDOGENESIS IN DIABETIC RATS.
P. Calders, W. Eechaute and E. Lacroix.

The effect of diabetes on the testicular steroidogenesis in adult male Wistar rats was investigated. Diabetes was induced by a single i.v. administration of alloxan (40 mg/kg) 14 days before experimentation. On day 14, diabetic and control rats were sacrificed and trunk blood was collected for LH, FSH and testosterone (T) measuring by RIA and for glucose measuring by an enzymatic method. In a number of testes the endogenous content of progesterone (P), 17α-hydroxyprogesterone (17α-OH P), androstenedione (δ4A) and T was measured directly. Other testes were incubated, as such, for 2h. Both, endogenous content and steroids produced by the incubated testes, were measured by RIA. Diabetes causes a slight decrease of body weight, while testicular weight was not affected. Plasma concentration of LH, FSH and T were significantly decreased, while glucose levels were strongly increased.
The endogenous content of all the measured steroids was significantly lower. These results were reflected in the steroid production after 2h incubation without LH stimulation in vitro and in vivo. When 10IU LH were added into the incubation vials just before incubation, P, 17α-OH P, δ4A and T production in controls was, as expected, significantly enhanced. After diabetes, there was no stimulation of the production of the measured steroids. Daily i.p. administration of 10IU LH, during the whole diabetic period, enhanced the production of the measured steroids in control animals. In diabetic animals, it produced only values which were comparable to the unstimulated control values. Accordingly, it can be concluded that without excluding a possible effect of the reduced plasma concentration of the gonadotrophins, diabetes has a direct inhibiting effect on steroid production.

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THE INCREASE OF THE CARDIODEPRESSANT FACTOR AND VASOPRESSIN CONCENTRATION IN THE SELLA TURCICA VENOUS BLOOD DURING ANGIOTENSIN II INFUSION INTO CEREBRAL ARTERIES IN RATS. A. Gorąca, M. Orłowska-Majdak and W.Z. Traczyk
It has been previously demonstrated that the cardiodepressant factor is present in the bovine hypothalamic extract and in the fluid incubating the posterior pituitary lobe "in situ" in rats (Acta Physiol Pol 1988, 39, 98-111). The present study was an attempt to elucidate if the cardiodepressant factor and vasopressin (AVP) were simultaneously released from the pituitary into blood. Six 30-min samples of venous blood flowing from the sella turcica were collected with a cannula inserted into the internal maxillary vein in anaesthetized rats. During 2nd sample collection angiotensin II (ANG II) was infused into the carotid artery (100 ng/100g b.w.). The concentration of AVP in blood plasma was determined by RIA and the activity of cardiodepressant factor by a biological test on spontaneously discharged pacemaker tissue of the right auricle of the right heart atrium of two-day-old rats. AVP concentration in control sample (I) of blood was 81.9 ± 17 pg/mL, while decrease of discharges of the pacemaker tissue was 10.2 ± 0.6%. After ANG II infusion concentration of AVP and decrease of discharges of the pacemaker tissue was greatest in the IIIrd sample of the blood (1632 ± 1285 pg/mL, 14 ± 0.6%, respectively). Infusion of ANG II into the internal carotid artery increased simultaneously the release of the cardiodepressant factor and AVP into the sella turcica venous blood.

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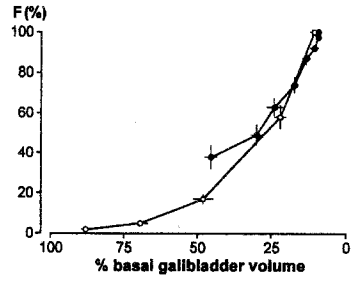
CONCURRENT STRAIN GAUGE AND ULTRASONOGRAPHIC ASSESSMENT OF CANINE GALLBLADDER (GB) CONTRACTION. K. Jonderko and L. Buéno
Repeated measurements of GB emptying were performed in two dogs equipped with a strain gauge implanted surgically onto the GB body in a position enabling to record the circular muscle contraction. GB emptying was induced by caerulein infused i.v.: (i) at stepwise increasing rates of 0.7 - 2.2 - 7.4 - 22.2 - 66.5 pmol·kg^{-1}h^{-1}, given each for 10 min [○], and (ii) at a constant rate of 22.2 pmol·kg^{-1}h^{-1} for one hour [●]. The maximum GB dimensions according to X-Y-Z coordinates were measured with a Toshiba SAL32B apparatus and a 5 MHz linear probe. Computation of GB volume was based on the ellipsoid approximation method.
Results. The relationship between the strain **F** recorded from the force transducer and GB volume was nonlinear. The reduction of GB volume to 50% of its basal value was accompanied by an increase in strain amounting to about 16% of the maximum response. [see the figure ▸]. Pronounced augmentation of the strain was observed with the GB volume ranging between 30 and 10% of the basal value. A linear relationship was found, however, between the contractile response of the GB registered by means of a strain gauge and the angle α contained between two radii passing from the GB centre towards the edges of the of the strain gauge: y = 0.852α - 30.97 (r=0.959, p<0.001) in the case of caerulein infusion at stepwise increasing doses, and y = 0,640α - 17.40 (r=0.869, p<0.001) for the constant rate caerulein infusion.



