1

FLUIDITY MEASUREMENTS IN WHOLE CELLS AND IN ISOLATED PLASMA MEMBRANES

M. Goppelt and W. Urbach

Fluorescence polarization measurements in intact cells are often used to get informations about the fluidity of cell membranes. Though the method is rather simple, there are contradictory reports in the literature as to whether the plasma membrane gets more fluid in response to growth stimuli. One example is represented by T-lymphocyte activation by mitogens such as ConA or succinylConA, which both bind to receptors of the plasma membrane and induce growth and proliferation.
T-lymphocytes prepared from calf thymus were incubated with either ConA or succinylConA for time periods up to 90 min. The hydrophobic label DPH (diphenylhexatriene) was incorporated into whole cells as well as purified plasma membranes. Its fluorescence polarization was measured and the order parameter calculated, which correlates inversely with the fluidity of the membrane.
Purified plasma membranes of stimulated thymocytes were more fluid than those of nonstimulated cells. This effect is caused by changes in the membrane lipid composition, predominantly in the degree of unsaturation of the phospholipid fatty acids.
However, fluorescence polarization measured in whole cells showed the opposite result: upon addition of the mitogen the calculated order parameter indicated a decrease of the membrane fluidity. The effect was reversible when the mitogen was removed by a competitive inhibitor and thus may be due to the binding of the mitogen on the surface of the cell.
Thus fluorescence polarization measurements in whole cells do not reflect truly biochemical changes of the plasma membrane. These can only be monitored in purified membranes.

Medizinische Hochschule Hannover, Zentrum Pharmakologie und Toxikologie, Abt. Molekularpharmakologie, D-3000 Hannover 61, FRG.

2

LOCATION AND MOTION OF UBIQUINONE 50 IN PHOSPHOLIPID LIPOSOMES (SMALL UNILAMELLAR VESICLES = SUV)

L. Michaelis, K.E. Wirth

It is well established that ubiquinone 50 (Coenzyme Q 10) functions in the electron transport chain of the inner mitochondrial membrane as well as in non-mitochondrial redox systems. There is still disagreement about the transverse diffusion ("flip-flop" movement) and location of this substance.

Ubiquinone could be incorporated into phosphatidyl choline liposomes (suv). A nonstoichiometric relation was observed up to 20 mole % ubiquinone. Electron microscope photographs showed no visible influence of ubiquinone on the membrane structure.

Fluorescence experiments with reduced ubiquinone showed a photochemical reaction of the quinol. Plastoquinol 45 from chloroplasts did not show this reaction.
Ubiquinone could be reoxidised with persulphate solutions by a copper catalysed reaction that was first-order with respect to ubiquinol. Either ubiquinol or electrons must cross the membrane at a rate, faster than the rate of oxidation, which was 0.07/sec.
The reduction of ubiquinone, and the reoxidation of ubiquinol, incorporated into dipalmitoyl phosphatidyl choline liposomes showed that both above and below the transition temperature of the membrane lipid, all the headgroups could react rapidly with reagents dissolved in the external aqueous phase. This suggests that regions of ubiquinone across the lipid bilayer might be liquid whatever physical state the membrane may have.

Institut für Pharmakologie der Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf

3

DETERMINATION OF PICOMOLE AMOUNTS OF CHOLINE IN PLASMA AND ERYTHROCYTES BY A CHEMILUMINESCENCE MEASUREMENT

O. Pleul

Choline becomes oxidized enzymatically producing betaine and H_2O_2. This oxygen consuming process is catalyzed by choline oxidase. H_2O_2 is detected by a luminescent reaction using luminol as reactant and peroxidase as catalyst. The procedure was shown by M. Israel and B. Lesbat(Neurochem. Intern. 3:81, 1981) to be suitable to measure acetylcholine in electric organ synapses.

Picomoles of choline can be determined corresponding to microliters of plasma or hemolysate. By adequate amplification of the chemiluminescence signal the determination was suitable also at the nanomole level.

Because of the specifity of the choline oxidase reaction no special purification of the samples was needed.

The influence of concentration and species of enzymes and pH on sensivity and reproducibility were tested. Blood and tissue concentrations of choline and choline derivatives in small animals are determined and the results are compared with other analytical methods.

Institut für Klinische Pharmakologie der Freien Universität Berlin, Hindenburgdamm 30 1000 Berlin 45

4

Isotachophoretic Determination of Acetylcholine

Haen, E.; Fiedler, I.; Gerbes, A.; Szinicz, L.

Present methods for measurement of acetylcholine (ACh) are based on the transformation of the molecule either enzymatically with subsequent labelling with radioactive isotopes (Goldberg et al. J. Neurochem. 20, 1,1973) or by a chemical reaction to form a volatile derivative for gas chromatography (Kilbinger, J. Neurochem. 21, 421, 1973; Jenden et al. Analyt. Biochem. 55, 438, 1973). Capillary isotachophoresis on the other hand permits the direct measurement of the unchanged substance. Isotachophoresis means the migration of ion species of the same sign in an electrolyte system placed in an electric field. They are separated on the basis of their different mobility. A conductivity detector was used giving a step - like signal, since the different ions are arranged in sharply separated zones. The relative height of the step is characteristic for a certain substance, whereas the length of the step is proportional to the amount of the substance.
In such a determination 50 pmol ACh is equivalent to a step length of 17.0 ± 1.2 mm (= 6.9% of the mean; n=4). The correlation between the amount injected into the capillary and the step length was linear in the whole range investigated (r=0.999). Two electrolyte systems were examined: 3 mM KOH/Cacodylic Acid, pH 7.0 - 10 mM Creatinine and 3 mM KOH/Acetic Acid, pH 4.6 - 10 mM ß-Alanine, the relative height for ACh being 18.7 ± 0.15 % (=0.8% of the mean, n=4) and 82.6 ± 0.7 % (=0.8% of the mean, n=4) respectively. The optimal injection volume was found to be 20 µl. Time for one analysis was 15 minutes.

Institut für Pharmakologie und Toxikologie der Universität München, Nussbaumstr. 26, D-8000 München 2

5

ENZYME IMMUNOASSAY OF THROMBOXANE B_2 (TxB_2) AT THE PICOGRAM LEVEL

M. Sawada

A very sensitive and reproducible enzyme immunoassay was developed for the measurement of TxB_2, instead of radioimmunoassay which poses the practical problems of exposure to radioisotopes, contamination and their disposal. TxB_2 as a label displacing radioactivity was coupled with ß-D-galactosidase by mixed anhydride reaction. The separation of immunocomplex from free TxB_2 was carried out by double antibody method. The precipitated enzyme was measured fluorometrically with 4-methylumbelliferyl-ß-D-galactoside as substrate. This method allowed to measure TxB_2 in the range of 0.001 - 5 pmol per tube. The cross-reactivity of the anti TxB_2 antiserum with α-dinor TxB_2 was about 20 %, but it was without significance for primary prostaglandins. The extracted TxB_2 from human urine was measured by both enzyme immunoassay (y) and radioimmunoassay (x) which has been compared to gas chromatography - mass spectrometry. Regression analysis of the data gave the satisfactory equation ($y = 0.996 x + 0.430$, correlation coefficient $r = 0.9947$). Intra-assay coefficient of variation was 3.7 % ($n = 10$). This enzyme immunoassay for TxB_2 could be regarded as potentially equivalent to radioimmunoassay, as far as assay precision, accuracy and sensitivity are concerned.

Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Auerbachstr. 112, 7000 Stuttgart 50

Ono Pharmaceutical Co., Ltd., Osaka / Japan

6

HPLC-PROCEDURE FOR THIAMIN DETERMINATION TO STUDY KINETICS IN HUMANS

W. Weber and H. Kewitz

After deproteinization and enzymatic hydrolysis of thiaminphosphates, thiamin is converted to highly fluorescent thiochrome by alkaline oxidation. The isobutanol extract, which contains the thiochrome was used for separation on HPLC, Lichrosorb-NH_2 column, eluted by ether/methanol 75/25, v/v. A spectrofluorometer was used for detection. Thiochrome phosphates were not soluble in isobutanol, allowing differentiation between thiamin and thiaminphosphates. Due to phosphatase activity in urine, there is free thiamin only.

Analytical recovery in plasma was 99.3 % and 97.9 % in urine with SD of 14.6 % and 6.6 %, respectively. Reference values of total thiamin in plasma of 91 volunteers were 4 - 30 pmol/ml, 1/3 the range in previous studies. Our results in whole blood agree with values reported by different authors.

After i.v. injection a three exponential elimination was found with $T/_2$ α phase 6 min, $T/_2$ ß phase 1 h and $T/_2$ γ phase 20 h. 6 h after 100 mg thiamindiphosphate i.v. 71 % of the dose were found in the urine and 77 % within 24 h. Respective values with 100 mg thiamin hydrochloride were 52 % and 54 %.

3 min after injection of thiamindiphosphate almost 50 % of the total concentration in plasma was hydrolysed to thiamin.

Institut für Klinische Pharmakologie, Freie Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45

7

HOMOGENIZATION OF MICE FOR INDUCTION EXPERIMENTS IN VIVO.

F. Heubel

In vivo induction parameters (relative liver weight, microsomal protein and cytochrome P450 content, metabolism of model substrates) are subject to considerable variability between individuals and between different consignments of animals, even if these are equal in respect to strain, sex, age and maintenance conditions. Therefore, if only slight differences of p.e. cytochrome P450 are to be demonstrated with statistical significance, inadaequately great numbers of animals are required. More homogeneous samples might decrease these numbers.

The effects of some homogenization measures on induction parameters were assayed in male Han:NMRI and C57BL/6JHan mice. Variability between consignments was present in NMRI (randombred) as well as in C57BL (inbred) mice. In mice of more than 6 weeks of age homogenization of age (maximal difference ± 0.5 days) had only little effect. "Purified diet" (a diet which consists of food components isolated from plants by technical processes) significantly decreased protein and cytochrome P450 content but also had rather little effect on variability. Equality of litter size markedly decreased variability within consignments as well as between consignments. Inbred mice from litters of equal size were more homogeneous than inbred ones from natural litters and randombred ones from equal litters. A difference of as little as 15 % of cytochrome P450 was significantly demonstrated, when inbred mice of equal age (72 ± 0.5 days), bred in litters of 6 animals each, were compared in a design containing a total of 16 controls and 16 treated animals. - Our findings suggest that animals of one consignment form a sample which, unintentional, is not drawn at random. This hypothesis should be tested by using an adaequate drawing scheme when consignments are put together by the producer.

Institut für Pharmakologie und Toxikologie, Philipps-Universität, Lahnberge, D-3550 Marburg.

8

QUANTIFICATION OF EXTREMELY VARIABLE FIRING RATES AS CONSECUTIVE HISTOGRAM WITH ADJUSTABLE BINWIDTH

K. Schmid

Measuring neuronal firing rates is a common problem in electrophysiology. In most cases counting pulses within a time window is not sufficient but it is necessary to compute frequency from interspike interval duration. In some applications however, this method has serious drawbacks especially when spike density shows abrupt changes and covers a wide range as is often observed with respiratory modulated neurons. We have developed hardware to solve this problem. This device has some additional useful features.

Our design is based on period-to-frequency conversion but avoids the annoying effect that the resulting frequency value from computation of an interval is necessarily displayed during the following interspike interval instead of in its matching interval. We solved this problem in using a random-access memory with 4096 locations and a wordlength of 12 bits, where successive values were continiously written and read out. This trick allows an exact matching of the frequency to its corresponding interval, which is in principal impossible being straight online. Digital to analog conversion leads to a sequential frequency histogram with binwidths that are identical with interspike interval length. Additionally, we made provision to generate a consecutive histogram with freely adjustable binwidths between 10 ms and 1 s in steps of 10 ms, by arithmetic computation of frequency content of subsequential bins. The output of this newly constructed device is either an analog voltage or a digital word with a resolution of 10 bits. To interface this instrument to a digital computer an appropriate handshake line is employed.

Physiologisches Institut der Universität Mainz, Saarstr. 21, D-6500 MAINZ

9

AN IMPLANTABLE ELECTRODE FOR RECORDING ELECTROMYOGRAPHIC ACTIVITY IN FREELY MOVING ANIMALS

G. Sigrist, D. Kleinebeckel

The construction of a small metal electrode for electromyography is described. The electrode consists of two small chlorided stainless steel pins 0.5 mm apart (diameter:0.15 mm; length: 1.5 mm with sharpened ends), mounted in a hemispherical polymer back, and two twisted stainless steel wires (0.05 mm) coated with varnish. Based on about 1000 electromyographic recordings from hindleg muscles of rats and frogs our experiences may be summarized as follows:
1) Galvanic plating of the electrode pins with gold, silver and chloride provides very reliable nonpolarizing electrodes; the impedance as measured in 0.9% NaCl-solution at 1 kHz varies between 2 and 4 kiloohms.
2) Preparation of the electrodes takes about 30 min/piece when made in a batch. The material is cheap and easily available.
3) The insertion of the two electrode pins into the muscle under investigation and the pressure of the neighbouring muscles on the polymer back assures stable fixation of the electrode.
4) When chronically implanted, good electromyographic potentials can be recorded up to five weeks.
5) Recordings have been done from muscles with a length of 10 mm and a thickness of 2 mm. Since the electrode can be made considerably smaller, recordings from even smaller muscles seem feasible.

Pharmakologisches Institut der RWTH Aachen, Schneebergweg, D-5100 Aachen
Institut f. Normale und Pathologische Physiologie Robert-Koch-Strasse 39, D-5000 Köln 41

10

A MULTIPLE ACTIVITY-MONITOR FOR SMALL ANIMALS AND ITS APPLICATION FOR DETERMINATION OF SLEEPING TIME CHANGES AFTER DIFFERENT TYPES OF LIVER TREATMENT

J.Lutz and M.Wagner

The determination of the recovery time after sleep induction by intraperitoneal injection of pentobarbital (30 mg/kg body weight) can be used as a sensitive test to judge the detoxifying function of the liver. As it is thereby preferable to use several animals at the same time, the construction of a low cost multiple activity-monitor became necessary.

Six swinging metal plates, each suspended with nylon threads from four rods, carry a ferrit magnet dipping in an inductive coil. A macrolon ^{R} cage with one experimental animal (rat) is placed on each plate. Through changing the length of the suspending threads the resonance frequency of the cages may be varied. Registration is performed on a 6-fold compensation recorder (Fa.Linseis, Selb,FRG).

The criterion used for the termination of the sleeping time is the appearance of the righting reflex (raising from the side position) which is combined with a distinct increase in the amplitude of the plot. By means of a comparison with control groups, enzyme induction effects under repeated measurements are compensated and relatively small changes in the recovery time can be discovered, as is evidenced by several examples. The method is used as a screening test for the microsomal function of the liver.

Physiologisches Institut der Universität Würzburg, Röntgenring 9, D-87 Würzburg

11

A MULTIPLE T-MAZE AS TEST FOR MEASURING THE EFFECT OF PSYCHOTROPIC DRUGS

U. -A. Jänicke

A multiple T-maze was used to prove higher integrative cognitive abilities. It is composed of straight and T-shaped light-proof tubes (ϕ 8 cm) and can be combined to max. 6 right-left choice possibilities. In a repeated distance of 45 cm there are gates with infra-red light barriers, the impulses of which are transferred to a computer system. It registers the running time between two gates, the total running time, the number of correct runs as well as the rate of errors and the time of decision to go either to the right or left direction. 32 male Wistar rats, 4 - 6 months old, were tested. The control group comprised 16 rats, the other 16 animals had to drink d-amphetamine solution (nearly 5 mg/kg/21 h). After a short pretraining period (straight runway) the learning procedure involved a 2-choice multiple T-maze, a 6-choice multiple T-maze and the reversed pattern of the 6-choice T-maze. Each maze training was given on 5 consecutive days with two trials per day. The running time (sec/100 cm), the number of correct runs and the rate of errors were recorded. The running time improves with increasing difficulty of the task in both groups, if the rats perform the task correctly. In the 2-choice maze the number of correct runs seems to be smaller in d-amphetamine treated animals. In the 6-choice maze, the control rats show a clear learning effect within the 5 days, which is not evident for d-amphetamine treated animals. The latter, however, attained much better performances in the reversed 6-choice maze. Similar results are obtained with regard to runs with errors. Accordingly the accuracy of the performance will be largely independent of the running time.

Institut für Neuropsychopharmakologie der Freien Universität Berlin, Ulmenallee 30, D-1000 Berlin 19

12

WHAT IS THE BASIS OF THE "LAW OF INITIAL VALUE"?

G. Pöch

One of the main issues of the "law of initial value" (LIV) is the general assertion that the lower the initial value the higher the response to "function raising stimuli" (Wilder J.: Stimulus and response. The law of initial value. Wright & Sons, Bristol 1967). As frequently done, Wilder expressed "response" as the difference (Δ) between basal and "drug values".

We have recently obtained experimental evidence in line with the LIV which can be explained by a change in baseline brought about by a mechanism which is independent from the action of the substance under study (Meth. Find. 4: 379, 1982). Under such conditions Δ-values of response appeared dependent on the baseline, whereas the affinity and intrinsic activity of the drug tested remained unaltered.

On the other hand, the contractile effects of carbachol, histamine or serotonin on isolated bovine tracheal muscle at normal (2.7 mM) and at elevated K^+ (20 mM) in recent experiments were not in accordance with the LIV. Halfmaximum effective drug concentrations at the lower baseline (2.7 mM K^+) either showed the same increase in tone (Δ) as at 20 mM K^+ (carbachol, serotonin) or even less pronounced increases (histamine). Under these conditions their EC_{50}-values were 5-10-fold lower at the higher baseline (20 mM K^+), i. e. potentiated.

Hence, the basis for the LIV can be seen in the actions of biologically active substances which are completely, or largely, independent from mechanisms affecting the baseline.

Inst.f.Pharmakodyn.u.Tox., A-8010 Graz,Univ.-Pl.2

13

ED_{50}-VALUES FOR EFFECTS OF ISOPRENALINE IN THE ANESTHETIZED RAT ARE INDEPENDENT OF BASELINES

F. Brunner

The determination of ED_{50}-values by means of dose response curves (DRC), particularly in the evaluation of receptor antagonists sometimes appears to be crucial when large differences in the control values (baselines) from one experiment to another are obtained. Therefore, the effect of baseline-variations on the ED_{50} was studied in urethane-anesthetized rats in which the ED_{50}-values of isoprenaline (Iso) for increases in heart rate (HR) and decreases in blood pressure (BP) were determined in the absence and presence of various beta blockers. The animals of various experimental series were divided into 2-3 groups with (significantly) different control values. All DRC were derived from absolute reading values (mm Hg or beats/min).

Systolic BP prior to application of Iso ranged between 95 and 120 mm Hg in the absence and between 80 and 120 in the presence of ß-blockers; the respective heart rates were 300-400 or 200-400 beats/min. In the absence of ß-blockers, ranges of ED_{50} (µg/kg) for Iso were 2.2-2.5 (BP) or 0.71-0.95 (HR); in the presence of 14 mg/kg practolol, these values were 1.6 and 1.9 (BP) or 7.1, 11.2 and 12.0 (HR); in the presence of 14 mg/kg metoprolol, they were 6.3 and 10 (BP), or 50 and 71 (HR); for a new structural analogue of metoprolol (Z 249), the respective values were 2.2 and 2.5 (BP) or 2.2 and 2.2 (HR).

The results indicate that in spite of significantly different control values the respective ED_{50}-values, as obtained from complete DRC, differ by less than a factor of 2, irrespective of a concomitant depression of the maximum response.

Inst.f.Pharmakodyn.u.Tox.,A-8010 Graz,Univ.-Pl.2

14

POLARIZABILITY AND DIPOLE MOMENT: DETERMINATION AND MOLECULAR IMPORTANCE FOR THE VERAPAMIL ACTION

R.Mannhold,R.Steiner,R.Rodenkirchen and R.Bayer

Electronic properties of drug molecules play an important role in receptor interactions. Thus, a complete data set of physicochemical parameters, which covers all aspects of charge dependent drug receptor interactions is compulsory for a detailed analysis of the underlying binding forces. Such a data set is presented here for a number of important benzene derivatives and is applied to precisely interpret the negative inotropic potency of verapamil congeners. Polarizability values have been calculated from the respective indices of refraction. Measurements have been performed with a sensitive laser refractometer. The determination of dielectric constants and indices of refraction for serial dilutions of the test compounds was used to calculate the dipole moments.
QSAR investigations with these electronic parameters yield a double correlation, which describes the variance in biological potency of verapamil congeners to 92% and combines the Hammett constant σ and the polarizability α:

$\log 1/EC_{50} = 0.97(\pm 0.5)\sigma + 0.46(\pm 0.23)\alpha + 4.29(\pm 0.14)$
$n = 9$; $s = 0.17$; $r = 0.96$; $F = 31.17$; $p < 0.01$

It is concluded that verapamil derivatives with rather small substituents exhibit a positive correlation with MR indicating polarizability dependent dispersion forces to be involved in drug receptor interaction. Substituent volumes exceeding the optimum lead to inverse correlations with MR indicating that the drug receptor interaction now is sterically hindered.

Physiol.Inst.,Lehrst.f.Klin.Physiol., Universität Düsseldorf, Universitätsstr.1, D-4000 Düsseldorf

Supported in part by grants of SFB 30, Kardiologie

15

ELASTOLYTIC ENZYMES IN ELASTIC TISSUES

I.Feuerbacher, J.Fingerle

The fragmentation of elastic fibres by elastolytic enzymes is involved in pathological processes, e.g.atherosclerosis or diseases of the connective tissue. Continuous, direct observation of the course of elastolysis may elucidate attempts in studying drug actions on its acceleration or inhibition. Elastolysis in intact, native elastic tissue "in vitro" was studied with a fluorescence technique which is based upon measuring the characteristic autofluorescence of the tissue (excit. 366 nm - emiss. 460 nm). For the measurements a Zeiss microscope fluorometer was used. Pancreatic elastase applied to the surface of bovine aortic intima or elastic ligaments caused a concentration dependent decrease of the autofluorescence intensity in these tissues. The elastolysis could be completely inhibited by p-toluoenesulfonyl-fluorid (p-TSF), partially by aprotinin and α-natrium-aescinat. Tissue samples from the elastic lig. which had been deep frozen at -20°C before use, exhibited a similar decrease in the autofluorescence without exogenously applied enzyme. This effect was pH-dependent (optimum at pH 8.0) and could also be inhibited correspondingly by the three substances used. The presence of elastolytic enzymes in the elastic ligament was additionally investigated by the use of an aequous extract of liquid N_2 frozen elastic ligament on $Suc(Ala)_3pNA$ as a specific substrate for elastase. The problem of whether this enzymatic activity derives from cellular enzymes or from enzymes bound to the elastic fibres will be discussed.

Institute of Physiology (I), University of Tübingen
Gmelinstr.5, D-7400 Tübingen 1

16

COMPOUND 48/80: A HIGHLY SPECIFIC AND POWERFUL ANTI-CALMODULIN DRUG

Klaus Gietzen and Peter Adamczyk-Engelmann

Calmodulin (CaM) is the major receptor for Ca^{++} in non-muscle cells and modulates a multitude of Ca^{++}-dependent enzymes and all functions. The CaM-dependent fraction of these enzymes can be inhibited by a wide range of chemically unrelated substances that share one common characteristic in being all cationic amphiphiles. However, all described inhibitors are more or less unspecific in that they also inhibit the basal CaM-independent activity of these enzymes. The present work is part of our search for a more specific inhibitor of effects mediated by CaM. Compound 48/80, a condensation product of N-Methyl-p-methoxy-phenethylamine with formaldehyde, is composed of a family of cationic amphiphiles differing in the degree of polymerization. Compound 48/80 was found to be a potent inhibitor of the CaM-activated fraction of phosphodiesterase and Ca^{++}-transport ATPase with IC_{50}-values of 0.2 and 0.85 µg/ml, respectively. However, the basal activity of both enzymes is not at all effected by the drug in concentrations $\leq$ 300µg/ml. Kinetic analysis revealed that the activation of Ca^{++}-transport ATPase and phosphodiesterase by CaM is inhibited competitively.
Comparison of the specificity of several anti-CaM drugs shows that compound 48/80 is the most specific inhibitor of CaM-mediated effects that has been described hitherto. Because of its high specificity compound 48/80 is proposed to be an ideal tool for studying CaM-dependent processes.

Department of Pharmacology & Toxicology, University of Ulm, Oberer Eselsberg, D-7900 Ulm, FRG.

17

SYNERGISTIC INHIBITION OF HUMAN PLATELET ADENYLATE CYCLASE BY ADRENALINE AND STABLE GTP ANALOGS

K.H. Jakobs and G. Gabel

The interaction of stable GTP analogs (SGA) and adrenaline (ADR) was studied on human platelet adenylate cyclase (PlAC). Whereas basal PlAC activity was increased by SGA, SGA decreased the PlAC activity stimulated by fluoride, purified, preactivated N_s-protein or forskolin by 60-70 %, with the potency order, GTPγS > GMP-P(NH)P > GMP-P(CH_2)P. The inhibition of the forskolin-stimulated PlAC was half-maximal and maximal at about 4 and 100 nM GTPγS, respectively. The inhibition occurred after a time lag period, which was inversely related to the GTPγS concentration, and was persistent wo washing the membranes. PGE_1-stimulated PlAC activity exhibited a biphasic response towards GTPγS, with a further (2-fold) activation occurring at low (1 nM) and an inhibition (60-70 %) at higher GTPγS concentrations. The inhibitory effect of GTPγS was competitively antagonized by the stable GDP analog, GDPβS, and by GTP. The antagonism by GTP was prevented by ADR, which inhibited the forskolin-stimulated PlAC in the presence of GTP to the same degree as observed with GTPγS alone. Similar to GTPγS-induced inhibition, inhibition of the PlAC by ADR was blocked by GDPβS, acting competitively towards GTP. ADR had two effects on GTPγS-induced inhibition. First, the time lag required for the inhibitory action of GTPγS was largely diminished by ADR. Second, ADR increased in a concentration-dependent manner the inhibitory potency of GTPγS, without increasing the maximal degree of PlAC inhibition induced by GTPγS. The data indicate that PlAC is under a stimulatory and inhibitory control by SGA and suggest that these guanine nucleotide actions are mediated by two separate guanine nucleotide regulatory sites, N_s and N_i, which apparently also mediate PGE_1 and ADR-induced PlAC stimulation and inhibition, respectively.

Pharmakologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg

18

INFLUENCE OF ISLET-ACTIVATING PROTEIN (IAP) ON ADIPOCYTE ADENYLATE CYCLASE (AC)

K. Aktories, Ch. Dallenbach, F. Blackkolb and L. Robbel

IAP is a B. pertussis toxin, which was recently found to interfere with the inhibitory system of the AC apparently by an ADP-ribosylation reaction. We studied the effects of partially purified IAP on the bi-directional regulation of AC in rat and hamster adipocyte membranes after in vivo and in vitro application of the toxin. IAP was partially purified by chromatography of the supernatant of a B. pertussis (1.5×10^{14}) suspension on a hydroxyapatite column (M. Yajima et al., J. Biochem. 83: 295, 1978). Rats were treated with one dose (i.v.) of 10-100 µl of the concentrated IAP solution (~1 mg protein/ml). Three days later, adipocytes and adipocyte membranes were prepared. Stimulation of adipocyte AC by ACTH (1 µM) was 2 to 3-fold increased after IAP treatment compared to controls. GTP inhibited basal and forskolin-stimulated AC by maximally 60 % at 1 µM ($IC_{50} \cong 0.2$ µM) in control membranes. After IAP treatment, the inhibitory effect of GTP (up to 30 µM) was abolished. Additionally, NaCl (150 mM), which reversed the inhibitory effect of GTP in control membranes, was without effect after IAP treatment. Accordingly, the IAP treatment reduced or abolished the GTP and NaCl-dependent inhibition of the adipocyte AC by nicotinic acid. In contrast, inhibition of the adipocyte AC by GTPγS (up to 70 % in control membranes) was largely unaffected in adipocyte membranes from IAP-treated rats. Treatment of hamsters with IAP also reduced or abolished inhibition of AC in adipocyte membranes by GTP, nicotinic acid and PGE_1, whereas GTPγS still caused an inhibition. Treatment of isolated rat adipocytes with IAP for 2 h abolished GTP-induced inhibition, whereas AC inhibition by GTPγS was not affected. The difference between GTP (± hormones) and its stable analog, GTPγS, suggests that an altered GTP hydrolysis is involved in IAP's action on the AC.

Pharmakologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, and Behring-Werke, D-3550 Marburg

19

ADENYLATE CYCLASE INHIBITION BY SOMATOSTATIN IN N_s-DEFICIENT S49 LYMPHOMA cyc^- VARIANTS

G. Schultz and Chr. Stannek

The cyc^- variants of S49 lymphoma cells are deficient in the guanine nucleotide (GN) regulatory site (N_s), mediating hormone- and GN-induced adenylate cyclase (AC) stimulation, but their AC can be stimulated by forskolin (FO). Stable GTP analogs, which stimulate AC in other cell types, inhibit FO-activated cyc^- AC, which findings suggest the presence of an inhibitory GN site (N_i) in these cells. The possible occurrence of an N_i-coupled hormone receptor was studied in cyc^- cells. Somatostatin (SSt) decreased the FO-stimulated cyclic AMP levels in cyc^- cells by maximally 35%, with half-maximal and maximal inhibitions occurring at 0.1 and 10 nM SSt, respectively. In the absence of GN, SSt had no effect on AC in cyc^- membranes. However, in the presence of GTP (1 µM), SSt inhibited the FO-stimulated AC by 20-30%, with half-maximal and maximal inhibitions occurring at about 1 and 10 nM SSt, respectively. Inhibition by the stable GTP analog, GTPγS, was accelerated and amplified by SSt. In addition, SSt increased the activity of a high affinity GTPase ($K_m \cong 0.2$ µM) by 30-40%, but had no effect on low affinity GTPase(s) ($K_m \cong 50$ µM). Stimulation of GTP hydrolysis by SSt was half-maximal at 3 nM. Similar data with regard to inhibition of AC and stimulation of a high affinity GTPase by SSt were obtained in membranes of S49 H21a variants, which exhibit GN effects on ß-adrenergic agonist binding but in which N_s coupling to AC appears to be defective. As studied in H21a membranes, SSt-induced GTPase stimulation was reduced or abolished by pretreatment of the membranes with N-ethylmaleimide or by stable GTP analogs. The data suggest that the N_s-deficient cyc^- membranes contain a N_i component, which mediates SSt-induced AC inhibition, and that the SSt- plus GTP-activated N_i is inactivated by a GTPase reaction.

Pharmakologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, Germany.

20

CALCIUMION-DEPENDENT STIMULATION OF ARTERIAL GUANYLATE CYCLASE BY 2',3'-DI-O-NITRO-5'-(N-ETHYLCARBOXAMIDO)-ADENOSINE (DINITRO-NECA) AND OTHER NITRO-COMPOUNDS

G. Steurer and W. Schütz

The adenosine analog dinitro-NECA is characterized by a delayed onset and prolonged duration of vasodilation in the intact dog, a finding which may be due to its complete denitration to NECA as the pharmacologically active metabolite (Wiener et al., Arch. Pharmacol. 319:R5). Since, however, dinitro-NECA can also relax isolated arterial strips in close correlation with increases in cyclic GMP levels (Kukovetz et al., Arch. Pharmacol. 310: 129), it was investigated whether this compound acts as a stimulator of vascular smooth muscle guanylate cyclase, as is well known for other nitro-compounds. In a 100,000 g supernatant of hog carotid arteries, dinitro-NECA markedly stimulated guanylate cyclase with an EC_{50} of 2µM. Similar to sodium nitroprusside and isosorbide dinitrate, this effect was enhanced by cysteine and almost completely abolished by methylene blue. NECA, a potent agonist at adenylate cyclase-coupled R_a-site adenosine receptors, was without any effect on guanylate cyclase activity. On the other hand, dinitro-NECA did not stimulate adenylate cyclase in adenosine-responsive tissues.

Further, the effect of Ca^{2+} on arterial as well as on hepatic guanylate cyclase has been studied using a metal buffer to regulate the free Ca^{2+} concentration. At 0.2µM Ca^{2+}, the guanylate cyclase stimulatory effect of all nitro-compounds investigated became significantly depressed; at 10µM Ca^{2+}, an 80% reduction in guanylate cyclase stimulation was observed. However, the basal guanylate cyclase activity proved independent of the free Ca^{2+} concentration. Hence, it may be supposed that guanylate cyclase stimulation induced by nitro-compounds can be modulated in vivo by free Ca^{2+}.

Pharmakologisches Institut der Universität, Währinger Str. 13a, A-1090 Wien

Supported by the Anton Dreher-Foundation

21

EFFECTS OF N-ETHYLMALEIMIDE ON R_i- AND R_a-ADENOSINE RECEPTORS IN ADIPOCYTES AND PLATELETS

D. Ukena, E. Poeschla, E. Hüttemann, U. Schwabe

Previous studies have shown that R_i- and R_a-adenosine receptors are labeled by several radioligands. Characterization of R_i-adenosine receptors has been achieved by (-)N^6-phenylisopropyl [^{3}H]adenosine ([^{3}H]PIA) and 1,3-diethyl-8-[^{3}H] phenylxanthine ([^{3}H]DPX), whereas labeling of R_a-adenosine receptors has been attempted with 5'-N-ethylcarboxamido[^{3}H]adenosine ([^{3}H]NECA). In the present study the effects of the sulfhydryl reagent N-ethylmaleimide (NEM) on the binding of [^{3}H]PIA and [^{3}H]DPX to R_i-receptors in rat adipocyte plasma membranes and the binding of [^{3}H] NECA to R_a-receptors in human platelet membranes were investigated. NEM pretreatment of platelet membranes had no effect on [^{3}H]NECA binding. Pretreatment of adipocyte plasma membranes with NEM inhibited the binding of [^{3}H]PIA to R_i-receptors with an IC_{50}-value of 2 µM, but not the binding of [^{3}H]DPX. The effect of NEM on the binding of [^{3}H]PIA was maximal after pretreatment for 30 min and was prevented by dithiothreitol. In the presence of 100 µM NEM the dissociation constant (K_D) for the binding of [^{3}H] PIA was reduced from 4.6 nM to 25.6 nM, whereas the maximal number of binding sites (B_{max}) was only marginally reduced from 2.0 to 1.3 pmol/mg protein. These results suggest that NEM uncouples R_i-adenosine receptors, probably by affecting the inhibitory guanine nucleotide-binding regulatory protein (N_i), whereas R_a-adenosine receptors are not affected.

Institut für Pharmakologie und Toxikologie der Universität Bonn, Reuterstr. 2 b, D-5300 Bonn 1, Fed. Rep. Germany

22

DIFFERENTIAL EFFECT OF LOCAL ANESTHETICS ON IN VITRO ABSORPTION OF GLUCOSE IN JEJUNAL AND ILEAL SEGMENTS OF RATS

G.Strugala, H.Fasol and W.Forth

The influence of lidocaine on the intestinal glucose absorption was investigated using the glucose analog 3-O-methylglucose. The experiments were performed on non blood perfused isolated intestinal segments in vitro of rats according to the method of Fisher & Parsons, modified by Rummel & Stupp (Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmak., 240, 79, 1960).

In ileal segments the glucose transport across the mucosal epithelium is markedly inhibited for about 70% even at concentrations of lidocaine as low as 10^{-8} mol/l. Surprisingly, no effect on glucose absorption with the proximal jejunum can be observed.
Within a wide concentration range from 10^{-8} - 10^{-3} mol/l lidocaine the water and sodium absorption in the small intestine are not affected, whereas the concentration of potassium on the serosal side of the ileum increased significantly at 5 x 10^{-4} mol/l lidocaine.

These findings are discussed with respect of the observation that some drugs such as local anesthetics (Lacko et al.,J.Cell.Physiol.,92,257,1977) and steroids (Lacko et al.,J.Cell.Physiol.,86,673, 1975), having no structural similarity to glucose, act as competitive or non-competitive inhibitors of glucose transport in human erythrocytes.

Institut für Pharmakologie und Toxikologie der Medizinischen Fakultät der Ludwig-Maximilians-Universität, Nußbaumstr. 26, D-8000 München 2

23

ORAL ABSORPTION OF VERAPAMIL WHEN GIVEN SIMULTANEOUSLY WITH CIMETIDINE AND DURING THERAPY WITH CYTOSTATIC DRUGS

J.Kuhlmann,B.G.Woodcock,J.Wilke,M.Wenchel and N.Rietbrock

Cimetidine has been shown to increase the absorption of propranolol by 40 - 90% due to a change in liver drug metabolism and/or liver blood flow. Cytostatic drugs reduce the rate and extent of absorption of digoxin attributable to reversible damage of the gastrointestinal mucosa but the absorption of digitoxin is not affected (Kuhlmann et al, Clin Pharm Ther 32:646,1982). Verapamil has a disposition in man similar to propranolol and under normal conditions, like digitoxin, is completely absorbed from the gut.
The effect of cimetidine and cytostatics on the absorption of verapamil ,obtained from the area under the serum concentration-time curve of verapamil(AUC) has been determined in patients having normal kidney and liver function.Verapamil, 160mg was administered as a single dose during the fasting state lasting from 12h prior to at least 2h after administration. From preliminary findings the AUC of verapamil during treatment with cimetidine 900mg daily was unchanged in 2 patients whereas an increase and a decrease were observed in a further two subjects.The first of a group of 8 patients with inoperable carcinomas who received verapamil during treatment with cytostatic drugs at dose rates known to reduce digoxin absorption by 50% showed no change in AUC and the time to peak concentration was also unchanged.These results suggest that verapamil absorption across the gut wall and the passage through the liver are not readily influenced by concomitant drug administration.

Department of internal medicine, University Clinic Würzburg and Department of Clinical Pharmacology, University Clinic, Frankfurt am Main, West Germany.

24

CONCENTRATIONS OF INDOMETHACIN IN INFLAMED EXSUDATE OF RABBITS DURING 10 HOURS INFUSION

P. Dittrich

The question whether or not nonsteroidal antiinflammatory drugs (NSAID) are accumulated in inflamed tissue is still a matter of controversy. Higher concentrations of NSAIDs in tissue than in blood were found after single dose applications of Indomethacin(Dittrich et al., N.S.Arch.Pharmacol. 321, R58, 1982) but may have been due to different elimination rates. This question was therefore studied more closely during a constant infusion of 5 mg/hr of Indomethacin (I) over ten hours in an inflammation model in rabbits. Inflammation was induced by injection of 2 ml of a 1 % carageenan solution into the lumen of a perforated, subcutaneously implanted teflon cylinder according to Bragt et al. (J.Pharmacol. Meth. 3, 51, 1980). Exsudate samples were collected from the cylinder by puncture every hour. Blood levels of I were 1.2 ± 0.25 µg/ml (mean ± SEM, N = 7) after 1 hour, 2.1 ± 0.22 µg/ml after 6 hours and 2.5 ± 0.32 µg/ml after 10 hours of infusion.Levels of I in the exsudate increased from 0.25 ± 0.09 µg/ml after 1 hour to 0.5 ± 0.17 µg/ml after 6 and 1.1 ± 0.33 µg/ml after 10 hours but never exceeded blood levels. Concentration of protein (measured by the method of Bradford) in exsudate and pH of exsudate did not change significantly over the entire period. The results indicate, that under the conditions of experimental inflammation employed there was no accumulation of I in the exsudate from the inflamed area, suggesting that the observed high tissue and exsudate levels of I after single doses are due to differences in uptake and elimination kinetics.

Inst.f.Pharmakodyn.u.Tox.,A-8010 Graz,Univ.-Pl.2

25

DEVELOPMENT OF PROTEIN BINDING DURING PREGNANCY AND THE NEONATAL PERIOD. STUDIES IN THE HUMAN AND IN EXPERIMENTAL ANIMALS ON DIAZEPAM, VALPROIC ACID, CARBAMAZEPINE, THEIR MAJOR METABOLITES AND INDOMETHACIN.

W. Kuhnz, R. Lorenz and H. Nau

Little is known on the pre- and postnatal development of drug protein binding. During pregnancy the serum protein binding of many drugs like carbamazepine (CBZ), diazepam (DZ), its major metabolite N-desmethyldiazepam (DDZ), valproic acid (VPA) and indomethacin (IND) is decreased compared to nonpregnant adults. A comparison of fetal and maternal protein binding at birth however, reveals that DZ, DDZ, VPA and IND are more extensively bound in fetal serum than in maternal serum, whereas CBZ is less extensively bound in fetal serum. It has been demonstrated that DZ and VPA cross the placenta and there have been indications that there is an accumulation in the fetus. Unbound fractions of DZ, DDZ and VPA in the fetus were similar to those in adults but lower than those in the mothers. A sharp increase in neonatal free fractions in the first postnatal day correlate with a concomitant increase in free fatty acid (FFA) levels in the newborn. Both, neonatal free drug fractions and FFA levels reach near control values at one week of age. We have extended these studies to experimental animals in order to gain a better understanding of interspecies differences. Our particular interest focuses on a possible relation between different protein binding of a particular drug, like indomethacin in man and experimental animal and observed differences in teratogenicity in both species.

Institut für Toxkologie und Embryonalpharmakologie, FU Berlin, Garystraße 9, D-1000 Berlin 33

26

DISPOSITION OF SULPHADIAZINE, SULPHADIMIDINE AND SULPHAMERAZINE IN DOGS.

M. Atef and S.A.H. Youssef

A single therapeutic dose of the sodium salts of sulphadiazine, sulphadimidine and sulphamerazine (100 mg/kg b.wt.) was injected intravenously into dogs. The half-life values showed that sulphadimidine was the compound most rapidly eliminated (6.35 h), followed by sulphadiazine (11.12 h) and sulphamerazine which showed the slowest elimination (12.05 h). The apparent volume of distribution was highest for sulphadimidine and lower for the other two sulphonamides. The body clearances confirmed better elimination of sulphadimidine (1.56 ml/kg/min) as compared to sulphadiazine (0.65 ml/kg/min) and sulphamerazine (0.52 ml/kg/min). A correlation existed between the elimination half-lives of the sulphonamides tested and their body clearances.

Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

27

TISSUE CONCENTRATION OF SOME SULPHONAMIDES IN DOGS

M.G.A. El-Sayed** and A.Y.I. El-Gendi*

Sulphadiazine, sulphamethazine and sulphamerazine were injected intravenously into 15 clinically healthy dogs at a dose of 100 mg/kg b.wt. Eight hours after injection the dogs were killed.The highest sulphonamide concentration in plasma was achieved by sulphamerazine (115 µg/ml), whereas sulphadiazine showed the highest concentration in tissues if compared with the other drugs. Sulphonamide concentrations in all tissues were lower than those in plasma. Kidney and liver had always higher sulphonamide concentrations than other tissues. The concentration of acetylated products of the three sulphonamides was very low in plasma (4.41 - 7.36%) but higher in tissues (up to 26.89%) except liver and kidney. Plasma proteins had a moderate tendency to bind sulphamerazine or sulphadiazine (17.85 and 16.94%, respectively) and a lesser one to bind sulphamethazine (11.69%).

*Department of Pharmacology,Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

**Department of Pharmacology, Faculty of Veterinary Medizine, Zagazig University, Zagazig, Egypt

28

PHARMACOKINETICS OF SULFONAMIDES IN RABBITS - QUANTITATIVE STRUCTURE - PHARMACOKINETICS RELATIONSHIPS IN COMPARISON TO RATS. A. Bruns*, J.-K. Seydel** and O. Wassermann*

For a series of 19 sulfapyridines (SP, Arch.Pharmacol. 307,R1,1979) the interdependence of structural and pharmacokinetic parameters was studied in rabbits. After i.v. injection volume of distribution, V_d, rate of elimination, k_{el}, metabolic rate of acetylation, k_m, total clearance, Cl_t, and protein binding constant, K_{ass}, have been determined, and the observed variation of these parameters was correlated to changes of the physicochemical properties of the substituted SP's. Electronic influence of the substituents is described by pKa, change in lipophilicity by ΔR_m or log k'_R (detd by HPLC). The regression equations obtained were compared to those previously obtained in humans and in rats. - Whereas for V_d and K_{ass} similar correlations were evaluated, large species differences were observed for the structure dependency of Cl_t. This observation was explained by different excretion mechanisms in rabbits and in rats. In rabbits the tubular secretion predominates for this type of drugs. In rats, however, only glomerular filtration occurs. - This type of correlation analysis proved to be an useful tool both for the differentiation of structural influences on excretion mechanisms and for the selection of the most suitable experimental animal for screening of new derivatives prior to application to humans. Furthermore, this correlation analysis renders possible the detection of exceptions. For example, some of the N_4-acetyl-derivatives were found to deviate significantly from the correlation equation derived for Cl_t. The reason proved to be a shift in the imido-amido tautomerism, which altered the excretion mechanism from predominantly tubular secretion to almost solely glomerular filtration.

* Dept. of Toxicology, University of Kiel, D-2300 Kiel, Germany

** Dept. of Biochemistry, Borstel Research Institute, D-2061 Borstel, Germany

29

PHARMACOKINETIC MODELLING OF INDOCYANINE GREEN: A SOURCE OF ERROR IN MEASURING LIVER BLOOD FLOW

P. Altmayer and E. Lang

Indocyanine green (ICG) is clinically and scientifically used to assess the excretoric liver function and liver blood flow. Because of some inconsistencies in the literature concerning the pharmacokinetics of ICG a study with intravenous bolus injections of ICG (0.5 mg per kilo body weight) was performed in 10 patients with liver cirrhosis. ICG condentrations in serum were monitored up to the limit of detection of the spectrophotometric assay (300 ng/ml). TheICG serum concentration time data were fitted to a biexponential function of the form: $Cp = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t}$ indicating an open two-compartment body model. The distribution half-life (α-phase) ranged from 1.3 to 9,8 min, the disposition half-life (β-phase) from 11.0 to 177.3 min, the volume of distribution in the centralcompartment from 1.3 to 4.4 l, the volume of distribution at steady state from 4.3 to 31.5 l, the total clearance from 87.4 to 399.3 ml/min.

The findings on ICG clearance will be discussed in view of the available clinical data for the patients studied.

It could be shown that monitoring only the early part of the plasma ICG curve and using an one-compartment body model for the determination of the clearance would significantly overestimate the liver blood flow. Our results suggest that ICG-clearance may not be a good parameter for estimating liver blood flow in cirrhosis.

Carl-Korth-Institute for Cardiovascular Research,
Div. of Clinical Pharmacology
Gebbertstraße 47, D-8520 Erlangen

30

Pharmacokinetic consequences of first-pass intrahepatic binding of methadone in the rabbit

P. Luder, J. Bircher

The hepatic first-pass effect usually is thought to represent presystemic drug elimination by hepatic metabolism resulting in a fraction of an oral dose which never reaches the systemic circulation. The idea, however, that the liver might also transiently bind a drug during its first passage has not as yet received adequate experimental support. In order to test this possibility, experiments have been designed in 5 nonanesthetized rabbits, equipped with permanent catheters in the portal vein, the aorta and the inferior vena cava. Bolus doses of 3H-methadone (0.48 µmol, 50 µCi) and for comparison of 14C-lidocaine (0.48 µmol,10 µCi) were injected i v or intraportally (i port) and blood sampled at intervals. Drug concentrations were assessed in arterial blood by solvent extraction followed by TLC and scintillation counting. Systemic availability of i port methadone averaged 22 %, time to peak concentration (tmax) 10 min, terminal elimination (β) 0.004 min^{-1} and mean residence time (mrt) 270 min. In contrast, after i v administration tmax was < 2.5 min, β 0.013 min^{-1} and mrt 95 min. The respective values for lidocaine were 33 %, < 2.5 min, 0.044 min^{-1}, 20 min, and < 2.5 min, 0.060 min^{-1} and 18 min. These data are consistent with nonspecific intrahepatic binding followed by later release resulting in delayed tmax, reduced β and prolonged mrt in the case of i port methadone. In contrast, there is no evidence for intrahepatic binding of lidocaine. It is concluded, that transient intrahepatic binding rather than slow absorption may for some drugs be responsible for delayed appearance in the systemic circulation.

Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH - 3010 Berne.

31

LOCATION OF FIRST-PASS METABOLISM AND MECHANISM OF EXTRACTION OF LORCAINIDE IN RATS

V. Plänitz

Lorcainide undergoes extensive first-pass metabolism in different species. In rats with surgical portacaval shunt bioavailability of unchanged lorcainide was significantly increased after oral administration from 13 to 45 per cent, because of exclusion of hepatic first-pass metabolism (Plänitz et al., Naunyn-Schm.'s Arch. Pharmacol. 311 Suppl., R 3, 1980).

After intraportal administration, i.e. elimination of intestinal first-pass metabolism, an availability of 36 per cent was found. Evidently, the first-pass metabolism of lorcainide is located in equal parts in both the liver and the intestinal wall. After intraperitoneal administration of the same dose bioavailability of unchanged lorcainide was significantly increased in rats with portacaval shunt to 27 per cent compared with 16 per cent in control rats. Comparison of bioavailabilities of total radioactivity (lorcainide and metabolites) shows a complete bioavailability only in rats with portacaval shunt both after oral and intraperitoneal administration and in rats following intraportal and intravenous administration of lorcainide. After oral and intraperitoneal administration of lorcainide in control rats, bioavailability of total radioactivity was only 53 per cent, respectively 63 per cent.
Concerning the mechanism of extraction of lorcainide the following conclusions could be drawn: Metabolites preformed in the intestinal wall, are absorbed and immediately eliminated by the liver.

Pharmakol. Inst., Obere Zahlb.Str. 67, 65 Mainz

32

DOSE-DEPENDENT PHARMACOKINETICS OF PHENPROCOUMON IN RATS

D. Trenk, B. R. Winkelmann and E. Jähnchen

Pharmacokinetics of phenprocoumon were investigated in two groups of 12 Sprague-Dawley rats in a pharmacologically relevant dose range (0.1 and 1.0 mg/kg). The rats in both groups differed with respect to their free fraction values in the plasma. The doses administered produced a maximal depression of prothrombin complex activity to 60 and 2 % of normal, respectively.
By increasing the dose from 0.1 to 1.0 mg/kg the half-life of phenprocoumon was shortened (from 29.1 ± 4.4 to 19.2 ± 3.0 hours, $p<0.001$) while total clearance declined only slightly (from 18.2 ± 12.3 to 14.2 ± 7.0 ml/kg·hr, N.S.). However, there was a decrease of about 33 % of the intrinsic clearance of phenprocoumon after administration of the higher dose, indicating saturation of hepatic metabolism. The volume of distribution decreased from 707 ± 387 to 367 ± 121 ml/kg ($p<0.05$). The liver/plasma concentration ratio and the ratio of total liver concentration to free concentration in plasma also decreased about two-fold after the higher dose. These results suggest that the uptake of phenprocoumon by the liver and by other tissues becomes saturated by increasing the dose. Although plasma protein binding was unaffected, tissue binding decreased following the higher dose. Total clearance was linearly related to the free fraction in plasma, whereas the elimination rate constant increased linearly with increasing free fractions in the tissue.

Thus, dose-dependent changes in pharmacokinetics of phenprocoumon observed in rats, result mainly from saturation of tissue binding and to some extent also from saturation of drug metabolism in the liver. Furthermore, these results provide experimental evidence that in rats the half-life of phenprocoumon is a function of tissue binding, whereas clearance is a function of plasma protein binding.

Pharmakologisches Institut der Universität Mainz,
Obere Zahlbacher Str. 67, D-6500 Mainz 1 (FRG)

33

COMPARATIVE PHARMACOKINETICS OF PROPRANOLOL, METOPROLOL AND ATENOLOL AFTER SINGLE AND MULTIPLE DOSING IN THE RAT

B. Lemmer, H. Winkler and M. Fink

Previously, daily variations in the pharmacokinetics of d,l-propranolol (30 µmol/kg) have been shown in synchronized rats with shorter elimination half-lives in plasma and organs in the dark period (D) than in the light period (L) (Lemmer, Bathe, J.Cardiovasc.Pharmacol. 4,635,1982). In order to evaluate whether this chronokinetic behavior is restricted to propranolol or is a characteristic for the group of ß-blockers, comparative studies after single and multiple doses (6 and 6 x 6 µmol/kg) of ß-blockers of different lipophilicity and different routes of elimination (propranolol, metoprolol, atenolol) were performed during L and D. Drug concentrations in plasma, heart, muscle, lung, brain, liver and kidney were determined by HPLC (Winkler et al., J.Chromatogr. 226,223,1982). After a single dose half-lives of all drugs in all organs were shorter during D than during L. Mainly by increasing the half-lives during D these daily variations were abolished in all organs after multiple dosing of METO and ATEN and in plasma, heart and kidney after PROP resp. Taking all kinetic data together they are influenced by drug-specific properties (e.g. lipophilicity, route of elimination, ß-receptor blockade) as well as by circadian variations in the dynamics of the ß-blockers, which in turn modify the kinetic behavior of these drugs.

Centre of Pharmacology, J.W.Goethe-University, Theodor-Stern-Kai 7, D-6 Frankfurt/M.
Supported by the DFG (Le 318/8-2).

34

PHARMACOKINETICS OF LEVOBUNOLOL AND DIHYDROLEVOBUNOLOL IN MAN

E.U. Kölle, H. Hengy, P. Thomann

The pharmacokinetics of levobunololol (LB) and its active metabolite dihydrolevobunolol (DHLB) following i.v. and p.o. administration has been investigated in a cross over study with 6 volunteers (8 mg infusion and 3x4 mg tablets). Blood levels and renal excretion data of LB and DHLB were determined using a sensitive HPLC method with fluorimetric detection.

After i.v. administration the following parameters were obtained for LB:
Cl = 11.0±1.6 ml/min/kg, V_β = 5.5±1.4 l/kg. LB and DHLB showed a very similar terminal time course with a mean half life of 5.7 h. 47.7±11.1 % of the i.v. dose was renally excreted as active compounds. 13.0±6.0 % accounted for LB and 34.7±11.0 % for DHLB. Renal clearances were 1.5±0.6 ml/min/kg (LB) and 3.9±1.5 ml/min/kg (DHLB). Following p.o. administration, LB was rapidly absorbed (C_m=22.9±8.1 ng/ml, t_m=3.2±1.5 h). DHLB showed a delayed concentration profile (C_m=14.9±5.0 ng/ml, t_m=3.2±1.5 h). The terminal half lives of LB and DHLB (6.2±6.5 h) did not differ significantly from the values obtained after i.v. administration. The absolute bioavailability of the sum of the active compounds LB+DHLB was 75±22 %.

The same subjects were given 9 oral doses of 12 mg at 24 h intervals. Comparison of the blood levels on the first and ninth day did not reveal accumulation or a change in the pharmacokinetic characteristics of LB and DHLB.

Another 12 volunteers received an oral dose of either 3x4 mg tablets or 3x4 mg capsules in a cross over design. Concerning the amount of absorption and the time course of LB and DHLB there was no difference between both formulations. The mean relative bioavailability of the tablets was 1.02±0.18.

Gödecke Research Insitute, Dept. of Biochemistry, Moos-waldallee 1-9, D-7800 Freiburg

35

INFLUENCE OF HYDRALAZINE ON PHARMACOKINETICS OF PROPRANOLOL AND LIGNOCAINE IN CONSCIOUS DOGS.

B.G.J. Heinzow+, A. Somogyi and A.J. McLean

Hydralazine (H) is known to increase the area under the plasma concentration curve (AUC) of orally coadministered d,l-Propranolol (P) in man (McLean et al., Clin.Pharmacol.Ther.24, 725, 1980). It was speculated that H altered presystemic elimination of P by increasing liver blood flow. Such a general cause should affect other drugs with hepatic first-pass elimination in a similar way.
In order to test this hypothesis 6 dogs were given orally a solution of d,l-Propranolol (2 mg/kg) and Lignocaine (L) (15 mg/kg) alone or simultaneously with 25 mg H. Plasma-concentrations of P and L were measured by specific HPLC methods.
Concomitant administration of H approximately doubled the peak concentration and the AUC_{0-8} of P from 34.3 ± 5.4 to 72.9 ± 9.6 (ng/ml) (mean + SEM) and from 142.4 ± 18.0 to 253.6 ± 56.3 (ng/ml · h) respectively. The peak concentration (.9 ± .2 to 1.09 ± 17 µg/ml) and the AUC_{0-8} (1.84 ± 29 to 2.09 ± .27 µg/ml · h) of L. were virtually unaffected by H.
These findings show that P and L, which both undergo extensive presystemic elimination, are not affected in a similar way by coadministration of H.
The change of first-pass clearance of P cannot be explained by a general underlying mechanism, such as an alteration in liver blood flow alone. It is speculated that other mechanisms (e.g. inhibition of P metabolism) are responsible for the observed interaction between P + H.

+Supported by DFG grant He 1178/1-2.

+Institut für Pharmakologie, Univ.Kiel, Hospitalstrasse 4-6, D-2300 Kiel,

Baker Medical Research Institute, Melbourne, Australia

36

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF HIGH-CEILING DIURETICS

W.P.Gluth, W.Hetz and M.Geldmacher-von Mallinckrodt

Several assays for the high-ceiling diuretics furosemide (F), bumetanide(B) and piretanide(P) have been published in the past. However, these assays suffer from several disadvantages, e.g. lack of appropriate internal standards(IS) or tedious sample preparation. For the evaluation of the pharmacokinetics of F, B and P we developed highly specific and sensitive assays, which allow quantitation in plasma, urine and bile. Preparation of samples is very rapid; for example 0.2 ml of plasma are precipitated with 0.4 ml of acetonitrile (CH_3CN) containing the IS. Separation was made possible on an Alltech ODS 10 µm 4.6 x 250 mm column with various mixtures of CH_3CN-water adjusted to pH 3.0 at a flow rate of 2.0 ml/min.

	solvent	retention substance	time IS	IS
F	35% CH_3CN	6.2	9.1	NMeF
B	40% CH_3CN	10.4	7.5	P
P	40% CH_3CN	7.5	10.4	B

For F the N-methyl-analog (NMeF) of F was used as IS. Detection was accomplished fluorimetrically at 235 nm excitation with a 370 nm cut-off emission filter. Sensitivity for F was 10 ng/ml and 1 µg/ml for plasma and urine respectively. The method was easily adapted to smaller volumes thus permitting studies in small laboratory animals like rats. The assay has already been successfully used in several studies. Using a WISP automated sample injector, we were able to measure up to 120 samples/day. Column lifetime was several thousand samples.

Carl-Korth-Institute for Cardiovascular Research, Div. of Clinical Pharmacology and Institut für Rechtsmedizin der Universität Erlangen-Nürnberg, D-8520 Erlangen, West-Germany

37

THE BINDING OF NDS-TEMPO TO RED CELL GHOSTS AND TO ISOLATED BAND 3 PROTEIN

K.F. Schnell

The binding of the spin label NDS-TEMPO (N-4-(2,2,6,6-tetramethyl-1-oxyl) piperidinyl-N'-4-(4'-nitro-2,2'-disulfonatostilbene)thiourea) to red cell ghosts and to isolated band 3, the anion transport protein, were studied. Band 3 was extracted according to the method of KÖHNE et al. (BBA: 664,108(1981)).The concentration of bound and of free NDS-TEMPO were determined by ESR - spectroscopy.

For intact ghosts the dissociation constant K_L of NDS-TEMPO (pH 7.2, 20°C) was 1.05±0.12 µM and the chloride dissociation constant K_s(Cl) was 30±5 mM(m ±SD, n=4). For isolated band 3, K_L(pH 7.8, 20°C) was 3.82±0.69 µM,K_s(Cl) was 45±9 mM and the maximal binding capacity 7.07±1.8µmoles/mg protein (m ±SD, n=5). The results indicate that the affinities of NDS-TEMPO and of chloride to the isolated band 3 are of the same order of magnitude as their affinities to the intact anion transport system of the red cell membrane.

Physiologisches Institut der Universität Regensburg, Postfach 397, D-8400 Regensburg

38

MEMBRANE POTENTIAL AND CHEMOTAXIS OF HUMAN LEUCOCYTES.

U. Jäger*, H. Gruler, and B. Bültmann*****

The membrane potential of polymorphonuclear leucocytes was determined using 20 MΩ-glass microelectrodes. The distribution function disclosed a single broad gaussian curve with an average of -8.7 ± 3.0 mV (±SD; n = 179). Exposure of the leucocytes to the chemotactic agent FMLP (N-formyl-methionyl-leucyl-phenyl-alanine) resulted in a directed migration and a significant hyperpolarization to a mean value of -17.1 mV (n = 76). The distribution function in the presence of FMLP was characterized by at least 3 superimposed gaussian curves. The autocorrelation function of membrane potentials measured over up to 10 min suggested an internal clock with a characteristic time T of about 1 min. This distribution function in the presence of FMLP reflects the periodicity of the membrane potential.

Treatment of leucocytes with Echo 9 virus resulted also in hyperpolarization: the distribution function of the membrane potential was similar as in the presence of FMLP, however, with a mean of -13.2 mV (n = 66) and T of about 5 min. When virus-pretreated leucocytes were exposed to FMLP they had lost their chemotactic activity. The characteristic time T was 5 min. The electrophysiological findings are in agreement with a selective inhibition of the chemotactic response of leucocytes induced by Echo 9 virus (Bültmann et al., Klin Wochenschr 59: 571, 1981).

***Abt. für Allgemeine Physiologie,**
****Abt. für Biophysik,**
*****Abt. für Pathologie,**
Universität Ulm, Oberer Eselsberg, D-7900 Ulm

39

DISCRIMINATION BETWEEN TIGHT AND LEAKY EPITHELIA FROM THE VIEWPOINT OF AC IMPEDANCE ANALYSIS

Paul Weskamp and Ulrich Hegel

So called tight epithelia are characterized by a virtually zero paracellular ion conductivity. The electrical equivalent circuit of such epithelia can be approximated by 4 parameters, namely resistances and capacitances of the apical and basal-lateral membranes, respectively. In this case transepithelial impedance measurements allow direct reduction of the measuring values to these 4 parameters.
However, in more leaky epithelia, where the tight junction exerts a significant ion conductivity, transepithelial impedance measurements result only in apparent data, which do not allow the determination of the underlying membrane parameters. So far it was not generally formulated how the limit between the tractable ideally tight and the - without further information - intractable leaky case could be defined. This, however, would be of importance for designing any AC impedance experiment on tight or leaky epithelia, especially with respect to the necessity of intracellular AC recordings.
Thus, we will present a set of formulae suitable for evaluation of the expected error of membrane parameters when evaluated from any individual apparent data set as a function of assumed shunt conductivities. Applications of this formalism to recently published data of rabbit urinary bladder, frog skin and rabbit colon will be demonstrated. It will be shown that frog skin epithelium cannot be considered as tight in the above defined sense.

Inst. f. Klinische Physiologie, Klinikum Steglitz, Freie Universität Berlin,D-1000 Berlin 45

40

EFFECT OF BICARBONATE ON INTRACELLULAR POTENTIALS OF CULTURED BOVINE CORNEAL ENDOTHELIAL CELLS

T.J.Jentsch,M.Koch,H.Bleckmann,M.Wiederholt

The corneal endothelium is known to transport bicarbonate and sodium from the stroma to the aqueous humour. Intracellular recordings (range: -40 to -80 mV) stable for hours could be obtained with cultured cells.

Removing HCO_3^- (46 mM) from the Ringer led to a depolarisation of 10 to 30 mV and to a transient hyperpolarisation upon readdition. Permeable buffers such as glycodiazine (25 mM) and butyrate (45 mM) could largely substitute for bicarbonate in the effect on the voltage, whereas an unpermeable buffer (HEPES, 45 mM) was ineffective. Ringer with 10 mM NH_4Cl led to a depolarisation and to a transient hyperpolarisation after removal, the latter increasing with the time of preincubation with NH_4Cl. The transient depolarisations were larger in HCO_3^--free than in bicarbonate-buffered solutions, suggesting an effect of internal pH.
Neither SITS nor acetazolamid alone (both 0.1 mM) significantly changed the PD, while a combination of both depolarised the cells by about 10 mV. SITS reduced the hyperpolarisation after addition of CO_2/HCO_3^- or removal of NH_4Cl .

It is suggested that corneal endothelial cells possess a SITS-inhibitable OH^- -conductance. Changes in conductivity for other ions may be involved in the steady-state depolarisation seen after removal of bicarbonate.

Institut für Klinische Physiologie,Klinikum Steglitz der Freien Universität Berlin,Hindenburgdamm 30,1 Berlin 45

41

OXYNTIC CELLS IN GASTRIC MUCOSA: Cl^- - AND K^+- NOISE FROM THE APICAL MEMBRANE.

W. Zeiske, W. Van Driessche and T.E. Machen+

Transport and conductance pathways for Cl^- and K^+ have been studied in frog gastric mucosa using noise analysis techniques. In Cl^--containing solutions, reduction in Cl^--transport (i.e. short-circuit current) by (i) reducing serosal $\{Cl^-\}$, (ii) increasing serosal $\{K^+\}$, or (iii) blocking the serosal Cl^-/HCO_3^- exchanger with SITS, were mirrored by a decrease in the overall current-noise power. In Cl^--free solutions a second, high-frequency Lorentzian component in the power spectrum was observed when a $\{K^+\}$ gradient was applied to the resting tissue in either transepithelial direction. The spontaneous Lorentzian noise could be enhanced by (i) appropriate voltage-clamping of the tissue and (ii) stimulation of proton secretion with histamine. Blockage of apical K^+ channels by mucosal Ba^{2+} led to the disappearance of the K^+ Lorentzian in the non-secreting gastric mucosa. In histamine-stimulated stomach, however, the K^+-relaxation noise was enhanced, or even induced. Both Cl^-- and K^+-related current noise seems to be generated at the apical membranes of oxyntic cells. A conductive cell-to-lumen movement of K^+ through spontaneously fluctuating channels, may be coupled to the neutral H^+-K^+ ATPase and account for apparent electrogenic H^+ transport in frog gastric mucosa. The characteristics of the K^+-channel noise in the stomach epithelium are very similar to the ones described recently for gallbladder (Van Driessche and Gögelein, Nature 275, 665 (1978)) and descending colon (Wills, Zeiske and Van Driessche, J. Membrane Biol. 69, 187 (1982)).

Labo voor Fysiologie, Campus Gasthuisberg, B-3000-Leuven, Belgium and +Dept. of Physiology-Anatomy, Univ. of California, Berkely, CA., U.S.A.

42

EFFECTS OF cAMP ON NECTURUS GALLBLADDER EPITHELIUM:INDUCTION OF A DOMINANT Cl PERMEABILITY IN THE APICAL MEMBRANE

K.-U. Petersen and L. Reuss

The effects of cAMP and equivalent agents on Necturus gallbladder epithelium were investigated using microelectrode techniques. Either cAMP (6 mM), 8-Br-cAMP (1 mM) or theophylline (3.3 mM) depolarized the cell membranes by about 15 mV and reduced the apparent ratio of membrane resistances (apical over basolateral) to virtually zero. These changes were due to a large increase in apical membrane Cl permeability (P^a_{Cl}) with no apparent changes in P^a_K or P^a_{Na}, as found in luminal ion substitution experiments. The effects of theophylline were dependent on luminal Cl, but not on luminal Na. Ion transference numbers (T_i) of the apical membrane were calculated from the effects of luminal ion substitutions on the apical membrane potential. T_{Cl} (n=6) and T_K (n=6) in the presence of cAMP were 0.88 and 0.18, respectively, values indicative of the dominance of P^a_{Cl}. Following addition of 8-Br-cAMP, intracellular Cl activity (aCl_i) fell from 20±2 to 13±1 mM (n=8), a value not different from that expected for passive Cl distribution. The rate of the initial change of aCl_i was 11.3 mM/min. This parameter allowed a second independent determination of T_{Cl}. The value obtained from this approach was 0.91. The large increase of P^a_{Cl} induced by cAMP may at least in part explain the inhibition of fluid absorption observed during serosal exposure to theophylline.

Supported by NIH Grant AM 19580 and DFG Grant I3-Pe 280/1-1.

Washington University, Dept. Physiol. & Biophys., St. Louis MO 63110; Abt. Pharmakologie, RWTH Aachen, D-5100 Aachen, FRG.

43

PROTON-MOVEMENTS IN BRUSH BORDER VESICLES FROM RAT JEJUNUM AND KIDNEY PROXIMAL TUBULE

G. Cassano, B. Stieger and H. Murer

The Acridine Orange fluorescence quenche technique was used to follow changes in internal acidification of brush border vesicles.

An outwardly directed K-gradient does not lead to a proton movement into the vesicles. The addition of valinomycin ($K_i > K_o$) leads to internal acidification due to proton conductance. Proton conductance in kidney is smaller than in the intestine. An outwardly directed Na-gradient provokes internal acidification via Na and H conductive pathways and via a Na-H exchange whose activity is measured in the presence of short circuited membrane potential (K-equilibrated plus valinomycin). Na-H exchange activity is higher in kidney than in intestine. Under short circuited membrane potential conditions, a preset ΔpH dissipates faster after injections of Na. This effect of Na in both membranes was reduced by 0.1 mmoles/l amiloride. At higher concentrations of amiloride, the inhibition is unspecific and also observed for ionophore induced K-H exchange.

In both membrane preparations no evidence for Cl-OH exchange was obtained with this technique. An inwardly directed Cl-gradient did not produce intravesicular acidification. Internal acidification provoked by Na-H exchange was not altered by the presence of different anions (gluconate or chloride).

Institute of Physiology, University of Zurich, Rämistrasse 69, 8028 Zurich (Switzerland)

Supported by Schweiz. Nationalfonds (H. Murer 3.226.082)

44

PROTON PATHWAYS IN RAT RENAL CORTEX BRUSH BORDER AND BASOLATERAL MEMBRANE VESICLES

I. Sabolić and G. Burckhardt

The quenching of acridine orange fluorescence was used to study the formation of pH gradients in brush border (BBMV) and basolateral (BLMV) membrane vesicles from rat kidney cortex. BBMV were prepared by a calcium precipitation method, BLMV by a Percoll density gradient centrifugation method. Na^+/H^+ or Li^+/H^+ exchange was demonstrated by diluting Na^+ or Li^+ loaded vesicles into Na^+ and Li^+ free buffers. The fluorescence signal due to a transient intravesicular acidification was decreased when the membrane pd was abolished by valinomycin and 100 mM K^+ (out=in). Therefore, $Na^+(Li^+)/H^+$ exchange is due to an electroneutral Na^+/H^+ exchanger and in addition to H^+ and Na^+ movements through conductance pathways. Intravesicular acidification in the presence of inside negative K^+ diffusion potentials is a further indication for a proton conductance in BBMV. As Cl^- gradients (out>in) did not lead to a detectable intravesicular acidification when membrane pd was abolished, an electroneutral Cl^-/OH^- exchanger does not play a significant role in transmembrane H^+ movements. As opposed to BBMV, a Na^+ (or Li^+) gradient (in>out) did not result in an intravesicular acidification in BLMV indicating that a Na^+/H^+ exchanger is not present in basolateral membranes. BLMV showed an H^+ conductance but unlike BBMV no detectable Na^+ conductance. As in BBMV no indication was found for an electroneutral Cl^-/OH^- exchanger. The asymmetric distribution of the electroneutral Na^+/H^+ exchanger can explain net H^+ secretion in proximal tubules. As H^+ and Na^+ conductances are not found in the luminal membrane in vivo, they may reflect altered properties of the isolated BBMV.

Max-Planck-Institut für Biophysik, Kennedyallee 70, D-6000 Frankfurt (Main) 70

45

ALTERATIONS OF Na^+ AND K^+ MOVEMENTS IN RAT ERYTHROCYTES DURING DIETARY K^+-DEFICIENCY

J. Duhm and B.O. Göbel

Previous studies on Na^+/K^+ transport across the red cell membrane of DOC-salt hypertensive rats indicated that the hypokalemia induced by the DOC treatment was responsible for the changes observed (Duhm et al.,Pflügers Arch.394: R28,1982). To test this hypothesis, a 1-6 week nutritional K^+-deficiency was studied as an alternate model of hypokalemia.

The fall in plasma K^+ below 2mM in K^+-depleted rats was accompanied by a loss of red cell K^+ and an increase in the Na^+ leak. Na^+-K^+ pump rates were elevated due to a rise in cell Na^+. The cation deficit of up to 30% of total Na^+ plus K^+ caused a cell shrinkage and an increase of mean cellular hemoglobin content. The Na^+-K^+ cotransport showed an up to ten-fold increase in its maximum capacity, and its normal function of a net Na^+ gain was converted to a net Na^+ extrusion in the K^+-deficient erythrocytes.

Osmotic shrinkage of normal cells in vitro induced a several-fold increase in Na^+-K^+ cotransport rates, suggesting that the cell shrinkage occurring in K^+-deficiency is largely responsible for the cotransport acceleration in vivo. However, the cotransport rates were reduced but not normalized when the cell volume of depleted cells was restored either by 1-3 days of in vivo and 3h of in vitro K^+-repletion or by osmotic means in vitro. In addition, in vitro shrinkage of normal cells accelerated the cotransport much less than a similar shrinkage in vivo due to K^+-deficiency. These findings indicate that other factors may participate in the changes occurring in K^+-deficiency, such as a lowering of red cell ATP (-20%) and 2,3-DPG (-17%) and of plasma inorganic phosphate (-38%), chloride (-11%) and H^+ concentration. It is concluded that the hypokalemia in DOC-salt hypertension is in fact responsible for the acceleration of Na^+-K^+ cotransport in rat erythrocytes.

Physiologisches Institut der Universität München, Pettenkoferstr. 12, D-8000 München 2

46

THE MECHANISM OF VANADIUM ACTION ON SELECTIVE POTASSIUM PERMEABILITY IN HUMAN ERYTHROCYTES

G.F. Fuhrmann, J. Hüttermann and P.A. Knauf

Recently H.Siemon et al.(Toxicology 29:271,1982) reported, that vanadium increased selective potassium permeability in human erythrocytes. Since added vanadate appears mostly as vanadyl in the cell interior, the suggestion was made, that vanadyl (VO^{++}) can open the "potassium channel" in the erythrocyte membrane similar to Ca^{++}, Mg^{++} or Pb^{++}. An alternate mechanism would involve vanadate inhibition of active outward calcium transport (J.P.F.C.Rossi et al., Biochim.Biophys.Acta 648:145,1981) leading to a build-up of calcium inside the cell which triggers the selective increase in potassium permeability, even in the presence of traces of calcium.

One problem in studying the effects of vanadyl cation as opposed to effects of vanadate anion is that vanadyl is readily oxidized to vanadate at physiological pH by atmospheric oxygen. In this investigation we have found that addition of small amounts of EDTA to the solution readily prevents or greatly reduces this oxidation process. In order to facilitate the entry of vanadyl into the cell we made use of the ionophore A23187. We found by EPR spectroscopy that this ionophore is also capable of transporting vanadyl ions. When EDTA is present in solutions along with A23187, vanadyl but not vanadate caused a very large increase in potassium permeability of the erythrocytes. The observed permeability change with vanadyl is sensitive to outside potassium and inhibited by quinidine or oligomycin. However, the mechanism of vanadyl on potassium permeability is an indirect one. Vanadyl is able to displace calcium from EDTA and already the small concentration of calcium which is always present in normal salt solutions is sufficient to trigger the increase in potassium permeability in the erythrocytes.

Pharmakologisches Institut der Universität Marburg, Lahnberge, D-3550 Marburg. Supported by DFG

47

ANALYSIS OF Na AND HCO_3 FLUXES IN THE GUINEA PIG GALLBLADDER UNDER SECRETORY CONDITIONS

J.M. Winterhager, C.P. Stewart, and K. Heintze

Under in vitro conditions, the guinea pig gallbladder absorbs Na and Cl electroneutrally. Prostaglandin E_1 (PGE_1) reverses this net absorption to an electrogenic net anion secretion which is Na-dependent and ouabain-sensitive, suggesting a secondary active transport process. To assess the underlying ion movements, titratable alkalinisation, assumed to be equivalent to unidirectional HCO_3 flux (J^{HCO_3}), was measured under pH-stat and voltage clamp conditions. Mucosal (m) or serosal (s) HCO_3 gave rise to an electroneutral HCO_3 flux of 1.5 umol/cm^2·h. After addition of PGE_1, the $J^{HCO_3}_{ms}$ was reduced by 0.9 umol/cm^2·h, with no change in short-circuit current (I_{sc}). However, in the presence of serosal HCO_3, PGE_1 induced a considerable I_{sc} which only partially reflected an increase in $J^{HCO_3}_{sm}$. Under these conditions, ouabain ($3\cdot10^{-5}$ mol/l) reduced the I_{sc} from 2.6±0.2 to 0.05±0.1 umol/cm^2·h, and the $J^{HCO_3}_{sm}$ from 2.8±0.2 to 1.0±0.1 umol/cm^2·h (n=19), indicating that the I_{sc} is not fully accounted for by the $J^{HCO_3}_{sm}$. The PGE_1-induced rise in I_{sc} was greater in the presence of Cl. Unexpectedly, Cl was required only on the mucosal side to stimulate concentration-dependently both the I_{sc} and the secretory HCO_3 flux. This may be explained in terms of a Cl/HCO_3 exchange mechanism at the apical membrane. Electroneutral Na absorption was inhibited by PGE_1 to values indistinguishable from zero, due to a reduction in J^{Na}_{ms}. Both processes, the stimulation of $J^{HCO_3}_{sm}$ and the inhibition of net Na absorption, contribute to the reversal of absorption to net secretion in the gallbladder.

This work was supported by the SFB 160, C2.
Abt.Pharmakol.,Schneebergweg,RWTH,D-5100 Aachen

48

SULFATE/ANION EXCHANGE AND Na^+-DEPENDENT SULFATE UPTAKE BY RAT RENAL BASOLATERAL MEMBRANE VESICLES

I. Löw

Rat renal basolateral membrane vesicles were prepared by a Percoll density gradient centrifugation method. The specific activity of the (Na^++K^+)-ATPase, a marker for basolateral membranes, was enriched 14.4 ± 1.9 fold (n=11) with respect to the starting homogenate. Leucine arylamidase, a marker for brush border membranes, was enriched 1.2i ± 0.2 fold (n=9). In the absence of Na^+, $^{35}SO_4^{2-}$ uptake was cis-inhibited by unlabeled sulfate indicating a saturable transport process. Gradients of SO_4^{2-}, SSO_3^{2-}, HCO_3^-, and Cl^- (in>out) trans-stimulated sulfate uptake which transiently exceeded the equilibrium value (overshoot). Overshooting uptake was also observed in the presence of a pH gradient (in>out). In the absence of pH- and anion gradients a Na^+ gradient (out>in) led to a transient intravesicular sulfate accumulation above the equilibrium value. As this overshoot was observed when membrane pd was abolished by valinomycin and 50 mM K^+ (in=out), a direct coupling of Na^+ and sulfate must be assumed. Our observations are not due to a contamination with brush border membranes which also contain a Na^+-dependent sulfate transport system, because D-glucose transport was not stimulated by Na^+ in basolateral membrane vesicles. In contrast to Na^+-independent sulfate transport, Na^+-dependent sulfate uptake was inhibited by L-lactate, acetate, succinate, and PAH. Our results demonstrate the presence of an anion exchange in the basolateral membrane of proximal tubular epithelial cells. This transport system exchanges SO_4^{2-} with SSO_3^{2-}, HCO_3^-, OH^-, and Cl^-. In addition, sulfate uptake across the basolateral membrane can be energized by a Na^+ gradient.

Arbeitsgruppe G. Burckhardt
Max-Planck-Institut für Biophysik, Kennedyallee 70, 6000 Frankfurt/Main 70

49

BIDIRECTIONAL ACTIVE TRANSPORT OF DICARBOXYLIC ACIDS IN THE PROXIMAL CONVOLUTION OF THE RAT KIDNEY

K.J. Ullrich, G. Rumrich and H. Fasold

Using ^{3}H-methylsuccinate, which is not metabolized, the short term (up to 3.5s) luminal and contraluminal influx into the proximal tubular cell in situ as well as the zero net flux transtubular concentration difference which is a measure of the active transport rate was determined. Furthermore, flux studies with brush border (BBM) and basolateral membrane (BLM) vesicles were performed (in collaboration with G. Burckhardt). Methylsuccinate is taken up into BBM as well as BLM vesicles by a Na^+-dependent transport mechanism, showing an overshoot in BLM only. Overall net transport results in active secretion from the interstitium into the tubular lumen. The secretory component is inhibited by other dicarboxylic acids as α-ketoglutarate, succinate and fumarate (trans-form), but not by the correspondent cis-form maleate. Furthermore it is inhibited by paraaminohippurate, DIDS and probenecid, but not by sulfate, thiosulfate, L-lactate, oxalate and urate. Three days starvation led to an increase in luminal transport, but to a decrease in net transtubular secretion. Li^+ (2 mmol/l) inhibited both luminal and contraluminal transport. The data indicate a Na^+-dependent contraluminal uptake mechanism for Krebs cycle intermediates in addition to a Na^+-dependent luminal reabsorptive mechanism. Both systems are regulated differently.

Max-Planck-Institut für Biophysik, Kennedyallee 70
6000 Frankfurt/Main 70

50

CURRENT-VOLTAGE RELATIONS OF APICAL SODIUM TRANSPORT IN RABBIT DESCENDING COLON: EFFECTS OF VARYING LUMINAL SODIUM CONCENTRATIONS

K. Turnheim, S.M. Thompson, and S.G. Schultz

The instantaneous transepithelial current-voltage (I-V) relations of isolated epithelia of rabbit descending colon were determined by passing brief current pulses of alternating polarity across the tissue sufficient to clamp the epithelium from 0 to ± 200 mV in steps of 20 mV. When the basolateral membrane is functionally removed by a serosal high-K solution (Fuchs et al., J.Physiol.267: 137, 1977), the I-V relation of the apical Na-entry step can be obtained from the difference between the I-V curves in the absence and presence of the Na-channel blocker amiloride. Since the I-V relation of the apical Na-entry step conforms closely to the predictions of the Goldman-Hodgkin-Katz constant-field flux equation, estimates of P_{Na}, the Na-permeability of the apical membrane, and of $(Na)_c$, the intracellular Na-activity, can be obtained.

When $(Na)_m$, the Na-activity in the luminal solution, was 100 mM, $(Na)_c$ averaged 12 mM. Decreasing $(Na)_m$ resulted in a decrease in $(Na)_c$, the relation resembling saturation kinetics. P_{Na}, on the other hand, increased in a curvilinear fashion as $(Na)_m$ was decreased. High values of $(Na)_c$ were always associated with low values of P_{Na}, but at low $(Na)_c$ both high and low values of P_{Na} were obtained. At a fixed value of $(Na)_m$ the rate of Na-transport was linearly related to P_{Na}. In contrast to apical Na-entry, which conforms to the kinetics of a single-site process, basolateral Na-extrusion has the properties of a multisite process which is far from saturation.

Dept.Pharmacol., Univ.Vienna, A-1090 Vienna, and
Dept.Physiol., Univ.Texas Med.Sch.,Houston, 77025

51

Na, K AND WATER TRANSPORT OF RAT RECTUM SPONTANEOUSLY INCREASE DURING THE COURSE OF GENERAL ANESTHESIA

Michael Fromm and Sieglinde Lüderitz

Previously we have demonstrated a pronounced increase of aldosterone and corticosterone plasma concentrations in thiobarbital or pentobarbital anesthetized rats. This increase was significant after one hour of anesthesia and both hormone levels remained elevated for at least 12 hours. In order to investigate how electrolyte balance is affected by corticosteroid hormones during prolonged general anesthesia we measured the time course of ion and water transport in rat upper colon and rectum under thiobarbital anesthesia. Net transepithelial fluxes of 90 min intervals were obtained for 12 hours. Fluxes are given in µmol/h·cm² or µl/h·cm²; secretion negative:

hour	1.5	3	4.5	6	7.5	9	10.5	12
UPPER COLON (n=6)								
J_{Na}	11.9	11.9	12.4	13.9	12.4	12.3	14.1	14.6
J_K	-0.9	-1.0	-1.3	-1.2	-1.4	-1.5	-1.5	-1.4
J_{H2O}	50.1	52.9	52.0	66.2	61.0	61.0	79.2	79.8
RECTUM (n=7)								
J_{Na}	-0.6	1.4	3.2	4.0	3.2	4.1	4.3	5.0
J_K	0.0	-0.3	-0.5	-0.7	-1.1	-1.2	-1.2	-1.2
J_{H2O}	-2.7	4.4	15.3	16.0	14.4	19.7	23.9	28.9

Thus, in upper colon, besides an increase of J_{H2O}, there is no significant change of J_{Na} or J_K. In contrast, in rectum all fluxes started at low levels around zero and then went up to high values. We conclude that general anesthesia plus abdominal surgery stimulate corticosteroid secretion and in turn Na, K and water fluxes in rectum but not in upper colon. This is in accord with the known difference in mineralocorticoid sensitivity of both gut segments. Possibly other mineralocorticoid target organs may respond in a similar way.

Institut für Klinische Physiologie, Klinikum Steglitz,
Freie Universität Berlin, D-1000 Berlin 45

52

STEREOSELECTIVITY OF STIMULATION BY PROPRANOLOL OF K-TRANSPORT INTO EHRLICH-ASCITES-TUMOUR-CELLS

P Geck and B Pfeiffer

It was shown by several groups that K-transport by Ehrlich cells, as measured by uptake of Rb-86, is stimulated by high concentrations of propranolol (0.1 - 1 mM). These experiments were performed using the racemate of the drug.

There is no influence on the flux via the Na-K-pump (characterized by the ouabain-sensitive portion of the Rb-86-uptake) or on the Na-K-2Cl-cotransport (characterized by the furosemide-sensitive portion of the Rb-86-uptake). The residual Rb-uptake not inhibitable by these two drugs is stimulated severalfold by propranolol. Maximal activation is obtained with approx. 250 uM for both isomers. The degree of activation, however, is quite different (16fold for d(+)-propranolol and 7-fold for l(-)-propranolol), halfmaximal activation is obtained by 25 uM of both enantiomers. Elevating the propranolol concentration above 250 uM leads to a reduction in Rb-86-uptake (at 1 mM the fluxes are approx. 35% of the maximal flux at 250 uM for the d(+)-isomer and approx. 50% for the l(-)-isomer. The influence of propropranolol on membrane potential and membrane potential dependent secondary active amino acid transport seems to be absolutely specific for the d(+)-isomer; while no effect of l(-)-propranolol is observed, hyperpolarization increases with increasing drug concentration up to 0.5 mM (highest concentration tested). Na-amino acid cotransport behaves equivalently (no effect by the l(-)-form but increasing flux with increasing concentration of the d(+)-isomer.

To explain these results, two models are discussed; either there is a stereospecific effect of the d(+)-isomer or this reversed specificity is mimicked by a specific influence of the l(-)-isomer antagonizing an unspecific influence of both isomers. Experiments to decide between these two possibilities are in progress.

Gustav-Embden-Zentrum der Biologischen Chemie der Universität
Theodor-Stern-Kai 7, D-6000 Frankfurt/Main 70

53

Ca^{++}-TRANSPORTING ATPase OF RAT KIDNEY BASAL-LATERAL PLASMA MEMBRANES

Piotr Gmaj and Ernesto Carafoli

The Ca^{++} uptake and the high-affinity Ca^{++}-ATPase activity were measured in a kidney basal-lateral plasma membrane fraction enriched 32-fold by Percoll gradient centrifugation. Both the Ca^{++} uptake and the ATPase displayed high calcium affinity ($Km_{Ca^{++}} \sim 0.2$ µM). Both reactions were inhibited by low concentrations of vanadate and of anti-calmodulin drugs R24571 and trifluoperazine (TFP). Calmodulin depletion of the membranes resulted in an inhibition of both the Ca^{++} uptake and the Ca^{++}-ATPase, and could be reversed by the addition of exogenous bovine brain calmodulin. The v_{max} of the ATPase was 80 nmoles/mg protein x min., or 6 % of the Na^+,K^+-ATPase activity. The ATP-dependent Ca^{++} uptake was almost completely inhibited by Na^+, presumably due to the Na^+/Ca exchange operating in parallel to the ATP-dependent system.

It is concluded that the basal-lateral plasma membranes of the kidney cortex contain a high-affinity calmodulin-dependent Ca^{++}-ATPase which may act as a low-velocity, high-affinity calcium transport system.

Institute of Physiology, University of Zurich, and Laboratory of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland

Supported by Schweiz. Nationalfonds (H. Murer 3.226.082)

54

CLASSIFICATION OF EFFECTORS OF THE SARCOPLASMIC CA^{2+}-TRANSPORT ATPASE.

G. Loer and M. Makinose

A screening test of a number of substances was carried out concerning to the functions of the sarcoplasmic Ca^{2+} transport enzyme with aim to find out useful effectors for the analysis of the reaction mechanism of this enzyme.

On the transport ATPase activity, 68 of 162 substances tested showed nearly no effect and 18 significant activation. Further 50 were proved to be weak and the other 26 strong ATPase inhibitors. The strong ATPase inhibitors were tested further with transphosphorylation activity of the enzyme. 6 showed no effect, 10 weak and the other 10 strong inhibition. The effects of the latter 10 agents were analysed by conventional Dixon-procedure varying few parameters. The affecting site on the transport enzyme of the classified substance groups could be successfully elucidated. A group of the substances (Maprotiline and Benzoctamine) effects competitively with MgATP and the other group (1-(2-Methoxy-6-chloracridine-9-ylamino)-1-methyl-4-diethylaminobuthan-dihydrochloride and 1-(2-Ethoxyacridine-9-ylamino)-3-diethylaminopropan-2-ol-dihydrochloride) competitively with Mg^{2+} ions on the sarcoplasmic transport system.

The pharmacological effects of the substances tested have no relation to the results of the screening. However, some interesting relationship between the screening results and the chemical structures of the test substances was observed, which may be useful for the search of new inhibitors in the future.

Max-Planck-Institut für Medizinische Forschung
Abteilung Physiologie
Jahnstrasse 29
D-6900 Heidelberg

55

PUTATIVE CALCIUM CHANNELS: SOLUBILIZATION, GLYCOPROTEIN NATURE AND IN SITU MOLECULAR WEIGHT DETERMINATION

H. Glossmann, D.R. Ferry and A. Goll

^{3}H-Nimodipine (^{3}H-NIM) labels putative calcium channels (CC) in guinea-pig skeletal muscle sarcolemma (SMM). The B_{max} was 2 pmol.mg^{-1} of protein, the K_D 1.5 nM (37°C). The pharmacological profile of these ^{3}H-NIM sites is typical of voltage-operated CC. Detergent-solubilized CC retained many of the characteristics of the membrane bound CC, including allosteric regulation by d-cis (but not l-cis) diltiazem. D-cis diltiazem increased the density of ^{3}H-NIM binding sites with a K_D of ≈ 3 nM by a factor of 2-3. ^{3}H-NIM binding to CC solubilized from partially purified SMM was sensitive to glycosidases. Appropriately we found that solubilized CC was retained by sepharose-coupled lectins. E.g. ^{3}H-NIM binding sites were quantitatively adsorbed by concanavalin A-sepharose, could not be eluted with salt but with α-methylmannoside. The average purification of the CC is 20-fold by this procedure. The in situ target-size analysis of the guinea-pig brain CC (K_D=0.6 nM at 37°C) with 10 MeV electrons at - 110°C yielded a D_{37} dose of 7.89 ± 2.12 Mrad, corresponding to a molecular weight of 227.000. Evidence for the subunit structure of the CC will be presented.

Rudolf Buchheim-Institut für Pharmakologie
Frankfurter Straße 107, D-6300 Gießen

56

IS THERE A HIGHLY PURIFIED CARDIAC SARCOLEMMA PREPARATION?

J.Schrader*and C.Londos

Isolation procedures for cardiac sarcolemma were refined in recent years towards the separation from various intracellular membrane systems. The possibility that cellular hetrogeneity may influence the purity of sarcolemma preparations has not been considered, although the heart is known to be highly vascularized and the surface area of coronary endothelium comprises as much as 30-40% of the total surface area of cardiomyocytes. We have therefore examined the contamination by endothelial membranes of a representative sarcolemma preparation from dog heart which has been characterized with regard to Na^+-Ca^{++} exchange and active Ca^{++} transport (Reeves,Sutko, Proc.Natl.Acad. Sci. 77, 6345,1980; Science 208, 1461,1980). Isolated membrane vesicles were subjected to density centrifugation on a very shallow Percoll gradient. On the basis of the distribution of angiotensin converting enzyme (ACE), a marker for endothelial membranes, and sarcolemmal Na^+-K^+ ATPase, two vesicular membrane populations were resolved which differed only slightly in their respective density. Enrichment of ACE and ouabain inhibitable Na^+-K^+ ATPase over the crude particulate fraction was 10 and 2-3 fold, respectively. Other marker enzymes, such as adenylate cyclase and 5'-nucleotidase were associated both with endothelial and sarcolemmal vesicles and are thus not sufficiently discrimatory. Furthermore, ATP-dependent accumulation of Ca^{++}, previously ascribed to cardiac sarcolemma, occured predominantly in endothelial vesicles. Our findings reveal, that conditions generally applied for the purification of cardiac sarcolemma result in a rather selective enrichment of endothelial membranes. Thus, previous conclusions drawn from in-vitro studies with "highly purified sarcolemma" need to be re-evaluated.

Natl.Inst.Arthr.Metabol. and Digestive Disease, Lab.Nutr. Endocrinol., NIH, Bethesda, MD 20205, USA
*adress: Dept.Physiology, University of Munich, Pettenkoferstrasse 12, 8 000 Munich, W-Germany

57

EFFECTS OF AN ANEMONIA SULCATA TOXIN (ATX II) ON MEMBRANE CURRENTS OF ISOLATED VENTRICULAR MYOCYTES

G. Isenberg and Ursula Ravens

Myocytes were isolated from guinea pig or bovine ventricles according to ISENBERG and KLÖCKNER (Pflügers Arch. 395:6, 1982). The cells were stimulated with conventional intracellular microelectrodes, the resulting action potentials (AP) and contractions (sarcomere shortening) were analysed. ATX II (2-20nM) prolonged the action potential duration (APD) and increased the extent of shortening severalfold. The effects were frequency-dependent and reversible after washing or upon addition of TTX (60µM). Since these effects of ATX II were similar as in multicellular preparations, the isolated myocytes were considered as a suitable model.

Membrane currents were studied with a single electrode voltage clamp technique applying firepolished suction pipettes (HAMILL et al., Pflügers Arch. 391:85, 1981) filled with 160 mM K-glutamate (resistance 2 M Ohm). ATX II (20nM) induced a slowly decaying (halftime>1s) inward current component which had a threshold at -60 mV, a maximal amplitude at -20mV and a reversal potential at +45mV. TTX (60µM) blocked the ATX II-induced current completely. It is concluded that this current flows most likely through modified non-inactivating sodium channels. The voltage dependence of inactivation of the sodium current (h_∞-curve) was not influenced by ATX II, the potential of half maximal inactivation was -63mV. Because of the slowly decaying ATX II-induced current component the net membrane current remains negative for periods as long as 0.5s and thereby retards the repolarization phase 2 of the AP. During the ATX II-prolonged AP the continuous sodium entry imposes a cellular sodium load severalfold surpassing the sodium entry under control conditions. It is suggested that this extra sodium load is responsible for the enhanced contractility because in Na-free, Li-substituted Tyrode solution ATX II prolongs the APD but it does not increase the extent of sarcomere shortening.

II. Physiologisches Institut, Univ. d. Saarlandes, 665 Homburg and Abt. Pharmakologie, Univ. Kiel, Hospitalstrasse 4-6, D-2300 Kiel

58

INTESTINAL SECRETION OF URIC ACID IN THE GUINEA PIG

G. Sprakties

Secretion of organic acids by the preparation of the isolated mucosa of guinea pig intestine (Lauterbach, Arch. Pharmacol. 297, 201, 1977) has previously been demonstrated for sulfonic acids as well as for salicylic acid. Intestinal handling of urate was now investigated by measuring transepithelial permeation and tissue uptake of 10 uM (2-^{14}C)uric acid added either to the luminal or the blood side of the isolated jejunal mucosa. (^{3}H)polyethylene glycol (PEG) (mol.weight 900) added simultaneously served as a marker for extracellular spaces and shunt permeability. Tissue content, referred to the intracellular space, amounted to 6,5% and 8,6% of the concentration administered at the luminal and blood side, resp., after 45 min. Transepithelial permeation increased linearly up to 180 min. In both directions, permeation was correlated with the simultaneous permeation of PEG. As compared with the regression line for the permeation lumen-to-blood the regression line for the permeation blood-to-lumen revealed a parallel shift to positive values indicating a secretory component in the total transepithelial permeation. From the difference between the two regression lines a net uric acid secretion of about 130 pmol·cm^{-2}·h^{-1} was calculated. Anaerobiosis abolished the difference between the two regression lines and increased tissue content to approx. 20%. The effect of several compounds known to inhibit renal urate transport was tested.

Institut für Pharmakologie und Toxikologie, Ruhr-Universität, D-4630 Bochum 1

59

INTESTINAL PERMEATION KINETICS OF DIGITOXIN

M. Misra

Investigations on the intestinal secretion of cardiac glycosides (Lauterbach, in: Handbook of Exptl. Pharmacology, Vol. 56/II, p. 105) were extended to the highly lipophilic digitoxin. Concentrations between 0.02 uM and 20 uM (^{3}H)digitoxin were administered either to the luminal or to the blood side of isolated mucosae of guinea pig jejunum or colon mounted in a flux chamber. Transepithelial digitoxin permeation as well as tissue uptake within 45 min were determined. Under all conditions, permeation blood-to-lumen exceeded permeation lumen-to-blood 1,5-3 fold indicating net secretion of digitoxin. This difference was completely abolished (jejunum) or greatly diminished (colon) by incubation at 7°. Per unit area, permeation rates in the colon were found 2-10 times higher than in the jejunum substantiating the greater potency of the colonic mucosa for the excretion of cardiac glycosides. Tissue content (referred to the intracellular space and expressed as percent of the concentration administered) in the jejunum amounted to 50-60% after luminal and to 100-110% after blood side administration. In the colon digitoxin was accumulated between 200% and more than 500% with a less pronounced dependency on the side of administration. Incubation at 7° reduced tissue content in all cases by at least one half. Virtually no digitoxin is taken up in the colon from 2 uM or 20 uM solutions administered at the luminal side. The critical role of the luminal membrane for digitoxin permeation was substantiated by efflux experiments.

Institut für Pharmakologie und Toxikologie, Ruhr-Universität, D-4630 Bochum 1.

60

COMPARISON OF METHOTREXATE TRANSPORT IN ISOLATED HEPATOCYTES OF RATS IN DIFFERENT METABOLIC STATE AND IN YOSCHIDA SARCOMA TUMOR CELLS

A. Leszczyńska and E. Pfaff

Methotrexate (MTX) uptake by isolated hepatocytes was decreased when rats were fasted 24-28 hours before preparation of cells as compared with liver cells from fed rat. In hepatocytes of starved rat intracellular GSH was 30% lower than in fed rat hepatocytes. Stronger depletion (80%) of endogenous GSH by phoron administration (25 mg/kg i.p. 2 hours prior to preparation of cells) caused greater inhibition of MTX uptake. Moreover, some important differences between hepatocytes of fed and fasted rats concerning the factors affecting MTX transport were found. In liver cells of fasted rat MTX transport was sharply decreased in Na-deficient choline-medium (contrary to hepatocytes of fed rat). On the other hand, blockade of membrane SH groups by p-CMBS caused significantly smaller inhibition of MTX transport in liver cells of starved rat than of fed one. Exogenous GSH had little effect on MTX uptake by starved rat hepatocytes, but the oxidating substance ferricyanide greatly enhanced MTX uptake which is contrary to GSH and ferricyanide effect in hepatocytes of fed rat. The same effect of ferricyanide and little effect of GSH administration on MTX transport was observed in liver cells of rat treated with phoron. Studies of MTX transport in Yoschida Sarcoma tumor cells brought out the likeness between cancer cells and hepatocytes of starved and phoron-treated rats, concerning the MTX transport. Examination of the level of membrane free SH groups of hepatocytes from fed, starved and phoron-treated rats as well as rat Yoschida Sarcoma tumor cells showed that membranes of liver cells of fed rat exposed 2-4 times less SH groups to the outside of the membrane than the other cell types examined.

Institut für Toxikologie, Universität Tübingen, Wilhelmstr. 56, D-7400 Tübingen

61

EVIDENCE FOR THE EXISTENCE OF PLASMA MEMBRANE-BOUND HEXOKINASE

B. Agrawal, K. Keller, K. Lange, U. Brandt, I. Monden and I. Reinsch

Hexokinase is a key enzyme of glucose metabolism. Its cellular localization is of special interest because of its functional implications. The presence of cytoplasmic and mitochondria-bound hexokinase has already been established (J. E. Wilson, in: Current Topics in Cellular Regulation, B. L. Horecker and E. R. Stadtman, eds., Vol. 16, p. 2, New York, Academic Press, 1980). The distribution between cytoplasma and inner surface of the plasma membrane, however, has scarcely been investigated so far. With the help of a phagocytosis technique we are able to isolate fractions containing large quantities of plasma membranes. The specific advantages of this technique lie in a definite orientation of the obtained membranes (inside out) as well as in the possibility of determining the exact surface area available. This method was applied to C 6-glioma and N2A-neuroblastoma cells. The observed hexokinase binding might permit drawing inferences on the possibility of a correlation between glucose transport and phosphorylation (K. Lange et al., J. Neurochem., 1982, in press).

Pharmakologisches Institut der Freien Universität Berlin, Thielallee 69/73, D-1000 Berlin 33

62

EVIDENCE FOR THE EXISTENCE OF PLASMA MEMBRANE-BOUND HEXOKINASE

B. Agrawal, K. Keller, K. Lange, U. Brandt, I. Monden and I. Reinsch

Hexokinase is a key enzyme of glucose metabolism. Its cellular localization is of special interest because of its functional implications. The presence of cytoplasmic and mitochondria-bound hexokinase has already been established (J. E. Wilson, in: Current Topics in Cellular Regulation, B. L. Horecker and E. R. Stadtman, eds., Vol. 16, p. 2, New York, Academic Press, 1980). The distribution between cytoplasma and inner surface of the plasma membrane, however, has scarcely been investigated so far. With the help of a phagocytosis technique we are able to isolate fractions containing large quantities of plasma membranes. The specific advantages of this technique lie in a definite orientation of the obtained membranes (inside out) as well as in the possibility of determining the exact surface area available. This method was applied to C 6-glioma and N2A-neuroblastoma cells. The observed hexokinase binding might permit drawing inferences on the possibility of a correlation between glucose transport and phosphorylation (K. Lange et al., J. Neurochem., 1982, in press).

Pharmakologisches Institut der Freien Universität Berlin, Thielallee 69/73, D-1000 Berlin 33

63

DIRECT OBSERVATION OF ANGIOTENSIN II (A II) MEDIATED CHANGES IN GLOMERULAR MICROCIRCULATION

M. Steinhausen, B. Zimmerhackl, N. Parekh, K.R. Wilhelm, H. Snoei and H. Kücherer

Recent micropuncture studies of the effect of A II on glomerular functions from different laboratories have been interpreted as showing an intraglomerular - probably a mesangial - contraction, effecting a reduction in filtering surface area. Using fluorescence labeled erythrocytes and video techniques we have studied the effect of A II on the microcirculation of single glomerular capillaries accessible in normal rats. A II caused a parallel reduction in glomerular and total kidney blood flow, but in contradiction to the contraction-hypothesis no change in capillary dimensions could be observed in vivo. Furthermore, we developed a postischemic hydronephrotic split-kidney in which the whole glomerular network, with afferent and efferent arterioles, is visible. With a three dimensional analysis of the glomerular network we could differentiate shorter pathways consisting of wide capillaries from longer narrow circuits. A mathematical model derived from these morphological observations showed that even a global reduction in blood flow would cause a reduction in effective filtration area since there would be then, simultaneously, filtration equilibrium in distal sections of longer pathways, but no equilibrium in short pathways. Thus A II could reduce effective filtering area, without intraglomerular morphological changes, by altered afferent and efferent lumen diameters only, as observed in vivo.

I. Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-69 Heidelberg

Supported by SFB 90

64

REVERSIBILITY OF TUBULAR CELL FUNCTION AFTER ANOXIA

G. Gronow, P. Benk, and H. Bertermann

The reduced ability of renal cells to recover after prolonged periods of ischemia may contribute to acute renal failure. We compared the aerobic recovery of cellular functions in collagenase-isolated tubular segments (ITS) of rat kidney cortex after different periods of normothermic anoxia. During anoxia, ITS were suspended either in Krebs-Ringer-Bicarbonate (KRB) or in modified Collins solution, without (MC) or with the addition of substrates stimulating mitochondrial anaerobic ATP production (MC+S). After anoxia, all ITS were resuspended aerobically for 30min in KRB+10mM lactate (recovery period). Absolute control values for aerobic functions under study were 3.68 ± 0.71meq intracellular k^+ per g protein (K^+), a gluconeogenesis rate (GNG) of 324 ± 49 micromoles glucose per g protein and hour, and a tubule-to-medium ratio of PAH of 82 ± 22.8 (means $\pm$SD, n=12). In the recovery period, all tested functions were significantly impaired after 20min of anoxia in KRB (=16-68% of aerobic control). However, as compared to KRB, the suspension of ITS in MC or MC+S reduced the anoxic decrease in cellular function markedly (MC:56-90% of control, MC+S: no significant difference in comparison to control). The reported increases in reversibility of cellular function are discussed in terms of physicochemical support of volume regulation in hyperosmolal "intracellular" solutions (MC) and in terms of additional mitochondrial energy production (MC+S) in the ITS during normothermic anoxia.

Physiologisches Institut der Universität Kiel, Olshausenstr. 40-60, D-2300 Kiel 1

65

MECHANISMS OF MALEATE-INDUCED FALL IN GLOMERULAR FILTRATION RATE (GFR)

K. H. Leser

Following maleate (MAL) administration GFR is reduced by about 50%. The present study investigates the mechanism of this fall in GFR using micropuncture techniques in the rat kidney. In group 1 (n=8) we measured hydrostatic pressures on the kidney surface in the proximal convoluted tubule under free flow (P_T) and during stop flow (SFP) conditions and in the glomerular capillaries (P_G) by means of a "servo-null" micropressure device. In group 2 (n=7) we determined single nephron glomerular filtration rate (SNGFR) from distal and then proximal collection sites in the same nephron. Chloride concentration was measured in the late proximal and early distal tubular fluid. Mean values ± SEM are shown in the table; CON: control period; MAL: 2 mmol/kg MAL i.v.; * $p < 0.05$.

	P_G	P_T	SFP	SNGFR nl/min		Cl mmol/l	
	mm Hg			prox	dist	prox	dist
CON	47.6 ±1.6	12.6 ± .5	33.6 ± .4	31.8 ±2.4	26.3 ±1.6	135 ±3.9	43.3 ±2.9
MAL	42.4* ±1.9	16.3* ± .8	33.1 ±1.3	24.3* ±2.2	15.2* ±1.6	121* ±6.4	61.9* ±6.0

It is obvious that MAL increased P_T due to inhibition of fluid reabsorption in the prox.tubule. The fall in P_G contributes also to the MAL induced fall in SNGFR. Since SFP was unchanged by MAL undisrupted distal tubular flow seems to be necessary for MAL to decrease SNGFR. In fact, the data of the group 2 demonstrate a greater difference between SNGFR from proximal vs. distal collection sites in the MAL period. This greater difference suggests an increased activity of the tubuloglomerular feedback after MAL administration.