Conclusion: The contractile response of the GB recorded by means of a strain gauge is linearly related to the angle α contained between two radii passing from the GB centre towards the opposite edges of the force transducer.

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ILEAL *E. COLI* STA ACTIVATES JEJUNAL BUT NOT COLONIC FLUID SECRETION BY A VAGAL REFLEX IN THE RAT IN VIVO. Vivien Rolfe & R. J. Levin.
Ileal enterotoxin STa causes secretion by a myenteric reflex activated by C-afferent fibres (Rolfe & Levin (1994) *J. Physiol.* **475**, 531-37). We have investigated whether the stimulation of these ileal afferents by STa also concomitantly activates secretion in jejunum and colon. Fluid transport was measured gravimetrically in jejunum (J), ileum (I) and colon (C) loops in anaesthetised fed rats. The I contained 0.9% NaCl with various substances while the J or C loop contained only saline. Some rats were pretreated with either L- or D-NAME (I.P., 40mg/kg b.wt.) 15 mins before luminal instillation of the fluids (Table 1) others were vagotomised.

Treatment	Ileal contents	Fluid transport	
		Ileum	Jejunum
Control A (4)	0.9% NaCl	-8.4 ± 1.5 a	-5.5 ± 1.5 b
D-NAME (8)	STa	0.8 ± 0.4 c	0.5 ± 0.2 d
D-NAME (6)	Carbachol (1mM)	2.0 ± 0.3 g	-1.5 ± 0.5 h
L-NAME (8)	STa	-1.6 ± 0.2 i	-1.1 ± 0.3 j
L-NAME (6)	Carbachol (1mM)	2.6 ± 1.0 k	-1.6 ± 0.6 l
Control B (6)	STa	2.3 ± 1.5 m	0.5 ± 1.1 n
Capsaicin (6)	STa + capsaicin	-2.1 ± 1.7 o	-4.3 ± 0.9 p

Table 1. Fluid transport (g fluid/g gut wt/hr as the mean ± S.E., brackets = number of rats) in I and J. Absorption = negative, secretion = positive. STa = 55ng/ml. Unpaired t tests; m v o = p<0.05; c v i, d v j, n v p = p<0.01; g v h, k v l = p<0.001. Ileal STa activated I and J secretion, L- but not D-NAME inhibited both. I-carbachol activated only I-secretion and was unaffected by L-NAME. I-capsaicin with I-STa completely inhibited both I and J secretion. Vagotomy did not affect STa-I secretion but inhibited J-secretion. C-secretion was not activated by I-STa. Ileal STa activates secretion in the J but not C by a vagal capsaicin-sensitive, nitrinergic reflex.

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EFFECT OF CHLOROQUINE ON MATURATION OF PROGASTRIN.
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Progastrin, the precursor of the acid stimulating hormone gastrin, gives rise to multiple peptides with distinct biological activities, notably amidated and COOH-terminal Gly-extended gastrins. Post-translational processing occurs in immature secretory granules and includes cleavage at Arg^{57}-Arg^{58}, Lys^{74}-Lys^{75} and Arg^{94}-Arg^{95}, Tyr-sulphation, Ser-phosphorylation and COOH-terminal amidation. The pH in secretory granules is thought to be acidic and in the present study we have used the weak base chloroquine (CQ) to examine the role of granule acidification in progastrin processing. Rat antral mucosa was incubated *in vitro* with [^{35}S] sulphate at 22°C for 2 hours and chased at 37°C in the presence or absence of CQ (200μM). In controls after 160min chase, 17-residue amidated gastrin predominated over 34-residue amidated gastrin (ratio 1.62 $\pm$ 0.13; mean $\pm$ SE, n = 6), but in the presence of CQ this was reversed (0.61 $\pm$ 0.09, $p < 0.01$)); similarly the relative proportions of Gly-extended gastrins, which are the immediate precursors of amidated peptides, were significantly shifted by CQ (G17-Gly/G34-Gly: control, 0.72 $\pm$ 0.07; CQ, 0.34 $\pm$ 0.05, $p < 0.01$). The total counts incorporated into amidated compared with Gly-extended gastrins were similar in control and CQ samples. **CONCLUSIONS.** 1. The acidic pH of secretory granules is required for cleavage of G34 and G34-Gly at Lys^{74}-Lys^{75}, but not for gastrin amidation. 2. Control of granule acidification may account for variation in the relative abundance of G17 and G34 in different physiological conditions.

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EFFECT OF CGRP ON PANCREATIC ENZYME SECRETION IN INTACT AND CAPSAICIN-DENERVATED RATS. J.Jaworek, A.Szlachcic, S.J.Konturek, A.Dembiński, Z.Warzecha.

Calcitonin gene-related peptide (CGRP) has been demonstrated in the pancreatic sensory nerves (SN), but its physiological role is unknown. This study was undertaken to compare the changes in pancreatic enzyme secretion produced by CGRP and by stimulation or destruction of SN. Methods: To stimulate SN, low doses of capsaicin (CP) were given intraduodenally (i.d.) to rats with chronic pancreatic fistula. To inactivate SN high doses of CP (100 mg/kg) were given s.c. 10 days before tests. For the *in vitro* experiments pancratic slices (containing nerve fibers) and isolated pancreatic acini were prepared from intact or CP-denervated rats. Results : 1. In conscious rats CGRP given s.c. (5-10 µg/kg), or CP given i.d. (0.25-0.5 mg/kg) reduced basal pancreatic enzyme secretion. CP (1.0 mg/kg i.d.) increased this secretion. 2. In pancreatic acini CGRP (10^{-10}-10^{-6}M), but not CP, stimulated amylase release. 3. In pancreatic slices CP (10^{-10}-10^{-6}M) increased enzyme secretion and this response was abolished by atropine (10^{-6}M) and by the destruction of SN. 4. CP-denervation did not affect the secretory response of pancreatic acini to caerulein, urecholine, or CGRP. 5. In conscious rats CP-denervation diminished secretion produced by feeding or diversion of pancreatic juice (DPJ) but not basal protein secretion. Conclusions: 1. Stimulation of SN by CP produced the secretory effects dependent in part on the release of CGRP. 2. Destruction of SN by CP did not change the secretory response of pancreatic acini to CGRP and pancreatic secretagogues but reversed the secretion produced by stimulation SN by CP. 3. In conscious rats CP denervation reduced the early secretory response to DPJ and meal.

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THE EXCITATORY EFFECTS OF CCK-8 ON MYENTERIC NEURONS IN THE GUINEA-PIG ILEUM ARE MEDIATED BY CCK-A AND CCK-B RECEPTORS. I.W.M. Schutte, A.B.A. Kroese and L.M.A. Akkermans.

Cholecystokinin (CCK-8) is involved in the neural regulation of the motility of the guinea-pig ileum and has been shown to have excitatory effects on myenteric neurons (Nemeth et al., Eur.J.Pharmacol. 116:263-269, 1985; Schutte et al., Eur J Physiol. 424:R8, 1993). The aim of the present study was to determine the CCK receptor subtypes involved in these effects. The results described were obtained by intracellular recordings from 15 S and 8 AH neurons, which all showed a concentration dependent excitatory response to CCK-8 (1-1000 nM). The CCK-8 (300 nM) induced excitation consisted in S neurons of a depolarization (10 ± 1 mV; mean ± s.e.m) and an increase in membrane resistance to 121 ± 3 % of control, often accompanied by a discharge of action potentials. In AH neurons, CCK-8 suppressed the after-hyperpolarization. Application of CCK receptor antagonists (25-1000 nM) caused in all neurons a reversible, concentration-dependent inhibition of the CCK-8 induced effects. In 9 neurons (5 S; 4 AH), application of the CCK-A antagonist L-364,718 (Merck, Sharp & Dohme; 250 nM) caused a complete blockade of the action of CCK-8. The CCK-B antagonist L-365,260 (500 nM) had no effect on these neurons. The CCK-8 induced response was completely antagonized by L-365,260 (100 nM) in 9 other neurons (5 S; 4 AH), on which L-364,718 (500 nM) had no effect. In the remaining 5 S neurons each antagonist caused only a partial blockade of the CCK-8 induced excitation and additive effects of the antagonists were observed. The CCK-B receptor agonist CCK-8NS (100 nM) induced excitatory effects comparable to those evoked by CCK-8, but only on those neurons in which the CCK-8 induced effects were antagonized by L-365,260. In conclusion, the findings show that the excitatory action of CCK-8 on myenteric neurons is mediated by both CCK-A and CCK-B receptor subtypes.

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CHARACTERIZATION OF OXYGEN SUPPLY AND OXYGEN-DEPENDENT GENE EXPRESSION IN DIFFERENTIATING EMBRYONIC STEM CELLS

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Blastocyst-derived pluripotential mouse embryonic stem cells can differentiate *in vitro* to form so-called embryoid bodies (EBs). These embryo-like structures consist of a variety of differentiating cell types such as neuronal, muscle and hematopoietic cells. Thus, EBs recapitulate several aspects of murine embryogenesis. We used this *in vitro* model to study oxygen supply and consumption as well as the response to a hypoxic environment during the earliest stages of development. EBs were found to grow equally well when cultured at 20% or 1% oxygen during the first 6 days of differentiation. Microelectrode measurement of pericellular oxygen tension within 13 to 14 days old EBs revealed shallow oxygen gradients preventing low oxygenation even in the core of the EBs (diameter 510-890 µm). This is rather remarkable compared to cancer cell-derived multicellular spheroids in which necrosis occurs at a distance of 50-300 µm from the periphery. Confocal laser scanning microscopy analysis of these EBs incubated with fluorescent dyes that specifically stain living cells confirmed that most if not all cells within an EB were viable. To determine the EBs' capability to sense and respond to low ambient oxygen tension, we quantified erythropoietin (EPO) mRNA, since this gene is regulated in mammals in an oxygen-dependent manner. Compared to the normoxic controls, we found increased EPO mRNA level after exposing 3 days old EBs to 1% oxygen, as measured by competitive reverse transcriptase-mediated PCR. In conclusion, our results suggest that EBs can grow in a poorly oxygenated environment. We propose that EBs behave similar to the preimplantation embryo *in vivo* which resides in an aquatic environment with low oxygen tension until implantation followed by angiogenesis.

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CONTROL OF NEOTENY IN A URODELE AMPHIBIAN. Per Rosenkilde.