Abt. Pharmakologie, RWTH Aachen, D-5100 Aachen

66

TISSUE LEVELS OF ATP AND ADENOSINE IN THE RAT KIDNEY UNDER VARIOUS EXPERIMENTAL CONDITIONS

H. Osswald, C. Linn, E. Juengling, G. Stoecker

The metabolic hypothesis of intrinsic regulation of renal function postulates that increased ATP hydrolysis leads to an increased generation of the vasoconstrictive metabolite adenosine. In the present study we compared tissue levels of ATP and adenosine (ADO) under different conditions: 1. renal ischemia; 2. maleate (1 mmol/kg i.v.) and 3. intrarenal infusion of hypertonic saline (HS). Sprague Dawley rats were anesthetized with thiobutabarbital, placed on a heated table and infused with 3 ml/h of 0.85% NaCl. Kidneys were exposed by flank incision and after a control period removed to be shock frozen. From the tissue extracts the adenine nucleotides were measured by HPLC (Juengling & Kammermeier, 1980) and ADO by an enzymatic assay (Osswald et al., 1977). Results are shown in the table (mean ± SEM).

Experimental Conditions		N	ATP µmol/g	ADO nmol/g	ATP/ADO
Controls		9	2.24±0.06	5.13±0.60	437
Ischemia (sec)	15	7	1.64±0.10	6.93±0.83	260
	30	6	1.28±0.16	15.0 ±1.31	85
	60	7	1.00±0.15	31.3 ±2.96	32
Controls		11	2.18±0.18	7.05±0.79	309
Maleate		10	1.31±0.16	13.6 ±1.12	96
Controls		6	2.55±0.05	5.69±1.2	448
HS		7	1.02±0.26	16.2 ±1.3	63

The data show an inverse relationship between ATP and ADO tissue levels and therefore support the hypothesis of a metabolic control of renal function.

Abt.Pharmakologie und Abt.Physiologie der Med.Fak.der RWTH,Schneebergweg,5100 Aachen

67

Barium reduces the transepithelial electrochemical gradient of Cl (E^{te}_{Cl}) in the diluting segment of frog kidney.

S.Neuman, W.Wang and H.Oberleithner

Previous studies suggest that the operation of the furosemide-sensitive Na, Cl, K-cotransport system is dependent on a high luminal K permeability which allows backflux of K from the cell cytosol into the luminal fluid and thus provides sufficient K for the cotransport process. Experiments were performed in diluting segments of the isolated perfused frog kidney to test the hypothesis that inhibition of the luminal K permeability by barium affects Cl reabsorption. The transepithelial potential difference (PD^{te}) and E^{te}_{Cl} were measured by conventional and Cl-sensitive microelectrodes, respectively, at zero net flux conditions in controls, after luminal furosemide ($5 \cdot 10^{-5}$ mol/l) and after addition of barium (5mmol/l) to the perfusates.

	control	furosemide	barium
PD^{te}	12.3 ± 0.7 (43)	0.2 ± 0.3 (15)	5.7 ± 0.5 (23)
E^{te}_{Cl}	39.5 ± 1.4 (63)	1.3 ± 0.5 (14)	16.3 ± 3.1 (17)

PD^{te} and E^{te}_{Cl} (mV) are lumen positive, ± SE, (n).

Control experiments in the frog diluting segment demonstrate that Cl reabsorption occurs against a steep E^{te}_{Cl}. PD^{te} and E^{te}_{Cl} are significantly reduced after application of barium on both sides of the tubule and abolished after luminal application of furosemide.

The data support our hypothesis that the function of the furosemide-sensitive luminal cotransport system depends critically on an intact luminal K permeability. However, barium could also act on the peritubular cell membrane by a mechanism which is not known at present.

Physiol.Institut, Fritz-Pregl-Str.3, A-6010 Innsbruck, Austria. Supported by Österr.Forschungs-Proj.No.:4366

68

EFFECT OF CELL MEMBRANE POTENTIAL AND INTRACELLULAR SODIUM ACTIVITY ON RENAL TUBULAR TRANSPORT OF PHENYLALANINE

G. Meßner, M. Paulmichl, F. Lang

In the proximal nephron, the transport of phenylalanine (Phe) and other amino acids across the luminal cell membrane is coupled to sodium and is driven by the electrochemical gradient for this ion (E_{Na}). Inhibition of Na/K-ATPase by ouabain eventually leads to an impairment of transepithelial amino acid transport. The present study was performed to test whether this effect is due to a dissipation of the electrochemical gradient for sodium or due to a regulatory inhibition of the carrier following enhancement of intracellular sodium activity. In the isolated frog kidney the lumen of proximal convoluted tubules was perfused alternatively with solutions containing either 0 or 5 mmol/l Phe. The potential difference across the peritubular cell membrane (PD_{pt}) was determined with conventional and E_{Na} with ion selective microelectrodes. PD_{pt} is (±SEM) 60.4 ± 2.0 mV and E_{Na} 103.0 ± 6.6 mV. Thus intracellular sodium activity approaches 10 mmol/l (extracellular sodium activity is 74 mmol/l). Both PD_{pt} and E_{Na} are decreased immediately ($t_{1/2} \approx 3$ s) by luminal application of Phe. Peritubular application of 10^{-4} mol/l ouabain leads to a gradual decline of PD_{pt} and E_{Na} ($t_{1/2} \approx 30$ min). The Phe-induced depolarization of PD_{pt} (ΔPD_{pt}) and E_{Na} (ΔE_{Na}) decrease in linear proportion to the absolute values of PD_{pt} and E_{Na} ($r = 0.902$). In a separate series, the lumped conductance of the luminal and peritubular cell membrane (G_{lu+pt}) was determined during application of ouabain. G_{lu+pt} is $2.3 \pm 0.3 \cdot 10^{-3}$ [S/m (tubule length)], which is almost identical to necturus proximal tubule. G_{lu+pt} decreases 26 ± 5 %, when PD_{pt} is decreased to half. G_{lu+pt} and PD_{pt} allow calculation of Phe transport rate (T_{Phe}) across the luminal cell membrane. A 50 % reduction of PD_{pt} decreases T_{Phe} to 44 %. The data suggest that T_{Phe} is not regulated but rather operates in linear proportion to the driving force.

Institut für Physiologie, Universität Innsbruck, Austria
Supported by Österr. Forschungsrat, Proj. Nr. 4366

69

INTRACELLULAR K^+-ACTIVITY IN ISOLATED PERFUSED CORTICAL THICK ASCENDING LIMB SEGMENTS OF RABBIT NEPHRON R.Greger

Recent modifications from our laboratory have made it possible to obtain stable intracellular electrical measurements in the small cells of the thick ascending limb (cTAL) In the present study we have used conventional microelectrodes filled with 1mol/l KCl and K^+-selective microelectrodes (KSM) filled with IE-190 exchanger. The electrode tips were <100nm, and the resistances were 150-200MΩ and 6-12GΩ for the conventional and ion selective electrodes, respectively. The selectivity of the KSM for K^+/Na^+ was 27±2(44). Isolated cTAL segments (n=72) were perfused in vitro as customary in our laboratory.

The PD across the basolateral membrane (PD_b) was -64±1mV (65). The PD read by the KSM (PD_K) was +9±2mV (44). Furosemide (10^{-5}-10^{-4}mol/l, lumen side) hyperpolarized PD_b from -63±3 to -75±2mV (14). PD_K fell from +10±2 to +2±2mV (26). From PD_K, the mean PD_b, and from the selectivity of the individual KSM, cellular K^+-activity (a_c^K) can be calculated. Under control conditions a_c^K was 110±12 mmol/l. Furosemide increases a_c^K slightly to 126±12mmol/l (26).

The data lead to the following conclusions: a) a_c^K in the cTAL segment is above Nernst distribution since PD_K was positive. b) Furosemide leads to a reduction in PD_K to values close to zero. This confirms our previous hypothesis that the furosemide induced hyperpolarization of PD_b is caused by a shift towards the zero current PD of K^+ (EMF_K). c) The a_c^K values reported here are in perfect agreement to the value previously predicted by ourselves on the basis of a resistance and circular current analysis.

Max-Planck-Institut für Biophysik, Kennedyallee 70, D-6000 Frankfurt/Main. Supported by Deutsche Forschungsgemeinschaft: Gr 480/7-2

70

PHLORETIN (PTN) INHIBITS ACTIVE NaCl REABSORPTION IN THE CORTICAL THICK ASCENDING LIMB SEGMENT (cTAL) OF RABBIT NEPHRON E. Schlatter

Previously we have shown that active NaCl reabsorption in the cTAL segment is energized by the basolateral uptake of any of the following substrates: D-glucose, L-lactate, pyruvate, acetate, butyrate, β-OH-butyrate. This prompted us to test wheter PTN inhibits basolateral substrate uptake. cTAL segments (n=89) were perfused in vitro as customary in our laboratory. Transepithelial PD(PD_t) and resistance (R_t) were measured to calculate the equivalent short circuit current (I_{sc}), which is linked stoichiometrically to the rate of active NaCl reabsorption. In some experiments also the PD across the basolateral membrane (PD_b) and the voltage divider ratio (VDR=lumen membrane resistance/basolateral membrane resistance) were recorded. PTN added to the bath inhibited I_{sc} rapidly (<2min) and partially reversibly with half maximal inhibition at 30-80umol/l irrespective of the used substrate. PTN had little effect from the lumen side. This inhibitory effect of PTN does not reflect inhibition of substrate uptake since it was also observed when butyrate was offered from the lumen side as the sole substrate. The inhibitory effect of PTN was paralleled by the following changes: Fall of PD_t to 0mV, increase in R_t by 31±11% (21), depolarization of PD_b from -66±2 to -26±3mV (17), and increase in VDR from 2.5±0.7 to 55±16 (4). PTN did not alter the fractional conductance to Cl^- of the basolateral membrane(3), nor did it induce a K^+ conductive pathway (3), and also not a measurable Na^+ conductive pathway (4). The depolarisatory effect of PTN on both cell membranes was grossly reduced when Na^+ was replaced on both sides by $choline^+$ (6). We conclude that PTN leads to a rapid increase in cellular Na^+ and a fall in cell K^+. The latter explains the increase in R_t and the dramatic increase in VDR. As yet we cannot decide whether PTN inhibits the $(Na^+ + K^+)$-ATPase or increases the Na^+ permeability of the basolateral membrane.

Arbeitsgruppe R.Greger,Max-Planck-Institut für Biophysik, Kennedyallee 70, D-6000 Frankfurt/Main. Supported by DFG.

71

MICROPUNCTURE STUDIES ON THE MODE OF TUBULAR ACTION OF MUZOLIMINE
J. Greven, and B. Kölling

In the present study the effect of muzolimine on sodium and chloride reabsorption by the rat kidney was studied. In addition to free flow micropuncture experiments, single short loops of Henle were perfused in vivo from the end-proximal tubular site. The perfusion fluid was a modified Ringer solution resembling endproximal tubular fluid, and containing ^{3}H-inulin. The perfusion rate was checked by in vivo calibration and amounted to 13 nl/min.

In contrast to furosemide, addition of 10^{-3} M muzolimine to the perfusion fluid did not significantly alter sodium and chloride reabsorption in the loops of Henle, indicating that luminal application of the drug does not affect the loops reabsorptive capacity. However, when muzolimine (50 mg/kg) was injected intravenously sodium chloride transport in the loops of Henle was markedly depressed.

Muzolimine did not share with furosemide and its chemically related analogues a common binding site on the Tamm-Horsfall glycoprotein. This protein is located in the surface membrane of the cells of the thick ascending limb and may play a role in electrolyte transport in this tubular segment.

It is concluded that the mode of tubular action of muzolimine is quite different from that of furosemide and its chemically related analogues.
Institute of Pharmacology, TH Aachen, Schneebergweg, D-5100 Aachen

72

SEX DIFFERENCE IN THE RENAL HANDLING OF INORGANIC PHOSPHATE IN THE RAT IN THE ABSENCE OF PARATHYROID HORMONE (PTH)
M. Neuweg, I. Durasin and A. Frick

In intact rats, the fractional excretion of inorganic phosphate (FE_{Pi}) in females was found to be higher than in males (1). From clinical studies, an increase in the parathyroid secretion secondary to estrogen therapy was reported (2). Thus the sex difference in the renal Pi-transport in the rat may be mediated by different parathyroid activities. In order to examine this possibility we excluded this influence of PTH in rats of both sexes.

Female and male adult Sprague-Dawley rats (210g(n=7), 240g b.w.(n=12), respectively) kept on a standard diet (Altromin; 0.90g Ca and 0.80g P/100g) were anesthetized with Inactin and prepared for clearance experiments. The parathyroid glands were removed 2.5 h before collection of urine samples. All animals were infused with a modified Ringer solution (either without or with Pi) to measure the Pi reabsorption both with control and elevated plasma Pi levels (Pi-titration). During stepwise phosphate loading, FE_{Pi} in females increased from 0.41±0.32(mean±S.D.) to 20.9±3.5 and finally to 53.2±4.2% of the filtered load, in males from 0.09±0.08 to 6.0±5.3 and to 31.7±8.6%, respectively. The differences in the respective FE_{Pi} between the both groups were highly significant ($P < 0.001$)

In conclusion, the present studies indicate a sex difference in the renal handling of Pi independent of PTH. The findings are consistent with the hypothesis that this sex difference is probably a result of an estrogen-induced inhibition of Pi reabsorption primarily in the proximal tubule.

Lit. 1) Harris et al.: Am.J.Physiol.(1974) 227, 972-976
2) Riggs et al.: J.Clin.Invest.(1972) 51, 1659-1663.

Deutsche Forschungsgemeinschaft Fr 239/9-1
Physiologisches Institut der Universität München
Pettenkoferstrasse 12, D-8000 München 2

73

CHANGES IN PLASMA ADH AND RENAL WATER EXCRETION BY INTRA-CEREBROVENTRICULAR OSMOTIC STIMULATION IN DUCKS.

R. Gerstberger and D. Gray

Osmoreceptive elements in the brain are known to contribute to osmoregulation in mammals and birds. At least a fraction of them was found to be located close to the wall of the third cerebral ventricle (III V). The characteristics of the juxtaventricular osmoreceptive system and its significance for the homeostatic control of renal water excretion have not been assessed under steady state conditions. Therefore, we perfused the III V of adult, conscious ducks, chronically implanted with a reentrant tube, with artificial cerebrospinal fluid (mock CSF) for periods of 15 min at rates of 15 $\mu l \cdot min^{-1}$. By changing its NaCl content, the mock CSF was made iso-, hypo- or hypertonic. A steady state diuresis was maintained by continuous intravenous infusion of 1.0 $ml \cdot min^{-1}$ saline at 200 $mOsm \cdot kg^{-1}$. Changes in urine flow were related to the steady state excretion rate. - A 15 min increase in osmolality by 32 ± 7 $mOsm \cdot kg^{-1}$ (mean ± SD) of the mock CSF caused a urine volume retention of 12 ± 2 ml associated with an elevated osmolality of the discharged urine. For different hypertonic perfusions, the increase of mock CSF osmolality and the urine volume retentions were linearly correlated with an average slope of 0.3 ml per $mOsm \cdot kg^{-1}$. Parallel to the antidiuresis, the plasma concentrations of the antidiuretic hormone (ADH), as determined with a radioimmunoassay, increased with increasing tonicity of the mock CSF, in average, by 0.2 $pg \cdot ml^{-1}$ per $mOsm \cdot kg^{-1}$. Reductions of the mock CSF tonicity during 15 min by 70 ± 4 and 116 ± 8 $mOsm \cdot kg^{-1}$ resulted in excess urine volumes of 8 ± 2 and 15 ± 3 ml respectively, indicating a sensitivity in the hypotonic range of about one third of that in the hypertonic range. Parallel to the enhanced diuresis, the plasma ADH levels decreased. Over the entire investigated range of hypo- and hypertonic mock CSF perfusions, the changes of plasma ADH and of urine output were closely correlated ($r = -0.93$).

Max-Planck-Institut f. physiol. u. klin. Forschung, W.G. Kerckhoff-Institut, D-6350 Bad Nauheim, West-Germany

74

APPARENT VOLUME DISTRIBUTION OF ^{22}Na IN PEKIN DUCKS.

R. Kaul and E. Simon

Osmoregulatory effector activities in Pekin ducks have been found to be sensitive to volume changes in the extracellular fluid (ECF) compartment(s). In order to estimate ECF volume, the exchangeable Na of Pekin ducks was labeled by infusing 5 μCi ^{22}Na in 2 ml isotonic saline intravenously (iv.). In 21 ducks (1.9-3.0 kg) which had been maintained for 30 or more days on salt water (SW) with an NaCl concentration of 600 $mOsm \cdot kg^{-1}$, 1 h after injection of the label its volume of distribution (V_d-^{22}Na) had reached a plateau value of $30.1 \pm 0.3\%$ (mean ± SE) of body weight. This was not different from the plateau value of $31.1 \pm 1.0\%$ found in 5 ducks maintained on fresh water. In the SW ducks the equilibration curve of ^{22}Na could be resolved into two exponential rates with half-times of 30 sec and 9 min, respectively. - The SW ducks were challenged with a continuous iv. infusion of 1000 $mOsm \cdot kg^{-1}$ NaCl solution at 0.4 $ml \cdot min^{-1}$, while collecting and measuring all salt and water excreted. Within 15 min after the start of loading the salt glands were activated to secrete salt and water at the same rate as infused. At this time, V_d-^{22}Na had increased by 36 ± 4 ml. This increase was sustained during salt loading, but V_d-^{22}Na returned to its pre-loading level when salt gland secretion ceased after the infusion had been halted. - The increase of V_d-^{22}Na was found to be about 3-times greater than the ECF volume changes calculated from the salt and water balance. One possible explanation for this was that ^{22}Na might have been treated differently in the ducks than was the naturally occurring Na. Because the salt glands have such exceptional capabilities of Na transport and excretion, they were thought to be a site where such differences might become detectable. Indeed, the specific activity ($cpm \cdot mol^{-1}$) of Na was found to be about 10% greater in the salt gland fluid than in the simultaneously sampled blood plasma.

Max-Planck-Institut f. physiol. u. klin. Forschung, W.G. Kerckhoff-Institut, D-6350 Bad Nauheim, West-Germany

75

INTRARENAL INFUSION OF 6-HYDROXY-DOPAMINE (6-OHDA) AS A NOVEL METHOD FOR RENAL SYMPATHETIC DENERVATION IN RATS.

L.M.L. le Noble and C.M. Kasbergen.

As an alternative for surgical denervation of the kidneys in rats, we have attempted to destroy sympathetic nerve terminals in rat kidneys by slow low dose (0.1 mg) intrarenal (i.r.) infusion of 6-OHDA. The effects were measured as the degree of inhibition of renal vasoconstriction during electrical stimulation of renal nerves. Under pentobarbital anesthesia (60 mg/kg i.p.) a midline incision was made in the abdomen of 6 spontaneously hypertensive rats (SHR). The right suprarenal artery was canulated. An electromagnetic flowprobe was placed on the right renal artery. The mean arterial pressure (MAP) was measured by a PE-50 catheter in the left femoral artery. Under a stereo microscope, renal nerves were dissected free of surrounding tissue. A monopolar silver electrode was placed around the nerve and insulated. The nerve was stimulated at frequencies of 1-10 Hz, and effects on MAP, renal blood flow (RBF) and heart rate (HR) were monitored. Before denervation, the MAP was 142 mmHg and increased by 22.5% maximally at 10 Hz. HR decreased by 10 b/min, and renal resistance (RR) increased by 163.2%. Then 6-OHDA was infused at a rate of 5 µg/min over a 20 min period (total dose 0.1 mg). This by itself resulted in minor hemodynamic changes (max effect on MAP: +2%, RBF: -13%, HR: +7 b/min. After the infusion, stimulations were repeated. Denervation appeared to be complete after 100 min. The effects of stimulation at all frequencies were significantly ($p<0.01$) diminished at this time. Electrical stimulation provoked a transient decrease followed by an increase in MAP, suggesting afferent nerve stimulation. Chemical analysis in a separate group of animals indicated a reduction of renal noradrenaline to less than 45% of control. These results suggest that i.r. infusion of 6-OHDA is an alternative for surgical denervation of rat kidneys and that denervation is complete 100 min after the start of the infusion.

Dept. of Pharmacology, University of Limburg, P.O. Box 616, 6200 MD Maastricht, The Netherlands.

76

Involvement of α_2-receptors in the diuretic effects of clonidine, moxonidine and related drugs.

ARMAH, I.B.

The diuretic effect of clonidine was first described by HOEFKE and KOBINGER [DRUG RES. 16 (1966) 1038].It has been shown that this diuresis is preceded by an increased PGE excretion [OLSEN,U.B., Europ. J. Pharmacol. 36 (1976) 95-101], but the mechanism of clonidine induced diuresis is still unknown. In this report an attempt has been made to identify intra-renal α_2-adrenoceptor stimulation as the primary source of clonidine diuresis in the rat. Parenteral dosis of 10-100 µg/kg clonidine results in a pronounced water diuresis with hyposmotic urine and minor increase in electrolyte excretion. Both ADH infusion and indomethacin pretreatment inhibit the diuretic effect of clonidine. In heterozygot Brattleboro rats clonidine induces a diuresis similar to its action in Wistar-rats. In homozygot male Brattleboro rats lacking ADH clonidine showed no diuretic effect. Clonidine-type diuresis was associated with moxonidine, guanfacine, tiamenidine, oxymetazoline, tramazoline and xylometazoline - all compounds of known α_2-agonist effect. The α_1-agonists phenylephrine and methoxamine were ineffective. Yohimbine and rauwolscine significantly reduced clonidine diuresis, whereas corynanthene and prazosin were ineffective. Pretreatment with 6-OH-Dopamine resulted in a slight increase in clonidine-diuresis. A hypothesis is being formulated to the effect that clonidine and related drugs cause diuresis through a mechanism involving intra-renal postjunctional α_2-adrenoceptors, PGE-synthesis and ADH inhibition.

Dept. of Pharmacology BDF, Unnastraße 48 2000 Hamburg 20

77

COMPARATIVE INVESTIGATIONS TO DEMONSTRATE DIURETIC EFFECTS IN MICE AND RATS USING THE NEWLY DEVELOPED BLOTTING-PAPER METHOD

O. Rohte and S. Magda

The diuretics Furosemide, Bumetanide, Clopamid, Polythiazide, Trichlormethiazide, Spironolactone, Acetazolamide, Etacrynic acid and Theophyllin have been given to mice (NMRI) and rats (Sprague-Dawley) placed in Macrolon cages the bottom of which was covered with blotting-paper. The paper was renewed after 1, 3 and 5 hours. The urine spots were made visible by using UV light (360 nm). The size of the spots was determined by cutting out and weighing them. The Na^+ and K^+ content was determined by elucidation in Tri-buffer. The results with the blotting-paper method were compared with those obtained with the classical method with water-loaded rats using metabolism cages. With the blotting-paper method it is possible to demonstrate the diuretic effects of all the compounds screened in both species. The mean of all the experiments indicates a higher sensibility of mice to diuretic effects. When comparing rats and mice in the blotting-paper method it is obvious that there is a smaller dependency of the curve on time in rats than in mice. A comparison of the rat paper and the rat metabolism cage method shows that both yield comparable results. In mice the paper method yields generally higher values compared to the metabolism cage method in rats. Using controls of the day resulted in a smaller variance than using overall controls. The possibility to use mice for screening diuretics offers new possibilities to screen this group of compounds in a hitherto neglected species.

Kali-Chemie AG, Pharmakologische Forschung, Postfach 220, D-3000 Hannover 1

78

STUDIES ON ANGIOTENSIN II "ESCAPE" IN CONSCIOUS DOGS

G. Kaczmarczyk, M.Marx, K. Lee, R. Mohnhaupt, B.Simgen and H.W. Reinhardt. Tech.Ass.: R. Jäckel, A. Schönenberg

I.v. Angiotensin II (A II) (4-5 ng·min^{-1}·kg^{-1}) (min^{-1}·kg^{-1}=:) decreases renal water and sodium excretion. This effect lasts less than one day (COWLEY et al.1981), when a certain amount of sodium and water has been retained. - An attempt was made to demonstrate an A II "escape" during extreme acute volume expansion (0.9% NaCl) (protocol 1) and by administration of higher dosages of A II without extreme volume expansion (protocol 2; control = C)
33 expts. have been performed in 5 female beagle dogs; sodium intake 4.5 mmol·kg^{-1}·d^{-1};standardized maintenance.
Protocol 1: In extremely volume-expanded dogs (UNaV̇>100 µmol·:, FENa%>16%, left atrial pressure > 18 cm H_2O) i.v. A II (4 ng·: over 120 min) still decreases sodium and water excretion by 40-50%.

Protocol:2	C	AII ng·:			
		1	4	20	200
HR b·min^{-1}	97±3	110±3	111±3	94±2	115±5
MABP mmHg	107±3	106±4	107±1	141±3§	172±4§
V µl·:	161±26	77±4	48±8§	68±13	88±16
UNaV̇ µmol·:	23±2	18±1	10±1§	13±2	19±3
UKV̇ µmol·:	2.3±0.3	1.8±0.3	1.4±0.2	1.5±0.2	2.1±0.2
GFR ml·:	5.5±0.4	5.5±0.2	3.8±0.1§	4.4±0.2	4.0±0.2
FENa%	2.9±0.2	2.1±0.1§	1.7±0.1§	2.1±0.2	3.1±0.4

§=p<0.025; x̄±SEM

The antidiuretic and antinatriuretic effect of A II is clear present at 4 ng·:. It decreases with increasing doses of A II, resulting in MABP increases to 141 and 172 mmHg, resp.
Therefore it is concluded, that, within a physiological range, A II has antidiuretic and antinatriuretic properties even during extreme acute extracellular expansion.

Arbeitsgruppe Experimentelle Anästhesie, Klinikum Charlottenburg, FU Berlin, Spandauer Damm 130, 1000 Berlin 19

79

INFLUENCE OF ANGIOTENSIN ON THE CHARACTERISTICS OF POSTJUNCTIONAL α-ADRENOCEPTORS IN ISOLATED BLOOD VESSELS OF THE RABBIT

I. Lues and H.J. Schümann

Mainly due to in vivo results, postjunctional vascular α-adrenoceptors are devided into the subtypes α_1- and α_2 which are suggested to be different also with respect to their anatomical localization. Thus, the α_2-receptor is discussed to be the target for blood-borne catecholamines. Working on the pithed rat the pressor response due to α_2-receptor stimulation was shown to be under the hormonal influence of angiotensin (De Jonge et al. Eur J Pharm 74: 385,1981). As observations from in vitro studies on this topic are lacking we investigated the influence of angiotensin on the characteristics of the postjunctional α-receptors of the isolated saphenous vein and aorta of the rabbit. In the saphenous vein where phenylephrine (α_1) and B-HT 920 (α_2) (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo[4,5-d]-azepine) both are potent agonists only the contractile response to B-HT 920 is markedly potentiated in the presence of 0.1 nmol/l angiotensin. Furthermore, the characteristics of the α-receptors as revealed by B-HT 920 are changed: only in the presence of angiotensin these receptors express the characteristics of the α_2-subtype (pA_2: prazosin 6.85±0.05; rauwolscine 8.46±0.07). In the aorta due to angiotensin B-HT 920 changed from being an antagonist (pA_2: 4.8±0.04) to a partial agonist (pD_2: 4.97±0.05; ISA 0.5 comp. to PE). In conclusion: while in the presence of angiotensin, B-HT 920 stimulates α_2-receptors in the saphenous vein, it stimulated α_1-receptors in the aorta.

Institute of Pharmacology, University of Essen Hufelandstr. 55, 4300 Essen, Fed.Rep.Germany

80

PREJUNCTIONAL α_2-ADRENOCEPTOR MEDIATED FEEDBACK IN THE HEART OF YOUNG AND ADULT SHR AND WKY.

A. de Jonge

In order to obtain more insight into the physiological role of cardiac prejunctional α_2-adrenoceptors, we studied the effect of B-HT 920, 2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo[4,5-d]azepine (0.1 mg/kg), and rauwolscine (1 mg/kg) on the increase in cardiac frequency to electrical stimulation (C7-T1, 2 ms, 50 V, 25 s, 0.1-10 Hz) of the cardiac sympathetic efferents in pithed young (7 weeks) and adult (20 weeks) spontaneously hypertensive rats (SHR) and progenitor Wistar Kyoto normotensive rats (WKY). In both SHR and WKY, B-HT 920 inhibited the increase in heart rate, which was significant ($P < 0.05$) up to 1 Hz of the stimulation frequency in young rats and up to 2 Hz in the adult animals. In young WKY, rauwolscine did not potentiate the cardiac response to electrical stimulation. However, in adult WKY a significant facilitation of the cardiac response to electrical stimulation by rauwolscine was observed from 0.2-10 Hz. In SHR, rauwolscine significantly potentiated the cardiac response to electrical stimulation from 0.2-10 Hz in young SHR and from 0.1-10 Hz in adult SHR. In both young and adult WKY, cocaine (10 mg/kg) produced an approximately 10-fold increase in cardiac response to i.v. noradrenaline. In young and adult SHR, cocaine produced an approximately 100-fold sensitization of the cardiac response to noradrenaline. The results indicate that the effectiveness of both endogenous and exogenous prejunctional α_2-adrenoceptor mediated feedback in the heart of SHR and WKY increases with age. In SHR, the endogenous feedback is much more pronounced. Paradoxically the neuronal recapture of noradrenaline in the heart of SHR is more effective as well.

Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands

81

INVOLVEMENT OF PROSTAGLANDINS IN THE IMPAIRED POSTJUNCTIONAL α_2-PRESSOR RESPONSE AFTER CHRONIC CAPTOPRIL ADMINISTRATION IN THE PITHED RAT.

J.T.A. Knape

The possible role of circulating Angiotensin II (AII) and activation of prostaglandin synthesis in the process of vasoconstriction mediated by stimulation of α_1- and α_2-adrenoceptors was studied in the pithed normotensive rat. As shown before, acutely administered captopril (5 mg/kg, i.v.) attenuates the vasoconstriction elicited by stimulation of postjunctional α_2-adrenoceptors with B-HT 920, 2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo (4,5-d)azepine, but not that of α_1-adrenoceptors by cirazoline. Restoration of the initial diastolic blood pressure, depressed by captopril administration, to the level of the untreated pithed rat by AII or vasopressin infusion, can abolish this impaired response. Inhibition of prostaglandin synthesis with indomethacin (25 mg/kg orally) does not influence this interaction. After chronic oral administration of captopril 100 mg/kg daily, the pressor response induced upon stimulation of α_2-adrenoceptors with B-HT 920 is further suppressed, whereas the effect of stimulation of α_1-adrenoceptors was not influenced. Inhibition of prostaglandin synthesis after chronic captopril or the restoration of the initial blood pressure by AII or vasopressin infusion alone resulted in a partial recovery of the suppressed dose hypertensive response curve of B-HT 920. Combined inhibition of prostaglandin synthesis and restoration of the depressed initial blood pressure, however, led to a complete restoration of the suppressed postjunctional α_2-pressor response. These findings suggest that the acute effects of captopril on the postjunctional α_2-pressor response in the pithed rat can be explained by vasodilatation, related to the inhibition of the plasma renin-angiotensin system, whereas in the situation of chronic converting enzyme inhibition by captopril activation of prostaglandins plays an important role as well.

Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, The Netherlands.

82

MEMBRANE SODIUM TRANSPORT IN ERYTHROCYTES OF DAHL HYPERTENSIVE RATS

A. Knorr, M. De Mendonca*, S. Kazda

Na^+pump activity, Na^+K^+cotransport, passive permeability, and cellular Na^+content were measured in erythrocytes of salt resistant (DR) and salt sensitive Dahl rats (DS), as well as Sprague-Dawley rats (SD) fed either a low Na^+ diet (0.4%) or a high Na^+diet (8%).
After the low Na^+diet, Na^+pump activity was significantly ($P < 0.005$) lower in DS (1.2±0.2 mmol/l cells x h; n=10) than in DR (2.2±0.2; n=10) and SD (2.9±0.2; n=14). After excess Na^+, the pump was almost completely inhibited in DS. In SD it was slightly reduced (2.1±0.3; n=7, $P < 0.025$), whereas it was unchanged in DR (2.0±0.3; n=9).

Na^+K^+cotransport was increased in SD under high Na^+diet, but not in DS.

These results suggest a) Na^+K^+cotransport does not function as a regulatory mechanism in Na^+-loaded DS, since it is not increased under this condition, b) Na^+pump inhibition can increase cellular Na^+, c) Na^+pump inhibition is involved in the development of Dahl rat hypertension.

BAYER AG, Inst. of Pharmacology, D-5600 Wuppertal 1, FRG.
*INSERM U7, Hôpital Necker, 75015 Paris, France

83

ELEMENT COMPOSITION OF RENAL CORTICAL CELLS IN TWO ANIMAL MODELS OF PRIMARY ARTERIAL HYPERTENSION*

F.Beck, G.Bianchi°, A.Dörge, R.Rick, M.Schramm, K.Thurau

Among the various animal models of primary hypertension, the Milan hypertensive rat (MHS) is characterized by the fact that transplantation of its kidney into normotensive controls induces hypertension in the latter. To investigate whether this causative role of the kidney is associated with an altered electrolyte composition of its tubular cells, electron microprobe analysis was carried out on proximal and distal tubular cells of MHS using energy-dispersive X-ray analysis. For comparison, cellular analysis was performed in spontaneously hypertensive rats of the stroke prone strain (SHRSP), in which hypertension appears not to be renally induced.
In SHRSP (mean systolic BP 211 mmHg cf. 160 mmHg in their appropriate normotensive controls) mean Na, Cl and K concentrations in proximal tubular cells were (± SEM) 23 ± 1, 22 ± 0.4 and 135 ± 1 mmole/kg ww, respectively, whereas in normotensive controls the concentrations were 18 ± 1, 22 ± 1 and 132 ± 2 mmole/kg ww, respectively. A similar alteration in cellular Na concentration was observed in the distal tubule. In MHS, systolic BP was 11 mmHg higher than in their respective normotensive controls. In contrast to the SHRSP, Na concentration in proximal tubular cells was 4 mmole/kg ww lower in the hypertensive animals than in the normotensive controls, cellular Cl and K concentrations being unchanged.
These data show, that the MHS, in which the hypertension is transplantable with the kidney, exhibits a diminished Na concentration in proximal tubular cells. In contrast, in that model, in which hypertension is of non-renal origin proximal tubular intracellular Na concentration is elevated. These observations indicate that specific changes in proximal tubular transport characteristics occur in MHS.
*Supported by the Deutsche Forschungsgemeinschaft
Physiologisches Institut der Universität München
Pettenkoferstr. 12, D-8000 München 2
°Istituto di Clinica Medica I, Università di Milano, Italy

84

ROLE OF PERIPHERAL CATECHOLAMINES IN RATS WITH CHRONIC SINO-AORTIC DENERVATION.

P. Dominiak, F. Kees and H. Grobecker

The participation of central noradrenergic and adrenergic neurones of elevated blood pressure in rats after sino-aortic denervation (SAD) has been shown (Chalmers et al., Circ. Res. 45, 519, 1979). Alexander et al. (Life Sci. 18, 655, 1976) reported an increased activity of plasma dopamine-beta-hydroxylase (DBH) in rats several weeks after SAD. Since plasma-DBH seems to be a poor biochemical parameter of rapid changes in activity of peripheral sympathetic nerves, we have measured plasma catecholamine concentrations radioenzymatically, tissue catecholamines of heart and adrenal medulla with HPLC and ELCD and determined blood pressure (BP), heart rate (HR) and increase in intraventricular pressure (dp/dt) in rats with chronic SAD (Krieger, E.M., Circ. Res. 15, 511, 1964).
Five weeks after SAD plasma noradrenaline concentrations (PNA) and plasma adrenaline concentrations (PA) reached significantly higher levels in SAD-rats (PNA : 357 pg/ml, PA : 278 pg/ml) when compared with sham operated rats (C) (PNA : 192 pg/ml, PA : 107 pg/ml). Noradrenaline content (NA) in the heart was significantly lower (796 ng/g w.w.) and adrenaline content (A) in the adrenal medulla was significantly higher (893 µg/g w.w.) when compared to C (heart-NA : 944 ng/g w.w., adrenal medulla-A : 676 µg/g w.w.). Plasma dopamine concentrations and dopamine content in tissue did not change. BP and dp/dt of SAD rats were raised significantly to 129/110 mmHg and 10474 mmHg/sec versus C (109/85 mmHg, 6910 mmHg/sec), whereas HR remained unchanged. A significant positive relationship was found between PNA and dp/dt and an inverse correlation between A in adrenal medulla and PA of SAD-rats. It is concluded that SAD produces a significant chronic hypertension possibly supported by enhanced release of NA from sympathetic nerves and A from adrenal medulla via stimulation of cardiac-B-receptors and possibly via stimulation of α-receptors of peripheral vessels.
Lehrstuhl für Pharmakologie, Universität Regensburg, Universitätsstraße 31, D-8400 Regensburg.

85

IN VIVO RELEASE OF ENDOGENOUS CATECHOLAMINES, HISTAMINE AND GABA IN THE HYPOTHALAMUS OF WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS

A. Yamatodani, L. Tuomisto, H. Dietl, U. Waldmann and A. Philippu

The release of endogenous dopamine (DA), noradrenaline (NA), adrenaline (A), histamine (HA) and GABA was studied in the posterior hypothalamic nucleus of conscious, freely moving Wistar Kyoto (WKy) rats and spontaneously hypertensive (SH) rats by using a push-pull cannula superfusion technique.

In SH rats the rates of resting release of DA and HA were higher than in WKy rats, while the rates of release of NA and A in SH rats were lower than those in WKy rats. No significant differences were found in the rates of resting release of GABA between SH and WKy rats.

In WKy rats superfusion of the posterior hypothalamic nucleus with KCl-rich CSF (60 mmol/l) significantly enhanced the rates of release of NA and A while those of GABA showed a tendency to be increased. In SH rats superfusion with KCl-rich CSF enhanced the rates of release of DA, NA, A and GABA. The KCl-induced release of neurotransmitters did not differ between SH and WKy rats. Superfusion with KCl-rich CSF did not affect the release of HA either in WKy or in SH rats. The results indicate the functional significance of the transmitters DA, NA, A and HA in the hypothalamic regulation of the arterial blood pressure.

Institut für Pharmakologie und Toxikologie der Universität Würzburg, Versbacher Straße 9, D-8700 Würzburg, FRG.

86

REGULATION OF ADENYLATE CYCLASE (AC) BY CA^{++}, CALMODULIN, AND GTP IN LOCUS COERULEUS (LC) AND OTHER SPECIFIC BRAIN AREAS OF SPONTANEOUSLY HYPERTENSIVE RATS (SH-R)

G. Schmid, A. Heidland (Med. Klinik) and A. Biber, K. Hempel.

Recently we showed that cAMP concentration is increased in some specific brain areas of SH-R in comparison with normotensive controls. Since these findings must be caused by an altered cAMP metabolism, we have studied the regulation of cAMP formation in specific brain areas of SH-R. Areas, removed by the punch technique (Palkovits), were LC (brain area with highest norepinephrine content and significant cAMP increase in SH-R), cingulate cortex (CC), hippocampus (H), and central grey (CG). AC activity was determined as function of Ca^{++} concentration (pCa 4-9) under the following conditions: 1. basal AC activity 2. Stimulation by calmodulin and/or GMP-PNP 3. Inhibition by Fluphenazin.

In all brain structures tested, Ca^{++} increased AC activity to about 400 % of basal. Half-maximal AC activity was found at pCa 7.6. Ca^{++} activated AC was stimulated 1.2-1.5 fold by the addition of calmodulin. At high concentration Ca^{++} strongly inhibited AC. Fluphenazin suppressed the Ca^{++} dependent stimulation of AC at low Ca^{++} (pCa 8-9). Calmodulin hardly increased the activity of GMP-PNP stimulated AC.

In SH-R, basal and calmodulin stimulated AC activity in CC, H and CG was the same as in controls, whereas the activity of GMP-PNP stimulated enzyme was significantly higher especially in H. In contrast, in LC basal and calmodulin stimulated AC activity was significantly higher in SH-R than in controls.

These results argue in favour of an enhanced sensitivity of the AC-system in hypertension.

Medizinische Klinik und Institut für Medizinische Strahlenkunde der Universität, Versbacher Str. 5, D-8700 Würzburg

87

24-HOUR BLOOD PRESSURE RECORDING IN AORTIC COARCTATION HYPERTENSIVE RATS

B. Garthoff

To evaluate the effect of antihypertensive drugs, the 24-hour profile is essential. Recently, we described a new system for the continuous direct recording of blood pressure and heart rate in the conscious rat (J.Pharmacol. Meth. 5, 275-278, 1981). We have improved upon this method by developing a new swivel connector, which is necessary to prevent twisting and obstruction of the pressure recording line as a result of the rotating of the rat.

The swivel connector, attached to the middle of an ordinary wire cage lid, consists of two steel parts which are connected by an adjustable rod. The lower part, connected to a steel wire bearing the catheter, rotates by means of a miniaturized ball-bearing. To avoid leakage of the saline filled pressure recording line, a plastic O-ring is fitted to a Teflon membrane in the upper steel part. The PE 50-catheters implanted into the aorta of aortic coarctation hypertensive rats are kept patent by infusing small amounts of saline every 12 minutes. System synchronizing and data output are done by means of a minicomputer system DAS 10/4 with floppy disc.

The method proved to be useful in performing 24-hour blood pressure profiles for calcium antagonists, converting enzyme inhibitors and vasodilators after oral or intraarterial administration.

Institut für Pharmakologie, BAYER AG, Postfach 101709, D 5600 Wuppertal 1, FRG

88

CHARACTERISATION OF VASODILATORS BY COMPARISON OF THEIR EFFECTS ON BLOOD PRESSURE AND COUNTER-REGULATION IN CONSCIOUS DOGS.

S. Bacher

Seven vasodilators dihydralazine (DHZ), propyldazine (PDZ), urapidil (U), molsidomine (M), isosorbide dinitrate (ISDN), isosorbide-5-mononitrate, isosorbide-2-mononitrate were tested in this study in order to evaluate their relative hypotensive potency. The effects of single oral doses on systolic and diastolic blood pressure were registered continuously over a period of 4 hours. Simultaneous measurements of heart rate (HR) and plasma renin activity (PRA) were performed to quantify reflex stimulation of the sympathetic nervous system and the renin-angiotensin system (RAS). The experiments were performed on 6 mongrel dogs, trained to submit to puncture of the femoral artery and to stand quietly in a special frame.
All compounds caused dose dependent hypotension, but differences were observed between the reduction in systolic and in diastolic blood pressure. While DHZ, PDZ (0.29, 1.42, 7.1 mg/kg) and U (0.4, 2.0, 10.0 mg/kg) lower both systolic and diastolic pressure, M (0.4, 2.0 mg/kg) and the nitrates (2.0, 10.0 mg/kg), known to act via venous pooling, decrease predominantly the systolic blood pressure. The not uniform reaction of HR and PRA permit further differentiation: DHZ and PDZ evoke a marked counterregulatory reaction, which is not seen with U. The importance of the RAS for development of tolerance to the hypotensive drug action is discussed.

Pharmakologisches Institut der Universität Wien, Währinger Straße 13 a, A-1090 W i e n

89

THE EFFECT OF SHORT-TERM ANTIHYPERTENSIVE THERAPY ON THE ENZYMURIA (THE OUTPUT OF gamma-GT and NAG) OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

Mályusz, M., Wrigge, P.

In male rats with experimental renal as well as with spontaneuous hypertension especially the early phase of the disease was followed by an enhanced urinary output of the enzymes gamma-glutamyl-transpeptidase (gamma-GT) and N-acetyl-beta-D-glucosaminidase (NAG). This was interpreted as hypertension-induced damage to the renal tissue.
In order to study the effect of antihypertensive therapy on the enzyme output, 17-32 weeks old SHR (mean RR=188 mmHg) were placed in metabolic cages and after a control period of 8 days treated for the same period with Piretanid (10 mg/kg x d), Propanolol (30 mg/kg x d) or Captopril (60 mg/kg x d) respectively. The substances were administered with the food. Urine flow, creatinine output (as reference for the GFR) as well as the excretion rate of gamma-GT and of NAG was checked daily.
The results are presented in the following table (x, S.D.)

	Control period		Piretanid		Propanolol	Captopril
RR mmHg	188	13	200	13	216* 13	152* 12
Urine flow ml/100 g x d	3.9	1.2	4.5	0.5	4.9* 1.3	15.7* 4.8
U x U creat. mg/100 g x d	4.0	0.3	4.1	0.1	4.3* 0.3	4.5* 0.2
gamma-GT output mU/100 g x d	999	248	1604*274		1370*165	1234* 217
NAG output mU/100 g x d	96	10	156 * 15		135 * 13	159 * 19

* means significant deviation from the control ($p < 0.05$).
All three substances tested elevated the output of gamma-GT and of NAG. The degree of elevation was independent of the urine flow. These results indicate that while it was possible to reduce the blood pressure of SHR by proper treatment (Captopril), the renal tissue did not respond favourably to the antihypertensive therapy applied.

Physiologisches Institut der Universität, Olshausenstr.40 D-2300 Kiel 1

90

EFFECTS OF INDOMETHACIN ON THE HYPOXIA OR COBALT INDUCED ERYTHROPOIETIN PRODUCTION

W. Jelkmann and J. Seidl

Recent findings indicate that prostaglandins (PG) mediate the process in the kidney which triggers the production of erythropoietin (Ep) following ischemic or hypoxic hypoxia (cf. J.Fisher, Nephron 25:53, 1980). To further test the dependence of Ep on PG production, we studied effects of the PG synthetase inhibitor drug, indomethacin, on the plasma Ep titer in rats in which Ep production was induced by different stimuli, namely by anemia, hypobaria exposure or the injection of cobalt. Plasma samples, which were assayed in the exhypoxic mouse assay for Ep, were obtained 8h after single injections of indomethacin (10 mg/kg i.p.). We found that indomethacin significantly lowered plasma Ep titers (.09± 0 versus .34±.10 IU Ep/ml in vehicle treated control rats, mean±SEM, n=4) in anemic rats (Hct.0.25), in which anemia was induced by previous injections of phenylhydrazine (30 mg/kg daily for 3d). Likewise, indomethacin reduced the plasma Ep titer (.82±.20 versus 1.54±.17 IU Ep/ml in controls) in rats exposed to hypoxic hypoxia (.42 atm. for 8h). However, indomethacin did not lower the Ep response (.27±.06 IU Ep/ml plasma) to cobalt (250 µmol/kg s.c.).
Since indomethacin lowered only the hypoxia-induced (anemia and hypobaria) Ep production, our findings are supportive of the concept that the renal PG system is involved in the mechanism by which tissue hypoxia leads to the elaboration of Ep.

Physiologisches Institut der Universität, D-8400 Regensburg

91

PROSTAGLANDIN E2 IS A SIGNAL MOLECULE FOR HYPOXIA INDUCED ERYTHROPOIETIN PRODUCTION IN RENAL MESANGIAL CELL CULTURES.

A. Kurtz and C. Bauer

Tissue hypoxia leads to an increased production of the hormone erythropoietin (Ep) in the kidney. Previously we have shown that renal mesangial cell cultures produce Ep and that the production of the hormone can be stimulated by lowering the O_2 concentration in the incubation atmosphere (Kurtz et al., FEBS lett.137:129;1981). This culture system can therefore be regarded as a model to study the hypoxia-induced Ep production on a cellular level.Because there is evidence from in vivo studies that prostaglandins(PGs) can elicit an Ep response (cf.Fisher,Nephron 25:53;1980) we investigated whether PGs are involved in the regulation of Ep synthesis in cultured mesangial cells. We found that addition of the cyclooxygenase inhibitor indomethacin (10^{-5}M) suppressed the rise in Ep production during hypoxia:

culture conditions	20%O_2	3%O_2	3%O_2+indo
Ep concentration in culture medium(mU/ml)	3.5±2.0	10.0±.6	4.2±.7

Stimulation of PG synthesis by arachidonic acid (10^{-5}M) enhanced Ep production at 20%O_2(530+200% of control). Addition of PGE 2 (10^{-6}M) also led to an increase in Ep production at 20%O_2(600±250% of control) whereas PGF 2α (10^{-6}M)lowered Ep production(30±20% of control).We infer from these results that PGE 2 is an important link in the sequence of events which leads to an enhanced Ep production under hypoxic conditions in cultures of renal mesangial cells.(Supported by the SFB 43 of the Deutsche Forschungsgemeinschaft).

Institut für Physiologie der Universität Regensburg, D-8400 Regensburg

92

CHLORIDE DISTRIBUTION AND INTRACELLULAR pH OF PRIMITIVE RED CELLS FROM CHICKEN BLOOD

R. Baumann and E.A. Haller

Very little is known about the ion transport properties of the first embryonic red cell population, the primitive red cells. Since these cells mature inside the circulation, they have retained the capacity for cell division, which property may not be compatible with a "passive" pH regulation as found in adult red cells. We have tried to assess the intracellular pH by measuring the chloride distribution and by direct pH measurements on red cell lysates prepared by freeze-thawing in liquid nitrogen. To eliminate errors in pH measurements resulting from CO_2 production aerobic metabolism was inhibited. To allow a theoretical calculation of the distribution ratio r for chloride, the buffer power and isoelectric points for embryonic hemoglobin were determined.
The results show that the distribution of chloride follows the Donnan equilibrium; r_{Cl} was 0.58 at pH 7.4 and 37° compared to a calculated value of 0.67. When extracellular chloride was substituted by the impermeant gluconate anion r_{Cl} changed in the manner expected for passive distribution of chloride.
While the pH_i calculated from r_{Cl} was 7.164 at pH_e 7.4, 37° direct pH measurements gave a value of 6.81. Likewise, r_{H^+} changed only from 0.26 (pH_e 7.4, 160 mmol Cl^-) to 0.6 (pH_e 7.4, 6 mmol Cl^-, 154 mmol gluconate), compared to an increase of r_{Cl} from 0.58 to 2.6 under the same conditions. These results indicate that the intracellular pH of primitive red cells is regulated by additional "active" processes aside from the Donnan effect. Preliminary measurements of the bicarbonate distribution support this concept.

Zentrum Physiologie, Medizinische Hochschule Hannover K.-Gustow-Str. 8, D-3000 Hannover 61.

93

STUDIES ON PLATELET BEHAVIOUR AND ARACHIDONIC ACID(AA) METABOLISM IN VITRO

H.B. Steinhauer, B. Günter and P. Schollmeyer

Platelet activation is followed by the release of prostaglandins(PGs) and thromboxane(TX)A2. As TXA2 is known to be the most potent inducer of platelet aggregation it is supposed that AA-induced platelet aggregation is mediated by TXA2. Recently it was shown that after selective inhibition of platelet TXA2 generation by imidazole or pyridine derivatives no correlation existed between TXA2 synthesis and platelet behaviour (Steinhauer et al., N.-S.Arch.Pharmacol.319:R31,1982). Further investigations were performed to elucidate AA-induced platelet aggregation.
Platelet rich plasma(PRP) of healthy volunteers was incubated with indomethacin, OKY-1581(Sodium-3-[4-(3-pyridylmethyl)phenyl]-2-methylacrylate), and/or phenidone. Platelet aggregation was induced by AA (1 µmol ml-1), PGE2 and TXB2 were determined by RIA, malondialdehyde(MDA) and MDA-reactive substance(MDA-RS) were measured according the method of Okuma et al.(J.Lab.Clin.Med.75:283,1975). OKY-1581 inhibited the AA-induced TXB2 generation up to 96%, the synthesis of MDA-RS up to 80% whereas the generation of PGE2 remained unchanged. In spite of the approximately complete inhibition of TXA2 synthesis, platelet behaviour was not influenced. Incubation of PRP with OKY-1581 and phenidone inhibited both, the generation of TXB2 and MDA-RS for more than 95%, simultaneously the AA-induced platelet aggregation was abolished. Corresponding results were obtained after incubation of PRP with indomethacin.
It is concluded that lipoxigenase products are involved in human platelet aggregation whereas TXA2 is not essential for AA-induced platelet aggregation.

Zentrum Innere Medizin der Univ. Freiburg, Abt.IV, Hugstetterstr. 55, D-7800 Freiburg i. Br.

94

CARBONIC ANHYDRASE (CA) ACTIVITY IN HUMAN PLATELETS

W. Siffert

Activity and kinetic parameters of carbonic anhydrase in human platelets were investigated.Platelet rich plasma (PRP) was obtained from buffy coat preparations by centrifugation at 150xg for 10 min at room temperature. Platelets were separated from PRP by centrifugation at 3000xg for 15 min and washed 3 times with a 10-fold vol. of 0.15 M NaCl. Platelets were lysed by the addition of a 10-fold vol. of distilled water and subsequent freezing in liquid nitrogen. Membranes were removed after thawing by centrifugation at 30.000xg for 30 min. After appropriate dilution in 0.015 M imidazole, 0.15 M NaCl, pH 7.3, the CA activity in the supernatants was determined by following the kinetics of CO_2-hydration in a pH-stopped-flow apparatus. CA activities were measured at CO_2 concentrations ranging from 1 to 5 mM. Plotting the data according to Lineweaver-Burk allowed us to estimate the Michaelis constant for CO_2, K_M, to be 1.4±0.2 mM. V_{max} was determined from this plot to be 0.22 mole s^{-1} per liter of pelleted platelets. From this parameters the intracellular CA activity in human platelets is estimated to be about 2400 (T=25°C, pH 7.3,$[CO_2]$=1.2mM). This means, that inside the platelet CO_2 hydration is accelerated by a factor of 2400. This activity appears to be negligibly affected by other blood cells. Microscopic investigation of several stained smears of pelleted platelets did not reveal leucocytes bud did show solitary erythrocytes. Quantitation of the contamination with erythrocytes by measuring hemoglobin concentrations indicated that less than 3% of the observed CA activities is due to residual red cells.

Institut für Physiologie, Universitätsklinikum Essen, Hufelandstrasse 55, D-4300 Essen 1, F.R.G.

95

HEMOGLOBIN-OXYGEN BINDING,CO_2 BUFFERING CHARACTERISTICS AND ELECTROLYTE CONCENTRATIONS OF ERYTHROCYTES WITH DIFFERENT AGE

W. Schmidt, D. Böning

In young erythrocytes the hemoglobin-oxygen affinity is lower than in old cells because of high$[DPG]$.This property and the steeper dissociation curve of the young erythrocytes improve the O_2-delivery to the tissue.

The Bohr coefficient for CO_2, calculated for plasma pH and erythrocyte pH, is clearly increased in old red cells compared to young cells. This may be explained by the high $[DPG]/[Hb]$ ratio. Although$[DPG]$ is high,$[H^+]$ of young cells is not increased compared to old red blood cells. Furthermore $[K^+]$ in old cells is decreased. Young and old erythrocytes show no differences in buffering characteristics against CO_2. Middle aged cells, however, react to acidification with smaller pH-changes than old and young cells.

Abt. Sport- und Arbeitsphysiologie
Zentrum Physiologie, Medizinische Hochschule Hannover, Konstanty-Gutschow-Str. 8,
D-3000 Hannover

96

INTRA-/EXTRACELLULAR ACID-BASE STATUS FOLLOWING NH_4CL APPLICATION

K.F. Rothe

Though buffers are widely used in daily practice, there is little information about their effect on intracellular pH (pHi). In this study the effects of NH_4Cl on intra- and extracellular acid-base equilibrium were examined. Intact Sprague-Dawley rats were infused with 3 mmol/Kg BW NH_4Cl. Arterial plasma pH and P_{CO_2} were measured at intervals for six hours. In addition the "mean whole body pHi", an overall estimate of the in vivo determinded plasma pH was calculated from the distribution of ^{14}C labelled DMO (5,5-dimethyl-2,4-oxazolidinedione). For evaluation of buffering, extra- and intracellular bicarbonate were calculated from the Henderson-Hasselbalch equation. Though a decrease of pHe by 0.12 pH units was found just after infusion of NH_4Cl, pHi increased over the six hours of investigation by 0.08 pH units. Extracellular bicarbonate was reduced but there was little change in the intracellular bicarbonate concentration. For the treatment of alkalosis therapeutic agents are given to reduce intracellular pH and intracellular bicarbonate concentration. However it was found in this study that NH_4Cl has no effect on intracellular bicarbonate concentration. In addition, infusion of NH_4Cl causes a tremendous increase of intracellular pH which cannot be detected by blood-gas measurements. The intracellular pH increase that we have demonstrated may have adverse consequences for patients and raises objections to the use of NH_4Cl in the treatment of metabolic alkalosis.

Department of Anaesthesiology, University of Tübingen, D 7400 Tübingen, Calwerstr. 7

97

The effect of anaesthesia and analgesia on blood gases,acid-base balance and haematological parameters in the rat.

J.Komarek,W.Gfeller,T.Gersl

It was observed recently(Brun-Pascaud et al., Resp.Physiol.,48:45,1982)that the blood gases and pH in rats anaesthetized with barbiturates were significantly different from those of unanaesthetized rats.In the present experiment,we compared the effects of ether,pentobarbitone sodium and fentanyl on blood gases,acid base balance and haematological parameters using adult rats of both sexes.The blood samples were taken by postorbital puncture,and the blood gases and acid-base status were determined with a Blood Gas Analyzer(Corning 168,USA);the haematological measurements were made in the Coulter S-Plus (USA).The results indicate that the use of ether and fentanyl had very little effect on blood gases and acid-base balance;however,the induction of pentobarbitone anaesthesia was followed by a significant increase in PCO_2 and TCO_2,while the pH values decreased.Furthermore,the data obtained here illustrate that the administration of all three drugs produced a decrease in RBC,PCV and Hb,pentobarbitone causing the most pronounced changes.Repeated blood sampling in conscious,manually-restrained rats had no significant effect(expect for the increase in PO_2)on the blood gases,acid-base balance,or the haematological parameters measured.

CIBA - GEIGY Limited,Basle,Switzerland.

98

INEFFICIENCY OF METHYLENE BLUE AS AN ANTIDOTE AGAINST CHLORATE POISONING

Ch. Steffen and Elke Singelmann

Methaemoglobinaemia is a prominent feature of chlorate poisoning. Treatment with methylene blue and ascorbic acid, however, fails to relieve this condition or to prevent haemolysis and renal failure (Steffen and Seitz, Arch. Toxicol. 48, 281-288, 1981).

Incubation of human erythrocytes with nitrite (10 mM) is followed by rapid, with chlorate (30 mM) by delayed methaemoglobin formation and glutathione oxidation. In contrast to the control experiment with nitrite, methylene blue is inefficient when methaemoglobin concentrations exceed appr. 50 %. Prolonged incubation with chlorate is followed by a decrease in potassium and an increase in sodium concentrations, and the erythrocytes become resistant to hypotonic haemolysis. Filtration time through polycarbonate membranes with 5 µm pore diameter as measured by the method of Reid et al. (J. Clin. Path. 29, 855-858, 1976) increases after a lag period of appr. 2 hr from 2.5 ± 0.26 sec to 100 sec suggesting a possible increase of erythrocyte rigidity. SDS-polyacrylamide electrophoresis reveals an increase of high molecular weight proteins, indicating membrane protein cross-linking. These membrane alterations explain several clinical aspects of chlorate poisoning that were hitherto poorly understood.

Pharmakologisches Institut der Philipps-Universität, Lahnberge, D 3550 Marburg

99

STUDIES ON PROTEIN COMPOSITION OF CARDIAC SARCOPLASMIC RETICULUM MEMBRANES (SR)

Dagmar Hartweg and Hermann Bader

Up to now, there are only a few studies on the protein composition of cardiac SR. This work is an attempt to compare data of cardiac SR with those of skeletal muscle SR and to investigate a possible relationship between structure and function of protein components of cardiac SR.

SDS-PAGE of dog heart SR vesicle proteins yield a major protein band (M_r100,000), representing the Ca(2+)-transport ATPase, in addition to three other major proteins (M_r64,000, 51,000, 31,000). Solubilization of vesicles by use of deoxycholate (detergent/protein 0.4-0.6, w/w) and high ionic strength (500-1000 mM K^+) selectively extracted these four protein components. Proteins (M_r100,000, 64,000, 51,000) precipitate with 4 mM Ca(2+),indicating probably their Ca(2+) affinity and function in Ca(2+) binding. Selective release of only the proteins with M_r64,000 and 51,000 can be achieved by treatment with EDTA at pH 8.6, demonstrating their attachment to the membrane is dependent on divalent metal ions. Several chemical and physical characteristics of these two proteins showed striking similarities to calsequestrin (M_r63,000) and the high affinity Ca(2+) binding protein (M_r55,000) of skeletal muscle SR.

The presence of calmodulin (CaM, M_r16,700) has been demonstrated in the cardiac SR vesicle preparation by boiling the SR membranes and quantifying the amount of CaM by a biological assay (phosphodiesterase). From the experiments a molar ratio of ATPase/CaM of 250:1 and 500:1 could be estimated for SR preparations obtained in the presence of Ca(2+) or EGTA, respectively. This indicates that the CaM content of the SR preparation is probably a contamination and that direct regulation of the ATPase by CaM might not occur.

Abt. Pharmakologie & Toxikologie der Universität Ulm, Oberer Eselsberg, D-7900 ULM, GFR.

100

CARDIAC EFFECTS OF VOLTAGE SENSITIVE DYES FOR PHOTOMETRIC MEASUREMENTS OF CARDIAC ACTION POTENTIALS

H. Windisch, W. Müller

A great number of voltage-sensitive fluorescent dyes are currently used to measure transmembrane action potentials in various tissues. The use of this photometric approach in cardiac muscles requires: i) A minimum of undesired side effects on excitation or contraction. ii) A good signal-to-noise ratio of the fluorescent dye. iii) Suppression of the distorsion of the optical signal due to the mechanical activity. We have performed electrophysiological and optical measurements with the well known dye Merocyanine 540 (M540) and the compound WW781*. Both dyes show potential dependent fluorescence. Using M540 with the guinea pig papillary muscle in concentrations of 10 to 30 µM we observed strong inotropic effects (500-2000%). In the concentration range of 30 µM the plateau phase of the action potential was markedly distorted and some types of afterpotentials were detected. When a fresh preparation was stained in 20 µM M540 for about 10 minutes (a usual way to prepare a muscle for optical detection) subsequent electrophysiological measurements in normal tyrode solution also showed positive inotropic effects and action potentials of slightly reduced duration. These rather unexpected effects which could not be found in the literature persisted even after long (1 hour) washout. Measurements on guinea pig atrial sheets after 10 minutes of staining in 0.5-1 mg/ml WW781* showed only little if any effects on the action potential shape. Optical detection of atrial actionpotentials with the prefered WW781* were carried out in normal tyrode solution. In some experiments the motion induced distorsion of the fluorescent light could be suppressed by using the backscattered light as a reference.

* pentamethin oxonol with {1,3 dibutyl barbituric acid (5)} and {1,(p-sulfophenyl)3-methyl, 5-pyrazolone (4)}

Institut für Medizinische Physik und Biophysik der Universität Graz, Harrachgasse 21, A-8010 Graz

101

EFFECTS OF INCREASED POTASSIUM CONCENTRATIONS IN CARDIOPLEGIC SOLUTIONS ON LEFT VENTRICULAR ULTRASTRUCTURE+)

Ph.A. Schnabel, M.M. Gebhard, D.H. Hunneman, Th. Pomykaj, J. Richter[1], B. Spieckermann[1], Ch. Schweickhardt, H.J. Bretschneider

Membrane depolarisation by increase of extracellular K^+-concentration is a commonly used principle in cardioplegic arrest. We compared the effects of clinically applied methods for myocardial protection on left ventricular ultrastructure. Canine hearts were perfused with cardioplegic solutions according to KIRKLIN (group I: 30 mM K^+), ST. THOMAS' hospital (group 2: 16 mM K^+) and BRETSCHNEIDER (HTK solution, group 3: 11 mM K^+). In group 4 we applied an increased concentration of 30 mM K^+ in HTK solution. The experimental procedure was described earlier (Pflügers Arch. ges. Physiol. 391, R 12, 1981 and 394, R 14, R 15, 1982).
Defined alterations especially of mitochondria and nuclei developed during ischemia. Compared to unprotected hearts at the same temperature, these alterations were postponed by a factor of about 3 in group 1 and 2 and by a factor of about 6 in group 3. Distinct inhomogeneities of the alterations were detected in group 1. Capillary cell swelling during ischemia and after resuscitation was slight in group 3, moderate in group 2, and severe in group 1. Alterations of capillary endothelial fine structure such as blebs, changes in mitochondria and other organelles were postponed in relation to alterations in myocardial cells only in group 2 and 3. The ultrastructural changes observed in group 4 were similar to those of group 1.
Thus, higher K^+-concentrations besides interactions with other ions and components of the different cardioplegic solutions may contribute to the accelerated occurence of morphological alterations. Correlations with the biochemical results (adenine nucleotides, lactate, FFA) are discussed.

Zentrum Physiologie und Pathophysiologie, Univ. Göttingen, Humboldtallee 7, D-3400 Göttingen
[1]Zentrum Anatomie, Univ. Göttingen
+)SFB 89

102

INFLUENCE OF TEMPERATURE ON MYOCARDIAL IMPEDANCE DURING CARDIOPLEGIC CARDIAC ARREST+)

L.J. Ulbricht, E. Gersing, C.J. Preusse

During ischemia in cardioplegically arrested hearts changes of electrical impedance of the myocardium could be observed at various frequencies. These time-dependant - and as shown - also temperature-dependant changes of the real part (Re), the imaginary part (Im) and thus the phase angle of the impedance were investigated using a frequency-range from 10 Hz to 100 kHz.
In the resulting impedance spectra changes of electrical impedance of the myocardium during global ischemia revealed a maximum at a frequency of 5 kHz. So the course of electrical impedance was observed at 5 kHz in 3 groups during ischemia.
All hearts of all groups were cardioplegically arrested using BRETSCHNEIDER's HTP or HTK solution. The hearts of group I were then incubated at a temperature of 35 °C, the ones of group II at 25 °C and those of group III at 15 °C. Parallel to the impedance measurements samples of myocardium were taken for analysis of ATP, CP and lactate.

In all groups the alterations of the real part, the imaginary part and the therefrom derived phase angle showed a typical course: In a first phase nearly no alterations of electrical impedance could be observed ("lower plateau phase"), then it increased rapidly until it reached a new level ("upper plateau phase"). The duration of the lower plateau phase varied with the incubation temperature: about 90 min at 35 °C, 180 min at 25 °C and 330 min at 15 °C. These times correlated well with the time of exhaustion of CP and the time, when a lactate accumulation of about 130 $\mu mol/g_{dw}$ was reached. On the other hand the correlations between impedance changes and decrease of the myocardial ATP-content were distinctly influenced by temperature.

Zentrum Physiologie und Pathophysiologie, Universität Göttingen, Humboldtallee 7, D-3400 Göttingen
+)Supported by the DFG, SFB 89 - Kardiologie Göttingen -

103

THE INFLUENCE OF THE ELECTROGENIC SODIUM PUMP ON THE DURATION OF THE CARDIAC ACTION POTENTIAL

J. Daut

Dihydro-ouabain (DHO) was found to be a useful tool for separating kinetically the inhibition of the electrogenic pump current and the resulting change in intracellular Na activity (a_{Na}) in guinea-pig ventricular muscle (J. Daut, J Molec cell Cardiol 14, 189, 1982). When the preparation was stimulated at a low rate (0.1-0.2 Hz) application of 4×10^{-5} M DHO produced a transient increase in action potential duration (APD). The lengthening of the action potential paralleled the inhibition of the pump current by DHO, the subsequent shortening coincided with the delayed rise in a_{Na}. During wash-out of the drug APD fell below its control value within 1 min, and then returned slowly to its control value. The shortening of the action potential paralleled the dissociation of DHO from the Na,K-pump, the subsequent lengthening coincided with the fall of a_{Na} towards its resting value. These results suggest that partial inhibition of the pump current may markedly prolong the cardiac action potential. The delayed fall of APD during application of DHO and the delayed recovery of APD after the wash-out may be explained by a stimulation of the turnover rate of the pump by the transient increase in a_{Na}. Since a_{Na} was found to rise not only after inhibition of the Na,K-pump but also after an increase in stimulation frequency (C.J. Cohen et al., Circ Res 50, 651, 1982) it is concluded that the electrogenic sodium pump may play an important role in the frequency-dependent regulation of APD in mammalian ventricular muscle.

Physiologisches Institut der Technischen Universität, D-8000 München 40. Supported by DFG

104

INWARD RECTIFYING Ba^{++}- SENSITIVE POTASSIUM CHANNELS IN GUINEA-PIG ATRIAL CARDIOBALLS.

M. Bechem, H.G. Glitsch and L. Pott

Myocytes from atria of adult guinea-pigs in cell culture develop to spherical cardioballs within 48 h after isolation and can be used during the following 6 days for single channel measurements using the patch clamp technique (Hamill et al., Pflügers Arch. 391, 85-100, 1981). In outside-out membrane patches from these cardioballs a K-conductance channel was analysed. At negative membrane potentials the I/V relation of this channel is linear with a slope of 16 pS at 2 mM K_o and 27 pS at 20 mM K_o (K_i=150mM). The corresponding reversal potentials are -95 mV and -50mV respectively. The mean open time is strongly voltage dependent ranging from 700 ms at -40 mV to 30 ms at +40 mV (K_o = 5 mM, T = 22°C). The probability of the open state (p_o) is about 0.9 at -40 mV and 0.1 at +40 mV. Taking into account both, the linear I/V relation and the voltage dependence of p_o a macroscopic I/V curve can be reconstructed, which shows inward going rectification.
In the presence of Ba-ions (0.2 mM) the opening events are interrupted by rapid closures, the single channel conductance being unaffected. This results in a linearization of the macroscopic I/V curve.

Institut für Zellphysiologie, Ruhr-Universität Bochum, Postfach 102148, 4630 Bochum, F.R.G.
Supported by Ministerium für Wissenschaft und Forschung, Nordrhein-Westfalen

105

DRUG-INDUCED I_{si} BLOCK: POTENTIATION BY COOLING AND FORMALDEHYDE

M.Kohlhardt

Taking the percent depression of $\dot{V}_{max}$ of I_{si}-mediated action potentials as an estimate for the strength of I_{si} block, the temperature-dependence and the modulation by the protein cross-linking formaldehyde of the verapamil action was studied in partially depolarized (E ~-50 mV) papillary muscles. Two types of block appeared, tonic and phasic inhibition. The installation of the former required higher drug concentrations ($>1x10^{-6}$ mol/l). In contrast to simple chemical reactions, the verapamil action exhibited a negative temperature coefficient. Cooling from 35°C to 30°C accentuated I_{si} inhibition and led to a decrease of apparent K_m's. K_m for tonic block declined from 1.7 to 1.2 x 10^{-6} mol/l, but the K_m for the phasic block reacted more strongly and fell from 8.8 to 4.4 x 10^{-7} mol/l (ISI 5s). The K_m change suggests an enhanced drug affinity of the subunit within the slow channel responsible for verapamil binding. A similar conformational rearrangement of the receptor could develop after chemical channel modification by formaldehyde. The I_{si} system became sensitized to bind verapamil preferentially under rested state conditions. This modification is unlikely related to delayed I_{si} inactivation as the shape of action potential normalized but block intensification persisted on formaldehyde washout. Nifedipine experiments excluded that this block modification is specifically related to verapamil. Again, 10 mmol/l formaldehyde potentiated drug action and decreased apparent K_m for nifedipine-induced I_{si} block. Obviously, slow channels possess drug receptor(s) of variable structural state.

Physiologisches Institute der Universität, Hermann-Herder-Str. 7, D-7800 Freiburg/Br.

106

DETERMINATION OF Ca UPTAKE RATE BY GUINEA PIG ATRIA AFTER RAISING THE EXTRACELLULAR Ca^{++} CONCENTRATIONS. H. Lüllmann

An increase of $[Ca^{++}]_o$ is accompanied by a gain of cellular Ca and an increase of contractile force of heart muscles. Small changes in tissue Ca can not be determined by spectrofluorometric methods properly enough to provide information on the initial transmembraneous Ca movements. To overcome the difficulties we used a radioactive procedure of keeping the specific ^{45}Ca activity constant while raising the chemical Ca concentration: isolated atria stimulated at 1 Hz were incubated for 2 h in Tyrode solution containing 0.45 or 0.9 mM Ca^{++} plus ^{45}Ca thus almost uniformly labelling tissue Ca. After this preincubation the $[Ca^{++}]_o$ was raised to 1.8 or 3.6 mM maintaining the specific radioactivity constant. Under this condition the increase of radioactivity in the tissue reflects net uptake of Ca but not Ca exchange. To determine the initial Ca uptake rate atria were removed in short intervals (minutes). After raising $[Ca^{++}]_o$ from 0.45 to 1.8 mM Ca^{++} the tissue Ca increased from 0.63 mM/kg w.w. to 1.23 mM/kg w.w. within 30 min. 50% of the uptake were already attained after 3-4 min. A similar time course of Ca uptake was obtained by elevating the $[Ca^{++}]_o$ from 0.9 to 3.6 mM. The adaption of contractile force to the increased $[Ca^{++}]_o$ proceeded with similar kinetics. - From the uptake rate within the first minutes the penetration of Ca through the plasmalemma per unit of time can be calculated, and amounts to about 0.45 µmoles/kg w.w. x 100 msec thus lying in the same order of magnitude as estimated for the Ca^{++} flux during the plateau phase of action potentials. The 4 fold increase of the $[Ca^{++}]_o$ and the initial uptake of Ca is not accompanied by significant changes of membrane or action potentials. It is suggested that the net gain of Ca under the given conditions proceeds without transmembraneous charge transfer, i.e. "non-electrogenic". Nifedipine, even at high concentrations depressing the contractile force by 90%, did not reduce this non-electrogenic Ca uptake.

Institut für Pharmakologie, Univ. Kiel, Hospitalstrasse 4-6, D-23 Kiel

107

OUABAIN INDUCED INHIBITION OF THE CARDIAC SARCOLEMMAL Ca-PUMP AND (Ca^{2+}, Mg^{2+}) TRANSPORT ATPase ACCOMPANIED BY A STIMULATION OF THE Mg^{2+}-ATPase ACTIVITY. J. Preuner

In sarcolemmal vesicles derived from cardiac ventricular muscle a highly active Ca-transport mechanism could be demonstrated (450 n mol Ca/mg x min at 35°C), which easily fulfilled the prerequisites to maintain Ca-homeostasis of the cardiac cell (Olbrich and Preuner, 1982). If the heart muscle was perfused with Tyrode solution containing 10^{-6}M ouabain the sarcolemmal Ca-pump was inhibited progressively during the time of perfusion (preformed effect). In guinea-pig ventricular muscle an inhibition of 72% was attained within 30 minutes. The Ca-transport was associated with an ATPase activity, -(Ca^{2+}, Mg^{2+})- ATPase - which was inhibited by ouabain to the same extent as the Ca-pump activity. The stoichiometry of the ouabain-inhibited fraction of the Ca-transport was found to be 1.1 ± 0.3 calcium ions transported per ATP hydrolyzed. The Ca^{2+}-ATPase activity could be separated into a Mg^{2+} dependent and a Mg^{2+} independent component. The latter component was not associated with Ca-transport and insensitive to ouabain. In the isolated heart both the (Na^+, K^+)-ATPase and the (Ca^{2+}, Mg^{2+})-ATPase were inhibited by ouabain. Nevertheless the ATP-consumption of the sarcolemma obtained from intoxicated heart muscle was increased up to 80% as compared to the control. This increase was due to a twofold stimulation of the basic Mg^{2+}-ATPase activity of the sarcolemma. It remains to be determined whether the Mg^{2+}-ATPase is at least some of the total (Mg^{2+}, Ca^{2+})-ATPase activity found in the sarcolemma. The specific effect of ouabain on the Mg^{2+} dependent sarcolemmal transport processes could be separated from inhibition of the sarcoplasmic reticulum Ca-pump, induced by hypoxia, ischemia or occuring during the Ca-paradoxon.

Abteilung Pharmakologie, Univ. Kiel, Hospitalstrasse 4-6, D-23 Kiel

108

Positive and negative inotropic actions of vanadate correlate to its action on $\dot{V}_{max}$ of the slow response

C. Hirth

The inotropic effects of vanadate are species- and tissue-dependent: vanadate increases contractile force in rat papillary muscle and left atria as well as in guinea-pig papillary muscle, but decreases contractile force in guinea-pig left atria. The action potential duration at 30% repolarisation (APD_{30}) is decreased in guinea-pig and rat atria but slightly increased in guinea-pig papillary muscle. In guinea-pig left atria the time course as well as the concentration dependence of the decrease in force parallels that of the shortening of the action potential (EC_{50} values for decrease in force and APD_{30} 5.3 and 4.7 µmol/l, respectively). In order to elucidate the species- and tissue-dependent differences with respect to the slow inward current we investigated the actions of vanadate on Ca^{2+}-dependent slow response of the K^+-depolarized muscle (resting potential approx. -40 mV after addition of 0.5 - 1.0 µmol/l Ba^{2+}): In guinea-pig papillary muscle vanadate (500 µmol/l) increases $\dot{V}_{max}$ by approx. 25% and also increases the APD_{30}. In guinea-pig left atria vanadate (10 - 25 µmol/l) decreases $\dot{V}_{max}$ by more than 40 % and slightly decreases the APD_{30}. In rat left atria vanadate (25-500 µmol/l) increases $\dot{V}_{max}$ by more than 40%, but reduces the APD_{30}. High doses of vanadate (500 µmol/l) cause a hyperpolarisation (up to 10 mV) in rat atria, but not in guinea-pig papillary muscle.
Our results support the view that the positive inotropic actions of vanadate are mainly due to an increase in slow inward current, whereas the negative inotropic effects are paralleled by a decrease.

Supported by the Deutsche Forschungsgemeinschaft, SFB 30 Kardiologie

Institut für Pharmakologie der Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf

109

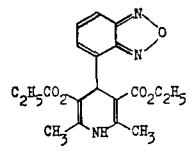
CARDIAC SLOW CALCIUM CHANNEL INHIBITION BY PY 108-068

G. Scholtysik

The dihydropyridine derivative PY 108-068 (PY) antagonizes calcium-induced contractions of rabbit aortic rings in depolarizing medium (Hof et al., J.Cardiovasc.Pharmacol., 4: 344-351, 1982), considered as a characteristic feature of a calcium antagonist. The influence of PY on calcium channels of cardiac cells have not yet been described and is therefore investigated with the following experiments.

Guinea-pig right papillary muscles were suspended in Tyrode solution in order to record transmembrane action potentials (AP) by means of standard intracellular microelectrode techniques. All preparations were electrically driven with 1Hz. After studying normal APs, slow APs were induced by DB-cAMP (0.25 mM) in pratially depolarized (KCl 16 mM) muscles. PY was added in a concentration of $3x10^{-7}$M to normal and partially depolarizing superfusion medium (5 experiments each).

Slow response APs of partially depolarized papillary muscles, which are carried by slow inward calcium - current, are effectively and reversibly suppressed by PY. This calcium antagonistic effect of PY on cardiac cell membranes is selective, since the AP upstroke velocity in normal APs, representative for the availability of fast sodium channels, is not influenced by PY.



Chemical structure of PY 108-068

Preclinical Research, Sandoz AG., CH-4002 Basel

110

DIFFERENCE IN ACTION OF THE CALCIUM ANTAGONISTS NIFEDIPINE AND DILTIAZEM ON THE SLOW RESPONSE IN VENTRICULAR MYOCARDIUM

U. Borchard and D. Hafner

Nifedipine and diltiazem are known to decrease slow inward current in guinea-pig ventricular myocardium without affecting the fast sodium channels. Both substances show similar effects on action potentials (AP) of human ventricular myocardium which consist of a shortening of the AP without changing the maximum upstroke velocity ($\dot{V}_{max}$) up to 10 µmol/l. In order to characterize their action on cardiac slow channels, slow responses (27 mmol/l $[K^+]_o$, 0.5 mmol/l $[Ba^{2+}]_o$) have been investigated in guinea-pig papillary muscles. Diltiazem = 0.3 µmol/l and nifedipine = 0.01 µmol/l reduce amplitude, duration and $\dot{V}_{max}$ of the slow response. In contrast to nifedipine, diltiazem delays the recovery of $\dot{V}_{max}$ after stimulus induced inactivation. Decrease in $\dot{V}_{max}$ by increasing stimulation frequency (use-dependence) is pronounced with diltiazem and very small with nifedipine as are the negative inotropic effects. Recovery from use-dependent blockade is almost complete within 2 min in experiments with nifedipine and 5 - 10 min with diltiazem. 0.01 µmol/l nifedipine or 1 µmol/l diltiazem reduce $\dot{V}_{max}$ under rested state conditions by about 25% or 15%, respectively.
We conclude that the primary mechanism of action of nifedipine on slow channels is a rested state blockade, whereas that of diltiazem additionally consists of a profound activation-dependent blockade.

Institut für Pharmakologie der Universität Düsseldorf, Moorenstr. 5, 4000 Düsseldorf

Supported by the Deutsche Forschungsgemeinschaft, SFB 30 Cardiology

111

CA-ANTAGONISTS INACTIVATE SINGLE CELLS BUT EXERT NEGATIVE CHRONOTROPIC EFFECTS ONLY IN MULTICELLULAR NETWORKS OF PACEMAKER CELLS IN CULTURE

B.Koidl, H.A.Tritthart

The inhibitory influence and the mode of action of D-600, diltiazem and bepridil were studied in single embryonic heart cells and in multicellular networks in culture using microelectrode techniques which included current injection via the potential measuring electrode. Action potentials of the cultured cells almost completely resemble those of pacemaker cells in the sinus node of adult myocardium but lack beta receptor responsiveness. After application of Ca antagonists the action potential amplitude started to decline through gradual reduction of rate of rise, overshoot and maximal diastolic potential leading to a stable potential of inactivation of about -40mV. Experiments with current injection proved that this inhibition is due to potential and frequency dependent block of the slow inward current. This dependence was strongest with D-600 and weakest with bepridil. However, these inhibitory effects were not accompanied by a decrease in the frequency of discharge in single cells. Negative chronotropic effects were only found in multicellular networks of cells, indicating that in these synchronized systems the inactivation of fibers with low potential and high intrinsic frequency shifts the pacemaker center of discharge to slower cells and thus decreases frequency of discharge.

Supported by Austrian Science Research Fund (Project No. 4662). Univ.Inst.f.Med.Physik u. Biophysik, A-8010 Graz.

112

IDENTIFICATION AND CHARACTERIZATION OF BINDING SITES FOR CALCIUM CHANNEL BLOCKERS AND OUABAIN IN DIFFERENT FRACTIONS OF BOVINE CARDIAC MUSCLE MEMBRANES.

P.Ruth, V.Flockerzi and F.Hofmann

The distribution of ^{3}H-Nitrendipine (N) and ^{3}H-Verapamil (V) binding sites in different membrane fractions of bovine cardiac muscle membranes has been studied and compared with ^{3}H-Ouabain (O) binding sites. The relative apparent abundance of binding sites in homogenates was 1(N):3(V):40(O). About 40-70% of these sites were recovered in a low speed pellet (LSP)(9000xg) with an 1-2-fold enrichment. Sites for N and O but not for V were enriched about 3-fold in the microsomal fraction (MS) (30 000xg). Sucrose density gradients of MS showed that N and O sites were enriched 5- and 2-fold in a fraction of density > 1.18 and 6-and 19-fold in sarcolemma(SL)(density 1.04). Competition curves for N and V sites present in SL and LSP with various unlabelled compounds indicated that N sites are specific for dihydropyridines and that V sites are specific for (-)D-600 and V. These studies indicate that N and V bind to different sites in bovine cardiac muscle membranes and that only part of these sites may be located at the SL.

Pharmakologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg

113

POSSIBLE MODE OF ACTION OF CAPSAICIN IN HEART AND UTERUS

G. Zernig, P. Holzer and F. Lembeck

Capsaicin (CAP) has been shown to exert specific neurotoxic effects on peptidergic primary afferent neurons. Effects of CAP on the isolated guinea-pig auricle and on the isolated rat uterus were used to elucidate its action with regard to implications on transmembr. Ca^{2+} flux. (1) In the electrically driven guinea-pig left auricle CAP increased the size of the contraction dose-dependently (0.03 to 6.5 µM). The effect of 0.33 µM CAP was reproducible at intervals of 15 min, tachyphylaxis occurred at shorter intervals. (2) The inotropic effect of 0.33 µM CAP was decreased when Ca^{2+} in the bath was doubled ($p < 0.01$), (3) but increased under 2.2 µM verapamil or 100 µM La^{3+} ($p < 0.05$). (4) The increase in contraction amplitude by 0.4 - 400 µM isoproterenol was greatly reduced in presence of 0.33 µM CAP ($p < 0.05$). (5) In the isolated anestrous rat uterus 0.03 - 0.33 µM CAP caused a transient inhibition of the spontaneous contractions in similar to the effect of 2.2 µM verapamil.
The observations are assumed to be based on an $[Ca^{2+}]_i$ increase by CAP: (1) This increase in $[Ca^{2+}]_i$ by CAP would explain the enhanced contraction of the auricle by CAP. (2) When $[Ca^{2+}]_i$ is already increased by $[Ca^{2+}]_e$ an additional $[Ca^{2+}]_i$ increase by CAP would be relatively lower and the size of the contraction be decreased. (3) Release of Ca^{2+} by CAP from stores inaccessible to either verapamil or La^{3+} which results in increased $[Ca^{2+}]_i$ would explain the enhanced effects of CAP under these drugs. (4) The diminished inotropic effects of isoproterenol in presence of CAP need further elucidation. (5) Inhibition of the spontaneous contractions of the uterus by CAP may result from an inhibition of the transmembranous Ca^{2+} influx comparable to that of verapamil.
The effects of CAP on muscular Ca^{2+} utilisation may serve as aid to explain the specific neurotoxic effects of CAP.

Institut für Experimentelle und Klinische Pharmakologie, Universität Graz, Universitätsplatz 4, A-8010 Graz, Austria.

114

β-ADRENOCEPTOR BINDING AND ADENYLATECYCLASE ACTIVATION IN LIVING RAT HEART CELLS AFTER CHRONIC TREATMENT WITH RECEPTOR BLOCKING DRUGS

H. Porzig and C. Becker

Beating monolayer cultures of neonatal rat heart cells were exposed for 5 days to one of the following β-adrenergic antagonists: [^{3}H]-(±)-carazolol (0.2 nM), [^{3}H]-(±)-CGP 12177(4-β-tert.buthylamino-2-hydroxypropoxy)-benzimidazol-2-on)(4 nM) or (-)timolol(10 nM). Receptor binding of a second radiolabelled antagonist and agonist-induced cAMP accumulation were measured 90 min to 48 h after washout. Within 90 min CGP and timolol dissociated completely from the receptor sites. Rebinding of ^{3}H-CGP revealed no change in receptor number or affinity compared to untreated controls. Isoprenaline(1 µM)-stimulated cAMP formation reached its control level within 90 min after removal of timolol. After 90 min of CGP washout the cAMP response was only 50% and reached 85% of controls only after 12 hours. Carazolol dissociation from specific sites followed a single exponential with $t1/2$ = 115 min. Yet, the cAMP response after 3 and 12 h of carazolol washout remained at 25% and 33% of controls even though more than 80% of the receptors were available for binding. Decoupling between receptor occupation and cyclase activation was not observed during recovery from agonist-induced desensitisation. Receptor binding and cAMP response both reached 83% of controls within 46 h. Our results provide a possible pharmacodynamic explanation for prolonged in vivo actions of β-adrenoceptor blockers that are not explained by their pharmacokinetics.

Pharmakologisches Institut der Univ. Bern
Friedbühlstr. 49, CH 3010 Bern

115

EFFECTS OF A ß-BLOCKING AGENT, SOTALOL, ON MEMBRANE CURRENTS AND CONTRACTION IN FROG ATRIAL FIBRES.

R.Kern, H.Müller, and Th.Schumacher

The ß-blocking agent sotalol exhibits in clinical tests an additional class III antiarhythmic effect, characterized by the prolongation of action potential duration. So the effect of sotalol on action potential, membrane currents and contraction were studied in double sucrose gap, voltage-clamped frog atrial fibres. Sotalol 5×10^{-7}M caused in preparations driven at a constant rate of 4/min an increase of the action potential duration (50% and 90% repolarization) of 15-20%; amplitude, maximum upstroke velocity and steady state Na-inactivation were not affected. A moderate positive inotropic effect, 10% increase, could be seen. The absolute refractory time was extended, the effective refractory period remained unaltered. In contrast, the effect of sotalol is invers in resting preparations: after a 3 min resting period, the action potential duration was reduced by 30%, the contraction was decreased by 30-50% and could not be restored. The alterations of the action potential are reflected in voltage-clamp measurements. In driven preparations, the Ca inward current and the related phasic contraction remained unaffected, the outward current was decreased by about 15-20% especially in the plateau potential range. The tension-voltage relation of the tonic contraction was a few mV shifted to more negative potentials. The positive inotropic effect is due to this shift and to the prolongation of the action potential duration, the antiarhythmic effect can be contributed to the extension of the absolute refractory time and the prolongation of action potential duration in absence of an influence on the Na-system.

Department of Physiology I, University of Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg

116

COMPARISON OF β-BLOCKING, INTRINSIC AND UNSPECIFIC CARDIODEPRESSANT ACTIVITIES OF DIFFERENT β-ADRENOCEPTOR BLOCKING AGENTS.

R. Lindner and R. Zahorsky

The influence of pindolol (pi), carazolol (ca), mepindolol (me), BM 14190 (an analogue of carazolol carrying instead of an isopropyl group at the aliphatic nitrogen a 2-methoxy-phenyl-ethyl residue) (bm), doxaminol (do) and prenalterol (pr) on frequency of contraction and on isoprenalin induced tachycardia without and with prior reserpinization was investigated in spontaneously contracting guinea pig atria. Frequency was reduced by 50% by the following concentrations: bm 6×10^{-6}, do 1.5×10^{-5}, ca 2×10^{-5}, me 1×10^{-4}, pi 6×10^{-4}, pr 8×10^{-3}M. ED_{50} values for 50% reduction of isoprenalin induced tachycardia were pi 3×10^{-9}, ca 5×10^{-9}, me 2×10^{-8}, bm 3×10^{-8}, pr 5×10^{-7}, do 8×10^{-7}M. As an index for the concentration difference between specific and unspecific effect the ratios were calculated from the ED_{50} values for depression of spontaneous frequency and the ED_{50} values for the antagonistic activity versus isoprenalin. The ratios obtained were: pi 200 000; pr 16 000, me 5 000, ca 4 000, bm 200, do 20. The intrinsic activity was found to be poorly developed in guinea pigs even after reserpin treatment of the animals (5mg/kgxday for 3 days). A moderate stimulation of frequency was observed in the case of pi and ca, not, however, after application of me and of bm. The concentration range necessary to induce this effect was higher and partly overlapped with the dose-response curves for ß-blockade and were terminated by the dose response-curve for unspecific frequency reduction. These results with respect to antagonistic properties support the assumption that in the guinea pig high affinity to the ß-receptor is at least in part related to a proper attachment of the indolic nitrogen to the respective part of the ß-adrenoceptor. The same molecular particularity appears to be responsible for agonistic activity, which is produced, however, in a higher concentration range. This suggests an interaction with a different receptor subtype. The unspecific membrane stabilizing effects, finally, simply reflect the overall hydrophobicity of ß-blocking agents.

Institut für Pharmakologie, Hospitalstr. 4-6, D-2300 Kiel

117

COMPARATIVE STUDIES ON THE CARDIAC ACTIONS OF DIFFERENT BETABLOCKERS ON ISOLATED GUINEA PIG HEARTS.

K. Güttler and Renate Podehl

The previously described procedure (K. Güttler et al., Pflügers Arch., Suppl. 382, R 53, 1979) for following continuously the atrio-ventricular conduction time of isolated hearts and for measuring the mechanical activity of these preparations provides a useful method to study simultaneously pharmacologically induced changes in the dromotropic and inotropic properties of the heart. By this means the myocardial activity of betaadrenergic blocking agents, such as acebutolol (ACE), carteolol (CAR), and propranolol (PRO), can be evaluated in more detail.
Isolated guinea pig hearts were perfused according to the Langendorff technique with modified Tyrode solution (0.9 mM Ca^{++}, 5.4 mM K^{+}) at a constant perfusion rate (1o ml/min). The contractile forces of both the right atrium and the left ventricle were measured by force-displacement transducers, the perfusion pressure was determined by a Statham transducer.
Results: 1. Marked concentration-dependent prolongation of the atrio-ventricular conduction time was produced by PRO beginning already in the lowest concentration range ($1o^{-10}$ M), whereas ACE and CAR caused only moderate prolongations beginning at appreciably higher concentrations ($1o^{-5}$ M and $1o^{-4}$ M resp.).
2. The negative inotropic activity of these betablockers increased concentration-dependently in the order CAR < ACE < PRO, without concomitant changes in the coronary perfusion pressure.
3. The sinus automaticity was only slightly influenced by PRO and ACE up to concentrations of $1o^{-5}$ M, whereas CAR induced a distinct positive chronotropic effect in the range of $1o^{-8}$ to $1o^{-5}$ M.

Pharmakologisches Institut der Universität Köln, Gleueler Str. 24, D-5ooo Köln 41

118

EFFECTS OF 5'-N-ETHYLCARBOXAMIDEADENOSINE (NECA) ON FORCE OF CONTRACTION AND CYCLIC NUCLEOTIDES IN THE GUINEA-PIG HEART

W. Schmitz, K. von Hadeln, W. Meyer, M. Nose and H. Scholz

Among several adenosine analogs NECA has been shown to be the most potent stimulator of adenylate cyclase activity in cell systems (e.g. liver or Leydig cells) possessing stimulatory R site adenosine receptors (R_a sites). N^6-phenylisopropyladenosine (PIA), another R site agonist, is less potent than NECA at the R_a sites. On the other hand, PIA is more potent than NECA in inhibiting adenylate cyclase activity in tissues in which the adenylate cyclase is coupled to inhibitory R site adenosine receptors (R_i sites; e.g. in fat cells or brain). In order to characterize the cardiac effects of adenosine (AD) further we investigated the effects of NECA, in comparison with those of PIA and AD, on force of contraction and the cAMP and cGMP contents in isolated electrically driven guinea-pig left auricles. NECA had a concentration-dependent negative inotropic effect (NIE) starting at 0.01 µmol l^{-1} and being maximal at 1 µmol l^{-1}. The IC_{50} was 0.040 ± 0.004 µmol l^{-1} (n=6). The concentration-response curve for the NIE of PIA was identical (IC_{50} = 0.06 ± 0.01 µmol l^{-1}; n=7). AD was much less potent (IC_{50} = 31.6 ± 10.8 µmol l^{-1}; n=7). At concentrations which reduced force of contraction by about 80 - 90 %, none of the substances changed the cAMP or cGMP levels of the intact contracting preparations. - The results indicate that the NIE of NECA, PIA and AD are not due to changes in the cyclic nucleotide contents of the intact preparations. Furthermore, the potency-ranking for the NIE of the adenosine analogs (NECA = PIA > AD) suggests that the structure of the presumed cardiac R site adenosine receptor, which is obviously not linked to the adenylate cyclase, differs from that of the adenylate cyclase-coupled R site receptors described for other organs and cell systems.

Abteilung Allgemeine Pharmakologie, Universitäts-Krankenhaus Eppendorf, Martinistrasse 52, D-2000 Hamburg 20

119

CHANGES IN CONTRACTILITY AND ELECTROPHYSIOLOGICAL PARAMETERS IN ISOLATED GUINEA PIG PAPILLARY MUSCLES DUE TO DIMAPRIT (H_2-AGONIST) AND HISTAMINE

U.J.Winter, W.Manteuffel, L.Reiher, U.Kebbel, Hj.Hirche

Recently, the newly developed H_2-agonist dimaprit (D) was shown to be an effective positive (pos.) inotropic agent (Baumann et.al.,Zschr.Kardiol.,Bd 71, 3, 1982). Papillary muscles (p.m.) (Ø ≦ 1mm) of Pirbright-White guinea pigs of either sex were used for isometric and electrophysiological measurements. Administration of histamine (H) and D (10^{-10} to 10^{-3}M) to the p.m. (n=5,each) in 10 minute periods produced a rapid increase in twitch contraction at 10^{-5}M H (by 500%) and 10^{-4}M D (by 350%). These doses of D and H caused arrhythmias in 40 % and 50 % of the preparations respectively. During the application of single doses of D (10^{-4},10^{-3}M) and H (10^{-5}, 10^{-4}M) a transitory dose-dependent maximum of contractility was quickly reached and followed by a slow decrease of contraction force, which reached a plateau above the control level. The pos.inotropic effects of H and D were shown to be strongly dependent on $[Ca^{2+}]_o$. During the maximal pos. inotropic effect of D 10^{-4}M and H 10^{-4}M (n=4,each), action potential duration $(APD)_{33}$ (-5%), APD_{50} (-11%) and APD_{90} (-12%), action potential amplitude (-3%) and effective refractory period (-9%) were reduced in comparision to the control values, whereas dV/dt_{max}, resting membrane potential and overshoot showed no significant change compared with control. All effects could be reversed by H_2-antagonist cimetidine 10^{-4}M(n=4). Conclusion: D and H are positive inotropic agents, whose effects are strongly dose dependent. The alteration of the action potential might be responsible for the frequent induction of arrhythmias.

Lehrstuhl für Angewandte Physiologie der Universität zu Köln, Robert-Koch-Str. 39, 5000 Köln 41

Supported by Deutsche Forschungsgemeinschaft, SFB 68 Cologne

120

DOES SODIUM CHANNEL BLOCKADE EXPLAIN THE NEGATIVE INOTROPIC EFFECT OF CLASS 1 ANTIARRHYTHMIC DRUGS?

P. Honerjäger[1], E. Loibl[1] and K. Ulm[2]

We have compared the negative inotropic effects of 4 class 1 antiarrhythmic drugs to that of a specific Na channel blocker, tetrodotoxin (TTX), at concentrations causing similar degrees of Na channel blockade.- Isometric contractions and transmembrane action potentials were recorded from guinea-pig papillary muscle stimulated at 1 Hz. In each muscle, the % reduction of peak force of contraction and of the maximum rate of depolarization of the action potential, $\dot{V}_{max}$, by a single concentration of one drug were determined at steady state. Subtracting the latter value from the former gives a difference, Δ, that is negative if the electrophysiological effect exceeds the inotropic effect and positive if the reverse applies. Only experiments in which $\dot{V}_{max}$ was reduced by 30-70 % were evaluated:

drug	conc.(µmol/l)	Δ(mean±SEM)	n
TTX	10-30	-24.0±6.2	5
aprindine	3-30	7.6±7.5*	6
AR-LH 31[3]	3-10	-23.7±3.7	6
mexiletine	100	30.1±5.7*	4
quinidine	30	18.4±3.6*	4

Under these conditions, aprindine, mexiletine and quinidine produced a significantly (*P<0.01) stronger negative inotropic effect than TTX or AR-LH 31. Thus, a mechanism additional to Na channel blockade appears to be involved in the negative inotropic action of some class 1 antiarrhythmic drugs. (Supported by DFG)
[3]8-(3-Diethylaminopropyl)-6,6-dimethyl-2-phenyl-1H-imidazo{4,5-h}isoquinoline-7,9(6H,8H)dione-dihydrochloride (Dr. Karl Thomae GmbH)

[1]Inst.f.Pharmakol.u.Toxikol.d.TUM, Biedersteiner Str.29,D-8000 München 40;[2]Inst.f.Med.Statistik u. Epidemiol.d.TUM, Sternwartstr.2,D-8000 München 80

121

ANTIARRHYTHMIC AND ANTIFIBRILLATORY EFFICACY OF DILTIAZEM IN ACUTE MYODARDIAL ISCHEMIA AND INFARCTION

J. Thale, H. U. Bramann, B. Jost, D. Rose

Following acute coronary artery occlusion (CO), three distinct phases of increased arrhythmic activity can be observed: two early phases Ia and Ib during the first 20 min. after CO, and a late phase beginning after 6 hours and lasting 2 — 5 days. The electrophysiologic mechanisms underlying these phases of arrhythmia are different. The aim of this study was to assess the antiarrhythmic and antifibrillatory efficacy of Diltiazem (D) during both early and late phases of arrhythmia following CO.

Methods:
Investigations were carried out on 10 dogs (BW 20 — 30 kg, Piritramide — N_2O — anaesthesia, CO of either the left descending coronary artery or the left circumflex coronary artery). In all animals, the time course of spontaneous ventricular arrhythmias and fibrillation and the changes in the ventricular fibrillation threshold of ischemic (VFT_{IM}) and non-ischemic (VFT_{NIM}) areas following CO were determined.

Results:
1. The incidence of ventricular arrhythmias were significantly reduced and sometimes even completely abolished during the early phases of arrhythmia, whereas late phase ventricular arrhythmias were not influenced.
2. Ventricular fibrillation was nearly completely prevented during the early phases of arrhythmia.
3. The fall in VFT_{NIM} immediately after CO was significantly reduced; the decrease in VFT_{IM} following CO was not changed.

Conclusions:
D exerts strong antiarrhythmic and antifibrillatory effects in early myocardial ischemia. The drug proved to be much more effective than ß-blockers and Class-I-antiarrhythmic agents. By contrast, D has no effect at all on late phase ventricular arrhythmias.

University Hospital, Department of Cardiology — Angiology, Domagkstr. 3, D—4400 Münster (FRG)

122

DIPRAFENON — A NEW CLASS-I-ANTIARRHYTHMIC COMPOUND FOR TREATMENT OF CHRONIC VENTRICULAR ARRHYTHMIAS

H. Gülker, R. Engberding, B. Olbing, F. Bender

Diprafenon (D) is a new Class-I-antiarrhythmic compound with close similarities to Propafenon regarding its chemical structure. To evaluate the antiarrhythmic profile of the drug, we assessed 1) the electrophysiologic effects of D in the normal nonischemic heart, 2) the antiarrhythmic and antifibrillatory actions of D during transient coronary artery occlusion, and 3) the antiarrhythmic efficacy of the drug during sustained ventricular tachycardia in acute myocardial infarction.

Methods:
Investigations were carried out on 20 dogs (BW 20 — 30 kg, Piritramide — N_2O — anaesthesia). D was given intravenously at increasing doses ranging between 0.1 — 3.2 mg/kg. Electrical testing of the heart was performed by determining a) the refractory periods of the atrium and the ventricle, b) the AV-conduction time (His-Bundle), c) the atrial (AFT) and ventricular (VFT) fibrillation threshold, d) the incidence of ventricular arrhythmias, especially fibrillation (VF) during the first 20 min. after coronary artery occlusion (CO) and during 1 hour following subsequent reperfusion (R), e) the time course of changes in the VFT during CO and following R, f) the inducibility of sustained ventricular tachycardia during early necrosis in acute myocardial infarction.

Results:
D caused a dose-dependent increase of both the atrial and ventricular refractory periods and of the AFT and VFT. The AH-interval was prolonged by 15 ms at a dose of 3.2 mg/kg. The incidence of ventricular arrhythmias following CO and R remained unchanged; the incidence of VF following CO was slightly reduced, but only at a dose of 3.2 mg/kg, whereas the incidence of VF following R was not diminshed. By contrast, sustained ventricular tachycardia occurring during early necrosis in acute myocardial infarction, could be prevented even at low doses ranging between 0.4 — 0.8 mg/kg.

Conclusions:
D exhibits typical Class-I-actions. Thus, it appears to be of particular value in the control of chronic ventricular arrhythmias, but not on acute ischemic ventricular arrhythmias.

University Hospital, Department of Cardiology — Angiology, Domagkstr. 3, D—4400 Münster (FRG)

123

ELECTROPHYSIOLOGICAL PROFILE OF AN INDOL-2-CARBON-AMIDE DERIVATIVE (KC 3791) WITH ANTIARRHYTHMIC PROPERTIES

S. Hohnloser, H. Antoni

KC 3791 (2-(3-(N,N-diethylamino)-2-hydroxypropylaminocarbonyl)-3-methoxy-1-phenyl-indole HCl) is a new compound which exhibited antiarrhythmic properties in various animal models (Naunyn-Schmiedebergs Arch. Pharmacol. Suppl. 391, 146 (1982). In order to examine the effects of KC 3791 on the basic excitatory processes of cardiac muscle the present studies were performed with intracellular microelectrodes in isolated papillary muscles of the guinea pig.

Under control conditions (32°C, K_e 5.4 mmol/l, frequency 0,5 Hz) KC 3791 (3 - 30 umol/l) reduced the maximal rate of rise of the action potential ($\dot{V}max$) in a dose-dependent manner without markedly affecting the resting membrane potential nor the amplitude or duration of the action potential. Increasing the frequency of stimulation enhanced the reduction of $\dot{V}max$ in the steady state (-34% at 0.2 Hz; -60% at 2 Hz). By contrast, the time constant of the recovery from inactivation of the fast Na channel following single premature beats was not essentially affected by KC 3791. The h_∞ curve relating $\dot{V}max$ to the membrane potential was shifted by KC 3791 (10 µmol/l) in the hyperpolarizing direction by about 8 mV. Action potentials of the slow response type are slightly reduced in their maximal rate of rise and duration by KC 3791 at a concentration of 50 µmol/l.

The studies characterize KC 3791 as a class I antiarrhythmic drug with specific time- and voltage dependence. The results are discussed in view of the antiarrhythmic potency of the substance.

Physiologisches Institut der Universität Freiburg i. Br., Hermann-Herder-Straße 7, D-7800 Freiburg i. Br.

124

STUDIES ON THE ANTIARRHYTHMIC ACTIVITY OF ASOCAINOL-HCl (Gö 4704A) IN CONSCIOUS DOGS WITH CORONARY ARTERY LIGATION

B. Wagner

Asocainol is a new antiarrhythmic drug with a high potency in different models and species (Wagner et al.1982,Naunyn-Schmiedeberg's Arch. Pharmacol. Suppl. 319, R37). The antiarrhythmic profile was now studied in more detail in conscious dogs with a coronary artery ligation.
To evaluate the antiarrhythmic efficacy of drugs, the total heart rate (HR) and percentage of sinus beats (SB) prior to drug administration are important parameters. Asocainol, cumulatively dosed 10 + 20 mg/kg p.o. (1 h apart) in tachycardiac dogs on day 1 post ligation (mean HR 176/min; 7 % SB), was thus found to be equieffective (maximal conversion to SB≈80 %) with 10 mg/kg p.o. in "low rate" dogs (mean HR 137/min; 22 % SB).
Comparative studies with reference drugs in tachycardiac dogs (day 1) allowed the classification of Asocainol according to the maximal conversion to SB (drug/mg/kg p.o.):

Asocainol/30 > mexiletine/20 > prajmaline/5 > propafenone/10.