Amphibian metamorphosis is induced by thyroid hormone, under control by the brain and pituitary. The Mexican axolotl, Ambystoma mexicanum, is a neotenic species: it does not metamorphose, but breeds and lives its whole life in the larval form. The study aims at elucidating the mechanism(s) which allows the axolotl to escape metamorphosis. Metamorphosis can be induced by thyroid hormone. Therefore, the mechanism behind neoteny is linked to the thyroid regulatory system. The young larvae experience a short period with intense thyroid secretion but do not respond with metamorphosis. Later in life, thyroid activity level is low, but the animals are responsive to thyroid hormone. In the present study, metamorphic responses to the two forms of thyroid hormone are compared during larval development. Early larvae react slowly or incompletely to the secreted, weakly active hormone form, thyroxine, but are very sensitive to the activated form, 3,5,3'-triiodothyronine. Later larvae respond more readily to thyroxine. It is concluded that early larvae posses or can develop receptors to triiodothyronine and can react to this hormone within few days, but they are unable or slow to perform the activation (peripheral deiodination) of thyroxine to 3,5,3'-triiodothyronine.

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THE ROLE OF NUCLEOSIDES IN PROLIFERATION OF LLC-MK_2 CELLS. R. Lemmens, L. Vanduffel, H. Teuchy and O. Culic

Extracellular ATP and adenosine regulate a whole diversity of cellular functions, including cell proliferation. Using the incorporation of [methyl-^{3}H]-thymidine as a proliferation marker, the effect of various nucleosides and nucleotides on endothelial LLC-MK_2 cells was studied. We found that ATP, ADP, AMP, and adenosine in concentrations of 10 µM or higher stimulate the proliferation of these cells.

Inhibition of ecto-ATPase and 5'-nucleotidase significantly diminished the stimulatory effect of ATP. This inhibition of proliferation is almost complete when in addition to 5'-nucleotidase, alkaline phosphatase is also inhibited. Therefore we conclude that the stimulatory effect on proliferation of LLC-MK_2 cells is primarily caused by extracellular adenosine and not by adenine nucleotides.

Purine nucleotides and nucleosides stimulate cell proliferation to a similar extent, while pyrimidine nucleotides and nucleosides inhibit proliferation.

The proliferative effect depends only on extracellular nucleosides, since blocking the nucleoside-uptake has no influence on proliferation.

The simultaneous presence of adenosine and any of the other purine nucleosides is not entirely additive in its effect on cell proliferation. At the same time any pyrimidine nucleoside, when added together with adenosine, has the same inhibitory effect as that of the pyrimidine nucleoside alone.

Apparently these proliferative effects are neither caused by any pharmacologically known P_1 purinoceptor, nor are they mediated by cAMP, cGMP, or IP_3 as second messenger, nor by extracellular Ca^{2+}

AP_4A and AP_5A were more potent in stimulating cell proliferation than any of the purine nucleosides. This effect is not due to degradation of the diadenosine polyphosphates to adenosine.

This study indicates that various purine and pyrimidine nucleosides can influence the proliferation of LLC-MK_2 cells by acting on putative purinergic and pyrimidinergic receptors, not previously described.

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MATURATION AND REGULATION OF DIGESTIVE ENZYMES IN THE SMALL INTESTINE AND SOME NON-DIGESTIVE ORGANS IN RATS. V.V.Egorova, V.G.Kassil. A.A.Nikitina, D.A.Shinder, N.M.Timofeeva, L.A.Vataeva

Maturation of digestive enzymes (carbohydrases, peptidases, alkaline phosphatase) and their regulation have been studied in various parts of the gut and in non-digestive organs (liver, kidney) of rats. As a result of this study the following new facts have been obtained: 1) high activities exerted by a number of enzymes were observed in the non-digestive organs of both adult and growing rats; 2) hydrocortisone (H) induced enzymatic activities in the small intestine to a greater extent than in other organs, whereas tyroxine (T4) had no effect; 3) detected in the colon of newborn rats high activities of some enzymes suggested their participation in the digestion at the early stages of ontogenesis; 4) changes in the regulatory properties of intestinal lactase and alkaline phosphatase in suckling rats were of adaptive character; 5) the intestinal sucrase induction caused by H-injection to 10-day-old rats whose mothers were subjected to immobilization stress or the combined action of injected H and T4, was decreased, while H-injection to 1-day-old rats left its level unaffected. It is possible that under stress in the mother's organism there seems to have been generated a factor, transmissible to her offspring and exerting an antistressor effect; 6) the body weight, the protein content, and the mucosal mass of the small intestine as well as the formation of a spectrum of digestive enzymes depended on whether the pups prematurely weaned were reared in isolation or with their litters-mates.

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OSMOTICALLY EFFECTIVE SODIUM BALANCE DETERMINES LONG-TERM VOLUME DEPENDENT CHANGES OF MEAN ARTERIAL PRESSURE

H.W. Reinhardt, E. Seeliger, W. Boemke, and M. Corea

Are there fixed relationships between changes of mean arterial pressure (MAP) and changes of sodium and water balances induced by experimental variations of the renin-angiotensin-aldosterone-system (RAAS)?

Experiments were performed in 6 groups of chronically instrumented dogs (ca. 15kg bw) over 4 to 5 consecutive days under standardised conditions (Am J Physiol 266, H650-H657,1994; intake: 91 ml water, 5.5 mmol Na and 3.5 mmol K /kg bw /day). RAAS changes were performed by: **1.** Servocontrolled reduction of renal perfusion pressure below the pressure dependent renin threshold (**rRPP**, n=7) **2.** rRPP and aldosterone infusion (**rRPP+Aldo**, n=7) **3.** rRPP+Aldo and angiotensin II infusion (**rRPP+AII+Aldo**, n=4) **4.** rRPP and Captopril infusion (**rRPP+Capto**, n=6) **5.** Infusion of Captopril and aldosterone (**Capto+Aldo**, n=6) **6.** Control (n=9).

Results regarding ΔMAP, total-body water (ΔTBW), Na (ΔTBNa) and K (ΔTBK) (obtained by cumulative balances after 4 (*5) days) were:

	mean	Δ MAP mmHg	Δ TBW ml/kg bw	Δ TBNa mmol/kg bw	Δ TBK mmol/kg bw
1	**rRPP**	+ 30	+ 32	+ 3.8	± 0
2	**rRPP+Aldo**	+ 30	+ 35	+ 8	- 2.5
3	**rRPP+AII+Aldo**	+ 40	+ 72	+ 11	- 3
4	**rRPP+Capto**	± 0	± 0	± 0	- 0.5
5	**Capto+Aldo***	± 0	± 0	+ 5	- 4.4

These results indicate: a) A chronic increase of TBW is a prerequisite for increased MAP. b) An increase of TBNa is essential for increased TBW. c) Howèver, if a Na retention is, at least partly, osmotically equilibrated by a K loss, than ΔTBW seems proportional the osmotically effective sodium balance (i.e. the balance of both cations).

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NATRIURETIC PEPTIDES HYPERPOLARIZE RAT MESANGIAL CELLS BY ACTIVATING A K^+ CONDUCTANCE. R. Cermak[1,2], W.G. Forssmann[2], R. Kleta[1], E. Schlatter[1].

Glomerular mesangial cells (MC) play a key role in regulating GFR. As natriuretic peptides relax MC and increase GFR, we examined the effects of these hormones on membrane voltage (V_m) of rat MC by using the fast whole cell patch clamp technique.

V_m of 145 MC was -38 ± 1 mV in control condition. Rat CDD/ANP-99-126 (ANP; n=25) or CDD/ANP-95-126 (urodilatin; n=9) both hyperpolarized V_m by 4 ± 1 mV or 3 ± 1 mV, respectively (160 nM each). The EC_{50} was at 400 pM, which is in the physiological range of plasma ANP levels. This hyperpolarization could be mimicked with 8-Br-cGMP (0,5 mM; n=10) and was blocked by Ba^{2+} (5 mM; n=6). Human BNP also hyperpolarized V_m significantly by 2 ± 1 mV (144 nM; n=8). CNP (227 nM; n=8) and the related peptides rat guanylin (301 nM; n=8) and human uroguanylin (300 nM; n=8) had no significant effect on V_m. 160 nM ANP could not enforce the hyperpolarization induced by 0,1 mM adenosine (n=7). The K^+ channel opener cromakalim had no influence on basal V_m of rat MC (10 µM; n=8). It also did not alter the hyperpolarization induced by 160 nM ANP (10 µM; n=8).

We could show that the natriuretic peptides ANP, urodilatin and BNP hyperpolarize V_m of rat MC mainly through activating a K^+ conductance, which can not be activated by cromakalim. This activation of a K^+ conductance is not additive to that by adenosine, indicating possibly the presence of a K^+ channel activated by cAMP and cGMP. These voltage effects are opposite to the depolarization of V_m induced by constrictors of MC like angiotensin II and ATP. Therefore this finding is in accordance to the diuretic and natriuretic action of these natriuretic peptides in the kidney.

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ATRIAL NATRIURETIC PEPTIDE (ANP) DURING LACTATION IN GOATS. K. Cvek, R. Gerstberger[1], H. Schütz[1] and K. Olsson.

Atrial Natriuretic Peptide (ANP) is well known to cause diuresis, natriuresis, haemoconcentration and vasodilatation. Lactation is a great challenge to fluid balance, since large amounts of water and electrolytes are secreted by the mammary gland. The mammary blood flow increases markedly at the onset of lactation. The aim of this study was to investigate the effects of ANP during lactation and whether the mammary gland is a target site for this hormone. Six Swedish domestic goats were infused with ANP or isotonic saline for two hours in random order. The ANP infusion caused significant increases in both plasma proteins and haematocrit, but no increase in urinary water or sodium excretion. Milk samples were collected every half hour during the experiments and were weighed and analysed for Na, K, and H_2O. None of the listed parameters changed during ANP infusion. Levels of ANP were measured in plasma and milk using radioimmunoassay. Naturally, there was a significant increase in plasma ANP during the infusion, but the analysis of milk did not give a clear answer. Biopsies from mammary tissue were obtained during general anesthesia from non lactating (N=3) and lactating goats (N=3). Tissue samples from the mammary glands of another two goats were collected during slaughter. Displacement studies were performed to charaterize the receptors. ^{125}I-ANP competed with unlabelled ANP (N=7), BNP (N=5) and cANP (N=4), respectively, in increasing concentrations. ANP was the only ligand that completely displaced ^{125}I-ANP, which shows that the receptors in the mammary gland are ANP-A receptors. High resolution film receptor autoradiography revealed that the binding sites are mainly located in the secretory tissue of the mammary gland both in lactating and non lactating animals. Since ANP has biologically active binding sites in the mammary gland and lactating animals showed no increased diuresis or natriuresis in response to ANP infusions, further studies on the function of ANP during lactation and on mammary blood flow are of great interest.