Infusion studies with Asocainol (10 mg/kg·h) revealed high therapeutic effects (>90 % SB) following doses ≥ 7.5 mg/kg i.v. without toxicity, whereas the arterial blood pressure remained unaltered up to doses > 40 mg/kg. Using lidocaine (40 mg/kg·h), such high values of % SB could only be reached with doses causing severe side effects (> 70 mg/kg i.v.). The mean ventricular conduction time (QRS duration) following 30 mg/kg i.v. Asocainol or 60 mg/kg i.v. lidocaine was prolonged 11.4 ± 2.7 % or remained unchanged, respectively.
In conscious dogs on day 4 post ligation, severe arrhythmias could be induced by bolus injections of 4 µg/kg i.v. epinephrine. Both Asocainol (5 mg/kg i.v.) and lidocaine (10 mg/kg i.v.), dosed prior to epinephrine, diminished these arrhythmias almost completely. However, the duration of the antiarrhythmic activity in this model appeared to be longer with Asocainol.

Goedecke Research Institute, D-7800 Freiburg

125

ELECTROPHYSIOLOGICAL EFFECTS OF A NEW ANTIARRHYTHMIC DRUG (ASOCAINOL) ON THE ISOLATED HEART AND ON VENTRICULAR MUSCLE PREPARATIONS AND PURKINJE FIBRES OF THE GUINEA PIG.

H.Langenfeld,K.Haverkampf

The studies are dealing with the electrophysiological effects of asocainol ((±)-6,7,8,9-tetrahydro-2,12-dimethoxy-7-methyl-6-phenethyl-5H-dibenz (d,f) azonine-1-ol (Goe 47o4-A)) on the isolated perfused guinea pig heart (Langendorff preparation) as well as on papillary muscles and Purkinje fibres of the same species.
In the isolated heart (n=6) the lowest effective concentration of asocainol is about o.2 µmol/l. At a concentration of 2 µmol/l the electrogram shows the following changes: A decrease in frequency (-23 %), a prolongation of PQ (+21 %), of QRS (+22 %), of QT (+9 %) and of the functional refractory period (+56 %).
In the papillary muscle (n=12) asocainol (3 µmol/l) prolongs the action potential (AP) by about 16 % while higher concentrations (1o µmol/l) exert an opposite effect. The resting potential and the amplitude of the AP remain unaffected. The maximal rate of rise ($\dot{V}max$) of the AP is reduced by asocainol in a dose-dependent manner (-31 % at 1o µmol/l).This effect shows marked use-dependence with the recovery from inactivation becoming complete after stimulus free intervalls of about 6o s. The $h\infty$-curve determined by variations of K_e is shifted by asocainol to more negative potentials. The percentage deviation of the $h\infty$-curve from control is more pronounced at lower membrane potentials.
In Purkinje fibres (n=5) the spontaneous activity occuring at K_e=2 mmol/l is suppressed by asocainol (1o µmol/l). As compared with its influence on papillary muscle asocainol affected the excitatory parameters of the Purkinje fibres in a essentially similar way.

Physiologisches Institut d.Universität Freiburg i.Br., Hermann-Herder-Str.7, D-78oo Freiburg i.Br.

126

PHARMACOLOGICAL DIFFERENTIATION OF ANTIARRHYTHMICS IN RATS

D. Thormählen and R. Oberbeck

A new simple and inexpensive screening model has been established.
Under oscilloscope-control, a purpose-made tripolar electrode catheter was inserted into the right heart of anaethetized Sprague-Dawley rats via the right jugular vein. The distal electrode was placed in the right ventricle, the medial one in the right atrium, and the proximal in the vena cava cranialis. Supraventricular (SAT) and ventricular arrhythmic thresholds (VAT) were determined by stimulating the heart electrically with increasing current. In addition, the maximum 1:1 conduction of the A-V node (CA-V) was determined by rapid atrial pacing to the point at which A-V Wenckebach occurred. CA-V was found to significantly correlate with atrioventricular conduction in man (J.K. Bissett et al., Cardiovasc Res 9:593-599, 1975). Blood pressure (BP) was measured from the left carotid artery, and heart rate (HR) was deduced from the ECG.
Results obtained with standard antiarrhythmics:

Class	dose M/kg i.v. free base	effect on				
		SAT	VAT	CA-V	BP	HR
Ia (Quinidine)	9.6 x 10^{-6}	↑	↑	→	↓	→
Ib (Lidocaine)	9.6 x 10^{-6}	→/↑	↑↑↑	→	→	→
II (Propranolol)	9.6 x 10^{-6}	↑↑	↑	→/↓	↓↓	→/↓
III (Amiodarone)	1.5 x 10^{-5}	↑	↑	↓	→/↓	→
IV (Verapamil)	1.2 x 10^{-6}	→	↑	↓↓↓	↓↓↓	→/↓

% change from control: →= 0; →/↑ or →/↓ = 0-15; ↑ or ↓ = 15-25; ↑↑ or ↓↓ = 25-35; ↑↑↑ or ↓↓↓ = > 35.
These findings are in agreement with known data and indicate that this model appears suitable to characterize antiarrhythmics and to differentiate between them.

Beecham-Wülfing GmbH & Co.KG, D-3212 Gronau/Leine, FRG

127

EFFECTS OF VENOUS SCLEROSING AGENTS ON THE ELECTRICAL ACTIVITY OF THE PERFUSED GUINEA PIG HEART AND OF ISOLATED MYOCARDIAL PREPARATIONS

J. Weirich, B. Oexle

Studies on the electrophysiological effects of the venous sclerosing agents sotravarix^R (polidocanol 60mg/ml) and variglobin^R (Na-jodate 80mg/ml) were performed on Langendorff-preparation and papillary muscle of the guinea pig heart.
Langendorff-preparation: During perfusion with sotravarix^R (concentration 1:10000) the electrogram of the isolated perfused heart showed a marked shortening of the QT duration and a prolongation of PQ and QRS. At a drug concentration of 1:8000 perfusion with sotravarix^R led to an irreversible cardiac arrest. Perfusion with a solution containing variglobin^R (1:4000) strongly shortened the QT duration of the electrogram. At a drug concentration of 1:2000 variglobin^R exerted additional effects resulting in a prolongation of PQ and QRS. Cardiac arrest occured at a drug concentration of 1:200. The perfusion rate of the isolated heart was not affected by both agents.
Papillary muscle: At a concentration of 1:1000 sotravarix^R shortened the duration of the action potential by about 24% and reduced the maximal upstroke velocity by about 41%. Both effects were irreversible. No or little influence of the drug was exerted on the resting membrane potential nor on the amplitude of the action potential. By contrast, variglobin^R (1:50) depolarized the resting membrane by about 7-8 mV and secondary to this reduced the amplitude and maximal upstroke velocity of the action potential by about 22% or 40%, respectively. At the same concentration variglobin^R shortened the action potential duration by about 80%. All effects caused by variglobin^R were reversible.

Physiologisches Institut der Univ. Freiburg i. Br. Hermann-Herder-Str. 7 D 7800 Freiburg i. Br.

128

TWO DIFFERENT BINDING SITES FOR 20,22-DIHYDRODIGITOXIN IN BEEF CARDIAC CELL MEMBRANES

Lindsay Brown and Erland Erdmann

20,22-Dihydroderivatives of digitalis are known for their low affinity for the cardiac glycoside receptor; high concentrations are needed to give positive inotropic effects.The binding of three cardiac glycosides ^{3}H-ouabain, ^{3}H-digitoxin and ^{3}H-dihydrodigitoxin to beef cardiac (Na^++K^+)-ATPase was measured to establish the specificity of receptor binding. Non-specific binding was defined as that in the presence of 0.1mM unlabelled compound,or in the absence of ligands. The dissociation constants (K_D) calculated from the inhibition of ^{3}H-ouabain binding were:ouabain,2.9x 10^{-9}M;digitoxin,8.1x10^{-10}M;dihydrodigitoxin,3.4x10^{-8}M. The IC_{50}-values for (Na^++K^+)-ATPase activity were: ouabain,5.9x10^{-9}M;digitoxin,1.6x10^{-9}M;dihydrodigitoxin 2.5x10^{-8}M.Ouabain and digitoxin showed straight Scatchard plots for one site of high affinity.However, dihydrodigitoxin binding gave a curved Scatchard plot. For Mg^{2+},Pi-supported binding,the K_D of the high affinity site was 1.6x10^{-8}M.The Bmax of this site was about 30 picomoles/mg protein,similar to the ouabain and digitoxin binding site values.Binding with Na^+,ATP, Mg^{2+}showed a high affinity site (K_D5.3x10^{-8}M) of similar Bmax.The high affinity site was not occupied in the presence of 200mM Na^+, or in the absence of ligands,had an assoc. rate constant of 1.8x$10^5$$M^{-1}$$sec^{-1}$ and a dissoc. rate constant of 3.5x$10^{-3}$$sec^{-1}$. The low affinity site (K_D 4.0x10^{-6}M for Mg^{2+},Pi; 5.5x10^{-6}M for Na^+,ATP,Mg^{2+})bound about 350 picomoles/mg protein, was occupied in the presence of 200mM Na^+,but not in the absence of ligands.There are two digitalis binding sites in beef cardiac (Na^++K^+)-ATPase; a dihydrodigitoxin high affinity site also occupied by ouabain and digitoxin,and a low affinity site,which can be occupied by dihydrodigitoxin,whose biological significance has to be further investigated.

Medizinische Klinik 1 der Universität, Klinikum Grosshadern,D-8000 München 70.

129

DIFFERENT EFFECTS OF CARDIAC GLYCOSIDES IN CARDIAC MUSCLE AND NON MUSCLE CELLS

K. Werdan, B. Wagenknecht, B. Zwißler, W. Krawietz

The role of sodium pump inhibition in the positive inotropic action of cardiac glycosides is still a matter of controversy. Especially the linkage between cardiac glycoside binding to its receptor and inhibition of the sodium pump in intact myocardial cells is disputed.

Results: In myocardial cells from chick embryos, (3H)-ouabain binding is characterized as following: binding occurs to a single class of binding sites; binding capacity 2.6(muscle cells) and 2.1(non muscle cells) pmoles/mg cell protein; dissociation constant $1.5(1.9)\times10^{-7}$M; association rate $3(2)\times10^{4}M^{-1}sec^{-1}$; dissociation rate $4(3)\times10^{-3}sec^{-1}$; binding is strongly temperature dependent and reduced by K^+ ions; it is prevented in heat shocked cells.
In non muscle cells, a stoichiometric relationship exists between occupation of these binding sites by ouabain and reduction of ouabain-sensitive ($^{86}Rb^+$+K^+) -influx or decrease of cellular K^+. Myocardial muscle cells, however, behave differently: up to 40 % of binding sites can bind ouabain with only minor reduction in sodium pump activity. Further increase in the percentage of binding sites occupied by ouabain then decreases ($^{86}Rb^+$+K^+)-uptake and cellular K^+ as expected.

Conclusion: In myocardial non muscle cells, binding of ouabain to its receptor inactivates the sodium pump in a stoichiometric manner. In muscle cells, however, ouabain binding is either not strictly coupled to sodium pump inhibition, or the non-inhibited portion of sodium pump molecules can in part compensate for the ouabain-inactivated pump molecules.

Medizinische Klinik I der Universität München, Klinikum Großhadern, Marchioninistr. 15, D-8 München 70

130

REFINED "ONE-WAY" E-C COUPLING MODEL CAN EXPLAIN RECENT "PROBLEMATIC" DATA ON LARGE ACTIVATOR CA FLUXES, I_{to},AND I_{si} INCREASE WITH HEART GLYCOSIDE INOTROPY H.J.Mensing

G.A.Langer et al.(Am.J.Physiol.237:H239,1979), possibly distracted, by the Fabiatos' postulates on SR function, from the potentialities of Langer's former "one-way concept", were unable to straightforwardly explain the rapid kinetics of activator calcium in beating heart cell cultures. Wohlfart (Acta Physiol.Scand.106:395,1979)concluded from interval-strength analysis in rabbit papillary muscles that most of the activator Ca must exchange with the extracellular space *within one beat*.Lewartowski et al. (J.Mol. Cell.Cardiol.12,Suppl.1:94,1980) found a huge ^{45}Ca influx of 0,25 mmol/kg w.w. *per beat* in guinea pig hearts; they suggested revision of the current e-c coupling models. Isenberg (Z.Naturforsch.37c:502,1982), working with isolated ventricular cells, reports integrals of "fast and large" Ca inward currents up to 0.1mmol/kg cell *in 100ms*. Other problem areas in myocardiology include: the unknown structure and function of the couplings ("feet") between sarcolemma and the SR; the still unknown mechanism(s) of Ca release from the SR; the mechanism of increase in Ca inward current/I_{si} with heart glycoside inotropy (Marban & Tsien, J.Physiol.329:589,1982); and the ionic nature and function of the transient outward current(s)/I_{to}.
All these "problems" can be resolved within the framework of a new e-c coupling model briefly outlined by the author in 1979 (this Arch.308:R35; see also TIPS 2:303,1981). In this model SR calcium is released to a diffusion restricted *extracellular space*: depolarization activates a passive Ca *outward* current (part of I_{to}) from the SR, through channels in the "feet", to the cleft beneath the external lamina of the glycocalyx, from where activator Ca enters via an early electrogenic $3Na_i/1Ca_o$ exchange (part of I_{to}),and via I_{si}. These three different Ca-dependent currents at present cannot be clearly separated. Glycoside inotropy augments the Ca load of the SR, and, by "extracellular recirculation" during activation, the Ca supply to the I_{si} channels.

Pharmakologisches Institut, Wilhelmstr. 56, D-7400 Tübingen.

131

EFFECT OF ELEVATION OF EXTERNAL CA CONCENTRATION ON TENSION DEVELOPMENT AND CALCIUM CONTENT OF ISOLATED RESTING GUINEA-PIG LEFT ATRIA UNDER THE INFLUENCE OF OUABAIN. T. Peters and Gundula Sievers

The investigation was aimed to study the influence of ouabain on net calcium movements into and across the plasmalemma under conditions where there is no excitation Ca-flux. Left atria of guinea-pigs were incubated in Tyrode's solution (KCl:2.7 mmol/l, 32°C, gassed by carbogen)and the Ca-concentration ($[Ca]_o$) was elevated from 1.0 to 4.0 mM after an equilibration period of 60 min in the absence (controls) or presence of ouabain (8×10^{-7} or 1.5×10^{-6}M). Subsequently, over a period of 30 min tension development, cellular calcium (Ca_c) and Na- and K-contents were measured fluorometrically and by atomic absorption spectroscopy, respectively. Under control conditions the elevation of $[Ca]_o$ did neither affect resting tension nor the cellular contents of Na or K (controls: K 137± 7; Na 45±6. Elevated Ca: K 139±11; Na 39±4 mmol/kg). Ca_c was found to be increased from 1.15 to 1.59 mmol/kg over a period of 30 min in elevated $[Ca]_o$. Raising the $[Ca]_o$ in the presence of 8×10^{-7}M ouabain resulted in an abrupt increase of resting tension within 1 min while the cellular contents of Na,K and Ca did not differ from values obtained in the absence of the drug (K 123±5; Na 43±8; Ca_c 1.80 mmol/kg). Under the influence of 1.5×10^{-6}M ouabain resting tension again increased immediately after raising $[Ca]_o$. After 5 min a slowly proceeding further increase of tension was observed. During the early phase of Ca-induced tension increment the cellular Na,K and Ca contents were unaffected as compared to the values obtained in the presence of ouabain alone. The second phase of slow tension enhancement was, however, accompanied by a progressive cellular loss of K(cellular content after 30min:92±6 mmol/kg). The results suggest that ouabain exerts an effect on the mediation between $[Ca]_o$ and Ca^{++} in the cytosol through a Ca-binding site, the properties of which may change independent of major alterations of intracellular Na or K concentrations.

Institut für Pharmakologie,Hospitalstr.4-6,D-2300 Kiel

132

ACTION OF OUABAIN ON RAT VENTRICULAR TISSUE

Stefan Herzig and Klaus Mohr

The effects of ouabain on contractility and on cellular Na- and K-content of rat isolated ventricular strips,the ouabain-induced inhibition of a rat cardiac Na/K-ATPase preparation and the ouabain-binding to rat cardiac cell membranes were investigated and compared with results reported for guinea-pig (g.p.) hearts.
At 1 Hz 3×10^{-7}M ouabain augmented isometrically recorded contractile force by 20%, 3×10^{-5}M by 60%. Intoxication was induced regularly at 10^{-4}M ouabain; in the great majority of experiments this was indicated by an unstable inotropic response, i.e. a transitory inotropic maximum mostly combined with a progressive increase in end-diastolic tension leading to contracture, while arrhythmia occurred only rarely. Under similar conditions a deterioration of Na/K-homeostasis was detected by flame-photometry only beyond 10^{-4}M ouabain. In g.p. heart the range of inotropic ouabain-concentrations is much smaller; the common toxic effect is an arrhythmia paralleled by marked loss of cellular K and gain of Na. The ouabain concentration required for a half maximum inhibition of rat Na/K-ATPase (60×10^{-6}M) was 20 fold higher than in g.p. (3×10^{-6}M). Scatchard analysis of[^{3}H]ouabain-binding revealed a high affinity binding with Kd~5×10^{-8}M (g.p.: 15×10^{-8}M); B_{max} amounted to 10% of B_{max} found in identically prepared g.p. cardiac membranes. The presence of additional ouabain-binding with low affinity and high capacity could not be excluded.

In rat and g.p. heart a high affinity ouabain-binding can be related to an inotropic response. Inhibition of rat Na/K-ATPase, however, becomes apparent in vivo and in vitro at far higher ouabain-concentrations than in g.p.. In agreement with this finding is the broad inotropic dose-response curve of ouabain in rat, which seems not to be terminated by a disturbance of cellular Na/K-homeostasis, but by an insufficient reduction of the diastolic cytosolic Ca^{2+}-concentration.

Institut für Pharmakologie, Univ.Kiel, Hospitalstr. 4-6, D-23 Kiel

133

THE DIHYDROOUABAIN-POTASSIUM ANTAGONISM ON FORCE OF CONTRACTION IN RELATION TO SODIUM

F. Ebner, A. Bachmaier, M. Reiter

The inotropic potency of dihydroouabain (DHO) on guinea-pig papillary muscle (Na^+ 140, Ca^{2+} 1.2 mmol/l; 1 Hz) was reduced by K^+ in a non-saturable and non-linear way with steeper slopes of the concentration-effect curves, which is inconsistent with an exclusive role of a K^+-regulated affinity of the DHO receptor. However, a model of the influence of $[K^+]_o$ on Na^+ fluxes could simulate the change of the DHO curves. Consistent with this model, the reduction of $[Na^+]_o$ to 70 mmol/l antagonized the inotropic effect of DHO predominantly at low $[K^+]_o$ with a marked steepening of the curves. Since K^+ depressed $\dot{V}_{max}$ of the AP only at high while saturating the Na pump already at low concentrations it appears that, in dependence on resting membrane potential, passive Na^+ influx during diastole contributes to the effect of K^+. Accordingly, an increase of $[K^+]_o$ should and actually was found to have a negative inotropic effect (1.2 - 4.5 mmol/l K^+). However, higher $[K^+]_o$ produced a positive inotropic effect (enhanced by elevated $[Ca^{2+}]_o$ or reduced $[Na^+]_o$) as expected in view of a depolarization-dependent impairment of Ca^{2+} elimination via Na-Ca exchange.

The results support the assumption that K^+ at least partly exerts its antagonistic effect by the interference with the relation of influx to efflux of Na^+.

Institut für Pharmakologie und Toxikologie der Technischen Universität München, Biedersteiner Str. 29, D-8000 München 40

134

MYOCARDIAL ACTION OF GITOXIN AND ITS FORMYL- AND ACETYL-DERIVATIVES IN GUINEA PIG.

E.Schellberg and U.Fricke

Contrary to other digitalis-glycosides, gitoxin (GT) is not used in man, particularly because of early reports of its poor absorption characteristics. This disadvantage, however, has been overcome by acetylation (pengitoxin, PAG) or formylation (gitoformat, PFG) of the steroid nucleus and the sugar chain. These drugs in part considered as "prodrugs" are now clinically available. Because of some reports (e.g. DOLPHEN & LESNE, Arzneim.-Forsch. 30, 614, 1980) of a greater safety margin of GT, one of the main metabolites of PAG and PFG, we studied the effects of GT, PFG, PAG and as further metabolites of these drugs the 16-formyl- (FGT) and 16-acetyl-derivative (AGT) of GT on isolated papillary muscles and NaK-ATPase of guinea pig hearts. In papillary muscles, concentrations for half maximal inotropic effects (ED50) increased in the following order: FGT (4.2) – PFG (4.4) – AGT (7.3) – GT (27.3) – PAG (48.0 x 10-7 mol/l). So did the concentrations for maximum inotropic effects (= toxicity threshold, TOX). Thereby calculated therapeutic indices (TOX/ED50) were in the range of 4.6-5.2 for PFG, FGT and GT. A lower index was obtained with PAG and AGT (3.0-3.1). Maximum inotropic effects were nearly identical, in the range of 70-90%, except FGT which increased contractility by only 55%. In the NaK-ATPase-assay, concentrations for half maximal inhibition (ID50) increased from FGT (1.5), PFG (3.4), AGT (7.4) to GT (19.1 x 10-7 mol/l). With PAG an exact ID50 could not be calculated, because this drug precipitated in concentrations higher than 5x10-5 mol/l. The maximum inhibition was nearly 40%. The estimated ID50-values were in the range of 1x 10-4 mol/l and exceeded >10x the usually obtained ratio of ID50/ED50 ≈1. Thus, esterification of GT considerably modifies the drug's affinity, the 16-formyl (-acetyl) derivatives being more effective than the pentaformylated (-acetylated) drugs. The therapeutic index, however, as measured as TOX/ED50, is quite within the range of other cardiac glycosides.

Pharmakol. Inst. Univ. Köln, Gleueler Str. 24, D-5000 Köln

135

SIGNIFICANCE OF FIBER DILATION FOR PROPRANOLOL AND EPINEPHRINE INDUCED ACUTE CHANGES IN CAPILLARY DENSITY IN THE RAT HEART.

F. Vetterlein

The present experiments have been designed to test whether a propranolol-induced decrease in coronary flow influences the number of perfused capillaries in the rat heart. In addition, the effects of an epinephrine-induced increase in blood flow were studied. - Rats were thoracotomized, and the ∝-globulin-conjugated fluorochrome FITC was injected intravenously in order to label the intravasal space. 10 min after injection of the dye the heart was excised and frozen for histology. Capillary densities were determined in cross sections of the subepicardium and the subendocardium. In the propranolol (1.0mg/kg i.v.)- as well as in the epinephrine (5.0 µg/kg x min) i.v. for 5 min)-treated rats significantly higher densities of dye-labelled capillaries/mm^2 were found as compared to the controls (subepicardium and subendocardium: controls 3,530±90 and 3,260 ± 90; propranolol: 3,890±90 and 3,410±100; epinephrine: 3,930±110 and 3,510±70). This increase was not induced by a rise in number of perfused capillaries as could be shown by the additional determination of fiber densities. Fibers were demonstrated by the intravital application of myoglobin, conjegated with the fluorochrome RB 200, which distributes itself in the extracellular space due to its low molecular weight. With this method it was found that the capillary/fiber relation was not changed by the drugs tested. Therefore the rise in capillary counts per square unit was due to an extension of the myocardial fibers produced by both propranolol and epinephrine.

Institut für Pharmakologie und Toxikologie, Universität Göttingen, Robert-Koch-Str. 40, D-3400 Göttingen

136

PERFLUOROCARBON EMULSION APPLIED TO THE ISOLATED PERFUSED GUINEA PIG HEART

W. Deutschmann and E. Lindner

Perfluorocarbon emulsions are in use as nutrient media for isolated perfused organs such as liver, kidney, and brain. Yokoyama et al. (Fed. Proc. 34, 1478, 1975) observed a prolonged survival time of an isolated heart perfused with such an emulsion. - In the present study a perfluorocarbon (FC-)medium (composition: Dirks et al., J. Pharm. Meth. 4, 95, 1980) and a Krebs-Henseleit (KH-)solution were used for perfusion of the isolated guinea pig heart. On switching the perfusion from KH-solution to FC-medium, the force of contraction increased from 63.5 to 76.3 mN (i.e. by about 20%, n=6), whereas the coronary flow decreased from 6.7 to 2.1 ml/min (i.e. by about 70%, n=6). These effects were reversible. Frequency and O_2-consumption did not change significantly. Ouabain (infusion rate $2.7x10^{-9}$ mol/min) increased the initial force of contraction by 35% in the FC-medium and by 28% in the KH-solution, while the percental increase in coronary flow caused by glyceryl trinitrate was the same for both nutrient fluids. Isoproterenol ($4.7x10^{-11}$ mol/min) increased the initial force of contraction by 55% in the FC-medium versus 37% in the KH-solution, but the increase in frequency was only 6% as compared to 26%. - Obviously, the FC-medium due to its fourfold greater O_2-binding capacity reduces the coronary flow to a more physiological level as compared to a simple salt solution. However, it does not alter classical drug effects in the isolated perfused guinea pig heart, except for the frequency.

Zentrum Pharmakologie und Toxikologie, Medizinische Hochschule Hannover, D-3000 Hannover 61
Hoechst AG, D-6230 Frankfurt (Main) 80

137

EFFECTS OF CATECHOLAMINES ON MYOCARDIAL EFFICIENCY AND EFFICIENCY OF CARDIAC PUMP-FUNCTION DURING HYPOTHERMIA+)

A. Hoeft,

Based on a mathematical model for estimating the optimal efficiency of cardiac pump-function under various hemodynamic conditions (ηm_{opt}) (Hoeft et al., Pflügers Arch. ges.Physiol. 394, R 9, 1982) hemodynamic data of 8 anaestized dogs with hypothermia were analysed. Compared to normothermia during hypothermia (30.8 ± 0.4 °C) myocardial energy demand and oxygen consumption decreased whereas myocardial efficiency (η) was improved. In both temperature ranges norepinephrine caused a decrease in η, however efficiency of cardiac pump-function (ηm) was very close to the optimal obtainable values ($\eta m/\eta m_{opt}$ = 99.1 ± 1.2 % in normothermia; $\eta m/\eta m_{opt}$ = 99.3 ± 0.4 % in hypothermia). In normothermia additional application of epinephrine and/or dopamine led to an increase in dP/dt exceeding values which would be necessary to achieve an optimum of caridac pump efficiency (dP/dt / dP/dt_{opt} = 140 ± 29 %). In contrast to this effect epinephrine and dopamine caused an insufficient increase in dP/dt during hypothermia (dP/dt / dP/dt_{opt} = 68 ± 12.7 %), leading to a decreased ratio of actual and optimal cardiac pump efficiency ($\eta m/\eta m_{opt}$ = 89 ± 10 %).

These data indicate that a benificial effect of hypothermia on myocardial efficiency is not seriously affected by norepinephrine but appears to be abolished by application of mainly ß-stimulating agents.

Zentrum Physiologie und Pathophysiologie, Universität Göttingen, Humboldtallee 7, D-3400 Göttingen

+)Supported by the Deutsche Forschungsgemeinschaft, SFB 89 - Kardiologie Göttingen -

138

EXAMINATION OF A NEW PRESSURE-WORK INDEX OF MYOCARDIAL OXYGEN CONSUMPTION

D.Baller, W.Jonas

The validity of the hemodynamic determinants of myocardial oxygen consumption($M\dot{V}O_2$) is still a controversial subject. Stroke volume(SV) and external cardiac work have been re-evaluated and integrated in a new index of $M\dot{V}O_2$: K_1(syst.blood pressure(SBP)x HR) + K_2(0.8SBP + 0.2diast.blood pressure)x HR X SV/body weight) + 1.43; K_1=4.08x 10^{-4}, K_2=3.25x10^{-4}(Circ.Res.50:273,1982).
In 16 intact anesthetized dogs this index was tested at moderate and extreme variations of $M\dot{V}O_2$ and hemodynamics(n=239). It was compared to other pressure-work indices and to the additive index $E_t=E_0+E_1+E_2+E_3+E_4$.Myocardial blood flow measurements(30-700ml/min·100g)were validated by three reference methods(pressure difference sinus catheter, argon technique,direct volumetric method) Physiologic sinus rhythm was maintained in all experiments. Hemodynamics were varied by use of catecholamines, atropine,ß-stimulators,ß-blockers and volume shifts. Results(n=150) at moderate and higher levels of $M\dot{V}O_2$(3-37ml/min·100g):PW-index: y=0.915x-2.4;r=0.914;$\bar{y}$=13.1±0.63,$\bar{x}$=16.92± 0.63; E_t:y=0.995x-0.4;r=0,958;$\bar{y}$=13.1±0.63;$\bar{x}$=13.5 ±0.6; external cardiac work:y=2.2x+3.9;r=0.85; $413xP_{art}xSV^{0.33}xHR$:y=0.7x;r=0.89;statistically derived new pressure-rate product:$(15P_{syst\ max} - 11P_{diast\ min})xHRx10^{-4}$:y=1.016x-1.3;r=0.92.
Results indicate similar corr.coefficients for pressure-work indices.However,after E_t, the best prediction of actual $M\dot{V}O_2$ is obtained by an easily measurable new pressure-rate product. E_t correlates even at extreme variations(2^{nd}set of exp., n=89)with $M\dot{V}O_2$(3-73ml/min·100g):y=0.9x+5.3; r= 0.90 supporting its theoretical concept.
Zentrum Physiologie und Pathophysiologie,Universität 3400 Göttingen, Humboldtallee 7 - FRG

139

QUANTIFICATION OF ISCHEMIC EPICARDIAL AREAS BY DESK-COMPUTER ASSISTED IMAGE ANALYSIS.

R.Rösen, B.Panzner, and W.Klaus

In electrically driven, Langendorff perfused rabbit hearts local myocardial ischemia was induced by ligation of a branch of the left coronary artery. Epicardial NADH-fluorescence was monitored by photographing fluorescence after flashlight excitation (Xe-lamp; UV-filter; 100 Joules; 120 µsec). The flash was synchronously triggered by the electrical stimulation of the heart. Pictures were taken with a camera (Rolleiflex) equipped with a 435 nm cut-off filter in front of the lenses (Kodak Tri-X pan, 27 DIN or Polaroid Typ 667, 36 DIN). Ischemic areas could be easyly identified by enhanced NADH-fluorescence. The pictures were digitized with respect to grey levels (grey-scale 0-255) into 256 x 256 pixels using a video A/D-converter (Hamamatsu) and stored on floppy disk for further data-processing with a desk-computer (Apple). Distribution curves for the grey levels of all pixels or of selected areas were evaluated. The grey levels of the ischemic area were determined. X- and Y-coordinates of the pixels with selected grey values were estimated and topographic maps were plotted. The number of these pixels directly corresponds to the ischemic area. Data-processing was performed with fast machine routines embedded in BASIC-programs, by this procedure the processing time was kept short.
The precision of the A/D-converter was very good. Digitizing the same picture for 5 times a variability in the number of pixels of ≦ 10% for a single or ≦ 5% (S.D.) for the sum of 4 grey levels was found.
Supported by DFG SFB 68/A18
Pharmakol.Inst.Uni Köln,Gleuelerstr.24,5 Köln 41

140

CORONARY REACTIVE HYPEREMIA: COMPARISON OF HYPOXEMIA AND OCCLUSION

A. Deussen

In 11 anesthetized,vagotomized dogs the left anterior descending coronary artery (LAD) was cannulated and perfused under constant pressure (88±4mmHg).The reactions of LAD-flow during a 60s-perfusion with tyrode and deoxygenated blood (O_2-content 2.6±0.6ml/100ml) were compared with the peak LAD-flow following a 60s occlusion.
Occlusion and perfusion with deoxygenated blood decreased left ventricular performance slightly. Perfusion with tyrode increased peak left ventricular pressure by 10% and dP/dt_{max} by 15%. The reactions of LAD-flow (ml/min·100g) are summarized:

	occlusion	deox.blood perfusion	tyrode perfusion
control	60±4	58±4	63±7
60s	0	202±28	425±65
10s after intervention	140±12	141±18	246±35

The difference between the flow increases during perfusion with deoxygenated blood and perfusion with tyrode is explained mainly by the low viscosity of tyrode and in addition by the increase of left ventricular performance. The comparable flow increases after perfusion with deoxygenated blood and occlusion indicate that coronary blood flow may increase maximally with arterial hypoxemia even without a stasis of dilating metabolites.

Physiologisches Institut I, Universität Düsseldorf, Moorenstr. 5, 4000 Düsseldorf 1

141

THE OCCLUSION OF A CORONARY ARTERY INCREASES THE EXTRAVASCULAR RESISTANCE OF AN ADJACENT CORONARY ARTERY.

J.D.Schipke

In 6 anesthetized dogs the left circumflex coronary artery was cannulated and perfused at a constant pressure of 113±3 mmHg. The left ventricular peak pressure (LVP), left ventricular enddiastolic pressure (LVEDP), dP/dt_{max}, dP/dt_{min}, systolic fiber shortening in the circumflex-perfused myocardium (SS_{CX}), mean circumflex coronary resistance (R_{CX}) and enddiastolic circumflex coronary resistance (R_{ED}) were measured. The reactions to a 60 s occlusion of the left anterior descending coronary artery during autoregulation and during maximal dilation of circumflex coronary artery by intracoronary infusion of adenosine were compared:

	AUTOREGULATION		ADENOSINE	
	0	60s	0	60s
LVP (mmHg)	99	95+	98	93+
LVEDP (mmHg)	6.4	8.1+	6.1	9.0+
dP/dt_{max} (mmHg/s)	1650	1500+	1600	1400+
dP/dt_{min} (mmHg/s)	1550	1300+	1500	1150+
R_{CX} (mmHg·min·100g/ml)	1.53	1.28+	0.52	0.56+
R_{ED} (mmHg·min·100g/ml)	1.05	0.90+	0.38	0.42+
SS_{CX}(%)	8.0	10.6+	8.5	10.0+

+p<0.05 vs. control

These data indicate that the intercoronary resistance decrease of autoregulating coronary arteries (A.Deußen, G. Heusch; Pflüg.Arch.Suppl.394,R16,1982) is reversed to a resistance increase in arteries with exhausted dilatory reserve. This increase of extravascular coronary resistance is mediated by increased systolic performance of the nonoccluded myocardial area as well as by an impaired relaxation of the left ventricle.

Physiologisches Institut I, Universität Düsseldorf
Moorenstr. 5, D-4000 Düsseldorf

142

CHANGES IN MYOCARDIAL UPTAKE OF LACTATE, GLUCOSE, AND FREE FATTY ACIDS (FFA) DURING SHORT-TERM CORONARY OCCLUSION+)

Th. Burdorf

On open-chest mongrel dogs (n = 11) repeated short (3 min) ischemia of relatively large parts of the myocardium was produced by proximal, intermittent ligation of the LAD artery. The vessel was occluded 1 to 3 times with recovery intervals of 45 min. Just before beginning and at the end of the occlusion and after 5 min of reperfusion arterial and coronary venous blood samples were simultaneously collected. Additionally during the first minute of reperfusion 5 ml of arterial and coronary venous blood were withdrawn by syringe-pumps. The blood samples were assayed for lactate and glucose enzymatically and for FFA by gas chromatography.
Under control conditions oxygen extraction ratios (OER) were about 60 % for FFA, 26 % for lactate and 4 % for glucose. During occlusion the arterio-venous difference (AVD), extraction (EX), extraction ratio (EXR) and OER of lactate decreased significantly. The AVD and EXR of FFA increased significantly accompanied by a slight but not significant increase of EX and OER due to the marked reduction of coronary venous O_2-saturation. The reactive hyperaemia in the first minute of reperfusion exerted a release of ischemia induced lactate and a marked increase in FFA-EX (+ 44 %) and FFA-OER (+ 33 %), while EXR and AVD of FFA returned to preocclusion values. After 5 min of reperfusion no further lactate release was seen but FFA-OER and FFA-EX remained slightly enhanced compared to control. Under all conditions there was no significant changes in the patterns of the individual FFA in the arterial or venous blood. It was demonstrated that during reactive hyperaemia the additional O_2-uptake and washout of lactate are accompanied by an increased FFA-uptake. These findings are discussed in view of metabolic and neurohumoral aspects of regional myocardial hypoxia.

Zentrum Physiologie und Pathophysiologie, Universität Göttingen, Humboldtallee 7, D-3400 Göttingen
+)Supported by the DFG, SFB 89 - Kardiologie Göttingen

143

CATECHOLAMINE INDUCED CARDIAC NECROSES ARE MEDIATED BY THROMBOCYTES

H. Kammermeier and M. Ober

According to our earlier observations catecholamines do not have deleterious effects in isolated hearts and therefore were discussed to induce necroses in intact animals by mediation of other mechanisms, e.g. the hemostatic system (Pflügers Arch. 349, 325 (1974). Thus influence of thrombopenia on the occurrence of necroses was investigated.

Methods: Administration of antithrombocyte serum (rabbit) 4 h prior to isoproterenol (40 mg/kg) in Sprague Dawley rats (female). Fixation of the hearts by formaldehyde 9 h after isoproterenol administration. Evaluation of 30 section of each heart (prependicular to axis) with respect to area and number of necroses.

Results: The total number and area of necroses are highly significantly reduced in thrombocytopenic rats to 34% and 23% (N = 8) resp. as compared to controls (number 426 ± 65 (SD), area 578 ± 77 · 10^3 μm^2/heart).

The results indicate that microcirculatory alterations are essential factors for the formation of catecholamine induced cardiac necroses.

The indispensable cooperation of Prof. Dr.Dr. G. Gillissen and Dr. K. Schweizer (Abt. Mikrobiologie), Prof. Dr. Chr. Mittermayer and Dr. H. Richter (Abt. Pathologie) and the support by the DFG (SFB 109) is gratefully acknowledged.

Abt. Physiologie der RWTH Aachen, Schneebergweg 211, D-5100 A a c h e n

144

EFFECTS OF CARDIAC SYMPATHETIC NERVE STIMULATION (CSNS) ON PERFUSION OF STENOTIC CORONARY ARTERIES. Gerd Heusch

The left inferior cardiac nerve was stimulated in 22 anesthetized, vagotomized dogs (1) with intact coronary arteries, (2) with an intermediate stenosis and (3) with a critical stenosis on circumflex coronary artery.Stenoses were produced by a wire snare and defined as intermediate by the reduction of peak reactive hyperemia repayment following a 15s occlusion from 430±70 to 130±10% of control blood flow;a critical stenosis was defined by only 20±3% reactive hyperemia repayment.CSNS decreased the resistance of the intact circumflex coronary artery from 1.20±0.18 to 0.90±0.09 mmHg·min·100g/ml ($p<0.01$) and the resistance of the moderately stenosed artery from 1.35±0.21 to 1.16±0.19 mmHg·min·100g/ml ($p<0.01$).With a critical stenosis, CSNS increased the coronary resistance from 2.31±0.31 to 2.90±0.49 mmHg·min·100g/ml ($p<0.01$).This stimulation resulted in net lactate production of the circumflex-perfused myocardium;4 dogs (18%) died by ventricular fibrillation.There was a significant hyperbolic correlation of the CSNS-induced change of coronary resistance to the degree of coronary hyperemic reserve (r=0.86).Phentolamine (2mg/kg i.v.) decreased the resistance of the severely stenosed coronary artery by 35% and prevented the resistance increase upon CSNS as well as the signs of myocardial ischemia. Conclusion: there is a continous unmasking of α-constrictive influences with increasing severity of a stenosis and thus decreasing coronary reserve. This α-vasoconstriction can induce myocardial ischemia distal to severe coronary stenoses.

Physiologisches Institut I, Univ. Düsseldorf

145

CULTURED ADULT HEART CELLS DURING ANOXIA AND REOXYGENATION [+)]

H.M. Piper, P. Schwartz

Under cell culture conditions progressing alterations in anoxic myocardial cells can immediately be followed by biochemical and morphological means. Ca stable adult cardiac ventriculocytes from rat were enriched under cell culture conditions (JMCC 14 (1982), 397) to almost 100 percent of intact cells and incubated in glucose free anoxic Tyrode solution up to 120 min. The influence of pH changes was excluded by high buffering capacity of the incubation medium.

The time course of metabolic changes depends on the relation of cell number to incubation volume: the smaller the volume the faster anoxic damage develops. After 60 min of anoxia ATP is only one third of control, lactate production and enzyme release (MDH) are slowing down. Lactate is produced from glycogen, but only half of the present can be used. Only 10 percent of total cellular MDH activity are released after 120 min, when ATP is at most one tenth of control. Loss of lactate production ability and MDH release both are highly correlated to ATP decay (r=0.98). Early onset of MDH release indicates that enzyme release is caused already by minor energetic disturbances. Energy turnover has declined to one tenth of aerobic control values after 60 min of anoxia.

Reoxygenation results in extensive recovery of CP, but ATP can only reach the value of total adenine nucleotides before reoxygenation. Electron micrographs reveal characteristic ultrastructural changes during anoxiy and partial recovery after reoxygenation.

These findings indicate that development of anoxic damage in cultured myocytes proceeds basicly as in whole ventricular tissue. The major difference consists in prolonged CP and ATP decay, due to mechanical rest of isolated ventriculocytes.

Zentrum Physiologie und Pathophysiologie, Abteilung Herzstoffwechsel, Universität Göttingen, Humboldtallee 7 D-3400 Göttingen

+) Supported by the DFG, SFB 89 - Kardiologie Göttingen -

146

EFFECT OF POCA, A NEW ACYLCARNITINTRANSFERASE BLOCKING AGENT, ON MYOCARDIAL FATTY ACID OXIDATION[+)]

J.F. Hütter, P.G. Spieckermann

Nonesterified fatty acids (NEFA) are oxidised after being transfered into mitochondria by acylcarnitintransferase. A possible way to change energy metabolism from NEFA to carbohydrate oxidation is to inhibit this transport system. POCA (sodium 2(5-(4-chlorphenyl)-pentyl)-oxiran-2-carboxylat) and analogous substances have been shown to work in this way. As NEFA oxidation is acompanied by a higher oxygen consumption than carbohydrate oxidation, this could be a useful attempt to save oxygen in situations of oxygen deficiency. The effect of POCA was tested on a computer assisted working rat heart. The RQ was used as a measure for the percentage of oxygen consumption which is attributable to NEFA oxidation.

It was shown that the relation between different albumin and NEFA concentrations and the resulting rate of NEFA oxidation can be described by a simple mathematical equation, which was derived under the assumption of an albumin-receptor mediated NEFA uptake. As POCA was found to be a competitive inhibitor, the rate law for NEFA oxidation (OXI) can be derived:

$$OXI = OXI_o \cdot [FA] \; / \; ([ALB] + Km \cdot (1 + [I] \; / \; Ki))$$

Where OXI_o, Km and Ki are constants, [FA], [ALB] and [I] are the concentrations of NEFA, albumin and the inhibitor POCA respectively. This relation was tested with different NEFA, albumin and POCA concentrations. The results obtained experimentally were found to be in good agreement with the rates calculated with the rate law.

Zentrum Physiologie und Pathophysiologie, Abteilung Herzstoffwechsel, Universität Göttingen, Humboldtallee 7, D-3400 Göttingen

+) Supported by the Deutsche Forschungsgemeinschaft, SFB 89 - Kardiologie Göttingen -

147

CHANGES IN FUNCTIONAL AND METABOLIC PARAMETERS IN THREE MODELS OF RAT HEART HYPERTROPHY

H.-G.Zimmer

There are only a few reports on heart function in intact small laboratory animals during the development of cardiac hypertrophy (Beznak,M., J.Physiol. 116, 74-83, 1952; Circ.Res. 6, 207-212, 1958). Therefore studies on left ventricular hemodynamics were performed in rats with three types of cardiac hypertrophy. Heart rate (HR), left ventricular systolic pressure (LVSP) and the maximal rate of rise in left ventricular pressure (LV dP/dt_{max}) were measured with an ultraminiature catheter pressure transducer which was inserted via the right carotid artery (Zimmer,H.-G., J.Mol.Cell.Cardiol. 14, 479-482, 1982). Cardiac hypertrophy was induced by constriction of the abdominal aorta to a final diameter of 0.65 mm, by administration of a single high dose of DL-isoproterenol-HCl (ISO, 25 mg/kg, s.c.) or by daily injections of 3,3',5-triiodo-L-thyronine-sodium salt (T_3, 0.2 mg/kg, s.c.). After aortic constriction, HR was decreased, whereas LVSP and LV dP/dt_{max} were both elevated by about 20% and 12%, respectively. Within 12 hours after ISO administration, HR and LV dP/dt_{max} were markedly increased, while LVSP was depressed by about 20%. In T_3-treated animals, all hemodynamic parameters were elevated during the first 72 hours. Comparison with the time course of changes in metabolic parameters revealed that the rise in LV dP/dt_{max} occurred at about the same time as the increase in the content of myocardial cyclic AMP. However, in all three hypertrophy models studied, cardiac contractility was enhanced before the maximal stimulation of myocardial adenine nucleotide and protein synthesis was observed.

Physiologisches Institut der Universität München, Pettenkoferstr. 12, D-8000 München 2

Supported by grants from the Deutsche Forschungsgemeinschaft (Zi 199/1-3, Zi 199/4-3)

148

MYOCARDIAL HYPERTROPHY AND FUNCTION AFTER LONG-TERM ANTIHYPERTENSIVE THERAPY WITH HYDRALAZINE, METOPROLOL AND NIFEDIPINE.

G.Ringsgwandl,W.Motz,H.Meyrl,B.E.Strauer

We investigated in SHR the influence of 40 weeks of therapy with hydralazine(H,15mg/kg/day),a combination of metoprolol(M)(50mg/kg/day)and H(M+H)and nifedipine(N,30 mg/kg/day)on blood pressure(BP),heart rate(HR),left ventricular(LV)-to-body weight-ratio(HW/BW), cardiac index(CI) and ejection fraction(EF).Therapy began at the age of 4 weeks,untreated SHR of equal age served as controls(Co). The contractile properties of isolated actomyosin fibers(AMF)from LV papillary muscles were determined as maximal force per mm^2 of cross sectional area(fiber stiffness ST, dynx100/mm^2)and velocity constant VC(s^{-1})of the stretch-induced isometric contraction,which is regarded as a measure of myosin cross bridge rotation rate.

All drugs lowered systolic BP from(mean)210+10 to 140+8mm Hg except M,which reduced BP only slightly.HR was slightly elevated in H(441),unchanged in N and M+H and decreased in M(295vs375 in Co).HW/BW was reduced under H,M+H and N,but not after M-therapy.LV-EF was increased in N,was normal in N and H(64%,66%), but reduced in M and M+H-therapy(55%, 57%).CI was slightly elevated in N and H, but reduced from 191ml/kg/min(Co) to 134 in the M-group.

VC was $20s^{-1}$ in the N-treated group,18 in the H-group,and 17 in the M+H-group vs. $16s^{-1}$ in Co(p 0.05) indicating a significantly faster velocity of isometric contraction of isolated contractile proteins in myocardium under antihypertensive therapy. There was no diffenrence in ST betweenthe three treatment groups and Co indicating equal force development per unit cross sectional area of myocardium.

We conclude that longterm antihypertensive therapy not only prevents the development of LV hypertrophy without impairing cardiac function, but also prevents the development of the alterations in contractile protein function observed in myocardium under untreated hypertension.

Med.Klin.I d.Univ.München,Marchioninistr.15, D-8000 München-70,West-Germany

149

THE RELAXING ACTIVITY OF A NEW STABLE PROSTACYCLINE (ZK 36 374) ON BOVINE CORONARY AND PULMONARY ARTERIES OF DIFFERENT AGE

H.-W.M. Breuer, J.M. Soriano Romero, R. Meschig, and G. Arnold

The aim of our study was to find out whether the effect of a stable prostacycline (carbacycline derivative ZK 36 374 (5-(E)-(1S,5S,6R,7R)-7-hydroxy-6-[(E)-(3S,4RS)-3-hydroxy-4-methyl-1-octen-6-inyl]-bicyclo[3.3.0]-octan-3-yliden-pentanoic acid), (Schering), is dependent on the animal's age and the origin of the investigated vessel.
Experiments were done on helicoids of bovine coronary and pulmonary arteries under isometric conditions. The resting tension of the helicoids was adjusted to 15-25 mN. Doses from $2x10^{-11}$ M up to $2x10^{-5}$ M were applicated to the strips. We formed two groups: animals younger than half a year (y) and animals older than 2 years (o).

Results:

	n	EC_{50} (µM)	SLOPE [50/log EC_{80}-log EC_{30}]	DECREASE IN TENSION (mN)
cor. art. (y):	64	0.100	24.32	8.99 ± 1.94
cor. art. (o):	62	0.119	27.03	10.85 ± 1.69
pul. art. (y):	42	0.0028	24.39	0.87 ± 0.06
pul. art. (o):	35	0.0022	25.00	1.20 ± 0.11

Conclusions: 1.: ZK 36 374 relaxes dose dependent bovine coronary and pulmonary arteries. 2.: There are no significant differences in the response of proximal, medial and distal segments of the investigated left coronary arteries (anterior descending and circumflex branch). 3.: There are no significant differences between the helicoids of young and old animals. 4.: The EC_{50} of the pulmonary arteries is significant smaller than the EC_{50} of the coronary arteries ($p<0.05$). The slope is similar. 5.: The decrease in tension of the coronary arteries is 9.6 times greater than the decrease in tension of the pulmonary arteries.

Institut f. Exp. Chirurgie der Universität Düsseldorf, Universitätsstr. 1, D-4000 Düsseldorf

150

INHIBITION OF MYOCARDIAL NECROSIS IN CORONARY LIGATED RATS BY DEXAMETHASONE.

W. Bernauer.

An important goal in the treatment of acute myocardial infarction is to prevent, as far as possible, the necrosis of ischemic myocardial tissue. Drugs suited for this purpose should decrease the percentage of ischemic heart muscle which becomes irreversibly damaged. In rats with coronary ligation it was investigated whether dexamethasone has such an effect. We developed a method to determine the relation of the ischemic area/area of necrosis. The extent of the ischemic area was calculated after perfusion of the coronary system with Evans blue, extraction and photometric determination of the dye. For the determination of the extent of the necrotic area the hearts were incubated in triphenyltetrazolium chloride containing phosphate buffer. Normally, the myocardial dehydrogenases convert the tetrazolium salt to the red formazan. In necrotic myocardium this ability is lacking. The determination of the formazan formation allowed to calculate the extent of the necrotic area and, hence, the percentage of ischemic myocardium which was necrotic. This percentage clearly increased with the time after the coronary ligation, and amounted to 56 ±1o.4 after 5 hours. Dexamethasone orthophosphate, 1.2 mg/kg given i.v. 20 min before and 2.5 h after coronary ligation, decreased this percentage at 5 hours significantly to 26 ± 6.5. Simultaneously, the lethality within the 5 hours after coronary ligation was significantly decreased from 81.8 % to 28.6 %.

Department of Pharmacology, University of Freiburg, Hermann-Herder-Str. 5, D-7800 Freiburg, Federal Republic of Germany.

151

EFFECTS OF 2-NICOTINAMIDOETHYLNITRATE (SG 75) AND ISOSORBID DINITRATE (ISDN; ISOKET^R) ON ISCHEMIC STRESSED MYOCARDIUM DURING SHORT-TERM CORONARY ARTERY OCCLUSION+)

H. Korb

The purpose was to compare the effects of two antianginal and preload reducing compounds, SG 75 and ISDN, on ischemic stressed myocardium.
Studies were carried out on 11 mongrel-dogs in conventional open-chest preparation in a model with short-term (3 min) repeated ligation of the LAD, allowing the intra- and interindividual comparison of control to therapy occlusions.

SG 75 (0.2 mg/kg bw, 10 min before each occlusion) caused a significant increase of O_2-debt during the occlusion period (+ 55.4 %; $p < 0.001$) combined with reduced amounts of inorganic phosphate (- 31.7 %; $p < 0.01$) and lactate (- 15.8 %; $p < 0.01$) released in the first minute of reperfusion.
Isoket^R (0.3 mg/kg bw; 30 min before each occlusion) led to a significant decrease of O_2-debt (- 43.12 %; $p < 0.01$) whereas lactate remained unchanged.
The released amounts of potassium were significantly increased by SG 75 (+ 34.3 %; $p < 0.01$) and by Isoket^R (+ 16.8 %; $p < 0.01$). The additional uptake of oxygen in the early reperfusion period was not affected.

From these data it becomes evident that the drugs, reported to exert protective actions in ischemia, influence the metabolic and energetic situation of ischemic stressed myocardium in a different extent and way. The mechanisms underlying these effects remain to be fully explored.

Zentrum Physiologie und Pathophysiologie, Universität Göttingen, Humboldtallee 7, D-3400 Göttingen

+) Supported by the Deutsche Forschungsgemeinschaft, SFB 89 - Kardiologie Göttingen -

152

COMPARATIVE HEMODYNAMIC PROPERTIES OF ISOSORBIDE DINITRATE (ISDN) AND ISOSORBIDE-2-MONONITRATE (IS-2-MN) IN RATS AND DOGS, AND ANTIANGINAL, PHARMACOKINETIC STUDIES WITH IS-2-MN IN RATS.

M.Leitold, H. Laufen, W. Fleissig

The effect of IS-2-MN was compared with that of ISDN in rats regarding the antianginal, hemodynamic and pharmacokinetic properties and in dogs regarding hemodynamic properties. The antianginal activity of IS-2-MN in equal oral dose persisted two times longer in the rat than did ISDN. In the same oral dosage, the two compounds caused the equal reduction of the number of deaths after an acute myocardial infarction. The intravenous or enteral administration of a single IS-2-MN dose caused an increasingly dose-dependent reduction of the systolic and mean arterial blood pressure in anaesthetized rats. The amounts of IS-2-MN required for the purpose were identical for both kinds of application. In conscious chronically instrumentated dogs ISDN and IS-2-MN lowered the systolic blood pressure by 20 mmHg after intravenous continuous infusion in a concentration of 100 µg/kg/min or in a concentration of 400 µg/kg/min. The drop of the mean arterial blood pressure due to ISDN and IS-2-MN in anaesthetized dogs was 15 times or 4 times weaker after intraportal bolus administration than it was after intravenous application. The hypotensive effect of ISDN or IS-2-MN could be antagonized by pretreatment of rats or dogs with i.v. dihydroergotamine (DHE). The complete bioavailability of IS-2-MN has been confirmed by comparison of plasma levels after oral and intravenous application in rats. The half-life of IS-2-MN in the rat was unchanged after increasement of the oral doses and was 3.6 times shorter than in humans. The accumulation of IS-2-MN found in the wall of arteries and veins in rats 10, 60, 240 minutes after i.v. application explain the dilating effect of the drug on the vascular system.

Dr.M.Leitold, Dept. of Pharmacology, Heinrich Mack Nachf., Chem.-pharm. Fabrik, Postfach 2064, D-7918 Illertissen.

153

INFLUENCE OF DIHYDROERGOTAMINE (DHE) ON FUNCTIONAL CHANGES CAUSED BY ß-ADRENERGIC STIMULATION IN THE NORMAL AND IN THE UNDERPERFUSED CANINE MYOCARDIUM.

R.Seitelberger, O.Schlappack, G.Raberger

The study was carried out in 6 anesthetized mongrel dogs. Regional myocardial segment shortening was measured using two pairs of miniaturized piezoelectric ultrasonic transducers which were inserted both in the normal and in the underperfused part of the myocardium. Under control conditions bolus i.v. injection of Isoproterenol (0.5 µg/kg) induced a marked decrease of enddiastolic segment length and an increase of segment shortening over a period of 3 to 5 minutes in both segments. After constriction of the R. circumflexus Isoproterenol induced an initial decrease of enddiastolic segment length and a small increase in segment shortening, which was followed by a fast return of enddiastolic segment length to control values and a distinctive decrease of segment shortening in the underperfused area, indicating regional myocardial dysfunction. Function in the normal myocardium was not altered by constriction of the R. circumflexus. Dihydroergotamine (2 µg/kg infused over a period of 5 minutes) prevented this functional deterioration, leading to a contraction pattern comparable to data assessed simultaneously in the normal area or in the underperfused area before constriction. This amelioration of myocardial function is seen over the whole experimental period of 60 minutes. It is concluded that Dihydroergotamine 2 µg/kg improves Isoproterenol induced dysfunction in the underperfused myocardium.

Pharmakologisches Institut der Universität Wien, A-1090 Wien, Währinger Straße 13a.

154

SMOOTH MUSCLE CELL PROLIFERATION IN THE INTIMA OF CHRONICALLY STIMULATED ARTERIES

W.Schlote and E.Betz

The space between the basement membrane of the endothelial lining of carotid arteries and the internal elastic lamina (IEL) of normal rabbits is free of cells. Mechanical or electrical stimuli applied to the adventitial side of a carotid artery cause a migration of smooth muscle cells (SMC) from the inner layer of the media through the IEL into the subendothelial space. Here they transform into a SMC-type which contains more organelles and less contractile fibres. The cells, however, are still able to exhibit contractions. In the intima the cells undergo mitotic division the extent of which depends on the intensity and the duration of the stimulation. If the stimuli are weak the endothelial lining remains in place and it is not destroyed.

The migrated cells arrange longitudinally along the axis of the vessel. Their length does not differ from that of normal SMC. Their diameter is less than that of media cells. If the stimulation is continued for some weeks the thickness of the proliferate increases considerably so that the vessel lumen becomes narrow. If the stimulating electrodes are removed after 4 - 6 weeks of repeated stimulation and the animal is subsequently kept under normal conditions for several months, the SMC rearrange preferentially at the luminal side into the normal spiral or circumferential pattern. They retransform in many instances into regular myocytes. They regain the capability to built up new orderly arranged internal elastic laminae. This is estimated to be an expression of an adaptation.

Abt.Submikroskop.Pathologie u.Neuropathologie und Physiologisches Institut(I),Universität Tübingen,Liebermeisterstr.8 bzw.Gmelinstr.5,D-7400 Tübingen 1

155

OESTRADIOL INDUCES STRUCTURAL DILATION OF UTERINE RADIAL ARTERIES IN GUINEA PIGS

W. Moll, R. Götz und S. Klappstein

Oestradiol benzoate (1-10 µg) induces a dose dependent dilation of uterine arteries as shown by in situ photograms: In dioestrous animals, the internal diameter increases from 130±10 µm to 210±3o µm ($\bar{x}$±SD) during the first 48 h after the injection on 10 µg. A similar effect was seen in ovarectomized animals. During the oestrous cycle, dilation of radial arteries occurs 2 d after a reported rise of plasma oestradiol, indicating that oestradiol plays an important role in the lumen control of these arteries. In order to study the mechanism of oestradiol induced dilation, the external arterial diameter was measured also in vitro in the presence of papaverine (4o mg/l). At the physiological intraarterial pressure of 6o mm Hg, the diameter was found to be the same in vitro and in situ: The diameter was 22o±2o µm and 300±3o µm in vitro and 200±2o µm and 310±3o µm in situ in untreated animals and 48 h after the injection of 10 µg oestradiol benzoate respectively. This finding shows that the dilation is not due to smooth muscle relaxation.- The time course of dilation was compared with that of weight increase and DNA synthesis (3-H-thymidine incorporation). A significant dilation was seen 18 h after the injection while weight and DNA synthesis were yet unchanged at this time indicating that, at this stage, the dilation is not based on arterial hyperplasia.- It is concluded that oestradiol dilates the uterine arteries by reducing the restraint of arterial connective tissue.

Institut für Physiologie, Universität Regensburg Postfach 397, D-8400 Regensburg.

156

THE INCREASE OF THE DEFORMABILITY OF ERYTHROCYTES INDUCED BY GLUCOSE IN VIVO

W.K.R. Barnikol°, D.P.F. Möller+, O. Burkhard•

When trying to assess the deformability of erythrocytes with the aid of filterability measurements, we got very unreproducible results. Only after quantitative separation of the thrombocytes and leucocytes from the erythrocytes [Pflügers Archiv 394 (1982) R 19] we got satisfying results. Studies with the improved method gave indication, that the deformability of the erythrocytes depends on the dietetic state of the probands. To study the phenomenon systematically, we carried out two kinds of experiments. First the test of glucose tolerance : We applied after a 20 hour abstinence of food 100 g glucose and measured afterwards hour by hour the relevant quantities. Second dietetic experiments : After a 12 hour abstinence of food, we measured immediately before and 1.5 hours after a breakfast the relevant quantities. The relevant quantities were the specific filtration time [see above Pflügers Archiv 394] , the viscosity of packed erythrocytes, the concentration of glucose in the plasma, the content of erythrocytes of ATP, of 2,3 diphosphoglycerate and of calcium. We found, that after applying 100 g glucose the viscosity and the specific filtration time decrease by approximately 30 % . The effect has a delay of 2 - 3 hours. Simultaneously the content of 2,3 diphosphoglycerate does not change significantly. The content of ATP increases and the content of calcium decreases. The consequences of our results with respect to normal nutritive perfusion are discussed, also the malfunction of the effect as a reason for disturbances of substrate supply to tissue.

° Physiologisches Institut der Universität Mainz, Saarstr.21, 6500 Mainz ; + Physiologisches Institut der Universität Bonn, Nußallee 11, 5300 Bonn; • Hämatologische Abteilung der Universitätskliniken Mainz, Langenbeckstr.1, 6500 Mainz, Bundesrepublik Deutschland

157

INFLUENCE OF 2,3-DIPHOSPHOGLYCERATE ON THE O_2-UPTAKE OF AN ISOLATED RABBIT HINDLIMB PERFUSED WITH HUMAN RED CELLS

K. Strein, I. Jaeschke, R. Jaeschke and J. Runge

The O_2-uptake ($\dot{V}_{O_2}$) of an organ depends besides other parameters on the O_2-dissociation curve (ODC). This curve is influenced by the concentration of 2,3-diphosphoglycerate (DPG) in the red cells (erys).
To examine the importance of DPG on the $\dot{V}_{O_2}$ an isolated rabbit hindlimb was perfused with human erys at different DPG concentrations. The erys were prepared by incubation of stored erys with inosine, phosphate and pyruvate. The erys from every donor were divided in two samples and the DPG-content was varied by different incubation times. After washing the erys were suspended in a buffer solution containing albumin and glucose. The hematocrit was adjusted to 20 %. The hindlimb was alternately perfused with the high and low DPG-erys-suspension in the sequence high-low-high-low or reverse. We used a constant flow of 6 ml/min. pH, p_{O_2} and p_{CO_2} were the same in both perfusates.
In a first series of experiments, erys-suspensions with mean DPG concentrations of 2.5 and 20.3 mmol/l erys were compared. The $\dot{V}_{O_2}$ was about 40 % higher when DPG was elevated.
In a second series with mean DPG of 5.5 and 8.1 mmol/l erys $\dot{V}_{O_2}$ was about 9 % higher with the elevated DPG. This difference in DPG caused a shift of the ODC to the right of about 4 mm Hg.
An increase of the DPG concentration produces an elevation of $\dot{V}_{O_2}$ under our experimental conditions.

Pharmakologische Laboratorien der Boehringer Mannheim GmbH, Sandhofer Straße 116, D-6800 Mannheim 31

158

THE RESETTING OF THE RELATIONSHIP BETWEEN LOCAL GLUCOSE UTILIZATION AND BLOOD FLOW IN THE BRAIN DURING GAMMA-HYDROXYBUTYRATE AND ITS POSSIBLE EXPLANATION.

W. Kuschinsky, C. Haller, S. Suda, L.Sokoloff

Local cerebral glucose utilization (LCGU) was quantified using the [^{14}C]deoxyglucose method (Sokoloff,L. et al., J.Neurochem.28, 897, 1977) and local cerebral blood flow (LCBF) by the iodo[^{14}C]antipyrine method (Sakurada,O. et al., Am.J.Physiol.234, H59, 1978). LCGU in the brain structures tested correlated well with the respective LCBF during control conditions (r=0.96) and during the action of gamma-hydroxybutyrate (GHB) (r=0.84). Due to a reduced global glucose utilization (-51%) at a grossly unchanged global blood flow (+12%) during GHB, the steepness of the correlation between LCGU and LCBF was increased. The mechanism of this resetting of the relationship between LCGU and LCBF during GHB was investigated by analyzing the cerebrovascular reactivity to changes in extravascular pH during control conditions and during GHB using the microapplication technique (Haller,C., Kuschinsky,W. Microcirculation 1, 141, 1981). During GHB, the slope of the concentration response curve for pH was increased thus indicating an enhanced sensitivity of pial arteries to changes in perivascular pH. Microapplication of GHB to pial arteries did not alter vascular diameter. This increased vascular reactivity during GHB may be responsible for the resetting of the metabolism/blood flow couple.

Lab. of Cerebral Metabolism, NIMH, Bethesda, Md. 20205, USA, Physiolog. Inst. d. Univ., Pettenkoferstr.12, D-8000 München 2, Physiolog. Inst. d. Univ. Nussallee 11, D-5300 Bonn 1
W.Kuschinsky was supported by a grant from the Deutsche Forschungsgemeinschaft

159

EFFECT OF AMPHETAMINE ON POWER OF THE ELECTROCORTICOGRAM, EXTRACELLULAR K^+ ACTIVITY, MICROFLOW AND LOCAL Po_2 OF THE BRAIN CORTEX IN THE CAT.

E.Leniger-Follert

This study was designed to contribute to the question of why extracellular cortical K^+ activity $[K^+]_o$ failed to increase after amphetamine injection as reported by Astrup et al. (1978), although Berntman et al. (1978) had registered an increase in cerebral blood flow (CBF) in rats after administration of amphetamine. Astrup et al. concluded from their results that $[K^+]_o$ cannot be a main factor in the control of CBF during activation. In our study amphetamine was twice injected in 13 cats and changes in ECoG, $[K^+]_o$, microflow and local Po_2 were continuously recorded together with changes in arterial blood pressure. The results show that microflow and local Po_2 only increased in those animals where a strong increase in arterial blood pressure occurred. $[K^+]_o$ remained constant independently from the behavior of blood pressure. Power analysis of the ECoG clearly showed that no real activation occurred in the brain cortex. Thus, we may state that the missing increase in cortical $[K^+]_o$ after amphetamine injection is no evidence against the assumption that $[K^+]_o$ is an important factor in the coupling of neuronal activity and cortical blood flow during activation.

Max Planck Institut für Systemphysiologie Rheinlanddamm 201, D-4600 Dortmund 1

160

BLOOD FLOW THROUGH MALIGNANT TUMORS DURING HEMODILUTION

P. Vaupel, C. Jung

Due to an inadequate and heterogeneous blood flow through most malignant tumors, the therapeutic efficiency of antitumor drugs and/or of a radiation treatment is already limited in early stages of tumor growth. One way to overcome these difficulties may be the use of a controlled hemodilution in order to improve the nutritive blood flow and, hence, the microenvironmental conditions of the cancer cells.
Material and Method: In order to study the effect of a controlled hemodilution on tumor blood flow (TBF) and on the supply conditions to the tissue, resp., experiments are performed using two different tumor models in rats:
(i) in situ perfusion of tissue- isolated tumors in the left kidney (DS- Carcinosarcoma), and
(ii) measurement of regional TBF in Yoshida sarcomas implanted at the hind foot dorsum using the 85-Kr(ß)- clearance technique.
Results: A controlled hemodilution distinctly improves blood flow through tumors. During in situ perfusion of tissue- isolated tumors, e.g., with decreasing hematocrit from 0.44 to 0.14, a 2.5-fold increase in TBF results if the perfusion pressure is kept constant. In this case, the flow improvement is paralleled by an increase in the oxygen consumption of the tumor tissue.
Conclusions: The results obtained are indicative for an improvement of nutritive tumor blood flow and, hence, of the metabolic status of the tumor tissue. As a consequence, the therapeutic efficiency of the modalities mentioned above should be enhanced.

Dept. of Physiology, University of Mainz, Saarstr. D- 6500 Mainz 1, W.- Germany

161

BLOOD HYPERVISCOSITY DURING MUSCULAR ISCHEMIA DUE TO ACCUMULATED LYSOPHOSPHOGLYCERIDES.

H. Rogausch, F. Steinhauer

In human healthy volunteers the blood viscosity increases during forearm ischemia of 3- 10 min duration. The increased viscosity cannot be satisfactorily explained by local processes, since in some of the experiments the viscosity also increases in the non-ischemic contralateral arm. We found an increased enzymatic activity of the plasma in the venous blood of both arms which lead to the accumulation of lysophosphoglycerides (lyso-P) and spiculated erythrocytes which contribute to the increased viscosity.
In another experiment, we were able to produce spiculated erythrocytes and an increased concentration of lyso-P by intravenous injection of norepinephrine (NE; 2-8 µg/ kg . min).
The data support the view that NE stimulates the production of lyso-P, and that this affects the erythrocytes and that these erythrocyte alterations contribute to the increased blood viscosity.

Physiologisches Institut der Universität, Deutschhausstraße 2, 3550 Marburg, FRG

162

INVESTIGATIONS IN THE INTRINSIC SYMPATHETIC ACTIVITY OF THE BETA 1-ADRENERGIC BLOCKING AGENT CELIPROLOL

H. Pittner, R. Smith, M. Leibowitz, R.G. Van Inwegen, P. Wolf

The beta 1-adrenergic blocking agent celiprolol [3-(3-acetyl-4-(3-tert.butylamino-2-hydroxypropoxy)-phenyl)-1,1-diethylurea hydrochloride] has positive chronotropic and inotropic actions on isolated guinea pig and cat atrial preparations, but it does not increase the developed tension of cat papillary muscles or the dog heart adenylate cyclase activity. Celiprolol increases the heart rate to the same extent as the beta-blocking agent pindolol both in reserpinized rats and in reserpinized, adrenalectomized, vagotomized cats. The beta-blocker propranolol blocks the positive chronotropic effects of celiprolol both in vitro and in vivo. Celiprolol has slight relaxing effects in isolated human arterial and venous preparations. Celiprolol increases the femoral arterial blood flow in anaesthetized dogs;this effect is evident also after pretreatment with propranolol.Celiprolol has only slight relaxing effects on the isolated guinea-pig trachea, but celiprolol decreases the airway resistance even after propranolol pretreatment in anaesthetized cats in which a bronchoconstriction is induced by serotonin; in the same preparation not only propranolol, but also the beta 1-selective blocking agents atenolol and metoprolol increase the airway resistance. Celiprolol increases the plasma insulin and glucose levels in awake dogs. It is concluded that the demonstration of intrinsic sympathetic activity of a beta adrenergic blocking agent largely depends on the choice of the test model.

Chemie Linz AG,Pharmakologie,A-4020 Linz,Austria
Revlon Health Care Group, Tuckahoe,N.Y.10707,USA

163

CHRONIC ADMINISTRATION ALTERS THE HYPOTENSIVE RESPONSE TO α-ADRENOCEPTOR BLOCKING DRUGS IN CONSCIOUS DOGS.

A. Beck

The effects of prazosin (P., 0.1; 0.5; 2.5 mg/kg, p.o.) and urapidil (U., 0.4; 2; 10 mg/kg, p.o.) on arterial blood pressure (BP), heart rate (HR) and plasma renin activity (PRA) were investigated in 6 normotensive dogs. The animals were trained to stand quietly in a rack. BP was measured after puncture of the femoral artery, HR by integration from the art. BP, PRA by RIA. The first administration of P. revealed a reduction in both systolic and diastolic blood pressure, increase in HR and PRA, which were not strictly dose dependent. U. showed similar changes of these parameters. After chronic administration of both drugs, twice a day for 4 days, the same parameters were measured. BP reduction, increase in HR and in PRA, as shown by P., were attenuated. The hypotensive effect of U. (10 mg/kg) was also attenuated. The hypotensive effect of U. (2 mg/kg) was not attenuated, but PRA was significantly decreased. U. (0.4 mg/kg) showed no hypotensive effect after both acute and chronic administration.
Since the decrease in BP caused by P. is not accompanied by an adequate increase in HR, an inhibitory effect of P. on the reflex activation must be assumed. U. also lacks an adequate reflex activation, which is probably due to its α_2 stimulatory activity. The attenuation of the hypotensive effect of P. and U. with chronic administration is not due to a persistent elevation of PRA.

Pharmakolog. Inst. d. Univ. Wien, 1090 Wien, Währingerstr. 13A, Austria.

164

POSTSYNAPTIC VASCULAR α_1- AND α_2-ADRENOCEPTORS INVOLVED IN SYMPATHETIC VASOCONSTRICTION OF THE CANINE FEMORAL BED

D. Elsner, M. Saeed, O. Sommer and E. Bassenge

It was hypothesized recently that postsynaptic vascular α_2-receptors (α_2-R) are activated mainly by circulating catecholamines, while α_1-R are innervated selectively. In the coronary bed, however, both circulating and neuronally released norepinephrine (NE) acts via vascular α_2-R. Since sympathetic constriction of this bed is moderate even under ß-blockade, we studied this issue in the femoral bed, which is under effective sympathetic control. In 24 anesthetized (0.2 ml/kg thalamonal, 80 mg/kg + 10 mg/kg/hr chloralose), spinalized, vagotomized dogs under ß-blockade (nadolol 2 mg/kg), femoral vasoconstriction was induced by stimulation of the decentralized lumbar sympathetic chain (SS, 0.1 - 3.0 Hz) or intra-arterial NE-infusion (0.001 - 1.0 µg/kg/min), while arterial pressure was kept constant. Arterial and femoral venous plasma NE was measured radioenzymatically. Following the control series, the complete trial was repeated after α_2-blockade (α_2-Bl, 0.03, 0.3 and 3.0 mg/kg rauwolscine, n = 8), after α_1-Bl (0.012, 0.12 and 1.2 mg/kg prazosin, n = 8), and after sham treatment. The α_2-Bl caused a dose-dependent, significant shift of the negative log EC_{50} (= arterial NE concentration during infusion causing 50 % decline in flow) from 7.43 ± 0.28 (control) to 5.57 ± 0.26 (3.0 rauw.); α_1-Bl from 7.36 ± 0.40 (control) to 5.85 ± 0.49 (1.2 praz.); sham treatment did not cause a significant shift (7.31 ± 0.38 vs. 6.98 ± 0.50). During SS with 1.0 and 3.0 Hz, α_2-Bl caused a significant augmentation of the veno-arterial NE-difference. This is in agreement with the theory of presynaptic modulation of NE-release by α_2-R. During SS at all frequencies studied, α_2-Bl caused a stronger or at least identical attenuation of constriction compared to α_1-Bl. - These data demonstrate the presence of both subtypes of vascular α-adrenoceptors in this bed, but they are not in agreement with the hypothesis of preferential intrasynaptic location of α_1-adrenoceptors.

Angewandte Physiologie, Hermann-Herder-Str. 7, 78 Freiburg

165

CARDIOVASCULAR PROFILE OF ERGOTAMINE IN PITHED RATS

H.O. Kalkman

The ergot alkaloid ergotamine (ET) is known to activate or antagonise both serotonergic- and α-adrenergic receptors. In pithed normotensive rats i.v. injections of ET (3-3000 µg/kg) caused a rise in blood pressure. The pressor effects of ET were analysed by means of selective antagonists, prazosin (P, 0.1 mg/kg; α_1), yohimbine (Y, 1 mg/kg; α_2) and methysergide (M, 50 µg/kg; S_2). It was concluded that ET is a mixed α_2/S_2 agonist. A pronounced difference in time necessary to reach the maximal diastolic pressure for 30 µg/kg ET was observed after α_2- or S_2-block respectively. After Y 12 min were required, after M it took 2 min only. After pretreatment with M, ET (30 µg/kg) induced a marked increase in cardiac output (thermodilution method). In contrast, after Y (leaving the S_2-component) an insignificant increase in cardiac output was observed. Heart rate remained unaltered throughout the experiments. The α_2-agonist B-HT 920, 2-amino-6-allyl-5,6,7,8-tetrahydro--4H-thiazolo{4,5-d}-azepine, (10-300 µg/kg) provoked a dose-dependent increase in cardiac output, which could be antagonised by Y. The selective serotonergic agonist 5--methoxytryptamine (30-300 µg/kg) caused a biphasic cardiac output response (increase followed by decrease). In conclusion, in pithed rats the α_2-component of ET is responsible for the rise in cardiac output, whereas the S_2-component appears irrelevant. One might speculate that the S_2--component of ET, like serotonin itself, causes both vasoconstriction and dilatation. When cardiac output does not contribute, the rise in blood pressure will then solely be due to the overall increase in peripheral resistance. This could explain the slow pressure response to 30 µg/kg ET after Y pretreatment.

Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands.

166

A CLASSIFICATION OF HEART SEROTONIN RECEPTORS

A.J.Kaumann

Serotonin receptors were classified by Peroutka & Snyder s_1 (K nM) and s_2 (K µM) (Mol.Pharmacol.16,687,1979). Experiments were carried out at 32.5°C on tissues from the heart of reserpine-pretreated kitten and on strips prepared from calf large coronary arteries. Spontaneously beating atria, electrically driven left atria (0.5 Hz) and papillary muscles (0.2 Hz) and the arteries were set up in Krebs solution containing 0.2 mM ascorbate and 6 µM cocaine. Serotonin causes positive chronotropic (EC_{50} 0.1 µM) and inotropic (EC_{50} 0.1 µM) effects on atria; maximum effects were 98% on right atria and 90% on left atria of those of (-)-isoprenaline. Serotonin did not stimulate atrial adenylyl cyclase. Serotonin causes positive inotropic effects in papillary muscles (EC_{50} 10 µM); maximum effects were 40% of those of (-)-isoprenaline. Concentrations greater than 10 µM serotonin elicited long-lasting aftercontractions in papillary muscles, probably due to a prolongation of the action potential. Serotonin produced contractures of coronary arteries (EC_{50} 0.05 µM). None of these effects was antagonized by 1 µM (-)-bupranolol or by 1 µM prazosin. Ketanserin (1-10000 nM), phenoxybenzamine (20-20000 nM) and yohimbine (60-10000 nM) were used to antagonize the effects of serotonin. Eq.diss.constants K (nM) are given in parentheses.

	ketanserin	phenoxybenzamine	yohimbine
Papillary muscle	-(no effect)	-	-
Left atrium	-	+ } irre-	-
Right atrium	-	+ } versible	-
Coronary artery	+ (0.6)	+ }	+ (50)

The K for serotonin, estimated from receptor occlusion with phenoxybenzamine, was 1 µM in left atria and 1-10 µM in coronary arteries. Conclusions: 1.Coronary arteries but not myocardium contain s_2-receptors. 2.Myocardial receptors do not appear to be s_1 because the affinity for serotonin is low. 3.It is proposed to designate atrial receptors as s_3 and ventricular receptors as s_4.

Klinische Physiologie, Physiologisches Institut der Universität, Universitätsstrasse 1, 4000 Düsseldorf, FRG

167

HEMODYNAMIC ACTIONS OF KETANSERIN AND THE ALPHA-ADRENOCEPTOR BLOCKERS PRAZOSINE AND PHENTOLAMINE IN THE CONSCIOUS SHR.

H.A.J. Struyker-Boudier and H. van Essen.

In recent studies, it was suggested that the blood pressure lowering effect of the new antihypertensive agent ketanserin (K) may be caused by peripheral alpha-adrenoceptor blockade rather than the originally claimed serotonin-2 receptor antagonism. In this study, we compared the hemodynamic effects of K (1 mg/kg, N=4; 3 mg/kg, N=8, i.a.) with those of the alpha-1 blocker prazosine (Pr, 0.1 mg/kg, N=9 i.a.) and the combined alpha-1 and alpha-2 blocker phentolamine (Ph, 1 mg/kg, N=6, i.a.) in conscious spontaneously hypertensive rats (SHR) with a chronic intra-aortic catheter for the continuous measurement of mean arterial pressure (MAP) and an electromagnetic flowprobe on the aorta for measurement of cardiac index (CI). Total peripheral resistance index (TPRI) and stroke volume index (SVI) were calculated according to standard procedures.

All 3 agents caused a rapid fall in MAP with a maximum of -30 ± 8 (mean ± S.E.; K, 1 mg/kg); -43 ± 2 (K, 3 mg/kg); -30 ± 4 (Pr) and -38 ± 8 (Ph) mmHg within 30 min. The fall in MAP was parallelled by a decrease in TPRI, amounting to -0.9 ± 0.3 (K, 1 mg/kg); -1.2 ± 0.1 (K, 3 mg/kg); -1.4 ± 0.2 (Pr) and -1.3 ± 0.3 (Ph) mmHg.min.100 g b.w./ml. Both alpha-blockers caused a reflex increase in heart rate (HR) of maximally +60 ± 8 (Pr) and +63 ± 17 (Ph) b/min. CI was clearly elevated after Pr (+3.6 ± 0.7 ml/min.100 g b.w.), but not after Ph, because of a reduction in SVI of -13 ± 3 µl/100 g b.w.). After K, both HR and CI did not change significantly. These data show that the acute hemodynamic effects of K differ from those of alpha-adrenoceptor blockers. Although an interaction with peripheral alpha-adrenoceptors cannot be excluded, additional mechanisms seem to play a role in the antihypertensive effect of K.

Dept. of Pharmacology, University of Limburg, P.O. Box 616, 6200 MD Maastricht, The Netherlands.

168

THE INFLUENCE OF KETANSERIN ON CENTRAL CARDIOVASCULAR REGULATION

E.Schneider and W.Felix

Ketanserin is a new and selective serotonin S2-receptor blocking agent with an additional and weaker α-adrenolytic activity. It produces hypotension when given i.v. Previous investigations have shown that this bloodpressure lowering effects is mainly due to a central mechanism suggesting that ketanserin influences baro- and chemoreceptor reflexes. The experiments were performed in cats anaesthetized with chloralose.

The baroreceptor reflex was evoked by noradrenaline (i.v. injection, 4 µg/kg) or by an injection of arterial blood with high pressure (200 mmHg) into the Aa.carot.comm. The response was a decrease of bloodpressure, heart-rate and respiratory minute volume. During bilateral carotid occlusion the three parameters increased. The chemoreceptors were stimulated either by an injection of 1-2 ml venous blood into the Aa.car. comm. or by inhalation of air with high pCO2. The reflex response was hypertension, bradycardia and hyperpnoe.

Ketanserin (.25-1 mg/kg,i.v.) had no influence on the chemoreceptor reflex. There was a differentiated effect on the baroreceptor reflex: no influence on respiratory response could be seen but a dose dependet inhibition of reflex induced change in bloodpressure and heart-rate. This occured during pressor response as well as during depressor response.

It is suggested that this observed reflex inhibition is not mediated by peripheral baroreceptor inactivation because respiratory response remained unchanged. Therefore, we concluded that Ketanserin interacts with vasomotor center. It does not affect its pressor area whereas the depressor area is inhibited. The fact that Ketanserin is a selective S2-receptor blocking agent indicates the participation of S2-receptors in cardiovascular regulation mediated by the depressor area of the vasomotor center in contrast to the pressor area.

Institut für Pharmakologie und Toxikologie der Med.Fakultät der Universität München

169

CENTRAL α-ADRENOCEPTOR SUBTYPES AND THE FUNCTION OF THE BARORECEPTOR REFLEX

G. Haeusler*

There is considerable evidence that clonidine facilitates transmission through the central part of the baroreceptor reflex (CPBR). This effect of clonidine is antagonized by α_2-adrenoceptor blocking agents. It was of interest to study whether the involved α_2-adrenoceptors are under the influence of central adrenergic neurones. The function of the CPBR was assessed in urethane anaesthetized cats by bilateral electrical stimulation of the carotid sinus nerves with single pulses and measurement of the resulting inhibition of the discharges (silent period) in the sympathetic preganglionic splanchnic nerve. In order to make the system more sensitive and to be able to locate drug effects to the nucleus of the solitary tract (NTS), the temperature of the NTS was reduced to approximately 10° C by placing thermodes to the exposed dorsal surface of the medulla oblongata at the intermediate parts of the NTS of each side. Clonidine and B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d)-azepine dihydrochloride) prolonged the induced silent period in the sympathetic discharge pattern, an effect which is readily explained by improvement of transmission through the CPBR. These effects of clonidine and B-HT 920 were antagonized by yohimbine and piperoxan. When given alone, the two α_2-adrenoceptor blocking agents considerably shortened the silent period, i.e. made the reflex less efficient. Prazosin and phenoxybenzamine on the other hand prolonged the silent period. The results suggest that α_1- and α_2-adrenoceptors at neurones of the CPBR are innervated by central adrenergic neurones and that their blockade leads to a facilitation and inhibition, respectively, of transmission through the CPBR.

Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel, Switzerland
*Present address: Pharmaceutical Research Department, E. Merck, D-6100 Darmstadt 1, FRG

170

SYMPATHICOLYSIS BY COMBINED CENTRALα_2-STIMULATION AND PERIPHERAL ß-BLOCKADE.

M.Hausen, B.Krämer, C.Nunhofer, W.Mäurer, W.Kübler.

Clonidine (C) induced sympathicolysis is due to stimulation of centralα_2-receptors whereas metoprolol (M) acts by blockade of peripheral ß-receptors.In order to investigate the effects of these different sympathicolytic mechanisms, plasma adrenaline (A) and noradrenaline (NA) were measured in 11 normotensive subjects at rest and during bicycle exercise (70-150Watts),following single and combined infusion of C (4µg/kgBW) and M(0.2mg/kgBW).C reduced A and NA at rest and during exercise.M did not influence A and NA at rest.During exercise, however,NA plasma levels were significantly more raised than those after placebo. After C+M, at rest and on moderate work load catecholamine levels were significantly lower than controls, at high work load,catecholamine concentrations were undistinguishable from those measured on placebo. M reduced heart rate (HR)at rest and during exercise, whereas the effect of C on HR decreased with increasing work load. In contrast to M,C lowered systolic blood pressure (BP) at rest and during exercise. The ratiosof HR/A and HR/NA were identical after placebo and C, indicating that the sympathicolytic effects of C are due to a reduction in catecholamine outflow. On M and C+M, HR/A and HR/NA were reduced due to blockade of ß-receptors.

	NA (ng/l)		RR syst.(mmHg)		HR (min^{-1})	
	rest	150W	rest	150W	rest	150W
P	240 ± 50	800±90	123±3	187±3	70±4	137±3
C	***70 ± 20	**560±90	***104±2	***153±4	***55±2	134±4
M	210 ± 40	*1040±130	120±2	***162±2	***54±2	***116±2
C+M	***70 ± 20	940±100	***107±2	***145±3	***52±2	***112±3

p vs. placebo ***=0.001, **=0.01, *=0.05

Simultaneous administration of C+M combines the reduction of HR produced by M with the reduction of BP produced by C at rest and during exercise. Combined administration of C+M also attenuates the excessive elevation of NA levels observed at high work load when metoprolol was given alone.

Med.Univ.-Klinik III, Bergheimerstr. 58,D-6900 Heidelberg.

171

CENTRAL GABA-ERGIC STIMULATION LOWERS BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS (SHRSP): ROLE OF THE SYMPATHO-ADRENAL AXIS.

Thomas Unger, Hans Becker, Rainer Rettig, Norbert Schwab: Department of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg

Gamma-amino butyric acid (GABA) has received increasing attention as an inhibitory transmitter in the brain and in central autonomic and cardiovascular regulation. In this study we have investigated the central antihypertensive action of the potent GABA-agonist muscimol (MU) in SHRSP, in which hypertension is known to be associated with stimulated sympatho-adrenal activity. All experiments were done in conscious unrestrained rats. Injection of MU (0.01 - 1 µg) into the lateral brain ventricle (i.c.v.) lowered mean arterial blood pressure (MAP) dose-dependently in SHRSP (n:13) with a maximal fall of -52.7 ± 5 mmHg from 192.1 ± 8.4 mmHg, lasting for about 90 min after 1 µg MU. This was accompanied by bradycardia and sedation. Pretreatment with atropin (2 mg/kg intraperitoneally or 15 µg/kg i.c.v.) did not significantly influence the MU induced fall in MAP. In WKY (n:12) the maximal decrease in MAP was -12.1 ± 1.6 mmHg from 109.3 ± 1.9 mmHg, and the effect lasted much shorter than in SHRSP. Following 1 µg MU i.c.v. plasma noradrenaline did not fall significantly in SHRSP and WKY, but in SHRSP plasma adrenaline was fully suppressed (from 118.1 ± 24.2 to 22.8 ± 5.7 pg/ml) throughout the depressor response. The sympathetic nervous activity as directly recorded from the n. splanchnicus was slightly reduced in SHRSP and WKY, whereas the adrenal nerve activity was reduced by 44% in SHRSP and only 24% in WKY.

Our results demonstrate that the antihypertensive action of central GABA-ergic stimulation in SHRSP is not mediated by an increase in vagal tone or a generalized sympathetic tone reduction but rather by a selective inhibition of the sympatho-adrenal activity.

172

IMPAIRMENT OF AUTONOMIC RESPONSIVENESS IN B.PERTUSSIS VACCINATED RATS DUE TO A HEAT LABILE COMPONENT IN THE VACCINE

D.J.de Wildt

Whether the cardiovascular system is primarily involved in the adverse reactions (shock, coma and convulsions) sometimes occurring after Bordetella pertussis vaccination has never been thoroughly investigated. In the present study autonomic receptor functioning within the circulatory system was established on basis of chronotropic and blood pressure changes due to i.v. infusion of various agonists in anesthetized rats.

A pronounced impairment of cholinergic and β_2-adrenergic transmission 4 days after inoculation with pertussis vaccine was demonstrated by the blockade of arecoline induced bradycardia respectively reduced blood pressure decrease elicited by salbutamol. While the pressor response evoked by the α_1-adrenergic agonist methoxamine was potentiated the pressor response induced by B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H thiazolo[4,5-d]-azepine dihydrochloride) was significantly reduced in the pertussis treated group. Moreover, the reflex bradycardia after methoxamine was nearly absent in vaccinated animals pointing towards an interrupted baroreceptor reflex which might be well explained by the strong atropine-like action of pertussis vaccine. It could be unraveled that the pharmacological effects of pertussis vaccine might be attributed to a heat-labile component which is assumed to be lymphocytosis promoting factor (LPF) which is evidenced by the absence of inhibitory effects after administration of heated vaccine. Furthermore comparable pharmacological effects were seen after administration of purified LPF. It is concluded that pertussis vaccination in rats induces autonomic impairment due to the protein constituent LPF.

Laboratory for Pharmacology, Section on Cardiovascular Pharmacology, National Institute of Public Health, P.O.Box 1, 3720 BA BILTHOVEN, The Netherlands

173

FLOW-DEPENDENT DILATION OF CANINE EPICARDIAL CORONARY ARTERIES IN VIVO AND IN VITRO: MEDIATED BY THE ENDOTHELIUM

J. Holtz, R. Busse and M. Giesler

Constrictions of epicardial arteries play a causal role in angina pectoris, but the trigger mechanism is unknown. Therefore, we looked for physiological factors modulating the tone of these vessels. In 9 conscious dogs the outer diameter (OD, ultrasonic technique) and the flow (CF, flowmeter) in one or two coronary branches (left circumflex and/or descending) was registered chronically. CF could be stopped or gradually limited by pneumatic occluders implanted distally to the site of OD measurement.
In the resting dogs, rapid increments in coronary flow by 300 % (postischemic reactive hyperemia; hyperinflation reflex; i.v. infusion of serotonin 20 µg/kg/min, isoproterenol 0.1 - 0.5 µg/kg/min, adenosin 1 - 3 µM/kg) caused a slowly developing dilation in both coronary branches (by 98 ± 26 and 115 ± 34 µm after 90 seconds), while mean arterial pressure was lowered by 12 ± 5 mmHg. When CF in one branch was kept constant during these stimuli by partial cuff occlusion, the slow epicardial artery dilation in this branch was abolished, while it was unaffected in the other branch. Stop of CF (for 30 s) caused a moderate epicardial constriction (by 32 ± 12 µm). The excised epicardial branches (OD 1.6 mm) were perfused and activated by 10^{-7} serotonin while the OD was registered photoelectrically. Variation of perfusion rate between 0.5 and 3.0 ml/min caused a parallel, but slowly developing variation in OD by 35 ± 8 µm, which could not be due to changes in transmural pressure. Enzymatical removal of the endothelium abolished the flow-dependent dilation, but did not affect the responsiveness to directly constricting or dilating stimuli.- The data demonstrate that epicardial coronary arteries in vivo are under a dilatory, flow-dependent control mediated by the endothelium. Reduction of this control during states of low myocardial metabolic demand and flow at night might facilitate enhanced constrictions of these arteries.

Angewandte Physiologie, Hermann-Herder-Str.7, 78 Freiburg

174

ROLE OF CYCLIC GMP IN ENDOTHELIUM-DEPENDENT RELAXATION OF CORONARY STRIPS BY ACETYLCHOLINE

S. Holzmann and W.R. Kukovetz

Recent evidence demonstrated that the contractile response of bovine coronary arterial strips to acetylcholine (ACh) was antagonized by concomitant rises in cGMP-levels (Kukovetz et al., N.S. Arch. Pharmacol. 319, 29, 1982). Since rabbit aortic strips with undisturbed endothelium responded to ACh with relaxation (Furchgott and Zawadzki, Nature 288, 373, 1980) it was investigated whether coronary strips behave similarly and whether the presence of endothelium influences the cGMP-response to ACh. Bovine coronary arterial strips prepared as previously by insertion of a metal probe into the vessel were suspended in tyrode solution and precontracted with 25 µM serotonin. These strips were contracted concentration dependently by 55-550 nM ACh and cGMP-levels raised up to the 3.5 fold control value. When strips were prepared with utmost care to keep the endothelium intact, e.g. by avoidance of metal probing of the vessel during dissection, they responded under otherwise similar conditions to ACh (5.5-550 nM) with concentration dependent relaxation and significantly larger rises in cGMP, up to the 7 fold control value. In the presence of the cGMP-PDE-inhibitor M&B 22,948 (2-o-propoxyphenyl-8-azapurin-6-one ; 370 µM) relaxation and rises in cGMP were significantly enhanced. Conversely, in the presence of 50 µM methylene blue which antagonized ACh-induced rises in cGMP but enhanced contraction of coronary artery strips (Kukovetz et al., 1982), the relaxing effects of ACh on endothelium-preserved strips as well as the rises in cGMP were abolished. The results suggest that endothelium converts the contractile effect of ACh into relaxation in coronary strips by augmenting rises in cGMP-levels.

Inst.f.Pharmakodyn.u.Tox.,A-8010 Graz,Univ.-Pl.2

175

EFFECT OF THROMBOXANE SYNTHETASE INHIBITION ON CORONARY ARTERY THROMBOSIS IN THE DOG.

M. Just, E. Schraven, P.A. Martorana

Coronary artery thrombosis was induced in anaesthetized dogs by electrical stimulation (9 V, 150 µA for 360 min) via a needle electrode placed on the intima of the left circumflex coronary artery. A specific thromboxane synthetase inhibitor UK 37248 (4-[2-(1-imidazol-1-yl)ethoxy] benzoic acid. HCl) at the dose of 0.1 mg/kg/min (n = 8) or saline (n = 8) was infused for the duration of the experiment.

In control dogs thromboxane (TXB_2; coronary sinus) increased significantly beginning with the 60th min of stimulation. 6-Keto-$PGF_{1\alpha}$ did not change until the 240th min after stimulation when a small but insignificant increase was found. Thrombotic occlusion occured in all control dogs after 158 ± 21 min and thrombus wet weight (measured at 360 min) was 74.4 ± 6.0 mg. Due to the thrombosis, myocardial infarction resulted in all animals. Infarct size was 23.8 ± 4 % of left ventricle weight.

In UK 37248-treated dogs the increase in TXB_2 was completely prevented. 6-Keto-$PGF_{1\alpha}$ on the other hand rose gradually to a level of significance. No thrombotic occlusion was found in 7/8 animals. However small nonocclusive thrombi were found at the site of stimulation. Thrombus weight was 28.1 ± 11.0 mg ($p < 0.005$). Myocardial infarction resulted in only 4/8 dogs (infarct size 6.6 ± 4.0 %, $p < 0.005$).

These data suggest that
1) thromboxane plays a significant role in the development of coronary artery thrombosis in this model,
2) the increase of prostacyclin, associated with thromboxane synthetase inhibition, may also be an important factor in the prevention of the thrombotic occlusion.

Cassella AG, Pharmaforschung, D-6000 Frankfurt/Main 61

176

POTENT AND STEREOSPECIFIC INHIBITION OF THROMBOXANE (TX) AND 12-HPETE FORMATION IN HUMAN PLATELETS BY PROSTACYCLIN AND PROSTACYCLIN ANALOGUES.

H. Darius

Arachidonic acid (AA) is released from platelet membranes following membrane stimuli and is metabolized by a cyclooxygenase giving raise to TXA_2 and a 12-lipoxygenase forming 12-H(P)ETE as final enzymatic products. The present study was designed to measure TX and 12-HPETE formation after treatment of the platelets with PGI_2 and a stable analogue ZK 36374 5{(E)-1S,5S,6R,7R)-7-hydroxy-6-[(E)-(3S,4RS)-3-hydroxy-4-methyl-oct-1-en-6-yn-yl]-bicyclo (3.3.0)octan-3-yliden}-pentanoic acid.

Stimulation of washed human platelets by thrombin (0.06-6 IU/ml) was followed by dose-dependent 12-HPETE and TX formation. The maximum TXB_2 concentration was 630 ± 130 pmol/l (n = 9). PGI_2 (0.3 - 300 nM) dose-dependently inhibited this thrombin- (0.6 IU/ml) induced TX and 12 HPETE formation. The IC_{50} was 13 ± 6 and 18 ± 8 nM (n = 4), respectively. ZK 36374 inhibited the eicosanoid formation with an IC_{50} of 4 ± 1 and 3 ± 2 nM (n = 6), respectively, whereas its 5(Z) stereoisomere ZK 36375 was 2-3 orders of magnitude less effective. In comparison, the IC_{50} for inhibition of 12-HPETE formation by 5,8,11,14-eicosatetraynoic acid was 2.0 ± 0.3 µM with thrombin (0.6 IU/ml). PGI_2 and ZK 36374 did not influence the AA-induced thromboxane or 12-HPETE formation.

It is concluded that PGI_2 and ZK 36374 are among the most potent inhibitors of thrombin-induced lipoxygenase and cyclooxygenase product formation in human platelets. This effect seems to be due to an decreased availability of endogenous AA rather than to specific inhibition of AA-metabolizing enzymes.

Pharmakologisches Institut der Universität Köln, Gleueler Str. 24, D-5000 Köln 41

177

STIMULATION OF VASCULAR PROSTACYCLIN BY GLYCERYL-TRINITRATE (NITROGLYCERIN) IN BOVINE CORONARY ARTERIES AND VEINS AND ITS INHIBITION BY DEXAMETHASONE

W. Rücker and B. Ahland

Previous studies have shown, that glyceryltrinitrate (GTN) stimulates coronary arterial PGI_2-formation (Schrör et al., Thromb.Res.23:59,1981) confirming data of Levine et al. J.Clin.Invest.67:762,1981) on isolated endothelial cells. This study was designed to evaluate possible mechanisms of this PGI_2-stimulating action on bovine coronary arteries (BCA) in comparison to bovine veins (BCV).

BCA's in Krebs buffer produce 20 ± 3 pmoles PGI_2/100 mm^2 surface and 10 min incubation at 37°C. This basal production is enhanced to 35 ± 5 pmoles/100 mm^2 in presence of 17 pmoles/ml GTN ($P < 0.01$). The basal release of BCV amounts to 38 ± 4 pmoles/100 mm^2 and is increased to 57 ± 7 pmoles/100 mm^2 in presence of only 0.6 pmoles/ml GTN ($P < 0.01$). Thus, BCV by comparison with BCA is not only more sensitive to GTN but also more efficient with respect to stimulated PGI_2-production. Pretreatment with indomethacin (11 nmoles/ml) reduces this stimulated PGI_2-formation of BCA and BCV to 6 ± 1 and 10 ± 5 pmoles PGI_2/100 mm^2, respectively ($P < 0.01$). Similar results are obtained after stimulating PGI_2 with arachidonic acid (9 nmoles/ml).

The GTN-stimulated PGI_2-formation of BCA is dose-dependently inhibited by dexamethasone (DEX), the IC_{50} being as low as 1 pmol/ml. In contrast, the concentration of DEX necessary for inhibition of basal PGI_2-production is 2-3 orders of magnitude higher. DEX up to 25 nmoles/ml does not change the net arachidonic acid-induced PGI_2-release.

The data indicate a greater responsiveness of BCV in comparison with BCA against GTN-stimulated PGI_2-formation. Remarkable is the higher sensitivity to DEX of stimulated rather than basal PGI_2-formation. This warrants further investigation.

Pharmakologisches Institut der Universität Köln, Gleueler Str. 24, D-5000 Köln 41

178

STIMULATION OF CORONARY VASCULAR PROSTACYCLIN (PGI_2) BY ORGANIC NITRATES

K. Schrör and P. Weiss

Recent studies have shown that glyceryltrinitrate is a potent stimulator of PGI_2 formation in bovine coronary arteries (BCA) (Schrör et al., Thromb.Res. 23:59, 1981). The present investigation was designed to study the effects of isosorbide-2-mononitrate (2-ISMN), isosorbide-5-mononitrate (5-ISMN), isosorbide-2,5-dinitrate (ISDN) theophylline (THE) and the new compound 5-(γ-theophylline-7-ylpropylamino)-5-desoxy-L-isoidide-2-nitrate-bifumarate (KC 046) on PGI_2 release in this system and to investigate the actions of these substances on coronary vessel tone.

Among the agents studied, KC 046 was the most active compound and produced a dose-dependent stimulation of PGI_2 release at concentrations between 0.2 - 200 nM. The basal release of PGI_2 at 10 min incubation of BCA in Krebs-buffer at 37°C amounted to 16 ± 2 pmol/100 mm^2 surface (bioassayed from inhibition of ADP-induced platelet aggregation) and was increased to 54 ± 9 pmol/100 mm^2 by 0.2 µM KC 046 ($n = 6$, $P < 0.01$). This effect was prevented by both indomethacin and methylene blue. 2-ISMN at 0.2 µM doubled the basal release, whereas 5-ISMN, ISDN and THE were almost ineffective at these low concentrations. A significant stimulation was obtained with 5-ISMN at 10 µM concentration. All of the agents studied relaxed BCA strips. The EC_{50} for 2-ISMN amounted to 5 µM, that for 5-ISMN to 100 µM. KC 046 tended to exhibit a biphasic response, having a maximum relaxation at about 60 µM.

It is concluded that stimulation of vascular PGI_2 is a more general property of organic nitrates and it seems to be the 2-nitro-group of the compounds studied which is particularly effective. By comparing 2- and 5-ISMN, this is also evident for the relaxation of the BCA strips.

Pharmakologisches Institut der Universität Köln, Gleueler Str. 24, D-5000 Köln 41

179

THE VASODILATING EFFECT OF NITRATES IS NOT MEDIATED BY PROSTAGLANDINS

W. BARTSCH, B. BRÄUNIG and J. JANICH

The hypothesis to explain the effect of organic nitrates by a release of prostaglandins has been raised in the last two years by MORCILLO et al. (Amer. J. Cardiol. 45, 53 - 57; 1980).

To check this hypothesis we investigated whether the vasodilating and blood pressure lowering effect of isosorbide-5-mononitrate (IS-5-MN), the main metabolite of isosorbide dinitrate, could be influenced by pretreatment with inhibitors of prostaglandin synthesis.

Experiments on isolated vessels were carried out on canine venous spiral strips (vena pedis dorsalis).

To make the isolated veins more sensitive for the relaxation, we increased the vasoconstriction tone with noradrenaline (2×10^{-7}M). The effect of IS-5-MN (4×10^{-5}M) was a relaxation of 346 ± 56 mg and after pretreatment with the cyclooxygenase inhibitor indomethacin (5×10^{-5}M) 585 ± 81 mg (M ± SEM; n = 7).

Conscious dogs ($\bar{n} = 8$) with chronically implanted arterial and venous catheters for continuous measurement of the blood pressure (BP mm Hg) furnished the following results.

Pretreatment twice daily for 3 days	BP initial value	BP decrease after IS-5-MN 10mg/kg iv.
Control	169	28
Acetylsalicylic acid 35 mg/kg p.o.	168	35
Control	172	37
Indomethacin 3 mg/kg p.o.	159	37

The fact that neither indomethacin nor acetylsalicylic acid reduced the vasodilating response to IS-5-MN in vitro and/or in vivo contradicts the hypothesis that the vasodilating action of organic nitrates is caused by an enhanced release of vasodilating prostaglandins.

Department of Cardiovascular Research, Boehringer Mannheim GmbH

180

Availability of organic nitrates from intravenous infusion sets

R. Bonn and W. Cawello

The loss of glyceryl-trinitrate (GTN) when infused at various rates through polyvinylchloride (PVC) tubing was measured against time by continuously monitoring the GTN concentrations at the outflow of the tubing using a direct photometric method.

The portion of GTN loss depends on the rate of infusion; at flow rates of 3-6 ml/hour, GTN losses of up to 60 % may be expected. Polyethylene (PE) tubing does not show this reduction of outflow concentration of GTN.

When infusing GTN through PVC tubing, the dosage cannot be controlled and any assessment of pharmacokinetic or absolute bioavailability becomes invalid. This effect of tubing can account for apparent losses of bioavailability of up to 50 %.

Other organic nitrates like isosorbide dinitrate are shown to be absorbed into PVC in a similar way. The losses encrease with larger lipophilicity of the drug.

SANOL SCHWARZ GMBH, Bereich Forschung, D-4019 Monheim

181

STIMULATION OF PROSTACYCLIN (PGI_2) SYNTHESIS BY NAFAZATROM (BAY G 6575)

E. Perzborn, F. Seuter

Nafazatrom (Naf) is a potent antithrombotic (Seuter et al., Arzneim.-Forsch./Drug Res. 29, 54, 1979) and antimetastatic drug (Honn et al., Prostaglandins and Cancer; Powles, T.J. et al. (eds.) Alan Liss Inc., New York, pp. 733-752, 1982). These effects were suggested to be due to its ability to stimulate vascular wall PGI_2 production. PGI_2 synthesis occurs primarily in the microsomal fraction of vessel wall homogenates. Therefore, we have examined the effect of Naf on the biosynthesis of PGI_2 from ^{3}H-arachidonic acid (AA) in a mixture of microsomes of sheep seminal vesicles (RSVM) and bovine aorta (BAM).

AA was incubated with RSVM and BAM in the presence and absence of Naf. The reaction was terminated by acidification to pH 3,5. The sample was extracted with ethyl acetate and separated by thin-layer chromatography. PGI_2 was assayed as 6-oxo-$PGF_{1\alpha}$, identified by cochromatography and a specific radioimmunoassay.

In the absence of Naf a small amount of PGI_2, PGE_2 and/or $PGF_{2\alpha}$ was generated. However, in the presence of Naf, the synthesis of PGI_2 was stimulated. A concentration dependent stimulation was observed within the range of 40 - 400 µmol/l. PGE_2 and/or $PGF_{2\alpha}$ synthesis was not significantly influenced.

These data support the idea that the pharmacological effects of Naf are caused by the enhancement of endogenous PGI_2 synthesis.

Institut für Pharmakologie, BAYER AG, Apratherweg 18, D - 5600 Wuppertal 1.

182

PHARMACOLOGY OF A NEW MONONITRATE: KC 046/264.

B. Gabard, S.S. Chatterjee

The pharmacological properties of KC 046/264 (5-(Y-Theophyllin-7 ylpropylamino)-5-desoxy-L-isoidid-2-nitrate bifumarate), a potential anti-anginal agent were assessed in various models and compared with those of Isosorbide-dinitrate (ISDN), -2-(2-ISMN) and -5-mononitrate (5-ISMN). In experiments with isolated organs (rat aortic strips and venous rings, dog coronary arteries, isolated guinea-pig hearts) all the tested compounds showed similar properties: noradrenalin-induced contractions were inhibited more strongly than KCl-induced contractions and KCl-depolarised coronary arteries were affected at much lower concentrations than needed in the other experiments. The orders of potencies were found to be KC 046/264 > ISDN ≫ 2-ISMN > 5-ISMN.