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NO SYNTHASE ACTIVITY IN THE DIFFERENT PARTS OF THE VASOPRESSINERGIC SYSTEM IN NORMAL RATS. N.A. Thorn

In a search for a possible role of NO synthase in regulation of the vasopressinergic system, we have employed the method of Hecker et al (1) to measure directly Ca^{2+}, calmodulin dependent NO synthase activity in the SON and PVN area of the hypothalamus as well as in the neurohypophysis (separated from the pars intermedia) and compared the activities with those found in the cerebellar cortex of the same normal PanWistar rats. A deep-frozen extract from the cerebellum served as a check that measurements done on different days did not differ.

The following activities were found (pmol•mg^{-1} protein min^{-1}): Cerebellar cortex: 85.6±3.1 (n=8), SON region: 43.3±5.2 (n=7), PVN region: 39.8±1.2 (n=7), Neurohypophysis: 107.2±6.1 (n=8). A Mann-Whitney two sample test showed that the values for the neurohypophysis were significantly different from those of the cerebellar cortex.

Conclusion: We have found that the Ca^{2+}, calmodulin dependent NO synthase activity in the terminal part of the hypothalamo-neurohypophysial system is roughly double as high as the activity in the proximal part and some 20% higher than that found in the cerebellar cortex of normal rats. It seems possible that NO may play a functional role at both the input and output part of the system.

Reference
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NATRIURETIC ROLE OF OXYTOCIN IN Na HOMEOSTASIS IN RATS M. Sjöquist, W. Huang, E. Jacobsson and S.-L. Lee

The body content of Na is crucially important for the well-being of the cells. Na^+ is the main extracellular cation and the Na salts determine the extracellular osmolality. For circulatory reasons and to prevent edema, regulation of extracellular fluid volume is also important. We have recently shown that oxytocin is a major mediator of osmotically stimulated Na excretion. Infusion of hypertonic NaCl into a lateral cerebral ventricle (ICV stimulation) of Brattleboro rats, causes an increased Na excretion. The natriuretic response was inhibited by and an OT antagonist (atociban, Ferring AB, Sweden). OT infusions that elevate plasma concentrations of OT to the same degree as that seen during ICV-stimulation, enhance Na output to a comparable extent. Renal Na excretion goes up by >15% / mM Na in cerebrospinal fluid, thus the mechanism is sufficiently sensitive to play an important role in the day-to-day regulation of sodium balance. Normal rats were infused with NaCl i.v. either as a hypernatremic (HNa) stimulation or as an isotonic volume expansion (VE). HNa caused a marked increase in sodium excretion from 0.25±0.06 µmol•min^{-1} to 10.04±0.62, while VE yielded a more moderate natriuresis from 0.25±0.04 µmol•min^{-1} to 1.64±0.15. Administration of OT-ant delayed and impaired natriuresis induced by HNa by 60%, but not that by VE. Plasma oxytocin concentrations were also significantly increased at the end of the HNa administration. A 24-hour dehydration caused hypernatremia and natriuresis. Plasma OT concentration rose from 15.5 ± 1.2 pg/ml to 23.8 ± 2.0. An intravenous infusion of atosiban blocked the dehydration natriuresis. WKY rats respond to ICV-stimulation with a more pronounced increase in Na excretion than SHR rats. In spite of enhanced oxytocin-release, the Dahl-S rats had an attenuated natriuretic responsiveness. These rats may have defect renal oxytocin receptors or there is a defect receptor coupling, that can contribute to their salt sensitivity. In micropuncture experiments, samples were taken from the proximal and distal tubules and from the final urine, before and after infusion of oxytocin. The results indicate that the main site of action of oxytocin in the kidney is beyond the distal tubule. In conclusion, OT is a nonhypertensive natriuretic agent involved in normal osmolar regulation.

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EFFECT OF INTRAVENTRICULAR INFUSION OF LOSARTAN ON FEEDING-INDUCED DRINKING IN SHEEP. M.L. Mathai[1], M.D. Evered[2] and M.J. McKinley.

There is evidence that the renin-angiotensin system elicits water drinking in response to feeding. In the rat, it has been shown that combined subcutaneous and intracerebroventricular (ICV) infusion of captopril inhibits drinking elicited by eating (Kraly F.S., *Prog Psychobiol Physiol Psychol* 14: 67-131, 1990). Our aim was to test if ICV infusion of losartan (an angiotensin AT_1 receptor antagonist) could inhibit drinking elicited by eating in sheep. A second aim was to test whether increases in plasma or cerebrospinal fluid (CSF) osmolality (as occur during feeding) might also induce water drinking via a central angiotensinergic mechanism.

Five cross-bred Merino ewes were trained to eat 800g of dry chaff in 30 min. Water access was withdrawn until the end of the eating period. The volume drunk following eating was measured at 15 min intervals for 90 min. Losartan or artificial CSF vehicle was infused at 0.1, 0.5 and 1 mg/h for 150 min in total, starting 30 minutes before the eating period and continuing until the end of the drinking period. ICV infusion at 0.5mg/h and 1mg/h reduced water drinking by 45% and 64% respectively ($P<0.05$) 30 min after water access was returned The influence of captopril on drinking elicited by eating was also examined. Either intravenous (IV; 40mg/h) or combined IV and ICV (2mg/h) infusions with captopril followed the same time course as with losartan infusion. IV captopril infusion did not inhibit drinking following eating. However, combined IV and ICV infusion decreased drinking by 53% ($P<0.05$) 30 min after water was returned.

In another experiment, the effect of ICV infusion of losartan (1mg/h) on water drinking in response to IV (4 mmol NaCl/kg) or ICV (500 mmol NaCl for 30 min) hyperosmotic stimuli was examined. We found that losartan treatment inhibited drinking in response to ICV hypertonic NaCl by 73% ($P<0.01$) but no inhibition was seen in response to IV hypertonic NaCl.

We conclude that a central AT_1 receptor-mediated mechanism is involved in the drinking response elicited by both eating and increased CSF tonicity. In contrast, at the same doses of losartan, we found no evidence that drinking in response to increased plasma osmolality is influenced by AT_1 receptors.

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EEG-EP MAPPING AND CURRENT GENERATORS OF CEREBRAL CORTEX POTENTIALS. S.Rjabov

EEG-EP mapping of cerebral cortex have been becaming odinary method for clinical and sientific neurophysiologists today. But basis of any type of mapping (potentials, power density, coherences and other) is a registration of extracellular potentials of brain cortex. In fact we speake of potential and current fields which are extensive in three dimensinal space.The questios concerning these date are: what do they reflect ? what neurophysiological mechanism is responsible for them ? where are actual active sites ?

EEG-EP mappig, electrical impedance, EEG-dispertion,current source-density was studed during spontaneous and evoked activity. Our studies showed that cerebral cortex is an omic, isotropical, laminar, inhomogeneous condactor with specific impedance 500-1000 om sm.The potential filds of EEG are created current dipols which have perpendicular orientation to cortex surface. More activy generators of EEG be placed in I-III and V-VI layers. The potential fild of somatosensory EP in cortex is created current dipols and quadrupols with maximum current source density in I-II and VI layers.

Mechanism of creation electrical dipols and quadrupols propose that cortex is composed of a heterogeneous population of neurons groups whose dendrites have specific morfological orientation, size and zons occuped by synapses.

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POSTNATAL DYNAMICS OF SIF-CELLS IN RAT AUTONOMIC GANGLIA. L.A.Knyaseva, I.G.Charyeva, S.A.Gaman and A.S.Pylaev

On the basic stages of postnatal ontogenesis of rat SIF-cells in cranial and peripheral ganglia were investigated. The following parameters were measured: the number of SIF-cells, areas of their preferable locations, ability to store of mediator, relationships between SIF-cells ahd ganglionic neurons or blood vessels. Three types changes in SIF-cells number were found. The first is observed in the main part of ganglia were investigated and consist in growing during initially postnatal weeks and slow reduction with ageing. Lumbar sympathetic ganglia lose practically all SIF-cells during first two weeks. The main pelvic ganglion is characterized by slow increase of this parameter with ageing. In each ganglion were found areas of preferable locations of SIF-cells. Their areas moved postnatally. In main pelvic ganglion tissue clusters of SIF-cells are foundation in zone occupied by adrenergic neurons but SIF-cells, situated alone, we can meet often in parasympathetic area. SIF-cells monoamine's level as a rule, independs from types of autonomic ganglion: from birth to two weeks failures exchanging rising in future. Clustered SIF-cells formed typical endocrine structures sometimes but not everytimes. The groups of SIF-cells are surrounded by satellite cells can be recognized far from the vessel. Efferent synapses from SIF-cells on profiles can be defined as neuronal dendrites relatively rare. This does not excluded possibility of transsynaptic modulation by SIF-cells mediator, because the distance of mediator diffusion is short. We can mark ultrastructural parameters of SIF-cells are different in cranial and peripheral ganglia not saving about relationship with blood vessels and neurocytes as postnatal variations of SIF-cells number. We proposed the following dynamics of SIF-cells function in time; pre- and early postnatally they can determinate ganglionic phenotype in details; in adult SIF-cells produce monoamines and peptides; in old age the most probably the function of compensation of reducing activity of sympatoadrenal system comes obligate.

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THE INFLUENCE OF THE NON-COMPETITIVE ANTAGONIST NMDA RECEPTORS (MK 801) ON CORTICAL EPILEPTIC AFTERDISCHARGES IN YOUNG RATS. D. Marešová

The influence of MK 801 (non-competitive antagonist of NMDA receptors) on cortical epileptic afterdischarges (Ads) was tested in freely moving young rats. Cortical afterdischarges were elicited by electric stimulation of the sensorimotor cortex in rats aged 12, 18 and 25 days. Duration and electrocorticogram pattern of Ads were age dependent. Repeated stimulation (6 times, interval between the end of Ads and next stimulation was 1 min) significantly prolongated the duration of Ads in 12-day-old rats. In 18-day-old rats the same stimulus had no effect. In 25-day-old rats the repeated stimulation significantly shortened duration of the 2nd, 3rd and 4th cortical afterdischarges

MK 801 in the dose 0.1 mg per kg of b.w., administered i.p. 30 min before stimulation, blocked the prolongation of Ads resulting from the repeated stimulation and shortened the third Ad. In 18-day-old rats, MK 801 shortened duration of the 1st, 4th and 5th Ads. In 25-day-old rats, such shortening was limited to the first afterdischarge only.