In anaesthesized cats, KC 046/264 like the other nitrates depressed the arterial blood pressure. After intravenous application (0,025 - 0,25 mg/kg) KC 046/264 was found to be about 10 times more potent and to have a longer duration of action than ISDN. Intraduodenal application of the substances gave similar results.

Following intravenous injection in anaesthesized dogs KC 046/264 (0,03 - 0,3 mg/kg) decreased systolic arterial pressure, pulmonary arterial pressure and left-ventricular enddiastolic pressure. This last parameter was maximally lowered by 50 % after 0,06 mg/kg. Similar effects could be observed only after higher doses of ISDN.

These results indicate that KC 046/264 possesses a pharmacological activity profile similar to that of ISDN, in addition to being more potent than this reference compound. Preliminary clinical studies confirm these findings.

Dept. of Pharmacology, Dr. Willmar Schwabe - Arzneimittel, Postfach 410925, D-7500 Karlsruhe FRG.

183

STUDIES ON THE MECHANISM OF THE VASODILATING ACTIVITY OF BM 14.190;

G. Sponer, B. Müller-Beckmann

The vasodilating and ß-blocking properties of BM 14.190 (=BM) 1-[Carbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethyl)-amino]-propranol-(2) have been reported at Arch. Pharmacol. 314, R 21, 1982). Investigations presented here were performed to elucidate the mode of the vasodilating activity of the drug.

I. The α-blocking potency of BM was quantified in pithed rats: Dose-response curves for methoxamine (M), clonidine (C) and angiotensin II (A) were established before and after i.v. injection of equipotent hypotensive doses of phentolamine (P; 2.8 mg/kg), dihydralazine (D; 1.1 mg/kg) and BM (0.37 mg/kg). The ED_{50} mm Hg (doses of M, C and A which increase the blood pressure by 50 mm Hg) was calculated. The influence of the vasodilating drugs on the effect of M, C and A, is shown in the table as the ratio of the ED_{50} mm Hg after treatment/before treatment. These results suggest that the α-blocking activity of BM is obviously not relevant for the blood pressure lowering effect of BM.

	D	P	BM
M	2.8	24.7	4.2
C	-	36.9	4.4
A	9.8	1.1	1.3

II. The vasodilating properties of BM and both its enantiomers were investigated in isolated perfused hind limbs of rabbits. In contrast to the lack of ß-blocking activity, R(+)BM possesses similar vasodilating properties to the S(-)BM or the racemic compound. It can be concluded that the vasodilating and ß-blocking activity is not mediated by the same (stereospecific) receptor-site; thus, the vasodilation is probably not induced by $ß_2$-stimulation.

III. The calcium-antagonistic properties of BM were investigated in K^+-depolarized spiral strips of rat aortas. Ca^{++}-induced contractions were inhibited in a concentration dependent manner by BM and verapamil (V), but not by hydralazine. The EC_{50} for V was 6.18×10^{-8} M, and for BM 2.28×10^{-6} M. Therefore, it can not be ruled out that calcium-antagonistic activity contributes to the vasodilating properties of BM.

Pharmakologische Laboratorien, Boehringer Mannheim GmbH, Postfach 31 01 20, 6800 Mannheim 31

184

REDUCED MICROVASCULAR DISTENSIBILITY IN OLD NORMOTENSIVE (WKY) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR). R.F.Hertel

Previous studies have shown, that topical verapamil applications on a mesentery preparation of rats (5 months old) were answered by a vascular dilatation between 14 to 38%. (Microvascular Aspects of spontaneous hypertension.Huber:Bern1982) To study microvascular functions in old rats we analysed as a part of total vascular activity microvascular distensibility in 12 or 17 months old SHR or WKY respectively. Experiments were performed in sexmatched pairs of WKY(mean arterial blood pressure,measured in the carotid artery:99±5mm Hg±SEM and SHR(163±5mm Hg). Using intravital microscopic techniques with a TV-equipment,the inner vessel diameters were measured before and after topical application of 0,125 mg verapamil. The microvessels were classified according to their branching order. The data represent percent increase in diameter,(15)=vessel number. In SHR, we measured in large arterioles A2 (15)5.3%, terminal arterioles A3 (19)8.9%, precapillary arterioles A4(19)10.8%, collecting venules V4(23)4.5%, large venules V3(20)4.6%, small veins V2(19)2.8%. An increasing tendency of dilatation with decreasing arteriolar size may suggest a relative augmentation of noncontractile materials. In WKY we found in A2(14) 5.8%,A3(16)5.4%,A4(19)5.5%,V4(9)4.3%,V3(18)3.8%, V2(13)4%,showing no significant differences at all. The reduced distensibility with increasing age correlates with an decrease of about 50% in intestinal lymph flow in 12 months old rats, we have shown before.

Fraunhofer-Institut für Toxikologie und Aerosolforschung, Nottulner Landweg 102, D 4400 Münster

185

EFFECTS OF VERAPAMIL ON ELECTROCORTICAL TOLERANCE TO SEVERE INSPIRATORY HYPOXIA IN THE RAT

C.F.Cartheuser

Severe progressive hypoxia ultimately causes the cessation of electrical activity of the brain. Occurrence of electrical silence is generally found at venous $PO_2 \leq 19$ mmHg of the brain and/or arterial glucose conc. of $\leq 1\mu g/g$,resp. As a simple electrophysiological approach in a screening model, tolerance time to silence (TTS)in the electrocorticogram (ECoG) was estimated in standardized experiments. Male albino rats, provided with permanent epicortex platinum or Ag/AgCl electrodes were exposed to progressive hypoxic hypoxia (constant rate N_2 addition mixed to normal air within a 25 l chamber). Actual FO_2 could be read continuously. During the experiments the ECoG was recorded for estimation of TTS, together with the electrocardiogram (ECG).
Since conscious and anesthetized rats revealed similar TTS mean values, Hexobarbitone anesthesia was used.
No difference was obtained for TTS in normotensive and hypertensive (SHR) rats. Different cerebral vasodilatory (hypotensive) drugs failed to alter TTS in normotensive and SHR rats - except for Ca^{++}-antagonists. This suggests that TTS does not depend on blood pressure within a wide range.
Verapamil as the most potent of the Ca^{++}-antagonists investigated, showed significant TTS prolongation in different strains of rats when applied i.v. (Wistar,5.0 mg/kg: +1o.5%,p<o.o1; Sprague Dawley, 2.5mg/kg: +21.6%, p<o.o1 ; Lewis, 2.5mg/kg: +12%, p<o.o5) while single oral administration remained ineffective (Wistar, 2o mg/kg p.os). Long term administration is under investigation.

It is concluded that the 'protective' effect by Ca^{++}-antagonists is not related to its vasodilatory action per se.

Zentrum Physiologie, MHH,K.-Gutschow-Str.8, D 3 Hannover

186

CONTINUOUS MEASUREMENT OF SYSTEMIC AND REGIONAL BLOOD FLOWS IN CONSCIOUS SHR: CHARACTERIZATION OF HEMODYNAMICS UNDERLYING VASODILATOR-INDUCED BLOOD PRESSURE CHANGES.

J.F.M. Smits

Arteriolar vasodilators primarily decrease total peripheral resistance. However, it is important to know the behaviour of the different vascular beds, because differential changes may lead to redistribution of flow. We are currently using methods for measurement of cardiac output as well as quantitation of regional flow through selected tissues in conscious spontaneously hypertensive rats (SHR). In this study, one group of male SHR (N=8) was equipped with an electromagnetic flow-probe on the ascending aorta (group I) Another group (N=6) was implanted with 3 Doppler-flow probes on the left renal artery, the superior mesenteric artery and the abdominal aorta (group II) for quantitation of the renal blood flow (RBF), mesenteric blood flow (MBF) and hindquarter blood flow (HBF). All animals had a catheter in a femoral artery. Vascular resistance changes were calculated from MAP and the blood flow changes. Hydralazine (0.3 mg/kg) caused a fall in MAP. It was reduced maximally at 1 h after injection (-21±3 mmHg in group I). TPR fell by 28±3% at that time. Heart rate (HR) increased by 66±8 bpm. CO increased by 4.7±0.7 ml/min.100 gr bw from a basal value of 27.1±3.1 ml/min.100 gr bw. In group II we found no evidence for a tissue-specificity for the vasodilation during the first 2 h after injection. In all tissues studied, a maximal reduction of resistance of 36-40% was observed between 0.5 and 1 h after injection.

The results indicate that the methods used, allow quantitation of flow-and resistance-changes, both systemically and regionally. Furthermore, in conscious SHR hydralazine dilates renal, mesenteric and muscular beds equally.

Dept.of Pharmacology, University of Limburg, P.O.Box 616 6200 MD Maastricht, the Netherlands.

187

CORRELATION BETWEEN INFLUENCE ON VASCULAR CONTRACTILE PROTEINS AND INHIBITION OF NORADRENALINE VASOCONSTRICTION IN VITRO

C.Kuthe, L. Criscione and P.R. Hedwall

The effects of chlorpromazine (C), diltiazem (D), felodipine (F), hydralazine (H), nifedipine (N), prenylamine (PE), and propranolol (PO) on superprecipitation (SUP) and myosin light chain phosphorylation (MLCPh) of bovine aortic actomyosin prepared according to Litten et al. (Arch. Bioch. Biophys. 182, 24, 1977) were studied. SUP was measured as increase in OD (660nm) after addition of ATP. MLCPh was determined by autoradiography and scintillation counting according to Silver et al.(J. Biol. Chem. 254/20, 9551, 1979). C, PE, and F caused significant inhibition of both parameters (C > PE≅F), whereas N, H, PO and D showed little or no inhibition (≪ 10 %) of SUP and MLCPh up to $3x10^{-4}$M.

The order of the potencies of the test compounds in these biochemical experiments correlates approximately with the order of their noradrenaline-antagonistic potencies in the isolated, perfused mesenteric arterial bed of the rat, which is: C>PE≅F>N>H>PO>D. The order of potassium-antagonistic potencies, however, is dissimilar: F≅N>PE≅D>C>PO>H.
Although considerably higher concentrations of the substances under investigation are required in biochemical as compared to pharmacological studies, biochemical studies on the contractile proteins are not without relation to responses in isolated arteries.

Biological Research Laboratories, Pharmaceuticals Division, CIBA-GEIGY Ltd., CH-4002 Basle

188

Relations of blood electrolyte concentration changes to effects on arterial pressure and hematocrit of eight vasoactive drugs in the conscious rat.

O. Aziz, W. Dittrich and E. Sommer

Blood electrolyte concentration (C_{el}) and hct using conductometry of blood ultrafiltrate and of whole blood respectively, and arterial pressure (p_a) were measured continuously in the alert rat 3-6 days after surgery. High intravenous doses of pressor drugs (catecholamines, angiotensin, vasopressin) produce biphasic changes of hct (primary fall) and C_{el} (primary rise). Similar changes are brought about by 2-5 mins of spontaneous activity. Smaller doses (e.g. 0.2 µg/min, 5 mins of angiotensin) elicit essentially a hct rise and C_{el} fall. When high doses of acetylcholine and serotonin (65 and 20 µg/min, 5 mins respectively) have similar biphasic effects on hct as pressor drugs, the effects on C_{el} are also similar. Histamine (5-20 µg/min) produced only hct increase and C_{el} decrease, whereas phentolamine (10-50 µg/min) produced only hct decrease and C_{el} increase at all doses studied. Low acetylcholine (5 µg/min behaves like histamine and low serotonin (10 µg/min) like phentolamine. Thus there is strong evidence of a relation of C_{el} changes to hct changes and not to p_a. C_{el} changes were in the order of +0.2-0.9 or -0.4-1.2 mmol/l NaCl when hct changed by -0.8-2.5 or +1.4-4.5 units. Noradrenalin abolished the previous C_{el} rise caused by phentolamine.

Institute of Applied Physiology, Philipps-Univ., Lahnberge, D-3550 Marburg/Lahn, FRG

189

IODECOL: EVALUATION OF THE ACUTE CARDIAC SIDE EFFECTS OF A NEW, NEARLY ISOOSMOTIC CONTRAST MEDIUM DURING CORONARY ARTERIOGRAPHY IN DOGS+)

R. Schräder, Th. Hoeft, H.G. Wolpers, G. Hellige

Effects of intracoronary injections (8 ml) of Iodecol (non-ionic, dimeric compound with iodine content of 350 mg/ml, osmolality of 0.34 osmol/kg, viscosity at 37 °C of 13.8 cps) and Iopamidol (non-ionic, monomeric compound, iodine content of 370 mg/ml, osmolality of 0.8 osmol/kg, viscosity at 37 °C of 9.5 cps) on hemodynamics, ECG, and cationic content and osmolality in coronary sinus blood were compared in closed-chest dogs (n = 9) with heart catheterization techniques.

Both contrast media produced comparable reactions but Iopamidol , as a rule, had stronger effects on parameters examined. Positive inotropism (dP/dt) and increase of systolic pressure was smaller after Iodecol ($p < 0.05$). That was true for increase of QRS- and QT-duration as well but differences did not reach significant levels. Iodecol produced smaller decrease ($p < 0.05$) of $[Na^+]$ and $[K^+]$ but similar decrease of $[Ca^{++}]$ and $[Ca_{total}]$. Increase of osmolality was stronger following Iopamidol ($p < 0.01$). The effects of Iodecol with respect to all parameters measured could be simulated by means of a 12 % sucrose solution with 15 % dextran (osmolality: 0.38 osmol/kg; visocity 37 °C: 14.5 cps).

Conclusion can be drawn that further slight reduction of acute cardiac side effects of coronary arteriography compared to other non-ionic compounds can be expected from this new contrast agent.

Zentrum Physiologie und Pathophysiologie, Abteilung Experimentelle Kardiologie, Universität Göttingen, Humboldtallee 7, D-3400 Göttingen

+) Supported by the Deutsche Forschungsgemeinschaft, SFB 89 - Kardiologie Göttingen -

190

CARDIOVASCULAR EFFECTS OF ACETALDEHYDE AND ETHANOL IN RATS ALONE AND IN COMBINATION WITH EMD 15 700 AND DISULFIRAM.

K.-O. Minck and J. Harting

It is generally agreed that most of the dramatic symptoms observed in patients after ingestion of disulfiram plus alcohol are due to the resulting increase in blood acetaldehyde level. However in view of the known sympathomimetic properties of acetaldehyde the hypotension observed is still obscure. (T.M.Kitson, J.Stud.Alc. 38, 96-113, 1977). It seemed therefore of interest to study the influence of ethanol and acetaldehyde on blood pressure in awake normotensive rats. Repeated i. v. application of 260.7 mg/kg ethanol (33 %) did not significantly influence blood pressure. However by pretreating the animals orally with 1 g/kg disulfiram or 30 and 300 mg/kg EMD 15 700 (2-methyl-4-nitro-1-(4-nitrophenyl)-imidazole) blood pressure was lowered. Infusion of acetaldehyde had a biphasic effect, lower doses (0.0415 mmole/min) decreased and higher doses (0.125 mmole/min) increased the blood pressure. Pretreating the animals with disulfiram (1 g/kg p. o.) potentiated the blood pressure lowering effect of acetaldehyde. This was also true for EMD 15 700 (300 mg/kg p. o.), which like disulfiram inhibits the low km acetaldehyde-dehydrogenase but does not inhibit the dopamine-ß-hydroxilase (L.A.Seyfried, Int.Congr.Pharmacol., Tokyo 1981, 827, O-31). The results demonstrate that the hypotension observed within the socalled disulfiram ethanol reaction can be explain by the blood pressure lowering properties of acetaldehyde per se.

Experimentell-medizinische Forschung, E.Merck, Frankfurter Str. 250, D-6100 Darmstadt

191

ANALYSIS OF THE KININ RECEPTOR MEDIATING RELAXATION OF FELINE CEREBRAL ARTERIES IN VIVO AND IN VITRO

M. Wahl and E.T. Whalley

In vivo studies were performed using perivascular microapplication to pial arteries (Pflügers Arch. 382, 203, 1979). For in vitro studies ring segments of basilar and middle cerebral arteries (m.c.a) under resting tone (500mg) and after contraction with 5-HT ($5x10^{-8}$ M) or KCl (18mM) were used. The basilar artery was insensitive to bradykinin (BK), des-Arg^9-BK, and methionyl-lysyl-BK (M-L-BK). The table contains the concentration (M) or dose (Moles) of each kinin producing 50% of the maximum obtained relaxation to BK and the relative potencies (R.P.) of each kinin compared to BK (100%) for m.c.a and pial arteries. The values shown are geometric means. Repeated administration of BK over 2-8 hr did not change the sensitivity of any of the preparations to the kinins.

	m.c.a.				pial arteries	
	resting tone		5-HT contracted			
	M	R.P.	M	R.P.	Moles	R.P.
BK	$5.6x10^{-7}$	100	$7.3x10^{-8}$	100	$2.2x10^{-12}$	100
M-L-BK	$1.1x10^{-6}$	32	$9.4x10^{-7}$	9	$1.3x10^{-11}$	54
des-Arg^9 BK	10^{-5}	6	10^{-5}	0.7	10^{-9}	0.04

In addition, the B_1-receptor antagonist des-Arg^9-Leu^8-BK did not antagonize the BK induced response. Thus, the receptor mediating relaxation of pial arteries and m.c.a to BK appears to be of the B_2-type. The basilar artery contains obviously no receptors for BK.

Physiologisches Institut, Pettenkoferstr. 12, 8000 München 2

192

ENKEPHALINS IN THE HEART

R.E. Lang, R. Reichl*, K. Hermann
Department of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, F.R.G.
*C.H. Boehringer KG, Department of Pharmacology, D-6507 Ingelheim, F.R.G.

Extracts of guinea pig hearts were subjected to high performance liquid chromatography (HPLC) and the eluted fractions monitored by radioimmunoassays (RIA) for their content of leucine^5-enkephalin (Leu-ENK) and methionine enkephalin (Met-ENK). Distinct peaks of both Leu-ENK and Met-ENK immunoreactivity were found corresponding to the position of synthetic Leu-ENK and Met-ENK respectively. The ratio of Leu-ENK to Met-ENK content was 1:4. Chemical sympathectomy with 6-hydroxydopamine (6-OH-DA) diminished the noradrenaline content of the heart by more than 99%; the concentration in Leu-ENK was reduced by 70%. The Leu-ENK content of the adrenal glands was not affected by this treatment. These observations point to an enkephalinergic innervation of the heart which appears to be of sympathetic origin.

In another series of experiments the synthetic ENK analogue 2-D-Ala,5-D-Leu-ENK exerted a negative chronotropic as well as inotropic action in spontaneously beating guinea pig auricles at concentrations of 10^{-8} to 10^{-6} M. Both effects were markedly reduced in the presence of naloxone (10^{-5} M).

The results show that enkephalinergic innervation exists in the heart. It appears to be associated with the sympathetic nervous system and to influence cardiac performance.

193

INCREASE IN THE HEART RATE CAUSED BY HYPOXIA IN A VASCULARLY ISOLATED HIND LEG OF RAT.

F. Thimm, E. Meier zu Verl

In preceeding experiments the hind legs of rats were severed from the body except for sciatic nerve and femur. A Tyrode solution, equilibrated with 95 % O_2 and 5 % CO_2 (pH = 7.4; T = 37°C) was perfused into the A.femoralis. Under test conditions, Tyrode solution was isotonically enriched by lactic acid or HCl. The following results were observed:

1) an increase in the heart rate when [lac] exceeded 5 mmol/l
2) the maximum heart rates were observed when $[H^+]$ and [lac] were simultaneously high (pH< 7.15; [lac] 9.5 mmol/l).
3) a low pH alone sufficed to increase heart rate (Thimm et al., Pflügers Arch. 394, R 53, 1982).

The problem arises whether an endogen lactic acid production influences the peripheral drive of the heart rate as well. Using the same technique as mentioned, an endogenous production was provoked by reducing the flow of the oxygenated Tyrode perfusion ($\dot{Q}$< 20 ml/100 g min) as well as by reducing the O_2 fraction of the gas mixture (80 % N_2, 15 % O_2, 5 % CO_2). In both cases the local oxygen uptake decreased ($V'o_2$< 0.2 ml/100 g min) while heart rate was enhanced. In the venous outflow [lac] was found to be increased up to 12 mmol/l. At [lac] of 8 mmol/l, e.g., the heart rate showed an increase of 39.8 ± 11.5 beats/min (n=6). The increases of heart rates due to 'endogenous lactic acid' production are of the same order as those increases that could be observed in the lac-perfusion experiments.

Physiologisches Institut der Deutschen Sporthochschule Köln, Carl-Diem-Weg, D-5000 Cologne, FRG

194

INTERACTION BETWEEN BEZOLD-JARISCH REFLEX AND ACTIVATION OF CHEMORECEPTORS

B.Mohr, W.Tilenius and V.Thämer

In anaesthetized cats activation of ventricular mechanoreceptors leads to a decrease in heart rate and blood pressure. Activity of sympathetic nerves , however , shows differing reactions. We found an inhibition as well as an activation of sympathetic activity. Inhibition was mediated via afferent fibres within the vagal nerve and disappeared after dissection of the nerve. The experiments were performed in order to find out the causes for these different reactions in sympathetic activity. Blood pressure, heart rate and both activity of renal and cardiac sympathetic nerves were measured in anaesthetized cats (60 mg/kg chloralose). Mechanoreceptors are activated by a distension of the left ventricular wall. This distension could be produced by either mechanical means, obstruction of cardiac outflow or coronary occlusion. An inhibition of sympathetic activity could not be observed when chemoreceptors were activated simultaneously by respiration with CO_2 or a fall in pO_2. Bradycardia , however, was not affected by changes in blood gases. These results hint to the fact that changes in sympathetic activity and bradycardia can be influenced separately.

Physiologisches Institut I der Universität Düsseldorf, Moorenstraße 5, D-4000 Düsseldorf

195

A NEW LINE OF SPONTANEOUSLY HYPERTENSIVE RATS WITH DIABETES INSIPIDUS: EVIDENCE THAT AVP IS NOT NECESSARY FOR HYPERTENSION.

U. Ganten, W. Rascher, D. Ganten; German Intitute for High Blood Pressure Research and Department of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, F.R.G.

The question whether arginine vasopressin (AVP) is necessary for the development of hypertension was investigated. Spontaneously hypertensive rats of the stroke-prone substrain (SHRSP) were crossbred with rats homozygous for hypothalamic diabetes insipidus of the Brattleboro strain (DI) which are unable to synthetize AVP. The successful introduction of the DI gene into the SHRSP strain in the F 7-generation was demonstrated by excessive water intake in the new SHRDI line, undetectable AVP in the plasma, in the hypothalamus and in the pituitary and reduced urine osmolality. Mean arterial blood pressure was markedly increased in SHRDI as well as in SHRSP (184 ± 9.7 vs 197 ± 5.2 mmHg). SHRDI and DI rats did not adequately concentrate their urine following water deprivation, but both strains of rats responded well with a fall in drinking and urine output and a rise in urine osmolality to subcutaneous administration of 1-deamino-8-D-arginine vasopressin (DDAVP), the non-pressor analogue of AVP.

The new line of SHRDI is the first substrain of spontaneously hypertensive rats with a single genetic defect in peptide hormone synthesis. The results demonstrate that AVP is not necessary for the development of spontaneous hypertension in rats which resolves a longstanding controversy.

Supported by the Deutsche Forschungsgemeinschaft, SFB 90 "Cardiovasculäres System".

196

ROOT EFFECT IN EEL BLOOD INDUCED BY CARBON DIOXIDE AND BY FIXED ACID

P. Scheid, C.R. Bridges, M.P. Hlastala

The Root effect, i.e. the reduction in O_2 affinity of blood with decreasing pH, has been reported for a number of fish species, including the eel. It is unknown whether the Root effect is evoked by changes in the H^+ activity or whether CO_2 exerts an influence on the Root effect which is independent of that of $[H^+]$. The Root effect was studied in whole blood of the European eel, Anguilla anguilla, at 15 or 25°C in which pH was varied in the range of 5.5 to 9 by either addition of acid or base (fixed acid Root effect) or by varying PCO_2 (CO_2 Root effect). Hemoglobin bound oxygen was independent of PO_2 above 150 Torr, and is hence referred to as O_2 capacity (O_2cap). At pH below about 8.5, O_2cap decreased sigmoidally with pH to attain, below pH 6.0, a value of about 50% of the maximum O_2cap, measured at pH above 8.5. Above pH of 6.5, the values of the CO_2 and fixed acid Root effects were identical. Below pH of 6.5, however, the CO_2 Root effect exceeded the fixed acid Root effect, indicating that in this range CO_2 did exert an independent influence on the Root effect. In this range, where the CO_2 Root effect exceeded the fixed acid Root effect, increase in PCO_2 resulted in an increase in pH, thus formally yielding a negative buffer value of true plasma. This may be explained by oxylabile carbamate formation which, under these conditions, results in a net H^+ binding upon increasing PCO_2.

Max-Planck-Institut für experimentelle Medizin 3400 Göttingen and Institut für Physiologie, Ruhr-Universität, 4630 Bochum

197

PROPERTIES OF AN EXTRAERYTHROCYTIC CARBONIC ANHYDRASE FACILITATING $^{14}CO_2$ EXCHANGE BETWEEN CAPILLARY BED AND TISSUE IN RABBIT SKELETAL MUSCLE.

C. Geers and G. Gros

We have studied the $^{14}CO_2$-$H^{14}CO_3^-$ exchange in skeletal muscle by comparing the washout curves of $H^{14}CO_3^-$ and of an intravasal indicator, ^{3}H-dextran. A bolus containing $H^{14}CO_3^-/^{14}CO_2$ (≈20/1) and ^{3}H-dextran was injected into the femoral artery of a blood-free perfused rabbit hindlimb. The outflowing perfusate was fractionated and analysed for [^{14}C] and [^{3}H]. Washout curves of the two isotopes were generated by plotting the fractional concentrations (isotope concentration per total amount of isotope injected) vs. time. Results: 1) ^{14}C was washed out much more slowly than ^{3}H, the peak height for ^{14}C being only 10% of that for ^{3}H. 2) Addition of acetazolamide (ACT) to the perfusate accelerated the washout of ^{14}C (but not of ^{3}H) and increased the peak height to 20% of that for ^{3}H. Similar results have been reported by Effros and Weissman and Zborowska-Sluis et al. 3) The ACT concentration necessary to achieve a half-maximal acceleration of ^{14}C washout is in the range of 10^{-6}M. 4) Perfusion with 10^{-6}M ACT for 1 min resulted in an acceleration of ^{14}C-washout that could not be increased by extending the perfusion time up to 1 hr. Conclusions: In the absence of ACT, $H^{14}CO_3^-$, while passing the capillary bed, is rapidly converted to $^{14}CO_2$ by an extraerythrocytic carbonic anhydrase (CA). As $^{14}CO_2$ can readily diffuse into the intracellular space the washout of ^{14}C is slow. With ACT this CA is inhibited, more ^{14}C remains in the form of $H^{14}CO_3^-$ which is largely confined to the extracellular space and thus washed out more quickly. The CA involved appears not to be located within the muscle cell: a) The sarcoplasmic CA is known to be inhibited by 50% at 10^{-4}M ACT whereas the present effect is achieved with 10^{-6}M ACT. b) Measurements from our laboratory have shown that ACT is taken up by muscle tissue with a half-time of 45 min while the present effect requires ≤ 1 min of exposure to ACT.

Institut für Physiologie, Universitätsklinikum Essen, Hufelandstrasse 55, D-4300 Essen 1, F.R.G.

198

A METHOD FOR MEASURING LOCAL OXYGEN CONSUMPTION OF THE PHYSIOLOGICALLY PERFUSED SKELETAL MUSCLE

H.-J. Meuer, C. Ranke.

The assumption that tissue oxygen consumption equals the PO_2 decrease during perfusion stop multiplied by the solubility coefficient holds only, if no spatial PO_2 gradient exists. In order to meet this condition tissue is perfused by a hemoglobin-free perfusate or blood with PO_2 values usually exceeding 300 Torr. These procedures cause a high tissue PO_2 that disturbs the microcirculation and may influence the measurements.
In order to determine the local O_2 consumption of a physiologically perfused muscle the time course of the tissue PO_2 during perfusion stop was computed using KROGH's model. Furthermore, it was taken into account that oxygen is liberated from capillary hemoglobin. In analogy to the solubility coefficient we defined a "consumption coefficient", which depends on the capillary supply volume and the capillary radius. To obtain these morphological data the tissue PO_2 during perfusion stop is measured by a double-barrelled microelectrode, whose second channel is filled with dye. After the measurement the electrode tip position is stained and the muscle is fixated by shock-freezing. From histological slices stained with the ATPase method, the perfused capillaries in the vicinity of the electrode tip are determined. From the data for the original geometrical arrangement of these vessels the stationary PO_2 field and the supply volume of each capillary are calculated.
Using this new method the oxygen consumption of white fibers of the rat gracilis muscle at rest was found to be 0,35 +/-0,15 ml/(100g · min).

Zentrum Physiologie der Medizinischen Hochschule Hannover, K.-Gutschow-Str. 6, D-3000 Hannover 61.

199

THEORETICAL ANALYSIS OF STATIONARY OXYGEN TENSION PROFILES IN MULTICELLULAR SPHEROIDS

W. Müller-Klieser

Oxygen supply to multicellular spheroids in stirred media under stationary conditions can be theoretically evaluated by establishing and integrating the differential equation of diffusion. This is performed separately for a diffusion-depleted layer in the medium surrounding the spheroids, for a zone of viable cells within the spheroids, and for a necrotic area in the center of larger spheroids. The oxygen tension (pO_2) gradient in the diffusion-depleted layer is a simple function of the specific O_2 consumption rate Q, of Krogh's diffusion constant K_M in the medium, and of the total volume of viable cells V_S. Since K_M and V_S are known from literature and from histological investigations, respectively, Q can be determined by measuring the pO_2-gradient in the diffusion-depleted zone outside the spheroids. Knowing Q, Krogh's diffusion constant K_S within the spheroids can be calculated from the pO_2-gradient in the zone of viable cells. Using the respective gradients determined with O_2-sensitive microelectrodes (W.F. Mueller-Klieser and R.M. Sutherland, Br. J. Cancer 45, 256-264,1982), pO_2-profiles can be calculated that are in a good agreement with spatial O_2-distributions obtained from microelectrode measurements. Corresponding interrelationships between spheroid size and central pO_2-values are found in the pO_2-measurements and in the theoretical evaluation.
The findings suggest that multicellular spheroids allow a quantitative experimental and theoretical analysis of O_2 diffusion under well defined supply conditions. Multicellular tumor spheroids may thus represent useful *in vitro* tumor models for studying diffusive O_2 transport in cancer tissue.

Department of Physiology, University of Mainz, Saarstrasse 21, D-6500 Mainz, FRG

Supported by the Deutsche Forschungsgemeinschaft (Mu 576/1)

200

CHANGES IN OXYGEN SENSITIVITY OF FLUORESCING MICRO- AND NANOPROBES DUE TO INFLUENCES OF THE MEDIUM TO BE MEASURED

N. Opitz and D.W. Lübbers

Oxygen sensitivity of fluorescence indicator molecules depends mainly on the mean lifetime of the excited state of the indicator molecule and on the oxygen permeability of the indicator solvent, since fluorescence quenching by oxygen is mostly a diffusion-controlled phenomenon. Deviations of this well known STERN-VOLMER behavior of oxygen sensitive fluorescence indicator molecules could be observed with thin indicator films (< 1/µm) and micro- and nanoencapsulated indicator molecules due to predominant boundary layer effects of the adjacent material. A theoretical interpretation of these observations is given with the aid of a model. The theoretical analysis shows that, for boundary layers, instead of STERN-VOLMER's equation ($I_o/I = 1 + k \cdot pO_2 \cdot \alpha_I$) one has to apply the following equation:

$$\frac{I_o}{I} = 1 + K \cdot pO_2 \cdot \left(\alpha_I \cdot \frac{13}{16} + \alpha_m \cdot \frac{1}{32} \cdot \left(\sqrt{\left(\frac{D_m}{D_I}\right)^3} + 5 \cdot \sqrt{\frac{D_m}{D_I}} \right) \right)$$

with: K = "overall quenching constant"; (α_I, α_m) = oxygen solubility coefficient of the indicator solvent and the adjacent material, respectively; (D_I, D_m) = oxygen diffusion coefficients as above; (I_o, I) = fluorescence intensities at zero and actual pO_2, respectively;

This equation states that, within boundary layers, the oxygen sensitivity of the fluorescence probes is additionally determined by the coefficients α_m and D_m of the adjacent medium.
The effect of boundary layers could be reduced by encapsulating solvents with low O_2 diffusion coefficients. In order to eliminate the influence of body fluids and tissue on capsule oxygen measurements, it is necessary to produce shell thicknesses greater than 0.1/µm.

Max-Planck-Institut für Systemphysiologie, Rheinlanddamm 201, 4600 Dortmund, FRG

201

CORRECTION OF ERRONEOUS PNEUMOTACHOGRAMS IN BREATH-BY-BREATH ANALYSIS BY MEANS OF THE INTRABREATH N_2 BALANCE.

U.Hoffmann, H.Bittner

When gas-exchange values are measured on a breath-by-breath basis, the nitrogen balance of each respiratory cycle may be utilized to detect changes in pulmonary gas stores and to monitor the random variations between in- and expiratory volumes. Moreover, a systematic inequality of the breath-by-breath nitrogen balance indicates instrumental or computing errors.

By means of the nitrogen-balance technique, we observed a systematic relative overestimation of the expired volume (V_E) (when compared to V_T) in exercising subjects. The differences between V_E and V_I ranged from 0 at rest to about 40 % at the highest ventilations observed (130 l min^{-1}). The flow measuring device (heated Fleisch sensor No. 3) and the connecting mask were found to be the major sources of this error. Intrabreath variations of temperature and gas composition could account for only a minor portion of the non-linearity. The influence of base-line shifts of the pneumotachograph could be minimized by an automatic base-line control and correction.

It is suggested that Fleisch type pneumotachographs, when inserted into common set-ups, deviate from linearity at much lower respiratory flow rates than expected from 'technical' calibration. In the latter case, gas flow is usually directed immediately into the capillary tubes of the Fleisch sensor whereas the geometry of both mask (or mouthpiece) and the upper airways will cause different directions and profiles of airflow. Such disturbances may influence the linearity of the Fleisch sensor to a greater extent than physical parameters (temperature, gas density) and will mainly affect the measurement of expiratory flows.

On-line corrections of single breath data may be obtained on the basis of the intrabreath nitrogen balance.

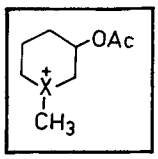
Physiologisches Institut der Deutschen Sporthochschule Köln Carl-Diem-Weg, D-5000 Cologne, FRG

202

STRUCTURAL AND STEREOCHEMICAL REQUIREMENTS FOR CHOLINERGIC ACTIVITY OF HETEROCYCLIC AMMONIUM AND SULFONIUM ANALOGUES OF ACETYLCHOLINE

G. Lambrecht, E. Mutschler and U. Moser

The experiments were carried out in order to clarify the structural and stereochemical factors influencing the interactions of compounds 1-7 with cholinergic receptors (muscarinic, nicotinic, acetylcholinesterase).



The cholinergic potency of 1-7 depends largely on the structure and stereochemistry of the onium centre, and on the configuration at C-3. Stereoselective Index: Muscarinic-5/6 = 890; Nicotinic-6/7 = > 1000; AchE-3/4 = 6.

Compounds 1-5 show higher muscarinic potency at ileal m_1 receptors than at atrial m_2 receptors.

No.	X	Configuration	Equiactive molar ratios[x] Guinea-pig Ileum	Atrium	Frog rect.	AchE
1	NH	R	27000	335000	xx	83
2		S	2100	6800	885	200
3	NCH_3	R	6400	13000	2600	12
4		S	480	3900	85	2
5	S-Cis	1R,3S/1S,3R	1700	8900	25	3
6	S-Trans	1R,3R	13	10	xx	14
7		1S,3S	700	460	89	3

[x]Ach = 1. [xx]Inactive at conc. up to 10 mmol/l.

Pharmakologisches Inst. f. Naturwiss., Theodor-Stern-Kai 7, Gebäude 75A, D-6000 Frankfurt/M

203

CARBACHOL STIMULATED PEPSINOGEN RELEASE FROM ISOLATED GUINEA PIG GASTRIC GLANDS IS Ca^{2+} DEPENDENT

W. Vivell, A.C. Bauer and K.-Fr. Sewing

Koelz et al. reported carbachol stimulated pepsinogen release both to be dependent (Am. J. Physiol. 243, G218, 1982) and independent (Gastroenterology 80, 1194, 1981) from extracellular Ca^{2+}. This controversy needed to be elucidated. Therefore in guinea pig isolated gastric glands pepsinogen release as measured by the hemoglobin technique (Berstad, Scand. J. Gastroent. 5, 343, 1970) was studied in response to graded concentrations of carbachol (10^{-7}-10^{-3} mol/l) and/or Ca^{2+} (10^{-5}-10^{-2} mol/l). Glands were prepared by incubation of a gastric mucosal scraping for 15 min in the presence of 0.05 % collagenase and for subsequent 45 min in the presence of 0.05 % collagenase and 0.05 % dispase. Incubation for pepsinogen release was carried out for 40 min in Hank's buffer (pH 7.4). Ca^{2+} in the range of 10^{-5}-10^{-2} mol/l stimulated basal pepsinogen release in a concentration dependent manner. Carbachol 10^{-4} mol/l enhanced pepsinogen release independently from the extracellular Ca^{2+} concentration, the differences between basal release and that in the presence of carbachol being most pronounced at appr. 3 x 10^{-3} mol/l Ca^{2+}. At high concentrations Ca^{2+} again reduced the carbachol response.

Experiments with calcium antagonists such as gallopamil seem to support a dual action of Ca^{2+} on gastric mucosal chief cells in which pepsinogen release in response to carbachol has now been shown to be calcium dependent.

Abt. Allgemeine Pharmakologie, Medizinische Hochschule Hannover, Konstanty-Gutschow-Str. 8, D-3000 Hannover 61

204

A SUBTYPE OF MUSCARINIC RECEPTORS SENSITIVE TO PIRENZEPINE IS INVOLVED IN VAGAL INDUCED GASTRIC SECRETION

A. Schiavone, A. Giachetti and R. Hammer

Pirenzepine (P) is a novel antimuscarinic discriminating between receptor subtypes. Muscarinic receptors with high affinity for P prevail in neural tissues (M_1), whilst those with low affinity are found mainly in smooth muscle and heart (M_2). In vivo, P inhibits selectively vagally mediated gastric secretion. To characterize muscarinic receptors involved in secretion, the isolated lumen perfused mouse stomach was used. Acid output was elicited either by exogenous bethanecol (10 μM) or by electrical field stimulation (10 V, 10 Hz, 0.5 msec) which mimicks vagal excitation. The tabulated values of P and atropine (A) are the nM conc. inhibiting by 50% the acid secretion (EC 50 and conf. limits).

	EC 50 (nM) Bethanecol	Field Stimulation
Pirenzepine (P)	840 (526-1220)	280 (189-408)
Atropine (A)	29 (18-46)	65 (42-100)
Ratio P/A	29	4

The results show that P is a potent antagonist of field stimulated secretion, being only 4 times less potent than A. On the other hand, P is a weaker antagonist of acid secretion evoked by exogenous muscarinic agonists. The data suggest that P-sensitive muscarinic receptors of the M_1 type are present in the gastric vagal pathway. It is postulated that these receptors are located at a site remote from the effector cells and are crucial in the neural regulation of gastric secretory function. Thus P should be viewed as a novel tool in understanding the physiology of gastric secretion.

Dpts. Pharmacol. & Biochem., Ist. De Angeli, Milan

205

INTRINSIC FACTOR AND R-PROTEINS OF ISOLATED RAT GASTRIC CELLS

H.-J. Ruoff and W. Schepp

Specific and nonspecific cobalamin binding capacity, i.e. the production and secretion of intrinsic factor plus R-proteins were studied using isolated rat gastric mucosal cells, which were obtained by treatment with pronase and subdivided into fractions containing 3 to 85 % of parietal cells by isopycnic centrifugation with Percoll. Incubation of these cells at 37°C lasted up to 240 min and was stopped by centrifugation. Cobalamin binding capacity was determined in the supernatant and the sonicated cell sediment by use of (^{57}Co)cyanocobalamin employing a charcoal assay. The intrinsic factor portion of the total binding capacity was estimated by means of specific antibodies from patients with pernicious anaemia and ranged between 45 to 50 %.
Maximal cobalamin binding capacity was found in fractions with less than 10 % of parietal cells, which showed as well the highest pepsin content. Within the other fractions, the degree of cobalamin binding decreased constantly with increasing number of parietal cells. In cell preparations with high cobalamin binding capacity, histamine, pentagastrin and hexoprenaline stimulated secretion into the medium up to 20 %, 10^{-3} mol/l dibutyryl cAMP enhanced the release by 40 % above the basal level. Carbachol proved to be the most effective stimulant, initiating the secretion process at 10^{-5} mol/l and leading to a maximal response up to 90 % by 10^{-3} mol/l. This effect of carbachol could be nearly abolished by 10^{-4} mol/l atropine or pirenzepine, whereas PGE_2 and somatostatin failed to exert significant inhibition.
The data support the concept that in the rat gastric mucosa the chief cells represent the site of intrinsic factor production and its secretion is mainly under cholinergic control.
Supported by the Deutsche Forschungsgemeinschaft
Pharmakologisches Institut der Universität Tübingen, Wilhelmstr. 56, D-7400 Tübingen 1, FRG

206

EFFECTS OF THE SUBSTITUTED BENZIMIDAZOLES PICOPRAZOLE AND OMEPRAZOLE ON PARTIALLY PURIFIED H^+/K^+-ATP-ASE FROM ISOLATED AND ENRICHED GUINEA PIG PARIETAL CELLS.

W. Beil and H. Hannemann

Protons are pumped from the parietal cells into the lumen by an H^+/K^+-ATPase which is localized in membranes of the intracellular canaliculi. H^+ secretion in response to activation of this enzyme can be inhibited by compounds which are referred to as substituted benzimidazoles.
It was the purpose of the present investigation to study the effect of typical substituted benzimidazoles such as picoprazole and omeprazole in an enzyme preparation partially purified by differential and density gradient centrifugation from isolated and enriched guinea pig parietal cells. Parietal depleted fractions did not contain an H^+/K^+-ATPase. In a 10-12 fold enriched enzyme preparation from parietal cells picoprazole and omeprazole inhibited the enzyme in a concentration dependent manner with an IC_{50}-value in the range of 1 µmol/l. Calcium inhibited the enzyme in a concentration dependent manner in the range of 1 to 100 µmol/l. Calcium-antagonists such as verapamil which have been shown to inhibit H^+ secretion by a mechanism close to the H^+/K^+-ATPase (Sewing and Hannemann, Pharmacology, in press) and the histamine H_2-receptor-antagonist cimetidine were ineffective. It can be concluded that in the stomach only the parietal cells contain H^+/K^+-ATPase and that the inhibitory effect of picoprazole and omeprazole on H^+ secretion reflects a true inhibition of the enzyme.

Abteilung Allgemeine Pharmakologie, Medizinische Hochschule Hannover, Konstanty-Gutschow-Str. 8, D-3000 Hannover 61, FRG.

207

ULCEROGENIC EFFECTS AND PHARMACOKINETICS OF ORAL AND I.V. ACETYLSALICYLIC ACID AFTER 16,16-DIMETHYL-PROSTAGLANDIN E_2.

U. Weißenborn and S. Mädge

16,16-Dimethyl-prostaglandin E_2 (PGE) in a nonantisecretory dose is known to prevent the formation of gastric lesions produced by various noxious agents (for review: T.A. Miller et al.: Gut 20, 75, 1979). The mechanism of this cytoprotection is not completely elucidated.
In female Wistar rats, which were fed ab lib., the effect of PGE on the mucosal concentration and pharmacokinetics of acetylsalicylic acid (ASA) and its main metabolite salicylic acid (SA) was studied. ASA and SA were determined by HPLC in plasma and fundic and jejunal mucosa. Gastric lesions appeared within 60 min after an oral dose of 150 mg/kg ASA but not after an i.v. dose of its sodium salt. After an oral dose unchanged ASA was still present in the fundic mucosa after 6 h. Only traces of ASA were found after the i.v. dose even at early time points. The development of lesions was prevented by pretreating the animals with 5 µg/kg PGE orally 30 min prior to ASA. PGE had no influence on the pharmacokinetics of ASA given i.v. but produced higher fundic concentrations of both ASA and SA after an oral dose of ASA within the first hour.
It is concluded that ASA reaches toxic gastric mucosal levels only when given orally, but not when given i.v.. In a nonantisecretory dose PGE prevents gastric mucosal damage although it enhances ASA tissue concentrations at early time points. ASA metabolism to SA does not seem to be influenced by PGE. SA concentrations however are much lower after i.v. than after oral administration. The longer plasma half-lives of SA after oral ASA compaired with after i.v. ASA cannot yet be fully explained.

Abteilung Allgemeine Pharmakologie, Medizinische Hochschule Hannover, Konstanty-Gutschow-Str. 8, D-3000 Hannover 61, FRG.
Supported by DFG (SFB 146 and Se 151/12)

208

SOME EVIDENCE FOR AN INDUCED CYTOPROTECTION BY ANTACIDS

S. Postius, H. Engler and I. Szelenyi

Certain antacids prove to be potent in the management of ulcer diseases. The buffer capacity combined with the ability to inactivate pepsin and to bind bile acids are partly responsible for the beneficial therapeutic effects of antacids. PGE_2 is known to exert a cytoprotective effect on gastric mucosa after intragastric instillation in non-secretion-blocking concentrations. Therefore, we measured mucosal PGE_2 synthesis and its liberation under defined conditions of the rat stomach.

The investigated antacid ($CaCO_3$ + $Al(OH)_3$) enhanced both, the mucosal synthesis of PGE_2 and its liberation into the gastric lumen. Concomitantly, the occurence of gastric lesions decreased. Unbuffered, neutral hyperosmolaric salt solutions show similar behaviour, however, with decreased ulcer protection. Unbuffered, acid hyperosmolaric salt solutions still enhance PGE_2 synthesis, but there was no ulcerprotection. The reason for these different observations can be sought in the acid instability of PGE_2: not more than 20% of PGE_2 were detected after 30 minutes at pH 2, whereas 50% survived at pH 3.5 - 4.5. The natural cytoprotective effect of PGE_2 is limited by its pH-dependent halflife. This can be counteracted by a regular intake of antacids.

Summing up, antacids exert their cytoprotective effect by an increase of PGE_2 synthesis, its liberation and a prolonged halflife in the gastric lumen.

Abteilung Pharmakologie, Heumann-Pharma, Heideloffstr. 18-28, 8500 Nürnberg

209

IS GASTRIC ACID SECRETION MODULATED BY A PERIPHERAL OPIOID MECHANISM?

W. Kromer, B. Skowronek, H. Stark and S. Netz

Contradictory results, mostly derived from in vivo studies, have been reported with regard to a possible action of opioids on gastric acid secretion. In preliminary experiments, we have investigated therefore the influence on acid secretion of D-ala^2-D-leu^5-enkephalin (DADLE, 1 µmol/l) and naloxone (NAL, 1 µmol/l). Acid secretion from isolated and enriched parietal cells from guinea pig gastric mucosa was stimulated by histamine (HIS, 10 µmol/l) and determined by means of ^{14}C-aminopyrine accumulation (Berglindh et al., Acta Physiol. Scand. 97, 401-414, 1976).

Although basal acid secretion was influenced neither by DADLE nor by NAL, DADLE enhanced HIS-stimulated acid secretion by appr. 15 % and NAL impaired it by appr. 17 %. NAL reduced acid secretion which was stimulated by HIS plus DADLE or by HIS alone to the same absolute level, corresponding to a 27 % inhibition ($p < 0.01$; $n = 13$) in the former instance.

Binding data obtained with membrane fractions of isolated parietal cells seem to indicate stereospecific, saturable and reversible opioid binding. The binding site, however, is highly labile and its validation therefore difficult.

Our data point to a probable peripheral modulation by exogenous and endogenous opioids of the stimulated gastric acid secretion.

Abteilung Allgemeine Pharmakologie, Medizinische Hochschule Hannover, Karl-Wiechert-Allee 9, D-3000 Hannover 61, FRG.

Supported by the Deutsche Forschungsgemeinschaft

210

INHIBITION BY INDOMETACIN OF INTESTINAL FLUID SECRETION AFTER MORPHINE WITHDRAWAL IN THE RAT.

E. Beubler

Morphine withdrawal by naloxone in morphine dependent rats reversed net water absorption into net secretion in jejunum and colon (Beubler, N.S. Arch. Pharmacol. 316 R 62 1981). Recently, the α_2-receptor agonist clonidine was shown to inhibit the morphine withdrawal syndrome and, in particular, withdrawal diarrhea (Gold, Lancet II, 599, 1978). To find out the mechanism of action by which morphine withdrawal induces fluid secretion, rats were rendered morphine dependent by morphine pellet implantation (75 mg morphine, 48 hrs). Net water flux in 20 min (and mucosal cAMP levels) were measured in the tied off colon. Morphine withdrawal was precipitated by naloxone (1 mg/kg s.c.).

Clonidine (100 µg/kg s.c.) inhibited intestinal fluid secretion caused by morphine withdrawal, but clonidine also abolished the increase in intestinal fluid volume induced by PGE_2 (20 µg/kg i.p., enteropooling assay). To find out whether endogenous PGE_2 is involved in the genesis of intestinal fluid secretion after morphine withdrawal, indometacin (4 mg/kg s.c.) was given 1 hr prior to naloxone-precipitation. In fact, inhibition of PG-synthesis by indometacin abolished intestinal fluid secretion induced by morphine withdrawal, indicating the involvement of PGE_2. Since PGE_2 has been shown to increase mucosal cAMP levels in previous experiments, cAMP levels are expected to be enhanced after morphine withdrawal. The dose-response curves for the effect of theophylline on net water secretion and mucosal cAMP levels were shifted to the left after morphine withdrawal, indicating the involvement of cAMP in the generation of fluid secretion after morphine withdrawal.

Conclusions. Intestinal fluid secretion after morphine withdrawal is supposed to be mediated by endogenous PGE_2-synthesis, which leads to an increase in cyclic AMP.

Inst.f.Exp.u.Klin.Pharmakol., Univ.Pl.4, A-8010 Graz.

211

THE INFLUENCE OF DITHIOCARB ON GASTRIC SECRETION AND GASTRIC EMPTYING

U. Peters

The inhibition of the ulcerogenic activity of nonsteroidal antiinflammatory drugs induced by the thio compound dithiocarb was accompanied by an increase in the stomach volumes (Hoppenkamps et al., this supplement). The present investigation deals with the gastric action of dithiocarb and with its mechanism.
In vitro, high concentrations of dithiocarb (10^{-2}g/ml) induced a long lasting (>90 min) contraction of gastric smooth muscle preparations (rat fundic strips, preparations of the corpus and pylorus). These contractions could not be prevented by the H1-receptor antagonist mecIastine but were inhibited by the H2-receptor antagonist cimetidine. In rats in vivo, dithiocarb (50 or 100 mg/kg i.p.) produced a strong increase in the stomach volume and a decrease in the gastric pH. Gastric emptying in vivo as investigated by administration of marker substances to rats (hexadecane, lidocaine and black ink) was found to be delayed after treatment with dithiocarb. Investigations on the gastric emptying in situ performed by the technique of Bertaccini et al. (Europ.J.Pharm. 22,320;1968) revealed that dithiocarb exerts a marked and long lasting spasmogenic effect on the rat pylorus sphincter. The effects of dithiocarb on gastric secretion in vivo as well as those on gastric emptying in vivo and in situ were antagonized by cimetidine but not influenced by antihistaminic drugs of the H1-type.
Conclusion: Dithiocarb, by a direct or indirect action on H2-receptors, stimulates gastric secretion and delays gastric emptying.

Institut für Toxikologie der Medizinischen Hochschule Lübeck, D-2400 Lübeck (FRG)

212

INFLUENCE OF HYDROSTATIC PRESSURE ON NET FLUID MOVEMENT ACROSS ISOLATED RAT COLONIC MUCOSA EXPOSED TO DIFFERENT SECRETAGOGUES

U.Karbach, R.Wanitschke

Subepithelial tissue pressure is a supposed driving force of intestinal secretion.With respect to paracellular permeability we investigated the effect of hydrostatic pressure (HP) applied on the serosal side on net water and sodium movement in everted sacs of stripped rat colon treated with deoxycholic acid (DA), bisacodyl (BI), etacrynic acid (EA), rhein (RH) and cholera toxin (CT).PEG was used as volume, erythrol as marker for paracellular permeability.
In controls hypertonic fluid (8,4+o,6ml/g d.w.) was absorbed against a 2cm HP.Absorption of hypertonic fluid was reduced after treatment with CT to 2,5+1,2ml/g d.w.Absorption of water and sodium was abolished in the presence of DA, BI, EA and RH.DA caused a 5-fold, BI a 2-fold and EA a 6-fold increase of erythrol movement from the serosal to the mucosal side.RH had no influence on paracellular permeability.The amount of erythrol moved across the mucosa pretreated with CT was 4o% smaller than in controls. At 7cm HP the net fluid transport was abolished in controls, sodium was still absorbed.There was a remarkable movement of isotonic fluid to the mucosal side when the tissue was exposed to DA (12,4+1,4ml/g d.w.), BI (13,6+1,9ml/g d.w.), EA (16,6+2,2ml/g d.w.) and less pronounced in the presence of RH (4,o+o,4ml/g d.w.).The fluid appearing at the mucosal side after treatment with CT (8,1±1,8ml/g d.w.) was hypotonic.
The results indicate that various secretagogues affect net fluid movement differently.Secretagogues acting by cellular mechanisms can be distinguished from compounds inducing secretion by an increase of paracellular permeability by the amount and the composition of the fluid transferred under an imposed serosal HP.

Institut für Pharmakologie und Toxikologie, Universität des Saarlandes, 665o Homburg/Saar

213

BUFFERING OF MEDIUM CALCIUM CONCENTRATION BY LEAKY ISOLATED EXOCRINE CELLS OF RAT PANCREAS

H. Streb

Isolated pancreatic acini made permeable by saponin take up $^{45}Ca^{2+}$ into intracellular organelles on addition of ATP (Wakasugi et al., 1982, J. Membrane Biol. 65, 205). Now we have investigated if this Ca^{2+} uptake regulates $[Ca^{2+}]$ of the extracellular medium, which in cells with permeable plasma membrane represents a kind of extended cytosol. Isolated acinar cells were incubated at 25°C in a medium containing MgATP, an ATP regenerating system and respiratory substrates (pH 7.4). The medium $[Ca^{2+}]$ was recorded continuously with a Ca^{2+}-specific electrode and adjusted by Ca^{2+} addition to a value between 0.7 - 6µmol/l. On addition of leaky cells, the medium $[Ca^{2+}]$ was reduced due to cellular uptake until a steady state value of 0.42 ± 0.01 (S.E.M.) µmol/l (n=54) was obtained, giving an estimate of the cytosolic $[Ca^{2+}]$ buffered by intracellular organelles. Changes in the medium $[Ca^{2+}]$ at steady state by addition of Ca^{2+} or EGTA were buffered by cellular uptake or release respectively until the steady state $[Ca^{2+}]$ was reestablished.
Experiments with mitochondrial inhibitors and the ATPase inhibitor vanadate showed that Ca^{2+} uptake into permeabilized cells is due to 1.) mitochondria, which determine the initial rate, and 2.) at least one nonmitochondrial structure, which determines the final steady state. Calcium uptake into this "nonmitochondrial steady state pool" is completely dependent on Mg^{2+} and cannot be stimulated by oxalate. Its rate of uptake is diminished in the absence of small monovalent cations (K^+, Na^+).
The data indicate that a cytosolic $[Ca^{2+}]$ of 4 x 10^{-7}mol/l can be maintained in exocrine pancreatic cells by the action of a nonmitochondrial MgATP-dependent calcium pool.

Arbeitsgruppe I. Schulz,
Max-Planck-Institut für Biophysik, Kennedyallee 70,
D-6000 Frankfurt (Main) 70

214

REGULATION OF ACETYLCHOLINE BINDING TO PANCREATIC MICROSOMES

F. Boege, L. Eckhardt, and I. Schulz

Using ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) and ^{3}H-oxotremorine (^{3}H-OT) binding of muscarinic antagonist and agonist was studied in microsomes obtained from bovine pancreas. Microsomes were incubated with ^{3}H-QNB or ^{3}H-OT and different test substances for 20 min at 30°C and separated from the incubation medium by filtration. Nonspecific binding was obtained in the presence of 10^{-5}mol/l atropine and each value for the dissociation constant (K_D) was evaluated by a Scatchard plot obtained from incubations at 7 different ^{3}H-QNB concentrations (10^{-10}-10^{-8}mol/l). In refrozen-thawn vesicles ATP in the presence of the phosphatase inhibitor NaF (10 mmol/l) increased the K_D of ^{3}H-QNB ($8x10^{-9}$mol/l) by 4-fold in a concentration dependent way. Pancreatic secretagogues which bind to different receptors, but exert the same effect on Ca^{2+} release and enzyme secretion such as cholecystokinin-pancreozymin (CCK), caerulein, and bombesin, act in the same way as the phosphatase inhibitor NaF, decreasing the affinity to ^{3}H-QNB in the presence of ATP. Similar to these results the Scatchard plot was linear for the binding of the agonist ^{3}H-OT in the absence of ATP (K_D 1,4 x 10^{-8}mol/l), whereas in the presence of ATP and NaF the plot was also linear, but the K_D increased to 5,5 x 10^{-8}mol/l. ^{3}H-OT binding in the presence of ATP but without NaF showed non-linearity with 2 slopes of similar K_D values as above. This suggests that the muscarinic agonist acts like the other peptide secretagogues, decreasing binding affinity to its receptor with increasing concentrations. The data indicate that binding of acetylcholine is regulated by a protein which is phosphorylated and dephosphorylated by a proteinkinase and phosphatase, respectively. In the phosphorylated form the protein decreases the affinity of the receptor to its ligand. Secretagogues inhibit the phosphatase, thus decreasing receptor affinity.

Max-Planck-Institut für Biophysik, Kennedyallee 70
6000 Frankfurt/Main 70

215

CIRCADIAN COURSE OF RAT PANCREATIC ACINAR CELL RESPONSIVENESS

V. Keim

Circadian variation of rat pancreatic secretion had been observed in conscious rats (1). Here we report the circadian change of rat pancreati acinar cell response to different amounts of Cerulein.
Methods: Rat pancreatic lobules are prepared by the technique of SCHEELE (2). The release of pancreatic enzymes into the incubation medium is stimulated by different doses of Cerulein (10^{-11}-10^{-8} M). At discrete times after the start of the incubation, samples are taken to measure amylase activity. After the end of the experiment the lobules are homogenized and the enzymes (Amylase, Trypsin, Chymotrypsin) determined. The release of Amylase is expressed as percent of total (= secreted + stored).
Results: It can be demonstrated, that pancreatic enzyme content changes with respect to the time of the day. At noon, about 30% of total Amylase can be released by 10^{-9} M Cerulein. At night only 12% of Amylase is secreted into the medium. The dose-response-curve seems to be shifted to higher values. When the dietary protein is substituted by equivalent amounts of aminoacids, so that pancreatic enzymes are not necessary for digestion of the food, stimulus-secretion-coupling changes only a little, but compared to the nighttime only very small amounts of enzymes are stored within the pancreatic acinar cell at noon.
Conclusion: Response of pancreatic acinar cells to Cerulein stimuli in release of enzymes depends on the time of the day. The sensitivity of cell receptors and/or secondary effects following effector-cell interaction may be involved.

(1) Keim & Haberich, Z. Gastroenterologie, Nr. 6, 20 (1982) 346-353
(2) Scheele, JBC, (1975) 250/7, 226-2270

Institute of Applied Physiology, Philipps-University, Lahnberge, D-3550 Marburg/Lahn, FRG

216

EFFECT OF INTRADUODENAL BILE UPON THE SPONTANEOUS EXOCRINE PANCREATIC SECRETION IN CONSCIOUS RATS

H.-J. Lübke and F. J. Haberich

Intraduodenal administration of bile increases pancreatic flow, protein and bicarbonate secretion in man, in the dog and in the cat. An adverse effect of bile upon pancreatic secretion was reported in the rat (Dig. dis. Sci. 24, 602-608, 1979). It was the objective of this study to determine the influence of intraduodenally infused bile and of bile diversion on the pancreatic secretory pattern in conscious rats.
Method: Separate diversion and recirculation of pancreatic juice and bile is possible in female Wistar rats by the previously described surgical technique (Z. Gastroent. 18, 427-435, 1980). During 90 min of bile diversion the following solutions were substituted: isotonic saline, 25 mmol bicarbonate and 25 mmol cholic acid. In additional experiments without diversion pooled bile was administered into the duodenum.
Results: Bile diversion increases pancreatic flow (+40%), the output of protein (+99%), bicarbonate (+46%), sodium (+17%) and potassium (+55%). This effect is partly inhibited by the presence in the duodenum of bicarbonate, more evident by cholic acid substitution. Intraduodenal perfusion of pooled bile decreases pancreatic flow (-10%), protein (-30%), bicarbonate (-9%), sodium and potassium output (-9%).
Conclusion: The spontaneous pancreatic secretion in conscious rats is partly controlled by intraduodenal bile; the absence of bile acids is implicated in an increased pancreatic secretory response.

Institute of Applied Physiology, Philipps-Univ., Lahnberge, D-3550 Marburg/Lahn, FRG

217

NO INCREASE OF BILIARY PERMEABILITY IN ETHINYL ESTRADIOL TREATED RATS. H.Jaeschke and H.Krell

Ethinyl estradiol (EE) - induced cholestasis was suggested to be due to increased biliary permeability with reflux of water and bile constituents. To test this hypothesis we compared biliary parameters after EE treatment in vivo and in isolated perfused livers (IPL) with those of controls (C) and after α-naphthylisothiocyanate (ANIT) treatment. EE (5mg/kg/d;s.c.) administered to male Wistar rats for 5 d decreased bile flow (BF) in vivo to 1.22±0.08µl/min/g liver and the basal BF in the IPL to 0.74±0.07 compared to resp. C-values (2.20±0.16; 1.34±0.12; p<0.001). The max. bile salt excretion rate (T_m) during taurocholate (TC) infusion was diminished after EE treatment although TC-conc. in bile was increased (p<0.001). C and EE rats developed almost identical biliary pressures (BP). The bile/perfusate ratio of (^{14}C)-sucrose and (^{14}C)inulin increased significantly, but this could be accounted for solely by the decline of BF as indicated by identical clearance (CL) rates. Using the integrated CL equation described by Forker (J Clin Invest 48:654,1969) the permeability coefficients K that statistically best fit the data were mathematically derived for all experimental groups. The analysis yielded K-values for sucrose and inulin of 0.243 and 0.037 in C and 0.273 and 0.038 in EE rats. In contrast, 9h ANIT treatment (250mg/kg;p.o.) caused cholestasis (basal BF:0.99±0.26) with diminished TC-conc in bile and TC-T_m, and reduced BP. Here, K-values calculated for sucrose and inulin were 0.807 and 0.175. These findings suggest that altered junctional permeability causes ANIT cholestasis but is not the primary event of EE cholestasis.

Toxikologisches Institut der Universität Tübingen, Wilhelmstrasse 56, D-7400 Tübingen

218

TRIS(HYDROXYMETHYL-)AMINOMETHANE STIMULATES INSULIN SECRETION IN RATS
Andrea Maria Lüders and H. Brasch

It is still unknown if the hypoglycaemic effect of TRIS is only due to an increased insulin secretion or if insulin-independent actions are equally important (Nahas,Pharmacol.Rev.14,447; 1962). To differentiate between these mechanisms in rats, we analysed the influence of TRIS on plasma glucose and insulin concentrations.Pentobarbital-anaesthetized (60 mg/kg i.p.) male Wistar-rats (250-300 g) received either a bolus injection (10 mM/kg) or a continuous infusion (0,5 mM/kg · min) of neutralized TRIS (3M solution; pH7,4) for 90 min.
Plasma insulin concentration increased from 36,7±13,7 µU/ml ($\bar{x}\pm s_{\bar{x}}$; n = 6) to 181,2±40,3 µU/ml 3 min after bolus injection and declined to predrug values after 10 min. Plasma glucose concentration was not changed. During infusion, TRIS plasma concentration rose steadily to 17,4±3,69 mg/ml after 90 min. Plasma insulin concentration was maximal after 10 min (178,4±58,5 µU/ml) and had returned to predrug levels after 30 min. During this time plasma glucose concentration dropped from 155±18,4 to 91±14,3 mg/100 ml, and then remained at this lower level. In diabetic animals (streptozotocin 75 mg/kg i.v.; 48 h prior to TRIS) TRIS infusion changed neither insulin nor glucose concentrations.
The results show that in rats:(a) TRIS induces hypoglycaemia by a brief stimulation of insulin secretion;(b) an insulin-independent action of TRIS, if present at all, is negligeable.

Institut für Pharmakologie der Medizinischen Hochschule Lübeck, Ratzeburger Allee 160, D-2400 Lübeck

219

EFFECTS OF ISOPRENALINE ON INSULIN RELEASE FROM ISOLATED MOUSE PANCREATIC ISLETS
S. Zielmann and U. Panten

Direct ß-adrenergic activation of pancreatic islet cells is generally believed to stimulate insulin secretion (Goodman and Gilman, 1980). However, this view does not stem from unequivocal experimental evidence.
Therefore we investigated the effects of isoprenaline on insulin release from isolated pancreatic islets.
Collagenase isolated mouse islets were perifused in order to keep the effects of secretory products on B-cells as low as possible.
0.1 or 1.0 µM isoprenaline had no effects on insulin release induced by 20 mM glucose.
Higher concentrations of isoprenaline inhibited the secretory response to 20 mM glucose.
The 80 % inhibition caused by 50 µM isoprenaline was antagonized completely by the α_2-adrenoceptor antagonist rauwolscine (1 µM).
α_2-adrenoceptor blockade by rauwolscine (1 µM) did not reveal any stimulatory effect of a low isoprenaline concentration (1 µM) upon insulin release elicited by 10 or 20 mM glucose.
These results argue against the general view that stimulation of ß-adrenoceptors of islet cells enhances insulin secretion.

Institut für Pharmakologie und Toxikologie der Universität Göttingen, Robert-Koch-Str. 40, D-3400 Göttingen

220

EFFECT OF REDUCED GLUTATHIONE (GSH) ON 45-CALCIUM UPTAKE INTO PANCREATIC ISLETS OF ADULT AND FETAL RATS.
W. Strölin, H.P.T. Ammon.

Glucose-induced calcium uptake into rat pancreatic islets has been suggested to be related to the redox state of islets thiols. In contrast to islets of adult rats in islets of fetal rats glucose does not initiate the secretion of insulin and formation of GSH.
Using collagenase isolated islets of adult or fetal rats uptake of lanthanum non displaceable 45-calcium was studied in the presence of 3.0 or 16.7 mM glucose with or without addition of GSH (0.1 mM) over a period of 15 minutes. Insulin release into the medium was measured under similar conditions by RIA.
In islets of adult rats elevation of glucose from 3.0 to 16.7 mM was associated with a significant elevation of 45-calcium uptake. Addition of GSH siginificantly increased 45-calcium uptake in the presence of 3.0 mM (160.4 ± 20.6 vs 259.6 ± 31.8 fmol/20 islets; n = 19; $p < 0.02$) and 16.7 mM (330.4 ± 44.6 vs 481.9 ± 44.4 fmol/20 islets; n = 22; $p < 0.05$) glucose. GSH augmented the secretion of insulin only in the presence of 16.7 mM glucose (487.4 ± 56.3 vs 697.3 ± 79.2 µU IRI/20 islets; n = 17; $p < 0.05$). In islets of fetal rats elevation of the glucose concentration from 3.0 to 16.7 mM was not associated with a significant increase in 45-calcium uptake or insulin secretion. In contrast to the data obtained with adult rats GSH failed to stimulate 45-calcium uptake or insulin secretion in islets of fetal rats in the presence of 3.0 or 16.7 mM of glucose.
In conclusion: From our data it may be suggested that in islets of fetal rats the failure of glucose to stimulate insulin release is at least in part due to its inability to raise calcium uptake. Whereas in adult rats GSH appears to be related to the calcium uptake, islets of fetal rats are suggested not being able to handle exogenous GSH for calcium uptake.

Department of Pharmacology, Institute of Pharmaceutical Sciences, University D-7400 Tuebingen, F.R.G.

221

DECREASED (125I)INSULIN BINDING TO PANCREATIC ISLETS OF GENETICALLY OBESE (FA/FA ZUCKER) RATS.

E.J. Verspohl and M.C.M. Melien

Recently in obese patients hyperinsulinemia was suggested to reflect at least in part decreased negative feedback of insulin on its own secretion (Elahi D et al, N Engl J Med 306, 1196, 1982). The hypothesis was tested whether hyperinsulinemia in islets of fa/fa rats is also associated with a decreased inhibitory action of insulin and whether such an effect might be explained by a decrease of insulin binding to islets in vitro.
Using collagenase isolated islets of fa/fa and normal Wistar rats (control) we studied the effect of insulin on glucose (16.7 mM)-induced insulin secretion (measured by RIA) during a 90 min incubation period and performed binding studies (displacement of (125I)insulin binding by increasing concentrations of unlabeled insulin) within 20 min incubations up to steady-state.
Fasting plasma insulin/glucose ratio was higher in fa/fa rats; ratio extremely increased during an i.v. glucose tolerance test (glucose bolus of 500 mg/kg rat body weight) compared to control rats. (125I)Insulin "specifically" bound was 50 % lower in islets of fa/fa rats ($p<0.01$). Scatchard plots of displacement studies revealed a parallel leftward shift of curvilinear plots. Insulin release was more pronounced in the presence of 16.7 mM glucose in islets of fa/fa rats (745±39 vs 581±41 µU/ml x 5 islets x 90 min; $p<0.001$). The inhibitory effect of increasing concentrations of exogenous insulin on glucose mediated insulin release was less in islets of fa/fa rats than in islets of controls.
In conclusion our data indicate a decrease in high affinity receptor number in islets of fa/fa rats. It is possible that hyperinsulinemia in fa/fa rats is due to a diminished negative feedback of insulin on its secretion in vitro as a result of a decreased number of high affinity receptors in pancreatic islets.

Department of Pharmacology, Institute of Pharmaceutical Sciences, University D-7400 Tuebingen, F.R.G.

222

RELATION BETWEEN CELLULAR ATP-LEVELS AND INSULIN RECEPTORS IN RAT FAT CELLS

H.G. Joost and H.J. Steinfelder

Several previous findings have suggested that insulin receptors may be regulated by metabolic events within the target cell. To further study the relation between the energy generating metabolism and insulin receptors, insulin binding was investigated in ATP-depleted fat cells. Insulin binding was reduced by theophylline and sodium azide in parallel to a concentration dependent decrease of the cellular ATP-content. In totally ATP-depleted cells the binding was half that of the controls. Binding curves revealed a decrease of receptor affinity rather than of receptor number. This conclusion was supported in kinetic studies of the receptor binding: in ATP-depleted cells the insulin-receptor complex dissociated faster than in control cells, whereas the association rate was unaltered.
Plasma membranes of azide-treated cells exhibited a similar reduction of insulin receptor affinity. The effect, once produced, thus apparently outlasted cell disruption, and internalization and/or translocation of the insulin-receptor complex cannot account for it. Cell integrity was necessary, however, for initiation of the effect, since sodium azide, as well as ATP, failed to alter insulin binding in a membrane preparation obtained from untreated cells.
In conclusion: The present results indicate that the maintainance of a normal insulin receptor affinity is ATP-dependent. It may be speculated, therefore, that receptor phosphorylation plays a significant role in the regulation of insulin receptor affinity.

Institut für Pharmakologie und Toxikologie der Universität Göttingen, Robert-Koch-Str. 40, D-3400 Göttingen

223

BLOOD FLOW IN THE ENDOCRINE AND EXOCRINE PANCREAS OF THE RAT DURING TOLBUTAMIDE APPLICATION.

G. Schmidt, D. Senske

The question was studied whether tolbutamide exerts circulatory effects in the endocrine and/or exocrine tissue of the pancreas of rats.Regional blood flow was measured by determining the distribution of microspheres within the pancreas gland. The application of the beads (8.8µm in diameter,non radioactive)was followed by injection of dithizone which served for intravital staining of the islet tissue.The organ was rendered transparent by glycerol,and the spheres were counted by microscopy for the two types of tissue separately.For quantification of the microsphere counts a reference sample was withdrawn during the microsphere injection. - Two concentrations of tolbutamide were studied (20 and 100mg/kg i.v) which showed dose dependent effects. Blood glucose levels decreased (67 and 61mg%,controls 87 mg%),whereas the blood flow to the entire pancreas increased (0.69± 0.09 and 1.01±0.05ml/(min x g);controls 0.55±0.04ml/(min x g)).Determination of the islet blood flow showed an overproportional increase:In the drug treated group islet flow rates were 1.84±0.14% and 2.44±0.13%,respectively,of the entire organ compared to 1.24±0.10% in control experiments. Absolute flow rates were 7.4 ±1.3 and 13.6±1.5 µl/min (tolbutamide),and 4.0± 0.5 µl/min (controls).All differences between tolbutamide-treated rats and controls were statistically significant. - The results indicate that tolbutamide,which stimulates insulin secretion, increases blood flow in islets of Langerhans to a higher degree than that in the exocrine tissue.

Institut für Pharmakologie und Toxikologie, Universität Göttingen, Robert-Koch-Str. 40 D-3400 Göttingen

224

EFFECTS OF FUELS AND TOLBUTAMIDE ON ATP PRODUCTION BY MITOCHONDRIA FROM PANCREATIC ISLETS IN OBESE MICE

J. Langer and J. Zünkler

Insulin releasing fuels may trigger insulin secretion by events coupled to enhanced electron flow in B-cell mitochondria, e.g. by an increase in ATP supply. However, sulfonylurea derivatives are believed to stimulate insulin release via direct activation of B-cell membrane receptors. Therefore we compared the effects of exogenous fuels, endogenous substrates and tolbutamide upon ATP production by B-cell mitochondria. A subcellular fraction containing B-cell mitochondria was prepared from pancreatic islets of obese hyperglycaemic mice. When the fraction was incubated in the presence of ADP, glucose and hexokinase, substrate-induced change of glucose-6-phosphate production indicated the rate of oxidative phosphorylation. Glycerol-3-phosphate, malate and pyruvate (in the presence of malate) enhanced ATP production by B-cell mitochondria. Glutamine-induced ATP production was small, but was enhanced severalfold by the non-metabolizable leucine analogue ± b-BCH (20 mM). Tolbutamide (0.1 or 0.5 mM) inhibited the effects of malate and pyruvate upon ATP production.

The results support the view that in contrast to B-cytotropic fuels tolbutamide does not stimulate insulin release by changes of B-cell energy metabolism.

Institut für Pharmakologie und Toxikologie der Universität Göttingen, Kreuzbergring 57, D-3400 Göttingen

225

CHARACTERIZATION OF MONOAMINE OXIDASE IN PANCREATIC ISLETS

S. Lenzen and H. Nahrstedt

Monoamine oxidase (MAO) was characterized in tissue homogenates from pancreatic islets and for comparison from exocrine pancreas and liver from rats. 2-Phenylethylamine was preferentially deaminated by pancreatic islet MAO while 5-hydroxytryptamine was preferentially deaminated by MAO from exocrine pancreas, and tyramine was a good substrate for both tissues. All three substrates were well deaminated by liver tissue. Clorgyline, a selective inhibitor of MAO-A, preferentially inhibited deamination of 5-hydroxytryptamine by all three tissue homogenates, while deprenyl, a selective inhibitor of MAO-B, preferentially inhibited deamination of 2-phenylethylamine. In the case of pargyline, a less selective MAO-B inhibitor, the preference in favour of 2-phenylethylamine was less pronounced. According to these results, MAO in pancreatic islets can be classified as predominantly type B enzyme species and MAO in exocrine pancreas as predominantly type A enzyme species while both types of the enzyme are present in the liver. Using the same three MAO substrates and compared with the effects of the selective enzyme inhibitors clorgyline and deprenyl, tranylcypromine can be classified as a potent unselective inhibitor of MAO in homogenates of all three tissues investigated with a slight preference in favour of the inhibition of the B-form of the enzyme, while in contrast amezinium can be classified as a weak unselective inhibitor of MAO with a slight preference in favour of the inhibition of the A-form of the enzyme.

Institut für Pharmakologie und Toxikologie, Universität Göttingen, Robert-Koch-Str. 40, D-3400 Göttingen

226

ß-CYTOTOXICITY STUDIES WITH THE RODENTICIDE PYRIDYLMETHYL NITROPHENYL UREA (PNU)

J. Beckmann and M. Thum

The rodenticide PNU (Vacor) has been found to be diabetogenic in man. Its molecule resembles nitrosomethylurea, the aglucone of the well known ß-cytotoxin streptozotocin (SZ). We have compared the ß-cytotoxic effects of PNU and SZ in order to analyze the functional relevance of the different molecular moieties of the latter. Isolated pancreatic mouse islets were preincubated for 40 min with PNU or SZ. Medium was changed and inhibition of insulin release measured by determination of the residual secretory capacity of the islets in the presence of high glucose for 60 min. 5 uM PNU or 1.2 mM SZ irreversibly inhibited glucose induced insulin release by ca.75%. When the pyridine nucleotide precursor nicotinamide or the radical scavenger dimethyl urea were employed in the preincubation medium the ß-cytotoxic action of either poison was reduced. This favours the view that the current explanation of SZ-ß-cytotoxicity - pyridine nucleotide depriviation in consequence of the generation of free radicals - also holds for PNU. Phlorizin which inhibits the glucose uptake and diazoxide which has been reported to hyperpolarize the ß-cell membrane, though reversibly inhibiting insulin release by their own, also protected the islets from SZ but not from PNU. From this it is concluded that active transport of the glucose moiety of SZ is essential for the toxic action of this drug and that also the antagonism between SZ and diazoxide may take place at the level of hexose transport.

Institut für Pharmakologie und Toxikologie der Universität Göttingen, Robert-Koch-Str. 40, D-3400 Göttingen

227

ACTION OF BRADYKININ ON MYOCARDIAL GLUCOSE METABOLISM.

P.Rösen and H.Reinauer

The action of Bradykinin (BK) on glucose metabolism as well as its dependence on insulin was studied in isolated rat hearts perfused with KR-bicarbonate buffer containing 5 mM glucose. In the absence of insulin (5U/l), BK (50 nM) increased coronary flow maximally, but had no influence on contractility and oxygen consumption. BK doubled the uptake and oxidation of glucose, whereas the triose production and myocardial glycogen were not affected. Thus, BK stimulated the basal, insulin-independent uptake of glucose. In the presence of insulin, BK increased coronary flow, but simultaneously contractility and oxygen consumption were enhanced. Glucose uptake was stimulated from 1016 to 1588 nmol/min (+ 50 %), glucose oxidation for 76 %. There was no change in the ratio between glucose and fatty acid oxidation in the heart by BK. The BK-mediated changes in glucose metabolism were not inhibited by preperfusion of the heart by indomethacin (2 ug/ml) to inhibit prostaglandin-biosynthesis. In the diabetic heart, neither BK nor insulin alone had any effect on glucose metabolism. However, both together accelerated glucose uptake and oxidation sixfold.

We conclude that the action of BK on myocardial glucose metabolism is not mediated by BK-stimulated synthesis of prostaglandins. In contrast we assume that BK changes the vascular permeability and, thereby, increases the concentrations of substrates and insulin available for myocardial tissue.

Diabetesforschungsinstitut, Auf'm Hennekamp 65, D-4000 Düsseldorf

228

MODULATION OF FUNCTION AND METABOLISM OF THE SUPERIOR CERVICAL GANGLION BY ENDOGENOUS SUBSTRATES UNDER CONDITIONS OF GLUCOSE DEFICIENCY

D. Kirsch and W. Kemmler

Under normal conditions nervous tissue is strictly glucose dependent. Since well controlled diabetics are prone to hypoglycaemic attacks, it is important to know, whether glucose concentrations alone or cellular conditions like the glucose transport system or endogenous substrates are limiting for neuronal function and metabolism. Both these parameters can be monitored simultaneously in the perfused (Krebs-Henseleit, 95% O_2, 5% CO_2, 37°C) superior cervical ganglion of the rat in vitro by measuring NAD(P)-H-fluorescence (F) and postganglionic action potential (AP). Using different frequencies of stimulation (1,3 and 6 Hz) glucose concentration was lowered stepwise from 5 to 0.25 mmol/l, each step lasting 20 mins. Whereas AP was only lowered by 15 % (±10%, n=4) at 0.25 mmol/l glucose at 1 Hz, 80 % of AP (±3%, n=4) were lost at 1.25 mmol at 6 Hz. F rose linear to stimulation frequency at 5 mmol/l glucose, and decreased with lower glucose concentration. At 0.25 mmol/l glucose F was lowered by 75% irrespective of the stimulation frequency and AP. So function depends more on frequency of stimulation at low glucose concentrations whereas metabolism is more glucose concentration dependent. Preincubation of the ganglion with high glucose concentration improves functional survival. If the medium is freed of glucose in one step a preincubation with 24.5 mmol/l glucose for 60 mins instead of 5 mmol/l increases the time until 50 % AP is reached from 18 to 27 mins. Preincubation with high glucose concentrations increase F at low glucose concentration indicating the activation of an endogenous substrate pool.
Even though neuronal function and metabolism depend on glucose concentration below 2.5 mmol/l both can be alleviated by endogenous substrates.

Forschergruppe Diabetes, Kölner Platz 1
D-8000 München 40

229

A KEY ROLE FOR PUTRESCINE IN EARLY MURINE EMBRYOGENESIS?

J.R. Fozard

Direct evidence of an essential rôle for ornithine decarboxylase (ODC) and/or the polyamines in early murine embryogenesis has been obtained using the highly selective inhibitor of ODC, DL-α-difluoromethylornithine (DFMO; MDL 71782) (J. R. Fozard et al., Europ. J. Pharmacol 65: 379, 1980). I now provide evidence that it is specifically the deficit in putrescine (put) which leads to the arrest of gestation.
DFMO, 50, 100 or 200 mg/kg injected s.c. every 6 h on day 8 of gestation inhibited murine embryonic development dose-dependently when assessed by the number of feti and resorption nodules present in the uterus on day 18. Co-administration of put, 200 mg/kg s.c., reversed completely the contragestational effects of DFMO, 100 mg/kg, and by ∿ 55% the effects of DFMO, 200 mg/kg. Put itself had no effects on gestation. Deciduomal ODC activity and the put and spermidine (spd) concentrations were reduced 6 h after the last dose of DFMO, 200 mg/kg. After combined treatment with DFMO, 200 mg/kg, and put, 200 mg/kg, the deciduomal put and spd concentrations were restored to control values although the ODC activity was further reduced. α-Monofluoromethyl-trans-dehydroputrescine (MDL 72197) reversed the contragestational effects of DFMO, 200 mg/kg, when co-administered i.p. at 80 mg/kg and despite itself inhibiting ODC.
Thus, the contragestational effects of DFMO in mice are readily reversed by co-administration of put as are the deficits in deciduomal put and spd. Since the effects of DFMO are also reversed by α-monofluoromethyl-trans-dehydroputrescine, a put analogue which cannot be converted to spd and for which no evidence of conversion to the spd analogue has been obtained, the results suggest a key rôle for put in murine embryogenesis.

Centre de Recherche Merrell International, 16, rue d'Ankara, 67084 - Strasbourg-Cedex, France.

230

Renal metabolism of Corticosterone (B) in vitro

D.Tsiakiras[1] G.A.Hoyer[2] H.Siebe[1] K.Hierholzer[1]

We have previously reported that rat renal tissue in vitro metabolizes B producing 20-dihydro-B (I) and 11-dehydro-20-dihydro-B (II)(Tsiakiras et al. Pfl.Arch. 392:R 12, 1982). The present experiments performed on isolated kidneys of male and female rats have revealed the production of two further metabolites (III and IV) which are less polar than B with the following properties as determined by HPLC:

	III	IV
UV-absorbance (∿250 ng)	+	0
chromat. identity with aldosterone	no	no
with 11-dehydro-B	yes	no
with progesterone	no	no
with 5-dihydro-B	no	yes
effect of metopirone	↑	-

We concluded that B is converted by a C11-hydroxysteroid dehydrogenase to form 11-dehydro-B and by a Δ4-reductase to form 5-dihydro-B. This hypothesis could be verified by mass spectrometric analysis. Furthermore HPLC was adapted to separate authentic 5α- and 5β-dihydro-B and to prove that metabolite IV is chromatographically identical with the 5α-isomer. - Renal metabolism of B is sex dependent with predominant formation of metabolites more polar than B in male rats.

1)Inst.f.Klinische Physiologie,Klinikum Steglitz Freie Universität Berlin, Hindenburgdamm 30, 1000 Berlin 45

2) Schering AG, 1000 Berlin 65

231

THE INFLUENCE OF HOUSING CONDITIONS AND MODE OF PRETREATMENT ON ENDOCRINE PARAMETERS IN RATS

H. Winterhoff

In toxicological screening, changes of body- and organ-weights of rats are considered to be indices of specific toxic effects. Thus it seems necessary to exclude unspecific influences on these parameters. When control collectives gained from different experimental designs using the same rats strain (WISW SPF cpd) were compared, only the weights of the pituitaries and the thyroid glands showed to be changed by the general experimental conditions. Thus, using male as well as female rats, the influences of housing conditions (single housed in wiremesh-cages and group housed 5 rats in macrolon-cages) were compared to the influence of different ways of pretreatment- none, daily gavage or i.p. injection and in some experiments together with vaginal smears.

Pronounced differences in organ weight were already seen between single- and group -housed animals in rats of both sexes the weights of the pituitaries were increased when single-housed, in male rats under the same conditions also the weights of the thyroid glands whereas the other organ weights remained unchanged.
Regarding the hormone levels, especially the female rats showed differences in basal values of TSH and T_4, this was also valid following a TRH stimulus.
The mode of different pretreatment exerted great influence on organ weights and especially on hormon parameters (TSH, T_3, T_4, LH and prolactin). These differences were more pronounced in female than in male rats.
In further experiments substances with endocrine side effects were tested under different experimental conditions: The results confirm that a proper evaluation is only possible in a control group handled and treated in the same way like the test-groups which in addition receive the respective test substance.

Institut für Pharmakologie und Toxikologie der Universität Münster, Domagkstr. 12, D - 4400 Münster

232

EFFECTS OF PROPYLTHIOURACIL (PTU), AND OF BACTERIAL ENDOTOXIN (LPS), ON SERUM-TRIIODOTHYRONINE (T3), RESPIRATORY RATE, SKIN AND RENAL BLOOD FLOW IN RABBITS

W. Riedel

In cold, or in the onset of fever, rabbits decrease respiratory rate, increase cutaneous vasomotor tone while simultaneously renal sympathetic activity decreases. Thyroid hormones are of importance in cold defence and evidence implicates oligopeptides in the dual control of pituitary and autonomic system activities. To assess cardiorespiratory activities associated with thyroid hormone changes, 8 rabbits, aged 120 days, were iv. injected 10 mg/kg PTU, dissolved in Tris-buffer, in warm environment (Ta 28 C). Ear skin temperature (EST) indicated cutaneous vasomotor tone, renal blood flow (RBF) was recorded with chronically implanted electromagnetic flow probes. Blood pressure was taken from an ear artery. Oxygen consumption (OC) was measured using open flow respirometry. T3 was estimated by radioimmunoassay. Injection of the vehicle caused small increases of all measured parameters, lasting for 5-10 min. 10-15 min after PTU, respiratory rate fell from 323 + 15 to 110 + 12 b/min, EST fell from 37.8 + 0.2 to 34.4 + 0.4 C, RBF rose from 56 + 4 to 81 + 4 ml/min, arterial pressure fell from 82 + 5 to 71 + 4 mm Hg, OC rose from 27.6 + 1.3 to 30.4 + 1.2 ml/kg/min, and core temperature rose from 39.4 + 0.1 to 40.5 + 0.2; the effects lasted about 60 min. T3 fell within 15 min, from 140 + 3 to 113 + 6 ng/dl 60 min after PTU. 6-8 days following thyroidectomy all responses to PTU were augmented, however, completely abolished 10 days later. T3 iv. (300,3000 ng/kg), but not T4, at Ta 24 C, immediately caused dose-related panting and cutaneous vasodilatation, at stable OC, in thyroidectomized rabbits, as caused 50 µg iv. thyrotropin releasing hormone (TRH) in intact rabbits. T3 rose from 140 + 3 to 262 + 14 ng/dl after LPS, and fell to 96 + 4 ng/dl when autonomic activities indicated heat stress. We assume TRH neurones being activated by falling T3, or by LPS, eliciting cardiorespiratory adjustments similar to those observed in cold stress.

MPI, W.G.Kerckhoff-Institut, Parkstr.1, D-6350 Bad Nauheim

233

ISOLATION OF TSH-INHIBITOR-COMPLEXES AND INVESTIGATIONS ON THEIR SECONDARY STRUCTURE BY CIRCULAR DICHROISM (CD)-SPECTROSCOPY

H.G. Gumbinger

The antithyrotropic efficacy of extracts from several plant-species and their constituents (e.g. caffeic acid, rosmarinic acid and their oxidation products) has been proven. For further elucidation of this antihormonal activity it should be scrutinized, whether this decrease in biological activity is essentially accompanied by a change in the secondary structure of TSH.

For these investigations fractions with high CD-activity were gained from Thyreostimulin® and Thyratrop® via exclusion chromatography on Sephadex G50 gel.
Following incubation with caffeic and rosmarinic acid, their oxidation products and several of their derivatives, the hormone-substrate-complexes were purified from excessive substrate by exclusion chromatography on Sephadex G100 gel.
Binding of the substrate to the hormone could be confirmed by UV-spectroscopy. The stability of some complexes was proven by in vitro tests in either buffer or rat serum. The TSH-fractions show distinct CD-active absorption bands in the region dominated by amide bond absorption, i.e. between 230 and 200 nm. Shape and size of the CD-spectra suggest a great share of α-helical parts in their structure.

Marked changes in CD-activity show complexes of TSH-fractions with oxidated chlorogenic acid as well as with oxidated and autoxidated rosmarinic acid. This is a strong hint on changes in the secondary structure of the TSH, induced by the inhibitor.
Correspondingly the biological activity of TSH was distinctly diminished following a preincubation of TSH with the inhibitors mentioned above.

Institut für Pharmakologie und Toxikologie der Universität Münster, Domagkstraße 12, D - 4400 Münster

234

THYROIDAL SECRETION IS INHIBITED BY PLANT EXTRACTS AND PLANT CONSTITUANTS, INDEPENDENT OF THEIR TSH-INACTIVATING EFFECT

A. Frömbling and U. Bensom

As it has been demonstrated earlier freeze dried extracts from Lycopus virginicus and L. europaeus are able to inhibit the stimulative effect of bovine TSH on thyroidal colloid droplet formation (endocytosis). Whether this effect is solely attributed to a change in the chemical and biological properties of the TSH-molecule or depends on a distinct intrathyroidal point of attack has now been investigated. TSH stimulates endocytosis within minutes after ip-administration. So rats have been injected with TSH and one hour later the plant extracts or constituants have been applicated; the rats were killed 4 or 6 hours after TSH-application. The effects were compared to those of a maximal effective dose of iodide. Endocytosis was markedly depressed by iodide and an even greater suppression occurred in the rats treated with plant extract. The blockage of secretion is also achieved by a number of plant constituants, which are phenol carboxylic acids and their oxidation products. A correlation can be drawn between the chemical structure of the substances and their physiological effect. p-Coumaric acid depresses the number of colloid droplets most, accompanied by a significant decrease of T_4-levels. Derivatives with two additional methoxy-groups show a weaker effect. With one methoxy-group missing or with o-diphenols the activity is even more reduced. Rosmarinic acid, a main plant constituant, shows endocytosis blocking properties as well, even in very low concentrations. It can be concluded that the thyroidal secretion is inhibited by plant extracts and constituants, and the mechanism is different from a direct TSH-inactivating process.

Institut für Pharmakologie und Toxikologie der Westfälischen Wilhelms-Universität, Domagkstr. 12, D-4400 Münster

235

OBSERVATIONS ON THE INTERRELATION BETWEEN THYROID AND THYMUS FUNCTION IN THE TOXICOLOGICAL SCREENING PROCESS

G. Uthe-Kassenbrock, H. Sourgens and F.H. Kemper

Hypothyroid states characterized by a decrease of circulating T_4 are known to influence thymus function. So investigations were performed to elucidate the interrelationship between these endocrine and immunological target organs in different experimental designs affecting the hypophyseal-thyroid feedback system. In intact male rats the application of different dosages of thyroxine (10 µg/kg - 2 mg/kg/d s.c.) induced a thymus weight increase, 10 µg representing already the maximal effective dosage. In thyroidectomized rats the thymus weights were only slightly decreased 3 weeks postoperative. T_4 serum content remained unaffected but circulating TSH was extremely increased. The qualitative examination of thymus gland morphology revealed an increased proportion of the connective tissue and a diminuation of the cortex region with a reduced number of lymphocytes. Whether these obvious changes reflect a lack of intrathymic T_4 or a distinct effect of TSH was further examined in intact rats. The application of exogenous TSH decreased thymus weights in a dose-related manner, the histological pictures showed a diminished cortex region even in the lowest applicated dose (2 x 0.1 I.E. TSH/kg/d). These effects of TSH could also be confirmed in thyroidectomized and T_4 substituted (10 µg/kg/d) animals; whereas the mere T_4-dosage restored thymus weights and histological picture to sham-operated levels. These findings show a dualistic effect of TSH and T_4; affecting the thymus gland: T_4 increase and TSH decrease lead to a stimulation of the thymus, whereas T_4 decrease and TSH increase result in depression of the gland. These dualistic effects have been found to be relevant in the process of toxicological routine screening. Substances with pharmacodynamic effects on TSH and T_4 serum content in the depicted way were found to influence the thymus, e.g. cinnamic acid and 4-CH_3-cinnamic acid. We thank the NIH for the generous supply with rat RIA kits.

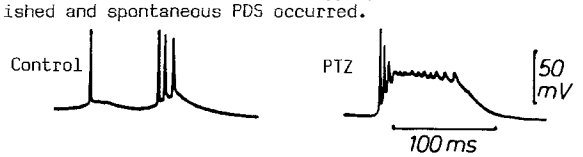
Institut für Pharmakologie und Toxikologie der Universität Münster, Domagkstraße 12, D - 4400 Münster

236

PAROXYSMAL DEPOLARIZATION SHIFTS (PDS) OF CA3 NEURONS IN HIPPOCAMPAL SLICES ELICITED BY PENTYLENETETRAZOL (PTZ)

D. Bingmann

Previous experiments on sensory spinal ganglion cells grown in tissue culture have shown that these neurons hyperpolarized transiently with the membrane resistance being reduced when they were exposed to 5-10 mmol/l PTZ. After a few minutes, this hyperpolarization turned over to a reversible depolarization (Bingmann et al., Neurosci. Lett. 4: 73, 1977). Similar shifts of the resting membrane potential were observed by Zeise et al. on neurons in hippocampal slices after application of PTZ (pers. com.). In both experimental series typical epileptiform reactions to this epileptogenic drug were missing.
With repetitive application of PTZ, however, ictal discharges appeared in CA3 neurons of hippocampal slices (300-400 µm thick, guinea pig) at a bath concentration of 15 mmol/l: During the first application CA3 neurons hyperpolarized transiently and bursts disappeared. With further administrations the transient hyperpolarizations were abolished and spontaneous PDS occurred.



After replacing Ca^{++} by Mg^{++} in the bath PDS activity stopped but still could be evoked by intracellular current injections. This may indicate that the synaptic input triggered the PDS of the tested neurons the membrane properties of which had changed during the repetitive application of PTZ.

Physiologisches Institut der Universität, Domagkstr. 6, D-4400 Münster

Supported by DFG (Bi 278/7-3)

237

HISTAMINE AND NORADRENALINE BLOCK CALCIUM ACTIVATED POTASSIUM CONDUCTANCE IN HIPPOCAMPUS

H.L. Haas and A. Konnerth

The hippocampus receives histaminergic (HA) and noradrenergic (NA) projections from the brain stem. Both amines produce neurotransmitter and -modulator actions on principal neurones in CA1 and dentate area. Strong excitation of hippocampal pyramids by depolarizing current injection or ionophoretically applied glutamate is followed by a slow afterhyperpolarization (AHP), which is due to gK(Ca). We report now a block of the slow (but not the fast) AHP in hippocampal slices of the rat perfused with micromolar concentrations of HA (H_2-receptor) and NA (beta-receptor). In TTX poisoned preparations Ca-spikes preceding the AHP were unaffected. The neurones were slightly depolarized by bath application while restricted ionophoresis or pressure ejection of HA and NA often hyperpolarized them. Dopamine and serotonin, which, in contrast to HA and NA, do not elevate cyclic AMP levels in hippocampal slices, enhanced the AHP and hyperpolarized the cells. Thus HA and NA work by at least two mechanisms in the hippocampus. The block of gK(Ca) is probably mediated by cyclic AMP and leads to a powerful potentiation of excitatory transmission. This mechanism may be in part responsible for its long lasting enhancement after tetanisation.

Neurophysiologie Labor
Neurochirurgische Universitätsklinik
CH 8091 Zürich, Switzerland

238

SINGLE GABA CHANNEL CURRENTS IN DORSAL ROOT GANGLION CELLS

R. A. Deisz

Chick dorsal root ganglion cells respond to application of GABA with an increase in conductance for Cl^- (Choi & Fischbach, J.Neurophysiol.45,605,1981). DRG cells were grown in tissue culture using the method of Barde et al. Proc.Nat. Acad.Sci.U.S.A.,77,1199,1980) to study the properties of single GABA channels with the patch clamp technique (Hamill et al. Pflüg.Arch.391,85,1981). Patch pipettes ($\leq$5 MegΩ) were filled with Hepes-buffered solutions containing in mM: 130 CsCl; 10 $CoCl_2$, 20 TEACl (to reduce or eliminate currents other than Cl^-) and GABA from 0.01 to 0.05. Seal resistances between 30 and 60 GΩ were regularly obtained, but single channel activity was quite rare, probably due to a low channel density. Recordings were stored on analog tape, digitized with at least 4 points/ms and analyzed with a LSI 11 computer linked to a DEC 20 system. A patch record analyzed in detail revealed the following. At resting potential single channel currents were inward with amplitudes of about 1 pA. Hyperpolarization of the patch by e.g. 70 mV increased the inward current amplitude to 3.6 pA. The mean open time of the channels was 0.9 ms the frequency of openings was 0.2 Hz. The open times were exponentially distributed with a time constant of 0.6 ms. Depolarization of the patch by e.g. 70 mV gave a mean outward current amplitude of 2.6 pA with a mean open time of 4 ms. The time constant of the exponential fit to the distribution of open time was about 3 ms. The mean frequency of the openings was 8 Hz. Depolarization of the patch by 90 mV increased the current amplitude to about 5 pA, the mean frequency of openings declined as did the mean open time. The zero current potential was about 15 mV less negative than resting potential, indicating that the currents were indeed carried by Cl^-. The slope conductance of these channels was about 50 pS. It is suggested that the probability of channel opening and the mean open time is non linearly voltage dependent with a maximum at depolarizations around 60 mV.

Dept. Neurophysiology, Max-Planck-Institute for Psychiatry, Kraepelinstr. 2, 8000 München 40, F.R.G.

239

FACILITATORY EFFECT OF SEROTONIN ON GABA EVOKED SPONTANEOUS TRANSMITTER RELEASE AT THE CRAYFISH NEUROMUSCULAR JUNCTION

Wolfgang Finger

As observed previously serotonin (5-HT) facilitates release of transmitter in opener muscles of crayfish (Dudel 1965, Naunyn-Schmiedebergs Arch exp Path Pharmak 249:515-528) and lobster (Glusman & Kravitz 1982, J Physiol 325:223-241). Spontaneous release of excitatory transmitter can be enhanced drastically by high concentrations of GABA applied by superfusion (Finger 1982, Neurosci Lett, in press). This effect of GABA is facilitated significantly by serotonin. In a typical experiment at small crayfish muscle fibres (length $\leq$ 0.8 mm) GABA evoked a maximal release rate $\tilde{n}_o$=900 quanta/s which decreased exponentially with a time constant of τ=21s. If the preparation was pretreated for 10 minutes with $2 \cdot 10^{-6}$ mol/l serotonin the rate of spontaneous release increased from 0.1 to 0.3 quanta/s in absence of GABA. On application of GABA, however, the rate of release $\tilde{n}_o$ was increased to $\tilde{n}_o$=3500 quanta/s and τ was decreased to τ=15s. As a consequence, the number of quanta released in total ($\tilde{s}$) was about 3 times larger subsequent to treatment with serotonin (before: $\tilde{s}$=24000 quanta; after: $\tilde{s}$=70000 quanta). This action of serotonin was reversible and could be induced several times at the same muscle fibre. In serotonin treated preparations rates of release up to about $\tilde{n}_o$=25000 quanta/s have been observed, corresponding to a total release of $\tilde{s}$=220000 quanta on a single application of GABA.

Physiologisches Institut der Technischen Universität München, Biedersteiner Strasse 29, D-8000 München 40, FRG

240

a^i_{Cl}-MEASUREMENTS IN CRAYFISH STRETCH RECEPTOR

H.Moser

Internal chloride activity (a^i_{Cl}) and effects of ions on it were investigated in the slowly adapting cell of the crayfish stretch receptor with Corning's new liquid ion-exchanger 477913. As determined in 16 cells in normal astacus saline the mean a^i_{Cl}-value was found to be 18.5 $\pm$ 3.2 mM/l at an E_m of -68.6 $\pm$ 5.6 mV S.D. These a^i_{Cl}-values are higher than reported by Brown et al. 1978 (15.2 $\pm$1.8 mM/l) or Deisz and Lux 1982 (12.7$\pm$1.3 mM/l). In a set of experiments Cl was substituted by gluconate, Na by choline or K.

In a log-log representation a^i_{Cl} depends on a^o_{Cl} close to straight lines with slopes of 0.144 each for three cells. The lowest a^i_{Cl}-values found are < 5.7 mM/l (95% of Cl being substituted).

a^i_{Cl} generally changes in the same direction as a^o_K does. The changes are much more pronounced at potentials more depolarized than threshold,which is about -60 mV. In superthreshold range E_m changes by about 60 mV/decade a^o_K, but 75 mV/decade a^i_{Cl}.

At low Na_o there is only a little increase of a^i_{Cl} from 0 to 3 mM/l, as seen in three cells.

Supported by the "Fonds zur Förderung der wissenschaftl.Forschung in Österreich",project no.4492.

Abt.Zoophysiol.,Peter-Mayr-Str.1a,A-6020 Innsbruck

241

INVESTIGATIONS AT A "PYRIDINE" SENSITIVE UNIT

H. Hatt and I. Schmiedel-Jakob

Recording action potentials from single afferent neurons in the walking leg of the crayfish Austropotamobius torrentium, we have identified "pyridine" sensitive units; i.e. among a small number of tested substances pyridine was most effective, but only at concentrations of 10^{-5}-10^{-4} mol/l (Hatt and Bauer, J.Comp.Physiol.1982: 148). Searching for a more active stimulus, in 21 preparations 87 pyridine derivatives or other related heterocyclic molecules were tested so far. Measuring with a microsuction pipette, the maximum response frequencies were evaluated by a computer program.

The most effective stimulus was pyrazine amide (10^{-7} mol/l), nicotinic amide (10^{-6} mol/l) and pyridine aldoxime, nicotinic acid methyl ester, acetylpyridine (10^{-5} mol/l) being still more effective than pyridine. For all tested substances the dose-response curves were linear over nearly 2 decades (log-log plot), showed a slope of 1 and a common saturation level of 50 s^{-1}, as far as measurable. The sequence of relative efficacies was the same for all units evaluated. The effectiveness of each molecule depends on the structure of the ring system and the position and structure of the side chain. The efficacy was maximal for drugs with a N-containing benzoide ring system whose side chain is similar to the amide group and located in position 3. Surprisingly, most of these receptors responded to both, chemical and vibratory stimuli.

Supported by DFG Schwerpunktprogramm Ha 1201/1-1
Physiologisches Institut der Techn.Univ.München
Biedersteiner Strasse 29, D-8000 München 40, FRG

242

INHIBITION OF SYNAPTIC TRANSMISSION BY TETANUS TOXIN IN CULTURED MAMMALIAN SPINAL CORD NEURONS

H. Bigalke [1], G. Bergey [2], P. Nelson [3]

Spinal cord neurons (mouse) in culture develop functional excitatory and inhibitory synapses forming a synaptic network. Intracellular recording from neurons show inhibitory and excitatory synaptic potentials. The latter frequently trigger action potentials. Recording from two synaptically connected neurons allows the study of monosynaptic potentials. Tetanus toxin (100 ng/ml), added to the culture medium, produces after about 40 min convulsant activity accompanied by a gradual disappearance of evoked inhibitory potentials. The evoked excitatory synaptic potentials remain unchanged. In a later stage of intoxication also the incidence of evoked excitatory potentials is reduced. They progressively diminish in amplitude concomitant with a decrease in frequency of convulsant activity till no synaptic potentials can be evoked. Action potentials can still be elicited by direct stimulation of the postsynaptic cell.

These findings show that tetanus toxin not only acts on inhibitory synapses but also on excitatory transmission although with less sensitivity. The higher sensitivity of inhibitory transmitter systems for tetanus toxin may explain the convulsant action of the toxin in the spinal cord in the early stage of the poisoning process.

[1]) Rudolf-Buchheim-Institut für Pharmakologie der Justus-Liebig-Universität Gießen, Frankfurter Straße 107, 6300 Gießen

[2]) Department of Neurology, The John Hopkins School of Medicine, Baltimore, Maryland, 21205 USA

[3]) Laboratory of Developmental Neurology, NICHD, Bethesda, Maryland, 20205, USA

243

DISSOCIATED NEURONAL PRIMARY CULTURES AS A MODEL SYSTEM: QUANTIFICATION OF ELECTROPHYSIOLOGICAL PARAMETERS.

W.Dimpfel, M.Wienrich and K.Reuss

Brain and spinal cord cells were taken from 16 day old rat fetuses (Wistar) and co-cultures were started and maintained according to modified standard methods (Reuss, unpub.). Intracellular recording was done in culture from day 36 to day 51. The membrane properties of 79 neurones were studied with respect to synaptic activity (EPSPs, IPSPs), spike activity (frequency histograms), membrane potential and current/voltage relation (I/V-curves, membrane resistance).
Neurones showed an average resting potential of -39 ± 9 mV. EPSPs were already present on day 36 whereas IPSPs did not appear until day 45 of culture. Excitatory and inhibitory synaptic potentials were quantified by means of frequency histograms. EPSPs had an amplitude of 5.8 ± 1.8 mV with a duration of 8.7 ± 2.9 ms and IPSPs exhibited an amplitude of 3.1 ± 1.3 mV with a duration of 10.4 ± 3.5 ms. 77% of all cells recorded demonstrated spontaneous spike activity and of these 25 % were continuously active, 38 % exhibited burst-like activity while the remainder could not be readily classified. Spike amplitude varied between 15 mV and 50 mV and spike duration between 1 ms and 4 ms. 23% of the cells recorded were not spontaneously active but in 11% of these cells spikes could be elicited by current injection with a threshold current of 0.8 - 2.0 nA. By means of I/V-curves the input resistance of neurones could be calculated and was found to vary between 25 MΩ and 70 MΩ . Drugs having influence on membrane properties should produce changes in the electrophysiological parameters described above. Therefore, electrophysiologically well-defined nerve cell cultures provide a potentially useful model system to test the direct effect of drugs at the level of the single neurone.