Our results indicate the MK 801 blocking of postsynaptically localized NMDA receptors leads to the decrease of natrium current, diminution of amplitude of EPSP and thus to the decrease of tissue excitability.

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THE EFFECTS OF MENTAL CALCULATION AND JENDRASSIC'S MANOEUVRE ON ACHILEEAN REFLEXOGRAM. Buraga M, Cârmaciu R, Papacocea R, Băcanu I

We have studied on a 69 subjects group between 19-23 years old (45 girls and 24 boys) the Achileean reflexogram in next conditions: basal status, mental activity and Jendrassic's manoeuvre. The Achileean reflexogram intervals were automatically registered with a reflexometer. We determined: T1, the latency period, T2, the reflex time, T3, the semirelaxation time.

RESULTS

1. Latency time is unmodified by mental calculation, but is significantly reduced by Jendrassic's manoeuvre application ($p<0.05$).
2. Semirelaxation time (T3) increases significantly during Jendrassic's manoeuvre, compared to T3 from rest and mental calculation.
3. Reflex time significantly decreases during mental calculation and rest.

The facility of Achileean reflexogram is more evident during Jendrassic's manoeuvre.

CONCLUSIONS

Although mental calculation decreases the reflex time, we consider that the primary effect of Jendrassic's manoeuvre does not consist of attention disturbance, but in activation of Proprioceptors' Control System.

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ARRHYTHMIAS INDUCED BY MAGNESIUM DEFICIENCY IN PATIENTS THAT UNDERWENT ABDOMINAL SURGERY. Dorina Bunea, Corneliu Zeana.

Magnesium (Mg) deficiency is frequent in the whole population, due to low intake and to excess occasional losses, that occur in stress.

Surgical operations represent a significant and prolonged stress and cause important Mg loss. In patients that have suffered an abdominal operation and are subsequently fed parentheraly for about one week, the Mg intake is drastically reduced and without a parentheral supplement of Mg, its plasma level is reduced, which can cause arrhythmias.

I have monitored 100 patients over an interval of three years - 1992-1994 - after a surgical operation. The patients were both males and females - 74 men and 26 women, 30-70 years old, operated for gastric, esophageal-gastric tumors and for acute pancreatitis.

Out of the total number of patients only in five cases severe arrhythmias were noted, occurring within 12 days from the operation: atrial paroxysmal tachycardia, atrial flutter, ventricular paroxysmal tachycardia and two cases with ventricular extrasystole. In these patients the plasma level of Mg was noted to be reduced up to 1.4 mg %.

These patients had no previous heart pathology and also no arrhythmias during the operation or within the first days after the operation.

Administration of antiarrhythmic drugs and or electric shocks provided no improvement for these patients. In all cases the arrhythmias were efficiently treated with Mg and there was no recurrence.

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RESPONSE OF RESPIRATORY REGULATION TO NON-ZERO AIRWAY PRESSURE. C.J. Caljouw, C.P.M. van der Grinten and S.C.M. Luijendijk

The contribution of pulmonary receptor output to ventilatory response was studied in spontaneously breathing anaesthetized cats. Receptor output was modulated by application of continuous positive or negative airway pressure in the range from -1.0 kPa to +1.0 kPa. At each tracheal pressure the steady state response to increased inspiratory CO_2 levels (4 to 6 from 0.0 to 5.0 kPa) was determined. During each measurement flow, transpulmonary pressure (P_{tp}), PCO_2 and EMG activity from the diaphragm and abdominal muscles were recorded. Additionally two arterial blood samples were taken for blood gas analysis. The entire protocol was repeated after bilateral vagotomy.

For each tracheal pressure CO_2 response curves were constructed as an indication of the state of the ventilatory control system. Despite large individual differences, there was no significant effect of applied tracheal pressure on the CO_2 response curve before vagotomy. We found the CO_2 sensitivity to be in the range of 0.5 to 1.2 $l \cdot min^{-1} kPa^{-1}$ and the apnoea point in the range of 3.0 to 4.0 kPa. After vagotomy there generally was a slight decrease in apnoea point (range 2.5 to 3.8 kPa), with the exception of all experiments with "high" (over 0.5 kPa) tracheal pressure. There we found the the apnoea point to be around 0.0 kPa. It could however clearly be seen from plotting P_{tp} vs. lung volume that, when volume increased, P_{tp} increased disproportionally, indicating mechanical limitation. The CO_2 sensitivity changed unpredictably with gains between 0.5 and 2.0 $l \cdot min^{-1} kPa^{-1}$.

We conclude that in steady state conditions pulmonary receptor output does not influence the CO_2 response, but does enable the respiratory control center to force the lung in the mechanically most desirable state, i.e. the "more compliant" part of the compliance curve.

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