Experimentell-medizinische Forschung, E. Merck, Frankfurter Str. 250, D-6100 Darmstadt

244

PROPERTIES OF SINGLE CHANNELS IN MOUSE NEUROBLASTOMA CELLS (N1E 115)

K. Nagy and T. Kiss

Single Na channel currents were studied in excised membrane patches (outside -out) from mouse neuroblastoma cells. Internal solution contained (mM) Cs^+ 125, Na^+ 12, EGTA 2, TEA 20. Temperature 6-8 °C. Holding potential: -100 mV. With 145 mM Na^+ outside the single channel current at -50 mV was 0.98±0.24 pA (n=1250). The conductance was 11±3 pS (n=13) similar to the value given by Quandt and Narahashi (1982). The amplitude histograms showed one peak with a hump near to it. The open time (OT) distribution was best fitted by two exponentials suggesting two different open states or two different populations of Na channels with almost the same conductance. At -45 mV τ_1 = 1.6, τ_2 = 6.6 ms.τ_1 increased linearly with potentials (V) between -70 and -30 mV, but decreased for V > -25 mV. τ_2 (which is observable at V > -50 mV) became shorter with increasing V. The mean OT increased linearly with V in the range -70 to -30 mV, but decreased at V > -30 mV.
Occasionally single channel currents of half the normal amplitude were observed. All types of single openings were abolished by 78 nM tetrodotoxin. Unitary amplitudes increased about two-fold between 5 and 35 °C. The Arrhenius plot of the conductance was linear (activation energy of 27.1 kJ/mole, Q = 1.28) with no indication for a phase transition.

Supported by SFB 38 and A.v.Humboldt-Stiftung.

I. Physiologisches Institut der Universität des Saarlandes, D - 6650 Homburg/Saar

245

SOME PROPERTIES OF SINGLE POTASSIUM CHANNELS IN CULTURED OLIGODENDROCYTES

H. Kettenmann and H.D. Lux

K^+ channels were studied in membrane patches of oligodendrocytes in explant cultures of embryonic mouse spinal cord using the giga-seal technique (Kettenmann et al., Neurosc.Letters, 1982, 32, 41-46). The conductance of the channel varied greatly, i.e. 6 to 125 pS. A more than threefold difference in conductance was found in channels from the same cell and also between channels in the same patch. In some patches there were up to three current levels of the same size. The mean open and closed time and percent time open also showed a large variation. For individual channels these times were not markedly affected by pulling the patch off the cell or by superfusing the isolated patch with bathing medium containing additional K^+, TEA or medium with EGTA and Ca^{++} free. Measurement of channel kinetics at different membrane potentials between -90 and -30 mV revealed a small but consistent effect of depolarization to decrease the percentage of time the channel was open. This decrease was the result of a decrease in the mean open time and an increase in the mean closed time. Large voltage steps, more than 100 mV, greatly decreased the percentage of time the channel was open. Current-voltage measurements on intact cells with conventional microelectrode technique also showed a striking decrease in membrane conductance at these large membrane potentials. The oligodendrocyte membrane contains K^+ channels with a large variation in both conductance and kinetics.

Inst. für Neurobiologie, Im Neuenheimer Feld 504, D-6900 Heidelberg and Abt. Neurophysiologie, MPI für Psychiatrie,Kraepelingstr.2, D-8 München

246

MANGANESE IONS INHIBIT ACETYLCHOLIN RECEPTOR SYNTHESIS IN CULTURED MOUSE SOLEUS MUSCLES.

H. Lorković and A. Feyrer

The role of Ca ions in the ACh receptor (AChR) synthesis is a matter of controversy. The proposal that the inhibition of AChR synthesis by mechanical activity is due to a high $[Ca^{2+}]_i$ could not be supported (Lorković, Pflügers Arch. 391: R41, 1981). However, Ca was shown to promote AChR incorporation into the membranes of cultured myotubes (McManaman, Blosser and Appel, J. Neurosci. 1: 771, 1981). We made similar experiments with thin bundles of excised mouse soleus muscles. Non-denervated muscles fastened to plastic support forks were kept in standard culture media at 37 °C (MEM, calf serum, antibiotics, fungicides). The 2 ml medium content of the culture vials was changed daily. After 3 days, the maximum contracture force provoked by 110 µM ACh and 330 mM K methanesulfonate was measured. Then the muscles were dried to disrupt the membranes, soaked in a Ringer solution containing ^{125}I α-bungarotoxin (BTX) for 40 min, washed, dried again, weighed, and their radioactivity was measured. Muscles which had been kept in culture media containing less than 0.02 mM Ca^{2+} were sensitive to ACh and bound BTX like their mates which had been kept in media containing 2 mM Ca. When virtually all of the Ca^{2+} in the culture medium had been replaced by Sr^{2+}, the ACh sensitivity and BTX binding were also about the same as in control muscles kept in Ca^{2+}- containing media. However, when Ca^{2+} had been replaced by 1.8 or 0.2 mM Mn^{2+}, ACh sensitivity and BTX binding were almost as low as in freshly excised muscles. The force produced by high K was about the same as in control muscles, indicating that the muscles were in a normal metabolic and contractile condition. It is concluded that Mn ions, which are known to block the Ca channel of the muscle membranes, interfere with AChR synthesis without otherwise damaging the muscles.

Abteilung für Allgemeine Physiologie der Universität Ulm, West Germany.

247

CHARACTERISTICS OF MINIATURE AND SUB-MINIATURE ENDPLATE CURRENTS AT THE MOUSE DIAPHRAGM ENDPLATE.

C. Erxleben and M.E. Kriebel

In addition to the classical miniature endplate potentials which form an overall bell-shaped distribution (bell-MEPPs), Kriebel, et al. (J. Physiol., 1982, 322, 211-222) have described a class of smaller MEPPs that skew towards the noise (skew-MEPPs) and have a mode about 1/12th that of the bell-MEPPs.

We have investigated endplate currents (MEPCs) with a 2-electrode voltage clamp technique and with a high signal-to-noise ratio (baseline RMS noise ≤ 35 pA). (Holding potentials were -140 mV and temperature was 30°C.) Characteristic MEPC parameters were determined by interactive computer programs. The modes of skew- and bell-MEPC amplitudes were the same in neonatal and adult junctions. However, the mean MEPC amplitudes varied greatly because of the large range in skew- to bell-MEPC ratios (1.7±0.7 S.D. to 5.0±1.2 nA). The junctions of newborn mice have broader distributions and the broad range of adult distributions is attributed to the high MEPC frequency resulting from the high $[Ca^{++}]_o$ needed to maintain stable and low holding currents. The mean bell-MEPC amplitudes (3.5 nA) of both young and old mice were the same. MEPCs of both classes were found to have the same ratio of area (charge) over amplitude and the same time constant of decay; whereas, the absolute values change with maturation (area/ampl ratio 4.5±0.8 in newborn to 1.2±.05 msec in the adult; ~ 5.6±1.2 in newborn to 0.8±.05 msec in adult.

The data show that both skew- and bell-MEPC classes are generated at the same site and result from the same post-synaptic mechanism(s).

Dept. of Physiology, Upstate Medical Center, Syracuse, New York 13210.

248

SUBSTANCE P SPEEDS ACh DESENSITIZATION IN SINGLE BOVINE CHROMAFFIN CELLS

D. Clapham and E. Neher

Substance P has been shown to inhibit the ACh-induced nicotinic response of bovine adrenal chromaffin cells in culture (Livett et al., Nature 228: 256, 1979). We have used patch clamp techniques (Hamill et al., Pflügers Arch. 391: 85, 1981) to study the change in ACh-induced current upon addition of substance P.

Single chromaffin cells (10 µ) were voltage clamped to -60 mV. The concentration of ACh or ACh plus substance P was changed within 100 ms and resulting ACh current recorded. Substance P in concentrations of 2 to 10 µM speeds the time course of desensitization approximately 5 fold. The decay of 20 µM ACh-induced current to half its maximum was reduced from 5 s to 1 s by 10 µM substance P. Power spectra of ACh-induced current fluctuations above 0.5 Hz were similar with or without substance P. Single ACh-channel amplitudes measured by outside-out and cell attached patches were also unchanged.

Substance P inhibits the ACh-induced response in cultured bovine chromaffin cells by increasing the rate of desensitization of ACh channels. It does not appear to affect single channel conductance or to block the open channel.

MPI f. biophysikal. Chemie, Postf. 968, D-3400 Göttingen

249

WHEN THE PHOTORECEPTORS IN THE RETINA OF THE HONEYBEE DRONE ARE STIMULATED, K^+ ACTIVITY IN THE GLIA RISES MORE THAN N_A^+ ACTIVITY FALLS

R. K. Orkand†, J.A. Coles and J.-L. Munoz

When drone photoreceptors are stimulated with light they gain Na^+ and lose K^+ into the extracellular space: most of this K^+ enters the glia (Coles & Tsacopoulos, 1981, J.exp.Biol. 95, 75-92). One possible mechanism for this K^+ entry is K^+/Na^+ exchange. To investigate this, we have made intracellular recordings with double-barrelled microelectrodes that measured membrane potential and either Na^+ or K^+. When the photoreceptors in a superfused slice of retina were stimulated with a train of light flashes, 1 per sec for 90 sec, K^+ activity in the glia rose by 8 mM, S.E.M.=1 mM. With the same stimulation, Na^+ activity fell by only 1.5± 0.3 mM. Hence, unless there is some other factor, such as the transfer of Na^+ from a bound to a free state in the glia, the efflux of Na^+ appears to be much smaller than the entry of K^+ and only a small part of the K^+ entry is by K^+/Na^+ exchange.

Laboratoire d'Ophtalmologie expérimentale et Département de Physiologie, Université de Genève

+ Institut für Neurobiologie, Heidelberg
Universität Heidelberg, Im Neuenheimer Feld 504
6900 Heidelberg

250

THREE LARGE OUTWARD CURRENTS IN RAT SYMPATHETIC NEURONES

M.Galvan and C.Sedlmeir

Rat superior cervical ganglia were maintained in vitro at room temperature. After treatment with collagenase (0.4%, 60min), superficial neurones were impaled with two KCl-filled microelectrodes and voltage clamped. In control solution, clamp commands from -40mV to potentials more positive than -25mV elicited large (≤150nA) outward currents, which showed little inactivation. In many cells, the current-voltage curve was "N-shaped" with a minimum at +80mV. Superfusion with Mn^{2+} containing solution reduced outward current at all voltages and abolished the "N-characteristic"; the remaining current slowly inactivated (τ>1sec). Depolarization from -70mV to -50mV and beyond elicited a transient outward current, which activated almost instantaneously (τ≤1ms) and inactivated rapidly (τ≈20ms). The amplitude of all the outward currents was dependent on the bath $[K^+]$ indicating that they are carried by potassium ions. It therefore appears that these neurones, like many invertebrate neurones, possess 3 large outward currents: a delayed rectifier (I_K), a Ca^{2+} activated potassium current (I_C) and a transient outward current (I_A). Tetraethylammonium ions (3mM) strongly reduced the amplitude of I_K and I_C and markedly prolonged the action potential. 4-aminopyridine (2mM) appeared to selectively block I_A and slightly prolonged the action potential. This suggests that I_A may contribute to spike repolarization in these cells.

Supported by the DFG, Ga 277/2-4.

Department of Physiology, University of Munich
8000 Munich 2, Pettenkoferstrasse 12, GFR.

251

CARBACHOL-INDUCED CHANGES OF FREE INTRACELLULAR Na^+ AND K^+ IN MAMMALIAN SYMPATHETIC NEURONES

K. Ballanyi, P. Grafe and G. ten Bruggencate

The typical action of carbachol on sympathetic neurones consists of a membrane depolarization followed by a post-carbachol hyperpolarization. We now have used double-barrelled ion-sensitive microelectrodes (Corning 477317, ETH 227, tip-size < 0.3 µm) to determine the precise relation between membrane potential and changes of the free extracellular and intracellular Na^+- and K^+-concentrations (Na_i, K_i, K_e). The experiments were performed on isolated, desheated rat superior cervical ganglia maintained in Krebs solution at 30 °C. Application of carbachol (50 µmol/l, 1 min.) via the Krebs solution induced a membrane depolarization of 23.6 ± 3.9 mV (n=21) from a resting potential of 43.6 ± 7.8 mV (n=24, action potential amplitude was 60.9 ± 12.3 mV). This was accompanied by an increase of Na_i from 10.9 ± 3.9 to 16.5 ± 5.6 mmol/l (n=10). Comparable GABA-induced membrane depolarizations did not elevate Na_i. K_i decreased from 110.6 ± 19.4 to 91.0 ± 13.5 mmol/l and K_e increased from 6 to 11 mmol/l. After the end of the application in general a membrane hyperpolarization was observed. During this phase Na_i and K_i slowly returned to their resting values and K_e undershot the baseline-level. The changes of the membrane potential, K_i, K_e and Na_i are consistent with the activation of an electrogenic Na^+-pump.
The data show that ion-sensitive microelectrodes can be used to measure changes of the free intracellular Na^+- and K^+-concentrations in small mammalian nerve cells. The kinetics of the ion movements resemble previous observations using the method of flame photometry (J. Physiol. 242, 1974, 307 f).
Supported by the Wilhelm Sander Stiftung.

Department of Physiology, University of Munich
D-8000 München 2, Pettenkoferstr. 12

252

THE CALCIUM SIGNAL IN PARAMECIUM: VOLTAGE-SENSITIVE ION-CHANNEL, CALMODULIN-CALCIUM DEPENDENT GUANYLATE CYCLASE AND CYCLIC GMP LEVELS.

J.E. Schultz, R. von Hirschhausen, S. Klumpp, M.K. Otto, U. Schönefeld

Calcium plays a pivotal role in the behavioral pattern of Paramecium. Voltage-sensitive ion-channels permeable for Ca, Sr, and Ba ions are localized in the ciliary membrane. Using 'pawn'-mutants defective in the Ca inward current as controls the Ca channel has been characterized biochemically. The kinetics of Ca, Sr and Ba influx into ciliary membrane vesicles are rapid and saturable. Ca influx is only marginally dependent on the experimental temperature indicating a pore as ion-channel. A guanylate cyclase of very high specific activity is localized in the excitable ciliary membrane. The enzyme is regulated by a calmodulin associated like a subunit with the catalytic entity thus conferring Ca sensitivity. Half maximal stimulation by Ca is achieved at 8 µM Ca, Sr is far less active, Ba inactive as Ca substitute. Calmodulin can be dissociated from the guanylate cyclase by lanthanum treatment (15µM). The inactive enzyme can be reactivated with calmodulin from various sources. However, stimulation kinetics for divalent cations are altered, the order of potency being Sr> Ca> Ba. The ciliary guanylate cyclase is proposed to serve as amplifier for the Ca signal. Cyclic GMP levels are very low in the resting state (2pmole/mg protein) and increase up to 10-fold through various stimuli. No relationship has been established yet between ion channel regulation or ciliary reversal and an increase in cyclic nucleotide levels in Paramecium. The available behavioral mutants are expected to aid toward this goal.

Pharmazeutisches Institut der Universität Tübingen, Morgenstelle 8, 7400 Tübingen, FRG

253

SINGLE CALCIUM CHANNELS IN HEART - KINETIC PROPERTIES OF ELEMENTARY CURRENTS.

D. Pelzer and R. Ochi

Elementary currents through single Ca channels in the membrane of adult guinea pig ventricular cells were recorded by the patch-clamp technique (cell-attached). Either 50mM Ba or Ca have been used as pipette filling solutions. Open time histograms of the single channel currents could be fitted with a single exponential having a time constant t_o. t_o became longer with increased depolarization. The shut time histogram showed a slow component which represents long intervals between channel openings (t_{shut} between bursts). In addition, an excess of short shut times due to interruptions within the channel openings was apparent (t_{shut} within bursts). We suggest that these short closing events represent sojourns of the channel in a closed state, C_2, placed intermediately on the opening pathway according to the scheme:

$$C_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C_2 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} O.$$

States C_1 and C_2 are both non-conducting and O is the open state. Following the mathematical treatment of the Castillo-Katz model by Colquhoun and Hawkes (Proc. R. Soc. Lond. B 211, 205-235, 1981), the rate constants can be calculated from t_o, t_{shut} within bursts, t_{shut} between bursts and the mean number of openings per burst. For 50mM Ba at +30mV pipette potential, the rate constants were: $K_1 = 35sec^{-1}$, $K_2 = 500sec^{-1}$, $K_3 = 540sec^{-1}$ and $K_4 = 700sec^{-1}$. Another measure of activation kinetics is the time interval from the voltage step to the time of the first channel opening (latency-to-first-event). For both, Ba and Ca, the histograms of the latencies have a maximum at a time later than zero, as expected for a process in which multiple closed states precede an open state. In addition, these histograms show a lack of events at later times. This observation is the expected result if Ca channels can inactivate before opening.

II. Physiol. Inst., 6650 Homburg/Saar

254

SINGLE CALCIUM CHANNELS IN HEART - ELEMENTARY AND RECONSTRUCTED MEAN CURRENTS.

A. Cavalié, W. Trautwein

Ca channels in the membrane of enzymatically isolated guinea pig ventricular cells were studied with the patch-clamp technique under cell-attached conditions. High concentrations of divalent cations (50 mM Ba and Ca, 90 mM Ba) had to be used in the pipette to increase the signal-to-noise ratio. On depolarization from the resting potential to different test potentials, channel openings occurred with various latencies singly or in closely spaced bursts, separated by longer shut periods. Openings became more probable with increased depolarization; at positive potentials, the probability of the open state (p) tended to saturate. Even at these potentials the channels did not always open, indicating saturation of p at a level below unity. For inward currents, the single channel current-voltage relation was linear. However, we failed to record clear outward currents, suggesting that Ca channels might rectify. For 50 mM Ba or Ca, the slope conductance was 9-12 pS. Elementary current amplitudes became only slightly larger when Ba replaced Ca, but they increased more than twofold when Ba was elevated to 90 mM (18-22 pS). The reconstructed mean currents mirrored the macroscopic I_{Ca} with peak amplitudes being 90 mM Ba > 50 mM Ba > 50 mM Ca. However, their current-voltage relation showed a positive shift along the voltage axis as Ba was increased or substituted equimolarly by Ca; the effectiveness for giving the shift was 90 mM Ba > 50 mM Ca > 50 mM Ba. Simultaneously, p was shifted parallely by the same amount suggesting either a screening effect of divalent cations on negative surface charges or block of the channel by permeant divalent cations.

Supported by the DFG, SFB 38.

II. Physiologisches Institut der Universität des Saarlandes, D-6650 Homburg/Saar

255

POTASSIUM CHANNELS IN ISOLATED PATCHES OF CARDIAC CELL MEMBRANE.

G. Trube and J. Hescheler

Currents through single inward rectifying potassium channels in the membrane of ventricular cells of the guinea pig were recorded previously by using the patch-clamp technique (Trube et al., 1981, Pflügers Arch. 391, R7). The patch of membrane can be isolated from the cell to expose its intracellular face to the bath (Hamill et al., 1981, Pflügers Arch. 391, 85). Similar currents as in cell attached condition are recorded in bathing solutions containing (in mM): KCl 133, Na_2ATP 4, $MgCl_2$ 4.5, EGTA 2, $CaCl_2$ 0.5. The single channel conductance is 28 pS for inward currents (for 145 mM KCl at the extracellular face of the membrane; T=22°C), outward currents are not observed (inward rectification). When Na_2ATP is replaced by NaCl, these currents disappear, and a new type of potassium currents is seen. Inward currents have a larger amplitude (conductance 80 pS), outward currents exist, but saturate at 1.5 pA. At negative potentials the channel flickers frequently between its open and closed state ($\sim 1000\ s^{-1}$ at -50 mV). The openings are grouped to bursts. Immediately after the change of solutions the bursts are long and frequent, and the time averaged current is larger than in the solution with ATP, but within about 10 min the activity decreases to a low level. The effect of withdrawing the ATP is reversible.

Supported by the DFG, SFB 38.

II. Physiologisches Institut der Universität des Saarlandes, D-6650 Homburg/Saar.

256

MECHANISM OF ACTION OF QUINIDINE ON MEMBRANE CURRENTS OF MOLLUSCAN PACEMAKER NEURONS

A. Hermann and A.L.F. Gorman

Quinidine is a prototyp of compounds which are known for their antiarrhythmic action on cardiac pacemaker activity. Its mechanism of action is, however, not well understood. We have studied the effects of the drug on membrane conductances of pacemaker neurons in the marine mollusc, Aplysia californica, using voltage clamp analysis. External quinidine blocks various K^+, Ca^{2+} and Na^+ current components of the neuronal soma membrane - but to different degrees. Its predominant effect is on the voltage dependent, delayed outward K^+ current. The apparent dissociation constant is 28 µM/l at V = +20 mV. The blocking actions is voltage- and time dependent and increases during a maintained depolarizing voltage step. The data is consistent with the block occurring about 70-80 % through the membrane electric field. The block proceeds with a biphasic time course after external application of the drug and is maximum after about 30 minutes. The blocking action is fast and monophasic after internal injection of the drug. A decrease of the external pH increases the maximum block. The data suggests that the drug passes through the membrane in its neutral form but exerts the blocking action in its charged form. We propose that the K^+ channels have a large, nonselective inner mouth extending about three quarters from the cytoplasmic side into the membrane.
The Ca^{2+}-activated K^+ current, the fast Na^+-current and the slow Ca^{2+} current are also suppressed by quinidine but at 10-50 times higher concentrations. Both, the molecular dimensions of the quinidine molecule and its lipophilic nature appear to play a role in its mechanism of action.

Universität Konstanz, Fakultät für Biologie, D-7750 Konstanz, FRG and Boston University Med. School, Department of Physiology, Boston, MA 02118, USA

Supported by DFG, SFB 138 and NIH, NS 11429.

257

BLOCK OF CALCIUM CURRENTS BY INTRACELLULARLY APPLIED D 890 IN SNAIL NEURONS

J. Walden, O.W. Witte, E.-J. Speckmann and C.E. Elger

The organic calcium channel blocker Verapamil and its methoxy-derivative D 600 were found to exert their effects not only on calcium but also on other membrane currents (BAKER et al., J. Physiol. 231: 511-526, 1973; KLEE et al., Pflügers Arch. 343: R 60, 1973). To find a substance which acts more selectively on calcium channels, the effect of D 890, the quarternary derivative of D 600, on neuronal membrane currents was tested. The drug has already been shown to block calcium currents of cardiac muscle after intracellular application (HESCHELER et al., Pflügers Arch. 393: 287-291, 1982).

With an intracellular concentration ranging between 10^{-6} and 10^{-4} mol/l D 890 had the following effects in the identified neurons B1, B2, and B3 of Helix pomatia: (1) The TTX resistent component of the inward current and the inward currents in Na^+ free sucrose or Tris solutions as well as in Ca^{2+} free Ba^{2+} solution were reduced in a dose dependent manner. (2) The steady state inactivation curve of the calcium inward current was not affected. (3) In Ca^{2+} free Co^{2+} solutions the inward current was not changed. (4) The late outward currents were strongly reduced in control solutions. This effect was mainly due to a reduction of the calcium dependent potassium current. Furthermore, the rate of inactivation of the late currents increased. (5) The amplitude and the steady state inactivation curve of the early outward current remained unchanged.

It is concluded that intracellularly applied D 890 exerts its effects predominantly on the calcium current.

Physiologisches Institut der Universität, Domagkstr. 6, D-4400 Münster

258

cAMP-PRODUCED MEMBRANE CONDUCTANCE CHANGES IN HELIX NEURONS

D. Swandulla

Fast pressure injections of cAMP in voltage-clamped identified neurons of Helix pomatia induce an inward current [I(cAMP)] which is not accompanied by a significant change in the cell input conductance near holding potential (-50 mV).
Intracellular measurement of $[Na^+]_i$ with ion-selective electrodes shows that $[Na^+]_i$ rises during I(cAMP). The inward current depends on $[Na^+]_o$ but is still present when Na^+ is substituted by Li^+ or $^oK^+$.
Divalent cations such as Ca^{2+} and Mg^{2+} as well as La^{3+} exert an inhibitory influence on I(cAMP), but Ca^{2+} itself is capable of carrying a comparatively small inward current. I(cAMP) is thus of nonspecific nature.
Changes in $[Cl^-]_i$ are not observed. The apparent lack of a change in the cell's input conductance during I(cAMP) is explained by a simultaneous decrease of the membrane permeability for K^+ ions (see also Deterre et al., Nature, 1981). Such effect becomes particularly obvious during long-lasting depolarizing voltage changes which activate K^+ currents. Measurements of $[K^+]_o$ near the cell surface show in fact that the outward $^oK^+$ flux is decreased following cAMP injection in this situation. The reduction of outward K^+ currents is much less pronounced if external Ca^{2+} is replaced by Ni^{2+}.
The nearly compensatory increase and decrease of two membrane conductances explain the observed lack of a change in the cell input resistance despite the considerable depolarizing action of intracellularly elevated cAMP.

Abteilung Neurophysiologie, Max-Planck-Institut für Psychiatrie, Kraepelinstr. 2, D-8000 München 40, FRG

259

THE EFFECT OF MEMANTINE ON THE ACTION POTENTIAL OF APLYSIA NEURONS

M.R. Klee

1,3-dimethyl-5-aminoadamantane (DMMA, memantine) in concentrations of 1-50 µM/l increases in Aplysia neurons the slope of the action potential and decreases its duration without changes in overshoot despite of a small reduction of the resting membrane potential (Drug Res., 32:1259, 1982). In neurons with a relative slow repolarization (S cells) increasing 2-5 times the calcium concentration of the arteficial sea water (ASW) can mimic the fastening of the voltage change during the action potential, especially that of the second repolarizing phase induced by the calcium dependent potassium current $I_{K,Ca}$. In voltage clamp experiments DMMA and increased calcium will reduce the time to peak of the early inward current and enhance the slope of the delayed outward current. In zero-sodium ASW plus TEA and 4-AP, DMMA reduces the calcium inward current while there is a slight increase in the outward current despite the presence of TEA and 4-AP. In double pulse experiments the recovery of the inward current system was measured. The response to the second pulse reaches 80% of the amplitude of the test pulse within 85 ms. This interval was reduced by 5, 25 and 51% respectively in 10, 100 and 1000 µM/l DMMA. The facilitatory effect DMMA has on the calcium dependent potassium current is also expressed by the increase of the amplitude of the hyperpolarization by $I_{K,Ca}$ that interrupts the inherent bursting in a special class of cells.

MPI Hirnforschung, Neurophysiologische Abteilung, Deutschordenstr. 46, D-6000 Frankfurt/M-71

260

POTASSIUM PERMEABILITY PROPERTIES OF NORMAL MYELINATED RAT NERVE FIBRES

T. Brismar and J.R. Schwarz

A potential clamp analysis was performed in order to further analyse the K permeability properties of normal rat fibres.
Measurements in Ringer solution (at 21°C) showed a max. $P_K = 0.28 \pm 0.12$ cm $s^{-1} \times 10^{-3}$ (in 13 fibres) at large positive pulses. This corresponded to a P_K/P_{Na} ratio of 0.08. Measurements in isotonic KCl showed a similar max. P_K, but in addition that the resting fibre had a relatively large steady state P_K (= 0.12 + 0.05 cm $s^{-1} \times 10^{-3}$. 10 mM TEA blocked 50 % of the max. P_K. The TEA induced block of P_K increased with more negative membrane potentials.
The leak conductance, estimated from the current associated with negative potential steps in Ringer solution was potential independent but showed a small (15 %) time dependent increase. The same was found at positive and negative potential steps in the presence of external TTX + TEA and internal CsCl.
In isotonic KCl negative potential pulses were associated with large transient inward currents which far exceeded (57 %) the size of the tail currents predicted from the resting (steady state) P_K. These currents were only recorded in high K outside, and were largely blocked by 10 mM TEA, which indicated that they were due to a transient increase in P_K at these conditions.

Department of Clinical Neurophysiology, Karolinska sjukhuset, S-10401 Stockholm and Institute of Physiology, Universitäts-Krankenhaus Eppendorf, D-2000 Hamburg 20

261

SLOW AND INCOMPLETE SODIUM CHANNEL INACTIVATION OF FROG NODES OF RANVIER BY GLUTARALDEHYDE

J. Schmidtmayer

In frog nerve fibres sodium channel inactivation is dephasic (τ_{h0} = 0.7 ms, τ_{h1} = 3.5 ms at V = 60 mV, 15°C) and complete for V > 40 mV (V = deviation from normal resting potential, depolarization positive). 10 mM glutaraldehyde (Glu) modulates Na inactivation. Onset of Glu action is a progressive and irreversible process but can be interrupted by washing with Ringer solution. Within 30 s a persistent I_{Na} appears and saturates at ca. 0.08 of the peak I_{Na} amplitude. In another 60 s the peak I_{Na} is reduced (to 0.70 of the control) and Na inactivation kinetics slowed (τ_{h0} = 1.0 ms, τ_{h1} = 5.6 ms) while the persistent I_{Na} component remains unchanged. Internally (by diffusion from a cut internode) applied 40 mM iodate has a very similar effect: peak I_{Na} = 0.65, persistent I_{Na} = 0.07 of control peak value, τ_{h0} = 0.9 ms, τ_{h1} = 4.8 ms (after 15 min in iodate). In nodes treated with internal iodate addition of 10 mM Glu externally leaves the persistent component unaffected whereas the peak current is further reduced and inactivation further slowed. Emergence of the persistent component and reduction of peak current plus slowed inactivation seem to be two independent effects of Glu since they proceed with different time course and add differently to iodate pretreatment.

Department of Physiology, University of Kiel, Olshausenstraße 40-60, D-2300 Kiel

262

SLOW RATE OF BENZOCAINE ACTION ON SODIUM CHANNELS KEPT OPEN BY VERATRIDINE

W. Ulbricht and M. Stoye-Herzog

The local anaesthetic benzocaine rapidly blocks peak I_{Na} of voltage-clamped Ranvier nodes of frog nerve fibres: tested at 10 Hz pulse rate half of the final reduction by 1 and 0.25 mM is observed within 2 and 3 periods. Hence even on a fast exchange of solutions benzocaine block appears to be limited by diffusional access to the nodal membrane. Nodes treated with veratridine (60 µM; pH 7.2, 20°C) show a second slowly (τ_s = 1-2 s) developing I_{Na} component, I_s, that persists as long as the membrane is depolarized and subsides slowly (τ_s' = ca. 1 s) on repolarization. Benzocaine is 2-3 times more effective in blocking I_s than peak I_{Na} at the start of each long pulse. While benzocaine does not significantly affect the onset rate of veratridine action the rate of benzocaine block of I_s during 10 to 14-s depolarizing (by ca. 40 mV) pulses is very much slowed. It proceeds in an approximately exponential fashion with a mean time constant, τ_{on}, of 8.0 and 6.2 s in 25 µM and 1 mM benzocaine, respectively. If benzocaine is applied shortly (0.5-1 s) before a pulse, I_s is already much reduced at its start. Likewise, onset of benzocaine action during a series of pulses (e.g. of 1-s duration every 2.5 s) is about twice as fast as during a long pulse of the same amplitude. The magnitude of τ_{on} and its little dependence on anaesthetic concentration suggest that the rate of benzocaine block of I_s is limited by the modified gating mechanism in channels kept open by veratridine. Interestingly, benzocaine block of the persistent I_{Na} produced by internal IO_3 is fast (half time ca. 60-80 ms).

Department of Physiology, University of Kiel, Olshausenstraße 40-60, D-2300 Kiel

263

INFLUENCE OF Ca, Mg, La ON EQUILIBRIUM EFFECTS OF TETRODOTOXIN ON MYELINATED FROG NERVE FIBRES

S. Grißmer

3.1 nM tetrodotoxin (TTX) in normal Ringer reduces the Na inward current I_{Na} of the node of Ranvier to 53 % of its normal size. In Ringer + 30 mM Ca the same TTX concentration reduces I_{Na} only to 66 % (in confirmation of Hille, Ritchie and Strichartz, 1975). Likewise, in Ringer + 30 mM Mg or 0.27 mM La 3.1 nM TTX reduces I_{Na} to 66 %. The diminution of the TTX effect by divalent and trivalent cations may be due to a reduction of the negative surface potential at the TTX binding site (as suggested by Hille et al., 1975). The shift of the negative resistance branch of the $I_{Na}(E)$ curve was 28.1±5.2 mV in 30 mM Ca (n = 5), 21.7±2.6 mV in 30 mM Mg (n = 5) and 20.3±4.3 mV in 0.27 mM La (n = 5). These shifts are 2-3 times larger than required to explain the reduction of the TTX effect; the shift is significantly larger for 30 mM Ca than for 30 mM Mg or 0.27 mM La while the reduction of the TTX effect is the same in all three cases. A possible explanation is that the negative surface charges at the TTX binding site differ from those affecting the m gates, e.g. have a lower density and a smaller affinity for Ca. Alternatively, it could be thought that the reduction of the TTX effect has nothing to do with surface charges but reflects competition between the cations and TTX for a common receptor, similar to the competition between H^+ ions and TTX discussed by Ulbricht and Wagner (1975).

I. Physiologisches Institut der Universität des Saarlandes, D - 6650 Homburg/Saar, FRG

264

EFFECTS OF THREE ANEMONE TOXINS ON SODIUM CURRENTS IN A CRAYFISH NEURONE.

Klaus Hartung and Werner Rathmayer

The sea anemone *Anemonia sulcata* contains three toxins (ATX I,II and III) of which only ATX II has been studied extensively. Crustacean neurones are sensitive to all three toxins. Their effects on the Na^+ current of an identified neurone (motor giant neurone of the crayfish *Astacus astacus* and *Orconectes limosus*) have been investigated employing voltage clamp techniques. All three toxins selectively affect the Na^+ current. The most obvious effect is a slowing of the inactivation process. Inactivation is still incomplete with depolarizing voltage steps lasting for 700 ms.

In contrast to other preparations the three toxins do not only slow inactivation but also influence the current voltage relationship of the Na^+ current as well as the tail currents. In the presence of ATX the negative resistance branch of the current-voltage relation of the peak Na^+ currents is shifted about 10 mV towards more negative potentials.

The decline of the tail currents is slowed by the toxins when the membrane is repolarized to potentials more positive than the holding potential (-70 to -80 mV). The half-time of the tail current at -45 mV is about 10 times longer compared to controls. It is concluded that the toxins also affect the closing of Na^+ channels upon repolarization.

If interpreted in terms of Hodgkin-Huxley kinetics, this indicates that ATX - besides its effect on the inactivation gate - also modifies the activation gate of the Na^+ channel in this preparation.

Supported by DFG (SFB 138). We thank Dr. Beress (Kiel) for kindly providing the anemone toxins.

Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz

265

THE EXCITABILITY THRESHOLD OF THE NERVE AXON MEMBRANE: EFFECT OF SMALL IONS AND n-ALKANOLS.

A. Tippe

Changes in the threshold voltage V_s of the nerve axon membrane (Rana esculenta) caused by changing the ionic environment are quantitatively determined by the stationary transition voltage V_{Tr} of the membrane (A. Tippe, Pflüg. Arch. Suppl. 394:R 47, 1982). The mechanism underlying this ionic effect on V_s is based on a coupling between V_{Tr} and the ζ-potential of the membrane surface (H. Müller-Mohnssen, H. Barske Amer. J. Physiol. 226:844, 1974).

V_{Tr}-shifts are also caused by n-alkanols (A. Tippe BBA, 598:200, 1980). The direction of these V_{Tr}-shifts depends on the alkanol chain length: depolarisation for chains $< C_4$, polarisation for chains $> C_4$. This effect corresponds to changes in the duration of the repolarisation phase of the action potential: increase of the duration for chains $< C_4$, decrease for chains $> C_4$. Contrary to the effect of small ions (H^+, Na^+, Ca^{++} etc.) these V_{Tr}-shifts are not correlated to the membrane ζ-potential (A. Tippe, BBA, 641:395, 1981), they are constricted to the stationary K^+-conductivity and were not observed in stationary current-voltage curves for Na^+-current (10^{-1} g/l Veratridine in normal Ringers' solution). Nevertheless, all alkanols applied (C_2-C_8) increase V_s. This increase correlates to an alkanol induced decrease in the stationary Na^+-conductivity.

The alkanol effect on V_{Tr} fit well to the calculated average position of the first gauche-conformation on the C_4-C_5 bond of the alkanols and support a recently proposed model about the interaction between alkanols and the lipid bilayer matrix of the membrane (G. C. Upreti, et. al., J. Membr. Biol. 55:97, 1980).

Abteilung für Physiologie, Gesellschaft für Strahlen- und Umweltforschung, Ingolstädter Landstr. 1, 8042 Neuherberg

266

ETOMIDATE REDUCES THE EXCITABILITY IN PERIPHERAL NERVE

V. de Haas and W. Vogel

In order to get information about the mechanism of action of hypnotics on the cellular level we tested some drugs at the nodal membrane of sciatic motor fibres of Xenopus at 15°C. For methods see Kniffki et al. 1981, J. Physiol. Lond. 313:37-48. When applying the short acting drug hypnomidate® (0.05 ml i.v. solution/ml Ringer solution corresponding to about 400 µmol/l or 0.1 mg/ml etomidate and 1.75 % propandiol) the stimulus amplitude necessary for eliciting an action potential had to be increased continuously and finally the fibre became almost inexcitable. After washing the node with drug-free Ringer solution the excitability recovered.
In voltage-clamp experiments the K outward currents were found to be reduced. The decrease of the Na peak current (run down) as controlled with standard pulses throughout the experiment was clearly hastened by the drug. Upon washout two thirds of this effect were reversed. An equilibrium of the effects was reached within about 2 minutes both for washin and washout of the drug. This value has been corrected for the time of fluid change in the nerve chamber. Single or averaged Na current recordings were approximated for kinetic analysis by a non-linear curve fitting programme and showed a slowed activation process. The decay of the Na current could be fitted best with only one time constant as opposed to two exponentials in the control. Additional experiments with propandiol-(1,2) revealed that the solubilizing alcohol also contributed to the reduction of K and Na peak currents but it did not affect the kinetics of the Na current.

Physiologisches Institut der Justus Liebig-Universität Giessen, Aulweg 129, D-6300 Giessen

267

PHARMACOLOGICAL REDUCTION OF METACAINE INDUCED BLOCKAGE OF IONIC CURRENTS IN MYELINATED RANA NERVE FIBRES

R.-G. Sommer, E. Koppenhöfer, R. Haller and U. Werner

The effects of 1 mM metacaine methanesulfonate (MMS) in Ringer solution on membrane currents of potential clamped Ranvier nodes were investigated. A freshly prepared test solution (pH=7.2; T=15°C) caused a reversible reduction of peak Na-currents to 47.8 ± 5.8 % (±S.D.; n=17) and of maximum K-currents to 80.1 ±4 % (n=16). Using a 1 day old test solution, the currents were reduced to 51.7 ±5.7 % (n=4) and 83.9 ±2.5 % (n=3), respectively. After 3 days the corresponding figures were only 58.4 ±0.7 % and 88.2 ±4.1 % (n=2). This loss of efficacy of the test solution was assumed to be due to a partial decomposition of the drug. Therefore, we separated the water-soluble components of a 6 day old 1 mM MMS solution by extraction of the metacaine base with chloroform after neutralization. The remaining aqueous solution (RAS) should contain sodium methanesulfonate and water-soluble decomposition products of the drug. We added RAS to Ringer's in an amount corresponding to an addition of 1 mM MMS. The observed reduction of Na- and K-currents to 96.7 ±2.9 % (n=11) and 98.5 ±1.3 % (n=10) might be due to some remaining MMS. When we added the same amount of RAS to Ringer's + 1 mM MMS, however, the Na- and K-currents were blocked to 55.5 ±5.4 % (n=10) and 82.5 ±2.7 % (n=8), respectively. This MMS induced blockage of Na-currents was significantly less effective than without addition of RAS. Comparing the effects of equimolar concentration of metacaine methanesulfonate with the corresponding hydrochloride, both in freshly prepared solutions, identical results were obtained. In 3 day old solutions containing metacaine hydrochloride, however, the reduction of the ionic currents remained unchanged. Thus, we conclude that the loss of blockage by stale solutions of MMS might be connected with the methanesulfonate ion.

Physiologisches Institut und Pharmazeutisches Institut der Universität Kiel, D- 2300 Kiel 1

268

BOTULINUM- AND TETANUSTOXIN-POISONED MOTOR NERVE ENDINGS RESPOND DIFFERENTLY TO AGENTS WHICH NORMALLY INCREASE THE RATE OF SPONTANEOUS TRANSMITTER RELEASE.

F. Dreyer and F. Rosenberg

Striking differences in the action of botulinum A toxin (BoTx) and tetanus toxin (TeTx) have been observed when the very low transmitter release probabilities of paralyzed nerve-muscle preparations were increased by nerve stimulation (F. Dreyer and A. Schmitt, Neurosci. Letters 26: 307, 1981). While BoTx did not change the short latency between nerve impulses and postsynaptic responses, TeTx produced a temporal dispersion of the quantal release suggesting that the toxins may act at different sites of the depolarization-transmitter release process.

The present studies were undertaken to obtain further evidences which support this hypothesis. One important effect of both toxins is the drastic reduction of the frequency of spontaneous miniature endplate potentials. On the other hand several procedures and agents like nerve terminal depolarization with high external potassium, black widow spider venom, Ca-ionophores and increase of osmotic pressure are known which all increase tremendously the rate of spontaneous release in unpoisoned nerve terminals. We compared the effect of these treatments on in vitro poisoned endplates by the means of electrophysiological recording and electron-microscopy. These experiments indicate that BoTx- and TeTx-poisoned endplates were affected quite differently by these treatments supporting our hypothesis of different sites of toxin actions.

Rudolf-Buchheim-Institut für Pharmakologie der Justus-Liebig-Universität Gießen, Frankfurter Straße 107, 6300 Gießen

269

SUCCINYLCHOLINE-INDUCED HYPERKALEMIA IS NOT PREVENTED BY IV LIDOCAINE

F. Schimek and B.R. Fink

IV lidocaine inhibits potassium release from ischemic brain (J. Astrup et al., Anesthesiology 55: 256, 1981) and from energy-deprived nerve (B.R. Fink, Anesthesiology 57: 167, 1982). Here, interactions between lidocaine and succinylcholine-induced release of potassium were studied.

Twelve rabbits were anesthetized with halothane 2-2.5% in oxygen. Catheters were introduced into femoral artery and inferior vena cava. Bolus administration of succinylcholine (5mg/kg) was preceded in six animals by lidocaine (10mg/kg) injected 20 min earlier. Venous blood samples (1ml) were drawn at intervals of 2 - 20 min for one hour. Potassium was measured by flame photometry and lidocaine by GLC.

The two groups showed no difference in degrees and time courses of succinyl-induced increase of plasma potassium. Blood gas analyses gave comparable readings in the groups and varied by no more than 0.02 pH units and pCO_2 3 torr throughout an experiment.

The damage to muscle fibre membrane produced by succinylcholine may comprise an element of mechanical trauma that exceeds the effect of energy deprivation and could explain why the dose of lidocaine tested in this study was ineffective.

Zentralinstitut für Anästhesiologie der Universität Tübingen, F.R.G.
and
Anesthesia Research Centre, University of Washington, Seattle, U.S.A.

270

INHIBITION OF Na^+, K^+ AND Mg^{2+}-ATPases BY ADENOSINE AND 2-CHLOROADENOSINE

E. Porsche

Na^+, K^+- and Mg^{2+}-ATPases interact with neurotransmitters. They can either stimulate the release of neurotransmitters or they can be inhibited by those. It is further known that adenosine (Ad) plays a role in synaptic transmission process.

We investigate a possible influence of Ad on the activity of Na^+,K^+- and Mg^{2+}-ATPases. Included is 2-chloroadenosine (2-ClAd) as a nonmetabolisable Ad-derivative.

We use cortex homogenate from rat brain. The enzyme activity is determined according to Gilbert & Wyllie (1975). First the effect of Ad and 2-ClAd on the nonstimulated ATPases activity is measured. Then the effects of noradrenaline (NA), dopamine (DA) and histamine (HA) are compared with effects which occur after simultaneous administration of a neurotransmitter and a purine derivative.

Ad and 2-ClAd inhibit the activity of Na^+,K^+- and Mg^{2+}-ATPases. As expected, 2-ClAd is more active than Ad, which is taken up easily and deaminated quickly. NA and DA show the known, doses dependent stimulation of Na^+,K^+- and Mg^{2+}-ATPases, but HA has apparently no remarkable effects. A significant inhibitory effect of 2-ClAd is observed in the presence of NA and DA. Their stimulatory effect is reduced in the same order of magnitude as in the case of the nonstimulated ATPases. Even in the presence of HA 2-ClAd and Ad act inhibitorily. Our results suggest that the activity of transport ATPases can be regulated either by Ad directly or by Ad mediated processes. In addition, Ad is obviously involved in the regulatory processes caused by NA and DA and thus in the transmitter release itself. These considerations may lead to a better understanding of neuroprotective effects of adenosine derivatives and xanthine derivatives (Porsche 1982).

HOECHST AKTIENGESELLSCHAFT, Frankfurt am Main
Werk Albert, 6200 Wiesbaden 12, FRG

271

RELATIONS BETWEEN BARORECEPTOR ACTIVITY AND FIRST ORDER NEURONES IN THE NUCLEUS TRACTUS SOLITARII (NTS)

M. Lambertz, G. Schulz, P. Langhorst

In the dorso-medial part of the NTS all afferents from baroreceptors terminate. In this area spontaneously active neurones with cardiac rhythm were recorded. With one electrode up to 3 neuronal activities were registered simultaneously under identical conditions. Action potentials of the different neurones were separated by amplitude discrimination and analysed by interval histograms, post-event-time histograms, auto- and cross-covariances. This led to detailed information concerning the integration of various inputs from cardiovascular and respiratory receptors and the intrinsic organization of this nucleus. By cross-covariance histograms of two pulse rhythmical activities the identification of first order neurones in the baroreceptor pathway was possible. Furthermore it could be shown that a temporal and spatial summation of the input from different receptors takes place at these neurones. The processing of such afferents by these neurones is not time invariant. Pressoreceptor afferents do not influence neighbouring neurones in that area of the NTS to the same extent. Neurones discharging with strict cardiac rhythm are intermingled with neurones exhibiting only pulse rhythmical modulations and neurones without cardiac rhythm. The implications of these results for the regulation of blood pressure will be discussed.

Langhorst, P., Schulz, G., Lambertz, M., Krienke, B.: Processing of the information from baroreceptors by neurones in the dorso-medial part of the nucleus of the solitary tract. In: Arterial baroreceptors and hypertension, ed. by P. Sleight. Oxford University Press 1980, p. 268.
Stroh-Werz, M., Langhorst, P., Camerer, H.: Neuronal activity with cardiac rhythm in the nucleus of the solitary tract in cats and dogs. Brain Res. 133, 65 (1977).

Institute of Physiology, The Free University of Berlin, Arnimallee 22, D-1000 Berlin 33, Germany

272

AN INTRACELLULAR STUDY OF THE SYNAPTIC INPUT TO SYMPATHETIC PREGANGLIONIC NEURONES OF THE CAT.

K. Dembowsky, J. Czachurski, and H. Seller

In chloralose-anaesthetized, paralyzed and artificially ventilated cats sympathetic preganglionic neurones (SPN) of the third thoracic segment were recorded intracellularly. Conduction velocity of SPNs as determined by antidromic stimulation of the white ramus T_3 ranged from 1.3 to 8.2 m/s (n: 53). Action potentials (AP) (up to 120 mV amplitude) of most SPNs showed a marked shoulder shortly after the peak followed by an afterhyperpolarization (3-15 mV) lasting from 30 to 650 ms. APs of a smaller group of SPNs displayed a fast early followed by a slow late repolarization. The subsequent afterhyperpolarization (2-10 mV) had a duration of 25 to 250 ms. Measurements of the input resistance of SPNs revealed values of 5-42 MΩ (n: 6). Synaptic input to SPNs was tested by electrical stimulation of descending spinal pathways at C_3 and somatic and visceral afferents (intercostal nerve T_4, white ramus T_4). In each case tested (n: 15), SPNs responded with an EPSP to stimulation of the respective afferents. The EPSPs were often subthreshold for eliciting an AP. EPSPs in response to somatic and visceral afferent stimulation had an early spinal (latency 7-15 ms) and a late supraspinal (latency 29-63 ms) component, the latter showing a high degree of temporal summation. By stimulation of descending pathways EPSPs with different latencies were evoked. The corresponding conduction velocities were: 9.3-25.9 m/s, 4.7-6.4 m/s, and 2.8-3.5 m/s.

The present results show that there are at least two types of SPNs which can be identified by the shape of their AP and that the excitatory synaptic input to these neurones is greater than that found with extracellular recordings.

I. Physiologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg, F.R.G.

This study was supported by the DFG within the SFB 90.

273

VISCERO-SYMPATHETIC REFLEXES IN POSTGANGLIONIC NEURONES SUPPLYING SKIN AND SKELETAL MUSCLE IN BRAIN-INTACT CATS.

H. Blumberg, K. Hilbers, W. Jänig

Reflexes were elicited, in postganglionic vasoconstrictor neurones supplying skin (CVC) and skeletal muscle (MVC) of the cat hindlimb and in sudomotor neurones (SM) supplying sweat glands in the hairless skin of the cat hindpaw, by stimulation of visceral afferents from urinary bladder and colon. The visceral afferents were excited by passive distension and by contraction of the organs. Activity was recorded from postganglionic axons which were isolated in bundles from peripheral nerves. The animals were anaesthetized with α-chloralose, immobilized and respired. 1) Distension and contraction of urinary bladder and colon lead to inhibition of activity in most CVC neurones and to excitation of MVC and SM neurones. Contraction of the organs was far more powerful than distension. 2) Rhythmic ongoing contractions of the organs induced rhythmic inhibitions and excitations in the sympathetic systems. 3) Slow filling of the bladder, without contractions of the organ, induced a slow decrease of activity in CVC neurones in part of the experiments. When the bladder finally contracted, the CVC activity increased (like a rebound) to the prefilling level after the end of the contraction. 4) These viscero-sympathetic reflexes did not change after cutting the hypogastric nerves; thus, they are produced by stimulation of sacral visceral afferent fibres. 5) The pattern of these viscero-sympathetic reflexes fit very well into the pattern of somato-sympathetic reflexes in brain-intact animals (excitation of SM- and MVC neurones and inhibition of CVC neurones). 6) Whether the viscero-sympathetic reflexes are elicited by excitation of sacral afferents which participate in micturition and defecation or by a special class of visceral afferents which participate in visceral nociception or by both is unclear.

Physiologisches Institut, Universität Kiel, FRG

274

VISCERO-SYMPATHETIC REFLEXES IN POSTGANGLIONIC NEURONES SUPPLYING THE HINDLIMB OF CHRONIC SPINAL CATS

H. Kümmel, Xu, H.

In brain-intact cats vasoconstrictor neurones supplying hairy and hairless skin (CVC) are inhibited by visceral stimuli, whereas vasoconstrictor neurones supplying skeletal muscle (MVC) and sudomotor neurones innervating the sweat glands (SM) are activated. The question is whether these reflex patterns are preserved in chronic spinal cats.
The activity in fine bundles of postganglionic axons supplying skeletal muscle and skin of the hindlimb and, as an indicator of the sudomotor activity, the skin potential on the hairless skin of the paw were recorded in chronic spinal cats 60-122 days after transsection of the spinal cord between T8 and T10. Visceral stimuli were induced by distension of bladder and colon, by bladder and colon contractions and by anal and vaginal stimuli. The following results were obtained: 1) All visceral stimuli led to reflex activations in MVC, CVC and SM neurones. The most powerful reflexes were elicited by contractions of the organs. 2) Rhythmic contractions of an organ induced rhythmic excitations of the sympathetic systems. 3) Slow filling of bladder or colon without inducing contractions was sometimes followed by a slow increase of the activity in the postganglionic vasoconstrictor neurones. 4) The major change occurring in the viscero-sympathetic reflexes in spinal animals, when compared to brain-intact animals, is the reversal of the reaction in CVC neurones from inhibition to excitation.

Physiologisches Institut, Universität Kiel, FRG

275

INHIBITION OF TRANSMISSION BY CLONIDINE IN GANGLIA OF THE CHOLINERGIC-SYMPATHETIC NERVOUS SYSTEM

A. Walland

Sweat secretion in the foot pads of the cat is totally controlled by the cholinergic-sympathetic nervous system and can be assessed by evaluation of the amplitude of the electrodermal potential (EDP). Clonidine (30 µg/kg, i.v.) inhibits EDPs induced by electrical stimulation of the hypothalamus at low but not at high rates of stimulation. This effect is due to blockade of the nicotinic ganglionic transmission by activation of somadendritic α_2-adrenoceptors (Walland, this J. 316, R57, 1981). Apparantly inconsistent with this mechanism, the effects of preganglionic electrical stimulation are not affected by clonidine. - It was assumed that the lack of an effect with preganglionic stimulation was due to maximal activation of the ganglion by this procedure. In order to keep ganglionic activation submaximal anaesthetized cats received a continous infusion of 0.08-0.3 mg/kg/min hexamethonium for partial blockade of ganglionic nicotinic receptors. Preganglionic stimulation of the right stellate ganglion at intervals of 1 min with trains (2 sec) of DC-pulses (2 msec, 0.6-0.9 mA, 0.5-128 Hz) induced EDPs which increased slightly with the rate of stimulation and were 30-50% smaller than in unpretreated controls. Under these conditions clonidine (30 µg/kg, i.v.)suppressed EDPs in 5 cats by 70-95%. A similar result was obtained upon topical application of 1 µg clonidine to the right ganglion in 5 cats. Yohimbine (200 µg/kg, i.v.) increased the amplitude of the EDPs above the values before clonidine, thus proposing some permanent supression of ganglionic transmission by endogenous catecholamines. - The results provide anew evidence for the inhibition of transmission in sudomotor ganglia by activation of ganglionic α_2-adrenoceptors and indicate that the uncritical use of preganglionic stimulation techniques causes underestimation of the importance of ganglionic α_2-adrenoceptors.

Department of Pharmacology, Boehringer Ingelheim KG, D-6507 Ingelheim, Federal Republic of Germany.

276

M_1 - AND M_2 -RECEPTORS IN SYMPATHETIC GANGLIA OF THE PITHED NORMOTENSIVE RAT; DIFFERENTIAL ROLE IN α-ADRENOCEPTOR-MEDIATED VASOCONSTRICTION.

D. Davidesko

In the pithed normotensive rat stimulation of ganglionic muscarinic receptors by 1,1-dimethyl-4-carboxypiperidine methylester (DMCPM) leads to activation of vasoconstrictor α_2-adrenoceptors. The present study was designed to determine the role of ganglionic M_1- and M_2-receptors in the DMCPM-induced vasoconstriction. Activation of M_1-receptors was assessed by the antagonism of the selective M_1-antagonist pirenzepine towards the pressor responses of this muscarinic agonist. M_2-receptor stimulation was demonstrated by a decrease in heart rate in pithed normotensive rats pretreated with atenolol. Consequently, DMCPM proved to be a mixed M_1/M_2-agonist. McN-A-343, 4-(m-chlorophenylcarbamoyloxy)-2-butynyl trimethylammonium chloride, is a selective M_1-agonist. The pressor responses of McN-A-343 were largely mediated by α_1-adrenoceptors. A very limited contribution of α_2-adrenoceptors was demonstrable. In the attempts to relate the small contribution of α_2-adrenoceptors to the absence of a M_2-agonistic effect of McN-A-343, the cholinergic effects of this agent were thorougly studied. A pronounced M_1-component was identified apart from slight nicotinic and M_2-agonistic activities. The M_2-component was present in the high dose-range of McN-A-343 only. A participation of nicotinic receptors in the increase in diastolic pressure caused by McN-A-343 was excluded by using hexamethonium. The results bring us to the hypothesis that both M_1- and M_2-receptors may be involved in ganglionic transmission. Stimulation of M_1-receptors elicits predominantly α_1-adrenoceptor mediated vasoconstriction (McN-A-343). M_2-receptor activation gives rise to activation of vascular α_2-adrenoceptors (DMCPM).

Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands

277

PROPERTIES OF β_1-AND β_2-ADRENOCEPTORS(R) IN RABBIT LUNG DETERMINED BY (±)-^{125}IODOCYANOPINDOLOL(ICYP) BINDING AT DIFFERENT TEMPERATURES(T).

O.-E.Brodde

In rabbit lung membranes properties of β_1- and β_2-R were studied by ICYP binding at T of 37°C and 18°C.T-changes did not affect density of ß-R while K_D for ICYP was slightly lower at 18°C.At both incubation-T β_1-R(practolol;metoprolol;noradrenaline and prenalterol in the presence of 0.1 mM GTP) and β_2-R(IPS 339=(t-butyl-amino-3-ol-2-propyl)oximino-9 fluorene;zinterol and procaterol in the presence of 0.1mM GTP) selective drugs inhibited binding with biphasic displacement curves and non-linear Hofstee-plots,while non-selective ß-R(propranolol;isoprenaline(IPN) and adrenaline in the presence of 0.1mM GTP) drugs showed monophasic displacement curves and linear Hofstee-plots.The "β_2-selective" agonist salbutamol,however,behaves at both T as a non-selective ß-R drug.With decreasing T affinity of antagonists increased only slightly,while affinity of agonists increased markedly.For all β_1- and β_2-R selective drugs a ratio of 80% β_1-R/20% β_2-R was calculated for rabbit lung membranes independent of the incubation-T.At 37°C displacement curve of IPN in the absence of GTP was biphasic with a non-linear Hofstee-plot indicating binding of IPN to high and low affinity state of ß-R.At 18°C,however,IPN displacement curve was monophasic independent of whether GTP(0.1mM) was present or not. It is concluded that a decrease in incubation-T of ICYP binding assay from 37°C to 18°C does neither alter the relative amount of β_1- and β_2-R in rabbit lung membranes nor the selectivity of β_1- or β_2-selective drugs.

Div.Renal & Hypertensive Diseases,Med.Klinik & Poliklinik, University of Essen, D-4300 Essen.

Supported by SANDOZ-Stift. für Therap. Forschg.

278

IMPORTANCE OF GLUCOCORTICOIDS IN MAINTENANCE OF HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RATS

J.B. Baumann, W. Ruch and J. Girard

Complete adrenalectomy of ten week-old spontaneously hypertensive rats (SHR) leads to a rapid, permanent reduction in systolic blood pressure, similar to that of normotensive Wistar Kyoto rats, provided that there is no regeneration of accessory adrenocortical tissue. Treatment of adrenalectomized SHR with the synthetic glucocorticoid betamethasone, but not with the mineralocorticoid aldosterone resulted in a dose-dependent restoration of high blood pressure within 12 hours. After cessation of treatment, the blood pressure returns to normotensive levels slowly, within one to three weeks. Concomitant treatment of adrenalectomized SHR with betamethasone and (-)-propranolol (2 mg/kg, twice daily) prevented the rise in blood pressure observed after 12 hours of treatment with betamethasone.

Our results indicate that glucocorticoids are a prerequisite for the maintenance of high blood pressure in SHR. The antagonizing effect of (-)-propranolol in our experiments suggests a close interaction between glucocorticoids and adrenergic neurotransmission in SHR.

Endokrin. Abt. Kinderspital and Dept. Forschung Kantonsspital, Universität Basel, CH-4031 Basel Switzerland

279

CHARACTERIZATION OF POSTSYNAPTIC α-ADRENOCEPTORS IN THE HUMAN SAPHENOUS VEIN

H.-R. Zerkowski, J. Wagner, N. Rohm
E. Rodrigues-Pereira

In experiments on isolated rabbit saphenous veins the postsynaptic α-adrenoceptor could not be associated with the known subtypes by means of selective agonists as well as antagonists (Lues,Schümann; NSAP 321, 256,1982). Since in contrast to vessels of animal origin only a few informations exist on postsynaptic α-adrenoceptors of human ones, especially of veins, we were interested to characterize even those of isolated human saphenous veins from the thigh obtained from patients undergoing elective coronary artery bypass grafting. The experiments habe been performed in Krebs-Henseleit solution (cocaine, $3x10^{-5}$,corticosterone, $4x10^{-5}$,pindolol, 10^{-7}mol/l) at 37°C.- Cumulative concentration-response curves (CRC) for noradrenaline (NA) were obtained under the influence of the selective antagonists prazosin (P) and BE 2254 (2-[ß-(4-hydroxyphenyl)-ethylaminomethyl]-tetralone; $\alpha_1$blocker) as well as of the α_2-antagonist rauwolscine (R). NA behaved as full agonist, its pD_2-value was 6.71±0.05 (n=22) while clonidine was a partial agonist with an intrinsic activity of 0.09 only.- The CRC for NA was shifted to the right by P as well as by BE 2254 but there was no indication of a competitive antagonism. Likewise, the α_2-antagonist R did not exert a competitive antagonism towards NA; after R, $3x10^{-7}$mol/l the CRC merged.- These results, therefore, do not allow to characterize the postsynaptic α-adrenoceptors of human saphenous vein as either α_1- or α_2-type.

Pharmakologisches Institut, Univ.-Klinikum Hufelandstr. 55, 4300 Essen 1, FRG

280

THE INTERFERENCE BETWEEN α_1 - AND β_2 -ADRENOCEPTOR-MEDIATED VASCULAR EFFECTS IN THE PITHED NORMOTENSIVE RAT. INDICATIONS FOR TWO POPULATIONS OF α_1 -ADRENOCEPTORS

B. Wilffert

Activation of α- and β_2 -adrenoceptors in the vascular wall elicits opposite effects. In the present study, the interaction between β_2 - and α_1 -adrenoceptor-mediated vasodilator and vasopressor effects in the pithed normotensive rat is reported. The increase in diastolic pressure induced by the α_1 -adrenoceptor agonists cirazoline, methoxamine and St 587, 2-(2-chloro-5-trifluoromethylphenylimino)imidazolidine, was differentially affected by pretreatment with the β_2 -adrenoceptor agonist salbutamol (1 mg/kg). Cirazoline-induced pressor responses were not attenuated in contrast to the responses of methoxamine and St 587. After pretreatment with the α_2 -adrenoceptor blocking drug rauwolscine (1 mg/kg) noradrenaline mainly stimulates vasopressor α_1 -adrenoceptors. The α_1 -adrenoceptor-mediated increase in diastolic pressure induced by i.v. noradrenaline was attenuated by pretreatment with salbutamol. Pressor responses of noradrenaline mediated by α_1 -adrenoceptors were also elicited by releasing this catecholamine from the neurones by the nicotinic agonist DMPP and the muscarinic ganglion stimulator McN-A-343, 4-(m-chlorophenylcarbamoyloxy)-2-butynyl trimethylammonium chloride. The former response was found sensitive to a pretreatment with salbutamol in contrast to the latter. A vasopressin infusion counteracting the vasodilation by salbutamol prevented the effects of this β_2 -adrenoceptor agonist. The results indicate the existence of two populations of postsynaptic α_1 -adrenoceptors. The vasoconstriction initiated by one population is sensitive to β_2 -adrenoceptor-mediated vasodilation in contrast to the other. The former population is predominently activated by St 587, methoxamine, i.v. administered noradrenaline and activation of nicotinic ganglionic receptors. The other population is preferably activated by cirazoline and activation of muscarinic M_1 ganglionic receptors.

Division of Pharmacotherapy, university of Amsterdam, Plantage Muidergracht 24, The Netherlands.

281

CARDIOVASCULAR EFFECTS OF SGD 101/75 IN ANESTHETIZED AND CONSCIOUS ANIMAL PREPARATIONS. M.J.M.C. Thoolen and M.J. Mathy

Sgd 101/75, 2-(2-methylindazol-4-imino)imidazolidine, is a clonidine derivative which differs from the parent drug in not reducing blood pressure upon i.v. administration in anesthetized rats, cats and dogs (Ismail et al., Br.J. Pharmacol.72,535P,1981). In the present study we examined the effects of Sgd 101/75 on blood pressure upon i.v. injection in conscious spontaneously hypertensive rats, intracisternal injection and infusion via the vertebral artery in chloralose-anesthetized cats as well as the pressor effects in pithed rats and cats. No dose-related hypotensive effects were observed after i.v. injection of Sgd 101/75 (1-10 mg/kg) in SH rats. After intracisternal injection and infusion via the left vertebral artery (up to 1 mg/kg) in cats but slight reductions of blood pressure were observed. In pithed rats, Sgd 101/75 elicited pressor responses. The dose-response curve was shifted to the right by pretreatment with the α_1-adrenoceptor blocking drug prazosin (0.1 mg/kg i.v., -15 min), but not by the α_2-adrenoceptor antagonist yohimbine (1 mg/kg i.v., -15 min). In pithed cats, the pressor responses were subject to tachyphylaxis and more susceptible to α_1-adrenoceptor blockade by prazosin (1 mg/kg,i.v., -15 min) than to α_2-adrenoceptor blockade by yohimbine (1 mg/kg, i.v., -15 min). Binding studies revealed, that Sgd 101/75 displayed but minor affinity for ^{3}H-clonidine binding sites. These results show that Sgd 101/75 is a selective agonist for α_1-adrenoceptors in the circulatory system of rats and cats. Its inefficacy in reducing arterial pressure in the various animal preparations is likely due to its low α_2-adrenoceptor agonist activity.

Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands.

282

DIFFERENTIAL INHIBITION OF α_1-ADRENOCEPTOR MEDIATED PRESSOR EFFECTS BY CALCIUM ENTRY BLOCKERS IN PITHED NORMOTENSIVE RATS. P.B.M.W.M. Timmermans and J.C.A. van Meel

In rat anococcygeus muscle, the compound Sgd 101/75, 2-(2-methylindazol-4-imino)imidazolidine, identifies an α_1-adrenoceptor different from that activated by noradrenaline (Coates et al., Br.J.Pharmac. 75,549,1982). We found its pressor effects in pithed rats sensitive to prazosin, but not to yohimbine. However, the log dose-vasopressor response curve to Sgd 101/75 is shallow, like that of all selective α_2-adrenoceptor agonists. It was therefore studied whether additional characteristics of α_2-adrenoceptor-induced vasoconstriction were shared by Sgd 101/75. In pithed rats, the log dose-pressor response curve to Sgd 101/75 was shifted to the right by captopril (5 mg/kg) and profoundly reduced by salbutamol (1 mg/kg). Nifedipine (0.1-3 mg/kg) markedly depressed both slope and maximum of the dose-response curve of Sgd 101/75. Similar results were obtained in pithed cats. This pattern of interactions is identical to that of the α_2-adrenoceptor agonists. However, the pressor effects of St 587, 2-(2-chloro-5-trifluoromethylphenylimimo)-imidazolidine, which displays extreme α_1-adrenoceptor agonistic activity (De Jonge et al., Life Sci.28, 2009,1981), were also sensitive to calcium entry blockade. On the other hand, the vasoconstriction to the preferential agonist of α_1-adrenoceptors, cirazoline (Van Meel et al., JPET,219,760,1981) was much less susceptible to these measures. The results suggest that Sgd 101/75 and St 587 elicit vasopressor responses in pithed rats which are dependent upon an influx of extracellular calcium, like those of α_2-adrenoceptor agonists. Conversely, cirazoline contracts vascular smooth muscle via a mechanism which is not primarily governed by an entry of calcium ions.

Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands
Present address of J.C.A. van Meel: Dr. Karl Thomae GmbH, D-7950 Biberach 1, B.R.D.

283

α_1/α_2-ADRENOCEPTOR BLOCKING ACTIVITIES OF THE PUTATIVE α_2-ADRENOCEPTOR ANTAGONIST RAUWOLSCINE, RX 781094 AND RS 21361
Qian Jiaqing, H.D. Batink and P.A. van Zwieten

The selectivities and the activities of the preferential α_2-adrenoceptor blocking drug rauwolscine, RX 781094, (imidazolinyl-2)-2-benzodioxane 1-4, and RS 21361, 2-(1-ethyl-2-imidazolyl)methyl-1,4-benzodioxane, were compared. Antagonistic activities at postjunctional (vascular) α_1-adrenoceptors were evaluated, pA_2 (post α_1), against the methoxamine-induced increases in diastolic pressure in pithed normotensive rats. The same animal model afforded the pA_2 (post α_2) values determined against the pressor responses to B-HT 920, 2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo[4,5-d]azepine. The blocking activities at presynaptic (cardiac) α_2-adrenoceptors, pA_2 (pre α_2), were quantified against the B-HT 920-evoked inhibition of electrically-induced tachycardia in pithed normotensive rats. Binding affinities, K_i, for ^{3}H-clonidine (α_2) and ^{3}H-prazosin (α_1) specific binding sites were measured in rat isolated cerebral membranes in vitro.

	pA_2			K_i (nM)	
Drug	post α_1	post α_2	pre α_2	α_1	α_2
Rauwolscine	5.24	7.54	7.04	3200	150
RX 781094	5.67	6.31	6.27	640	15
RS 21361	<3.4	5.17	4.98	38500	2340

The results of the present study demonstrate that all of the three drugs, but especially rauwolscine and RS 21361, act as selective antagonists of α_2-adrenoceptors. In functional studies on α_2-adrenoceptors, rauwolscine was more potent than RX 781094 and RS 21361. However, RX 781094 possessed considerably higher in vitro binding affinities than rauwolscine. RX 781094 was found about 3 times more active than rauwolscine in counteracting the centrally-mediated hypotension of clonidine in anaesthetized normotensive rats.

Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, The Netherlands.
Present address of Qian Jiaqing: Wuhan Medical College, Wuhan, People's Republic of China.

284

CHOLINERGIC HISTAMINE RELEASE FROM PERFUSED GUINEA PIG LUNG
H. Kleinjung and W. Schmutzler

Cholinergic histamine release depends either on special genetic conditions or on active immunization of individuals as could be demonstrated in isolated mast cells from rat, guinea pig, dog and man (Schmutzler et al.,Int. Archs. Allergy, in press).

The present experiments were designed to find out whether histamine release is involved in the mechanism of cholinergic bronchoconstriction which is thought to contribute largely to allergic asthma (Gold et al., J. appl. Physiol. 33, 719, 1972). In the saline perfused lung of normal guinea pigs metacholine (10^{-8} moles/kg) caused bronchoconstriction and histamine release. Both effects were found to be significantly stronger in lungs from actively sensitized animals. Atropine (10^{-4} M) inhibited the effects of metacholine in the lungs from normal animals significantly better than in those from the sensitized animals. Atropine (10^{-7} M) rather enhanced the allergic histamine release but did not induce bronchoconstriction or histamine release by itself.
Electric stimulation of the N. vagus did not evoke histamine release.
We conclude from these results that cholinergic histamine release is one of several mechanisms of "pseudo-allergy". Cholinergic mechanisms, however, do not contribute essentially to anaphylactic bronchocostriction in the guinea pig.

Abt. Pharmakologie, RWTH Aachen, Schneebergweg, D-5100 Aachen

285

DIFFERENCES IN THE ACTION OF ACETYLCHOLINE AND CARBACHOL IN GUINEA PIG ATRIA AND TAENIA COLI.

F. Mitchelson and A. Ziegler

In isolated guinea pig atria and taenia coli the actions of carbachol (CCH) and acetylcholine (ACh) were compared. In both tissues the rate of response to ACh was faster than to CCh. Treatment of either tissue with paraoxon (10 µM for 20 min) did not eliminate differences in the rates of onset for the two agonists. The half life of disposition for CCh as estimated from the negative inotropic effect in atria driven at 1 or 3 Hz was found to be 48 and 15 sec, respectively. The corresponding values for ACh were 8 and 5 sec, and after pretreatment with paraoxon, 23 and 21 sec, respectively. In the taenia coli contractions, recorded isotonically, were evaluated by measuring the initial peak of the phasic response and the magnitude of the maintained response after 4 min contact with the agonist. ACh usually produced both a rapid phasic and a slowly developing tonic contraction, whereas with CCh a phasic response was not clearly evident. Furthermore, effects of comparable size for the two agonists could be differentially influenced by reducing the Ca^{++} concentration in the bath solution from 1.8 mM to 0.18 and 0.06 mM or by the use of the Ca antagonists nifedipine (3-10 nM) and gallopamil (30-300 nM). With any of these treatments the tonic response to ACh was reduced to a greater extent than the phasic response. The tonic response to CCh was inhibited to a significantly smaller degree than the corresponding response to ACh. For example, in paraoxon-pretreated taenia coli the tonic response to ACh 0.1 µM and CCh 0.1 µM were reduced by nifedipine 10 nM to 29.4 ± 7.4 (5) (mean ± S.E.M. (n)) and 68.7 ± 12.4 (5)% of the corresponding controls, respectively. These findings with reduced Ca^{++} and the Ca-antagonists suggest, that the part of the Ca necessary for the contractile activation in taenia coli by CCh is not derived from the extracellular space. The results for the two agonists in both tissues suggest differences in the disposition of the drugs and in the taenia differences in the interaction at the receptor.

Depts. Pharmacol. Kiel and Melbourne, Hospitalstrasse 4-6, D-23 Kiel

286

THE INTERACTION OF CARBACHOL WITH THE POSITIVE INOTROPIC EFFECT OF CATECHOLAMINES

M. Korth

In the guinea-pig papillary muscle driven at 0.2 Hz, carbachol antagonized the positive inotropic effect of isoprenaline (ISO) but hardly affected that of noradrenaline (NA). NA (0.1 - 100 µmol/l) produced a positive inotropic effect which was accompanied by a partial decline of the contraction steepness, S_1, as could be seen from the large increase of S_1 associated with contractions interpolated between regular beats. ISO had a dual action, up to an EC_{80} the positive inotropic effect was exclusively due to an increase of S_1 while at higher concentrations partial decline of S_1 became apparent. Derivatives of cAMP produced qualitatively the same effects as ISO. In the presence of NA, carbachol (0.1 - 3 µmol/l) enhanced S_1 without affecting force of contraction. When neuronal uptake of NA was inhibited by 20 µmol/l cocaine or became saturated in the presence of β-adrenoceptor blockers, ISO-like inotropic effects were produced by NA, and carbachol had a strong negative inotropic effect. Conclusions: cAMP has two distinct actions: first, it enhances force of contraction by increasing S_1 and then, at high concentrations, it antagonizes this effect by inducing a loss of Ca^{++} from the rapid releasing sites. That both mechanisms operate simultaneously in the presence of NA can be explained by assuming that neuronal uptake produces concentration gradients for NA in the extracellular space which are large enough to allow high and low receptor occupancy at the same time. Carbachol fails to antagonize the positive inotropic effect of NA since its negative inotropic effect is compensated by its simultaneously induced increase of S_1. Both carbachol effects can be explained by inhibition of adenylate cyclase.

Institut f. Pharmakologie u. Tox. d. Techn. Univ. München, Biedersteinerstr.29, D-8000 München 40

287

THE PROTEIN COMPOSITION OF THE SYMPATHETIC NERVE-VESICLES (CO-EXISTENCE OF DOPADECARBOXYLASE, DBH AND SMALL POLYPEPTIDES)

H. Husseini and H. Balzer

With HPLC TSK-G 3000 SW column and a mobile phase of 0.1 M phosphate buffer, pH 6.8, containing 0.1 M NaCl 1-4 main fractions of proteins were found in preparations of sympathetic splenic nerves. The protein fractions were 1=150,000 Mr; 2=75,000 Mr; 3=27,000 Mr and 4=small polypeptides with < 13,500 Mr. The 150,000 Mr protein (1) showed dopadecarboxylase activity. The dopamine-ß-hydroxylase (DBH) was not found in tetrameric form; the 75,000 Mr subunit was only detected. The fraction 4 showed small polypeptides with Mr of 13,500 - 1,000 as it is known for opioid peptides. The proteins were separated by preparative electrofocusing and reseparated by HPLC, SDS-HPLC and SDS-polyacrylamid-gel-electrophoresis. These proteins were found in the nerve-vesicles in soluble and insoluble forms with different distribution. Small and large vesicles were ultracentrifugally separated by 0.3-1.1 M sucrose gradient. The 150,000 Mr protein (dopadecarboxylase) was found in large vesicles in soluble and membrane-bound form with equal distribution (20 %). In contrast to that the 75,000 Mr fraction was detected in soluble form with high amounts (44 %).

These results showed the co-existence of dopadecarboxylase, DBH and small (opioid) polypeptides in the nerve-vesicles of the sympathetic nerve.

Zentrum für Pharmakologie, Abt.I, der Universität Frankfurt/M., Theodor-Stern-Kai 7, D-6000 Frankfurt/Main

288

BLOCKADE OF HOMOGENIZATION-INDUCED RELEASE OF DOPAMINE ß-HYDROXYLASE (DBH) INTO THE SUPERNATANT OF HEART TISSUE BY GADOLINIUM CHLORIDE

F.J. Spira

Rabbit isolated hearts were perfused with Tyrode's solution at 20 ml/min. After an equilibration period of 60 min the hearts were suspended in 5 - 10 vol of ice-cold 0.25 M sucrose in 50 mM Tris buffer (pH 6.0) containing either Na_2EDTA or $GdCl_3$, and homogenized using an Ultra-Turrax and subsequently a glass homogenizer. The homogenate was centrifuged for 1 h at 100,000 g. DBH activity was determined in samples of the crude homogenate and of the 100,000 g supernatant. In the presence of Na_2EDTA (1 mM) no reduction of the soluble DBH fraction was found. On the other hand, increasing concentrations of $GdCl_3$ (0.01 - 0.2 mM) reduced the soluble DBH fraction of the homogenate in a concentration-dependent manner. At 0.2 mM $GdCl_3$ only 67.4 ± 9.1 (n = 3) units DBH were found in the 100,000 g supernatant which corresponds to about 25 % of the total soluble DBH (277 ± 20, n = 7) in the absence of $GdCl_3$.

$GdCl_3$ inhibits the catecholamine secretion from cultured chromaffin cells by blockade of calcium channels (G.W. Bourne and J.M. Trifaro, Neuroscience 7: 1615, 1982). Therefore, it is concluded that a calcium influx into the terminal nerve fibres is required to induce the DBH release from the adrenergic vesicles into the soluble phase of the homogenate during the homogenization procedure. - Supported by DFG.

Pharmakologisches Institut der Universität Obere Zahlbacher Str. 67, D-6500 Mainz

289

DOES THE GRANULAR CARRIER TRANSPORT NEUTRAL OR PROTONATED CATECHOLAMINES ?

G. Kobold and A. Burger

As there are contradictory reports whether the substrate of the granular carrier is neutral (Scherman & Henry 1981: Eur.J.Biochem.116,535-539) or protonated (Knoth et al 1981: J.Biol.Chem.256,6541-6543) catecholamine (CA), we studied the pH-dependence of Km of CA uptake into ghosts of chromaffin granules (CG) of the bovine adrenal medulla. CG were prepared by density gradient centrifugation. Ghosts (∿125 nmoles CA/mg prot.) were obtained by lysis of CG in a hypotonic medium containing urea. Ghosts were incubated at various pH for 10 min (30°C) with six conc.'s of CA (0.5-100 µM CA: 70% adrenaline/30% noradrenaline) which was labelled with ^{3}H-(-)noradrenaline. The uptake of ^{3}H into ghosts was measured after membrane filtration of incubated samples. To calculate the rate of uptake of CA, the ^{3}H-activity and the conc. of CA were measured in the medium at the beginning (addition of ^{3}H-CA) and the end of incubation. In spite of CA net uptake into ghosts, the CA conc. was nearly constant during incubation, because the conc. of membrane protein was kept low (∿20 µg/ml). In the absence of ATP the uptake increased with increasing pH and amounted to 5-12% of the uptake in the presence of ATP. Reserpine (0.5 µM) completely blocked the ATP-stimulated uptake. At various pH the ATP-stimulated uptake showed the following Km and Vmax (n = 5, each) given in µM and nmoles CA/(mg protein · min) respectively:

pH :	6.2	6.6	7.0	7.4	7.8	8.2
Km :	93±12	33±6	11±1	7.4±2.5	2.7±0.4	1.9±0.2
Vmax:	9.5±1.5	16±3	21±1	13±2	16±1	9.7±0.6

From the marked pH-dependence of Km is concluded that neutral CA is the substrate for the granular carrier. In fact, after calculation with pk = 8.57 the true Km of the neutral CA was constant at each pH and amounted to 0.41 ± 0.04 µM (n = 6). Supported by the DFG (Bu 296/4-2).

Institut für Pharmakologie und Toxikologie der Universität Versbacher Str. 9, D-8700 Würzburg

290

THE CHLORIDE DEPENDENCE OF THE NORADRENALINE TRANSPORT INTO PC-12 CELLS.

H. Bönisch and U. Friedrich

The neuronal transport of noradrenaline (NA) is absolutely dependent on Na^+ (Sammet and Graefe, N.-S. Arch. Pharmacol., 309, 99-107,1979) and on Cl^- (Sanchez-Armass and Orrego, Life Sciences, 20, 1829-1838, 1977): increasing concentrations of Na^+ increased Vmax and decreased Km of NA transport while increasing concentrations of Cl^- only increased Vmax. It was the aim of this study a) to examine the effect of various Cl^--substitutes on the NA-transport of PC-12 cells (rat pheochromocytoma cells which possess many characteristics of adrenergic neurones) and b) to compare the effect of Cl^- and of Na^+ on the kinetics of NA transport in these neuronal cells. Vesicular amine storage and monoamine oxidase were inhibited.

Initial rates of uptake of 0.01 µM ^{3}H-7-NA were reduced when Cl^- was replaced by other anions. The halogenide Br^- and the pseudohalogenide SCN^- substituted for Cl^- with 42% and 23% effectiveness, respectively, whereas other anions were poor substitutes irrespective of their low (isethionate and sulphate) or high lipophilicity (nitrate). Substitution of Na^+ by $Tris^+$ or sucrose completely inhibited uptake of ^{3}H-NA. A half-maximal activation of uptake of ^{3}H-NA (0.1 µM) was observed at about 15 mM Cl^- (isethionate as substitute) and at about 30 mM Na^+ ($Tris^+$ as substitute).
The transport of ^{3}H-NA showed saturation by increasing concentrations of NA when determined at several fixed concentrations of Cl^- or Na^+. Progressive increases in the concentration of Cl^- or Na^+ decreased the apparent Km and increased the Vmax of ^{3}H-NA transport.
Conclusion: Under normal conditions both, Na^+ and Cl^-, must bind to the amine carrier for transport of NA to occur.

Supported by the Deutsche Forschungsgemeinschaft
Institut für Pharmakologie und Toxikologie der Universität, Versbacher Str. 9, D-8700 Würzburg

291

THE ISOTOPE EFFECT OF TRITIUM IN (-)-NORADRENALINE

U. Trendelenburg, M. Grohmann and F. J. E. Stefano

Under conditions which do not saturate the system, rates of neuronal (arterio-venous difference in rabbit perfused heart) and extraneuronal uptake (rat perfused heart) were higher for unlabelled than for labelled noradrenaline (NA) (factor of 1.4 to 2.0). In both preparations ring-labelled NA was a poorer substrate of uptake than was side chain-labelled NA.

In "metabolizing systems" (i.e., in systems in which the substrate is transported by an uptake mechanism to the intracellular enzyme), the steady-state tissue/medium (T/M) ratio decreases with decreasing k_{uptake} (= Vmax/Km for uptake) and increases with decreasing k_{enzyme} (= Vmax/Km for enzyme).

On incubation of the rat vas deferens with 0.1 µmol/l ^{3}H-NA for 60 min, the T/M ratio was 0.65 ml/g for ^{3}H-7-NA and 3.63 ml/g for ^{3}H-7,8-NA ($P < 0.01$). Hence, in the "neuronal deaminating system" NA labelled in position 8 is a very poor substrate of monoamine oxidase (MAO).

In the extraneuronal O-methylating system of the rat heart (MAO and neuronal uptake inhibited) exposed to 0.01 µmol/l ^{3}H-NA the steady-state T/M ratio was 2.34 ml/g for ^{3}H-7,8-NA, but it was higher for ^{3}H-2,5,6-NA (3.02 ml/g; $P < 0.01$). Hence, side chain-labelled NA is preferred not only by extraneuronal uptake (see above) but also by extraneuronal catechol-O-methyl transferase (COMT).

In analogous experiments with the extraneuronal deaminating system (COMT and neuronal uptake inhibited) the steady-state T/M ratio was 4.22 for ^{3}H-7-NA and 5.96 ml/g for ^{3}H-2,5,6-NA ($P < 0.05$). Thus, if there is virtually no tritium in position 8, also extraneuronal MAO prefers the side chain-labelled to the ring-labelled NA.

It is concluded that most isotope effects are small (except that tritium in position 8 greatly hinders deamination by MAO) and that NA labelled in position 7 is clearly superior to ring-labelled NA.
Supported by the Deutsche Forschungsgemeinschaft.
Pharmakologisches Institut, VersbacherStr.9,8700 Würzburg

292

THE TEMPORAL RELATIONSHIP BETWEEN VAGUS AND SYMPATHETIC STIMULATION FOR THE MUSCARINIC INHIBITION OF NORADRENALINE RELEASE

E. Muscholl and A. Muth

Rabbit atria with the extrinsic right sympathetic and vagus innervation intact were isolated and perfused with Tyrode solution at 3.6 ml/min and 36° C. The noradrenaline (NA) stores were labelled with ^{3}H-NA and the acetylcholine (ACh) stores with ^{14}C-choline. The radioactive compounds were separated on columns and determined by scintillation spectrometry. The stimulation-evoked overflow of ^{3}H-NA and ^{14}C-ACh was calcium-dependent and abolished by tetrodotoxin; the inhibition of evoked ^{3}H-NA release by vagus stimulation was blocked by muscarinic antagonists (see Muscholl and Muth, this journal 320: 160 - 169, 1982).
Extrinsic stimulation of the vagus (VNS) and sympathetic nerves (SNS) was carried out by pulses of 1 ms, maximal strength (6 - 10 mA) and a frequency of 3 Hz for 3 min. When both nerves were stimulated simultaneously, the individual VNS pulses always preceded the SNS pulses; pulse intervals of 0.3, 1.0, 3.0, 10, 30 and 100 ms were studied. The ^{3}H-NA overflow evoked by SNS was maximally inhibited when the VNS pulses were applied 3 ms before the SNS pulses. A significant inhibition ($P < 0.01$) was observed also when the SNS pulses were delayed 10 ms compared with VNS. However, at all other intervals the ^{3}H-NA overflow did not differ from that of the controls carried out in the absence of VNS. The inhibition of evoked ^{3}H-NA overflow by VNS at a 3 ms interval was correlated with the evoked overflow of ^{14}C-ACh ($r = 0.723$; $n = 10$; $P < 0.05$), indicating that the latter is a valid measure of ACh liberated onto adrenergic terminal fibres.
The present results strengthen the idea that the cholinergic-adrenergic interaction in the atrium occurs at discrete sites, possibly axo-axonic synapses.

Pharmakologisches Institut der Universität Mainz
Obere Zahlbacher Str. 67, D-6500 Mainz

293

THE EFFECT OF PHENOXYBENZAMINE (POB) ON THE INHIBITION BY METHACHOLINE (MCH) OF THE ^{3}H-NORADRENALINE (NA) RELEASE EVOKED BY NERVE STIMULATION. H. Fuder and P. Fuchs

The occurrence of a receptor reserve in the activation of presynaptic receptor mechanisms has not been investigated previously. The aim of the present study was to estimate the dissociation constant Ka of a muscarinic agonist by determining the maximum inhibition of ^{3}H-NA release and the EC50 before and after partial irreversible blockade of presynaptic receptors. Isolated rat hearts with the right sympathetic nerves attached were perfused with Tyrode's solution (35°C) containing cocaine 10, corticosterone 10, and propranolol 0.1 µM. After loading the heart with ^{3}H(-)-NA, the nerves were stimulated with trains of 10 pulses (0.1 Hz, 1 ms, SNS). ^{3}H-NA was determined in the overflow after separation from the ^{3}H-metabolites.
Exposure of the heart to POB 5 µM for 15 min (beginning 60 min before SNS 1, washout of 45 min) neither affected the basal (0.036 ± 0.003, $\bar{x}$ ± SEM, no POB, and 0.032 ± 0.002 pmol/min after POB) nor the stimulation-evoked ^{3}H-NA overflow (0.107 ± 0.017 vs. 0.101 ± 0.021 pmol at SNS 1, each n = 17). The decline of the evoked release in the course of the train series in the group with POB exposure was not different from controls. MCH suppressed the ^{3}H-NA release by 96 ± 2 % (1 µM) concentration-dependently with an EC50 of 0.072 µM (0.05 - 0.1, 95 % confidence limit, n = 6). After POB exposure MCH decreased the release maximally by 51 ± 6 % and its EC50 was 2.9 µM (1.0 - 7.8, n = 9). A double reciprocal plot of equieffective concentrations from unexposed vs. exposed tissue revealed that 98 % of the receptors were eliminated. Ka of MCH calculated from the intercept was 3.6 µM.
It is concluded that the EC50 of a muscarinic agonist with high intrinsic activity does not reflect the true dissociation constant at the presynaptic receptor. Thus, no linear relation exists between receptor occupancy and the response under the present release conditions.

Pharmakologisches Institut der Universität Mainz
Obere Zahlbacher Str. 67, D - 6500 Mainz

294

COMPARATIVE EFFECTS OF α_1 AND α_2 ADRENOCEPTOR BLOCKERS ON CATECHOLAMINE OVERFLOW AND CARDIAC RESPONSES IN SYMPATHETICALLY STIMULATED RABBIT HEARTS.

E.F. Smith III, R. Schaffran, M. Kluth

Studies were performed to compare the α adrenergic antagonists phentolamine (PHA), yohimbine (YOH) and prazosin (PRA) on noradrenaline (NA) overflow and cardiac responses in sympathetically stimulated Langendorff perfused rabbit hearts. The right sympathetic nerve was stimulated at 10 V 10 Hz for 0.5 min at time 0 (S_1) and 13 min (S_2).

PHA concentration dependently (0.3to 3 µM) increased NA overflow (S_2/S_1) ($P < 0.01$) at concentrations greater than 0.3 µM compared to control hearts (n = 15) where the NA overflow was 0.75 ± 0.04. At 1 and 3 µM PHA, NA overflow was increased 165%(n = 4, $P < 0.01$) and 65%(n = 4, $P < 0.001$), respectively. YOH at 0.03 and 0.3 µM increased NA overflow by 65 (n = 5, $P < 0.01$) and 85 percent (n = 9, $P < 0.001$), respectively, whereas PRA (0.001 and 1 µM) only increased NA overflow between 35 and 40 percent. Left ventricular pressure, dp/dt and heart rate were significantly increased ($P < 0.05$) by 3 µM PHA while 1 µM YOH only significantly increased left ventricular pressure and dp/dt.
These data indicate that cardiac α_2 blockade by phentolamine or yohimbine results in a significant increase in NA overflow and myocardial responses by interrupting a negative feedback mechanism. Comparatively, 0.001 and 0.1 µM prazosin resulted in smaller increases in NA overflow (approximately 35 percent) and changes in myocardial responses (5 to 10 percent). It is concluded that presynaptic receptors with pharmacological α_2 properties have a significant role in regulating cardiac responses but that the presence of functional presynaptic α_1 receptors cannot be excluded.

Pharmakologisches Institut der Universität Köln, Gleueler Str. 24, D-5000 Köln 41

295

EFFECT OF ENDOGENOUS ADRENALINE ON NORADRENALINE RELEASE IN THE ANAESTHETIZED RABBIT

H. Schmidt, L. Hedler and H. Majewski

Exogenous adrenaline facilitates noradrenaline release from sympathetic nerves in vivo by activating presynaptic ß-adrenoceptors (Majewski et al., Naunyn-Schmiedeberg's Arch Pharmacol 321: 20-27, 1982). The present study, in the pentobarbitone-anaesthetized rabbit, was to determine whether adrenaline released from the adrenal medulla can be accumulated in, and released from, sympathetic nerve terminals in sufficient amounts to activate presynaptic ß-adrenoceptors and therefore increase noradrenaline release. The adrenaline content of sympathetically innervated tissues (atria, ventricle) was significantly increased after stimulation of the left splanchnic nerve (5 Hz, 5 min). Plasma adrenaline had returned to basal levels 30 min after stimulation whilst at this time the noradrenaline release rate was significantly increased to 149 % of control. When rabbits were pretreated with propranolol HCl (2 mg/kg, i.p.) to block ß-adrenoceptors or desipramine HCl (1 mg/kg, i.v.) to block neuronal uptake of adrenaline, the effect of splanchnic nerve stimulation on the noradrenaline release rate was abolished. These findings suggest that adrenaline released from the adrenal medulla can be accumulated in sympathetic nerve terminals and that its subsequent release from the nerves enhances noradrenaline release through activation of presynaptic ß-adrenoceptors.

Pharmakologisches Institut der Universität, Hermann-Herder-Strasse 5, D-7800 Freiburg i.Br.

296

INHIBITION BY OPIATES OF NORADRENALINE RELEASE IN THE RABBIT EAR ARTERY

N. Pfeiffer, P. Illes and K. Starke

The opioid peptides Met- and Leu-enkephalin reduce vasoconstrictor responses of the rabbit ear artery to sympathetic nerve stimulation (J. Knoll, Eur J Pharmacol 39:403, 1976). In order to find out whether this is due to an inhibition of transmitter release, ear arteries were preincubated with ^{3}H-noradrenaline 2.2 µM, and both the increase in perfusion pressure and the overflow of tritium elicited by field stimulation (1 Hz, 200 mA, 0.3 ms pulse duration) were measured. Subtype-selective opiates were used, namely the µ-receptor agonist morphine, the delta-receptor agonists Leu-enkephalin and D-Ala^2-D-Leu^5-enkephalin, and the kappa-receptor agonist ethylketazocine. The delta- and kappa-agonists depressed both the stimulation-evoked overflow of tritium and the vasoconstrictor responses, the maximal inhibition being about 40 %. The IC_{50} values of D-Ala^2-D-Leu^5-enkephalin, Leu-enkephalin and ethylketazocine for depression of tritium overflow (2.2, 20 and 140 nM) were similar to the IC_{50} values for depression of the mechanical response (3.5, 12 and 280 nM, respectively). Naloxone 3 µM prevented the effects of Leu-enkephalin 1 µM and ethylketazocine 1 µM. Morphine 10 and 100 µM exerted no inhibitory effect. In conclusion, the sympathetic terminal axons of the rabbit ear artery seem to possess delta- and/or kappa-receptors. The enkephalins and ethylketazocine probably reduce the vasoconstrictor response solely by inhibition of the release of noradrenaline.

Pharmakologisches Institut der Universität, Hermann-Herder-Strasse 5, D-7800 Freiburg i.Br.

297

Rhythmic activity to trunk muscles during spinal locomotion

E. D. Schomburg and H. Steffens

Pharmacologically induced fictive spinal locomotion resembles several characteristics of normal locomotor patterns and has therefore been used as a model for the investigation of phase dependent reflex transmission (Forssberg et al.,Brain Res. 132:121, 1977; Schomburg et al.,Neurosci. Lett. 4:249,1977). Since trunk muscles show a coordinated rhythmic activity in the normal walking cat (Carlson et al., Acta physiol. scand. 105:251, 1979) it was of interest to check if a similar participation of trunk muscles in locomotor rhythmic activity takes place during fictive spinal locomotion.
In anaemically decapitated high spinal paralyzed cats neurograms were taken bilaterally from nerves to the longissimus dorsi, the obliquus abdominis externus and various hindlimb muscles. Fictive spinal locomotion was induced by injection of nialamide and L-DOPA (Viala and Buser, Brain Res. 35:151, 1971).
During stable efferent rhythmic activity to the limb muscles the efferents of trunk muscles took part in such a way that the back and abdominal muscles on one side were synchronously active in phase with the flexor muscles of the ipsilateral hindlimb. But, a bilateral synchronous activity of the back muscles in phase with the limb extensor muscles was also observed. This is in agreement with patterns occurring during normal walking or galloping.
The results indicate, that the activity of trunk and limb muscles can be coordinated within the spinal cord to a meaningful pattern without need for peripheral sensory feedback.

Supported by the Deutsche Forschungsgemeinschaft, SFB 33

Physiologisches Institut der Universität Göttingen, Humboldtallee 7, 3400 Göttingen

298

RESPONSE CHARACTERISTICS OF RENSHAW CELLS ACTIVATED BY A SMALL NUMBER OF MOTOR AXONS

D. Bergmann, S. Cleveland

In decerebrate, deafferented cats the time course of Renshaw cell responses to step changes in input were determined by stimulating only a few α-motor axons. To this end, the ventral root was divided until the Renshaw cell responded reliably to antidromic shocks (0.5-1 Hz) with either 1 or 2 spikes. Thereafter, this portion of the ventral root was stimulated at frequencies between 0.2 and 100 Hz for periods of 5 s or more. Above about 2 to 5 Hz the response probability begins to drop. At frequencies of 5 Hz and above there is a recognizable initial overshoot in response probability. These step responses are characterized by a fast and a slow component of adaptation. The time constant of the fast component decreases with increasing stimulus frequency.
The latency histogram for a given stimulus frequency is asymmetrical; it can be quite narrow or broader. Nevertheless, with increasing stimulus frequency the median latency increases, as does the spread of values, although both tend toward a maximum at higher frequencies.
The static discharge rate of a cell can be described by a rectangular hyperbola that approaches saturation with increasing stimulus frequency.
The present results demonstrate a qualitative similarity of the response properties of Renshaw cells obtained using only a small number of axons with those found with synchronous stimulation of many motor axons.

Physiol. Inst. (Lehrstuhl II), Universität Düsseldord, D-4000 Düsseldorf, Moorenstr. 5

299

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF SUBSTANTIA GELATINOSA (SG) NEURONS IN AN IN VITRO PREPARATION OF THE DORSAL HORN OF THE SPINAL CORD OF ADULT RATS

W. Zieglgänsberger and B. Sutor

Neurons of the SG have been implicated in most sensory mechanisms in the spinal cord as a pool of predominantly inhibitory interneurons. The small size of their cell bodies have precluded a more detailed electrophysiological analysis in vivo. In the present study, extra- and intracellular recordings of SG neurons were obtained from adult spinal cord slices. The traverse slices were placed in oxygenated CSF kept at 35°C. Dorsal and ventral roots were preserved and were stimulated with bipolar electrodes. About half of the SG neurons encountered were spontaneously active. They responded to large and small fiber input activated through dorsal and ventral roots. Some of the spontaneously firing neurons displayed "wind-up": Repetitive stimulation with low intensity stimuli which did not cause a direct response led after a certain delay to an increased activity which outlasted the stimulation for up to 10 min. Stimulation of the dorsal (and in several cells also of the ventral root) with high intensity stimuli inhibited this activity. Bath applied bicuculline inhibited this effect. Most of the non-spontaneously active neurons responded with a short burst of action potentials (on a polysynaptic EPSP) to low intensity stimulation. Increase in stimulation increased their firing covariantly. Some SG neurons became spontaneously active following high intensity stimulation. The present study shows that SG neurons in vitro display similar properties as those recorded in vivo and that stable recordings can be obtained also from adult spinal cord slice preparations.

Max-Planck-Institut für Psychiatrie, Abteilung Neuropharmakologie, Kraepelinstraße 2, D-8000 München 40, F.R.G.

300

LONG LATENCY REFLEX MECHANISMS OF HUMAN FINGER MUSCLES

H.-H. Friedemann, H.R. Matthews, J. Noth

To investigate the gain of the stretch reflex of human finger muscles sinusoidal movements were imposed to the index finger according to the method introduced by Rack (In: Handbook of Physiology, The Nervous System II, 229, 1981). A constant voluntary force (20% of maximal voluntary force) was exerted with the fingertip pressing against a sinusoidally moving lever and the resulting force was measured at various frequencies between 3 and 16 Hz. The gain and phase of the sinusoidal modulated force were plotted for each frequency in a vector diagram.

20 adult subjects were investigated. The individual vector plots indicated a reflex pathway longer than the known spinal transmission. The resonance frequency of this reflex loop was 3.5 - 4.0 Hz and thus half of the resonance frequency of the spinal reflex. Patients with Friedreich's ataxia, a degenerative disease of the central and peripheral nervous system, showed no resonance frequency, which proves that the resonance phenomenon of normal subjects is of reflex origin and not due to musculo-mechanical oscillations. Although a long latency spinal reflex route cannot be ruled out, our results are in agreement with the long loop hypothesis according to which a transcortical reflex loop plays a functional role in the control of distal arm muscles.

Supported by the Deutsche Forschungsgemeinschaft, SFB 200

Neurologische Klinik der Universität Düsseldorf, Moorenstraße 5, D-4000 Düsseldorf

301

FEEDBACK EFFECTS OF UNFUSED CONTRACTIONS OF MOTOR UNITS ONTO HOMONYMOUS ALPHA MOTONEURONES OF THE CAT.

W. Koehler, R. Enoka, T. Hamm, D.G. Stuart, U. Windhorst

It is well documented that contractions of single motor units (MUs) can modulate the discharge patterns of muscle receptors. Also, the postsynaptic effects of discharges of single muscle afferents on homonymous α-motoneurones (MNs) have been studied extensively. However, these studies have been confined to small samples of afferents only. Hence, the compound effects on central neurones could not easily be predicted. We have studied the feedback effects from MUs of the cat medial gastrocnemius (MG) muscle onto homonymous MNs.
In anaesthetized cats, MG MNs were impaled to record membrane potential fluctuations in response to combined stimulation of three MG MUs via their axons. Stimuli patterns were uncorrelated and random at physiological mean rates (ca. 9-11 pps). The membrane potential fluctuations in conjunction with muscle tension changes (at isometric length) were averaged with respect to the stimuli.
The comparison of evoked MN PSPs with average MU twitches revealed that the effects on single MNs of contractions of different MUs varied quantitatively and qualitatively. The amplitudes of evoked PSPs lay between zero and about 300 µV. Mostly, these PSPs consisted of a hyperpolarization during tension rise of the respective MU. In some cases, the hyperpolarization was preceded and/or followed by depolarizing phases, the first being brief and the second being of prolonged duration stretching over the period of MU relaxation. The first depolarization could be due to "early" discharges of spindle afferents, the second to "relaxation" discharges. The hyperpolarization may be caused by increased Golgi tendon organ discharge and decreased spindle discharge. Linear and nonlinear summation of the effects from several MUs will also be reported.

Dept. of Physiology, University of Arizona, Tucson/Arizona 85724, U.S.A.; and Zentrum Physiologie und Pathophysiologie der Univ. Göttingen, Humboldtallee 7, 3400 Göttingen, FRG

302

CAN THE LANGUAGE DOMINANT HEMISPHERE BE RECOGNIZED DURING LANGUAGE PROCESSING BY SLOW BRAIN POTENTIALS IN MAN?

R. Jung and A. Hufschmidt

Brain potential shifts during writing show unilateral negativity over the language dominant hemisphere.These are also influenced by the contralateral writing hand/1/.Slow potentials during speech are complicated by large respiration artifacts/2/. Both tests need exclusion of additional complications and are made by backward averaging.
Therefore, various language tasks were used without speaking and with forward averaging: During completion or construction of sentences as well es during translation, memorizing and association of words preceding the writing act, we measured the averaged slow brain potential fields over both hemispheres and midline.
When language processing precedes script performance, their EEG-accompaniments can be distinguished and the hand effect may be excluded. In righthanders negative potentials during these language tasks are larger over the left cerebral hemisphere than over the right. Verbal evokation of figure drawings also shows larger negativities over left precentral and parietal regions when compared with mere visual copying of figures. Evidence of hemispheric predominance is presented in righthanders and in two groups of lefthanders, writing with the right and left hand respectively.

1/Jung R,Hufschmidt A,Moschallski W: Arch Psychiatr Nervenkr 232 (im Druck,1983)
2/Grözinger B,Kornhuber HH,Kriebel J,Szirtes J, Westphal KTP:Progr Brain Res 54,798-804(1980)

Abt. Neurophysiologie, SFB 70, Universität Freiburg, Hansastr. 9, D-7800 Freiburg i.Br.

303

CAN CHRONICAL LESIONS IMITATE AGEING PROCESSES IN MOTOR PERFORMANCE?

B. Jänicke and G. Schulze

Reduced oxygen consumption as well as a diminished cerebral metabolism induce a loss of CNS controlled functions in old organisms as e. g. deficits in motor coordination. It was examined to what extent the consequences of a surgically induced chronical lesion in an adult animal might imitate ageing processes during life span. We performed two surgical lesions in adult rats: 1. ligature of both carotid arteries, 2. portacaval anastomosis. These animals (4-6 weeks after surgery) as well as old ones were tested in the following procedures: 1. open field, 2. tilting plane, 3. climbing on a vertical ladder, 4. rotarod test. Adult (6 months old) male Sprague Dawley rats were used. A group of carotid ligated rats and a group with portacaval anastomosis were compared to sham operated controls and to a group of senile rats (30 months old). In a previous study it has been shown that with this test battery a differentiated loss of performance of old animals can be disclosed. Our results reveal that both chronical lesions do not influence the performance of rats in the chosen test procedures. In open field test only a reduced locomotion was obvious in old rats. In the tilting plane test all adult rats lost their balance at 51^{o}, whereas old animals failed at 44^{o}. Also in the climbing test as well as in the rotarod test a difference was only seen between adult and aged rats. It is obvious that ageing processes or their consequences on motor behavior cannot be studied sufficiently in adult animals with lesions as described.

Institut für Neuropsychopharmakologie der Freien Universität Berlin, Ulmenallee 30, D-1000 Berlin 19

304

SLEEP-PATTERN RECOGNITION BY CLUSTER ANALYSIS

P.Grass

The aim of this study is the development of an automatic on-line sleep stage classificator.Pattern recognition with the aid of cluster analysis has proved to be a suitable method for the simultaneous analysis of interactive parameters for describing sleep. Input variables of our algorithm are the spectral power of the EEG, quantity and duration of sleep spindles, quantity of REM, EMG-power, heart and respiration period. These features are extracted from the data stream by hardware pre-processors, they are cleaned from artefacts and normelized by Z-transformation. For the definition of sleep patterns the data of one night are processed off-line in a divisive, hierarchic cluster analysis comparable to the adaptation of the system to an individual subject. The control of the classification is performed by changing features and weighting functions on various levels. On the lowest level the most weighted feature is the delta-band, the classes being arranged under the aspect of growing delta activity.On higher levels nonlinear functions are used to adapt the classes according to alpha and sigma activity.Once centre and standard deviation of each cluster are established for one subject under normal conditions, the data from following nights can be classified on-line by computing the smallest Euclidian distance between the measurement vector and the cluster centres. Moreover, the vector is examined to be within a confidence-interval around the cluster centre. Results of this sleep analysis show the peculiarities of individual sleep with high resolution.Time resolution is limited only by filters and memory size, typical epoche length for a whole night recording being 10 to 30 seconds. In a normal course of sleep the analysis is confined to 12 sleep patterns. If unexpected patterns appear, a new class is defined automatically.For comparison with the coarse conventional classification our analysis can be properly condensed.

Institut of Physiology, Universität Marburg, Deutschhausstr.2, D-3550 Marburg

305

EARLY INSPIRATORY INHIBITION IN BULBAR EXPIRATORY NEURONES

D. Ballantyne and D. W. Richter

In anaesthetised, paralysed and artificially ventilated cats recordings from caudal expiratory neurones reveal an augmenting depolarisation during expiration and a rapid onset of declining hyperpolarisation during inspiration. This hyperpolarisation is associated with a declining intensity of synaptic noise, which is minimal during late-inspiration and post-inspiration but which increases again during the subsequent expiratory depolarisation. Chloride injection into cells initially converts this declining hyperpolarisation to an augmenting pattern of hyperpolarisation and finally to a declining pattern of depolarisation. This suggests a prominent i.p.s.p. input to expiratory neurones in early-inspiration but it does not reveal whether the associated gradual reduction in synaptic noise is due to cessation of synaptic inputs or to the attainment of the i.p.s.p. reversal potential.
A composite i.p.s.p. was evoked in the cell and used as a test i.p.s.p. This i.p.s.p. reversed polarity in early-inspiration. Subsequently, and correlated with the period in which spontaneous synaptic noise was minimal, the test i.p.s.p. was entirely suppressed but reappeared during the expiratory phase, again correlated with the reappearance of spontaneous p.s.p.s.
This result suggests that the diminution of synaptic noise is not due to coincidence of membrane potential with i.p.s.p. reversal potential but due to antecedent "gating out" of this input. This interpretation is consistent with the pattern of changes in input resistance which is smallest in early-inspiration and largest during late-inspiration. We suggest that the rhythmic inhibition of expiratory neurones is confined to early-inspiration and that this inhibition is followed by a significantly long-period in which the neurones are inaccessible to all synaptic inputs.

Supported by the Deutsche Forschungsgemeinschaft
I. Physiol. Inst. Univ. D-6900 Heidelberg, INF 326

306

SARCOMERE LENGTH DETERMINATION USING LIGHT DIFFRACTION IS LESS ACCURATE THAN OFTEN ASSUMED.

B. Fleischmann, R. Rüdel, and F. Zite-Ferenczy

Meassurement of the angle of 1st order light diffraction is commonly used for the determination of sarcomere length (SL) of striated muscle. Some authors (cf. Iwazumi and Pollack, IEEE Transactions BME 26, 86, 1979) believe that the limiting factor in the resolution of SL signals can be pushed to about 2 nm (= 0.1%) by using sophisticated electronics for angle measurement. We have already stressed that left and right 1st orders of light diffraction may be different in such important aspects as intensity at rest (J. Physiol. 272, 31P, 1977) and diffraction angle during active shortening (Nature 278, 573, 1979). We now report that in single frog muscle fibres at rest the diffraction angles of the two 1st orders (positions of the maximum intensities) may differ by up to 3%. During sinusoidal passive length changes (0.1 < f < 10 Hz; 2.3 < SL < 2.9 µm) the intensity distribution within each 1st order line displays complicated changes which seem uncorrelated but which are reproducible and independent of frequency. When light itensity is integrated using position-sensitive diodes the angle changes during passive length changes display uncorrelated steps (Pollack et al., Nature 268, 757, 1977). It is unlikely that these steps are caused by synchronized force generators. Rather, Bragg angle effects (J. Physiol. 290, 317, 1979) seem to limit resolution and interpretation of diffraction experiments.

Abteilung für Allgemeine Physiologie, Universität Ulm, Oberer Eselsberg, D-7900 Ulm, West Germany

307

INFLUENCE OF VIBRATION ON CONTRACTILE FORCE OF ISOLATED GUINEA PIG CARDIAC TISSUE.

H. Lange-Asschenfeldt

Left guinea pig auricles or atrial strips were mounted horizontally in an organ bath between a strain gauge and a hook fixed on the membrane of an electrodynamic vibrator. The preparations were bathed in normal Tyrode solution continuously gassed by carbogen (95% O_2, 5% CO_2). A preload of 5 mN was applied and the muscles were stimulated by rectangular pulses at frequencies between 0.2 to 3 Hz. When an equilibrium state in contractile force was attained the muscles were vibrated at 200 Hz for periods up to 20 min (changes of length less than 100 µm at the vibrator hook). The mechanical irritation caused a sudden decrease in contractile force by almost 50% which was more or less maintained even when the vibration period was prolonged for more than 10 min. After ending the vibration a restitution of the force to pre-vibration values occurred without delay. This response pattern was found altered when the oxygen pressure in the bath was considerably lowered. Thus, gassing with a mixture of 15% O_2, 80% N_2, and 5% CO_2 reduced the contractile force by 80 to 90%. Upon vibration the force was initially further depressed as under normoxic (control) conditions. It was then, however, followed by an increase which at least compensated the vibration-induced depression. The rate of this increase depended upon the beat frequency. The increase was accomplished within 2 to 3 min at 3 Hz and within about 15 min at 0.2 Hz. Under hypoxic conditions the stop of vibration caused a sudden increase in force to a level much higher than before starting the vibration. Pre-vibration amplitudes were reached again in the course of about 15 min. The adaptation of the contractile force to vibration in the presence of hexobarbital and D 600 producing negative inotropic responses similar to hypoxia did not differ from the control pattern. As a possible mechanism a change in oxygen utilisation is discussed.

Institut für Pharmakologie, Univ. Kiel, Hospitalstr. 4-6, D-2300 Kiel

308

Ca^{2+}-ACTIVATION OF SMOOTH MUSCLE CONTRACTION: NONCORRELATION OF ATPase ACTIVITY, TENSION DEVELOPMENT AND MYOSIN PHOSPHORYLATION

U. Mrwa, K. Güth and M. Gagelmann

Although contradictory results have been reported, a Ca^{2+} and calmodulin-dependent phosphorylation of the regulatory light chain of myosin is thought to be the critical event in Ca^{2+}-activation of smooth muscle. We present evidence that the Mg^{2+}-dependent actin activated ATPase activity of highly purified chicken gizzard myosin which is phosphorylated up to 75 % is low (2 - 5 nm Pi/mg/min). It can be activated by an as yet unidentified protein factor up to 70 nm Pi/mg/min at 10^{-5} M Ca^{2+}. Calmodulin and Tropomyosin enhance this effect. A similar activation occurs when the degree of phosphorylation is as low as 25 %. In chemically skinned chicken gizzard fibres maximal activation of isometric tension development and shortening velocity is found at 10^{-5} M Ca^{2+}, under these conditions 50 % of the myosin is phosphorylated. Interestingly, the same activation can be achieved at 7×10^{-8} M Ca^{2+} in presence of 3 µM calmodulin and at a Ca^{2+} 10^{-8} M with 50 µM calmodulin. In both cases, the phosphorylation does not exceed 20 % of the available sites. We conclude that phosphorylation alone is not sufficient to activate smooth muscle.

Supported by the DFG

II. Physiologisches Institut der Universität
Im Neuenheimer Feld 326, D-6900 Heidelberg

309

EVIDENCE FOR DIFFERENT Ca^{2+} REQUIREMENT OF MYOSIN PHOSPHORYLATION AND ACTIN-ACTIVATED ATPase ACTIVITY IN CHICKEN GIZZARD SMOOTH MUSCLE

L. Merkel, K.D. Meisheri, G. Pfitzer and J.C. Rüegg

As myosin phosphorylation may be a prerequisite for ATPase activation, it is important to establish the quantitative relationship between these two processes. In view of previous controverses, we reexamined this relationship by measuring the Ca^{2+} dependences of myosin phosphorylation and ATPase activity over the physiological Ca^{2+}-concentration range of 0.01 μM to 10 μM under identical experimental conditions. We found a significant ($p \leq 0.05$) difference in the Ca^{2+}-requirement for half maximum activation:

	Free Ca^{2+} for:		
activation of	threshold	halfmaximal	maximal
myosin phosphorylation	$1,35 \times 10^{-7}$M	$2,25 \pm 0.18 \times 10^{-7}$	8×10^{-7}M
ATPase activity	4.6×10^{-7}M	$6.6 \pm 0.6 \times 10^{-7}$M	9×10^{-6}M

The Ca sensitivity (EC 50) of the phosphorylation is higher than that for ATPase activity. No significant ATPase activation could be obtained up to about 60 % phosphorylation. At intermediate $[Ca^{2+}]$, phosphorylation and ATPase activity are non-linearly related. A further increase in Ca^{2+} induces further activation without change in phosphorylation which raises the question whether, in addition to phosphorylation, other calcium-dependent activating mechanisms may be involved.

II. Physiologisches Institut der Universität Im Neuenheimer Feld 326, D-6900 Heidelberg

310

The Effect of Ionophore A 23 187 on the Spontaneous Mechanical and Electrical Activity of the Isolated Portal Vein of the Guinea-Pig.

K. Kölling, M. Ernst, H. Schwarz, M. Michailow, L. Schachinger, J. Riemer

The effect of ionophore (A 23 187) on the spontaneous activity of the isolated portal vein was studied using isometric tension devices and intracellular recording techniques. Adding 10^{-8} mol/l of the substance to normal Krebs solution leads to an increase of the amplitude and frequency of the phasic contractions of the vessel. Higher concentrations induce the muscle to contract more frequently but less strongly. 5×10^{-6} mol/l of the ionophore causes a well-sustained tonic tension with superimposed small phasic contractions of high frequency. The drug-induced changes of the mechanical activity are accompanied by a dose-dependent depolarization of the membrane and a shortening of the bursts and the spike-intervals. In the presence of high concentrations of the drug the electrical activity tends to regular spiking which is not modulated by bursts. The shape of the spikes is minimally changed by the drug. In calcium-free solutions the ionophore does not affect the maximal relaxation of the vessel. Similarly, the ionophore is not able to change the relaxing effect of high doses of the calcium-antagonistic drug nifedipine. It is concluded that the ionophore potentiates the contractions of the vessel by depolarizing the cell membrane and improving the spike activity.

Department of Physiology, University of Munich Pettenkoferstraße 12, D-8000 Munich 2, Germany

311

SPONTANEOUS TONE OF GASTRIC SMOOTH MUSCLE (FUNDUS STRIPS FROM CATS AND DOGS) AND ITS DEPENDENCE ON ENDOGENOUS PROSTAGLANDIN SYNTHESIS

K.Boev[x], A.Bonev[x] and K.Golenhofen

Fundic muscle strips usually develop a strong spontaneous myogenic tone which was nearly completely suppressed by blockade of the endogenous prostaglandin synthesis with indomethacin in canine preparations (K.Milenov and K.Golenhofen, Prost. Leuk. and Med. 8, 287-300, 1982). Longitudinal (outer layer) and circular muscle strips (inner layer) were dissected from the upper fundus region, and the mechanical activity was recorded under isometric or near-isotonic conditions. Indomethacin (IND) and 5,8,11,14-eicosatetraynoic acid (ETA) both suppressed the spontaneous activity, and they shifted the dose-response-curve for the excitatory effect of arachidonic acid (10^{-7} to 10^{-4} mol/l) to the right. At a concentration of 10^{-6} mol/l the inhibitory effect of IND was near maximal, and an increase of the concentration to 10^{-5} mol/l produced only little further suppression. The activity which persisted at this concentration (determined by application of papaverine 10^{-3} mol/l or nitroprusside sodium, NP, 10^{-5} mol/l) can therefore be called "IND-resistant component". This component was small in 19 strips from 2 dogs (around 10% of the control value). In 10 strips from one other dog, however, this component was large: 10-30% in the longitudinal and 25-50% in the circular strips. In strips from 10 cats the IND-resistant component was altogether larger. It was between 20 and 50% in most circular strips, and between 0 and 20% in the longitudinal strips. The effects were essentially the same during tetrodotoxin or nifedipine treatment. ETA was about 10-times less potent than IND. The IND(10^{-5}mol/l)-resistant and the ETA(10^{-4} mol/l)-resistant components were of similar size when compared in one and the same strip. It can be concluded that in gastric fundus muscle another mechanism - in addition to the endogenous prostaglandin synthesis - can contribute to the spontaneous tone, particularly in circular strips, and that individual and species differences exist.
(Supported by the Deutsche Forschungsgemeinschaft).

Department of Physiology, University of Marburg, Deutschhausstr. 2, D-3550 Marburg/Lahn, FRG

[x]Institute of Physiology, Bulgarian Academy of Sciences, Acad. G.Bonchev Str., Bl. 23, 1113 Sofia, Bulgaria

312

FAST AND SLOW COMPONENTS IN TENSION INCREASE OF RAT TRACHEAL MUSCLE

U.Peiper, R.N.Speden, E.Donker and C.F.Vahl

After onset of stimulation, smooth muscle preparations show a biexponential increase in tension with an initial fast and a subsequent slow component which has been related to the release of intracellularly stored calcium and to the increased transmembraneous calcium flux, respectively (Bohr, 1963). The post-vibration tension recovery of the isolated rat tracheal muscle is also biexponential (time constant τ_1 for the fast component 0.92 ± 0.05 sec ($n = 50$), τ_2 for the slow component 6.79 ± 0.29 sec. Both time constants depend on the duration of the stimulation period prior to vibration (electrical field stimulation square wave 30 Hz, 0.3 msec). τ_2 which reflects the kinetics of actin-myosin interaction, is at a minimum at about 32 sec after the start of stimulation and becomes greater at shorter or longer pre-vibration periods. As the myosin light chain kinase activity is likewise time-dependent (Aksoy, M.O., Amer.J.Physiol. 242, C 109-C 116 (1982)), the observed time course of τ_2 may be explained by respective changes in the kinetics of cross-bridge cycling due to the activity of the protein kinase; the kinetics become faster after onset of stimulation, reach maximum values at about 30 sec and become slower during sustained activation. In contrast to this time course, the developed tension continued to rise to steady-state maximum values.

Universitäts-Krankenhaus Eppendorf, Physiologisches Institut, Martinistrasse 52, D-2000 Hamburg 20

313

EFFECTS OF HYDROPEROXIDES ON VASCULAR SMOOTH MUSCLE CONTRACTION.

H. Heinle

The formation of hydroperoxides (HP) in living cells can be considered a general attribute of aerobic life, and increased levels in tissues and blood were found under various pathological conditions (Chance et al., Physiol.Rev. 59, 527, 1979). Although it was shown recently, that HP stimulate glycogen phosphorylase a activity in vascular smooth muscle (VSM) probably by an Ca^{2+}-dependent mechanism (Heinle, Biochem.Biophys.Res. Comm. 107, 597, 1982), little is known about the general effects of hydroperoxides on VSM. Therefore experiments were performed elucidating the effect of HP on the contractility of VSM.
Segments of rabbit carotid arteries were isometrically mounted and circumferential wall force was measured. Using oxygenated Tyrode solution, the arteries were superfused either with glucose (5 mM) and/or pyruvate (3 mM) or substrate free. Hydrogenperoxide and t-butylhydroperoxide were applied from 0.1 to 20 mM. The results show, that both hydroperoxides similarily induce reversible contractions. The sensitivity was highest in substrate-free solution and lowest with pyruvate as substrate. Addition of EGTA or verapamil (10^{-6} - 10^{-5} M) influences the contraction and shows the Ca^{2+}-dependency of this type of contraction. Although the underlying mechanisms remain to be elucidated, an important prerequisite for the induction of the HP-dependent contraction seems to be a limited ability of ATP-regeneration.

Physiologisches Institut(I) der Universität Tübingen, Gmelinstr. 5, D-7400 Tübingen.

314

EVALUATION OF $V'o_2$ KINETICS ON THE BASIS OF PSEUDO-RANDOM BINARY SEQUENCES OF WORK LOAD.

D.Eßfeld, J.Stegemann

The determination of oxygen-uptake kinetics may be used as a non-invasive and specific method to assess the state of endurance training of different muscle groups. In addition to a high resolution of $V'o_2$ measurements (breath-by-breath analysis), appropriate test signals are important prerequisites for the reliability of this method.
In this respect, single step changes of work load, which have been used most frequently as input signals, seem to be disadvantageous. With this type of signal, the number of $V'o_2$ readings during the transient period is restricted by the respiratory frequency. Thus, the evaluation of $V'o_2$ kinetics is susceptible to the large interbreath fluctuations of data that are inevitable in common breath-by-breath techniques. In addition, the power spectral density of the step signal shows a relatively small bandwidth and an accentuation of the low frequency range. Sinusoidal inputs, which are an appropriate alternative from a theoretical point of view, are rather time-consuming if a complete polar plot is to be obtained. Statistical signals of the PRBS type (pseudo-random binary sequence) offer a convenient compromise since they permit a 'tailoring' of both the duration of the test and the power spectral density of the input. Due to the essentially statistic nature of the evaluation, the PRBS technique is less susceptible to random fluctuations of breath-by-breath analyses.
We have evaluated the $V'o_2$ kinetics of 12 subjects on the basis of both single step changes of work load (20 W - 100 W - 20 W) and PRBS sequences (20 W to 100 W, 15 bits of 30 s duration per bit, 3 cycles). The comparison of results shows that the relation between $V'o_2$-on kinetics and PRBS evalutation are closer than the $V'o_2$-off to PRBS relation. Due to its lower degree of reliability, however, $V'o_2$-on kinetics should not be utilized to predict the results obtained from PRBS techniques.

Physiologisches Institut der Deutschen Sporthochschule Köln, Carl-Diem-Weg, D-5000 Cologne, FRG

315

EXERCISE HEMOCONCENTRATION AND OSMOLALITY

N. Maassen

Alterations of acid-base-balance are accompanied by changes in blood osmolality. In vitro, in oxygenated blood the following relationship was found: $\Delta Osm = -31.7 \Delta pH + 0$ (Böning , Maassen 83). During physical activity (cycle ergometer, increasing workload beginning with 50 watt, steps of 50 watt every 3 min; n=10) the in vivo relationship is $\Delta Osm = -136.6 \Delta pH + 1.9$. A pH decrease of 0.2 units results in an increase in osmolality of 29.2 mosmol/kg H_2O. Only 6.5 mosmol are due to acid-base-balance changes because of compensating the rise in lactic acid concentration by CO_2 delivery. The remaining increase of 22.7 mosmol/kg H_2O is caused to a large extend by a water shift into the working muscles due to osmotic effects which are related to the anaerobic metabolism ($\Delta Osm = \Delta 2.05 [Lac^-] + 2.7$). More than 50% of the exercise hemoconcentration can be explained by these osmotic effects.

Abt. Sport- und Arbeitsphysiologie, Zentrum Physiologie, Medizinische Hochschule Hannover, Konstanty-Gutschow-Str. 8, D-3000 Hannover

316

BIOMECHANICAL INVESTIGATIONS ON THE HUMAN FOOT SOLE UNDER ASPECTS OF OCCUPATIONAL MEDICINE

B. Kurz, W. Diebschlag

With respect to Debrunner (1972) in cause of the variety of forms of feet they will be classified as 'healthy' respectively 'without pathological findings', when functioning normally. After a mass examination on the feet of more than 1.000 young German soldiers we diagnosed only 0.2-0.4% as orthopedically unconspicuous and nearly 80% were in need of treatment. These results are approximately valid for the population. Foot troubles and foot deformities as well as unfitting or outworn shoes enhance the danger of accidents at the working place and may influence negatively the biomechanical course of action and the efficiency of men.

For the physiological and biomechanical assessment of the load capacity under the human foot sole the knowledge of its force- and pressure distribution is one of the most important criterias. With former testing methods some fundamental datas were found out, however those methods were insufficiently adapted and too inaccurate.

With a novel device we registered and interpreted the pressure distribution under the foot sole of each 5 men and women during standing and walking, barefoot and in shoes. The results show in detail the extent of existing foot deformities and their effects on the different phases of walking.

With a corrected form of the inside of shoe soles or an arch support standing and walking may be influenced positively for prevention or treatment of foot troubles. These fundamental investigations in our laboratories are not yet finished, and call for a transformation that is used in general practice, according to improved shoe insoles and -uppers as well as a more adapted choice of their materials.

Institut für Arbeitsphysiologie der Techn. Universität München, Barbarastraße 16, 8000 München 40

317

TIME COURSE OF TRAINING EFFECTS ON POSTURAL INSTABILITY PRODUCED BY STANDING ON ONE LEG

H. Knaup and W. Büchele

Standing on one leg causes a physiological instability of postural balance, which is due to both a reduction of the area of foot support as well as a reduction of somatosensory input from ankle joint receptors. Imbalance becomes particularly apparent when the eyes are closed, and the multi-loop control of posture relies mainly on the evaluation of vestibular and somatosensory afferences. The improved performance of balancing tasks by training indicates that postural reflexes are not optimized under daily life conditions.

Fore-aft and lateral body sway of 10 healthy subjects were continuously measured and analyzed by means of posturography while balancing on one foot for intermittent daily training phases of a total of 32 min over a period of 5 days:
1. postural sway activity (RMS-values) in all subjects improved up to 50% within the 5 days; 2. a 30% exponential rapid improvement was obtained within the first day; 3. a daily short-term training-effect and a long-term training-effect, together, form a typical sawtooth curve of increasing postural stability with time, reaching an asymptote after 3 days; 4. after termination of training learned balance skill is preserved for weeks.

The process of sensory-motor re-arrangement underlying the improvement of balancing is closely related to the degree of initial instability. Clinicians should make it a strategy in ataxia therapy to expose patients to increasingly instable body postures in order to facilitate re-arrangement and recruitment of control capacities. The amount of rapid improvement by balance training within one hour seems to be a reliable measure for the expectation of the overall benefit of physical therapy in an ataxic patient.

Neurologische Klinik mit klinischer Neurophysiologie, Alfried Krupp Krankenhaus, D-4300 Essen

318

CHARACTERISTICS OF WARM FIBRE DISCHARGE IN BOA CONSTRICTOR.

K. Schäfer

Discharge pattern of Boa warm fibres was analysed 1, at constant temperatures, 2, following rapid warming steps, and 3, during calcium or EDTA application.

1, With increasing constant temperatures a temperature dependent monotonic shift to shorter intervals is apparent. The reduction of mean discharge rate at higher temperatures results obviously from omitted impulses: the longer intervals are multiples of the shorter ones. An underlying periodic process is therefore supposed.

2, The adaptation phase of the dynamic response to rapid warming varies considerably with adapting temperature: A gradual decline at lower temperatures changes to an undershoot and transient inhibition following the overshoot at higher temperatures. Sometimes the dynamic response is suppressed completely at higher temperatures.

3, Generally calcium application induces a decrease and EDTA application an increase in receptor activity. A discharge in regular groups of impulses (burst discharge) is apparent in some fibres during EDTA infusion. It is concluded that Boa warm receptors possess a calcium dependent outward current comparable to that supposed in mammalian cold fibres.

Supported by the Deutsche Forschungsgemeinschaft

Department of Normal and Pathologic Physiology, University of Marburg, Deutschhausstr. 2, D-3550 Marburg, Federal Republic of Germany

319

CAPSAICIN FAILS TO IMPAIR AUTONOMIC HEAT DEFENCE IN AN AVIAN SPECIES, THE DUCK.

Elisabeth Geisthövel

Capsaicin has been shown to impair heat defence in several mammals. This effect is ascribed mainly to the destruction of warm receptors. Birds employ the same autonomic mechanisms of heat defence as mammals, but their thermoreception seems to be differently organized. This difference could be further substantiated by testing the effects of capsaicin on autonomic heat defence of ducks (1.9 - 2.5 kg body weight).- Compared with rats capsaicin was much less toxic in ducks: while 150 mg·kg^{-1} administered in the usual way to rats caused severe hypothermia and was often fatal, ducks tolerated single intravenous (i.v.) injections of up to 500 mg·kg^{-1} from the same batch without significant irritation, showing neither consistent vasodilatatory nor hypothermic responses.- Autonomic heat defence by panting was tested in three ducks before and after 1 g·kg^{-1} capsaicin given in repeated i.v. injections. The animals were exposed to a hot (35-37°C) environment and their core temperature (T_c, esophageal) was controlled by a cooling thermode inserted into the colon. At the threshold of panting (breathing frequency, BF, 50 min^{-1}), T_c after capsaicin was 41.67 ± 0.05°C (mean ± SE; n = 14) and not significantly different from the threshold T_c of 41.59 ± 0.04°C (n = 10) before capsaicin. The rise of BF with T_c in the capsaicinized animals was at least as steep as in the non-treated ducks, and the same maximum BF rates were observed. The strict linearity between BF (X) and respiratory evaporative heat loss (REHL, Y) observed in non-treated ducks ($\bar{r}$ = 0.95, n = 10) was maintained after capsaicin ($\bar{r}$ = 0.97, n = 14), although the slope was reduced. However, the rise of REHL with BF was always sufficient to keep T_c after capsaicin in the same range as before capsaicin. It is concluded that the nervous control of autonomic heat defence in ducks is not impaired by 1 g·kg^{-1} capsaicin. At most, evaporation from the respiratory surfaces might be reduced to some extent.

Max-Planck-Institut f. physiol. u. klin. Forschung, W.G. Kerckhoff-Institut, D-6350 Bad Nauheim, West-Germany

320

TEMPERATURE SENSATION, THERMAL COMFORT, SKIN BLOOD FLOW THRESHOLDS AND FREQUENCY ANALYSIS OF SKIN VASOMOTOR RHYTHMS

K. Issing, F. Kossivi, E. Fuhr

11 students in bathing suits were exposed for 30 min to 12 °C room temperature (RT). The temperature linearly increased within 45 min to a final RT = 45 °C. Only the right hand (arm) was thermally insulated from the RT by a water circulated plastic cylinder kept at 33 °C.
At various sites of the body local temperature sensation (LTS) and local thermal comfort (LTC) can be perceived. LTS depends linearly on local temperature (T_{local}), whereas local thermal comfort (LTC) depends both on T_{local} and mean skin temperature ($\bar{T}_S$). General temperature sensation (GTS) and general thermal comfort (GTC) dissociate above $\bar{T}_S \approx 33$ °C.
The right and left hand show nearly the same high skin blood flow increase (SBFI)($\Delta\lambda \approx 1.6$ units) but different thresholds for rhythmic skin blood flow (32.58 °C resp. 25.24 °C). SBFI is moderate at the forehead and lowest at the foot ($\Delta\lambda \approx 0.7$ units), because of the low initial foot temperature ($T_{Foot} \approx 21$ °C). The rhythmic vasodilatation of the foot begins only at high $T_{Foot} \approx 27.41$ °C, whereas the forehead shows a small vasomotion already from the beginning. Fourier analysis of skin blood flow rhythms has shown that the greatest amplitudes in the power spectra occurred at local temperatures between 33 °C and 36 °C. In the higher local temperature range vasomotor rhythms had shorter periods.

Supported by the Deutsche Forschungsgemeinschaft

Institute of Physiology, Deutschhausstr. 2, 3550 Marburg, Federal Republic of Germany

321

THREE TYPES OF SHORT-TERM COLD ADAPTIVE EFFECTS IN THE GUINEA-PIG

P. Hinckel, K. Pfizenmaier and K. Brück

Shivering evoked by external cooling was shown in man as well as in the non-cold-adapted guinea-pig to start at a lower mean body temperature during the second cooling phase in comparison with the shivering threshold during the first cooling if the intermittent rewarming period lasted no longer than ca. 30 min. In cold adapted guinea-pigs, whose initial shivering threshold was by 0.5°C (on average) lower than in the non-cold-adapted animals, no significant short-term shivering threshold displacements were observed (Pfizenmaier et al., Pflügers Arch. 394, R38, 1982).

These results could be reproduced in another series of experiments in the guinea-pig. Moreover, in both the non-cold and cold adapted groups a significant gain reduction of the thermogenic response was found during the second cooling compared to the first cooling phase. Normal gain was demonstrated to be restored when the rewarming period lasted longer than 100 min., whereas the restoration of the initial shivering threshold was shown in cases in which the rewarming period lasted longer than ca.30-40min. Another type of cold adaptive modification was seen in the cold adapted group. These animals exhibited a strong non-shivering-thermogenic (NST) response during the second cooling, whereas in the first cooling period NST did not seem to be evoked.

In conclusion, the missing short-term shivering shift and the sensitization of NST through a priming cold exposure in the cold adapted animals are interpreted to represent specific components of stabilization of body temperature under cold load developed in the course of chronic cold acclimation, although the short-term shivering gain reduction is not altered and the body temperature is maintained at a slightly reduced level.

Physiologisches Institut, Univ. Giessen, Aulweg 129, D-6300 Giessen (Supported by the DFG, Br 184/16)

322

THE EFFECT OF AMINOPHENAZONE ON THE THERMOREGULATION OF UNTREATED AND FEVERISH RABBITS

J. Haan, J. Cordes, G. Götze and B. Nitz

The set-point of thermoregulation of feverish animals is adjusted to a higher control-level. The mode of action of antipyretic substances should be in normalizing the shifted control-system. Solving this problem experiments have been done with untreated and febrile rabbits. Lipopolysaccarides (LPS 1 µg/kg iv.) were used to pyretogenesis, aminophenazone (75 mg/kg im.) to decrease in temperature. The thermoregulation was stressed differently: animals were exposed to various ambient temperatures (between 10°and 33°C) in a calorimeter - chamber for 6 hours. Measured were the rectal-, ear- and skintemperatures, the respiratory- and heartrate, the heat-production and heat-emission.

It was observed at cold-stress a decrease, at warmth an increase and at indifferent-temperature no change in body-temperature. Similar results were received in combined experiments with aminophenazone and LPS. Pyrogen strengthened the decrease in temperature at cold environment - initiated by aminophenazone - at heat a distinct fever reaction was recorded, at indifferent temperature no pyrogen - reaction was noticed. These fact agree with the theory of resetting the elevated set-point of thermoregulation. It demonstrates the influence of heat-stress and cold-exposure to thermoregulation and the change of pharmacon effect. As it would be expected the animals react "normal" only to indifferent-temperature.

Institut für Pharmakologie und Toxikologie der TU Braunschweig, Mendelssohnstraße 1, D-3300 Braunschweig

323

ENDOGENOUS PYROGEN (EP) AND CHEMICAL ANTIPYRESIS: A COMPARISON OF THEIR EFFECTS UPON IMMUNE RESPONSE IN RABBITS

U.-E. Wieland, M.D. Merkel and H. Hensel

With the presented examination the effects of single i.v.-injections of EP and a suitable antipyretic were to be compared to those of control solutions (denaturated EP and a buffer solution) in regard to their influence upon the primary immune response. For the test 12 male domestic rabbits (weight approx. 3.5 kg) were used.

To immunize the rabbits for the determination of specific humoral antibodies all animals were injected once, with sheep red blood cells intravenously at the beginning of the test. Of the 4 groups, each containing 3 animals, the 1st received intravenous injections of native EP solution, the 2nd additionally, prior to, and after applying EP, an antipyretic, the 3rd denaturated EP and the 4th NaCl-phosphate buffer solution for comparison. During the 5 days of the test, only - as expected - within the 1st group a EP fever of approx. 1.4 °C occurred, so that contamination with endotoxin could be excluded.

Every 2 days, up to the 2nd day blood samples were taken and subjected to an agglutination and haemolysis test. The results of both tests indicate that the animals injected with EP had, in comparison, by far the highest antibody titre (1:30 compared to control animals of the agglutinine test and 1:130 compared to control animals of the haemolysine test). This shows a definite immune stimulating effect of the EP fever in rabbits. The level of antibodies that was somewhat above that of the control animals in both tests among the group treated with an antipyretic (Metamizol-Na) to the extent of 1:8 suggests that the antipyretic used, succeeded in suppressing the rise in temperature whereas possible immune stimulating components of EP were only restrained in part.

Supported by the Deutsche Forschungsgemeinschaft

Institut für Physiologie, Universität Marburg, Deutschhausstraße 2, D-3550 Marburg 1, FRG

324

IMMUNOCYTOCHEMICAL REACTION OF VASOPRESSIN- CONTAINING NEURONS DURING DEVELOPMENT OF FEVER IN THE GUINEA PIG.

G. Merker, E. Zeisberger, S. Blähser and M. Krannig.

According to Cooper et al. (J.Physiol.(Lond.) 295, 33-45, 1979) centrally released arginine-vasopressin (AVP) might be responsible for fever suppression in the ewe. From previous immunocytochemical investigations on the reactivity pattern of vasopressinergic neurons in pregnant guinea pigs we concluded, that activation of AVP-neurons in the medial portion of the paraventricular nucleus (PVN) and an increased immunoreactivity of AVP-terminals in the lateral septum (LS) and in the amygdala (AM) might be functionally involved in fever suppression (Merker et al., Cell Tissue Res. 212, 47-61, 1980). In our recent immunocytochemical study the distribution and reactivity of AVP-containing neurons and fibers was examined in the brains of 9 male guinea pigs during the development of fever. Fever response to i.m. injection of bacterial pyrogen (Salmonella enterititis, 40 µg/kg body weight) was examined in each animal. A week later the fever experiment was repeated and the animals were sacrificed for the immunocytochemical examination. The first group was examined during fever rise (+0.7 to +0.9°C), the second at maximal fever (+1.3 to +1.5°C) and the third during fever decrease (+1.2 to +0.9°C). Controls were examined one week after a fever test. While the AVP-neurons in the controls reveal the usual reactivity pattern, the AVP-reaction during fever rise strikingly resembles to that observed in pregnant animals, characterized by increased AVP-reactivity in the medial PVN, the LS and the AM and also in perikarya and fibers of the suprachiasmatic nucleus. At maximal fever the reaction is moderately reduced. Fever decrease of 0.3°C only, reveals a reactivity pattern corresponding to that of the controls. It is concluded, that the similarity of changes in the AVP-neuronal system at term of pregnancy and during fever rise signalizes the involvement of AVP in fever reduction also in nonpregnant animals.

Physiologisches Institut und Institut für Anatomie und Cytologie, Justus Liebig-Universität, 6300 Giessen.

325

TEMPERATURE REGULATION DURING SLEEP DEPRIVATION

M. Scarperi

During sleep deprivation (SD), deep-body temperature decreases whereas its circadian periodicity remains almost unaltered (for ref.see J.A.Horne, Biol.Psychol.7: 55, 1978). The aim of our study was to investigate the effects of SD on the systems of autonomic temperature regulation and thermoregulatory behavior. Methods: 5 healthy male volunteers rested for 45 min and exercised (constant work load at ca.5o-6o% $\dot{V}_{O2max}$) for another 45 min totally immersed in a well stirred water bath, the temperature of which they had to adjust continuously to comfort temperature. Rectal temperature(T_{re}), water temperature (T_{comf} = mean skin temperature $\overline{T}_s$), Oxygen uptake ($\dot{V}_{O2}$), local sweat rate on the forehead (LSR),and the calculated total heat conductance (k) were recorded in 1 min intervals. On each subject 6 control experiments and 6 experiments after 24 h SD were carried out early in the morning. Results: At the end of rest and exercise , T_{re} is lower after SD than in the control experiments ($p < .01$, Wilcoxon test). During rest and exercise, k is higher after SD than in controls ($p < .05$). All other recorded values were not significantly different in both experimental conditions. Conclusions: 1. Autonomic thermoregulatory responses are altered after SD: despite the lower thermal inputs, there is no significant difference in LSR between SD- and control experiments and k is even higher after SD than in control experiments. This suggests a lowered thermal threshold for skin blood-flow and sweat secretion. 2. Since, after SD, thermal comfort is achieved at a lower temperature level than in control experiments, the thresholds for both behavioral and autonomic thermoregulatory reactions seem to be shifted in the same direction.

Physiologisches Institut der Universität, Martinistr.52, D-2ooo Hamburg 2o

326

CORTICOTROPIN RELEASING FACTOR (CRF) EXCITES HIPPOCAMPAL NEURONS.

J.B.Aldenhoff *, C.L.Ehlers, D.L.Gruol, and G.R.Siggins

We investigated the effect of synthetic CRF (W. Vale et al. Science 213: 1394, 1981) on the electrical activity of CA1 and CA3 neurons of the rat hippocampus in vitro. Hippocampal slices were completely immersed and continuously superfused with carbogenated artificial CSF solution. Microelectrodes for intracellular recording contained 4 M K-acetate.
CRF (15-500 nM) produced a marked increase in spontaneous discharge activity. This effect was due partially to a decrease in size and duration of the post-burst after-hyperpolarizations due to CRF. Concentrations above 250 nM also depolarized the neurons at 3 to 15 mV. In the presence of TTX (100 nM) CRF still reduced the after-hyperpolarizations which followed "calcium-spikes", generated by current-injections.
As an explanation of these effects of CRF on the electrical activity of these hippocampal neurons we propose an action on the calcium-mediated processes which are assumed to play an important role in hippocampal pyramidal cells. In an in vivo study CRF (10 - 1000 nM) was applied intraventricularly in rats with stereotaxically implanted EEG-electrodes. After a delay of 15 to 20 min. long-lasting EEG-activation was observed in the amygdala and the dorsal hippocampus. Simultaneously typical changes in the behavior of these rats occurred.
These data were discussed in respect to the hypothesis of an activating function of CRF in the CNS.

A. V. Davis Center, The Salk Institute, La Jolla, Ca. 92037
* present address: BKH Kaufbeuren, D-895 Kaufbeuren
supported by the Alexander v. Humboldt Ges.

327

ENHANCEMENT OF MACROPHAGE OXIDATIVE AND ARACHIDONIC ACID METABOLISM BY SUBSTANCE P

H.P.Hartung and K.V.Toyka

Substance P (SP) has recently been found to augment phagocytosis by exudate cells. We examined whether SP induces heightened cellular activity in macrophages (mø) as reflected by stimulation of an oxidative burst and enhancement of arachidonic acid metabolism. Peritoneal mø were collected from Hartley guinea pigs 4 or 14 days after i.p. injection with human serum albumin (HSA) or C.parvum. Following purification by adherence they were challenged with SP and incubated for up to 18 h. Supernatants were examined for release of O_2^-, H_2O_2 (photometrically), and thromboxane B_2 (TXB_2) (by RIA) (Hartung et al, J. Immunol.; in press). Oxygen radical release from C.parvum-activated mø was maximal within 1 h and at 6×10^{-7} M amounted to 13 nmol O_2^-/6 nmol H_2O_2/h/10^6 cells. TXB_2 generation by HSA-elicited mø reached a plateau at 12 hwith 7 ng TXB_2 /10^6 cells. Oxygen species generated in the oxidative burst are cytotoxic, mediate endothelial injury and partake in inflammatory responses. TXA_2 induces platelet aggregation and release reaction. Liberation of oxygen derivatives and TXB_2 from mø in response to SP appears relevant to the concept of mø - platelet interaction in inflammation. In the context of neuroinflammatory disease SP may play a decisive role in that its release from nerve terminals initiates not only antidromic vasodilation, plasma extravasation, and augmented phagocytosis but also, as indicated by our data, the generation of phlogogenic mø products.
Supported by DFG, SFB 200, B 5.

Neuroimmunology Lab., Dept.of Neurology, Univ.of Düsseldorf, Moorenstr.5, D-4000 Düsseldorf 1

328

CORRELATION BETWEEN SUBSTANCE P CONTENT OF PRIMARY SENSORY NEURONS AND PAIN SENSITIVITY IN RATS EXPOSED TO ANTIBODIES TO NERVE GROWTH FACTOR

U. Otten

Nerve growth factor (NGF) is a naturally occurring trophic substance for substance P (SP)-containing primary sensory neurons. Chronic NGF treatment of neonatal rats results in dose-related increases in SP-content in dorsal root ganglia and in dorsal spinal cord. Administration of either purified heterologous or monoclonal anti-NGF antibodies to neonates causes a temporary, marked reduction of SP-content in spinal ganglia and in spinal cord. However, these effects are permanent if the antibodies are injected directly into the embryo.

Rats exposed to maternal anti-NGF antibodies in utero and in milk have been used to investigate the role of SP in pain perception. Anti NGF-treated rats show a significantly diminished sensitivity towards pressure- and chemically-induced pain stimuli. The reduced responsiveness to pain correlates with a marked, selective decrease of SP in the dorsal horn of the spinal cord. The results suggest that SP plays a role in nociceptive sensory transmission.

Dept. of Pharmacology, Biozentrum, University of Basel, CH-4054 Basel, Switzerland

329

MODULATION OF THE RELEASE OF CHOLECYSTOKININ FROM SLICES OF RAT NEOSTRIATUM BY BIOGENIC AMINES

D.K. MEYER, A. HOLLAND and U. SCHNEIDER

Veratridine stimulates the release of cholecystokinin-immunoreactivity (CCK-IR) from nerve-endings in the neostriatum of rats in vitro. Dopamine (DA) in low concentrations enhances the release of CCK-IR, while high concentrations do not. After destruction of the neurons intrinsic to the neostriatum with kainic acid also high concentrations of DA enhance the release of CCK-IR. Thus, it can be reasoned that DA enhances the release via D_2-receptors and attenuates it via D_1-receptors (Meyer and Krauss, Naunyn-Schmiedeberg's Arch. Pharmacol. 321, R 22).

In the present study selective D_2-antagonists and agonists were used.

(-)Sulpiride abolished the enhancement of the release of CCK-IR induced by DA (10^{-7}M), while the selective agonist RU 24926 (N-n-propyl-di-ß(3-hydroxy phenyl) ethylamine hydrochloride) increased the release of CCK-IR in a dose-dependent way. The agent did not show inhibitory effects at any dose used. In separate experiments it was shown that serotonin (5-HT), another transmitter in the neostriatum, caused a dose-dependent increase in the release of CCK-IR. It is possible that DA and 5-HT modulate the release of CCK-IR also in vivo. Since CCK-neurons seem to connect structures of the limbic system with the neostriatum, the data may be of importance in future studies on the physiology and pathophysiology of the basal ganglia.

Pharmakologisches Institut der Universität Freiburg i.Br., Hermann-Herder-Strasse 5, D-7800 Freiburg i.Br.

330

PITUITARY OPIATES REDUCE VASOPRESSIN RELEASE FROM RAT NEUROHYPOPHYSIS IN VITRO

W. Knepel and D. Nutto

It has been concluded previously that an endogenous opiate, released by electrical stimulation of the isolated neurohypophysis, inhibits the release of oxytocin but not of vasopressin (Bicknell and Leng, Nature 298, 161, 1982). Since the pattern of stimulation used in that study resembles the electrical activity of oxytocin rather than vasopressin neurones, we re-investigated the effect of an opiate antagonist on vasopressin release. Rat posterior pituitaries were superfused in vitro and stimulated electrically. When the pulses (24 mA; 2 msec) were applied in 10 sec trains with 10 sec intervals, vasopressin release per pulse increased progressively over the frequency range of 3 - 12 Hz. The release was blocked by addition of tetrodotoxin or by removal of calcium ions from the superfusion medium. The opiate antagonist naloxone 1 or 10 µM, introduced before a second period of stimulation, enhanced vasopressin release from neurointermediate lobes after phasic stimulation at 9 Hz. However, after removal of the intermediate lobe naloxone 10 µM had no longer an effect on vasopressin release from isolated neural lobes; yet the addition of camel ß-endorphin 2 µM inhibited vasopressin release in a naloxone-reversible manner.

We conclude that the evoked release of vasopressin from the neurointermediate lobe is reduced by an endogenous opiate of intermediate lobe origin, possibly ß-endorphin. Stimulation conditions appropriate to vasopressin neurones are necessary for this mechanism to function.

Pharmakologisches Institut der Universität Freiburg, Hermann-Herder-Str. 5, D-7800 Freiburg i. Br.

331

INHIBITION OF NOCICEPTIVE RESPONSES IN CAT SPINAL DORSAL HORN NEURONES BY MORPHINE MICROINJECTION IN THE NUCLEUS RAPHE MAGNUS

J.G.Thalhammer, H.-J.Du, L.M.Kitahata and M.Zimmermann

Morphine microinjection (MOR) and focal electrical stimulation (Stim) in the nucleus raphe magnus (NRM) produce naloxone reversible behavioral analgesia. It has been shown that Stim in the NRM exerts its inhibitory effect via descending pathways from the NRM to the dorsal horn of the spinal cord. It is unclear, however, whether the analgesic effect of MOR is due to the raphe-spinal descending inhibitory system, since Le Bars et al. (Brain Res 189, 467, 1980) failed to demonstrate any inhibitory effect of MOR in the NRM at the dorsal horn neurones.

Lumbar dorsal horn neurones responsive to noxious radiant heat stimulation of the foot or toe pads and/or to electrical stimulation of cutaneous hindlimb nerves were studied in anesthetized and paralysed cats. MOR markedly reduced the response elicited by electrical stimulation of A-fibers (2,5 V, 0,1 msec) in three out of eight neurones and by electrical stimulation of C-fibers (25 V, 1,0 msec) in seven out of eight neurones. The response to noxious heating (50°C, 10 sec) of the skin was attenuated in all seven neurones studied. The MOR induced inhibition was naloxone reversible.

It is concluded that MOR in the NRM exerts an inhibitory effect on the transmission of noxious information at the dorsal horn of the spinal cord, which may in part explain the mechanism of MOR induced analgesia.

Supported by the Deutsche Forschungsgemeinschaft, DAAD and NIH

II. Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg, G.F.R.

332

THE NOVEL OPIOID PEPTIDE PRECURSOR PRO-ENKEPHALIN B IS DIFFERENTIALLY PROCESSED INTO VARIOUS OPIOID PEPTIDES IN DISTINCT REGIONS OF BRAIN AND PITUITARY

B.R. Seizinger, C. Grimm and V. Höllt

The complete amino acid sequence of a novel opioid peptide precursor, pro-enkephalin B, has recently been elucidated (Kakidani et al., Nature 298, 245-249, 1982). Pro-enkephalin B contains the sequence of three "big-leu-enkephalins", dynorphin (DYN), neo-endorphin (neo-E) and rimorphin (= dynorphin B). In order to evaluate putative differences in the post-translational processing of pro-enkephalin B between various tissues, levels of particular opioid peptides contained within the sequence of this precursor, were determined in various brain and pituitary areas by the combined use of HPLC, gelfiltration and radioimmunoassay.

Within the rat hypothalamo-hypophyseal system, α-neo-E, ß-neo-E (= α-neo-E_{1-9}), DYN_{1-32}, DYN_{1-17}, the N-terminal octapeptide DYN_{1-8} and rimorphin were found to occur in similar amounts.

In contrast, within the rat adenohypophysis, only high-molecular weight forms (MW ∿ 6000) of DYN and rimorphin, but no DYN_{1-32}, DYN_{1-17}, DYN_{1-8} or the tridecapeptide rimorphin could be detected. Moreover, the major part of α-neo-endorphin-immunoreactivity consisted of a high-molecular-weight 8000 dalton species.

However, the rat striatum contained predominantly DYN_{1-8} and α-neo-E as major endproducts of processing, while DYN_{1-32}, DYN_{1-17} and ß-neo-E were present in only minor quantities.

The selective post-translational processing of pro-enkephalin B may reflect specific functions of the various endproducts of this biosynthetic pathway as neurotransmitters, neurohormones or hormones within different tissues.

Max-Planck-Institut für Psychiatrie, Abt. Neuropharmakologie, Kraepelinstrasse 2, D-8000 München 40, F.R.G.

333

BINDING OF HUMAN β-ENDORPHIN TO THE TERMINAL SC5b-9 COMPLEX OF HUMAN COMPLEMENT IS MEDIATED BY ITS COOH-TERMINUS

L. Schweigerer[1], S. Bhakdi[2] and M. Lederle[3]

Human β-endorphin (β_H-EP) binds specifically to nonopiate binding sites on the terminal SC5b-9 complex of human complement (Schweigerer et al., Nature 296: 572, 1982). In a previous study we have demonstrated the importance of the C5b, C6 and/or C7 subunits of SC5b-9 for high-affinity binding ($K_D \sim 3$ nM) (Schweigerer et al., Life Sci. 31: 2275, 1982).

To delineate the β_H-EP sequence region necessary for binding, a series of COOH-terminal β_H-EP fragments were tested for their potency to inhibit ^{125}I-β_H-EP binding to SC5b-9. The COOH-terminal dipeptide Gly-Glu was found to represent the minimal effective sequence. Extension of COOH-terminal β_H-EP fragments towards the NH_2-terminus led to an increased binding affinity, whereby NH_2-acetyl-β_H-EP and β_H-EP had the highest affinities.

No inhibition of ^{125}I-β_H-EP binding was observed in the presence of a series of opioid-unrelated peptides or the opoid antagonist naloxone or of some NH_2-terminal β_H-EP fragments.

β_H-EP binding to SC5b-9 was dependent on temperature, pH and on the presence of intact disulfide groups and appeared to involve both, hydrophobic and ionic interactions.

It is suggested that the β_H-EP/SC5b-9 interaction might be of biological significance.

[1]) Rudolf-Buchheim-Institut für Pharmakologie, and [2]) Institut für Medizinische Mikrobiologie der Justus-Liebig-Universität, 6300 Gießen and [3]) Pharmakologisches Institut der Albert-Ludwigs-Universität, 7800 Freiburg

334

TARGET SIZE ANALYSIS OF MULTIPLE OPIATE RECEPTORS

S. Ott, B. Hietel*, F. Schulz* and A. Herz

The present concept of a multiplicity of opiate receptors indicates the distinct existence of μ-, δ- and κ-receptors in the central nervous system. We have used the radiation inactivation technique to determine the molecular weight of the particular opiate receptors in the rat brain. This method has the advantage of allowing the determination of the minimal functional size of a membrane protein - such as the opiate receptor - in situ. The molecular weight can be derived from the loss of biological activity upon irradiation by a high energy beam. In the present study, lyophilized rat brain membranes were irradiated by electrons 2.5 MeV from a linear accelerator with doses ranging from 0.1 to 30 MRd. The residual opiate binding capacity was measured by a conventional radioreceptor assay, employing either tracers such as ^{3}H-oxymorphone and ^{3}H-D-Ala^2,D-Leu^5-enkephalin to selectively monitor μ- and δ-binding sites, respectively, or of using ^{3}H-diprenorphine as an overall label for μ-, δ- and κ-sites in combination with the sequential blocking technique. The decay curves obtained for opiate binding capacities were curvilinear and accordingly indicate the existence of at least two different molecular sizes of opiate binding sites, one of about 100 000 daltons and another with an at least 8 times higher molecular weight. Identical data were obtained for each of the separately monitored μ-, δ- and κ-binding sites; thus, the different molecular weights cannot be attributed to particular types of opiate sites but are rather a characteristic inherent within each of the receptor classes investigated. The experiments performed so far have revealed varying proportions of high vs. low molecular weight form. It is suggested that the apparent size of the opiate receptor is depending on its functional state at the time of irradiation.

Supported by the Deutsche Forschungsgemeinschaft, Bonn.

Max-Planck-Institut für Psychiatrie, Abt. Neuropharmakologie, Kraepelinstrasse 2, D-8000 München 40, F.R.G.
*Gesellschaft für Strahlen- und Umweltforschung, Physikalisch-technische Abteilung, D-8042 Neuherberg, F.R.G.

335

RECEPTOR AFFINITIES AND BIOLOGICAL EFFECTS OF DIMERIC ENKEPHALIN ANALOGUES DO NOT VARY IN PARALLEL

T. Costa and Y. Shimohigashi*

Hormonal molecules dimerized with spanners of varying length in order to produce bivalent ligands may be helpful tools for the study of the spatial organization of membrane receptors. Two series of dimeric enkephalin analogues have been synthetized consisting of monomeric peptide amides linked at the C-terminus by methylene chains of increasing length. The monomer in the first series is the pentapeptide Tyr-D-Ala-Gly-Phe-Leu NH_2 and in the second series the tetrapeptide Tyr-D-Ala-Gly-Phe NH_2. In previous studies, these peptides showed an increase in affinity as a function of the chain length only for the "delta" type of the opiate receptor as estimated by radioreceptor assay. Affinities for μ-receptors were little changed or reduced as compared to the parent monomers.

We have, now, evaluated the biological activities of these compounds in isolated smooth muscle preparations, the mouse vas deferens and the longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum. A general agreement between binding and biological activity was found in the pentapeptide series. However, for the compounds of the tetrapeptide series, the increases in receptor affinities produced by dimerization did not result in increases in activity of a comparable extent.

These data indicate that the enhanced receptor affinity of a bivalent ligand, which presumably results from an increase in multiple interactions with the receptor (bridging) is not necessarily translated into an enhanced biological effect.

Max-Planck-Institut für Psychiatrie, Abt. Neuropharmakologie, Kraepelinstrasse 2, D-8000 München 40, F.R.G.
*NICHD-ERRB, National Institutes of Health, Bethesda, Md., U.S.A.

336

ENKEPHALINERGIC INTERNEURONS OF THE SUBSTANTIA GELATINOSA (SG) MAY TONICALLY INHIBIT DORSAL HORN CELLS VIA POSTSYNAPTICALLY LOCATED OPIATE RECEPTORS

N. Mercuri and P. Stanzione

Evidence for both, pre- and postsynaptic interactions between enkephalin-containing interneurons of the SG, primary afferents and neurons of spino-fugal pathways in the dorsal horn of the spinal cord have been reported. In the present study, an enkephalin-analogue (D-ala^2-D-leu^5-enkephalin, DADL) and the excitatory neurotransmitter l-glutamate (GLUT) were ejected from micropipettes by pressure into the neighbourhood of single neurons the activity of which was recorded extracellularly. This novel technique permits, in contrast to microiontophoretic applications, the control of the concentration reached in the tissue. Preparation of the animal (halothan or urethan anaesthesia), data acquisition and analysis employed conventional techniques. DADL inhibited spontaneous, synaptic and GLUT (10^{-6}-10^{-7}M) induced neuronal activity in concentrations as low as 10^{-8}M. These effective concentrations compare favourable to those found in bioassays designed for opiate actions. Naloxone (10^{-6}-10^{-7}M) antagonized the inhibitory action of DADL without affecting the spike-generating mechanism. At these concentrations, naloxone itself displayed moderate excitatory actions in the majority of neurons suggesting an enkephalinergic inhibitory tonus excerted probably by spontaneously active interneurons. The finding that GLUT which excerts its excitatory actions via postsynaptically located receptors is antagonized by DADL and the excitatory action of the opiate antagonist suggest that spino-fugal pathways are under a tonic inhibitory influence mediated via postsynaptically located opiate receptors. The notion of a postsynaptic location of opiate receptors in the spinal cord dorsal horn is totally consistent with most recent ultrastructural and histochemical findings which could not find any indication for axo-axonic interaction, e.g. of primary afferent fibers containing substance P and enkephalin-containing terminals.

Max-Planck-Institut für Psychiatrie, Abt. Neuropharmakologie, Kraepelinstraße 2, D-8000 München 40, F.R.G.

337

IS cAMP INVOLVED IN ANTINOCICEPTIVE EFFECTS OF MORPHINE IN THE RAT SPINAL CORD?

I. Jurna

The effect of intrathecal (i.t.) injections of morphine, aminophylline, dibutyryl cAMP (DBcAMP) and naloxone were studied in motor and sensory responses to stimulation of nociceptive afferents in rats with the spinal cord transected at the lower thoracic level. Responses were evoked by electrical stimulation of Aδ and C fibres in the sural nerve and recorded as flexor reflex activity in the electromyogram of the tib. ant. muscle (motor response) or activity in ascending axons of the spinal cord (sensory response). Morphine (2 mg/kg i.v. or 10 µg i.t.) depressed the motor and sensory responses. Aminophylline (25 mg/kg i.v. or 50 µg i.t.) abolished the effect of morphine on the motor but not on the sensory response. The effect of morphine on the sensory response was antagonized by naloxone (10 µg i.t.). DBcAMP (100 µg i.t.) depressed the motor response like morphine, and this effect was antagonized by aminophylline. In contrast to morphine, DBcAMP did not reduce the sensory response but facilitated it, and this facilitation was abolished by morphine.

It is concluded that morphine depresses the motor and sensory responses of the spinal cord to noxious stimulation by different mechanisms. The depression by morphine of the motor response may involve cAMP.

Supported by a grant of the Sonderforschungsbereich 38 "Membranforschung"

Institut für Pharmakologie und Toxikologie der Universität des Saarlandes, 6650 Homburg/Saar (F.R.G.)

338

ADAPTIVE PROCESSES AS A CONSEQUENCE OF CHRONIC OPIATE RECEPTOR ACTIVATION IN A CLONAL HYBRID CELL LINE

M. Wüster and C. Gramsch

Neuroblastoma x glioma hybrid cells (NG 108-15) have been extensively employed as a model preparation for the investigation of molecular mechanisms of opiate action. These studies have revealed a critical significance of the intracellularly localized enzyme adenylatecyclase, which is acutely inhibited by opiates. Subsequent studies demonstrated a compensatory activation of this enzyme as a result of chronic opiate action, which has been related to the chronic effects of opiates in vivo, that is, the development of tolerance and dependence. We have studied the adaptive processes in NG 108-15 cells after treatment with morphine or D-Ala^2,D-Leu^5-enkephalin for different periods of time. Both ligands dose-dependently induced a state of reduced sensitivity to a further acute opioid challenge. Unlike the phenomena in vivo or in isolated tissues, this desensitization was not expressed by a shift in the dose-effect curves of opioids but rather resulted in a decreased maximal effect upon adenylatecyclase inhibition. Such cell preparations were tested, furthermore, for their expression of opiate dependence, as revealed by the ability of the opiate antagonist naloxone to precipitate a withdrawal sign in the form of elevated cAMP levels. Such "withdrawal signs" were relatively small in slightly desensitized cells, resulting in a maximally 50 percent increase over baseline cAMP levels. Unexpectedly, such increases were completely absent in "highly tolerant" cells.

These results are in conflict with the generally accepted assumption of a common cellular mechanism underlying both opioid tolerance and dependence. They rather indicate that the state of "tolerance" is achieved independently from an induction of adenylatecyclase-hypertrophy, possibly by an uncoupling of opiate binding sites from the subsequent effector system.

Supported by the Deutsche Forschungsgemeinschaft, Bonn.
Max-Planck-Institut für Psychiatrie, Abt. Neuropharmakologie, Kraepelinstrasse 2, D-8000 München 40, F.R.G.

339

(D-MET^2, PRO^5) ENKEPHALINAMIDE ELICITS BRADYCARDIA, BRADYPNOEA AND SLEEP-LIKE STATES FROM THE FOURTH CEREBRAL VENTRICLE IN DOGS

D. Knolle and J.O. Arndt

The opiate fentanyl when perfused continuously through the cerebroventricular system of awake dogs, elicits bradycardia with sleep-like states via the fourth, but not the third ventricle (E. Freye, J.O. Arndt, Naunyn Schmiedeberg's Arch.Pharm. 307, 123, 1979). Do endorphins, the natural ligands of opiate receptors, act similarly?

In five trained, unanesthetized dogs with chronically implanted cannulae (d-met^2, pro^5) enkephalinamide (=enkephalin) was perfused continuously through the fourth cerebral ventricle, the effects being observed on the circulation, respiration, EEG and behaviour.

With increasing enkephalin concentrations between 25 and 400 µg/ml, all animals showed sleep-like behaviour with delta-activity in the EEG, bradycardia (-40 beats/min), bradypnoea (-20 beats/min), a decrease in arterial PO_2 (25 mmHg) and an increase in PCO_2 (+10 mmHg). All effects saturated at and around enkephalin concentrations of 100 µg/ml and were reversed by naloxone.

(D-met^2, Pro^5)-enkephalinamide elicits sleep-like states from the fourth cerebral ventricle in unanesthetized dogs with cardio-respiratory manifestations resembling that of slow wave sleep. Opiate receptors in the medulla presumably mediate these effects.

Abt. Experimentelle Anaesthesiologie, Universität Düsseldorf, Universitätsstr. 1, 4000 Düsseldorf

340

(D-MET^2, PRO^5) ENKEPHALINAMIDE ACTIVATES CARDIOINHIBITORY EFFERENTS IN ANESTHETIZED DOGS

B. Nashan and K. Inoue

Endorphins and opiates elicit bradycardia when injected into the neighbourhood of the nucleus ambiguus (M. Laubie, H. Schmitt, M. Vincent, Europ.J.Pharmacol. 59, 287, 1979). Neurophysiological proof for the suggested vagal origin of the bradycardia is lacking. We therefore recorded the activity of cardioinhibitory vagal efferents and studied their response to (d-met^2, pro^5) enkephalinamide (= enkephalin).

The data is derived from seven anesthetized dogs (chloralose/urethane). Cardioinhibitory efferents were isolated from the central stump of the cervical vagus nerve and their average discharge rate (spikes/s) recorded along with heart rate and blood pressure. Enkephalin was perfused at concentrations between 50-400 µg/ml through the cerebroventricular system via cannulae, one in a lateral ventricle, the other in the cisterna magna.

With increasing intraventricular concentration of enkephalinamide, the vagal discharge rate increased from 3.1 ± 0.8 to 8.8 ± 1.4 spikes/s, heart rate decreased from 134 ± 15 to 95 ± 17 beats/min, while there was little change in blood pressure. All effects were fully developed at and around concentrations of 100 µg/ml and were reversed with naloxone.

(D-met^2, pro^5) enkephalinamide strongly activates cardioinhibitory vagal efferents and leads to bradycardia in dogs perhaps via opiate receptors.

Abt. Exp. Anaesthesiologie, Universität Düsseldorf, Universitätsstr. 1, 4000 Düsseldorf

341

INFLUENCE OF OPIATE RECEPTOR ANTAGONISTS ON THE HYPOTENSIVE ACTION OF CLONIDINE, PRAZOSIN AND CAPTOPRIL IN SPONTANEOUSLY HYPERTENSIVE RATS

W. Gaida, W. Hoefke and K. Kraft

It is known that opioid antagonists are able to attenuate hypotensive effects of shock and also of antihypertensive compounds (Farsang, C. et al: J. Pharmacol. Exp. Ther. 214, 203, 1980). Pretreatment of conscious SHR with naltrexone or naloxone (2 mg/kg i.p. or 0.1 mg i.c.v.) antagonized the hypotensive effects of clonidine (0.015 mg/kg i.v. or 0.3 mg/kg i.m. or 0.003 mg i.c.v.). In normotensive rats no antagonism was observed between clonidine (0.3 mg/kg i.m.) and naltrexone (2 mg/kg i.p.). In order to test wether or not naltrexone would be effective in antagonizing the action of antihypertensive drugs that do not act primarily via central mechanisms we administered prazosin (1.0 mg/kg p.o.) or captopril (50 mg/kg p.o.) into conscious SHR. Both drugs effectively lowered the blood pressure. Pretreatment with naltrexone (2 mg/kg i.p.) did not affect the hypotensive action of either prazosin or captopril. These results suggest that the interference of opioid antagonists with antihypertensive drugs is mediated centrally. The adrenergic system seems to be involved.

German Institute for High Blood Pressure Research, Im Neuenheimer Feld 366, D - 6900 Heidelberg and Department of Pharmacology, Boehringer Ingelheim KG, D - 6507 Ingelheim

342

IS THE CLONIDINE POTENTIATION OF JUMPING IN PRECIPITATED MORPHINE WITHDRAWAL MEDIATED BY α_1-RECEPTORS?

J.W.van der Laan

Clonidine has a dual action on naloxone-precipitated morphine withdrawal symptoms in rats: a suppressive action on body shakes and body weight loss and a potentiating action on jumping and aggression.
It has been suggested that this potentiating, excitatory action is mediated by α_1-receptors. More specific α_2-agonists should therefore have a less excitatory effect on the latter symptoms. This hypothesis has been studied in rats dependent on morphine using "Slow release" preparations. Withdrawal was precipitated using naloxone. Prior to naloxone the α_2-agonists clonidine, guanfacine, azepexole, B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H thiazolo-[4,5-d]-azepine dihydrochloride) or UK 14.304 (5-bromo-6-[2-imidazolin-2-ylamino]quinoxaline tartrate) or the centrally acting α_1-agonist ST 587 (2-(2-chloro-5-trifluoromethyl-phenyl-imino)imidazoline nitrate) were administered. All α_2-agonists but not the α_1-agonist potentiated the jumping and decreased body shakes and body weight loss. The effect of B-HT 920 on jumping could not be antagonized by prazosine nor by yohimbine.
In conclusion, there seems to be no involvement of α_1-receptors in the potentiation of morphine withdrawal jumping in rats by α_2-agonists.

Laboratory for Pharmacology, Section on Psychopharmacology, National Institute of Public Health, P.O.Box 1, 3720 BA BILTHOVEN, THE NETHERLANDS

343

THE PARTIAL REVERSAL OF NARCOTIC EFFECTS BY NALOXONE IN PAIN RATINGS AND IN PAIN RELATED CEREBRAL POTENTIALS

B. Bromm and E. Scharein

The dosis dependent naloxone blockade of opioide induced pain relief was investigated in 15 healthy subjects between 21 and 29 years of age, measuring subjective pain estimation and cerebral potential amplitudes in response to randomly applied painful and non-painful electrical skin stimuli; each session lasting 9o minutes. The following drugs were orally administered (double blind) 3o min before stimulation in a repeated measurement design of 3 independently randomized latin squares of 5(treatments) x 5(sessions): Tilidine (Valoron^R) loo mg; naloxone: 32 mg; TN8 (Valoron N^R): loo mg tilidine and 8 mg naloxone; TN32: loo mg tilidine and 32 mg naloxone; placebo. In a recent study (Bromm and Scharein, EEG Journal 52, 94-1o3, 1982) the evoked cerebral potentials were decomposed by means of principal component analysis into 6 independent basic wave forms, two of which were shown to correlate highly significantly with the subjective pain sensation. The same components reflect the effects of tilidine induced analgesia, as shown now. No component arose or vanished under any of the applied drugs. Both variables, the cerebral potential and the subjective pain rating, were markedly depressed by about 25 % under pure tilidine. The high dosage of naloxone (32 mg) markedly antagonised the actions of tilidine. These findings indicate that naloxone in the high oral dosage arrives clearly at its sites of action to block the tilidine effects, whereas no significant differences were found between the TN8- and the tilidine treatments. Pure naloxone influenced, on the average, the evoked potential parameters, with little or no effects on the subjective pain ratings. These findings were discussed with regard to a suppression of stress induced analgesia due to naloxone.

Physiologisches Institut der Universität Hamburg Martinistr. 52, D-2ooo Hamburg 2o

344

STUDIES ON THE PHYSIOLOGICAL SIGNIFICANCE OF β-CASOMORPHINS

H. Teschemacher, M. Umbach, J. Svedberg, U. Hamel and H. Bostedt

β-Casomorphins are peptide fragments contained in the amino acid sequence of β-casein and behave like opioid agonists of the μ-type when liberated from β-casein (Brantl et al., Life Sci. 28: 1903, 1981).

We tried to obtain information on an eventual physiological role of these opioids. As a first step, radioimmunoassays were developed for determination of β-casomorphin immunoreactive materials in extracts from plasma or from enzymatic milk digest; using these techniques, after ingestion of bovine milk, plasma or contents of the gastrointestinal tract were assayed for the presence of β-casomorphin immunoreactive materials in new-born calves or adult humans, resp.

In adult humans, considerable amounts of β-casomorphin immunoreactive materials were found in the gastrointestinal tract, but no such material was found in the plasma. In new-born calves, a β-casomorphin immunoreactive material was found in the plasma; however,as chromatographical analysis showed, that material was not identical with any β-casomorphin, but rather might represent a precursor thereof. The absence of any β-casomorphin in the plasma as found in humans or calves is compatible with another finding raised by our group; β-casomorphins proved to be degraded in human or bovine plasma very fast.

In summary, in new-born mammalia, from a β-casomorphin precursor absorbed from the gastrointestinal tract β-casomorphins might be liberated to elicit effects via any opiate receptors, e.g. in the central nervous system.

Rudolf Buchheim-Institut für Pharmakologie und Geburtshilfliche Veterinärklinik der Justus-Liebig-Universität Gießen, Frankfurter Str. 106/107, 6300 Gießen, FRG

345

LOPERAMIDE INHIBITS DEOXYCHOLIC ACID INDUCED RAT COLONIC SECRETION BY PERMEABILITY REDUCTION

U.M. Farack and K. Loeschke

The antidiarrheal effect of the opiate analogue loperamide (lop) has been attributed to an inhibition of intestinal motility. Recent studies have also demonstrated an inhibitory effect of lop on the secretory action of prostaglandin E_2 and choleratoxin. The aim of this study was to examine the effect of lop on deoxycholic acid (DOC) induced rat colonic secretion, epithelial permeability and mucosal Na-K-ATPase.
2 h prior to laparotomy female Wistar rats received intragastrically lop (4 mg/kg) or its solvent (4 ml/kg), ethanol: H_2O, 1:20 (V/V)). Ligated colonic loops were filled with saline with and without 3 mmol DOC.-For permeability studies 5 µCi ^{14}C-erythritol was injected intravenously. - After an incubation time of 45 min, content and length of the colonic loops were measured and the mucosa scraped off for determination of Na-K-ATPase. ^{14}C-erythritol clearance was calculated from the ^{14}C-radioactivity in the loop content and blood.
Lop did not significantly affect basal fluid absorption (lop: 100±13 (7); controls: 89±13 (7)) but significantly reduced DOC induced net fluid secretion (-15±4 (8); controls: -41±5 µl/45 min.cm (7)). This was accompanied by a significant reduction of the DOC augmented ^{14}C-erythritol clearance (236±24(7); lop: 172±15 $\times 10^{-5}$ µl (7)). Na-K-ATPase was not affected by lop.
In conclusion, DOC induced colonic secretion is in part inhibited by lop which acts via a reduction of DOC augmented epithelial permeability.

Medizinische Klinik Innenstadt, Universität München, Ziemssenstr. 1, D-8000 München 2

346

THE EFFECT OF A VASOPRESSOR RECEPTOR ANTAGONIST ON VASOPRESSIN-INDUCED ß-ENDORPHIN RELEASE FROM RAT ANTERIOR PITUITARY IN VITRO

L. Homolka and M. Vlaskovska

The circulating hormone vasopressin is known to induce antidiuresis and vasoconstriction. These actions of vasopressin are thought to be mediated by two different types of receptors: antidiuresis is brought about by occupation of the so-called V_2-receptor coupled to adenylate cyclase, whereas vasoconstriction is mediated by the so-called V_1-receptor, which does not appear to be coupled to adenylate cyclase. Several lines of evidence suggest that vasopressin acts directly on the anterior pituitary gland to release ACTH and ß-endorphin. In order to gain insight into the receptor type involved, we studied in vitro the effect of the vasopressin analogue d$(CH_2)_5$Tyr(Me)AVP on ß-endorphin release from rat anterior pituitary quarters stimulated by vasopressin. d$(CH_2)_5$Tyr(Me)AVP antagonizes the pressor response to vasopressin (Kruszynski et al., J. Med. Chem. 23, 364, 1980).
Arginine vasopressin elicited the release of ß-endorphin-like immunoreactivity (ß-EI) in a concentration-dependent manner (ED50=10nM), whereas d$(CH_2)_5$Tyr(Me)AVP had no effect in concentrations up to 100 µM, when given alone. However, d$(CH_2)_5$Tyr(Me)AVP inhibited the release of ß-EI induced by vasopressin 10 nM (IC50=2 µM). d$(CH_2)_5$Tyr(Me)AVP diminished also the vasopressin-induced accumulation in the medium of cAMP, which is considered as to be the second messenger of ß-endorphin release.
These findings will be discussed with respect to the relation of the receptor type involved to the existing categories of vasopressin receptors.

Pharmakologisches Institut der Universität Freiburg, Hermann-Herder-Str. 5, D-7800 Freiburg i.Br.

347

TONIC ENDORPHINERGIC CONTROL OF LUTEINIZING HORMONE (LH) SECRETION IN PREPUBERTAL RATS

R. Schulz, A. Wilhelm and K.M. Pirke

Both the opiate antagonist naloxone and the α_2-adrenoceptor agonist clonidine stimulate the release of LH in adult male and female rats. In contrast, prepubertal rats display highly sex-specific effects. They involve either endorphinergic or adrenergic control mechanisms for the LH secretion. While naloxone increases LH secretion in female prepubertal rats, clonidine fails to do so. On the other hand, clonidine considerably increases plasma LH levels in males, while naloxone does not. This apparent lack of a tonic endorphinergic inhibition of LH secretion, being confined to males, suggests a role of testosterone in the regulation of LH release. To study the effect of this androgen, male and female rats were gonadectomized on the 1st day of life and tested on the 10th day for the response to naloxone. Under these conditions, both male and female rats increased LH secretion upon challenge by naloxone, while clonidine increased the LH level only of male castrates. Replacement of testosterone in gonadectomized rats prevents the naloxone effect on LH secretion only in male litters. In contrast, testosterone given to females only moderately reduced the naloxone stimulation of LH release. These findings suggest that endorphins tonically inhibit LH release both in intact male as well as female rats. But testosterone, being predominantly present in males, masks the endorphinergic mechanism in males. Furthermore, it is unlikely that the sexual differentiation of the brain, occurring in rats during the first two days of life, is responsible for the tonic inhibitory effect of endorphins in the control of LH secretion.

Max-Planck-Institut für Psychiatrie, Abt. Neuropharmakologie, Kraepelinstrasse 2, D-8000 München 40, F.R.G.

348

TWO KINDS OF DOSE-RESPONSE RELATIONSHIPS OF ANESTHETICS IN VERTEBRATE RETINA: SIGNAL PROCESSING VS. ENERGY METABOLISM

W. Sickel

Several local and central anesthetics were applied in the perfusate of isolated frog retinas and the effects on light responses and on oxygen uptake measured simultaneously (Hdb.Sens.Physiol.VII,2).

The ratio of the efficacies seen in the two criteria reflects a margin of safety and goes parallel with the extent to which the drug effects are reversible. The span was large with local anesthetics but narrow with central anesthetics.

Depending on the neural pathways activated, i.e. on the state of adaptation, the dose-response curves differed. Pentobarbital depressed the light responses more in dark adaptation, whereas chloral hydrate was more potent in light adaptation.

On a log plot the curves deviated from a straight line. Thus, within ranges, high doses of both procaine and pentobarbitale showed less effective than lower ones when judged by the responses to light flashes, but not so from the oxygen uptake. Hence, excitatory and inhibitory processes were affected, respectively. Desinhibition was observed with pentobarbital in light adaptation, with chloralose in dark adaptation.

The selective effects of drugs were investigated to better understand the retinal network; they might also furnish a rationale for the therapeutic selection and combination of anesthetics.

Physiologisches Institut der Universität Köln
Robert-Koch-Strasse 39, D - 5000 Köln 41

349

EFFECTS OF ACH AND SPECIFIC CHOLINERGIC ANTAGONISTS ON DISCHARGE PROPERTIES OF RESPIRATORY MODULATED NEURONS

G. Böhmer and Pia Schmidt

Ach, the muscarinic antagonist atropine and the nicotinic antagonist hexamethonium were applied to respiratory modulated neurons (RMN) by iontophoretic techniques. Two barrels of 4- or 5-barreled microelectrodes were filled with 2 M NaCl and were used for extracellular recording and for ballance of iontophoretic currents respectively. The latter was also used to test the effects of currents. Drugs were ejected by cationic currents of 30-100 nA. Discharge density of most RMN was increased by Ach. When affected burst duration was predominantly shortened by Ach. In a great potion of RMN tested atropine decreased spike density and shortened burst duration. In all but one RMN tested hexamethonium increased spike density, while burst duration was shortened or remained unaffected in 50 % each. Effects of the drugs could be classified as mediated by excitation of muscarinic or nicotinic receptors. Excitation of muscarinic receptors mainly resulted in increase of firing rate and shortening of burst duration. Excitation of nicotinic receptors resulted in decrease of firing rate, while alteration of burst duration seems inconsistent.

Physiol. Inst. Univ. Mainz, Saarstr. 21, D-6500 MAINZ

350

LOCALIZATION OF DRUG EFFECTS IN THE CNS BY THE DEOXY-GLUCOSE METHOD.

Th. Beck, J. Krieglstein and P. Vogg

In recent years the localization of functional activity in the CNS by measuring glucose utilization with radioactive 2-deoxyglucose (Sokoloff et al., J. Neurochem. 28: 897, 1977) has become a widely accepted method in neurochemistry. In the present study we used this technique for characterizing the effects of some centrally active drugs. The local cerebral glucose utilization (LCGU) was determined after administration of diethylpemoline, tifluadome, pentobarbital and dihydroergocristine in various cerebral structures of the rat. According to earlier experiments pentobarbital reduced LCGU in all structures of the brain. Only minor changes could be observed after dihydroergocristine. Particularly interesting results were obtained using diethylpemoline and tifluadome. Similar to apomorphine, diethylpemoline, described as an indirect dopaminomimetic drug, changed LCGU in the frontal and sensorimotor cortex. Perpendicular to the surface of the brain, alternating areas with decreased and increased LCGU were demonstrable in these cortical structures. In layer IV and V of these structures as well as in the nuclei subthalamici and the substantia nigra LCGU clearly increased. Tifluadome is a newly synthesized benzodiazepine with agonistic properties at kappa opiate receptors. In contrast to morphine, this drug did not reduce LCGU in brain tissue but enhanced the activity of the nucleus accumbens. The results suggest the ^{14}C-2-deoxyglucose technique to be useful for localizing drug effects in the CNS.

Institut für Pharmakologie und Toxikologie im FB 16 der Philipps-Universität, Ketzerbach 63, D-355 Marburg

351

DEACETYLATION OF ACETOPHENETIDINES AND ACETAMIDOPHENOLS IN RAT BRAIN, LIVER AND KIDNEY

J. Baumann, F. v. Bruchhausen and G. Wurm*

p-Phenetidine and p-aminophenol, the deacetylation products of the analgesic-antipyretics phenacetin and paracetamol, had proved to be more potent inhibitors of the prostaglandin biosynthesis than their parent compounds (v. Bruchhausen and Baumann, Life Sci. 30:1783, 1982) and may contribute to the central effects of these drugs. In monkey brain, a so-called phenacetin deacetylase was described (Oommen et al., Life Sci. 26:2129, 1980). We studied the enzymic deacetylation of phenacetin, paracetamol as well as of their o- and m-substituted analogues using tissue preparations from rat brain, liver and kidney. Although, in general, all acetyl-aminophenol derivatives were deacetylated, especially 2-acetophenetidine was strongly split in all tissues, whereas paracetamol was a poor substrate. N-Deacetylase activity was high in liver and kidney in comparison with cerebrum and cerebellum which possessed low enzyme activity. The deacetylation of acetyl-aminophenolic compounds was inhibited by the organophosphorus diester bis(p-nitrophenyl)phosphate which is a rather specific inhibitor of the acetanilide-cleaving carboxylesterase amidase pI 5.6. On the other hand, aminophenols and phenetidines were rapidly N-acetylated in brain, liver and kidney when Acetyl-Co A was added as an acetyl donor. We, therefore, suppose that N-acetylating and N-deacetylating activities contribute to the routes of biotransformation of aminophenolic compounds in brain and other tissues.

Pharmakologisches Institut der Freien Universität Berlin Thielallee 69/73, D-1000 Berlin 33
*Institut für Pharmazie der Freien Universität Berlin, Königin-Luise-Straße 2-4, D-1000 Berlin 33

352

CENTRAL ACTIONS OF PROSTAGLANDINS D_2, E_2 AND $F_{2\alpha}$ IN THE RAT

U. Förstermann and R. Heldt

PGD_2 is the most prevalent PG in rodent brain. Therefore the effects of intracerebroventricular (icv) administration of this PG were investigated and compared to those of PGE_2 and $PGF_{2\alpha}$. PGD_2 and PGE_2 caused sedation and significantly reduced spontaneous motor activity at 2 ug icv. $PGF_{2\alpha}$ produced comparable effects only at 20 ug icv. PGD_2 and PGE_2 (20 ug icv) attenuated convulsions induced by pentylenetetrazol. Also in this respect $PGF_{2\alpha}$ was less effective. These data indicate, that PGD_2, like PGE_2, exerts depressant effects on the central nervous system, whereas $PGF_{2\alpha}$ is less depressant. Thus the inhibition of the synthesis of the predominant PGD_2 by aspirin-like drugs may explain some of the recently described proconvulsive effects of these compounds (Förstermann et al., Brain Res. 240 (1982) 303).

PGE_2 and $PGF_{2\alpha}$ (2 ug - 20 ug icv) dose-dependently caused a marked increase in arterial blood pressure and concomitantly increased body temperature. PGD_2 (20 ug icv) had only a weak pressor effect and a small pyrogenic action. PGD_2 (2 ug icv) had no significant effect on blood pressure and even slightly decreased body temperature. Thus there might be a correlation between the central effects of PGs on blood pressure and body temperature. The intense peripheral vasoconstriction caused by PGE_2 and $PGF_{2\alpha}$, which enhances blood pressure, may lead to a decreased capacity to loose heat, resulting in hyperthermia. On the other hand PGD_2, which gives rise to only a small increase in blood pressure, results only in a small rise in body temperature.

Department of Pharmacology, University of Freiburg, Hermann-Herder-str. 5, D-7800 Freiburg

353

TETRAHYDROBIOPTERIN AND TOTAL BIOPTERIN CONTENT IN VARIOUS CELL CLONES AND THE RELATIONSHIP BETWEEN TYROSINE HYDROXYLASE ACTIVITY AND INTRACELLULAR TETRAHYDROBIOPTERIN CONTENT IN THE PHEOCHROMOCYTOMA CLONE PC-12

M. Bräutigam and R. Dreesen

Tetrahydrobiopterin content was determined in several clonal cell lines with reversed-phase high pressure liquid chromatography and subsequent electrochemical detection. Total biopterin was measured with fluorimetric detection as described by Fukushima et al. (Anal. Biochem. 102:179, 1980). The catecholamine producing clones neuroblastoma N1E-115 and pheochromocytoma PC-12 contained 96 ng and 60 ng tetrahydrobiopterin/mg protein, respectively. The corresponding amount for the neuroblastoma clone N2A was 36 ng/mg protein. The tetrahydrobiopterin content in C 6-glioma cells was below the limit of detection. The total biopterin in tetrahydrobiopterin containing clones was about 20% above the tetrahydrobiopterin content. Addition of 2,4-diamino 6-hydroxypyrimidine (DAO-Pyr) - an inhibitor of D-erythro-q-dihydroneopterin triphosphate synthetase - to the culture medium of PC-12 cells resulted in a dose-dependent decrease of tetrahydrobiopterin and total biopterin content within 4 h, suggesting that the cells are capable of synthesising the found biopterin. A significant decline of tyrosine hydroxylase activity (measured as DOPA-production) after blockade of biopterin synthesis with DAO-Pyr was only seen after the intracellular tetrahydrobiopterin content was reduced by at least 70% of the control levels.

Pharmakologisches Institut der Freien Universität Berlin
Thielallee 69/73, D-1000 Berlin 33.

354

STIMULATION OF THE SUPERIOR CERVICAL GANGLIA ALTERS ACTIVITY OF SINGLE PINEAL CELLS IN THE RAT

St.Reuss and L.Vollrath

In the mammalian pineal gland melatonin formation has been clearly shown to be regulated by sympathetic fibres originating in the superior cervical ganglia (SCG) (SCG). In addition central commissural fibres reach the gland but their function is not known.(For review see L.Vollrath: The Pineal Organ, Springer Verlag, 1981). Recently it has been demonstrated that electrical stimulation of the SCG stimulates pineal melatonin formation, as assessed by measuring one of the melatonin forming enzymes (serotonin-N-acetyltransferase) (C.W.Bowers, R.E.Zigmond,J.Physiol.330, 279,1982). The aim of our study was to examine the effect of SCG stimulation (1-10 c/sec; 0.5-5 mA) on single unit activity in the pineal gland of urethane-anesthetized rats.
Extracellular recordings revealed that one group of cells tested was not affected by SCG stimulation. A second group of spontaneously active cells was inhibited, while a third group of "dormant" cells was stimulated.
These findings suggest a complicated innervation pattern of the intrinsic pineal cells.

Anatomisches Institut, Neurophysiologisches Labor,
Johannes Gutenberg-Universität, Saarstr.19/21,
6500 Mainz

355

MEMBRANE POTENTIAL-DEPENDENT ACCUMULATION OF ^{3}H-IMIPRAMINE INTO INTACT SYNAPTOSOMES FROM RAT CEREBRAL CORTEX.

G. Schmalzing

Purified synaptosomes were incubated at 37°C for 10 min under various conditions and separated from medium by silicon oil centrifugation (50 000 g for 20 min) at 4°C or 37°C, respectively. Steady-state binding of ^{3}H-imipramine (^{3}H-IP) was markedly temperature-dependent: high-affinity sites (K_D 2 nM) which were easily demonstrable by 4°C centrifugation were barely detectable by separation at 37°C.
The accumulation of ^{3}H-IP at 37°C was found to depend on free Ca^{2+}, which was adjusted with Ca/OH-EDTA buffers. The accumulation ratio $(^3H\text{-IP})_i/(^3H\text{-IP})_o$ (2 nM, 76 Ci/mmol) increased from 440 without Ca^{2+} to maximally 580 at 12 µM Ca^{2+}. The synaptosomal plasma membrane potential $\Delta\Psi_S$ was calculated from the distribution of ^{3}H-triphenylmethylphosphonium$^+$ (^{3}H-TPMP$^+$) in the presence of tetraphenylboron anion + CCP and from $(K^+)_i/(K^+)_o$. $\Delta\Psi_S$ showed a Ca^{2+}-dependent increase from 52 mV at 0 Ca^{2+} to 83 mV at 12 µM Ca^{2+}. The uptake of ^{3}H-serotonin and ^{3}H-norepinephrine was augmented by increasing Ca^{2+} in a similar manner as the accumulation of ^{3}H-IP, ^{3}H-TPMP$^+$ and K^+.
The membrane potential-dependent ^{3}H-IP accumulation was diminished at higher IP concentrations and disappeared above 1 µM IP. Addition of valinomycin or A 23 187 led to a decrease of $\Delta\Psi_S$ and a concomitant reduction of the ^{3}H-IP accumulation ratio to 350 or 240, respectively. ΔpH as determined by ^{14}C-dimethyloxazolidine distribution remained unaffected by free Ca^{2+}.

It is concluded that under certain conditions IP penetrates the synaptosomal plasma membrane in its cationic (IP·H$^+$) rather than in its uncharged form. The role of $\Delta\Psi_S$ as a driving force of ^{3}H-IP accumulation was confirmed by addition of 52 meq/l KCl, which induced a marked efflux of both, ^{3}H-TPMP$^+$ and ^{3}H-IP·H$^+$.

Institut für Toxikologie der Universität Tübingen

356

COMPARISON OF DICLOFENSINE (Ro 8-4650) WITH NOMIFENSINE FOR NEUROCHEMICAL EFFECTS IN RAT BRAIN

H.H. Keller and M. Da Prada

Diclofensine (DICLO, rac-4-(3,4-dichlorphenyl)-1,2,3,4-tetrahydro-7-methoxy-2-methylisoquinoline), an effective antidepressant in man, has been shown to inhibit potently , in vitro and ex vivo, the synaptosomal uptake of dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) , whereas, in this respect, nomifensine (NOMI) was less effective by a factor of at least 10, 2 and 100, resp. (Burkard et al., Front. Neuropsychiatr. Res., E.Usdin, ed., Mac Millan Press, 1982). DICLO at 10 mg/kg i.p. reduced homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) by 20 - 30 % for at least 6 h and attenuated their accumulation after probenecid. These effects may be the consequence of marked inhibition of amine uptake , leading to increased synaptic DA and 5-HT concentration and stimulation of synaptic receptors, ultimately resulting in reduced DA and 5-HT turnover. In contrast, NOMI (10-30 mg/kg i.p.) increased HVA and enhanced the accumulation of HVA and 5-HIAA after probenecid. - In vitro, the K^+-stimulated ^{3}H-DA release from striatal slices in the presence of cocaine was increased by NOMI but little,if any, by DICLO. Also, the spontaneous ^{3}H-DA release from striatal slices of reserpinized rats was elevated by NOMI, but much less by DICLO.
The increment of 5-HIAA after NOMI cannot be due to a 5-HT-releasing effect since NOMI (as well as DICLO) did not enhance spontaneous and K^+-induced ^{3}H-5-HT release from cerebral cortex slices.
In conclusion, DICLO is a potent uptake inhibitor for all of the 3 amines and devoid of releasing action at lower doses, whereas NOMI at higher doses induces DA release.

Pharm. Res. Deptm., F. Hoffmann-La Roche & Co., Ltd.,
CH 4002 Basel, Switzerland

357

EFFECTS OF DESIMIPRAMINE AND NOMIFENSINE ON THE UPTAKE OF ^{3}H-DOPAMINE INTO DOPAMINERGIC AND NORADRENERGIC NEURONES OF THE ISOLATED NEUROINTERMEDIATE LOBE (NIL) OF THE RAT

K. Racké

The NIL has a rich dopaminergic innervation. However, little is known about the regulation of the dopamine (DA) release from the nerve terminals in the NIL. Since the NIL contains also noradrenergic nerve fibres, the present study was performed to test whether the stores of the DA-fibres in the NIL could be selectively labelled with ^{3}H-DA.

NILs were isolated from male rats fixed at the stalk by a platinum wire clip and incubated for 30 min in 0.5 ml Krebs solution containing ^{3}H-DA (0.1 µM). After superfusion with ^{3}H-DA free medium for 60 min the tissue was analysed for ^{3}H-NA and ^{3}H-DA. NA, DA and the metabolites were separated on alumina and Dowex 50WX4 columns.

In control experiments 416±50 fmol ^{3}H-DA and 133±15 fmol ^{3}H-NA (n=5) were found in the NIL homogenates. ^{3}H-DA and ^{3}H-NA represented 92±2 % of the total tissue radioactivity. Addition of 1 µM desimipramine (DMI) to the medium during the labelling period had no effect on the storage of ^{3}H-DA (394±34 fmol, n=3) in the NIL but reduced the formation of ^{3}H-NA by 86 %. When DMI (1 µM) was added 15 min prior to the labelling period and was also present throughout the superfusion, the formation of ^{3}H-NA was reduced by 97 %, whereas the storage of ^{3}H-DA was unaffected (397±19 fmol, n=3). Nomifensine (10 µM) reduced the storage of both ^{3}H-DA and ^{3}H-NA by more than 95 %.

The present results show that in the isolated NIL ^{3}H-DA is taken up in both, dopaminergic and noradrenergic nerve terminals. DMI selectively blocks the uptake into noradrenergic fibres. After labelling in the presence of DMI, tritium release from the NIL is supposed to reflect DA release from dopaminergic nerve terminals.

Pharmakologisches Institut der Universität Mainz, Obere Zahlbacher Str. 67, 6500 Mainz

358

ACTION OF THE α_2-ADRENOCEPTOR AGONIST B-HT 958 ON BRAIN DOPAMINE AND NORADRENALINE TURNOVER.

H.Hörtnagl, U.Petsche and O.Hornykiewicz

In addition to their well established modulation of noradrenaline (NA) release, α_2-adrenoceptor agonists influence the dopamine (DA) turnover in the central nervous system. We therefore investigated the modulating effects of B-HT 958 (2-amino-6-(p-chlorobenzyl)-4H-5,6,7,8-tetrahydrothiazolo [5,4-d] azepine) on aminergic neurons in the rat and mouse brain. The central effects of B-HT 958 are of special interest since at peripheral α_2-adrenoceptors this compound is characterized by a high pre/postsynaptic activity ratio (Pichler et al., N.S.Arch.Pharmacol. 320, 110, 1982).

The turnover of DA and NA was studied by measuring the disappearance of the amines after inhibition of their synthesis with α-methyl-p-tyrosine (α-MpT, 250 mg/kg,i.p./4 hrs). Groups of 6 animals were injected with either saline or B-HT 958 (in a dose range of 0.2-20 mg/kg,s.c.) or clonidine (0.1 mg/kg,i.p.) 15 min before the injection of α-MpT. DA and NA levels in various brain areas were measured by HPLC with electrochemical detection.

B-HT 958 caused dose-dependently a considerable inhibition of the α-MpT induced decline of DA in the striatum (35% vs 72% of control values) and in the hypothalamus (21% vs 35%) of the rat and in the whole mouse brain (40% vs 72%). In brain areas with a relatively dense NA innervation (hypothalamus, hippocampus, amygdala-pyriform cortex) B-HT 958, in contrast to clonidine, did not inhibit the NA turnover. However, in the striatum, which receives a sparse NA innervation, B-HT 958 almost completely inhibited the NA decline produced by α-MpT.

The present data indicate that B-HT 958 differs from clonidine by its failure to modulate noradrenergic activity in several NA rich brain areas while strongly affecting DA turnover.

Institut für Biochemische Pharmakologie, Universität Wien, Borschkegasse 8 a, 1090 Wien.

359

Demonstration of Central α_2-Adrenoceptor Effects Via Apomorphine Induced Hypermotility

H.-P. Kley, U. Brand, H. Müller

The influence of noradrenergic mechanisms on dopaminergic motor activity, e.g. in Parkinson's disease, is still a matter of discussion (S.M. Antelman et al., Science 195:646(1977)). Postmortem brains of patients with Parkinson's disease not only show a decrease of dopamine (DA) in several regions but also a decrease of norepinephrine (NA), e.g. in l. coeruleus, s. nigra, and n. accumbens (I.J.Farley et al. in: Advances in Parkinsonism, Eds. W.B.Birkmayer and O. Hornykiewicz, Roche, Basel, p.178(1976)). There is evidence that direct or indirect increase in NA leads to an increase in motor activity (MA) mediated by direct stimulation of DA receptors (N.-E.Anden et al. Naunyn-Schmiedeberg's Arch. Pharmacol. 302:299 (1978)).

Our own experiments with the α_2-receptor-stimulating agent clonidine showed a potentiation of MA in non-reserpinized mice after 1 and 4 mg/kg apomorphine (Apo) s.c. and a diminution of MA in naive mice. Mianserin, an α_2-receptor-blocking agent with antidepressant properties, antagonized MA after 1 mg/kg Apo and did not influence MA after 4 mg/kg Apo. The α_2-receptor-blocking agent yohimbine did not influence MA after 1 mg/kg Apo but antagonized the effect of 4 mg/kg Apo. Further, studies with other agents acting on α_2-receptors revealed that, in intact animals, it is possible to demonstrate whether this action is of a blocking or stimulating type.

The influence of noradrenergic mechanisms on different DA-receptors (DA_i, DA_e) (A.R.Cools et al., Psychopharmacologia 45:243 (1976)) is still open for discussion.

From the Research Laboratories of Byk Gulden Lomberg Chemische Fabrik GmbH Byk-Gulden-Str. 2, D-7750 Konstanz

360

2-BROMOLISURIDE: AN ERGOT DERIVATIVE WITH POTENTIAL NEUROLEPTIC ACTIVITY

H. Wachtel

2-Bromolisuride, a derivative of the ergot dopamine (DA) agonist lisuride, was investigated in rodents with regard to its influence on DA related behaviour, cerebral DA metabolism and prolactin (PRL) secretion.

2-Bromolisuride produced catalepsy (ED_{50} in mice 3.3 mg/kg i.p.; in rats 3.9 mg/kg i.p.), antagonized apomorphine-induced stereotypies (ED_{50} in mice 0.4 mg/kg i.p.; in rats 0.2 mg/kg i.p.), inhibited locomotor activity in rats (0.025 - 6.25 mg/kg i.p.), antagonized the hyperactivity induced by various DA agonists in rats (0.1 - 1.56 mg/kg i.p.) and inhibited the apomorphine-induced hypothermia in mice and rats (0.025 - 6.25 mg/kg i.p.). 2-Bromolisuride (0.03 - 10 mg/kg i.p.) stimulated DA biosynthesis and DOPAC formation in the striatum and the DA rich limbic system of rats. Furthermore, 2-bromolisuride (0.025 - 6.25 mg/kg s.c.) enhanced PRL secretion in intact male rats.

These findings indicate DA antagonistic properties of 2-bromolisuride presumably due to blockade of central DA receptors. 2-Bromolisuride is the first ergot compound with definite antidopaminergic properties suggesting its potential usefulness as a neuroleptic.

Dept. of Neuropsychopharmacology, Schering AG, Müllerstr. 170-178, D-1000 Berlin 65

361

HALOPERIDOL-INDUCED INCREASE OF SPIPERONE BINDING: INFLUENCE OF PHENOBARBITAL, DIAZEPAM AND SUPIDIMIDE

H.-H. Hennies and L. Flohé

It has been reported that chronic treatment with neuroleptics elevates DA receptor binding capacity in brain (Burt et al., Science, 196, 326, 1977). The influence of simultaneously administered phenobarbital, diazepam or supidimide [2-(2-oxo-3-piperidyl)-1,2-benzisothiazoline-3-on-1,1-dioxid; Friderichs et al., Arzn.Forsch./Drug-Res., 32II, 898, 1982] on the haloperidol-mediated increase in rat striatal [^{3}H] spiperone binding was investigated.
Chronic oral application of haloperidol for 14 days (one application per day of 10 mg/kg) followed by a drug free period of 5 days results in a significantly enhanced amount of [^{3}H] spiperone bound per mg striatal receptor protein. In contrast to phenobarbital (50 and 75 mg/kg i.p.) and diazepam (10 and 20 mg/kg i.p.), supidimide ($\hat{=}$ 300 mg/kg i.p.) antagonized the haloperidol-mediated increase in radioligand binding. The results appear consistant with the previous observation that supidimide in mice chronically treated with haloperidol inhibits or at least delays the development of tolerance (Wilsmann and Friderichs, Arzn.Forsch./Drug Res., 32II, 900, 1982), an effect which is believed to be associated with increased DA binding capacity and DA supersensitivity.

Grünenthal GmbH, Center of Research, Zieglerstraße 6, D-5100 Aachen-Eilendorf

362

EFFECTS OF DOPAMINE ON HIPPOCAMPAL CELLS IN THE DENTATE GYRUS AND THE PYRAMIDAL CELL LAYER.

G. Gmelin and T. Dunwiddie

The effects of Dopamine (DA) were studied in the hippocampal slice preparation. Coronal slices of male Spraque-Dawley rat brains were cut at 350 - 400 µm and incubated at 36°C in two types of perfusion chambers: in one the slices were totally superfused with the medium and in the other the medium reached only a level just below the surface of the slice. Population spikes recorded from granule cells were induced by stimulation of the perforant pathway and those from CA_1 pyramidal cells were induced by stimulation of the stratum radiatum near the border of CA_1 and CA_2. When (10 ... 50 µM) was applied in the bath, the amplitude of the population spike measured in the granule as well as in the pyramidal cell layer was markedly increased. In both superfusion conditions, the DA effect was quantitatively the same except that it appeared earlier when the slices were totally submerged. In the granule cell layer the action of DA was seen more often than in the CA_1 layer. When fluphenazine (100 mM) was applied locally with short pressure pulses using a micropipette near the recording electrode, the increase in the amplitude of the population spike could be blocked in both layers. When fluphenazine (100 µM) was added to the superfusion medium, even with 30 min pretreatment, no action on the DA-effect could be seen. One explanation could be that fluphenazine had local anaesthetic effects when applied with the micropipette. Experiments with other DA-antagonists are in progress.

Preclinical Research, SANDOZ AG, CH-4002 Basel and Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262, USA.

363

MONOAMINE METABOLITES IN THE CSF OF UNRESTRAINED CATS.

M. Spindler, I. Degrell, K. Zenner, G. Stock

The concentration of dihydroxyphenylacetic acid (dopac) and homovanillic acid (HVA), the metabolites of dopamine (DA) and 5-hydroxyindoleacetic acid (5-HIAA), the metabolite of serotonin, were measured repeatedly in the CSF of unrestrained cats. Animals were chronically equipped with cortical and subcortical EEG-electrodes. Samples of 10 µl CSF were collected and immediately analyzed by a HPLC method with electrochemical detection. Concentrations varied considerably between subjects but were stable within the same subject for periods of up to 150 days.

n = 8	dopac	HVA	5-HIAA
ng/ml			
mean ± S.E. (range)	70.3±30.6 (30 - 130)	346.6±168 8 (180 - 750)	220.2±65.7 (110 - 340)

Under control conditions a marked gradient for monoamine-metbaolites was found when CSF samples from frontal sites of the lateral ventricle were compared to samples from dorsal sites. After application of neuroleptic drugs (haloperidol, pimozide), there was a marked increase in dopac and HVA but not in 5-HIAA. In contrast, application of 3-PPP (4 mg/kg, i.p.), a DA-agonist with preferential activity on presynaptically located DA-receptors markedly decreased the CSF-levels of dopac and HVA for a period of 4 hours. The same result was obtained with apomorphine (1.5 mg/kg i.p.).
The proposed animal model can be used to obtain electrophysiological, behavioural, and biochemical observations in parallel.

I.Physiol.Inst., Univ. Heidelberg, INF 326, 69 Heidelberg

This study was supported by the DFG within the SFB 90

364

RELATIONSHIP BETWEEN THE DOPAMINERGIC SYSTEM AND ORAL DRUG TAKING BEHAVIOR

V. Fuchs and H. Coper

Naive rats having the free choice between an etonitazene solution of 3 µg/ml and tap water show after a few days a preference for the opioid source. In contrast to etonitazene, 3 mg/ml barbital are rejected when water is available simultaneously. After 14 days of forced barbital drinking and repeated withdrawal cycles, however, in the free choice procedure vs water 33% of the rats ingested an average of 120 mg/kg barbital per day. The remaining animals rejected the barbiturate. Addition of 7 and 14 µg/ml haloperidol to the water bottles reduces the "voluntary" etonitazene intake from 70% of daily fluid consumption to 47% and 30% respectively, whereas under the same conditions the spontaneous barbiturate ingestion is not affected by the neuroleptic. In a second experiment rats received 0.3 mg/kg/day etonitazene for 6 days. Then they were treated with 1 mg/kg apomorphine and the stereotypic licking, gnawing, head shaking and foot stepping were recorded from the 10th to 60th min after the apomorphine injection. In the etonitazene rats the number of the stereotypic movements was not significantly altered (88% compared to controls). On the second day of etonitazene withdrawal, the stereotypies were 84% of control and 111% at the 6th day. In the barbital treated rats, however, the apomorphine stereotypies are reduced to 32%. At the 6th day of barbital withdrawal the control level is reached. Further experiments are necessary to clear up the involvement of the dopaminergic system in drug taking behavior of different addictive drugs.

Institut für Neuropsychopharmakologie der Freien Universität Berlin, Ulmenallee 30, D-1000 Berlin 19

365

EFFECTS OF DU 24565 (6-NITROQUIPAZINE) AND QUIPAZINE ON SEROTONINERGIC AND NORADRENERGIC NEURONES OF THE RAT BRAIN CORTEX

E. Schlicker, K. Classen and M. Göthert

The effects of DU 24565 and quipazine were compared in uptake studies and superfusion experiments on rat brain cortex slices.- DU 24565 blocked tritium accumulation in slices incubated with ^{3}H-serotonin more markedly than in slices incubated with ^{3}H-noradrenaline (IC_{50} values: 0.08 and 89.1 µmol/l, respectively); by contrast, quipazine exhibited no marked preference (IC_{50} values: 5.25 and 4.90 µmol/l, respectively).- In slices preincubated with ^{3}H-serotonin, DU 24565 facilitated the electrically evoked tritium overflow in the presence or absence of paroxetine (a selective inhibitor of serotonin uptake) to the same degree; however, it had no effect on slices exposed to paroxetine and pargyline. Quipazine increased the evoked overflow from slices superfused with paroxetine or paroxetine plus pargyline to a similar extent (Schlicker and Göthert, Naunyn-Schmiedeberg's Arch. Pharmacol. 317, 204, 1981). The concentration-response curves of unlabelled serotonin (obtained in the presence of paroxetine) and of noradrenaline for their inhibitory effects on the evoked overflow were shifted to the right by quipazine but not by DU 24565.- It is concluded that, by contrast to its parent compound quipazine, DU 24565 is a highly potent and selective inhibitor of neuronal serotonin uptake; DU 24565, which may inhibit monoamine oxidase, is at least less potent than quipazine in blocking presynaptic serotonin and α-adrenoceptors on the serotoninergic neurones.

Pharmakologisches Institut, Universität Essen, Hufelandstr. 55, 4300 Essen 1, Fed.Rep.Germany

366

^{125}I-LSD, A NEW USEFUL LIGAND FOR THE CHARACTERIZATION AND LOCALIZATION OF $5HT_2$-RECEPTORS.

G. ENGEL, F. v.AMSTERDAM and J.M. PALACIOS

According to PEROUTKA and SNYDER (Mol Pharmacol 16: 687, 1979), two distinct types of serotonin (5HT) receptors have been postulated. $5HT_1$-receptors are labelled by ^{3}H-5HT, and $5HT_2$-receptors can be identified by ^{3}H-spiperone or ^{3}H-ketanserin (LEYSEN et al., Mol pharmacol 21: 301, 1982). LSD was iodinated with ^{125}I-Na and chloramine T to get the radioligand ^{125}I-LSD (^{125}IOL) and with N-succinimide to get the non-radioactive compound 2-I-LSD (IOL) for comparative pharmacological studies. The introduction of iodine in position 2 of LSD leads to an increase in selectivity for $5HT_2$-receptors. In rat cortex membranes, ^{125}IOL possesses a K_D = 0.9±0.1 nM, Bmax = 240± 20 fmoles/mg, and a non-specific binding of <40% in presence of 10^{-7} M ketanserin. The rate constants for association and dissociation are 1.6×10^8 M^{-1} min^{-1} and 0.042 min^{-1}, respectively. In competition experiments, 5HT antagonists showed monophasic displacement curves with K_I-values which correlate with pD_2-values, for inhibition of 5HT-induced contraction of canine basilar artery. The competition curves performed with 5HT are biphasic and insensitive to GppNHp (10^{-4}) M. In a routine binding screen with various ligands, the inhibition constants of IOL for a alpha, beta, histamine and cholinergic receptor are ≥ 100 nM with the exception for dopamine receptors, 39 nM. In the longitudinal muscle of guinea pig ileum, $5HT_2$-receptors could be identified which are different from those in CNS. For Ketanserin, a weak inhibition constant of 0.6 µM was found in comparison to 2 nM in the cortex. Because of the high specific activity (2175 Ci/mmol) of ^{125}IOL, it is a favourable ligand for autoradiographic localization of its binding site, requiring only short exposure time. The localization of ^{125}IOL-binding sites in the rat brain will be also presented.

Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland.

367

HIGH AFFINITY BINDING SITES OF (3H)-TETRAHYDRONORHARMANE (TETRAHYDRO-ß-CARBOLINE) AND (3H)-TRYPTAMINE ON BRAIN MEMBRANES OF RATS.

Hans Rommelspacher and Gerold Brüning

Tetrahydronorharmane (THN) occurs in vivo and exerts pharmacological effects. It displaces (3H)-spiroperidol (IC50 26 µmol/l), (3H)-5-hydroxytryptamine (IC50 4µmol/l, (3H)-naloxon (IC50 353 µmol/l) and other ligands in binding experiments (Müller et al. Pharmacol. Biochem.Behav. 14, 693, 1981). However, its affinity towards the various binding sites seems not sufficient enough to explain the biological activity. Thus, we investigated whether THN binds to receptors of the structurally related tryptamine and/or to its own receptors. Scatchard analysis of (3H)-tryptamine binding to rat brain membranes in the concentration range from 0.5 to 50 nmol/l revealed two equilibrium dissociation constants of 3±1.5 and 2 5±10 nmol/l. THN displayed high affinity inhibition (IC50 22nmol/l at 1.5 nmol/l (3H)-tryptamine). 5-Hydroxytryptamine and spiroperidol were found to be less potent (IC50 400 nmol/l and 10 µmol/l respectively). Further studies were conducted using (3H)-THN as a ligand. A high affinity binding site (kD 2 nmol/l) was identified. Low concentration of tryptamine displaced (3H)-THN (1nmol/l, IC50 20 nmol/l). It is very well conceivable that these high affinity binding sites represent the molecular site of the action of THN.

Institut für Neuropsychopharmakologie der Freien Universität, Ulmenallee 30, 1000 Berlin 19, F.R.G.

368

EFFECTS OF ACh, NICOTINE AND MUSCARINE ON RAT NEOSTRIATUM CELLS IN SLICES

U. Misgeld, M.H. Weiler, D.K. Cheong

Using sliced tissue we characterized previously an intrinsic excitatory cholinergic synapse in the rat neostriatum which is furnished with postsynaptic nicotinic and pre- and postsynaptic muscarinic receptors(Misgeld et al.,Exp.Brain Res.39:401,1980;Brain Res.:in press). In the same preparation,the effects of ACh, nicotine and muscarine injected locally into the tissue in 0.5pmole to 0.5nmole quantities were investigated. The higher doses of ACh depolarized the neuronal membranes, sometimes a fast and a slow component could be differentiated. Muscarine induced only slow depolarizations. The input resistance increased in parallell to the membrane depolarization. Small amounts or injections repeated at short intervals elicited membrane hyperpolarizations associated with membrane conductance increases. In all instances the amplitudes of the EPSPs evoked by electrical stimulation of the neostriatal tissue near the recording electrode decreased. Addition of nicotine (1mM) or muscarine(100uM) to the bath led to reduction of EPSP-amplitudes only. EPSP-amplitudes reduced by muscarine or muscarinic agonists could be restored by atropine or scopolamine (10uM). Addition of atropine alone to the bath resulted in a marked increase of locally evoked EPSP-amplitudes. Although the principle of "identity of action" is not violated, it is not possible to predict cholinergic synaptic function in rat neostriatum from the effects of extrasynaptically applied ACh because of the presence of so many different receptor sites.

Max-Planck-Institut f. Hirnforschung, Deutschordenstr. 46, D-6000 Frankfurt/M-71

369

INTERACTION OF BARBITURATE AND GABA SITES IN RAT CEREBELLAR AND CEREBRAL CORTICAL MEMBRANES

U. Quast and K.-O. Vollmer

Modulation of ^{3}H-muscimol binding by picrotoxin, pentobarbitone and (+)-etomidate was investigated in rat cerebellar and cerebral cortical membranes. In cerebellum, at 37°C in the presence of 150 mM chloride, picrotoxin and picrotoxinin decreased specific ^{3}H-muscimol binding to 43±3 % of control with midpoint (EC_{50}) at 1.2±0.1 µM. ^{3}H-muscimol saturation experiments in the presence and absence of picrotoxin indicated that the picrotoxin effect was primarily due to a loss of high affinity muscimol sites with $K_D \approx 10$ nM.

Pentobarbitone enhanced specific ^{3}H-muscimol binding to 259±3 % of control with EC_{50}=292± 37 µM and etomidate increased binding to 298± 18 % with EC_{50}=7.1±1.0 µM. The influence of temperature and chloride ion concentration on these effects was investigated at constant ionic strength. The results indicated that studies at 0°C underestimate the coupling between GABA receptors and barbiturate sites and that they greatly overestimate the importance of chloride ions in this phenomenon. In cerebral cortical membranes (37°C, 150 mM Cl^-), the effect of picrotoxin was similar to that observed in cerebellum whereas the effects of pentobarbitone and etomidate were greater but occured at higher concentrations.

Gödecke Research Institute, Dept. of Biochemistry Mooswaldallee 1-9, D-7800 Freiburg

370

GABA DIRECTLY DEPOLARIZES CULTURED OLIGODENDROCYTES

P. Gilbert

GABA depolarizes approximately one third of immunocytochemically identified oligodendrocytes in cultures of mouse spinal cord. During GABA application the depolarization is followed by a partial repolarization to a stable plateau. After GABA has been washed out the cell repolarizes to the original membrane potential. The GABA effect is dose dependent. The depolarizing effect of GABA does not result from the release of K^+ from adjacent neurons; no increase in extracellular K^+ could be measured along the surface of the reacting cell. Furthermore, no neurons were detected by immunolabeling with tetanus toxin in the vicinity of the studied oligodendrocytes. The depolarization was not accompanied by a change in cell input resistance. Muscimol produces a similar depolarization as GABA. Biuculline, picrotoxin,pentobarbital and chlordiazepoxid all reduce the GABA induced depolarization.

Inst. für Neurobiologie, Im Neuenheimer Feld 504 D-6900 Heidelberg

371

EFFECTS OF PENTYLENETETRAZOLE ON ELECTRICAL PROPERTIES AND SYNAPTIC RESPONSES OF HIPPOCAMPAL RAT NEURONS IN VITRO

M.L. Zeise, U. Preisendörfer and L. Heymann

Effects of pentylenetetrazole (PTZ) were investigated in slices (300 µm) of the rat hippocampus. Compared to other convulsants an about 100 times higher concentration (2-10 mM/l) was necessary to yield repetitive population spikes in field responses of the CA3 pyramids and the granule cells. Intracellular recordings from granule cells with bath application of 2 mM/l PTZ revealed an abolition of the IPSP and a remarkable increase in amplitude and duration of the EPSP. RMP and spike amplitude increased. 10 mM/l PTZ reduced these parameters to control values while the spike duration increased. Repetitive discharges were elicited only with tenfold threshold stimulus strength. Even with more than 1 h application of PTZ (10 mM/l) neither spontaneous spikes nor PDS appeared. Significant changes of membrane resistance were not observed with PTZ (10 mM/l).
GABA applied locally (10 mM/l) reduced membrane resistance, EPSP amplitude and membrane potential in granule cells. These effects were only slightly decreased by PTZ (2 mM/l). PTZ influenced GABA effect on membrane potential most, the change of EPSP was least affected by PTZ. With 10 mM/l PTZ GABA effects were reduced by approx. 65%. Although PTZ reduced the amplitude of the GABA response, GABA effects persisted for a longer time than in control.

Max-Planck-Institut für Hirnforschung, Neurophysiologische Abt., Deutschordenstr. 46, D-6000 Frankfurt 71

372

CARDIOVASCULAR EFFECTS OF INTRAVENOUSLY-INJECTED GABA, THIP AND ISOGUVACINE IN THE ANAESTHETIZED RAT

E. Mesdjian, F.V. DeFeudis, G. Jadot, M. Valli, B. Brugerolle, and P. Bouyard

Arterial blood pressure and heart rate were monitored in pentobarbitone-anaesthetized rats after i.v. injections of GABA (0.03-100 mg/kg), THIP (4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridine-3-ol, 0.1-100 mg/kg), or isoguvacine-HCl (0.1-100 mg/kg). GABA produced dose-dependent hypotension and bradycardia, THIP had no appreciable effect on either parameter, and isoguvacine (1.0-100 mg/kg) produced hypotension, but no bradycardia. The hypotension and bradycardia produced by GABA (1.0 mg/kg) and the hypotension produced by isoguvacine (1.0 mg/kg) were partially antagonized by pretreatment of the rats with bicuculline (1.0 mg/kg, s.c.) or bicuculline-methobromide (1.5 mg/kg, s.c.). As THIP passes the blood-brain barrier more readily than GABA or isoguvacine, as GABA and isoguvacine exerted more pronounced cardiovascular effects than THIP, and as bicuculline-methobromide antagonized the effects of GABA and isoguvacine, it is suggested that a peripheral mechanism(s) is involved in the cardiovascular effects of GABA. Further study of isoguvacine and its derivatives might lead to the development of an hypotensive (anti-hypertensive) agent that acts on peripheral GABA-ergic systems.

Laboratoire de Pharmacologie Médicale, Université d'Aix-Marseille, Faculté de Médecine, 13385 Marseille Cedex 4, France.

373

ON THE ROLE OF GABAERGIC MECHANISMS IN STRIATUM AND SUBSTANTIA NIGRA IN MEDIATING MUSCULAR RIGIDITY

U. Havemann, L. Turski, M. Schwarz and K. Kuschinsky

The effects of injections of the GABAergic agonist muscimol into various areas of the nigrostriatal system were studied in rats. Unilateral injections of muscimol (25 or 50 ng) into the ventral striatum produced both catalepsy and a tonic activity in the EMG, which was recorded from the gastrocnemius-soleus muscle. The latter effect appears to be a sign of muscular rigidity. Injections of similar doses into the dorsal striatum produced a less pronounced EMG activity and no catalepsy. Co-administration of the GABAergic antagonist bicuculline (500 ng) antagonized the effects of muscimol. Unilateral injections of muscimol (25 ng) into the substantia nigra pars compacta (SNC) produced a tonic activity in the EMG as well, whereas injections into the substantia nigra pars reticulata (SNR) produced contraversive turning and no EMG activity. Administration of bicuculline (12.5-50 ng) into the SNR also led to a tonic activity in the EMG.

The results might be interpreted in the following way: Injections of muscimol into the ventral striatum inhibited the striato-nigral GABAergic neurones, leading to a dis-inhibition of efferent neurones in the SNR and by this mechanism to catalepsy and muscular rigidity. Similar effects were produced by administration of bicuculline into the SNR, whereas injections of muscimol into the SNR led to a contraversive turning behaviour, suggesting an inhibition of the efferent neurones in the SNR. The EMG activity after injection of muscimol into the SNC might be due to an inhibition of nigro-striatal dopaminergic neurones. Our results do not exclude the possible relevance of the globus pallidus in the effects described.

Max-Planck-Institut für Experimentelle Medizin, Hermann-Rein-Str. 3, D-3400 Göttingen

374

SUBSTANTIA NIGRA AS A STATION THAT NOT ONLY TRANSMITS, BUT ALSO TRANSFORMS INCOMING SIGNALS FOR ITS BEHAVIOURAL EXPRESSION: STRIATAL DOPAMINE AND GABA-MEDIATED RESPONSES OF PARS RETICULATA NEURONS

R. Jaspers[1], W. Kolasiewicz[2], A.R. Cools[1], S. Wolfarth[2] and K.-H. Sontag[3]

In order to investigate whether striatal dopaminergic mechanisms are involved in the behavioural expression of the GABA-ergic mechanisms within the pars reticulata (pr) of the substantia nigra (SN), apomorphine or haloperidol were bilaterally administered into the caudate nucleus (NC) of cats pretreated with a unilateral injection of picrotoxin or muscimol into the nigral pr. Although the doses selected for the NC injections have been shown to be maximally effective in affecting the behavioural expression of the NC function, the pharmacological treatment of the NC did not produce any significant change in the behaviour elicited from the SN: neither the picrotoxin-induced asymmetric posturing, asymmetric circling, freezing and hind leg disorders nor the muscimol-induced asymmetric posturing, asymmetric spinning and stereotyped licking were significantly affected. The latter behaviour was absent in animals with a partial or total destruction of the nigral pr. As the behavioural expression of the SNpr differed completely from the asymmetric head twisting known to be characteristic for the NC, it is suggested that the behavioural expression of the NC requires a main output station elsewhere in the brain. Furthermore, the present results demonstrate that the SNpr does not form part and parcel of a feedback system that simply transmits incoming signals from the NC towards the pars compacta. Finally the present study demonstrates that the dopaminergic activity within the caudate nucleus may only modify, but certainly not determine, the behavioural expression of the SNpr. It is concluded that the SNpr not only transmits, but also transforms its incoming signals.

[1]Inst. of Pharmacol., Nijmegen, [2]Inst. of Pharmacol., Nauk, Krakow, [3]Max-Planck-Inst. of exp. Medicine, Goettingen

375

INDICATIONS FOR AN INVOLVEMENT OF THE GABA-SYSTEM IN THE MECHANISM OF ACTION OF GABAPENTIN

G.D. Bartoszyk, E. Fritschi, M. Herrmann, G. Satzinger

Gabapentin (1-(aminomethyl)-cyclohexan acetic acid) is a GABA-analogue with very low toxicity in mice and rats (LD_{50} = 8000 mg/kg p.o.) which easily crosses the blood-brain barrier.
Seizures were provoked in mice by various chemical and physical methods, most of which are known to have an effect on synthesis or degradation of GABA. Gabapentin was given orally at different times before seizure provocation. ED_{50} values for protection against tonic seizures were calculated as listed below.

	ED_{50} (mg/kg) premedication (min)			
provocation	30'	60'	120'	240'
semicarbazide	6		10	80
isoniazide	58	32	20	
3-mercaptopropionic acid	110	30	20	
bicuculline		38	42	
pentetrazol	30	52	76	323
audiogenic seizures	18	3		

Dose-dependent inhibition of polysynaptic but not monosynaptic reflex in cats as seen with diazepam or L-dopa as well as decreased muscle tone in rats also refer to supraspinal activity of gabapentin, indicating a possible antispastic action.

The fact that gabapentin protects mainly against convulsions provoked by inhibition of GABA-synthesis, in addition to the similarities in action to GABAergics (e.g. cf. Reimann, this meeting), indicates that gabapentin acts on the GABA-system.

Gödecke Research Institute, Dept. of Pharmacology 7800 Freiburg, FRG

376

INHIBITION BY GABA AND SOME GABA-ANALOGUES OF DOPAMINE RELEASE FROM RABBIT CAUDATE NUCLEUS

W. Reimann

GABA and baclofen attenuate dopamine release from rabbit caudate nucleus in a bicuculline insensitive manner (e.g. Reimann et al., J.Neurochem.39,961,1982) and thus might act at the proposed $GABA_B$ receptor. The GABA analogue gabapentin shows anticonvulsive effects resembling those of baclofen (Bartoszyk et al., this meeting). We investigated whether these substances impair dopamine release at the same site.

Slices of rabbit caudate nucleus were preincubated with ^{3}H-dopamine, superfused and electrically stimulated twice (S_1;S_2). 0.1 and 1 mM gabapentin, added 15 min before S_2, decreased the stimulation-evoked overflow of tritium by 13 and 16 %, 0.1 and 1 mM baclofen by 16 and 17 %, respectively. The inhibition by gabapentin was bicuculline and strychnine insensitive. The inhibition by GABA in the presence of nipecotic acid was confirmed. In order to investigate whether GABA, baclofen and gabapentin act at the same site, 1 mM GABA was added throughout superfusion. Under these conditions neither baclofen nor gabapentin up to 1 mM displayed release-inhibiting activity. In the presence of 1 mM baclofen, 0.1 and 1 mM gabapentin inhibited the stimulation-evoked overflow by 10 and 12 % and, vice versa, baclofen was active in the presence of gabapentin.

The results show that prolonged occupancy by GABA of inhibitory presynaptic receptors on dopaminergic terminals impairs the effect of both baclofen and gabapentin. However, at a high concentration of either agonist the other still has an additive effect, indicating that they seem to act at different sites.

Gödecke Research Institute, Dept. of Biochemistry, Moos-waldallee 1-9, D-7800 Freiburg

377

SOME PHARMACOLOGICAL EFFECTS OF RO 5-4864, A SPECIFIC LIGAND OF THE PERIPHERAL TYPE OF BENZODIAZEPINE BINDING SITES

L. Pieri, P. Polc, E.P. Bonetti, W. Burkard, R. Cumin, and W. Haefely

Ro 5-4864, the 4'-chloro derivative of diazepam, was inactive in classical tests used to reveal the characteristic effects of benzodiazepines (protection of electroshock and chemical convulsant-induced seizures in mice, reduction of muscle tone and coordination in mice, depression of locomotor activity in mice and rats, increase of punished responses in a rat conflict test). Ro 5-4864 was proconvulsive in mice at 30 mg/kg p.o. (a dose having no action per se), i.e. it increased the convulsive effect of a threshold dose of pentetrazole. After a latency of 50 to 60 min Ro 5-4864 at 70-100 mg/kg p.o. induced mild clonic seizures in mice. In rats a dose of 100 mg/kg i.p. induced long-lasting clonic seizures and head-shaking after a latency of 30-40 min. In cats Ro 5-4864 was inactive at 1 mg/kg i.p.; at 3 mg/kg i.p. it induced behavioural stimulation, hypersalivation, muscle rigidity and piloerection for about 30 min; intravenous Ro 5-4864 reduced segmental dorsal root reflexes and increased gamma motoneurone activity. In squirrel monkeys alternating phases of muscular rigidity and ataxia were present for 24 h after 30 and 100 mg/kg p.o. The level of cGMP in the cerebellum of rats was increased after 30 mg/kg Ro 5-4864 p.o.; this and the proconvulsive effect in mice was not affected by the specific benzodiazepine antagonist Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazol(1,5a)(1,4)-benzodiazepine-3-carboxylate).
Ro 5-4864 produces central effects opposite to those of benzodiazepine tranquillizers. The mechanism of action remains to be clarified.

Pharma Research Dept., F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel, Switzerland

378

EFFECT OF BENZODIAZEPINE- AND GABA-ANTAGONISTS ON ATAXIC, MUSCLE RELAXANT, SEDATIVE, ANTICONVULSANT AND ANTIHYPOXIC ACTIVITY OF DIAZEPAM IN MICE

H.J. Kruse, W. Leitzbach and G. Müller

The effect of ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo (1,5-a)(1,4) benzodiazepine-3-carboxylate (Ro 15-1788), ethyl ß-carboline-3-carboxylate (ß-CCE), bicuculline (BIC), picrotoxin (PTX), caffeine (CAF) and strychnine (STR) on major actions of diazepam (D) was investigated in mice. Ataxia, muscle relaxation, sedation, anticonvulsant and antihypoxic effects were induced by D at 20, 20, 20, 15 (all ED97) and 5 (ED+50) mg/kg p.o. respectively. Evaluation took place by measuring rotarod performance (RR), traction test behavior (TR), spontaneous motor activity (SMA), prevention of electroshock-induced convulsions (ECS), and prolongation of asphyxia survival time (AST), respectively. ID50 values were defined as doses reducing the effect of D by 50 %. For SMA, the doses enhancing motor activity by 500 % over D-vehicle controls were determined (ED+500). The following ID50 and ED+500 values were obtained for Ro 15-1788 p.o., ß-CCE i.p., BIC, PTX, CAF and STR (all s.c.), respectively:

1. RR : 1.5 , 24.8, 1.3, 0.79, 8.2 and > 0.75 mg/kg
2. TR : 1.7 , 10.4, 2.1, 0.92, 13.1 and > 0.75 mg/kg
3. SMA: 0.64, 17.5, > 2, > 2.5, 2.9 and > 0.50 mg/kg
4. ECS: 1.8 , > 150, 1.3, > 2.5, > 50 and 0.46 mg/kg
5. AST: 1.1 , 18.0, 1.1, 0.56, 7.5 and > 0.50 mg/kg

Thus, ataxia, muscle relaxation and prolongation of AST were antagonized by all test drugs except STR. Sedation was reversed by Ro 15-1788, ß-CCE and CAF but not by BIC, PTX and STR. Anticonvulsant (anti-ECS) activity of D was counteracted only by Ro 15-1788, BIC and STR.
These results suggest that ataxic, muscle relaxant and antihypoxic (anxiolytic) effects of benzodiazepines are linked to GABA-ergic neurotransmission, whereas sedation may involve adenosinergic and anticonvulsant (anti-ECS) activity glycinergic functions as well.

HOECHST AG, D-6230 Frankfurt/M. 80, Germany

379

EFFECTS OF L-CYCLOSERINE ON THE SLEEP OF RATS, CATS AND RABBITS. A ROLE FOR GABA IN THE CONTROL OF THE SLEEP-WAKEFULNESS CYCLE.

R. Scherschlicht, M. Steiner, J. Marias and J. Schneeberger

L-cycloserine, in contrast to D-cycloserine, was found to inhibit pyridoxal phosphate dependent enzymes, but to have a particular high affinity to the GABA degrading enzyme GABA-transaminase (GABA-T). Consequently, in whole mouse brain, 30 $mg \cdot kg^{-1}$ L-cycloserine i.v. raised the GABA concentration to 350% of the pre-drug value, but only slightly altered the concentrations of alanine, glutamate and aspartate (R. Scherschlicht et al., Abstr. 646, 13th CINP Congr. 1982). L-cycloserine was less toxic than most other GABA-T inhibitors. The rise of the GABA concentration developed slowly, peak values were attained not before the 3rd hour after administration. In chronically instrumented cats, L-cycloserine augmented total sleep time (TST) with 3 $mg \cdot kg^{-1}$ i.p., in rabbits with 30 $mg \cdot kg^{-1}$ i.v. and in rats with 30 and 100 $mg \cdot kg^{-1}$ i.p. The time course of TST increase was parallel to that of the brain GABA augmentation. While in cats rapid eye movement sleep (REMS) was increased, in rabbits both REMS and non-REM sleep (NREMS) were augmented. In rats, both doses increased NREMS, but only the low also augmented REMS. The high dose suppressed REMS, but augmented a state with synchronized hippocampal θ-activity and slow (8-12 Hz) spindles in the brain cortex, which is normally found in transition from NREMS to true REMS. The species specific effects of elevated brain GABA concentrations closely resembled those of benzodiazepines and strongly suggested that GABA may play an important, not yet sufficiently elucidated, rôle in the control of the sleep cycle.

Pharmaceutical Research Department, F. Hoffmann - La Roche & Co., Ltd., CH 4002 Basel, Switzerland

380

STUDIES ON THE MECHANISM OF CENTRAL ACTION OF CYCLOSERINE IN THE RAT

M.A. Dańko

Since tuberculostatic therapy with cycloserine (CS) is frequently complicated by side effects from the CNS, the present study was performed in order to determine whether or not CS induces CNS alterations in rats. CS (10 and 100 mg/kg ip) was administered daily for four days. Behavioural and biochemical experiments were performed 2 hs after the last injection of the drug. CS in the "open field" test depressed exploratory activity of rats, which was reflected by a decrease in ambulation and rearings. CS also strongly depressed the spontanous locomotor activity. After administration of CS (100 mg/kg), the stimulatory action of L-DOPA (100 mg/kg ip) and amphetamine (2.5 mg/kg ip) on the locomotor activity markedly decreased, while reserpine (5 mg/kg sc) induced hypoactivity was enhanced. CS (100 mg/kg) decreased the stereotypy produced by amphetamine (5 mg/kg ip) and enhanced haloperidol (2 mg/kg ip) induced catalepsy. CS (10 and 100 mg/kg) produced a significant and long lasting depression of body temperature of rats. Reserpine pretreatment failed to influence the hypothermic action of CS administered at the dose of 10 mg/kg and 100 mg/kg. Moreover, CS had no effect on the immobilization time of rats in the behavioural "despair test", decreased the number of wet dog shakes produced by L-5-HTP and enhanced the number of wet dog shakes produced by carbachol. CS (100 mg/kg) pretreatment considerably decreased the brain concentration of NA, while did not either significantly affect the brain concentrations of DA or those of 5-HT and 5-HIAA, but increased the elimination-rate constant of NA and DA after α-methyl-p-tyrosine and shorthend the NA and DA turnover time. CS neither affected the elevation of brain 5-HT nor the disappearance of 5-HIAA after tranylcypromine, and consequently failed to affect 5-HT turnover time. These results demonstrate that CS has a profound depressant effect on the function of the CNS in the rat.

Department of Pulmonary Diseases, Institute of Medicine, Medical School, Jaczewskiego 8, PL 20-090 Lublin, Poland

381

PHARMACOLOGICAL PROPERTIES OF AN ANXIOGENIC ß-CARBOLINE DERIVATIVE

W. Kehr, D.N. Stephens and H. H. Schneider

Recent investigations of ß-carboline carboxymethylamide (ß-CCA) revealed that the compound causes severe attacks of intense anxiety in human volunteers. In the present communication attempts are made to correlate experimental findings in animals with those reported in humans. ß-CCA has been shown to displace ^{3}H-flunitrazepam from cerebral membrane binding sites in vitro and mouse brain in vivo (Petersen et al, Europ J Pharmacol 82, 217, 1982) as well as ^{3}H-lormetazepam in rat brain in vivo (ED_{50} 10 mg/kg i.p.). However, ß-CCA failed to exert anticonflict activity in various conflict situations. Instead, ß-CCA reversed the anticonvulsive, anticonflict and motordepressant effects of benzodiazepines without inducing convulsions itself. When administered to rats which were habituated to the test procedure ß-CCA elevated serum corticosterone levels dose-dependently with a maximum 4-fold increase at 3 mg/kg i.p. In rats trained to discriminate pentylentetrazol (administered at a subconvulsive dose of 15 mg/kg i.p.) from saline in a food-reinforced lever pressing paradigm, ß-CCA at doses of 2.5-15 mg/kg i.p. was recognized by most of the animals as pentylentetrazol-like. The benzodiazepine antagonistic action in conjunction with increased serum corticosterone and the induction of a pentetrazol-like discriminative stimulus in rats may be related to the anxiomimetic properties of ß-CCA.

Dept. of Neuropsychopharmacology, Schering AG, Müllerstr. 170-178, D-1000 Berlin 65

382

A NOTE ON NEUROTOXIC EFFECTS OF HEXACHLOROCYCLOHEXANE (HCH)

J. Portig, K. Stein and H. W. Vohland

Major neurotoxic effects of HCH in rats require the following mean (S.D. 10 to 20 % with N = 14) conc. in brain (mmol/kg dry matter): (myo)clonic seizure due to γ-HCH 0.085; anaesthesia due to δ-HCH 1.5; generalized gross tremor "at rest" due to α-HCH 2.1.

The effective conc. of γ-HCH is identical with that of penetrazol (0.077 mmol/kg dry matter), a structurally different convulsant the motor effects of which, however, are indistinguishable, and the effective conc. of δ-HCH is close to that of general anaesthetics (3 mmol/kg dry membrane; P. Seeman, Pharmac. Rev. 24: 583, 1972). The correspondent effective conc. indicate conformity with Ferguson's principle if it is assumed that γ- as well as δ-HCH act through mechanisms the nature of which is physical.

α-HCH is less soluble in water than its isomers yet the failure to produce behavioural signs of convulsant or anaesthetic action is clearly not due to "cut-off". Obviously, the excitatory activity is too low for convulsions to be elicited. It might, however, be sufficient to counteract any "non-specific" depressant effects on function which the compound would be expected to exert like any other indifferent chemical on attaining a conc. of the order of millimolar. An independent factor is a configuration-specific affinity to white matter, presumably to some component(s) of myelin. The proportion present in this part of the tissue may not contribute to "non-specific" depression.

Institut für Toxikologie und Pharmakologie Pilgrimstein 2, D-3550 Marburg a. d. Lahn

383

RESPONSE PROPERTIES OF THE TYPE I SLOWLY ADAPTING MECHANORECEPTORS TO TEMPERATURE STIMULATION IN MAN

F.Konietzny

The functional properties of the Type I slowly adapting mechanoreceptors under combined thermal and mechanical stimulation were investigated by single fiber recordings with percutaneously inserted tungsten microelectrodes from the superficial branch of the radial nerve in 18 conscious human subjects (7 female, 11 male, aged 20-58 years).
A force-controlled mechanostimulator (Burchard et al., 1967) which drove a water circulated miniature thermode (1 to 5 mm^2 contact area) connected with seven thermostats set at 12, 17, 22, 27, 32, 37, and 42 ° C was used for the bimodal stimulation. By means of a multiway tap, cooling steps of 5° C between 12 and 42° C were applied to the most sensitive point within a receptive field of a mechanoreceptor.
SA I mechanoreceptors were silent in the absence of mechanical stimulation. Radiant heat or radiant cold was ineffective in initiating afferent discharge in any of the receptors. Steady-state activity could be elicited at all temperatures studied, when constant force stimuli were applied to the receptive field. A maximum static discharge of about 12 imp/10 s occurred at static skin temperatures between 32 and 37° C.
Dynamic responses to cooling of the receptive field were not obtained. Warm stimulation resulted in a decrease or transient inhibition of the steady-state activity in the SA I mechanoreceptors.

Supported by the Deutsche Forschungsgemeinschaft

Institut für normale und pathologische Physiologie der Universität Marburg, Deutschhausstr. 2, D-3550 Marburg

384

ELECTRICAL TRANSMISSION THROUGH A PERIPHERAL NERVE BRANCH POINT IN THE RAT.

M. Mizutani, Fr.-K. Pierau and D.C.M. Taylor

In intracellular recordings from the 5th and 6th lumbar dorsal root ganglia about 50% of the sensory neurones responded to stimulation of 2 or 3 different nerves. In all cases tested, the action potential produced by peripheral nerve stimulation could be collided with the antidromic action potential due to intracellular current injection (Pierau et al.,Neurosci.Lett.31,123,1982). This excludes the possibility that the double response is transmitted by chemical synapses, indicating that many of the neurones possess peripheral fibres which dichotomize into different peripheral nerves. If this conclusion is correct we would expect that action potentials elicited in one branch of the peripheral process should travel via the branching point to the other peripheral process. Consequently, an action potential produced by stimulation of the second branch should be subject to collision by the first one. This collision should be detected by intracellular recordings in the sensory ganglion, avoiding single fibre recordings in the peripheral nerve. In a number of experiments action potentials produced in the sciatic and pudendal or in the tibial and peroneal nerves could be collided with a collision time slightly larger than the maximum predicted by the equation 2d/v(d;distance between recording and stimulating electrodes,v;conduction velocity), but varied in different experiments. This might suggest that the branching point is quite near to the ganglion but that the delay of conduction at this point differs according to the characteristics of the ramification. The latter is supported by the observation that in some experiments collision only occurred in one direction,i.e. only when the first stimulus was applied to the tibial nerve and not when the peroneal nerve was first stimulated. An unidirectional conduction between the two branches of a dichotomizing peripheral process would fit into the model usually used to demonstrate an axon reflex.

MPI für physiol.u.klin.Forschung, D-6350 Bad Nauheim.

385

STATIONARY TEMPERATURE DEPENDENCY OF SPIKE-TRIGGERING PROCESSES IN THERMOSENSITIVE RECEPTORS

H.A.Braun, H.Wissing

The stationary discharge rate of thermosensitive afferents usually follows a maximum curve. The maximum curve of cold receptors e.g. is regarded to be produced by the interaction of two antagonistic receptor processes (Hensel 1952), possibly by the difference of temperature-dependent changes of active and passive membrane currents (Pierau et al. 1975).

Discharge pattern analyses, however, show that the impulse sequences of bursting and nonbursting cold fibers are obviously triggered by oscillating processes, the frequency of which (OF) not necessarily coincides with the mean discharge frequency (MF). While OF almost monotonically increases with increasing temperature, the number of spikes per oscillation simultaneously decreases. At higher temperatures the shortened periodic processes more and more fail to trigger even single spikes. The previously increasing MF now decreases, in spite of a still increasing OF.

The same principles of temperature dependent discharge pattern variations are also found in cold sensitive afferents of the ampullae of Lorenzini of the dogfish and even in warm fibers of the Boa constrictor, indicating that the bell-shaped mean frequency curve of thermosensitive fibers generally results from a temperature dependent frequency increase of endogenously oscillating receptor processes (corresponding perhaps to those of molluscan neurones). Statistical components of the spike-triggering processes are necessary to ensure a more or less gradual frequency decline at higher temperatures.

Hensel (1952) Ergebnisse der Physiol. 47: 166-368
Pierau, Torrey and Carpenter (1974) Brain Res. 73: 156-160

Department of Normal and Pathologic Physiology
Deutschhausstraße 2, D-3550 Marburg FRG
Supported by the Deutsche Forschungsgemeinschaft.

386

CLASSIFICATION OF MUSCLE RECEPTORS WITH SLOWLY CONDUCTING AFFERENT FIBRES.

S. Mense and H. Meyer

Previous studies of this laboratory have shown that not all thin-fibre receptors in skeletal muscle can be considered to be nociceptors. In order to determine the number and proportion of different receptor types amongst these units, afferents in the gastrocnemius-soleus nerve having a conduction velocity below 30 m/s were tested with a variety of graded stimuli: local pressure, muscle stretch, isometric contractions and temperature changes. Bradykinin, as a painful chemical stimulus, was injected into the receptive field.
The classification was based on the most effective innocuous stimulus; only if no clear response occurred to weak stimuli was the unit considered nociceptive. Four receptor types could be distinguished: 1) Nociceptors (38%) which had a mechanical threshold in the noxious range and were often also responsive to bradykinin. 2) Low-threshold pressure sensitive units (32%) which could be activated by touch or innocuous deformation of the tissue. 3) Contraction sensitive units; these responded to contractions of moderate force (1-3 kp) and to innocuous stretching of the muscle but had a relatively high threshold to local pressure. 4) Thermosensitive units (9%) which had a high threshold upon mechanical stimulation and responded vigorously to innocuous warming or cooling of the receptive field. With the exception of type 4) bradykinin excited most of the afferent units tested.
The nociceptive afferents could mediate deep pain during trauma and inflammation of the muscle, the low-threshold pressure sensitive units pressure sensations from subcutaneous tissues. The contraction sensitive receptors appear to be well-suited to induce cardiopulmonary reflexes during exercise. The thermosensitive afferents might constitute an additional input for thermoregulation.

Physiologisches Institut der Universität Kiel, Olshausenstr. 40/60, D-2300 Kiel, FRG.

387

RESPONSES OF UNMYELINATED NOCICEPTIVE UNITS IN A HUMAN SKIN NERVE AND CONCOMITANT PAIN SENSATIONS EVOKED BY REPEATED RADIANT HEAT STIMULATION

H.O.Handwerker, H.Adriaensen, J.Gybels, and J.VanHees

From superficial radial nerves of human volunteers spike discharges of single afferent C-fibres were recorded percutaneously with tungsten microelectrodes. Eight units were studied, which responded to heat radiation and also to squeezing the skin at their receptive fields, and thus were classified as "polymodal nociceptors" (MH-nociceptors) They were identified as C-fibres by the latencies of their responses to transcutaneous electrical stimulation revealing conduction velocities below 2 m/s. Controlled radiant heat was used to raise the skin temperature by a step function to an individually adjusted painful level above 40^{0} C for 16 s periods. Whereas stimulus size and duration was uniform in each experiment, stimuli followed each other at three different intervals (35s, 70s, 105s) ocurring in random sequence. Each unit was tested with a series of at least 20 stimuli. In none of the C-fibres studied, we observed signs of sensitization during such a sequence of heat stimuli. Instead, all of them showed a diminished response upon repeated heat stimulation. Depression of firing was most pronounced after shortest intervals. Post stimulus depression of C-fibre responses affected mainly the early dynamic response of the nociceptors following the step increase of skin temperature to noxious level. Together with C-fibre discharges we recorded the pain sensations evoked by the heat stimuli, which were rated by the subjects by manipulating continuously the length of a light bar. It was found that pain responses had also a tendency to raise slower after short than after long intervals. Differences in ratings can possibly be explained by differences in C-fibre spike discharges.

II.Physiologisches Institut, Universität Heidelberg, and Lab.exp.Neurologie, Universität Leuven, Belgien

Supported by the Belgian FGWO and by the DFG

388

THE POSTERIOR ARTICULAR NERVE: AN ELECTRON MICROSCOPIC EXAMINATION

L.A. Langford and R.F. Schmidt

The Posterior Articular Nerve, a branch of the tibial nerve, supplies the lateral and posterior structures in the knee joint of the cat. In this study the axonal components of PAN were determined by using ablative surgery and then counting the remaining axons in an electron microscope. Unilateral intradural ventral rhizotomy (L_4-S_3) was performed to remove efferent axons and a unilateral sympathectomy (L_3-S_3) was used to remove all autonomic axons. Sections of the nerve were taken as it arises from the tibial nerve. Gold sections were examined in an electron microscope and all axons, myelinated and unmyelinated, were counted at a magnification of 7,000. The operated limb is compared to the contralateral normal limb.

	MY	UN	TOTAL
Ventral Rhizotomy L_4-S_3 (n=1)			
op	240	789	1029
nor	238	768	1006
Sympathectomy (L_3-S_3) (n=3)			
op	253	401	659
nor	274	944	1218

As with the Medial Articular Nerve (Pflügers Archiv 394: R 57, 1982) all axons in PAN are of dorsal root ganglion cell origin or sympathetic origin since no degeneration was noted after ventral rhizotomy. The sympathetic system contributes about one-half of the unmyelinated axons and no myelinated axons. All myelinated and one-half of the unmyelinated axons are sensory in origin.
In respect to axonal components PAN and MAN are almost identical.
Supported by Alexander von Humboldt Stiftung and the Deutsche Forschungsgemeinschaft.
Physiologisches Institut, Röntgenring 9, 8700 Würzburg

389

BRADYKININ SENSITIVITY OF POLYMODAL FINE AFFERENT JOINT UNITS. SENSITIZATION BY PROSTAGLANDIN E1

H.-G. Schaible

Fine afferent units of the medial articular nerve to the knee joint of the cat in the Group III (Aδ) and in the Group IV (C) range are very often sensitive both to mechanical stimulation (local touch or pressure, passive joint movements) and to close i.a. injection of algesic substances such as bradykinin, serotonin or potassium ions. Performing passive joint movements four types of units can be recognized: (a) activated by non-noxious movements; (b) weakly activated by non-noxious movements; (c) activated only by noxious movements; (d) not activated by movements. Most units of types (a) and (b) have lower von Frey thresholds than units of types (c) and (d) (Kanaka et al., Neuroscience 7, S109, 1982, Schaible, Schmidt, J.Neurophysiol., in press). All four types of units can be excited by close i.a. injection of bradykinin (0.26-26µg). Injection of prostaglandin E1 (0.3-30µg) did not excite the units or evoked a few spikes. But in most units of types (c) and (d) and with high von Frey threshold (5 Group III-, 7 Group IV- units) the sensitivity to bradykinin was dramatically increased for about 15-30 min after the preceeding PGE injection. 7 Group III- and 4 Group IV- units of the type (a) and (b) and with lower von Frey thresholds did not show any increase in their bradykinin responses after a PGE application. Such results give support to the assumption that prostaglandin E1 preferentially, if not specifically, increases the bradykinin sensitivity of nociceptive joint units whereas units involved with reception of everyday non-noxious mechanical events remain unaffected. This sensitization of articular nociceptive units by prostaglandin E1 (and possibly by other prostaglandins) may play a major role in the development of tenderness (allodynia) and hyperalgesia of injured and inflamed joints.

Physiologisches Institut der Universität Würzburg, Röntgenring 9, D-8700 Würzburg

390

RESPONSES OF CAT PULPAL NERVE FIBRES TO STIMULI THAT CAUSE PAIN WHEN APPLIED TO DENTINE IN MAN.

W. Kollmann, B. Matthews and H. Suda

Pulpal fibres from cat canine teeth are activated by temperature stimuli (10-50°C) applied to the tooth tip (W. Kollmann, B. Matthews in: Anatomical, Physiological and Pharmacological Aspects of Trigeminal Pain; Eds: B. Matthews, R.G. Hill, Amsterdam, Elsevier, 1982). In order to see whether these afferent units are temperature sensitive only and whether temperature insensitive fibres respond to other stimuli, we tested the responses of fibres from tooth pulps to mechanical, probing, thermal, chemical and drying stimuli applied to dentine. These stimuli evoke pain in humans. Recordings were made from pulp fibres teased out of the inferior alveolar nerve just central of the mandibular foramen in cats anaesthetized with sodium pentobarbitone. Bipolar electrical stimuli were used to identify the fibres and to determine their conduction velocity. 1) Of 264 units studied with thermal stimulation only, 88 (c.v. 27.3 ± 9.6 ms^{-1}, mean ± 1 S.D.) gave a sustained response to cooling and often a transient to heating, with latencies being of 150 ms or longer. A second group of units (c.v. 7.1 ± 9.2 ms^{-1}; N = 20) responded vigorously to 50°C, generally with a longer latency than above. Occasionally, they also responded to cooling. 2) 33 out of 82 units were activated by mechanical stimuli and/or drying of the exposed dentine etched with acid. 3) When the responses of pulp fibres to all stimuli were tested, 21 out of 33 units responding to cooling also responded to mechanical stimuli and/or drying. Some of these also reacted to heating and chemical stimuli. The second group of thermosensitive pulp fibres, which reacted to heating, rarely responded to the other stimuli. Thus, there appear to be two classes of receptors in cat teeth, one responding potentially to all stimuli and the other activated mainly by noxious heat. Many fibres did not respond to any stimulus.

Dept. of Physiol., Medical School, Bristol BS8 1TD

391

PAIN-RELATED ELECTRICAL POTENTIALS OF THE HUMAN NASAL MUCOSA ELICITED BY CHEMICAL STIMULI

G. Kobal

Negative potentials of the human respiratory nasal mucosa that correlated to sensations of pain were discovered, while studying the electroolfactogram. They were recorded from the surface of the mucosa by tubular electrodes (teflon tube, ringer-agar, silver-chlorided silver-wire) referenced to the contralateral medial eye angle. A newly developed olfactometer offered chemical stimuli within a constantly flowing air stream of controlled temperature and humidity (transition time to any chosen level of concentration < 20 ms). 4 subjects were tested. The amplitude of the negative potential (several hundred µV) increased with higher concentrations of the stimulants (CO_2, isoamyl-acetate, eucalyptol, linalool, eugenol, menthol, benzaldehyde). When interstimulus intervals of paired stimuli were in the range of 6 s to 1 min, the second responses showed adaptation; when interstimulus intervals were shorter than 6 s, however, the second responses' amplitudes increased. All changes in amplitudes correlated to changes in the subjects' estimation of intensity. The negativity only showed on the stimulated side, did not depend on the psychogalvanic skin response, and disappeared, when the local anesthetic tetracaine hydrochloride was applied. Moreover, it was diminished by intravenous application of the analgesic drug pentazocine. Using non-invasive recordings this method offers an objective and quantitative measurement of biological phenomena correlated to painful sensations and of their inhibition by analgesic drugs.

Institut für Pharmakologie und Toxikologie der Universität Erlangen, Universitätsstraße 22, D-8520 Erlangen

392

NOCICEPTIVE NEURONES IN CYTOARCHITECTURALLY IDENTIFIABLE REGIONS IN THE VENTROLATERAL PERIPHERY OF THE CAT'S VENTROPOSTEROLATERAL NUCLEUS (VPL).

K.-D. Kniffki, A.D. Craig and K.J. Berkley

Recent reports have demonstrated that neurones responsive to noxious stimuli are located in the periphery of the cat's VPL (Kniffki & Mizumura, J. Neurophysiol., 1983; Honda, Mense & Perl, ibid.). Using HRP, Craig & Burton found dense spinal input to the ventrolateral portion of VPL and also reported nociceptive neurones in this region (Soc. Neurosci. Abstr. 5: 705, 1979).

These results raised the possibility that the regions containing nociceptive neurones have identifiable cytoarchitectural characteristics. To investigate this possibility, responses of single neurones in the ventrolateral periphery of VPL were studied in nembutal-anaesthetized cats. Whenever a nociceptive neurone was encountered, its location was marked with a small lesion. Parallel cytoarchitectural studies were performed on sections cut in different planes from unoperated cats.

Neurones responsive to noxious stimulation of skin or muscle were located at VPL's most ventral and ventrolateral edges, beginning just lateral to VPI. In every plane of section, this region contained small, pale-staining neurones of various shapes. These neurones were distinguishable from the dark-staining, larger and round neurones usually associated with VPL, and were contained within a 75 µm to 150 µm-thick region apposed to VPL.

These results suggest that neurones in identifiable regions of the ventrolateral periphery of VPL may take part in nociception.

Physiologisches Institut der Universität Kiel, Olshausenstraße 40-60, D-2300 Kiel.

393

AGE-RELATED THRESHOLD CHANGES OF CUTANEOUS VIBRATION, TEMPERATURE, AND PAIN SENSITIVITY.

L. Habermann, P. Heintz, J. Mißler

The aim of this study was to determine cutaneous sensitivity in normal subjects and its changes with age.
146 supposedly healthy male and female individuals aged 10 - 93 years participated in the study. From all a brief medical history was taken,and a short neurological examination was made. The following parameters were bilaterally measured: thresholds to a 100 Hz vibration at second metacarpal,tibial and first metatarsal bones; skin temperature together with warm,cold,cold pain and heat pain thresholds at the upper lip,thenar eminence and lateral aspect of the foot (for methods see J.Neurcl.Neurosurg.Psychiat. 42,793, 1979, and 39,1071,1976).Values from subjects with probable local or general neurological disturbance were partly or totally omitted from further analysis.
The results show that vibration thresholds progressively increase with age whereas temperature and thermal pain thresholds are little affected.The deterioration of vibration sense is more prominent in the legs than in the hands Warm and cold thresholds are clearly dependant on skin temperature. The thermally indifferent range between warm and cold threshold is not affected by skin temperature,and it shows little interindividual variation. In contrast, thermal pain thresholds vary greatly interindividually; they correlate,however,significantly with each other, thus indicating that the criterion for the pain thresholds seems to be the same irrespective of modality or locus of stimulation.

Institute of Physiology, University of Marburg, Deutschhausstrasse 2, D-3550 Marburg, Germany.

394

SUITABILITY OF RETINYL ESTERS FOR THE REGENERATION OF BLEACHED RHODOPSIN IN ISOLATED FROG RETINAE

G. Nöll, E. Röcker[+] and Ch. Baumann

The regeneration of rhodopsin can be studied in isolated, totally bleached retinae when lipid vesicles are used to deliver retinal or retinol to the photoreceptors(Yoshikami & Nöll, Methods in Enzymology 81, 1982). The intact frog eye contains not only retinal and retinol but also considerable amounts of 11-cis- and all-trans-retinyl ester stored in the pigment epithelium (Hubbard & Dowling, Nature 193, 1962). The present study was carried out in order to see whether or not retinyl esters are accepted by the photoreceptors for the regeneration of bleached rhodopsin.
11-cis-retinyl palmitate was produced in two ways: (i) by photoisomerization of the commercially available all-trans retinyl palmitate and (ii) by synthesis from 11-cis-retinol and palmitoyl chloride. The yield of the first method was never larger than 0.9% although various solvents, inert gases and temperatures were tried. The synthesis was more productive. Its reaction products were separated and cleaned by HPLC.Impurities of the end product 11-cis-retinyl palmitate were smaller than 2 %. Extracting the pigment epithelia of 20 frog eyes resulted in two fractions of retinyl palmitate. Co-chromatography with the synthesized esters revealed a proportion of 31% 11-cis- and 60% all-trans-retinyl palmitate. The absorbance spectra of these isomers had maxima at 315 nm (11-cis) and 326 nm (all-trans), respectively. We also synthesized 9-cis-retinyl palmitate with a maximum absorbance at 321 nm. When the photoreceptors were provided with either of the isomers of retinyl palmitate, the regeneration of bleached rhodopsin was not promoted as it was with 11-cis-retinal(ol).
Our results suggest that the eye stores retinol (including the 11-cis-isomer) in esterified form, but the esters are not accepted by the photoreceptors for the resynthesis of rhodopsin.

Physiologisches Institut und [+]Institut für Organische Chemie der Justus Liebig-Universität, D-6300 Gießen

395

THE EFFECT OF NEUROTRANSMITTERS ON LUMINANCE AND PATTERN RESPONSES OF THE ISOLATED CAT EYE

R.P. Schuurmans and T. Berninger

In 43 enucleated and arterially perfused cat eyes graded potentials were simultaneously recorded from the retina (ERG) and summed action potentials from the optic nerve (ONR) in response to the reversal of spatial patterns as well as to luminance stimulation. An oxygenated calf serum medium was used for perfusion. Checkerboard stimuli were produced on a TV-monitor with a mean luminance of 20 cd/m² and 96% contrast.
The ONR and ERG show striking similarities in response characteristics to each reversal of the pattern, exhibiting an initial negative and slow positive component. Furthermore, both responses show a linear increase of amplitude from check size of 9' to 38' followed by a saturation of response up to 74'. This indicates that the ONR and pattern reversal ERG reflect ganglion cell activity as well as antagonistic centre-surround mechanisms.
Using luminance stimuli, strychnine in a concentration of 15 µM revealed reversible, dose-related decreases of the b-wave and oscillatory potentials while the initial fast component of the ONR increased. This indicates the absence of electric coupling between the ERG and ONR recordings as well as a lack of ganglion cell contribution to the luminance ERG and glycine mediated inhibitions in the inner nuclear layer of the cat's retina. Injection of 2.5 µM DL-2-amino-4-phosphono-butyric acid (APB) known to block reversibly on-responses in the retina was followed by a decrease of b-wave and a polarity inversion of the responses. The off-response, however, was not affected. During transient interruption of perfusion, the b-wave and the negative off-effect decreased in amplitude. However, pattern reversal responses, increasing with check size, could still be recorded.

Max-Planck-Institut für Physiologische und Klinische Forschung, W.G. Kerckhoff-Institut, D-6350 Bad Nauheim, FRG

396

LIGHT FROM FLUORESCENT TUBES EVOKES FLICKER RESPONSES IN VISUAL NEURONS OF THE CAT

U. Eysel and U. Burandt

The different spectral composition of artificial light and daylight is usually made responsible for possible biological effects of fluorescent tube light. The different temporal information of artificial light (10 ms periodicity) is thought to be negligible because of the low-pass properties of the afferent visual system.
We now present evidence that first and second order neurons in the cat central visual system respond differently to daylight,light of incandescent lamps and fluorescent tube light due to the periodicity and depth of modulation of the artificial light sources.Under normal room illumination conditions (150cd·m^{-2}) single on- and off-center neurons in the optic tract and lateral geniculate nucleus displayed increased impulse rates and strong phase locking when stimulated by fluorescent tube light. Many off-center neurons showed a functional reversal when stimulated with fluorescent tube light: units which were inhibited by large field stimulation with the other light sources were excited instead. Some neurons (on- and off-center) followed the 100 Hz frequency of the fluorescent tubes with 1:1 responses, and thus had not yet reached their individual critical flicker fusion frequency. The phase locking to fluorescent tube light results in an uneven impulse probability distribution which is absent in daylight and not present to this extent with incandescent light.
The results suggest that dependent on the respective light sources different impulse patterns and impulse rates are transmitted in the subcortical visual system.

Institut für Physiologie, Universitätsklinikum Essen, Hufelandstrasse 55, D-4300 Essen 1, F.R.G.

397

ACTIVITY OF VESTIBULAR NUCLEI NEURONS IN THE ALERT MONKEY DURING OPTOKINETIC AND SMOOTH PURSUIT EYE MOVEMENTS

R. Boyle and U. Büttner

The vestibular nuclei play an important role in the generation of compensatory slow eye movements induced by head rotation and optokinetic stimulation. Furthermore single unit recordings in the alert monkey show activity changes in relation to smooth pursuit eye movements. The present study was undertaken to further investigate the role of the vestibular nuclei in both smooth pursuit and optokinetic nystagmus.

Prior to experiments monkeys (Macaca fascicularis) were trained to track a small light spot (smooth pursuit eye movements). During experiments the monkey sat upright in a primate chair with its head fixed. For vestibular stimulation he was rotated on a turntable about a vertical axis. The optokinetic stimulus consisted of a large striped drum that could be rotated in front of the monkey. Vestibular neurons receiving an input from the horizontal semicircular canals were tested during optokinetic and smooth pursuit eye movements. Virtually all neurons responded to the optokinetic stimulus. If they responded during smooth pursuit the response was usually smaller than that during optokinetic nystagmus at the same eye velocity. These results will be discussed in relation to current concepts of smooth pursuit and optokinetic nystagmus generation.

Supported by Deutsche Forschungsgemeinschaft SFB 200 A 2 and Humbold-Stiftung (R. Boyle).

Neurologische Klinik der Universität, Moorenstr. 5, 4000 Düsseldorf, FRG

398

MONKEY VISUAL CORTEX: CROSS-CORRELATIONS BETWEEN SPIKE TRAINS RECORDED BY 30 MICRO-ELECTRODES.

K. Jacobi and J. Krüger

Single or multiple unit activity was recorded with a 5x6-array of microelectrodes (Krüger and Bach, Exp. Brain Res. 41:191-194,1981) in the striate cortex of three anaesthetized,paralysed monkeys. Spikes were collected in any one layer for about 2-3 hours during application of various visual stimuli. Cross-correlogram structures a few milliseconds wide were rare, and mostly observed between neighbouring electrodes only (tip distances 160 microns). However, clear peaks about 60 msec wide straddling zero mutual delay,sometimes laterally displaced by 5-30 msec, were observed frequently. The reduced magnitudes of peaks at a mutual delay of one stimulus period proved that the former peaks were not entirely due to external synchronisation resulting from common stimulation. Interaction strength was taken to be proportional to the peak area.Typically it fell off over distances of roughly 0.5 mm, with strong inter-individual variations.Correlations predominantly between neurones driven by the same eye, including frequent bridging of the contralateral eye stripe, was seen whenever interactions were fairly localized. In some recordings, however, the fall-off with distance was less clear, and then, interaction strength did not follow the eye dominance stripes. We conclude that one component of the interactions is due to common LGN input (or to activity-dependent correlation strength, revealing eye dominance columns as a consequence of monocular stimulation). Another component may be a larger scale synchronization related to the state of alertness.

Neurol. Univ.-Klinik Hansastr.9, 7800 Freiburg

399

RESPONSE OF ACCOMMODATION TO A CONTINOUSLY MOVED STIMULUS

J. Zülch, H. Krueger

Any variation of viewing distance in the depth of field results in a change of convergence, accommodation and pupil size to get a single, sharp retinal image. Fusional convergence is reduced to accommodative convergence alone in monocular vision. Accommodation stimulated by a continously moved target shows some particular characteristics, compared to a stepfunctional moving target.
Methods: The test set-up used the optometer-principle (Badal-lense). The target (Landolt-ring surrounded by a radial grid of low spatial frequency) was moved within a range of distances from 0D (oom) to 9D (0.11m) with various velocities (0.1D/s-40D/s; 0.01m/s-10m/s). Accommodative convergence, pupil size and subjective depth of focus were measured for monocular viewing conditions.
Results: 1. High velocities give a smooth response of accommodation. 2. Low target velocities result in a step-like response of accommodation. 3. The time course of pupil-near-reflex determines the actual depth of focus, which influences the height of the steps. 4. Therefore velocity and accuracy of accommodation is determined by the actual depth of focus of the eye. 5. The accommodation follows the target to higher velocities for far-to-near movements than for near-to-far by means of the time course of pupil-near-reflex. 6. The deviation of accommodation from the accurate value increases with increasing amount of accommodation, that means accommodation is allways shifted towards the resting point of accommodation.

Institut für Arbeitsphysiologie der Technischen Universität München, Barbarastr. 16, 8 München 40

400

HUE SHIFTS IN COLOR GRATINGS DEPENDENT ON SPATIAL FREQUENCIES *

C. Fach and L. Spillmann

Color induction is usually investigated with inducing fields and single test spots, though little is known about the reciprocal interaction between such stimulus configurations. To maximize the possible interactions we used square-wave color gratings, made up of all combinations of the following Munsell papers: 5R (red), 5Y (yellow), 5G (green), 5B (blue) and N6 (grey). All were approximately equal in lightness and saturation (Munsell 6/6). The gratings were surrounded by a neutral field (N6) and illuminated by CIE source C (MacBeth). The mean luminance was 16.5 ftL. Spatial frequency was varied from 2.5 to 10 cycles/degree (cpd) equivalent to single stripe widths of 11 to 3 arc min. Subjects with normal color vision and acuity foveally matched the hues of the gratings using a large set of Munsell color swatches.
At 10 cpd, hue shifts were most prominent: yellow shifts in hue towards the other grating color when combined with red (appears orangish) and green (appears yellow-greenish) but not when combined with blue, where it shows contrast (appears reddish). Green shifts in hue only when combined with blue. Blue and red shift in all color combinations. When combined with grey only red shifts in hue (towards purple). All hue shifts weaken with decreasing spatial frequency and disappear below 4 cpd. These results cannot be completely explained by chromatic interactions (Ware and Cowen, Vision Res. 22, 1353; 1982). Chromatic organization of perceptive fields as well as small field tritanopia may need to be considered.

Neurologische Universitätsklinik, Hansastr. 9, 7800 Freiburg i. Br., West Germany

* Supported by DFG (SFB 70, Teilprojekt A6)

401

MESOPIC VISION OF DEUTERANOPES STIMULATED BY LARGE VISUAL FIELDS

Peter Scheufens and Horst Scheibner

In photopic foveal vision of deuteranopes, the "blue" sensitive cones do not contribute to luminance; they contribute only to chrominance (blueness-yellowness). By means of a visual tristimulus colorimeter with a 10° visual field confusion lines, the missing primary and the alychne were determined at a mesopic level, L =(0.2 - 2.0) cd/m^2. Results: The deuteranopes proved to be "complete" deuteranopes in the sense of Jaeger and Kroker (Kl. Monatsber. Augenheilk. 121 (1952), 445-449). The missing primary (copunctal point) contained a strong negative blue component with the consequence that the monochromatic character of foveal vision at the long wavelengths has disappeared with large fields. The alychne and the spectral brightness sensitivity derived from it suggest that a medium or short wavelength mechanism participates in brightness vision. The confusion lines in the "yellow" region of the chromaticity chart show a steeper descent in comparison to foveal vision. This suggestes that a single univariant long wavelengths mechanism (Paulus and Scheibner, Ber. dtsch. ophthalmol. Ges. 75 (1978), 518-521) is no more working alone, rather, that an additional medium wavelength mechanism cooperates with blue cones. It is suggested that this additional medium wavelength mechanism is formed by rods.

Physiologisches Institut II, Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf

402

QUANTITATIVE STUDY OF HORIZONTAL CIRCULAR VECTION IN MAN

K. Bötzel and O.-J. Grüsser

A "planetarium projector" was used to produce a rotating visual field projected to a vertical cylinder of 240 cm diameter. The subject was placed in the center of the cylinder; the visual field(random light dots) was rotated at 9 different angular velocities V_S between 1.8 and 180 degrees · s^{-1} around the subject. The subject indicated circular vection by means of a handle connected to a potentiometer. When the subject fixated a stationary target (LED) the apparent speed of circular vection V_{CV} depended on the angular velocity of the visual field V_S according to the following equation:

$$V_{CV} = \frac{aV_S}{1 + KV_S} \text{ [subjective units]}$$

A graduated increase in V_S led to an exponental time function of V_{CV} increase, while a graduated decrease in angular velocity changed in most subjects V_{CV}, corresponding to a second order differential equation. Circular vection was reduced in all subjects when they pursued one of the dots (optokinetic nystagmus). Three types could be discriminated:
(a) V_{CV} was reduced by 20–30 percent
(b) V_{CV} was reduced by 60–100 percent
(c) The direction of circular vection changed.
In most subjects additional stationary stimuli facilitated circular vection. The latter findings indicate that not only movement-sensitive neuronal systems, but also relative movement (with respect to a stationary "frame")and gaze pursuit movements have an effect on the visual input to the vestibular system.
Supported by the DFG, Gr 161
Physiologisches Institut der Freien Universität Berlin, Arnimallee 22 1000 Berlin 33

403

DIFFERENTIAL EFFECTS OF CENTRAL AND PERIPHERAL VISUAL FIELDS ON POSTURAL BALANCE IN MAN

A. Straube, Th. Brandt and S. Krafczyk

Optimal balance requires the continuous evaluation of the reafferent visual-vestibular-somatosensory consequences of self-generated body movements. Within this multiloop control, vision plays a major role for postural stabilization. The body sway is visually detected by the relative retinal shift of viewed stationary targets in the environment. This serves as an input for motor compensation.

The differential effects of central versus peripheral vision on postural stabilization have been tested by means of posturography. Root mean square values of the lateral and fore-aft body sway in free upright stance (on a slab of foamrubber, which reduces the reliability of the joint somatosensors) revealed the following:
1. binocular visual control attenuates body sway by a factor of v = 0.36 as compared to eyes closed; 2. binocular vision is significantly (v = 0.81) more effective than monocular vision; 3. with monocular vision,occlusion of the fovea (2°) has only a slight diminishing effect; 4. pure foveal vision does not significantly stabilize fore-aft sway; 5. a 30° central visual field has a significantly greater stabilizing effect than a 30° paracentral field; 6. the stabilizing effect of a 30° central field quantitatively roughly corresponds to that of the entire peripheral visual field with occlusion of a 30° central area

Thus - as opposed to circularvection - visual stabilization of posture involves the two modes of visual processing "focal" and "ambient" with the central visual field being more effective than the peripheral. The relative contribution of the foveal region seems to be insignificant for postural balance.

Neurologische Klinik mit klinischer Neurophysiologie, Alfried Krupp Krankenhaus, D-4300 Essen

404

DISSOCIATION BETWEEN SENSORY AND MOTOR MAPS IN THE HUMAN VISUAL SYSTEM: VISUAL FIELD COORDINATES ARE PREWIRED, THE OCULOMOTOR MAP IS NOT

E. Pöppel, W. Fries and Petra Störig

Lesions within the geniculo-striatal projection system result in areas of blindness in the visual field. Unilateral striatal lesions produce homonymous field defects, i.e. corresponding retinal points loose their cortical target. What happens if a patient with a geniculo-striatal lesion has had a squint prior to the lesion; does the cortical lesion destroy the visual field representation in the squinting eye with respect to the fovea or with respect to a retinal position that corresponds to the fovea in the good eye ("pseudo-fovea")? It was possible to answer this question in a patient who had a convergent squint in the left eye since early childhood and who has suffered a striatal lesion in the left hemisphere as an adult. As expected, a hemianopsia in the right visual field was observed. The area of blindness in the right eye went right to the vertical meridian. However, the area of blindness in the left eye did not start at the vertical meridian but approximately 17 degrees nasally of the vertical meridian. The blind spot of the left eye was found between 1 and 5 degrees in the nasal visual field. Measurements of increment threshold along the horizontal meridian indicated an increase of sensitivity between the point of fixation and more peripheral positions in the nasal visual field. These results demonstrate that a striatal lesion destroys the visual field representation with respect to the fovea and not with respect to the pseudo-fovea. The striatal representation of the visual field is prewired and cannot be altered by the functional development of a pseudo-fovea. However, although visual field representation is prewired, oculomotor control appears to be rearranged with respect to the pseudo-fovea. Under monocular viewing conditions the patient used his pseudo-fovea and not the anatomical fovea as primary position.

Institut für Med. Psychologie, Schillerstr. 42,München 2

405

INTEROCULAR TIME THRESHOLDS AND LATENCY DIFFERENCES IN MULTIPLE SCLEROSIS*

W.H. Ehrenstein, K. Manny and G. Oepen

Interocular time thresholds were measured psychophysically in patients suspected of multiple sclerosis (MS) and in normal controls. The stimulus was presented foveally and consisted of a small cross (30') formed by 4 rectangular LEDs (λ_d = 650 nm). By means of polarizing filters one eye was exposed only to the horizontal bar, the other to the vertical. Observers had to indicate whether the horizontal or the vertical bar appeared first. The stimulus onset asynchrony ranged from 0 to $\pm$ 300 ms (the horizontal bar preceeding or following the vertical).

Normals showed little interocular differences; their mean thresholds were 51.8 ms (right before left) and - 49.0 ms (left before right). Patients exhibited higher time thresholds (ranging from 45 to 187 ms) and had considerable interocular latency differences (up to 33.5 ms) indicating unilateral or asymmetric impairment of the visual pathways.
The psychophysical data were compared to VEP-latencies and their lateral differences obtained with foveal stimulation. The diagnostic reliability based on psychophysical measurements was equal to or, for Mc Alpine's class II of probable MS-patients, superior to that based on VEP-recordings.

Foveal interocular time thresholds and latency differences thus appear to be useful indicators of MS, especially in its early stage of development.

Neurologische Universitätsklinik, Hansastr. 9,
7800 Freiburg i.Br., West Germany

*Supported by DFG (SFB 70, Teilprojekt A6)

406

BINAURAL INTERACTION IN CAT MEDULLA FIELD POTENTIALS(MFPs)

D. Caird and D. Sontheimer

In the cat the Brain Stem Evoked Potential (BSEP) recorded by a gross electrode on the dura and the MFP, recorded with a microelectrode in the Superior Olivary Complex (SOC) were compared during monaural and dichotic click stimulation (max. interaural delay (ΔT), 2048 µs. A closed stimulation system with condensor microphone drivers was used. The MFP consists of a fast afferent volley (2 ms latency) followed by a slow (4.3 ms) wave of opposite polarity to ipsilateral and contralateral stimulation. Binaural interactions were studied by comparing the dichotic MFPs with the sum of the monaural MFPs. At no point in the medulla or at any ΔT value did these components of the MFP show any binaural interaction, i.e. the dichotic MFP was always equal to the algebraic sum of the monaural MFPs. We assume that this is because the generators of the MFP, postsynaptic potentials in the dorsal (ipsi MFP) and ventral (contra MFP) dendrites of the Medial Superior Olive cells are spatially separated, although on the same neurons. The MFP at this level does not therefore represent the binaural interactions that are present (Brugge and Geisler, Ann Rev Neurosci 1:363-394, 1978). A small binaural component corresponding in latency (3.6 ms) to wave IV of the BSEP could be seen at small ΔTs (256 µs). Wave IV was also the first BSEP component to show (inhibitory) interaction (Huang, Brain Res 184: 215-219, 1980). Wave IV is faster than the FP recorded in the Superior Colliculus (5.5 ms) and this wave probably represents the output from the SOC (i.e. the lateral lemniscus, Huang 1980). Thus, wave IV represents two types of binaural interactions (excitation/excitation and Excitation/inhibition) occurring 'presynaptically' and the earlier waves assigned to the SOC do not show existing binaural interactions. The BSEP is therefore not a reliable indicator of binaural processing.

Z.Physiologie,Theodor-Stern-Kai 7, D-6000 Frankfurt/M. 70

407

PARAMETERS OF ACOUSTICAL SPEED PERCEPTION - EXPERIMENTS AND MODELLING

S.W. Droste, M. Falkenstein, D.E.W. Trincker

Most of the studies in human auditory perception have only dealt with experimental sound sources in fixed positions. While the real world includes a great many of moving sources the related sensory processes are not sufficiently understood. Directional hearing in the case of fixed sources has been analysed mainly in terms of binaural time (Δt) and intensity (ΔI) differences (with frequency-dependent relationship). Perception of source movements was thought as the rather pure consequence of Δt and/or ΔI changes, excluding the physically understood Doppler shift and the 1/R-rule (R, source distance). Following this concept, an increase of the functional interaural distance (d) should reduce the velocity discrimination (caused by the higher degree of lateralization as yet provoked by small angles of deviation from zero position). But our experiments showed the opposite result: an improved speed perception. Further experiments with amplitude and/or frequency modulation of the moving acoustical stimuli led us to a new model of the involved sensory functions (related to the Colburn-Jeffress model) with a detector system for changes of a loudness-weighted binaural cross-correlation and with (weighted) two-variables operations for the target course projection into the perceptual space.

Inst.f.Physiologie(II,MA4),Ruhr-Universität,D-4630 Bochum

408

BRAINSTEM AUDITORY EVOKED POTENTIALS (BAEP) AND PRE- TO POST-OVULATORY SHIFT OF TEMPERATURE IN HUMAN.

TAGHAVY A., FÜNFGELDER J. AND LÖSSLEIN H:

Previous works showed shorter latencies of peaks (sig. for III to VI) and interpeak-latencies (sig. for I-III and I-V) and sig. higher amplitudes of BAEP in female comparing to male subjects. (Taghavy A. a. Lösslein H., Pflügers Arch. 394 (1982) R 227).

Temperature as a factor influencing this difference has not yet been ruled out, since hypothermia is known to increase interpeak-latencies (Stockard JJ. et al., Ann. Neurol.3, (1978)368). We measured BAEP in 10 Women (39 ± 7 ys) twice in pre- and post-ovulatory period using monaural stimulation of 70 dB above SL to r. and l. ears separately in each subject. Other recording parameters as reported previously.

A shift of basal temperature of a mean value of 0.49°C occured in all subjects.
The latencies of the peaks I-VI and of interpeak-latencies as well als the amplitudes of I and V were The same in pre- and post-ovulatory comparison.
Both values in this group of women were almost identical to previous ones (above reference).

The sex differences in BAEP must be attributed to some other factors, such as size.
The BAEP is unaffected by temperature within the physiological range.

Neurologische Klinik der Universität Erlangen
(Kopfklinikum), Schwabachanlage 6,
D-8520 Erlangen.

409

EVALUATION OF A PERFUSION SYSTEM OF THE WHOLE HUMAN TERM PLACENTA FOR METABOLIC AND PHARMACOKINETIC STUDIES.

U.-W. Wiegand, R.C. Chou, D. Maulik, and G. Levy

It has been well established that essentially all drugs can be transferred across the placenta from mother to fetus. The fetal exposure to drugs and other xenobiotics acquired by environmental exposure or diet is of major concern. Since the knowledge of the processes involved is still limited, the perfusion of the human term placenta can give useful information. The purpose of this study was to evaluate a dual perfusion system of the whole human term placenta that has been developed earlier (D. Maulik et al., Placenta, Suppl. 3, 353-365, 1981) to investigate physiological processes in the placenta for studies of drug metabolism and placental transfer. Several modifications of the original experimental conditions (fetal circulation, perfusate composition, temperature system, oxygenator) permitted perfusions with drug solutions for up to 360 minutes. Important parameters could be kept in an acceptable range for the maternal and fetal side, respectively (mean±SD, n=7): Temp.: 34.5±1.5°C; pH: 7.44±0.09, 7.34±0.09; pO_2: 129.7±80.8, 41.8±14.6; pCO_2: 21.7±2.4, 32.9±7.9; flow rate: 436.3±60.6, 27.8±7.7 ml/min; O_2 consumption: 7.66±3.34 ml/min. These data indicate that the placentae remained viable. The following tentative results were obtained with propoxyphene (PPX) and acetaminophen (APAP): a. PPX metabolites and APAP conjugates were not detectable in the perfusion fluids and placental tissues except for a trace of norPPX in the placental tissues; b. PPX showed a higher uptake into the placental tissue than did APAP; c. the effective (net) transplacental clearances were low relative to the perfusion rates (5.9±3.3 and 7.2±1.5 ml/min for PPX and APAP, respectively.

Department of Pharmacology, University of Mainz, F.R. of Germany, Departments of Pharmaceutics, Gynecology and Obstetrics, State University of New York at Buffalo, USA

410

CONVENIENT METHOD FOR THE DETERMINATION OF THE METABOLIC CONVERSION OF POLYCYCLIC AROMATIC HYDROCARBONS

K.L. Platt and E. Schmid

The metabolic conversion of polycyclic aromatic hydrocarbons (PAH) is usually determined by incubation of the radioactively labelled PAH in the presence of a metabolizing system, isolation of the metabolites and the unreacted PAH by extraction with an organic solvent, separation by high-performance liquid chromatography and quantification of the components by liquid scintillation counting, the whole procedure being laborious and time-consuming.
We, therefore, developed a rapid and sensitive method for determination of the metabolic conversion of PAH, which avoids the need for radioactively labelled substrate. The incubation (2 ml) of the PAH (10-150 nmol/ml) is stopped with acetonitrile. Then an internal standard is added. After thorough mixing and centrifugation the supernatant is drawn through a short reversed phase column (500 mg, C_{18}). PAH, internal standard and metabolites are concentrated on the column which after drying is eluted with acetone. The eluate is concentrated and applied to a short silica gel column (500 mg). Elution with petroleum ether-chloroform (4:1) yields the PAH and the internal standard free of polar metabolites. Quantification of the PAH is achieved by gas liquid chromatography on a packed column. The whole procedure takes about 30 min per sample. We tested the described method with dibenz(a,h)anthracene (DBA) using 5,6-dihydro-DBA as the internal standard. This standard accompanies DBA through all liquid separation steps, due to the very similar polarity of the two hydrocarbons, but is easily separated from DBA by gas chromatography. The recovery of DBA after the two liquid chromatographic steps is about 90 %. The detection limit is 200 pmol DBA. Our method was successfully applied to the determination of the metabolic conversion of DBA under different incubation conditions.

Pharmakologisches Institut der Universität, Obere Zahlbacher Strasse 67, D-6500 Mainz

411

EFFECTS OF DIETARY PROSTAGLANDIN PRECURSORS ON PLASMA LEVELS OF ESSENTIAL FATTY ACIDS AND PLATELET AGGREGATION IN THE RABBIT

B.A.Schölkens, D. Gehring and M.S. Manku

Corn oil (CO) and evening primrose oil (EPO) are both rich in polyunsaturated essential fatty acids which are biosynthetic precursors of prostaglandins. Whereas CO contains only linoleic acid, EPO contains both linoleic and γ-linolenic acid. To evaluate the influence of dietary supplementation on plasma levels of essential fatty acids and platelet aggregation, rabbits were treated for 30 days with identical diets, supplemented orally with 1 ml/kg/day of CO, EPO or tap water. Essential fatty acids in plasma phospholipids were measured by gas chromatography, platelet aggregation ex vivo was monitored in a Born aggregometer.
Plasma linoleic acid levels rose slightly but not significantly in the CO and EPO treated groups. There was a marked significant rise in dihomo-γ-linolenic acid on days 8 and 22 of the EPO treated group, but there were no changes in the CO or water group. Arachidonic acid showed small, irregular and non-significant changes in all three groups.
Collagen induced platelet aggregation ex vivo was significantly inhibited by oral treatment with EPO and CO, however antiaggregatory effects were more marked in the CO group at days 15 and 29 of treatment than in the EPO group.
The results indicate that rabbits have little or no ability to convert linoleic acid to γ-linolenic acid or dihomo-γ-linolenic acid. Rabbits also appear to have little delta-5-desaturase activity as indicated by failure of EPO to raise arachidonic acid levels. These data show that in the presence of such lipid changes high doses of CO have a more marked inhibitory effect on platelet aggregation than do similar amounts of EPO.

Hoechst AG., 6230 Frankfurt(M)80, FR Germany, and Efamol Research Institute, Kentville, Canada

412

INFLUENCE OF EMD 34 853 ON THE TURNOVER OF ^{14}C CHOLESTEROL IN RATS

H.W. Diekmann, H. Nowak

EMD 34 853 (2-Phenyl-2-[6-ethoxy-benzothiazolyl (2)-thio]-propionic acid) is an effective plasma cholesterol lowering agent in the rat (H. Nowak, E. Schulze: Poster presented at the 6th Int. Symp. on Atherosclerosis, Berlin 1982). To elucidate its mechanism of action we investigated the influence of EMD 34 853 on the turnover of [4-^{14}C]-cholesterol injected into rats. After a 7 day interval of equilibration of the radiotracer with the body cholesterol a 10 day period of administration of i) 30 mg/kg/die EMD 34 853 (n=6), ii) application vehicle only (n=6), iii) 0.2 g/kg/die cholestyramine (n=3) followed. The experiment was terminated after an additional 12 day period without drug administration. The parameters investigated were a) plasma concentration of ^{14}C, b) plasma concentration of total cholesterol, c) specific activity of total plasma cholesterol d) excretion of ^{14}C with urine and feces and tissue distribution of ^{14}C after 28 days e) proportion of neutral and acidic sterols in feces.
Our results showed no differences between EMD 34 853-treated and control animals with respect to the elimination from plasma and excretion of ^{14}C as well as the time course of the specific activity of total plasma cholesterol; the proportion of neutral and acidic sterols in feces was unaffected likewise.
EMD 34 853 obviously does not influence cholesterol catabolism or excretion, but it lowers plasma cholesterol very effectively. We propose a mechanism, where only cholesterol distribution is affected, possibly via lipoprotein receptors.

E. MERCK, Darmstadt; Inst. f. exp. Arzneimittelforschung, Am Feld 32, D-8018 Grafing/München

413

INFLUENCE OF EMD 34 853 ON ELIMINATION FROM PLASMA AND UPTAKE INTO LIVER OF ^{3}H-CHOLESTEROL LABELLED PLASMALIPOPROTEINS IN RATS

H. Sailer, A. Garbe

EMD 34 853 (2-Phenyl-2-[6-ethoxy-benzothiazolyl (2)-thio]-propionic acid) has been shown to be an effective plasma cholesterol lowering agent in the rat (H. Nowak, E. Schulze: Poster presented at the 6th Int. Symp. on Atherosclerosis Berlin 1982). To elucidate its mechanism of action we investigated the influence of EMD 34 853 on plasma elimination and tissue uptake of ^{3}H-cholesterol-labelled plasma lipoproteins in rats. Plasma lipoproteins were labelled in vivo with ^{3}H-cholesterol and injected into rats (n=12 each group), pretreated with i) 4 x 30 mg/kg/die EMD 34 853 or ii) 4 x application vehicle only. Plasma levels of ^{3}H-radioactivity were measured up to 6 hrs, then the tissue distribution of the ^{3}H-radioactivity was determined.

The data obtained show, that the elimination of plasma lipoproteins from the blood is markedly accelerated by EMD 34 853: $t_{1/2}$ for EMD 34 853-pretreated rats = 3.4 hrs, control animals = 5.5 hrs ($p < 0.01$). At the end of the experiment 5.9 % of the injected dose were present in the total plasma of EMD 34 853-pretreated animals compared to 18.3 % in control animals ($p < 0.001$). The lipoproteins eliminated from plasma were selectively taken up by liver tissue. At the end of the experiment the livers of EMD 34 853-pretreated animals contained 27.0 % of the injected dose compared to 17.5 % in control animals ($p < 0.0025$).

Conclusion: EMD 34853 lowers plasma cholesterol by activating hepatic lipoprotein receptors.

E.MERCK, Darmstadt; Inst. f. exp. Arzneimittelforschung, Am Feld 32, D-8018 Grafing/München

414

DISTRIBUTION AND METABOLISM OF FENTANYL IN THE RAT

E. Schneider

Fentanyl is widely used in neuroleptanalgesia since it is an opioid with immediate onset and short duration of action. Recently the phenomenon of fentanyl rebound has been reported (Becker L.D. et al., Anesthesiology 44, 291, 1976), i.e. the appearance of a second phase of respiratory and mental depression following wakefulness. One reason for the incidence of this phenomenon may be redistribution of active fentanyl from storage sites, into the brain after some latency. We have investigated the possibility that such redistribution may occur using radioactive labelled fentanyl ([^{3}H-phenethyl] - fentanyl) in connection with whole body autoradiography and chemical analysis. We found that active fentanyl appeared in brain immediately following injection and disappeared within minutes. At the same time the concentration increased in the stomach and the lower GI-tract, the kidney and connective tissue including fat. A second peak of radioactivity was not found in brain. TLC analysis showed that the initial high concentrations of radioactivity in brain were almost exclusively due to genuine fentanyl. The same holds true for the stomach. The kidney and the lower GI-tract contained both fentanyl and the labelled acidic metabolites. From these results it is unlikely that the fentanyl rebound may result from redistribution of genuine fentanyl into the brain. It remains open, however, that not labelled but pharmacologically active metabolites of fentanyl are produced in the brain. Experiments using fentanyl labelled in the anilinopiperidine part, i.e. the structure which may lead to such active metabolites, are in progress.

Institut für Pharmakologie und Toxikologie der Universität Erlangen-Nürnberg, Universitätsstraße 22, D-8520 Erlangen

415

MUTAGENICITY OF N-HYDROXYUREA IN V79 CHINESE HAMSTER CELLS

K. Ziegler-Skylakakis, H. Homfeldt and U. Andrae

N-Hydroxyurea (HU), used as antineoplastic agent in the chemotherapy of various types of tumours, has recently been shown to induce DNA repair in human cells in culture during incubation with a microsomal activation system from rodent liver (Andrae and Greim, Biochem. Biophys. Res. Commun. 87:50, 1979). The DNA damage detected by the repair test was apparently caused by reactive oxygen species formed during the metabolism of HU. Since very little is known about the mutagenic potential of oxygen-radical induced DNA lesions, we reinvestigated the genotoxicity of HU in a test system for the induction of specific locus mutations in V79 Chinese hamster lung cells.

V79 cells were incubated for 3 hours with HU (1-8 mM) and with or without liver microsomes from phenobarbital-treated rats (0.5 mg protein/ml) and a NADPH-regenerating system. After an expression time of 6 days cells were replated in normal medium for the determination of the surviving fraction and in selective medium containing 30 µg 8-azaguanine/ml for the determination of the number of mutants deficient in the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT).

In the presence of the microsomal activation system, HU caused a concentration dependent increase in the number of azaguanine-resistant mutants. HU was not mutagenic in the absence of microsomes. The cloning ability of the cells was reduced to approximately 60% compared to untreated controls irrespective whether the activation system had been present or not.

These findings show that reactive oxygen species produced during microsomal metabolism of HU are capable to induce point mutations in V79 cells. Cytotoxicity, however, is not mediated by the formation of oxygen radicals but appears to be a consequence of the well known killing of cells in S-phase by the compound.

Gesellschaft für Strahlen- und Umweltforschung (GSF), Abteilung für Toxikologie, D-8042 Neuherberg

416

INTERACTION OF PEPLEOMYCIN AND NEOCARZINOSTATIN WITH BLEOMYCIN: DIFFERENTIAL EFFECTS ON CELL GROWTH AND DEGRADATION OF DNA. H.C. Schröder, R.K. Zahn and W.E.G. Müller

In the present study, the interactions of the peptide antitumor antibiotics pepleomycin (PEP) and neocarzinostatin (NCS) with bleomycin (BLM) were investigated both on cellular- (L5178y mouse lymphoma cell system) and molecular level (DNA fragmentation).

Dose-response experiments revealed that the slopes at the ED_{50} concentrations for NCS and BLM (both pure BLM-A_2 and BLM-B_2 and the clinical mixture) are nearly identical. However, the dose-response curve of PEP was characterized by a significantly steeper slope at ED_{50}, indicating that this antibiotic inhibits cell proliferation within a closer concentration range. Combined treatment of L-cells with BLM and NCS shows that both drugs act in an additive way. In contrast, PEP potentiates the cytostatic effect of BLM considerably. In this case, the calculated fractional inhibitory concentrations amounted to values significantly lower than 0.5, indicating a highly synergistic interaction of both compounds. Alike BLM, PEP inhibits proliferation of synchronized L-cells both in G_2- and in S-phase to the same extent. All three drugs cause "unbalanced growth" of L-cells.

NCS and BLM were found to act additively also on molecular level, both with respect to thymine liberation from DNA and DNA strand scission. On the other hand, the molecular mode of action of PEP and BLM, which are distinguished only by their terminal moieties, towards DNA was found to be different. While BLM causes at higher concentrations preferentially double-strand breaks, the ratio between the number of single- and double-strand scissions of DNA, isolated from PEP-treated cell cultures (at concentrations above ED_{50}), is approximately 13:1.

Supported by grants from the Deutsche Forschungsgemeinschaft and from the Fonds der Chemischen Industrie.

Institut für Physiologische Chemie der Universität Mainz, Duesbergweg, D-6500 Mainz.

417

INTESTINAL PHENOL METABOLISM AND METABOLITE TRANSPORT: STUDIES WITH 14C-LABELLED 1-NAPHTHOL AND PHENOLPHTHALEIN IN THE ISOLATED GUINEA PIG MUCOSA.

R.B.Sund and F.Lauterbach

The above drugs were incubated for 45 min with jejunal and colonic mucosal sheets (Lauterbach, Arch. Pharmacol. 297,201, 1977) in a medium containing 0.9 mM sulphate ion. The following examples serve to illustrate the complexities in metabolite distribution pattern: With 50 µM 1-naphthol added at the lumen side, metabolism and metabolite efflux were nearly complete;both main metabolites (sulphate conjugate = I = 50-60%, glucuronide = II = about 30% of dose) were predominantly distributed to the luminal fluid. When added at the blood side, more II (30-40% of dose) than I (15-25%) was formed. In this case, II accumulation on the blood side was greater than on the lumen side; however, the distribution was less asymmetrical than by lumen side administration. I tended to distribute evenly on the lumen and blood chamber. In the colon, 2-3 x as much I than II was formed regardless of side of administration. I again showed a fairly uniform distribution, whereas II independently of side of administration accumulated predominantly on the blood side. Phenolphthalein was metabolized at a much slower rate than 1-naphthol, and a substantial tissue accumulation was noted already at 20 µM. Glucuronides were in all instances the main metabolites found; their distribution was in principle as described for 1-naphthol II. - The results suggest that at least in the jejunum two different compartments are responsible for the metabolism of xenobiotics administered at the lumen or blood side.

Institut für Pharmakologie und Toxikologie, Ruhr-Universität Bochum, D-4630 Bochum 1.

418

PHARMACOKINETICS AND BIOTRANSFORMATION OF HYMECROMONE AFTER INTRAVENOUS ADMINISTRATION TO PATIENTS WITH IMPAIRED LIVER FUNCTIONS

U. Femfert, H.D. Kuntz, B. May

The biliary antispasmodic agent Hymecromone (7-hydroxy-4-methyl-2H-1-benzopyran-2one) is metabolized in human adults mainly via glucuronidation of the 7-hydroxy function. Other transformations, namely O-methylation, sulfate conjugation, and additional phase I oxidations are only of minor importance.
A rapid ion pair reverse phase HPLC method for simultaneous determination of the parent compound and the major metabolites will be presented.
The disappearance of Hymecromon from serum is rapid and biphasic, corresponding to an open two compartment model. The total set of pharmacokinetic parameters of this model was calculated from patients with various degrees of impaired liver function.
In molecular terms the conception was developed that the transforming enzyme UDP-glucuronosyltransferase EC 2.4.1.17 will be present in an active, already glucuronidated state, which is responsible for the rapid first part of the elimination process. The much slower second part will then represent the de novo formation of the active enzyme species from the UDP pool.

Ruhr Universität Bochum, Medizinische Universitätsklinik "Bergmannsheil", Abt. Gastroenterologie und Hepatologie.
Hunscheidtstr.1, D-4630 Bochum 1

419

ISOLATION OF GLUCURONIDES BY ION-PAIR REVERSED PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

W. Lilienblum

A general method for the isolation of enzymically formed glucuronides by use of HPLC is presented. The system consists of a C-18 column and mixtures of methanol and phosphate buffer containing 5 mM tetrabutylammonium as lipophilic counterion, adjusted to pH 7. The usefulness of this system is demonstrated by two examples: 1) When 3-H-diethylstilboestrol (DES, 0.5 mM) was incubated in presence of detergent-activated rat liver microsomes and 3 mM 14-C-UDP-glucuronic acid (UDPGA) the monoglucuronide was formed at a rate of 2 nmol/min/mg protein whereas the diglucuronide was not detected. DES glucuronidation was slightly induced (1.9fold) by pretreatment of rats with phenobarbital whereas pretreatment with 3-methylcholanthrene had no inducing effect. 2) Glucuronidation of pentachlorophenol (PCP) under the above incubation conditions occured at a rate of 5 nmol/min/mg protein. Formation of PCP glucuronide could be established by incubation either with 14-C-PCP or 14-C-UDPGA. The conjugate was stable at pH 7 but it was hydrolysed in weak acidic medium (half-life at pH 5 about 1 h). It was also hydrolysed by ß-glucuronidase at pH 7. Thus the low recovery of PCP glucuronide in urine may not reflect total PCP glucuronidation in vivo.
Glucuronides of several arylamines and of monohydroxybiphenyls were also isolated by this method. Therefore this system may be generally helpful to obtain pure glucuronides for reference or calibration purposes.

Institut für Pharmakologie und Toxikologie der Universität, Kreuzbergring 57, 3400 Göttingen.

420

HUMAN PLATELET PHENOLSULFOTRANSFERASE (PST): SEPARATION OF THERMOLABILE AND THERMOSTABILE FORMS

C.Reiter,J.Dunnette,J.Van Loon,R.Weinshilboum

PST catalyzes the sulfate conjugation of a variety of phenolic and catechol drugs and endogenous substrates, e.g. catecholamines. Human platelets contain at least two forms of PST, which are regulated independently. In platelet homogenates one form is relatively thermolabile and on is relatively thermostabile. Dopamine is a model substrate for the thermolabile form while p-nitrophenol (4 µmol/l) serves as a substrate for the thermostabile form (Reiter et al, Fedn.Proc.41:1478,1982). Experiments were performed to separate the two forms of platelet PST. The thermolabile and the thermostabile forms present in a pooled platelet homogenate were separated by Ion Exchange chromatography (DEAE-Sepharose CL 6B) using a linear gradient ranging from 50 to 225 mmol/l NaCl. The purification was for the thermolabile form 10.5 fold and for the thermostabile form 7.4 fold. The separated forms exhibited a thermal inactivation pattern similar to that found in the platelet homogenate. p-Nitrophenol, phenol, 6-OH-melatonin, and paracetamol were substrates for both partially purified forms of platelet PST. K_m-values (mmol/l) for the thermolabile/thermostabile forms were 1.5/.00038, 2.3/.014, .36/.018, and 2.9/4.5, respectively. The ratios K_m-thermolabile/K_m-thermostabile indicated, that p-nitrophenol (4 µmol/l) was the superior substrate to measure the thermostabile form of platelet PST.

Medizinische Universitätsklinik,D-5300 Bonn(C.R.) and Mayo Foundation, Rochester, MN 55905, USA

421

GLUTATHIONE CONTENT AND CONJUGATION OF CHLOR-2,4-DINITROBENZENE WITH GLUTATHIONE IN THE RAT DUODENUM

N. Wichmann and H. P. Büch

Duodenal segments of female Sprague-Dawley rats were perfused in a modified Fisher and Parsons apparatus with Krebs-Henseleit solution (37°C; pH =7.4;) containing Chlor-2,4-dinitrobenzene (A). After a 2 h lasting circulation of the perfusion medium gassed with carbogen A and its glutathione conjugate (GA) were measured in the absorbate and in the duodenal mucosa at 254 nm after HPLC; glutathione (G) in the mucosa was measured colorimetrically.

A mM offered	absorbate µmole/g wet weight A	GA	mucosa µmole/g wet weight A	GA	G*
0.025	n.d.	0.14	n.d.	0.04	3.46
0.20	0.04	1.72	0.05	0.33	2.60
1.00	0.69	0.89	1.57	0.80	1.37

$\bar{x}$, n=4-9; n.d.=not detectable, < 0.004 µmole/g; *G of control = 4.50 µmole/g;

Up to 0.025 mM A in the perfusion medium the unchanged substrate neither in the absorbate nor in the mucosa was detectable. In contrast, GA was found in both absorbate and mucosa. In the absorbate GA was maximal at 0.2 mM A. In the mucosa GA increased linearly with increasing substrate concentration in the perfusion medium. A higher concentration of A (1.0 mM) caused a considerable decrease of GA in the absorbate (48%) and a corresponding decrease of G in the mucosa (47%) compared with the respective values at 0.20 mM A.

Pharmakologie und Toxikologie, Universität des Saarlandes, D-6650 Homburg;Supp. by SFB38, A3;

422

BILIARY EXCRETION OF GLUTATHIONE AND ITS MODIFICATION BY PHENOBARBITAL, GSH-DEPLETORS, CARBON TETRACHLORIDE AND ETHANOL. U. Jeß

Biliary excretion of glutathione (GSH) was studied in bile-fistula-rats under urethane anesthesia (1.2 g/kg ip). Total GSH (GSH+2GSSG) was estimated after incubating bile with GSH-reductase and then using Ellman's reagent. No differentiation between GSH and GSSG was made because of rapid autoxidation of GSH during sampling. Bile was collected hourly for 5 hrs after pretreatment with different compounds known to alter hepatic GSH-concentrations; results are compiled in the table ($\bar{x} \pm$ S.E.M.; n = 6 each):

Treatment groups	Dose/kg	Bile flow (ml/kg·5 hrs)	GSH+2GSSG (µmol/kg·5 hrs)
Olive oil ip	10 ml	9.63 ± 0.10	16.51 ± 0.31
Phenobarbital ip	0.075 g	14.78 ± 0.15	43.93 ± 0.75
CCl_4 ip	0.5 ml	7.14 ± 0.19	4.49 ± 0.31
Phorone ip	0.25 g	17.37 ± 0.22	23.13 ± 0.37
Diethylmaleate ip	1.0 ml	11.18 ± 0.18	7.26 ± 0.29
Vinylidene chlor.ip	0.2 ml	11.02 ± 0.36	15.17 ± 0.69
1 % Tylose po	2 ml	10.54 ± 0.21	19.65 ± 0.55
Paracetamol po	1 g	21.61 ± 0.56	13.78 ± 0.39
Saline po	15 ml	12.45 ± 0.25	21.77 ± 0.74
Ethanol 40 % po	4.8 g	9.55 ± 0.15	9.56 ± 0.29

Total GSH concentration in rat bile amounted to 25 % of the hepatic content. No correlation was seen between the changes in GSH-levels in the liver and those found in bile after either treatment. This lack of correlation as well as the observations that CCl_4 and ethanol inhibited the biliary excretion of GSH without affecting the hepatic GSH content may suggest the participation of an active transport mechanism for the biliary excretion of glutathione.

Institut für Toxikologie der Medizinischen Hochschule Lübeck, Ratzeburger Allee 160, D-2400 Lübeck (FRG)

423

FORMATION OF GLUTATHIONE CONJUGATES DURING MICROSOMAL METABOLISM OF p-BROMOPHENOL AND BROMOCATECHOL.

O. Cumpelik and K.-H. Oliv

Studies on the activation of bromobenzene by liver microsomes have demonstrated that phenolic metabolites are intermediates in the formation of reactive species which irreversibly bind to protein (Wolff a. Hesse in "Micros. Drug Oxid. a. Drug Toxicity", p.513, 1982). To explore one of the major deactivation mechanism, we have studied the formation of reactive metabolites and conjugates from p-bromophenol and bromocatechol in the presence of glutathione (GSH).

The NADPH-dependent binding of ^{14}C-p-bromophenol and ^{14}C-bromocatechol to microsomal protein was inhibited by 80% at 1 mM GSH. Concomitantly, the amount of nonextractable metabolites in the aqueous phase increased 3fold. Moreover, a decrease of GSH-concentration at a rate of about 1 nmol/min was measured photometrically. GSH-conjugates were detected by HPLC-analysis of the incubates containing either ^{3}H-GSH and unlabeled p-bromophenol or ^{14}C-p-bromophenol and unlabeled GSH. Both assay mixtures showed the radioactive peak of dinitrophenyl-derivates of GSH-conjugates eluting at the same retention time from the kationic ion exchange column. Incubation of p-bromophenol with GSH in the presence of rat liver cytosolic fraction which containes GSH-transferases did not increase GSH-conjugate formation. When ^{14}C-bromocatechol, isolated from incubation mixtures of ^{14}C-p-bromophenol, was re-incubated with microsomes and NADPH the amount of GSH-conjugates was tripled. Presence of liver cytosol neither altered the amount of GSH-conjugates nor the extent of binding to microsomal protein. The results indicate, that oxidative metabolites from p-bromophenol are effectively conjugated with GSH, presumably via bromocatechol. The finding that cytosolic GSH-transferases did not increase the formation of conjugates suggests that the reactive intermediates spontaneously react with GSH.

Ges. f. Strahlen- u. Umweltforschung, Abteilung f. Toxikologie, D-8042 Neuherberg, Ingolstädter Landstr. 1

424

THE ELEVATION OF PARTICLE-BOUND GLUTATHIONE-S-TRANSFERASE ACTIVITY BY SOME XENOBIOTICA IN VIVO

A. Cassebaum and P. Kraus

Iodoacetamide (IAA) and N-Ethylmaleinimide (NEM) have been shown to increase microsomal Glutathione-S-transferase (GST) activity in vitro (MORGENSTERN et al., Biochem Biophys Res Comm 87:657, 1979). Similar experiments in vivo have not yet been described. Therefore we treated rats once resp. three times on following days with doses of 2o mg/kg IAA or 5 mg/kg NEM. Single doses of either substance elevated microsomal GST activity by 25-5o%, whereas cytosolic activity remained unchanged. Repeated applications of both compounds did not yield uniform changes in microsomal GST activity but constantly decreased cytosolic activity by 15-2o%.

In another series of experiments, infant and adult rats were treated with α-Hexachlorcyclohexane (α-HCH) or Phenobarbital (PB) as described previously (KRAUS et al., Biochem Pharmacol 3o:355, 1981; MUKHTAR et al., Xenobiotica 11: 367, 1981) or with both compounds simultaneously. The single substances increased GST activity in microsomes by 15-45% and in mitochondria by 2o-9o%. When both compounds were given simultaneously, their effects summarized. Since it has been found out that there are two separately inducible mRNAs each coding for one subunit of the GST molecule (PICKETT et al., Arch Biochem Biophys 215:539, 1982) it may be supposed that PB and α-HCH exhibit their increasing effect on GST activity by inducing different mRNAs.

Institut für Toxikologie und Pharmakologie der Philipps-Universität, Pilgrimstein 2, D-3550 Marburg

425

THE MECHANISM OF FERRIHEMOGLOBIN-FORMATION IN VITRO AND IN VIVO BY N-ARYLACETOHYDROXAMIC ACIDS

H. Sterzl

We have found that the oxidation of purified human hemoglobin (=$HbFe^{2+}$) in vitro by N-hydroxy-N-(4-chlorophenyl)acetamide (=NOH-4ClAA) at 37° followed second order kinetics with a velocity constant of 3.45 ± 0.23 $mol^{-1}sec^{1}$ and that NOH-4ClAA oxidized several equivalents of $HbFe^{2+}$, indication for the catalytic activity of the active molecule. The activity of NOH-4ClAA increased from 1 : 16 to 1 : 45 on decreasing its concentration fom 1.1×10^{-4} to 1.1×10^{-5}M, indication for a higher stationary concentration of the active molecule at lower concentrations of NOH-4ClAA. Analysis of a reaction mixture containing $HbFe^{2+}$: NOH-4ClAA=3 : 1 incubated at 37° for 2 and 60 min gave: $HbFe^{3+}$(45,100%), NOH-4ClAA (85,75%), 4-ClAA (2.2,4.0%), 4-ClNOB (1.3,0.6%), and 4-$ClNO_2$B (1.0,0.2%). This is indication for the oxidation of NOH-4ClAA by the dioxygen species from oxyhemoglobin which yielded acetyl 4-chlorophenyl nitroxide, which sustained the oxidation of $HbFe^{2+}$, but whose self-reaction gave the 3 products.
Analysis of the blood of a rat 3 min after i.p.-injection of 50 mg NOH-4ClAA/kg gave 34.5% $HbFe^{3+}$, 17.8 µg NOH-4ClAA/ml, 1.3 µg 4-ClAA/ml, 1.3 µg 4-ClA/ml, 0.4 µg 4-$ClNO_2$B/ml, and 3.2 µg 4-ClNOB/ml. Analysis of the blood of a rat 3 min after i.p.-injection of 8 mg NOH-4ClA/kg gave 32.4% $HbFe^{3+}$, 0.4 µg 4-ClA/ml, and 3.0 µg 4-ClNOB/ml. These results indicated that $HbFe^{3+}$in vivo was not produced by NOH-4ClAA itself, but after oxidation to 4-ClNOB and subsequent reduction to NOH-4ClA, whereas in vitro, 4-ClNOB was not activated, because it was not reduced to NOH-4ClA.

Institut für Pharmakologie und Toxikologie der Universität München, Nußbaumstr. 26, 8000 München 2

426

HYDROXYL RADICAL - MEDIATED OXIDATIVE DECOMPOSITION OF N-NITROSODIMETHYLAMINE

H.-J. Haussmann, H. Kuthan and J. Werringloer

The formation of nitrite associated with the aerobic metabolism of N-nitrosamines by liver microsomes and reconstituted cyt.P-450 monooxygenase systems has been ascribed to reductive reactions contributing to the biotransformation of such compounds (Schrenk et al., Adv.Exp.Med.Biol. 136: 1157, 1982; Appel and Graf, Carcinogenesis 3:293, 1982). Although a participation of superoxide in these *reductive* reactions has been suggested by Schrenk et al., an involvement of hydroxyl radicals as primary reactive agents in an *oxidative* type of reaction has as yet not been considered. Using N-nitrosodimethylamine(NDMA) as a substrate and xanthine/xanthine oxidase in the presence of Fe^{3+}-EDTA as an hydroxyl radical generating system a rapid formation of both nitrite *and* formaldehyde was observed. Compounds like mannitol, ethanol, benzoate and tris-(hydroxymethyl)-aminomethane inhibited the formation of both products indicating that hydroxyl radicals generated via an iron-catalyzed Haber-Weiss mechanism mediate these reactions. This interpretation was substantiated by the demonstration of a high sensitivity of the formation of both products to inhibition by superoxide dismutase as well as catalase. Further, methylviologen and menadione enhanced the decay of NDMA to nitrite and formaldehyde. The pronounced lag phase preceding the rapid formation of both products was found to be either attenuated or enhanced in the presence of added hydrogen peroxide or catalase, respectively. The ratio of the products formed (formaldehyde:nitrite) using the xanthine/xanthine oxidase system (2:1) was found to be considerably smaller than in the reaction catalyzed by liver microsomes (10:1) - a discrepancy readily explained by the contribution of a direct *α*-hydroxylation of NDMA in liver microsomes. These studies suggest that additional pathways of NDMA metabolism remain to be evaluated regarding the formation of reactive intermediates.

Institut für Toxikologie der Universität Tübingen, Wilhelmstrasse 56, D-7400 Tübingen

427

BIOTRANSFORMATION OF NITROSO-CHLORAMPHENICOL IN RAT LIVER

K.-G. Eckert, P. Eyer, and H. Kampffmeyer

Nitroso-chloramphenicol (NOCAP), which is more toxic than chloramphenicol (CAP) to cultured human bone marrow cells, is accused to be responsible for aplastic anemia (Yunis et al.). Although biotranformation of CAP to NOCAP has not been shown hitherto, evidence has been presented for amino-chloramphenicol (NH_2 CAP) formation in rats, bacteria, and mammalian liver fractions including human liver. Therefore, the disposition of the suspected NOCAP intermediate was investigated.
NOCAP was hemoglobin-free perfused once through isolated rat livers. More than 90% of 0.46 mM prehepatic NOCAP was metabolized. This extraction rate was even higher at lower NOCAP concentrations. The metabolites of 0.46 mM NOCAP were 0.32 mM NHOHCAP, 0.08 mM NH_2 CAP and 0.02 mM glutathione sulfinamidochloramphenicol (GSONHCAP). At 0.2 mM prehepatic NOCAP, 0.1 mM NHOHCAP, 0.08 mM NH_2 CAP and 0.015 mM GSONHCAP were formed. Correspondingly, the liver glutathione content was depleted by NOCAP.
The liver's capacity to reduce NOCAP was investigated in postmicrosomal supernatant. As revealed by GPC, at least 2 NADPH-dependent and 2 NADH-dependent reductases were involved, one of the latter being alcohol dehydrogenase (K_m = 8 µM, V_{max} = 0.13 U / mg protein). It is calculated that at steady state concentrations of 0.1 mM NOCAP, 0.1 mM NADH, and 0.1 mM NADPH, NOCAP is reduced by cytosolic enzymes at a rate of 60 U / g liver. NHOHCAP formed thereby is either reduced to NH_2 CAP or reoxidized. This latter reaction is enhanced by oxyhemoglobin. Therefore, the further fate of NOCAP is determined by reactions within red cells.

Institut für Pharmakologie und Toxikologie, Med. Fakultät der Ludwig-Maximilians-Universität München, Nußbaumstr. 26, D-8000 München 2, F.R.G.

428

THE ELECTROCHEMICAL BEHAVIOUR OF N-ARYLACETOHYDROXAMIC ACIDS

W.Lenk and M.Riedl

We have measured 3 types of oxidation-reduction potentials of the following N-arylacetohydroxamic acids which may help to elucidate the mechanism of the autocatalytic formation of ferrihemoglobin: N-hydroxy-[-acetanilide,-4-chloroacetanilide, -3.4-dichloroacetanilide,-phenacetin,-4-acetylaminobiphenyl,-2-acetylaminofluorene, and -2-acetylaminophenanthrene]. The potentials of the reversible one-electron oxidation, leading to N-acetyl-N-aryl nitroxides, were found between +550 and +630 mV by voltammetry in H_2O-CH_3OH (9:1) at pH 7.4. This result supports the assumption that hydroxamic acids can be oxidized to nitroxides by the dioxygen species of oxyhemoglobin. In the voltammograms we found a second oxidation potential between +800 and +1100 mV, which is related to the irreversible withdrawal of the second electron and caused the formation of nitrosoarenes as was indicated by an additional potential wave between 0 and +200 mV in the voltammogram. Nitrosoarenes are also formed by self-reaction of nitroxides. This supports our idea that they are formed in the same way from hydroxamic acids with oxyhemoglobin in vitro.
Since the potential of the irreversible reduction of hydroxamic acids to N-arylacetamides was polarographically inactive, we have determined it indirectly by using a series of reversible organic redox couples in 0.8 M HCl-actone (1:3) and found potentials between +280 and +300 mV. Attempts to determine the potentials at pH 7.4 have failed. This result indicates that the N-arylacetamides found as metabolites of hydroxamic acids by purified hemoglobin in vitro were not formed by reduction.

Institut für Pharmakologie und Toxikologie der Universität München, Nußbaumstr. 26, 8000 München 2

429

STRUCTURE AND ROTATIONAL DIFFUSION OF CYTOCHROME P-450 IN A RECONSTITUTED MEMBRANE AND REGULATION OF DRUG BIOTRANSFORMATION

R. Greinert and A. Stier

Rotational diffusion of cytochrome P-450 LM_2 in reconstituted vesicle membranes was investigated by a measurement of time-dependent polarized emission of delayed fluorescence. Purified cytochrome P-450 (17-18 nm/mg) protein), labelled with diiodofluorescein iodoacetamide, was reconstituted with phosphatidylcholine, phosphatidylethanolamine from egg, phosphatidic acid (2:1:0.06 w:w, protein/lipid ratio 1:10 w:w) by cholate dialysis technique. The cytochrome exhibited strict uniaxial rotation about the normal to the membrane at all temperatures measured between 8°C and 37°C. The rotational relaxation time decreased from 8 to 24°C from 193 µs to 110 µs and increased slightly above 24°C. Binding of benzphetamine and reduction of the cytochrome greatly changed the conformation of P-450 as evidenced by large reorientation of the label in relation to the rotational axis. Rotational relaxation time changed from 129 to 204 µs after benzphetamine binding and to 51 µs after reduction of the substrate complex. Strict uniaxial rotation is preserved under these conditions. As P-450 LM_2, monomerized by n-octylglucoside, has a spherical structure (W.L. Dean and R.D. Gray, Biochim. Biophys. Res. Commun. 107: 265, 1982) from our data an oligomer (hexamer?) which forms a disc-like structure being immersed into the membrane to a depth, related to the conformational state of the protein, is most likely. Results demonstrate three states of immersion of the rotamer differing for the unliganded cytochrome, its substrate bound form and the reduced substrate complex. The transition between these states may control the transfer of the first and second electron during mixed function oxygenation.

Max-Planck-Institut für biophysikalische Chemie, Am Faßberg, D-3400 Göttingen

430

THE INDUCTION BY PHENOBARBITAL IN RABBIT LIVER OF A SECOND FORM OF CYTOCHROME P-450, FORM 5, ACTIVE IN THE METABOLISM OF AROMATIC AMINES TO MUTAGENIC PRODUCTS

I.G.C. Robertson*, C.J. Serabjit-Singh, J.E. Croft and R.M. Philpot

The major cytochrome P-450 isozymes induced in the rabbit liver by phenobarbital (PB) and by polycyclic hydrocarbons are forms 2 and 4, respectively. Form 4 is active in the metabolism of aromatic amines to mutagenic products. We have also shown (Mol. Pharmacol. 20: 662, 1981) the importance of form 5, a major pulmonary but a minor hepatic isozyme, in this activation.
Treatment of rabbits with PB increases the mutagenic activation of 2-aminoanthracene and 2-aminofluorene by hepatic microsomal preparations. In preparations from PB treated animals this activity is inhibited 85 % by antibody to form 5 compared to 55 % inhibition in control preparations, and the increase in form 5 mediated activity with PB treatment is 10-12 fold. The induction of form 5 was confirmed by increases in single radial immunodiffusion, and immunostaining of form 5 on nitrocellulose paper that contains microsomal proteins transferred from polyacrylamide gels. Treatment with ß-naphthoflavone decreases the hepatic microsomal content of form 5 to less than detectable levels and has little overall effect on the mutagenic activation of these aromatic amines.
We conclude that: 1) treatment of rabbits with PB increases the hepatic microsomal concentration of two isozymes of cytochrome P-450 (2 and 5) that are structurally, immunochemically, and catalytically distinct; 2) the hepatic microsomal concentrations of two isozymes (4 and 5) active in the mutagenic activation of aromatic amines are increased by different inducers.

National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, N.C. 27709, USA.
* Present address: Pharmakologisches Institut der Universität Mainz, Obere Zahlbacher Strasse 67, D-6500 Mainz.

431

WHICH SPECIES OF CYTOCHROME P-450 HYDROXYLATES DEBRISOQUINE? ATTEMPTS TO CHARACTERIZE THIS REACTION IN THE RAT.

M. Strecker and T. Wolff

About 10 % of the European population are unable to metabolize the antihypertensive drug debrisoquine (DQ) by 4-hydroxylation (Sloan et al., Br. med. J., 2, 655, 1978). There are indications, that the impaired oxidation of this compound is the result of a functional deficiency of a hepatic cytochrome P-450 isoenzyme. Since it is not known, which species of cytochrome P-450 mediates the DQ-4-hydroxylation we studied the effects of different inhibitors and inducers of cytochrome P-450 on the activity of the DQ-4-hydoxylase in rat liver microsomes. Similar to other monooxygenase reactions, the DQ-4-hydroxylase was dependent on NADPH and was inhibited by CO, metyrapone, SKF 525-A and 7,8-benzoflavone. A strong strain dependent difference of DQ-4-hydroxylase was noted between rats of the Wistar and DA strain (250 resp. 15 pmol/mg P/min), whereas the metabolism of aldrin, ethylmorphine and benzo(a)pyrene showed little variation. No sex-difference in DQ-4-hydroxylation was found in contrast to the 3 other monooxygenase activities studied, which were considerably higher in males than in females.
To induce microsomal DQ-4-hydroxylase, Wistar rats were pretreated with phenobarbitone, 3-methylcholanthrene, Chlophen A 50, dexamethasone and pregnenolone 16α-carbonitrile. In contrast to the other monooxygenase activities neither treatment did induce DQ-4-hydroxylase. Autoinduction with the enzyme substrate DQ could not be achieved by both p.o. and i.p. administation. Apparently no correlation exists between DQ-4-hydroxylase and the other monooxygenase activities in rats. Also in human liver biopsies no correlation was found between the activities of aldrin epoxidase and DQ-4-hydroxylase. Our findings suggest, that the DQ-4-hydroxylase is a special form of the cytochrome P-450.

Gesellschaft für Strahlen- und Umweltforschung, Abteilung Toxikologie, D-8042 Neuherberg

432

DIFFERENCES IN THE MECHANISM OF PEROXIDATIC AND MIXED-FUNCTION N-OXIDATION OF 4-CHLOROANILINE BY RABBIT LIVER MICROSOMAL CYTOCHROME P-450

I. Golly and P. Hlavica

Cytochrome P-450, which is known to mediate organic hydroperoxide-supported C-oxidation of a vast variety of substrates was tested for its ability to catalyze cumene hydroperoxide-dependent N-oxidation of 4-chloroaniline (4-CA). Structural modification, by treatment with mercurials or deoxycholate, of cytochrome P-450 to yield the P-420 form renders the hemoprotein a more powerful peroxidase. Pretreatment of the experimental animals with phenobarbital only slightly elevates the level of CHP-sustained N-oxidase activity, but strongly increases the coupling efficiency in the NADPH-driven process. There is a dramatic decrease in affinity for cytochrome P-450 of 4-CA when peroxide substitutes for NADPH/O_2 (K_m=20 mM), which, in part, results from competition between the arylamine and the oxidant for the same binding site on the enzyme. Rapid reaction studies suggest that, in both the NADPH- and the CHP-sustained N-oxidation reaction, product release might limit the overall rate of cytochrome P-450 cycling. With CHP, the metabolite profile is shifted from the preponderate production of 4-chlorophenylhydroxylamine to the formation of 4-chloronitrobenzene. These findings raise serious question as to the commonness of the oxygenating mechanism operative in the NADPH- and CHP-supported system respectively. The CHP-dependent process is proposed to involve one-electron oxidation of the arylamine by cumyloxy radicals generated through homolytic cleavage of the peroxide. The amino free radicals produced are likely to collapse with iron-bound hydroxyl radicals (Compound II) to give the corresponding hydroxylamine; the latter is carried through a sequence of one-electron oxidations to finally yield the aromatic nitro derivative.

Institut für Pharmakologie und Toxikologie der Universität, Nussbaumstrasse 26, D-8000 München 2, F.R.G.

433

EFFECT OF PEROXIDASE AND MONOOXYGENASE ON THE ABILITY OF SALICYLIC ACID AND ITS METABOLITE, GENTISIC ACID, TO BIND COVALENTLY TO PROTEIN AND DNA.

A. Stelzer and M. Metzler

Salicylates are among the most widely used drugs and are frequently taken during pregnancy. At high doses, salicylates are teratogenic in the rat. As the structures of salicylic acid (SA) and some of its known metabolites imply that these compounds may undergo metabolic activation, we have studied covalent binding to protein and DNA in vitro of SA and its 5-hydroxy-derivative, gentisic acid (GA), after oxidation with peroxidase and monooxygenase.

SA and GA, ^{14}C-labelled in the carboxyl group, were incubated with bovine albumin or calf thymus DNA in the presence of either peroxidase (from horseradish and mouse uterus)/hydrogen peroxide or microsomes (from phenobarbital-induced rat liver)/NADPH. In control experiments, enzyme was omitted. Albumin and DNA were recovered by precipitation with trichloroacetic acid or ethanol, respectively, and were reprecipitated twice. Albumin was further purified by dialysis and gel filtration.

Under these conditions, a high degree of apparently covalent binding of radioactivity to protein was observed for GA, but not for SA after activation with peroxidase. Peroxidase-mediated DNA-binding was marginal for GA and absent for SA. Monooxygenase did not cause protein binding of SA or GA.

These data indicate that a metabolite of salicylic acid, gentisic acid, can damage protein by means of peroxidatic activation, and may have implications for the mechanism of teratogenicity of salicylates.

Institute of Toxicology, University of Würzburg, Versbacher Strasse 9, D-8700 Würzburg, F.R.G.

434

INFLUENCE OF ANTIOXIDANTS ON THE CONCENTRATION OF OXYFERRO CYTOCHROME P-450 AND ON THE FORMATION OF H_2O_2 IN RAT LIVER MICROSOMES

R.Kahl and A.G.Hildebrandt

The action of commonly used food and feed antioxidants on the cytochrome P-450 redox cycle was studied. The steady state concentration of oxyferro cytochrome P-450 as measured by its absorbance at 440 nm was determined in liver microsomes from untreated and from ethoxyquin (EQ)-fed male Wistar rats (14 days, 1%). EQ feeding decreased the concentration from 0.119 ± 0.030 to 0.061 ± 0.013 nmoles per mg protein at pH 7.85 and from 0.044 ± 0.007 to 0.024 ± 0.015 nmoles per mg protein at pH 7.0 (means ± S.E., n=3). In a preliminary experiment, butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) fed for 8 weeks at 2% also lowered the oxyferro cytochrome P-450 concentration (BHA to 40%, TBHQ to 70% of control values).
The influence of antioxidants in vitro on the NADPH-dependent production of H_2O_2 was measured according to A.G.Hildebrandt and I.Roots (Arch. Biochem.Biophys.171:385, 1975). EQ activates H_2O_2 formation over a concentration range from 50µM (+ 20%) to 2 mM (+ 85%). Activation was also obtained with BHA (+ 65%), propyl gallate (+ 40%) and octyl gallate (40%) but not with butylated hydroxytoluene and dodecyl gallate in concentrations up to 2 mM. If such activation processes also occur in vivo at biologically effective antioxidant concentrations remains to be studied.
Max von Pettenkofer-Institut, Bundesgesundheitsamt, Thielallee 88-92, D-1000 Berlin 33

435

INHIBITION IN VITRO OF HEPATIC DRUG OXIDATIONS BY VANADATE, MOLYBDATE, AND REINECKATE

F.E.Beyhl

The inhibitory action of sodium ortho vanadate, ammonium hepta molybdate, and ammonium reineckate, on some microsomal mixed-function oxidases of rat liver, namely ketamine N-demethylase (I), 4-methylaminoantipyrine N-demethylase (II) aminopyrine N-demethylase (III), papaverine O-demethylase (IV), 4-methoxybiphenyl O-demethylase (V), and anisic ester O-demethylase (VI), and of guinea pig liver, namely methylayapanine O-demethylase (VII) and 7-ethoxycoumarin O-deëthylase (VIII), was studied in vitro, with inhibitor concentrations between 0.01 and 1.0 mM.

Sodium vanadate is an inhibitor of I, IV, V, VI, VII, and (to a minor extent) of VIII but it does not inhibit I and II. Ammonium meta vanadate and vanadyl sulphate are also inhibitors of microsomal mixed-function oxidases.

Ammonium molybdate is an inhibitor of VIII only but nor of II, III, IV, V, and VI. VII is inhibited only weakly, at higher concentrations.

Ammonium reineckate only inhibits III and IV, but none of the other mixed-function oxidases.

These differential inhibitory effects of these three salts on mixed-function oxidases could be used for the discrimination between the different cytochrome P-450 species of liver microsomes.

HOECHST AG, D-6230 Frankfurt am Main 80, F.R.G.

436

COBALT AND WARFARIN AS MODIFIERS OF CYTOCHROME P450: INFLUENCES OF DIFFERENT INDUCERS ON O-DEMETHYLATION OF THE TWO RADIOISOMERS OF ^{14}C-SCOPARONE IN VIVO

W. Legrum and E. Funke

In previous studies we found that several coumarin derivatives are sensitive to a pretreatment of mice with $CoCl_2$ (Legrum and Netter, Xenobiotica, 10:271,1980) showing an accelerated metabolism. This peculiarity was used to investigate O-demethylation of labelled herniarin in living cobalt-pretreated mice by measuring exhaled $^{14}CO_2$ (Legrum and Frahseck,J.Pharmacol.Exp.Ther.,221:790,1982) revealing a higher metabolism rate although the hepatic cytochrome P450 content is diminished.
A new coumarin derivative might possibly better detect not only cobalt-induction but also better recognize new and standard inducers. The two radioisomers of scoparone, [6-O-methyl-^{14}C]scoparone(R6) and [7-O-methyl-^{14}C]scoparone (R7) served as tools because the ratio of the demethylations occurring in microsomes is inverted using phenobarbital-induced rats (Müller-Enoch et al., Hoppe-Seyler s Z. Phys. Chem., 362:1091,1981).
The present work used the following pretreatments in male C57BL/6JHan mice: saline (1day), PB (3d x 80mg/kg, i.p.), 3-MC(1d x 30mg/kg i.p.),warfarin-Na(3d x 120mg/kg, p.o.), and $CoCl_2$(2d x 40mg/kg,s.c.). R7 was administered 2 hours after giving R6. Warfarin and 3-MC elevate the ratio R6/R7 whereas cobalt lowers it and PB has no effect. The in vivo molecular activity towards both demethylations together (calculated using the cytochrome P450 content determined immediately after the breath-test by preparing microsomes) decreases in all inducers used exept for cobalt. Female mice of the same strain show a clearly distinct behaviour regarding the ratio in normal individuals as well as after the different pretreatments. The breath-test presented here using the two scoparones describes a sensitive tool in detecting induction stages.
Institut für Pharmakologie und Toxikologie der Philipps-Universität, Lahnberge, D-3550 Marburg/Lahn

437

COBALT AND WARFARIN AS MODIFIERS OF CYTOCHROME P450: IDENTIFICATION OF CYTOCHROME P450 FORMS BY PAGE AFTER PARTIAL PURIFICATION ON OCTYL-SEPHAROSE CL-4B

L. Kling and K.J. Netter

As previously shown oral applications of sodium warfarin (3d x 120mg/kg) lead to an increase in microsomal protein and cytochrome P450 content in male C57BL/6J Han mice. This induction phenomenon was confirmed by enzyme kinetics (in vitro and in vivo) and ultrastructural data (Kling, Naunyn-Schmiedeberg's Arch. Pharmacol. 319 Suppl.: R 9, 1982). Experiments determining hexobarbital sleeping time and zoxazolamine paralysis time indicated a significant shortening ($p < 0.001$) of both times after warfarin pretreatment. In contrast cobalt pretreatment (2d x 40mg/kg) reduces microsomal cytochrome P450 content but enhances molecular activity for 7-ethoxycoumarin metabolism (Legrum et al., Toxicol. Appl. Pharmacol. 48: 195, 1979).

Now it is of interest if the above mentioned modifiers manipulate cytochrome P450 pattern in liver microsomes: Liver microsomes were solubilized with 0.75% Na-cholate in 20% glycerol (cyt. P450 yield: 55%-92%) and purified by hydrophobic interaction chromatography on Octyl-Sepharose CL-4B (column recovery of cyt. P450: 55%-67%). The specific content (nmol cyt. P450/mg protein) in the pooled fractions obtained by washing the column with a phosphate buffer, pH 7.4, containing 2% emulgen 913 and 0.1% Na-cholate was five- to eightfold. SDS-polyacrylamide gel electrophoresis was performed according to Laemmli (Nature 227: 680, 1970) using a 12% slab gel.

After warfarin pretreatment PAGE shows a phenobarbital-like cyt. P450 pattern. In cobalt-pretreated material an inversion of the intensities of the bands within the lower molecular weight range of the cyt. P450 family is observed in comparison to controls.

Institut für Pharmakologie und Toxikologie der Philipps-Universität, Lahnberge, D-3550 Marburg/Lahn

438

CHARACTERIZATION OF CIMETIDINE INTERACTION WITH LIVER MICROSOMES IN VITRO.

J.C.Jensen and R. Gugler.

Cimetidine (CI) has been shown to inhibit liver microsomal oxidative drug metabolism, with a spectral dissociation constant a magnitude lower than the inhibition constant (K_i), suggesting formation of a reactive intermediate producing an inactive CI-cytochrome P-450 (P450) complex (Rendic et al., Xenob. 9: 555, 1979). To investigate this possibility, rat liver microsomes were preincubated with CI and NADPH prior to the addition of 7-ethoxycoumarin (7EC) as substrate. Preincubation led to a decrease in product formation greater than that observed when CI and 7EC were added to microsomes together. This decrease was 47.4% after 5 min., 71.4% after 10 min., and 90% after 20 min. preincubation. Preincubation of microsomes with NADPH alone, CI alone, or with EDTA added had no effect on the decrease in product formation from 7EC. P450 content was also decreased in a time dependent manner, by 37.7% after 5 min., 67.7% after 10 min., and 73.6% after 20 min. preincubation with CI and NADPH. Addition of FeCN to the preincubation mixture reversed the decrease in P450 content. In addition, Ki of CI decreased from 0.254 mM to 0.202 mM and the type of inhibition changed from competitive to non-competitive when microsomes were preincubated for 10 min. with 0.25 mM CI and NADPH prior to the addition of 7EC. It is concluded that the inhibition of 7EC metabolism by CI involves the production of an intermediate which inactivates cytochrome P-450.

Medizinische Klinik, University of Bonn, 5300 Bonn-Venusberg, F.R.G.

439

ON THE CLEAVAGE OF DIGITOXIN (DT-3) BY HUMAN LIVER MICROSOMES

A. Schmoldt,* W. Herzberg,+ H. van Ackeren,+ H.F. Benthe ‡

In previous studies on the dt-3 metabolism in rats we could show that the cyt.P450 system is involved in both the cardenolide hydroxylation and the stepwise sugar chain cleavage yielding the bis - and monodigitoxoside of digitoxigenin (dt-2 and dt-1, resp.) (Schmoldt and Ronloff, 1978, this journal 305, 167).

The purpose of the present study was to find out whether the same mechanism can be confirmed for man. Liver microsomes were prepared from samples after liver operation of two patients. With the complete incubation system (with 40 µM ^{3}H-glycoside) we found the following oxidation and cleavage rates (values of two patients given in pmoles/mg micr. prot./min.): 15'-dehydro-dt-3: 16.0/4.9; dt-2: 17.3/7.0 (substrate: dt-3); 9'-dehydro-dt-2: 205/80; dt-1: 214/71 (substrate: dt-2); 3-dehydro-dt-1+dt-0: 11.2/29.8 (substrate: dt-1).

No metabolites were found in the absence of NADPH or O_2 and the presence of CO caused an inhibition by 50%

From the results it can be concluded that also in man digitoxigonin digitoxosides can only be cleaved after a cyt.P450 catalysed oxidation of the terminal digitoxosyl. Obviously, the rate limiting step is the initial oxidation of dt-3 yielding the intermediate 15'-dehydro-dt-3.

Inst. f. Rechtsmed. (*), Inst. f. Pharmacol. (‡) Univ. Hamburg, D 2 Hamburg 20. Marien-Krankenhs. (+) D 2 Hamburg 76

440

EXPRESSION OF POLYCYCLIC AROMATIC HYDROCARBON-INDUCIBLE AND PHENOBARBITAL-INDUCIBLE MONOOXYGENASES IN CONTINUOUS HEPATOMA CELL CULTURES

F.J. Wiebel*, F. Kiefer*, S.S. Park+, and H.V. Gelboin+

Previous observation have suggested that cell lines derived from H-4-II-E rat hepatoma cells express polycyclic aromatic hydrocarbon (PAH) inducible cytochrome "P-448"-dependent aryl hydrocarbon hydroxylase (AHH) and/or cytochrome "P-450"-dependent aldrin epoxidase (Wiebel et al., BBRC 94: 466, 1980). - The present studies were aimed at characterizing more conclusively the monooxygenase species expressed in differentiated and dedifferentiated variants of the H-4-II-E cells by their response to inducers and their inhibition by monoclonal antibodies.

PAH induced the AHH-activity in the dedifferentiated H5 cells more than 30-fold. Aldrin epoxidase activity increased about 5-fold in the differentiated C2Rev7 cells after exposure to dexamethasone and less than two-fold after phenobarbital. Monoclonal antibodies which were prepared to inhibit PAH-induced AHH (MAb MC P448) and phenobarbital-induced AHH (MAb PB P450) specifically inhibited the monooxygenase activities in the dedifferentiated H5 and differentiated C2Rev7 hepatoma lines, respectively. MAb PB P450 did not distinguish between the cytochrome P-450-dependent AHH and aldrin epoxidase activities of the C2Rev7 cells; however, their different sensitivity to 7,8-benzoflavone indicated that they represent two different monooxygenase forms. The results strongly support the previous notion that cells in continuous culture are capable of expressing cytochrome P-450 as well cytochrome P-448-dependent monooxygenases.

* Dept. Toxicology, Gesellschaft f. Strahlen- u. Umweltforschung, 8042-Neuherberg-München, F.R.G.;

+ Lab. Molecular Carcinogenesis, Natl. Cancer Inst., NIH, Bethesda, MD, USA.

441

DRUG-INDUCED HYDROCARBON RELEASE FROM ISOLATED PERFUSED MOUSE LIVER.

A.Wendel and M.Thelen

Livers of male phenobarbital-pretreated mice were perfused after using a cytochrome c pulse as a quality criterion for the perfusion. Without any additions, the organ evolved 1.1 pMol min^{-1} $gram^{-1}$ ethane while pentane was metabolized with a rate of 0.6 pMol min^{-1} $gram^{-1}$. Upon infusion of $FeCl_2$ 9 pMol min^{-1} $gram^{-1}$ ethane were released after 6 min. Perfusion of paracetamol led to a dose-dependent increase of ethane release up to 6 pMol min^{-1} $gram^{-1}$ at 2 mM paracetamol infused. From 2 to 10 mM paracetamol ethane rates decreased to basal. In contrast the rate of the perisinusoidal paracetamol-induced glutathione efflux continued to increased up to 10 mM paracetamol. Infusion of up to 100 mM ethanol was without effect on either parameter. We showed previously that paracetamol administration causes lipid peroxidation in vivo while the drug quenches this event in vitro. The implication of the findings in the perfused liver for studying drug toxicity mechanisms are discussed.

Physiologisch-chemisches Institut der Universität Tübingen
Hoppe-Seyler-Straße 1, D-7400 Tübingen

442

IS OXYGEN ACTIVATION INVOLVED IN ANTHRACYCLINE-INDUCED CARDIOMYOPATHY ?

H. NOHL

Anthracycline-induced cardiomyopathy is nearly exclusively discussed on the basis of mechanisms involving activated oxygen species. EPR investigations provided evidence that adriblastin is reduced to its semiquinone form (ADQ$^{\cdot}$) both, in the presence of electron transferring rat-heart mitochondria (RHM) and in the presence of NADPH supplemented NADPH-P_{450}-oxidoreductase from rabbit liver. The latter system was found to be autoxidizable itself but adriblastin additionally stimmulated $O_2^{\cdot}$-radical formation. On the other hand autoxidation of ADQ$^{\cdot}$ could not be observed when adriblastin reduction occured by electron transfer from respiring RHM. The search for a reducing system in the heart having the characteristic of liver microsomal electron transferring particles led to the detection of CO-binding pigments with an absorption maximum at 420 nm and an absorption minimum at 436 nm. Reduction with dithionite resulted in another type of difference spectra exhibiting a maximum at 433 nm and a minimum at 409 nm. Subcellular fragments both from rat- and from beef hearts containing these absorbing pigments were not able to generate activated oxygen species in the presence of reduced pyridine nucleotides (NAD[P]H) and also failed to reduce adriblastin to ADQ$^{\cdot}$. These results do not support the "activated oxygen theory" as the basic mechanism of anthracycline-induced cardiomyopathy. (supported by the DFG)

Institute für Pharmakologie und Toxikologie der Universität München, Veterinärstr. 13, 8 München 22 und Nußbaumstr. 26, 8 München 2.

443

THE RELATIONSHIP OF INTERINDIVIDUAL VARIATION OF CYTOSOLIC AND MICROSOMAL EPOXIDE HYDROLASE IN MAN TO OTHER DRUG-METABOLIZING ENZYME ACTIVITIES

I. Mertes, R. Fleischmann, H.R. Glatt and F.Oesch

Epoxides are the active forms of various carcinogens, mutagens and other toxic compounds. Many epoxides can be inactivated by enzymatic hydrolysis. Mammals have at least two epoxide hydrolases with a broad significance in drug metabolism, with one enzyme localized in the endoplasmatic reticulum and other membranes, and the other in the cytosol. We now report that human individuals greatly differ in the activities of these enzymes in liver. The activity in hepatic microsomes from 166 subjects (most of them patients suffering from hepatic diseases) was measured with benzo(a)pyrene 4,5-oxide as the substrate. The highest and lowest specific activities differed by a factor of 63. The activity in the cytosol, determined with trans-stilbene oxide as substrate, varied 539-fold among 145 subjects. Microsomal, but not cytosolic, epoxide hydrolase activity was increased in tuberculosis patients treated with rifampicin, ethambutol and isoniazid.

Microsomal epoxide hydrolase activity correlated weakly (Spearman's correlation $r = 0.17 - 0.29$, $p < 0.05$) with hepatic aryl hydrocarbon hydroxylase, ethoxycoumarin O-dealkylase, cytochrome c reductase, (1-naphthol) UDP glucuronosyl transferase and (2,4-dinitrochlorobenzene) glutathione transferase activities, but did not correlate ($p > 0.05$) with hepatic ethoxyresorufin O-dealkylase, cytosolic epoxide hydrolase activity and plasma activity levels of γ-glutamyltranspeptidase and glutamate pyruvate transaminase. Cytosolic epoxide hydrolase activity was unique among drug-metabolizing enzymes not only in that it showed the largest interindividual variation, but also in that its activity did not correlate with any other measured enzyme activity.

Pharmakologisches Institut der Universität Mainz und Medizinische Universitätsklinik Tübingen

444

TILIDINE: RELATIONSHIP BETWEEN ANTINOCICEPTIVE ACTIVITY AND RATE OF N-DEMETHYLATION IN VIVO AS DETERMINED BY ANALYSIS OF $^{14}CO_2$-EXHALATION

W. Christ, Karin Gindler, W. Hecker, and I. Roots+

The analgesic drug tilidine requires metabolic activation. To establish a relationship in vivo between rate of tilidine-N-demethylation and the antinociceptive activity, N-[$^{14}CH_3$]-tilidine was synthetized and administered i.p. (60 mg/kg b.w.) to female Lewis rats (200 g). $^{14}CO_2$-exhalation was continuously monitored as a measure of N-demethylation rate. These data were controlled by g.l.c. determination of nortilidine. Antinociceptive activity was evaluated by effect/time profiles using the hot plate test. Maximum exhalation rate of $^{14}CO_2$ was 0.30 % of the dose per min (SD=0.09) and occurred during the time span from 15 to 30 min. Within 3 h, 30 % (SD=7.8) of the radioactive dose was exhaled. This rapid metabolism corresponded to the maximum analgesic effect in the hot plate test observed 30 min after tilidine injection.
Modification of cytochrome P-450 activity by an inhibitor (metyrapone, 100 mg/kg orally, 2 h prior to tilidine inj.) decreased maximum exhalation rate and cumulative excretion of $^{14}CO_2$ by about 70 %. This was parallelled by decreased and delayed antinociceptive efficacy as tested on the hot plate. Conversely, the enzyme inducer phenobarbital (40 mg /kg i.p., 4 days) effected a threefold increase in maximum exhalation rate of $^{14}CO_2$ and a corresponding increase in analgesia. - Pretreatment with ketoconazole (80 mg/kg orally, 4 days) whose enzyme inducing effect has been described, pronouncely potentiated the antinociceptive effect of tilidine. However, $^{14}CO_2$-exhalation curves were similar to those of controls. This may point to an inhibitory effect of ketoconazole on further steps of $H^{14}CHO$ metabolism. - The experiments show that the time course of analgesia after tilidine is greatly subjected to changes in the rate of its metabolism.

Institut für Arzneimittel des Bundesgesundheitsamtes, Dept. klin. Exptl. Pharmakologie, Seestr.10,D-1000 Berlin 65
+ Inst.f.Klin.Pharmakologie, Freie Universität Berlin

445

PATHWAYS OF BIOTRANSFORMATION FOR THE HEXACHLOROBENZENE (HCB) AND PENTACHLORONITROBENZENE (PCNB) METABOLITE N-ACETYL-S-(PENTACHLOROPHENYL)CYSTEINE (PCC), AND FOR SOME OF ITS DERIVATIVES. POINTS AT THE MECHANISMS OF THE REDUCTIVE DECHLORINATION OF HCB AND THE REDUCTIVE DENITRATION OF PCNB.

G.Renner, P.-T.Nguyen and C.Hopfer

Studies on the metabolism of the fungicides HCB and PCNB indicated similarities in their pathways of biotransformation. Sulphur-containing metabolites of both fungicides, such as PCC, pentachlorothiophenol, pentachlorothioanisole, 1,4-dimercapto-tetrachlorobenzene and/or 4-methylthio-tetrachlorothiophenol, and 1,4-bis(methylthio)tetrachlorobenzene, but also non sulphur-containing metabolites, such as phenols and chlorinated benzenes, are identical. Studies on the metabolism of the mercapturic acid PCC in rabbits and rats revealed the above-mentioned sulphur-containing metabolites which were further metabolized. Of interest was the finding of pentachlorobenzene after administration of pentachlorothiophenol and of 1,2,4,5-tetrachlorobenzene after administration of S,S'-(tetrachloro-p-phenylene)dicysteine, a degradation product of PCNB and of its metabolite 2,3,5,6-tetrachlorothiophenol, respectively, in hexane extracts of excreta by g.l.c. analyses. These results elucidate the mechanisms of reductive dechlorination of HCB and denitration of PCNB. Replacement of one chlorine in HCB or of the nitro group in PCNB by the acetylcysteinyl moiety originating from glutathione leads via PCC to pentachlorothiophenol which forms pentachlorobenzene by reductive desulphuration.

Institut für Pharmakologie u. Toxikologie der Universität München, Nußbaumstr.26, D-8000 München 2

446

FORMATION AND DISPOSITION OF A METABOLITE OF DOXYCYCLINE

R. Böcker and C.-J. Estler

Contrary to earlier findings doxycycline undergoes biotransformation in experimental animals and humans, at least one metabolite is formed (R Böcker, J Chromatogr in press, 1983). This metabolite has hardly any antibacterial activity and therefore cannot be detected in biological test system but can be measured by means of high performance liquid chromatography (R Böcker CJ Estler A Weber, The Lancet 1155, 1982). In mice 6 h after injection of 50 µg/g doxycycline small amounts of the metabolite can be found. Maximum concentrations are reached after 7-9 hours. The formation of the metabolite can be enhanced by pretreatment of the animals with phenobarbital: in the livers of phenobarbital pretreated animals the metabolite reaches about 3-5 times higher concentrations than in the control animals. In the kidneys of normal and phenobarbital pretreated animals the concentration of the metabolite is about 2-3 times higher than in the livers of the animals. The metabolite was not detectable in the feces. Most of it seems to be excreted via the urine. Within 15 h after the injection of doxycycline the amount excreted (corresponding to ca 3% of the dose of doxycycline applied) is six times higher in phenobarbital treated mice than in the control animals.

Institut für Pharmakologie und Toxikologie der Universität, Universitätsstraße 22
D 8520 Erlangen

447

ASSESSMENT OF HEPATIC DRUG HYDROXYLATION AND CONJUGATION ACTIVITY IN MAN BY URINARY METABOLITE PROFILES OF ANTIPYRINE

H. Bässmann, J. Böttcher and R. Schüppel

Urinary profiles of phase-I- and phase-II-metabolites of antipyrine (A.) have been used to assess hepatic MFO and conjugation activity in male and female volunteers (n= 2 x 5, mean age: 24.8±3.0 y). Eleven different metabolites have been followed for 72 h after ingestion of 1200 mg of A.
Urinary glucuronides have been measured directly by HPLC against authentic glucuronide standards. Sulfates of norantipyrine (NORA) and of 4-hydroxy-antipyrine (4-HA) have been assayed using selective hydrolysis and quantitative TLC. Phase-I-metabolites and unchanged A. have been quantitated by TLC. Elimination kinetics of A. have been followed from saliva samples (over 48 hrs.).

Results in this group are as follows ($\bar{x}\pm$s.e.m. as % of the dose): unchanged A.: 3.5±1.1 %, free phase-I-metabolites: 11.0±1.8 %, comprising 3-hydroxymethyl-antipyrine (3-HMA): 4.7±1.2 %, 3-carboxy-antipyrine: 4.5±1.3%, and as minor metabolites up to 1 % each, 4-HA, 4'(p)-hydroxy-antipyrine and 4,4'-dihydroxy-antipyrine (4,4'-DHA). Total glucuronides were found to represent about 69.7±4.2 %. This fraction consisted of 3-HMA gluc.: 13.1±1.1 %, 4-HA gluc.: 29.4±2.5 %, NORA gluc.: 23.3± 2.3 % and 4,4'-DHA gluc.: 3.9±1.0 %. Sulfate esters were within the range of 8-13 %.
Clearance values for A. were calculated as follows: 0.0394±0.0064 l/h/kg.

Comparing both sexes, no significant sex differences were found in any of the parameters tested. Results obtained in this pilot study utilizing HPLC and TLC techniques are in excellent agreement with own data reported earlier from a study using 3-^{14}C-antipyrine in man. (Naunyn-Schmiedeberg's Arch. Pharmacol. 316, R5 1981).

Inst. für Pharmakologie u. Tox., TU, D-3300 Braunschweig

448

RENAL ELIMINATION OF SODIUM VALPROATE AFTER A SINGLE ORAL DOSE APPLICATED IN 3 DIFFERENT FORMULATIONS TO HEALTHY VOLUNTEERS UNDER FASTING AND NONFASTING CONDITIONS

M. Theisohn, I. Herma, H. Hahn

1o healthy volunteers participated in the study (f:m=4:6, age: 19-29 y, b.wt.:55-75 kg). A single dose of 600 mg sodium valproate (VPA) in form of syrup, enteric coated dragee, and time delay capsule (resorbable in the stomach) was given orally in a sequence with an interval of 2 weeks. The volunteers took their dose in the morning either after breakfast (5) or on an empty stomach with additional fasting until lunch.
Serum levels of VPA were determined by GLC for 72 h after each dose. The urine was collected for 1 week in periods of 8-24 h. The urinary concentrations of VPA and its saturated metabolites 3-OH-VPA and 3-Keto-VPA (ß-oxidation), 4-OH-VPA (ω2-oxidation), and 5-OH-VPA (ω1-oxidation) were determined by the GLC method of Schäfer and Lührs (Drug Res. 28:657, 1978) before and after hydrolysis by ß-glucuronidase/arylsulfatase (24 h at 37°C, pH 4.7).
From the administered dose of VPA (600 mg) 49.3% were recovered in the urine. 13.5% of the dose (of d.) were found as unchanged VPA of which 92% was conjugated. 32% of d. were recovered as metabolites of the ß-oxidation pathway: 3-Keto-VPA (29% of d., 7% conjugated) and 3-OH-VPA (3.o% of d., 6o% conj.). Only 3.8% were metabolized by ω-oxidation: 5-OH-VPA (o.9% of d., 23% conj.) and 4-OH-VPA (2.9% of d., 28% conj.).
The metabolic pattern was identical after the intake of VPA as syrup or enteric coated dragee (fast resorbable preparations). However, after the intake of the time delay capsule the elimination as VPA (8.5% of d.) and by ω-oxidation (3.o% of d.) was reduced in favour of the ß-oxidation pathway (35.7% of d.). Additionally, the recovery in the urine was slightly but significantly reduced (47.2%). Intake of the VPA dose under fasting or nonfasting conditions had no influence neither on the metabolic pattern nor the recovery rate.

Pharmakol.Inst.Uni Köln, Gleuelerstr.24, D-5000 Köln 41

449

BIOTRANSFORMATION OF ^{3}H-LEVOBUNOLOL IN RAT, MOUSE, DOG AND MAN

A. von Hodenberg and W. Klemisch

Levobunolol is a potent nonselective ß-adrenoceptor antagonist (Kaplan in: Pharmacology of Antihypertensive Drugs, Raven Press, 1980). Comparison is made of biotransformation of the tritiated compound after oral administration to rat, mouse, dog and man.

The metabolite profiles in urine were established by means of hplc combined with a radioactivity flow-through detector. Separation was achieved on a C-18-reversed-phase column after injection of the urine sample without prior clean up.

After oral administration of 10 or 50 mg/kg to rat, mouse and dog and 12 mg to human volunteers 54-82 % of the radioactivity were found in 0-24 h urine.

In principle the following routes of biotransformation were observed:

1. Reduction of the carbonyl group yielding dihydrolevobunolol
2. Hydroxylation of the ring system
3. Oxydative degradation of the side chain
4. Conjugation of the unchanged substance and the metabolites

The metabolite pathways showed pronounced species differences. In rat and mouse urine the ring-hydroxylated metabolites dominated. The introduced hydroxyl group was conjugated with ß-D-glucuronic acid. In rat urine a further metabolite occured with an additional hydroxyl group in the ring system. In dog urine both hydroxylation of the ring system as well as oxidative degradation of the side chain were observed. Biotransformation in man was less intense. The major part of urinary radioactivity could be attributed to levobunolol and dihydrolevobunolol and/or their conjugates.

Gödecke Research Institute, Dept. of Biochemistry, Mooswaldallee 1-9, D-7800 Freiburg

450

MASS SPECTRAL STUDIES ABOUT THE METABOLISM OF DITAZOL IN MAN*

H. Maurer, I. Kleff and K. Pfleger

Ditazol (4,5-Diphenyl-2-bis(2-hydroxyethyl)-aminooxazol) was introduced as a platelet aggregation inhibitor with antiinflammatory and analgesic properties. Marchetti et al. [1] have described the metabolism in rat, rabbit and man employing TLC, GC, PC, UV and radio activity measurements. They have identified the following metabolites in man: Ditazol, N-desalkyl-ditazol and benzyl.

In addition we found employing mass spectrometry N,N-bisdesalkyl-ditazol, hydroxyphenyl-ditazol, N-desalkyl-hydroxyphenyl-ditazol, N,N-bis-desalkyl-hydroxyphenyl-ditazol.

METHODS: The studies were carried out using the urine of healthy volunteers, which had taken a single dose of 400 mg of ditazol. After enzymatic hydrolysis (ß-glucuronidase and arylsulphatase) the urines were extracted twice with a mixture of two parts dichloromethane, two parts isopropanol and six parts ethylacetate at a pH of about 8-9. The residue was acetylated (acetanhydride in pyridine) to improve the gas chromatographic volatility of the hydroxy and amino compounds. The acetylated extract was analysed using a computerized gas chromatographic-mass spectrometric technique (GC-MS-conditions see Ref. [2]).

REFERENCES: [1] E. Marchetti, G. Mattalia, and G. Bergesi (1973) Arzneim.-Forsch. 23: 1291-1295
[2] H. Maurer and K. Pfleger (1981) J. Chromatogr. 222: 409-419.

*This work is part of the MD thesis of I. Kleff.

Institut für Pharmakologie und Toxikologie der Universität des Saarlandes, D-6650 Homburg/Saar

451

DIFFERENTIAL INTERACTION OF SODIUM 2-MERCAPTOETHANE SULFONATE (MESNA) AND ITS OXIDATION PRODUCT DIMESNA WITH THE ANION EXCHANGE PROTEIN IN RED BLOOD CELLS

D. B. Wildenauer, M. Kohl and H. Reuther

Mesna (Uromitexan^{R}) has been introduced recently as an antidot against the urotoxic effects of oxazaphosphorine cytostatics (Scheef et al. Cancer treatment reports, 63: 501-505, 1979). Studying the influence of mesna and its oxidation product dimesna on the transport of ^{35}S-labeled sulfate in human red blood cells in vitro, the following results were obtained:

1. Countertransport and transmembrane effect indicated that mesna serves as substrate for the carrier.
2. Dimesna was found to inhibit the carrier mediated transport of sulfate in a competitive manner (50% inhibition: 60 mM dimesna, 5 mM sulfate, 37°C, pH 7.2). Inhibition was also observed when the transport of mesna was studied in erythrocytes suspended in plasma. Oxidation of mesna occurs under these conditions.

This conversion of a substrate into an inhibitor by components of the plasma simply by oxidation seems to be the reason that the compound can reach the kidney without interfering with the antitumor effect of cytostatic agents. It is shown by Ormstad et al. (Cancer Res.1982, in press) that dimesna is taken up by the kidney and reduced to mesna. Mesna is supposed to bind the renally excreted urotoxic metabolites of oxazaphosphorines.

Institut für Pharmakologie und Toxikologie der Universität München, Nussbaumstr. 26, D-8000 München 2.

452

ANTIDOTAL EFFECT OF SODIUM THIOSULFATE (ST) ON FREE IONIC AND ON SODIUM NITROPRUSSIDE (SNP)-BOUND CYANIDE IN RABBITS

J. Pill, P. Engeser and M. Höbel

SNP has a dose-dependent hypotensive effect, which disappears soon after stopping the infusion. This is explained by a rapid decay and/or decomposition of SNP (Smith, Kruszina, J. Pharmacol. exp. Ther. 191:557, 1974, Höbel et al. Klin. Wochenschr. 56 (Suppl.I): 147 and 153, 1978). It is not known, however, whether iron and cyanide containing complexes are generated as intermediates or complete decomposition to iron and cyanide ions in blood or vessels takes place. To clear up this question rabbits (group I) were infused with 7.5 mg/kg/h SNP (LD 100 61 min, Höbel et al.) and with 31.25 mg/kg/h of the cyanide antidote ST and group II with comparable amounts of iron ($FeSO_4$, IS), cyanide (NaCN) and ST. We observed pH, pCO_2 and base excess (BE) in arterial blood as well as survival time ($\bar{x}$+s$_{\bar{x}}$).

		pH	pCO_2 (mmHg)	BE (mmol/l)
I	before	7.44 ± 0.026	32.6 ± 1.34	-1.0 ± 1.92
(n=5)	after	7.45 ± 0.016	26.3 ± 1.95	-4.3 ± 0.90
II	before	7.46 ± 0.013	28.9 ± 1.89	-1.5 ± 0.42
(n=6)	after	6.75 ± 0.057	60.3 ± 9.20	-30.9 ± 2.74

The animals in group I showed only slight changes in blood gas values and no changes in behaviour during 8 h infusion and 48 h observation period. In contrast, if cyanide is infused simultaneously with IS and ST the average survival time in group II was 66.7 ± 13.8 min after starting the infusion. Severe metabolic acidosis was observed in blood obtained by cardiac puncture post mortem. The different behaviour of free and complex bound cyanide leads to the conclusion that in SNP bound cyanide is not broken down in blood. These findings are compatible with plasma iron changes (Höbel et al.).

Department of Medical Research, Boehringer Mannheim GmbH Sandhoferstraße 116, D-6800 Mannheim

453

GENERATION AND FUNCTIONAL CHARACTERIZATION OF MONOCLONAL MOUSE ANTIBODIES AGAINST SURFACE ANTIGENS OF RAT BASOPHILIC LEUKAEMIA (RBL) CELLS.

G. Micklefield, A. Bohn

The close relationship between rat mast cells and rat basophilic leukaemia cells (RBL) as to IgE-receptor binding and the presence of Fcγ-receptors led us to generate monoclonal antibodies directed against cell surface antigens. Hybridomas were generated by the fusion of NS-1 mouse myeloma cells with murine spleen and lymphnode cells. The culture supernatants were assayed by two Elisa systems: 1) for the production of mouse immunoglobulin in general and 2) for antibodies directed against surface antigens of RBL-cells. Therefore RBL-cells were attached to polyvinylchloride microtiter plates. Nine hybrids produced antibodies directed against surface antigens of RBL-cells.Hybrids were cloned and characterized with regard to their isotype and light chains. All nine clones secreted IgM withκ light chains. Monoclonal antibodies were further purified by immunoadsorption. A immunofluorescence performed with RBL-cells revealed, that all nine antibodies are able to show a specific fluorescence. Furthermore seven of these nine antibodies also show a specific fluorescence with purified rat mast cells. These seven antibodies were tested as to their ability of interacting with the IgE-receptor of RBL-cells and purified rat mast cells. Four of these seven antibodies inhibited the binding of radiolabelled rat IgE to rat mast cells; the inhibition rate ranged from 40 to 70 percent. These four antibodies also revealed an inhibition of the passive cutaneous anaphylaxis (PCA) reaction induced with mouse reaginic serum.

Lehrstuhl Medizinische Mikrobiologie und Immunologie, Ag. Infektabwehrmechanismen, Ruhr-Universität, 4630 Bochum

454

IN VITRO STIMULATION OF MACROPHAGES BY BIOLOGICAL RESPONSE MODIFIERS (BRM) TO SECRETE COLONY STIMULATING FACTOR(S) AND PROSTAGLANDIN E.

E. Schlick, K. Hartung, A. Bartocci and M.A. Chirigos

In vitro growth and differentiation of bone marrow granulocyte-macrophage precursor cells in semisolid agar cultures requires the presence of colony stimulating factors (CSF). Prostaglandin E serves as a negative feedback regulator in this system. CSF and PgE are elaborated mainly by monocytes and macrophages. Here, we report the evidence of an augmented CSF and PgE secretion by macrophages after in vitro incubation with murine L-cell Interferon, Poly ICLC (Polyriboinosinic-polycytidylic acid poly-L-lysine), BM41.332 (2-cyan-1-[(2-methoxy-6-methyl-pyridin-3yl)-methyl]-aziridine and LPS. Optimal effects with an up to 3-fold increase in CSF activity were obtained by incubating the cells with 500 U/ml IF, 50 µg/ml Poly ICLC, 25 µg/ml BM41.332 or 10 µg/ml LPS for 2 to 4 days. This effect was paralleled by a similar strong increase in PgE secretion. Time studies revealed a termination of the PgE secretion after 24 hrs, whereas the CSF secretion continued for up to 4 days. Histological examination of the colonies induced indicate the presence of at least two types of CSF. Incubation of macrophages for 3 days in the presence of neutralizing antibodies against murine L-cell IF abolished the CSF inducing effect of IF, but not of Poly ICLC or LPS. Other drugs tested (Diethyldithiocarbamate, Maleic anhydride divinyl ether, Azimexone) failed to stimulate the in vitro secretion of CSF and PgE. The results indicate that some BRM not only stimulate macrophages to secrete the myelopoietic growth factor CSF, but also the inhibitor PgE. The increased CSF secretion does not appear to be associated with the ability of the active drugs to induce IF.

Immunopharmacology Section, Biological Research and Therapy Branch, DCT, National Cancer Institute, NIH, Frederick, MD. 21701, USA.

455

SUBCELLULAR CHARACTERIZATION OF ENZYMES INVOLVED IN LEUKOTRIENE FORMATION WITHIN HUMAN POLYMORPHONUCLEAR GRANULOCYTES

J. Brom[x], K.D. Bremm[x], B. Spur[xo], A. Crea[xo], W. König

We studied the enzymatic distribution of lipoxygenase, S-glutathionyltransferase and L-gamma-glutamyl-transpeptidase within human granulocytes. Cells were disrupted. Differential centrifugation was performed at 400-, 3000-, 20000- and 200000xg (fractions 1-5). Individual fractions were incubated with arachidonic acid (0.16mM) in the presence of indomethacine (10^{-7}M). Chemotactic and slow reacting substance (SRS) -activity was recovered from fractions 4 and 5. S-glutathionyltransferase activity was determined with 1-chloro -2,4 dinitrobenzene (7,5mM) in the presence of glutathionine (30mM) or with synthetic LTA_4. Gamma-glutamyl-transpeptidase which transforms LTC_4 to LTD_4 was determined using 7-amino-4-methyl-coumarine as substrate. Gamma-glutamyltranspeptidase activity was present in the 200000xg precipitate while S-glutathionyl transferase was recovered from the 200000xg supernatant. By isopycnic sucrose-gradients the various enzymes (lipoxygenase, S-glutathionyltransferase, gamma-glutamyltranspeptidase) could be separated. Our results indicate a compartmentalisation of these enzymes involved in leukotriene formation within the cells.

Lehrstuhl Med. Mikrobiologie und Immunologie,[x] Ag Infektabwehrmechanismen, Ruhr-Universität Bo.[xo] Universität Düsseldorf, Institut für Chemie

456

IMPROVEMENT OF CORNEAL EPITHELIAL REPAIR BY BAY O 8276, A NOVEL LIPOXYGENASE INHIBITOR AND ANTIINFLAMMATORY AGENT

M. Mardin[1], W.-D. Busse[1], R. Grützmann[1], R. Rochels[2]

Bay O 8276 (N-1,2,4-Triazol-3-yl-p-chlorphenylsulfenamid) inhibits the formation of lipoxygenase (LO) products (5-HETE, 12-HETE, 15-HETE, LTB_4 and its isomers) in rabbit and human PMN leukocytes at concentrations of 1-10 µg/ml as shown by the HPLC-analysis of the metabolites. Its selectivity towards lipoxygenase (LO) pathway seems to be more pronounced than the references NDGA and phenidone. This was demonstrated with human platelets showing that Bay O 8276 inhibits the conversion of ^{3}H-arachidonic acid to 12-HETE more effectively than to HHT and TXB_2 at concentrations of 1-10 µg/ml, as detected by thinlayer radiochromatography. In a model of corneal re-epithelialisation in New Zealand rabbits, the effect of Bay O 8276 on leukocyte infiltration and epithelial repair was investigated by histological determinations after abrasio corneae. Upon daily topical application of a 1% solution of the compound in Tween 80 a complete repair of epithelium was achieved after 2.5 days. The control as well as the CO inhibitor indomethacin (1%) treated groups (n=5 each group) on the other hand showed a significant longer duration of the epithelium regeneration of approx. 5 days. It was also demonstrated that the infiltration of PMNs into the injured areas was reduced by the treatment with Bay O 8276 but increased by indomethacin. The results indicate that leukocyte infiltration may play an important role in corneal inflammation which is effectively inhibited by the LO-inhibitor Bay O 8276.

1) Institut für Pharmakologie, Bayer AG, 56 Wuppertal 1
2) Universitätsaugenklinik, Langenbeckstr. 1, 65 Mainz

457

RATE OF PROSTAGLANDIN SYNTHESIS IN VARIOUS CELLS IS NOT CONTROLLED BY PHOSPHOLIPASE A ACTIVITY BUT BY REINCORPORATION OF RELEASED FATTY ACIDS INTO PHOSPHOLIPIDS

C. F. Körner, G. Hausmann, D. Gemsa and K. Resch

The arachidonic acid (AA) metabolism has been studied in isolated rat thymocytes and rat peritoneal macrophages. Resting and growth stimulated rat thymocytes preferred ^{14}C-AA incorporation into membrane phospholipids, a significant conversion to prostaglandins (PG) was not found. Rat peritoneal macrophages known to release large amounts of PG's in response to various stimuli showed an increase of ^{14}C-AA conversion to PG's when stimulated by zymosan particles, whereas ^{14}C-AA incorporation into phospholipids was not affected or decreased.
Inhibition of lysophosphatide acyltransferase (LAT) by 50 µMol/l merthiolate led to an increase of all determined PG's (PGE_2, $PGF_{2\alpha}$, TXB_2, 6-keto-$PGF_{1\alpha}$) in stimulated, as well as in unstimulated rat thymocytes and peritoneal macrophages. This augmentation of PG production was more than two fold and showed a maximum within 60 min.
In order to proof these results on endogenously produced PG's we chose the same incubation procedures without adding ^{14}C-AA. PG's were measured in cell supernatants by radioimmunoassay methods. PG's formed from endogenously released arachidonic acid increased up to seven fold when LAT was inhibited.
These results suggest that the amount of endogenous PG's is not controlled by phospholipase A_2 activity but rather by LAT activity regulating the substrate concentration for the cyclooxygenase and probably lipoxygenase pathways. It is generally accepted that release of arachidonic acid from cellular phospholipids is the rate limiting step in prostaglandin synthesis.

Medizinische Hochschule Hannover, Zentrum Pharmakologie und Toxikologie, Abt. Molekularpharmakologie,
D-3000 Hannover 61, FRG

458

HISTAMINE RELEASE: EFFECTS OF CLOXACEPRIDE, A CALMODULIN-ANTAGONIST, ON MAST CELLS

E. Sánchez-Delgado* and G. Metz**

Since the discovery of cromoglycic acid (DSCG), the substances that inhibit the release of mediators from mast cells have a key position in the therapy of immediate hypersensitivity diseases. In this context we tested the potential antiallergic properties of cloxacepride. Isolated rat mast cells were preincubated 5 min at 37°C with different concentrations of cloxacepride. Then the degranulating agent (compound 48/80, 1µg/ml)was added and the samples were incubated for further 5 min. The released histamine was assayed fluorometrically. For comparison the same was done with DSCG and ketotifen.

Cloxacepride inhibited significantly rat mast cells secretion with an IC_{50} of 52 µM. In other experiments we found that cloxacepride inhibits the calmodulin-dependent activity of the phosphodiesterase. It is known that the elevation of c-AMP in the mast cells inhibits histamine release. This could explain the action of cloxacepride as histamine release inhibitor. Other calmodulin-dependent functions could also be involved. Other effects of cloxacepride and their possible explanation will be discussed.

*Abt. Pharmakologie & Toxikologie der Universität Ulm, Oberer Eselsberg, D-7900 Ulm.
** Abt. Forschung und Entwicklung, Merckle GmbH, D-7902 Blaubeuren.

459

RELEASE OF SLOW-REACTING SUBSTANCE OF ANAPHYLAXIS (SRS-A) FROM GUINEA PIG COLON.

R.H. Wölbling, U. Aehringhaus, K. Morgenroth, B.M. Peskar and B.A. Peskar

SRS-A, now known to consist of a mixture of leukotrienes (LT), is a potential mediator of inflammatory reactions. We have investigated the release of SRS-A from guinea pig colon. The colon of ovalbumin-sensitized guinea pigs was separated into mucosa, muscularis propria and subserosa as confirmed histologically. The tissues were preincubated in Tyrode solution at 37° C for 5 min and then challenged by the addition of ovalbumin (1 mg/ml). Incubation was continued in the presence of the antigen for 2o min. SRS-A in the incubates was determined by bioassay using the isolated guinea pig ileum and results were expressed in terms of histamine equivalents (HE). Data on SRS-A release were confirmed using a radioimmunoassay for LTC4 (Aehringhaus et al., FEBS Letters 146, 111, 1982). No SRS-A was released from the colonic tissues during the preincubation period, but considerable amounts (o.52 ± o.o9 HE/g wet weight/2o min,mean ± S.E.M., n = 17) were found in mucosal incubates after challenge. Much smaller amounts of SRS-A were released from challenged subserosa and muscularis. The biological activity of the SRS-A formed could be antagonized by the SRS-A antagonist FPL55712 (sodium 7-(3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-2-hydroxy-propxy-4-oxo-8-propyl-4H-benzopyran-2-carboxylate). Furthermore, the dual inhibitor of cyclooxygenase and lipoxygenase BW755C (3-amino-1-(3-trifluoromethylphenyl-2-pyrazoline hydrochloride) abolished release of biologically active SRS-A and immunoreactive LTC4. The results suggest that SRS-A synthesized in the mucosa may play a role as mediator of colonic inflammatory reactions, particularly those of immunological etiology.

Department of Pharmacology and Toxicology and Department of Pathology, Ruhr-Universität Bochum, and Department of Gastroenterology, University Essen.

460

DENERVATION INCREASES THE SENSITIVITY OF RAT SKELETAL MUSCLE TO ATX-II

I. TESSERAUX AND J. B. HARRIS

ATX-II From anemonia sulcata depolarises mammalian slow-twitch muscle fibres and prolongs the muscle fibre action potential (Alsen, Harris and Tesseraux, Br. J. Pharmac., 1971, 74, 61-71). Fast-twitch muscle fibres are relatively insensitive to ATX-II (Harris and Pollard, Br. J. Pharmac., 1981, 74, 249P). Experiments were designed to see if the removal of a neural influence results in a rat fast-twitch muscle becoming sensitive to the toxin. Denervation rendered soleus muscle fibres slightly more sensitive to ATX-II, threshold concentration falling from 10^{-7}M to 3 x 10^{-9}M. The change in sensitivity of EDL was much greater, the threshold concentration falling from 10^{-6}M to 3 x 10^{-9}M.
The proportion of EDL fibres exhibiting prolonged action potentials after ATX-II (10^{-8}M) increased with time following denervation over the period of 3-8 days; there was no correlation between action potential duration and time after denervation in individual muscle fibres.
The effects of the toxin were Na^+ dependent, enhanced by high frequency stimulation (50 pulses at 10 H_z) and readily reversed by washing the affected muscles.

Muscular Dystrophy Group Laboratories
Newcastle General Hospital
Newcastle upon Tyne NE4 6BE England

461

THE ENRICHMENT OF VOLATILES FOR TOXICOLOGICAL AND ENVIRONMENTAL TRACE ANALYSIS

W.Dünges

The determination of further, not yet identified compounds is important in toxicological and environmental research. Volatile organics in ground and surface water are part of this "pool of unknowns" which threatens one of our most vital resources.

Even with most sensitive instruments as capillary-GC/MS in most cases enrichment is necessary for the analysis of environmental toxicants. Acetone solutions could be concentrated under partial reflux -see W.Dünges, "Prä-chromatographische Mikromethoden", Heidelberg, 1979- if the solutes had boiling points above 140°C.

Experimental conditions have now been established for the investigation of lower boiling solutions. For instance 100 ng of toluene in 2 ml of n-pentane were retained quantitatively as the solvent was evaporated to 5 µl; so far end volumes had to be above 200 µl because of losses.

The search for volatile unknowns is thus facilitated greatly as e.g. extracts from water can be enriched to much higher extent.

Außenstelle Frankfurt des Instituts für Wasser-, Boden- und Lufthygiene (Institutsleiter Prof. Dr.K.Aurand) Kennedyallee 97 D-6000 Frankfurt/M

462

ON THE IMPORTANCE OF FUNCTIONAL AND BEHAVIORAL TESTS DURING THE POSTPARTAL DEVELOPMENT OF RATS.

H. Frohberg, J.Gleich and H.D. Unkelbach

Reproductive toxicity studies include - apart from the parameters usually examined in toxicity studies, e.g. survivability, body weight development, and food consumption - also parameters on the physical and functional development. In addition, the behavior in the open field, locomotion and learning behavior are tested. It was the aim of the present investigation to find out the amount of additional information gathered from these extensive tests. For this purpose the following investigations were carried out:

1. Restriction of food (50 %) during gestation, with and without restriction to 8 offspring/litter after birth;
2. Administration of vitamin-A-acid, a well-known teratogen, during organogenesis;
3.+4. Administration of biphenylethinylcarbinol, a nonsteroidal antiphlogistic, and of fencamfamin, a psychostimulant, from days 15 post conceptionem to 21 post partum.

The total of the parameters on the functional and physical development of the offspring was compared with the parameter body weight development of the offspring and the influence of the individual treatments on the mothers.

The body weight development of the offspring resulted to be the most sensitive of all parameters tested.

Hauptleitung Medizin/Institut für Toxikologie, E. Merck, Frankfurter Straße 250, D-6100 Darmstadt

463

EFFECT OF IONIC ENVIRONMENT ON THE ACTION OF A PSEUDOMONAS AERUGINOSA CYTOTOXIN (PAC) ON EHRLICH ASCITES TUMOR CELLS

E.Weigert and F.Lutz

Ionic groups are involved in molecular interactions between membranes and proteins. We tested whether changes of the ionic composition of the incubation medium might influence the binding of PAC (IEPs:5.0-6.4) to plasma membranes from Ehrlich cells and its toxicity against the cells. The enzymatically iodinated PAC was bound to isolated plasma membranes (prepared according to M.S.Kilberg and H.N. Christensen, Biochemistry 18:1525,1979) to an extent of 1.5 µg/100 µg of membranes in TRIS buffer pH 7.4 within a few min and without saturation characteristics. The binding showed only a small dependence on temperature. At pH 5.1 the binding was increased threefold and it decreased to about 50% at pH 8.0.
Ehrlich cells suspended in PBS pH 7.4 ($3x10^7$ cells/ml, prepared 7-15 days after transplantation into HAN-NMRI mice) released 8.6, 17 and 42 mmol/l of cellular K^+ within 1.5, 5 and 60 min, respectively, at incubation with PAC at a conc. of 5 µg/ml at 37°C. The respective Na^+ uptake was 7.8, 15 and 46 mmol/l. Addition of NH_4Cl in the range of mmol/l to the medium resulted in an enhancement of the breakdown of the cellular cationic gradient depending on concentration and time of addition. 20 mmol/l NH_4Cl added before or simultaneously with the PAC caused a doubling of toxicity. The PAC toxicity was also changed by varying the pH of the incubation medium. 50% of cellular cationic gradient breakdown within 60 min was found at toxin conc. of 0.27, 6.5 and 34 µg/ml at pH 5.1, 7.4 and 8.0, respectively. Ruthenium red (50 µmol/l) caused a moderate inhibition of cationic breakdown. Cepharanthine (100 µmol/l) and A23187 (50 µmol/l) were without influence on PAC toxicity.

We conclude from these experiments that the negative charge of the cell surface at physiological pH is an impediment for the interaction of the negatively charged PAC with Ehrlich cells.

Institut für Pharmakologie und Toxikologie der Universität, Frankfurter Straße 107, D-6300 Giessen, F.R.G.

464

IN VITRO EFFECT OF DITHIOCARBAMATES OR TETRAZOLE THIOL - CONTAINING BETA - LACTAM ANTIBIOTICS ON ALCOHOL DEHYDROGENASE

E. Schreiner, U. Christmann-Kleiss, K. J. Freundt

Certain dithiocarbamates (DTCs) (fungicides, rubber accelerators, alcohol deterrent) may impair ethanol elimination from blood in the laboratory rat (Freundt, K. J., Netz, H.: Arzneimittel-Forsch. 27 / 1977 / 105-108) or in man (Peachey, J.E.: Lancet 1981 , I , 943 - 944). In the present investigations, enzyme preparations purified from livers of adult, female Wistar rats were used to study the influence of various DTCs on alcohol dehydrogenase (ADH). The inhibition of ADH induced by dimethyldithiocarbamate, diethyldithiocarbamate, and Maneb depending on ethanol as the variable substrate only became significant at concentrations of 10^{-3} M and above, or in the case of tetramethylthiuram disufilde, tetraethylthiuram disulfide or tetramethylthiuram monosulfide at 10^{-4} M. In contrast, CS_2 (metabolite of DTCs), Ziram or Zineb had no effect even at concentrations of 10^{-3} M. Depending on NAD as the variable substrate, all agents studied inhibited ADH at concentrations of 10^{-3} M upwards, whereas the same concentration of CS_2 or Ziram did not cause inhibition. The inhibition was mainly noncompetitive. Ineffectiveness of the DTC-Zn compounds (Ziram, Zineb) and CS_2 points to chelate binding of cofactor Zn to the monomeric DTCs as the cause. Beta-lactam antibiotics (BLAs) with a tetrazole thiol group (NCS structure analogous to that of DTCs), i. e. MOX, CMD, CPZ, CTM, did not inhibit ADH at concentrations of 10^{-3} M with ethanol or NAD as the variable substrate. Using 10^{-2} M of the particular BLAs, very mild inhibition was suggested. This finding rules out a participation of ADH in BLA-induced alcohol intolerance.

(The investigations with DTCs were supported by the "Hauptverband der gewerblichen Berufsgenossenschaften e.V." in Bonn-Bad Godesberg / FRG).

Institute of Pharmacology and Toxicology, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Maybachstraße 14 - 16, D-6800 Mannheim 1 / FRG

465

VIABILITY OF JEJUNAL MUCOSA CELLS ISOLATED FROM CHRONICALLY ETHANOL-TREATED GUINEA PIGS

E. Hegazy , M. Locher

It is well known that ethanol exhibits direct toxic effects on various functions of the intestinal mucosa. It is less known whether chronic ethanol treatment affects the viability of mucosacells in the upper gastrointestinal tract.

Three groups of male guinea pigs (5 per group) were used. All animals had free access to standard chow. Control group 1 obtained water ad libitum, control group 2 an aqueous sucrose solution (5%) and the test group obtained aqueous solutions with stepwise increasing ethanol concentrations (from 5% to 15% in 6 weeks, then 15% for another 6 weeks). Before sacrification (after 12 weeks) the mean body weights were 611 g (control 1), 709 g (control 2) and 489 g (test group).

Jejunal cells were isolated as described (Hegazy et al., Eur. J. Cell Biol. in press). Cell viabilities, as measured by trypane blue exclusion, LDH-release, oxygen consumption rates and mitochondrial energetic coupling were identical in all 3 groups. However, cells from chronically ethanol treated animals were more susceptible to the direct toxic effects of ethanol, added to the incubate. Furthermore, hydroxycumarin conjugation and ethoxycumarin hydroxylation rates were higher in control 2 (180% and 160%) and test group (164% and 260%) than in the control 1.

Thus chronic ethanol treatment had no effect on the basic viability of jejunal mucosa cells, but it exhibited a clear inducing effect on the microsomal drug metabolizing activities.

Inst. of Toxicology, University, 7400 Tübingen

466

INHIBITION OF THE ULCEROGENIC ACTIVITY OF SOME NONSTEROIDAL ANTIINFLAMMATORY DRUGS BY DITHIOCARB

R. Hoppenkamps, O. Strubelt, R. Pentz

Sulfhydryl-containing chemicals like cysteine or cysteamine protect the gastric mucosa against the ulcerogenic activity of nonsteroidal antirheumatics (NSA) (1). Dithiocarb (DTC), a thio compound with a low toxicity, in a dose of 100 mg/kg i.p. also inhibited the development of gastric lesions induced by phenylbutazone, indometacin, flufenamic acid, and diclofenac, but did not significantly attenuate those induced by aspirin, paracetamol, or piroxicam. DTC caused an increase in the rat stomach volume and in the amounts of phenylbutazone remaining in the stomach 4 hr after treatment, whilst the plasma levels of phenylbutazone were 3 times lower in the DTC-treated rats than in the controls. The concentration of phenylbutazone in the stomach contents did not change significantly after DTC treatment, suggesting that the gastric lesions caused by phenylbutazone are mainly due to the systemic action of this substance. The pharmacokinetics of paracetamol, however, were not significantly influenced by DTC.
Thus the protective effect of DTC against NSA-induced gastric lesions seems to be rather a pharmacokinetic than a pharmacodynamic one. The protective role of GSH and cysteine depends on a different mechanism of action, because these thio compounds had no influence on the absorption of phenylbutazone.
(1) Hoppenkamps and Strubelt, Arch. Pharmacol. 316, Suppl. R 18, 1981.

Institut für Toxikologie der Medizinischen Hochschule Lübeck, D-2400 Lübeck (FRG)

467

OTOTOXIC SIDE EFFECT OF LOOP DIURETICS: FUROSEMIDE, PIRETANIDE, BUMETANIDE, AZOSEMIDE AND OZOLINONE IN THE CAT.

R. Klinke, K.-H. Göttl, A. Roesch

Ototoxic side effects of Furosemide (FUR), Piretanide (PIR) and Bumetanide (BUM) were compared in cats. The comparison was based on determination of ED_{50} for a defined hearing loss, namely complete suppression of the compound action potential (CAP) of the auditory nerve, elicited by clicks 30 dB above threshold. Recovery functions of CAP and serum levels of the drugs were additionally determined and evaluated. ED_{50} were 19.20 mg/kg for FUR; 4.37 mg/kg for PIR and 2.21 mg/kg for BUM. As mean equipotent diuretic doses were 2.61 mg/kg for FUR, 0.26 mg/kg for PIR and 1.16 mg/kg for BUM it becomes clear that PIR has more than twice the therapeutic range of FUR whereas BUM only has 0.26 the therapeutic range of FUR. After application of doses near ED_{50} recovery of CAP to 50% of the control takes less than half an hour with FUR and PIR whereas it takes nearly ten-times as long with BUM. Serum levels were determined when recovery of CAP to the 50% value had occurred. If the dose applied was not greater than $1.5 \cdot ED_{50}$ the 50% CAP recovery occurred when FUR and PIR serum levels had dropped to about 65% of the initial value. With BUM a decrease of serum level to 10% was necessary before the 50% CAP recovery could be observed. This may be an indication that BUM may be more retained in the cochlear spaces than FUR and PIR.
Slow application of the drugs reduce the risk of ototoxic side effects.
Azosemide (AZO) and Ozolinone (OZO) were tested qualitatively. ED_{50} for AZO is <10 mg/kg. With OZO the diuretic (-)OZO is also ototoxic. The (+)OZO has no diuretic effect and is not ototoxic.

Zentrum der Physiologie, Theodor-Stern-Kai 7
D-6000 Frankfurt/Main 70, Germany

"Ototoxische Nebenwirkungen von Schleifendiuretika: Furosemid, Piretanid, Bumetanid, Azosemid und Ozolinon bei der Katze".

468

STOP-FLOW INVESTIGATIONS, IN THE RAT, OF THE TARGET OF RENAL ATTACK BY α-AMANITIN AND OF THE ANTAGONIZATION BY SILYMARIN.

G. VOGEL and I. GEORGE

α-Amanitin, from the deathcap fungus (Amanita phalloides Fries.), leads to renal damage. Previous investigations have revealed: increase in plasma urea level, reduction of the GFR, reduction in the absolute and fractional absorption of water, Na^+ and glucose, as well as a drastic reduction in the PAH clearance (Agents and Actions 9, 221 (1979)). The investigations now presented consisted of: 4 mg/kg α-amanitin i.p., treatment with silymarin, 100 mg/kg i.v. 1 hour before poisoning with α-amanitin, ca. 64 hours later stop-flow investigation under mannitol diuresis, 20 urine samples of ca. 30 µl per kidney. After α-amanitin: the urine creatinine concentration is halved and the typical concentration maximum in the 7th and 8th urine samples disappears. Silymarin causes a partial approximation to the normal concentration profile. The picture for Na^+ is a mirror image of the creatinine pattern. Intact animals exhibit a K^+ minimum in the 10th sample: α-amanitin inverts the profile so that there are concentration maxima in samples 7-10. The distal PAH concentration maximum almost wholly disappears with α-amanitin, which does not affect the shape of the urea concentration profile. As far as can be deduced from the stop-flow curves, α-amanitin affects both the distal and the proximal active transports; passive transport of substances remains unaffected. On treatment with silymarin all parameters either undergo a normalization of their measured concentration profiles or a displacement in the direction of normality.

Department of Pharmacology Dr. Madaus & Co.
Ostmerheimer Straße 198
D-5000 Cologne 91
Federal German Republic.

469

HEPATIC METALLOTHIONEIN INDUCTION BY CARBON TETRACHLORIDE: INTRACELLULAR REDISTRIBUTION OF ZINC

H.E. Heilmaier and K.H. Summer

Metallothionein (MT), a unique protein of low molecular weight and high cysteine and metal content, can be induced by several factors including metals, hormones, various stresses and organic chemicals like diethylmaleate, iodoacetate and carbon tetrachloride (CCl_4). Zinc is required for these elevations since MT levels have been shown to be not increased in zinc-deficient animals.
In the present study we investigated the redistribution of intracellular zinc and the changes in the ratio of MT- and glutathione(GSH)-derived sulfhydryl groups in mouse liver due to pretreatment with CCl_4 in vivo.
Single i.p. administration of CCl_4 (0.4 and 2 ml/kg bw) increased mouse hepatic MT up to 23 nmol/g ww. This could be demonstrated either by the analysis of the cytosolic zinc- or sulfhydryl-content. Eighteen hours after CCl_4-treatment cellular GSH was depleted by 80%.
Consequently, the ratio of MT- and GSH-derived sulfhydryl groups rose from 0.03 to 0.6. Under these conditions, electrophilic metabolites of CCl_4 might increasingly be sequestered by MT.
After the administration of CCl_4 the zinc-content of liver and hepatic cytosol remained unchanged. However, a decline of zinc occured in the cytosolic high molecular weight proteins (MW > 10 000) which fully accounted for the zinc increase in the cytosolic low molecular weight metallothionein. Concomitantly, the hepatic activity of the zinc-containing alcohol dehydrogenase (MW 83 000) was inhibited by 40%.
These results suggest that the CCl_4 -induced redistribution of intracellular zinc - although necessary for MT induction - might be fatal.

Inst. Toxicol. Biochem., GSF, D-8042 Neuherberg

470

GLUTATHIONE PEROXIDASE AND GLUTATHIONE S-TRANSFERASE IN BLOOD PLATELETS FROM CHILDREN WITH DIFFERENT SE STATES

H.Menzel, G.Steiner and I.Lombeck

The Se content of human platelets is 3-4 times higher than that of erythrocytes (ref. to w.wt.). The activity of the Se-Glutathione peroxidase (Se-GSHPx) shows about the same relation.
5 patients (4-8 years old) with low Se intake, due to dietary treatment of phenylketonuria (PKU) or maple syrup urine disease (MSUD), revealed a low Se state. It was investigated if during Se supplementation there was a change of Se-GSHPx activity in platelets and plasma because of the importance of this enzyme for the hydroperoxide metabolism.

During administration of yeast rich in Se (dosis 75-100 µg Se/d) for 17-23 weeks, a rapid increase of Se-GSHPx in plasma (2 days) and platelets (7 days) occurred. The values in plasma and platelets reached a plateau within 1-3 weeks. 2-3 weeks respectively. After the end of the supplementation there was a slow decrease. The activities in platelets reached a low plateau again after 24 weeks. Glutathione S-transferase (GSHTf) measured with 1-chloro-2,4-dinitrobenzene did not show a significant difference between platelets of healthy and dietetically treated patients and could not be detected in plasma.
Though in rat liver non-Se-GSHPx compensates Se-GSHPx activity during Se deficiency, in the patients platelet GSHTf did not change in different Se states and failed to exhibit GSHPx activity.
The absence of a non-Se-GSHPx and the dependence of GSHPx activity on the Se state, stress the importance of Se for hydroperoxide metabolism in platelets.

Institute of Toxicology, University of Düsseldorf, Moorenstr. 5, 4000 Düsseldorf

471

COMPARATIVE EVALUATION OF THE HEPATOTOXICITY OF TETRACYCLINE IN OLD AND MEDIUM-AGED FEMALE MICE

G. Hopf

Earlier studies on mice gave evidence for an age-sex-dependency of the hepatotoxicity of tetracycline. Recent investigations by Böcker et al (unpublished) have demonstrated that the body distribution of tetracycline in mice is also sex and age dependent: tetracycline accumulates in the livers of old mice to a much lower degree than in the livers of medium-aged animals.
In order to elucidate whether this difference in drug distribution is reflected by a lower hepatotoxicity in old individuals we have comparatively measured the effects of tetracycline (100 µg/g i.v.) on some parameters representing liver function in female mice of 18-22 and ca 3-6 months of age. It appeared that in the senescent mice most parameters were less affected by tetracycline: e.g. the accumulation of triglycerides and cholesterol in the liver and the increase of serum transaminases were less pronounced. On the other hand, the serum urea level which is dependent also on renal function rose higher in the old group.

Institut für Pharmakologie und Toxikologie der Universität, Universitätsstraße 22
D 8520 Erlangen

472

COENZYME A DEPLETION BY VINYL CHLORIDE METABOLITES

P. Simon and H.M. Bolt

Based on preliminary investigations we have advanced the theory that alkylation of coenzyme A by reactive metabolites of various xenobiotics leads to disturbances of carbohydrate and fatty acid metabolism (Biological Reactive Intermediates II, p. 667, 1982); as a result an enhanced formation of acetone is observed (Arch. Toxicol. 49, 107, 1982). In order to chemically establish this reaction and depletion of coenzyme A by metabolites of vinyl chloride we have incubated 1,2-^{14}C-vinyl chloride (substrate saturation) with NADPH, rat liver microsomes and ^{3}H-labelled CoA. Control incubations did not contain NADPH or vinyl chloride. The reaction mixtures, after protein precipitation with ethanol, were subjected to HPLC on RP-18. From control incubations 25 % of the total CoA could be recovered in the reduced form. In presence of NADPH and vinyl chloride 3 doubly labelled peaks were eluted from the column; the isotope ratio (^{14}C/^{3}H as introduced into the incubation) was 2:1 in one and 1:1 in two other products. This demonstrates inactivation of CoA by vinyl chloride metabolites in vitro. Further experiments were designed to demonstrate CoA depletion in vivo. After exposure of male Sprague-Dawley rats for 14 h to 2,500 ppm vinyl chloride analyses of both mitochrondrial and cytosolic CoA were performed. An HPLC method for CoA analysis was developed for this purpose. It was evident that depletion of CoA in both intracellular pools was disproportional in that vinyl chloride exposure led to CoA depression only in the cytosol. Mitochondrial CoA was not affected. These data strongly support the above mentioned theory which explains the increased formation of acetone on the basis of cytosolic CoA depletion.

Abt. Toxikologie, Pharmakologisches Institut, Obere Zahlbacher Str. 67, 6500 Mainz

473

A SIMPLE METHOD FOR MONITORING LIPID PEROXIDATION IN THE ISOLATED PERFUSED RAT LIVER.

A.HASHEM AND F.R.UNGEMACH

It has been well established,that the peroxidation of membrane phospholipids is a main primary mechanism through which a variety of agents exert their toxicity.In isolated cells,tissue homogenates and subcellular organells,this process can be easily and directly monitored[e.g by following the formation of Malondialdehyde(MDA)or measurement of conjugated dienes in the lipid extracts].In the isolated perfused organs,however,the monitoring of the lipoperoxidative process(L.P.)is rather troublesome.Apart from two recent approaches,in which either chemiluminescence or the evolution of volatile short chained hydrocarbons respectivly have been measured,the evaluation of L.P. was only possible after ceasing the perfusion and homogenising the organ,making it difficult to determine the time course of the process.In the isolated perfused rat liver[closed recirculating mode]we could extract a substance released by the liver into the effluent perfusate,which,like peroxidsed fatty acids,absorbs in the region of 232-235 nm.Furthermore we could monitor the formation of MDA in the perfusate by an Iron-catalysed TBA-reaction.Using Paraquat,$CBrCl_3$, ADP.Fe^{+3},Ethanol and $FeCl_2$as inducing agents,we could observe a well correlation between the release of this U.V. absorbing material and the formation of MDA,both increasing 4-5 fold above the control values.A responce as early as 10 minutes after infusion of the inducing agent could be observed.An effect on the bile flow could also be shown with some of the inducing agents,while with others the bile flow has not been affected.With this model,it is possible to follow the early events of L.P. and its time course throughout the whole perfusion period[up to 3 Hours]. Attempts will be made to characterise the nature of this U.V. absorbing substance,which is supposed to be a lipoperoxidative breakdown product with diene configuration.

Institut für Pharmakologie und Toxikologie.FB Tiermedizin Veterinärstr,13 D-8000 München 22

474

DOSE-RESPONSE CHARACTERISTICS OF CHEMICAL CARCINOGENESIS AND TUMOUR PROMOTION

H.A. Tennekes, L. Edler and H.W. Kunz

Druckrey and his associates have established clear dose-response relationships for a variety of carcinogenic chemicals. These investigations demonstrated that the effects of carcinogens are irreversible and cumulative in nature. Moreover, there does not appear to be a threshold level for carcinogenic action. The present investigations have been focussed on the hypothesis that enhancers of carcinogenesis could exhibit different dose-response characteristics. Experimental evidence in favour of this concept was first derived from a dose-response analysis of TPA-mediated tumour promotion in mouse skin. The action of a putative enhancer of mouse liver tumourigenesis, dieldrin, was subsequently investigated. The dose-response characteristics of this latter compound were also found to be at variance with that invariably demonstrated for chemical carcinogens, but similar to the dose-response characteristics of TPA. The dose-response characteristics of other enhancers of liver carcinogenesis are currently under investigation. The evidence obtained sofar indicates that the effects of enhancers of carcinogenesis are determined by exposure concentration, and not, as in the case of carcinogens, by the sum of all consecutive doses. There is also evidence for the existence of threshold levels for tumour-promoting action. The toxicological implications will be discussed.

The German Cancer Research Centre, Institute of Biochemistry (H.A.T. and H.W.K.), and Institute of Documentation, Information and Statistics (L.E.), Im Neuenheimer Feld 280, D-6900 Heidelberg.

475

INFLUENCE OF LIVER TUMOR PROMOTERS ON CELL DEATH BY APOPTOSIS IN RAT LIVER

W. Bursch, and B. Lauer

Liver tumor promoters (cyproterone acetate (CPA), phenobarbital) induce liver growth in adolescent rats. Rat liver enlargement after CPA is essentially due to parenchymal cell replication and is thought to be an adaptation to the functional load imposed on the organ. Continuous CPA treatment leads to a new steady state of liver size and liver DNA (cell) content at a higher level. Cessation of treatment causes a partial regression of the DNA surplus within a few days, indicating death of cells. Histological investigation of the involuting liver revealed no necroses.Instead, apoptosis appears to be the predominant mechanism of cell elimination during de-adaptation of liver size. The incidence of apoptoses can be modified by signals provided by feeding.

Continuous ^{3}H-TdR infusion during CPA treatment was used to differentiate between proliferating(labelled) and non-proliferating (unlabelled) hepatocytes.During liver involution, no labelled apoptotic hepatocytes were found. Obviously, those hepatocytes which undergo apoptosis did not proliferate during treatment and, therefore, appear to be in the stage of terminal differentiation (G_T), i.e. they may be programmed for death.

Apoptosis is also observed in putatively preneoplastic lesions in rat liver. The incidence of apoptoses in these lesions can be modified by liver tumor promoters.

Institut für Toxikologie und Pharmakologie der Universität Marburg, Pilgrimstein 2, D-3550 Marburg/Lahn

476

TESTING FOR ACTIONS OF TUMOR PROMOTERS IN CULTURED RAT HEPATOCYTES;INFLUENCE OF THE CELLULAR ENVIRONMENT.

W.Parzefall, P.Galle

Numerous drugs, insecticides and some hormones act as mitogens in rodent liver. Several seem to be tumor promoters. We were interested to study effects of tumor promoters on differentiation and cell replication in liver cells. Hepatocytes isolated according to Seglen (Meth.Cell Biol.XIII,1976) with modifications were grown on collagen gel in William's medium E supplemented with hormones, newborn calf serum (NCS),or rat serum (RS).Within 24 hs after plating hepatocytes formed cord like structures. This morphology did not alter during the 5 days observation period. Lower serum concentrations allowed earlier flattening and confluency. ^{3}H-Thymidine incorporation into nuclear DNA was evaluated autoradiographically. DNA labeling was low in serum free cultures. It could be stimulated by raising NCS or RS concentration up to 20%, was lower again with 50% serum and was nearly absent at 96% NCS. Epidermal growth factor (EGF) stimulated DNA synthesis under all conditions tested. Labeled mitoses were observed in EGF treated cells.Of the liver tumor promoters Cyproterone acetate (CPA), α-Hexachlorocyclohexane and Nafenopin, only CPA was able to increase labeling significantly. The effect was further enhanced by sequential treatment with EGF.

Institut für Toxikologie und Pharmakologie, Pilgrimstein 2 , D-3550 Marburg

477

ABERRANT ADAPTATION OF PUTATIVE PRENEOPLASTIC CELLS AS A POSSIBLE CAUSE OF FORMATION AND PROMOTION OF LIVER TUMOR BY NON-GENOTOXIC COMPOUNDS

R. Schulte-Hermann, J. Schuppler, I. Timmermann-Trosiener

Many compounds cause the appearance of liver tumors in rodents without exhibiting detectable genotoxic/mutagenic activity. Several of these agents appear to p r o m o t e tumor development from putative preneoplastic liver foci which are formed either spontaneously or after administration of initiating carcinogens. Previous studies have shown that promoters such as phenobarbital or α-hexachlorocyclohexane lead to selective growth of the foci, but the cause of this effect remained unclear.

It has been known since long that in normal liver the agents mentioned induce expression of a set or program of adaptive responses consisting of increased cell proliferation and enhanced activity of drug-metabolizing enzymes. We have now found that putative preneoplastic foci may express the adaptive program, coupled to certain regulatory defects ("Aberrant adaptation"). Switching on the program by an inducer/promoter therefore results in unbalanced and excessive multiplication of focal cells.

Institut für Toxikologie und Pharmakologie der Universität Marburg, Pilgrimstein 2, 355 Marburg/Lahn.

478

DOSE-DEPENDENT PROMOTING EFFECT OF PCBs ON PRENEOPLASTIC ISLANDS, AND ITS CORRELATION WITH THE INDUCTION OF MICROSOMAL ENZYMES IN RAT LIVER.

E.Deml and D.Oesterle

The promoting effect of low doses of Clophen A 50, a PCB mixture on preneoplastic islands in livers of 3 weeks old female Sprague-Dawley rats was studied by the histochemical demonstration of ATPase-deficiency, emergence of GGTase, and glycogen storage after 12 weeks.
Clophen A 50 was given p.o. one week after a single treatment with 8 mg DEN/ kg b.wt. three times a week for 11 consecutive weeks at doses of 0.1, 0.5, 1, 5, 10, and 50 mg /kg b.wt respectively. Controls received DEN or Clophen A 50 in the doses applied above. The activities of ethylmorphine demethylase, aldrine epoxidase, and aryl hydrocarbon [BaP] hydroxylase were measured in the livers of the animals treated with Clophen A 50 only.
DEN induced about 7 islands/cm^2 with a total area of 0.01 mm^2/cm^2.The increase in island number and area in rats treated additionally with Clophen A 50 up to 10 mg/ kg b.wt. was dose-dependent. Up to 1 mg Clophen A 50/kg b.wt. no significant increase in number and area was observed, indicating a no-effect level between 1 and 5 mg. Treatment with 10 mg Clophen A 50 enhanced island number about 7-fold and area 120-fold. 50 mg/kg b.wt. were less effective, indicating a toxic effect. Clophen A 50 itself initiated only few islands.
The enzyme induction was dose-dependent over the total dose range. With 50 mg Clophen A 50 it amounted up to 3-fold for ethylmorphine demethylase, 40-fold for aldrine epoxidase, 230-fold for aryl hydrocarbon [BaP] hydroxylase. The content of cytochrome P 450 was enhanced 4-fold.
In conclusion, promotion of preneoplastic islands by Clophen A 50 is dose-dependent and correlates with the induction of microsomal monooxygenases within the dose range between 1 and 10 mg/kg b.wt. Lower doses are ineffective in respect to promotion.

Inst. Toxikol. Biochem., Abt. Toxikol., Ges. f. Strahlen- u. Umweltforschung, D-8042 Neuherberg

479

EFFECTS OF ETHANOL ON EARLY STAGES IN NITROSAMINE CARCINOGENESIS IN LIVER.

M. Schwarz, G. Wiesbeck

Excessive alcohol consumption is associated with an increased risk in cancer of various organs including liver. The mechanism of action of alcohol is unknown but ethanol by itself does not seem to show any carcinogenic potential. Therefore, this compound may possess co-carcinogenic or tumour-promoting activity. An interference of ethanol with the activation metabolism of carcinogens has been postulated as a possible explanation. In fact, the metabolism of the hepatocarcinogen dimethylnitrosamine by rat liver microsomes is markedly enhanced after pretreatment of the animals with ethanol. This increase in activation metabolism, however, was not observed in vivo, where ethanol caused only an enhancement of DNA synthesis.

Number and especially size of ATPase deficient islets in rat liver induced by either N-nitrosomorpholine or diethylnitrosamine were found to be strongly enhanced in animals treated with a combination of ethanol and these carcinogens. This increase was dependent on the treatment schedules and the carcinogen and ethanol dose levels. Administration of ethanol following cessation of carcinogen treatment (sequential feeding protocol) slightly reduced the extent of ATPase deficient foci in liver.

It is concluded that a causal correlation exists between the enhancement of pre-neoplastic response and the increase in DNA synthesis following ethanol administration. The results also suggest that ethanol acts by modifying tumour initiation and does not possess tumour promoting capacity in liver.

Institut für Biochemie, Deutsches Krebsforschungszentrum Heidelberg, Im Neuenheimer Feld 280, D-6900 Heidelberg 1

480

EFFECT OF PHENOBARBITAL ON THE EXTENT OF DNA SINGLE STRAND BREAKS INDUCED BY HEPATOCARCINOGENIC NITROSAMINES

A. Buchmann, K. Karmaschek, K.H. Schlemmer

Simultaneous treatment of rodents with hepatocarcinogens and inducers of the microsomal monooxygenase system has been found to result in a decrease in liver tumour response. It is generally assumed, that this inhibitory effect is due to interference with carcinogen activation. This concept is supported by the observation that phenobarbital (PB) pretreatment of rats results in a reduction of dimethylnitrosamine (DMN)-induced alkylation of cellular macromolecules both in vitro and in vivo.
To quantitate the initial subcellular effectiveness of three different nitrosamines and its modification by PB, DNA single strand breaks, which result from DNA alkylation products, were analyzed with the method of the alkaline elution assay. Pretreatment of rats with PB resulted in a decrease of DMN-induced DNA damage. Inhibition was most pronounced at the lowest DMN dose level used (1 mg/kg) and became less effective with increasing DMN dose. A comparison of the effect of PB on DNA damage induced by equipotent dose levels of DMN, diethylnitrosamine or N-nitrosomorpholine showed very similar degrees of inhibition.
Daily injections of DMN for a period of up to ten days resulted in an accumulation of DNA single strand breaks. Reduction by simultaneous PB treatment remained constant throughout the entire treatment period.
Determination of DNA single strand breakage shows the character of a concentration measurement and is therefore influenced by alterations in the size of the target pool. Since PB increases the relative liver weight and the amount of liver DNA, the observed decrease in the extent of DNA single strand breakage is only small if related to total liver DNA. The frequency of DNA single strand breaks per cellular genome is however diminished in both cases.

Institut für Biochemie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg.

481

DIMETHYLHYDRAZINE METABOLISM IN ISOLATED COLONOCYTES OF GUINEA PIG

G.Hauber, H.-J.Burger, M.Schwenk, H.Remmer

Dimethylhydrazine (DMH) is a carcinogen, which acts mainly in the colon. Specific metabolism of the drug in the colon might be the cause for this organotropy. We therefore studied DMH-metabolism in isolated mucosa cells from the colon of guinea pig and compared it with that in isolated hepatocytes.

Colonocytes were isolated by a method, previously used for small intestinal cells (Hegazy et al. Eur. J. Cell Biol, in press). The isolated colonocytes were morphologically (electron microscopy) and functionally (oxygen consumption, drug metabolism, transport) intact.

Colonocytes were incubated with ^{14}C-DMH (48 µM). The compound was taken up by the cells. The final intracellular DMH-concentration, reached within 15 s, was 5-fold above the extracellular concentration. DMH-metabolism proceeded at a constant rate for 60 min. Some 240 pmol/mg prot were metabolized to $^{14}CO_2$ within 60 min; another 45 pmol/mg prot were covalently bound to macromolecules. The metabolizing rates in isolated hepatocytes of guinea pig were not any faster than those in colonocytes, although their p 450 content and aminopyrine hydroxylation rate was 15-20 times higher .

The efficient DMH-metabolism in colonocytes could therefore be an important cause of its organotropy.

Inst. of Toxicology, University, D-74 Tübingen

482

DEFINED SMOKE CONDENSATE FRACTIONS AND THEIR INFLUENCE ON MICROSOMAL OXYGENASES IN VITRO AND IN VIVO.

A. Sternitzke

Earlier studies have shown, that the major phenolic compounds occurring in smoke condensate inhibit the dealkylation of model substrates such as 7-ethoxycoumarin, 7-ethoxyresorufin and p-nitroanisole in rat as well as in mouse hepatic microsomes. Respective experiments with the original phenolic smoke condensate fractions showed a slightly more intensive inhibitory effect. Mice hepatic microsomes were exposed to final dilutions of purified phenolic fraction (without polycyclic aromatic hydrocarbons). monohydroxyphenol fraction and dihydroxyphenol fraction ranging from $2x10^{-8}$ - 2x10-3. For 7-ethoxycoumarin the mean IC_{50} value for the individual phenols is $3x10^{-4}$M, and for the three fractions a dilution of $4x10^{-5}$ fold. The monohydroxy fraction shows the most intensive inhibition.

Pretreatment with purified phenolic fraction (3x50mg/kg) increases its own and the other inhibitory effects, whereas pretreatment with phenobarbital-Na (3x80mg/kg) or with 3-methylcholanthrene (2x30mg/kg) exerts no discernible influence. Using 7-ethoxyresorufin as model substrate the same results were obtained. However, the inhibition of deethylase activity is more marked after pretreatment with the purified phenolic fraction. The dose-response-curve for the dihydroxy fraction is especially steep. A pretreatment with the purified phenolic fraction (3x50mg/kg) shows a significant decrease in 7-ethoxyresorufin metabolism. Compared to controls only 78% of the deethylase activity were obtained. A single dose of purified phenolic fraction corresponding to 20% of the LD_{50} (188.2mg/kg) leads to a moderate increase of the microsomal cytochrome P450 after 3 hours. 9 hours after application the effect is most pronounced. 7-ethoxycoumarin deethylase activity is only slightly increased.

This work was supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad-Godesberg. Department of Pharmacology. Philipps-University, Lahnberge, D-3550 Marburg/Lahn.

483

DOSE DEPENDENT INDUCTION OF ATPase-DEFICIENT FOCI IN LIVERS OF RATS EXPOSED TO VINYL CHLORIDE.

R.J. Laib, U.M. Wünschel, T. Pellio and N. Zimmermann

The dependence of vinyl chloride (VC)-induced angiosarcoma in rats with the exposure concentration is linear in a range of 500-50 ppm (Gehring et al., Toxicol.Appl.Pharmacol. 44, 581 (1978). Below 50 ppm the dose-response curve is not yet established. Studies on hepatic non-protein sulfhydryl content of rats after exposure to VC (50-2000 ppm) revealed a progressive depression with increasing exposure concentration. No depression was observed after exposure to 10 ppm VC (Watanabe et al., Toxicology, 6, 1 (1976)). This led to conclude that this level of exposure may not induce tumors. To study the dose-dependence of the genotoxic effect of VC over an extended dose range hepatocellular preneoplastic ATPase-deficient foci were evaluated after subchronic exposure of newborn rats. Wistar rats were exposed, immediately after birth, to 10,40, 70, 150 und 500 ppm VC (5 days/week, 8h/day). The animals were sacrificed after 10 (14) weeks of exposure. Cryostat sections of the liver were stained for ATPase-deficient foci and quantitated morphometrically. For a more detailed investigation in the lower dose range the experimental schedule was modified. Newborn Wistar and Sprague Dawley rats were exposed for 21 days to 2.5, 5, 10, 20, 40, 80 ppm VC.After cessation of exposure for 10 weeks the animals were sacrificed and the livers examined for hepatocellular ATPase-deficient foci. Both sets of experiments revealed a linear relationship between the dose of VC and the % of ATPase-deficient foci induced. Within the accuracy of the method no obvious threshold for the induction of preneoplastic foci by VC was observed.

Pharmakol. Institut, Abt. Toxikologie, D-6500 Mainz

484

INFLUENCE OF HYPOXIA AND GLUTATHIONE DEPLETION ON THE METABOLISM AND HEPATOTOXICITY OF VINYLIDENE CHLORIDE AND CARBON TETRACHLORIDE IN RATS

C.-P. Siegers and W. Horn

The metabolism of vinylidene chloride (VDC) and carbon tetrachloride (CCl_4) was investigated by measuring the removal of the compounds from the atmosphere of a closed exposure system occupied with male rats. Hepatotoxicity was evidenced in the same rats by determining serum enzyme activities of the aminotransferases (GOT, GPT) and sorbitol dehydrogenase (SDH) before, at the end of the exposure time and 24 hrs later. Control rats exposed to VDC concentrations up to 4000 ppm did not show any sign of (hepato) toxicity; hypoxic conditions (10 % oxygen) slightly depressed the metabolism of VDC (500 or 2000 ppm concentration in the atmosphere), but failed to evoke hepatotoxicity. GSH-depletion in the liver (<10 % of control value) due to pretreatment with phorone (diisopropylidene acetone, 250 mg/kg i.p.) markedly decreased the metabolic degradation of VDC which was followed by high increments of liver-specific serum enzyme activities. Phenobarbital-pretreatment (0.1 % solution instead of drinking water for 7 days) markedly accelerated the VDC-metabolism without evoking hepatotoxic effects. In contrast to VDC, CCl_4-metabolism was increased under hypoxia and consequently hepatotoxicity was aggravated. Phenobarbital pretreatment also accelerated the metabolism of CCl_4 (150 ppm) without enhancing the hepatotoxicity at this low CCl_4 concentration. Hepatic GSH-depletion decreased the metabolism of CCl_4 but markedly increased the hepatotoxic response to CCl_4.

These results are discussed in terms of the influences of GSH and oxygen in lipid peroxidation and/or covalent binding of reactive intermediates of both CCl_4 and VDC.

Institut für Toxikologie der Medizinischen Hochschule Lübeck, Ratzeburger Allee 160, D-2400 Lübeck (FRG)

485

FORMATION OF LIPIDS WITH CATABOLIC STABILITY DURING CARBON TETRACHLORIDE METABOLISM.
AN ALTERNATIVE HYPOTHESIS TO EXPLAIN ITS TOXICITY.

Bernhard Link, Heinz Dürk and Hartmut Frank

CCl_4-metabolism in vivo, formation of volatile hydrocarbons and incurred losses of polyunsaturated fatty acids (PUFA) in hepatic microsomal lipids of rats were determined. Release of transaminases was measured and the extent of pericentral necrosis was determined. Correlation of the total quantity of CCl_4 metabolized with the amounts of hydrocarbons generated and the loss of PUFA revealed that more than 2 molecules of CCl_4 must be activated to destroy 1 molecule of PUFA. The time course of CCl_4-metabolism and hydrocarbon exhalation indicate that lipid peroxidation ceases when CCl_4-metabolism has ended. Also, the depression in PUFA is strongest at this stage. However, the development of liver injury in vivo follows a different pattern, with little changes during the first hour. Release of transaminases becomes apparent only after about twelve hours.
Incubation of hepatic microsomes with ^{14}C-CCl_4 leads to formation of covalently modified lipids. Isolation of the labelled lecithin and incubation with phospholipase A_2 from different sources reveals that a fraction of about 60% of the labelled lipid is stable towards enzymic cleavage. In context with the role of the endoplasmic reticulum in phospholipid synthesis these results suggest, that the cause for toxicity lies in the formation of pathological lipids with heavily altered geometry in the acyl chains, which escape metabolic control of phospholipid turnover. As an alternative to better understand the consecutive derangements of different cell organelles we propose the incorporation of these altered lipids into the respective membranes.

Institut für Toxikologie der Universität Tübingen, Wilhelmstraße 56, D-7400 Tübingen

486

INTERRELATION BETWEEN LIPID PEROXIDATION, LYSOSOMAL ENZYME RELEASE, LOSS OF MICROSOMAL Ca^{2+}-SEQUESTRATION ACTIVITY AND HEPATOCELLULAR DAMAGE

M. Younes and M. Albrecht

To study the relationship between lipid peroxidation (LPO) and cellular damage we chose three compounds known to evoke LPO (cumene hydroperoxide, CHP), hepatocellular injury (thioacetamide, TAA) or both (carbon tetrachloride, CCl_4). In a first series of experiments pre-mitochondrial supernatants of phenobarbital (Pb)-induced rat liver homogenates were used. After incubation in the presence of either agent and an NADPH-regenerating system LPO was assessed by measurement of malondialdehyde (MDA) formation and correlated with the release of lysosomal β-glucuronidase. While CCl_4 and CHP promoted both events in a time and concentration dependent manner, TAA did not evoke either LPO or lysosomal enzyme release.In a second series of experiments Pb-induced male rats were treated with one of the three agents and LPO was monitored via the measurement of exhaled ethane. Again, treatment with both, CCl_4 and CHP resulted in an increased ethane exhalation. When liver-specific serum enzyme activities (GPT, SDH) were investigated 24 h later, however, hepatotoxicity was evident only in rats treated with either CCl_4 or TAA. Thus, CHP evoked LPO but no hepatotoxicity, while with TAA the opposite was true. Recently, loss of ATP-dependent Ca^{2+}-sequestration activity of microsomal membranes was suggested to be a first common step leading to cellular death. This activity was assayed in liver microsomes isolated from control rats or from rats treated with either agent. 2 h after treatment with either CCl_4 or TAA a clear inhibition was seen which persisted after 24 h only in the case of CCl_4. CHP, which clearly evoked LPO, did not alter the Ca^{2+}-uptake by microsomes at all. Thus, a clear correlation between cellular damage and LPO cannot be expected in every case.

Institut für Toxikologie der Medizinischen Hochschule Lübeck, Ratzeburger Allee 160, D-2400 Lübeck (FRG)

487

IMMUNOHISTOCHEMICAL AND BIOCHEMICAL DEMONSTRATION OF INCREASED UDP-GLUCURONYLTRANSFERASE IN PRENEOPLASTIC RAT LIVER FOCI

D. Ullrich, G. Fischer*, N. Katz and K.W. Bock

Preneoplastic liver foci were produced by two methods: 1)Continuous feeding of basal diet containing 2-acetylaminofluorene (0.03%, w/v) for 25 weeks, followed by feeding basal diet without carcinogen for 10 weeks. 2) Single i.p. injection of N-nitrosomorpholine (75 mg/kg), 24 h after partial hepatectomy, followed by phenobarbital-treatment (0.05%, w/v, in tap water) for 34 weeks. Phenobarbital was withdrawn 2 weeks before liver examination.
Increased UDP-glucuronyltransferase (GT) could be visualized immunohistochemically in focal lesions which were ATPase-negative and γ-glutamyltranspeptidase-positive. Detection of GT was possible using rabbit IgG against rat liver GT as first antibody and peroxidase-labeled swine anti-rabbit IgG as second antibody. Histochemical results were substantiated by assaying 1-naphthol-GT activity in microdissected focal and extrafocal tissue (0.3 to 0.6 µg). Compared with extrafocal tissue GT activity was 5.1-fold and 2.6-fold higher in focal tissue obtained by methods 1 and 2, respectively. The results extend earlier findings with hyperplastic liver nodules and Morris hepatomas (Cancer Res. 42, 3747 (1982)) demonstrating permanently increased GT activity in preneoplastic tissue whereas cytochrome P-450 dependent reactions were decreased. The altered pattern of drug metabolizing enzymes is consistent with increased resistance of preneoplastic cells to the cytotoxicity of chemical carcinogens.

Zentrum Pharmakologie und Toxikologie und *Zentrum Pathologie der Universität, Robert-Koch-Str. 40, D-3400 Göttingen

488

STUDIES ON THE MECHANISM OF ACTION OF S-(DICHLOROVINYL)-L-CYSTEINE

A. Schmid, W. Beuter, L. Mayring

Trichloroethylene-extracted soybean oil meal (TCESOM) causes an intoxication of cattle and horses that manifests itself as hemorrhagic diathesis and as aplastic anemia (W. Rundles, Blood 13: 899, 1958).
S-(dichlorovinyl)-L-cysteine (DCVC) and S-(dichlorovinyl)-L-glutathione were found to be the toxic factors of TCESOM (L. L. McKinney et al, J Am Chem Soc 81: 909, 1959). Their mode of action is not understood; yet it seems to be correlated with the dichlorovinyl moiety.
We synthesized DCVC (L. L. McKinney et al, J Am Chem Soc 79: 3932, 1957) and studied its radical-inducing properties in vivo. Test animals were male mice weighing 40 g, which were given DCVC i.p. in aqueous solution. Ethane expiration (C. Riely et al, Science 183: 208, 1974) and formation of malonic dialdehyde (J. Terao and S. Matsushita, Lipids 16: 98, 1981) in the kidneys 2 hours after application of DCVC served as indicators of free radical formation. Results:
a) DCVC (40 - 200 mg/kg) causes a dose-dependent increase of ethane expiration and of formation of malonic dialdehyde ($p < 0,01$).
b) Both indicators are reversely proportional to a reduction of pO_2 in inspired air from 20 to 15 vol%.
c) Pretreatment of test animals using metyrapone (100 mg/kg i.p. 1 hour before DCVC) lowers the values of both indicators ($p < 0,01$).
These results indicate metabolic radical formation by DCVC in mice.

Institut für Pharmakologie, Toxikologie und Pharmazie der Tierärztlichen Fakultät München, Veterinärstr. 13, D 8000 München 22

489

CHEMICAL AND BIOLOGICAL REACTIVITY OF HEXACHLORO(1,3)BUTADIENE

S. Schütz and D. Reichert

Hexachloro(1,3)butadiene (HCBD) is a by-product in the synthesis of many chlorinated aliphatic hydrocarbons and is used as an industrial solvent. HCBD is chemically highly stable and a well known environmental pollutant; it acts as a selective nephrotoxic compound and is nephrocarcinogenic in rats (Kociba et al., Am.Ind.Hyg. Ass.J. 38:589, 1977). To evaluate the molecular mechanism of the toxicity of HCBD, we investigated the chemical and biological reactivity of this compound.

Chemical oxidation of HCBD was achieved by three methods: 1)photooxidation, 2)oxidation under a stream of oxygen above 180°C, 3)substitution reaction with ethanolic KOH and consecutive addition of chlorine. Two HCBD-oxidation products were unequivocally identified by GC/MS: 3-perchlorobutenic acid chloride and tetrachlorosuccinic acid chloride. The chemical oxidation reactions of HCBD should correlate, at least in part, to the compound's biologic activation. The identification of 2-perchlorobutenic acid in the urine of rats after HCBD gavage supports this assumption. The first metabolite in HCBD metabolism is the monoepoxide, which rearranges to the corresponding acid chloride. The intramolecular migration of the double bond is plausibly explained by an allylic rearrangement.

Institut für Pharmakologie und Toxikologie der Universität, Versbacher Str. 9, D-8700 Würzburg

Supported by the Deutsche Forschungsgemeinschaft

490

INVESTIGATION OF ALLYL BROMIDE METABOLISM IN THE RAT: SIGNIFICANCE FOR IN VIVO GENOTOXICITY

E. Eder and G. Freitag

Allyl halides exerted direct genotoxic activities (without metabolic activation) in the Salmonella typhimurium mutagenicity test (Ames), as well as in unscheduled DNA synthesis (UDS) in HeLa-cells. Addition of metabolizing enzymes (S-9 mix) to the microbial assay led to a significant decrease or even total loss in mutagenicity.

The significance of a possible metabolic epoxidation for genotoxic effects in the whole animal was investigated in this study of the metabolism of allyl bromide: 125 mg/kg ^{14}C labelled allyl bromide (4.16 mCi/mmol) in corn oil were administered to a rat by gavage. The total 24h elimination of urine, feces, expired air and CO_2 was collected separately; 41.4% of the administered radioactivity were excreted in urine, 3.1% in expired air, 0.6% as CO_2 and only 0.1% in the feces. About 30% remained in the carcass. Allyl mercapturic acid and carboxyethyl mercapturic acid, dimethyl ester have been identified as urine metabolites. Because carboxyethyl mercapturic acid, dimethyl ester is a main metabolite of acrolein, it is very probable that acrolein is an intermediate in the allyl bromide metabolism: (CH_2=CH-CH_2Br + H_2O → CH_2=CH-CH_2OH + HBr, CH_2=CH-CH_2OH $\xrightarrow{ADH}$ CH_2=CH-CHO).

No mercapturic acid formed via epoxidation of allyl bromide could be found in rat urine yet.

Institut für Pharmakologie und Toxikologie der Universität, Versbacher Str. 9, D-8700 Würzburg

Supported by the Deutsche Forschungsgemeinschaft

491

PHARMACOKINETICS OF ETHENE AND 1,3-BUTADIENE AND THEIR REACTIVE METABOLITES IN THE RAT

J.G. Filser, F. Störmer, H.P. Rolzhäuser, K. Lieser and H. Peter

The mutational effects of ethene oxide and butadiene monoxide are well known. Both compounds are also weakly carcinogenic; the question of DNA alkylation at specific sites has also been addressed (Ashby et al., Mut. Res. 103, 257, 1982). On the basis of hemoglobin alkylation data Ehrenberg et al. (Mut. Res. 45, 175, 1977) have put forward the theory that ethene, when taken up by the organism, is partly converted to ethene oxide which is reactive towards macromolecules. However, so far no direct proof of epoxide formation from ethene or butadiene in vivo has been presented.
We have exposed rats to either ethene or 1,3-butadiene in a closed desiccator jarchamber, as previously described (Arch. Toxicol. 47, 279, 1981). Both olefins are metabolized by rats; metabolic elimination can be inhibited by dithiocarb (200 mg/kg) pretreatment. Above atmospheric concentrations of 1.000 ppm ethene or butadiene (closed chamber) metabolism shows a saturation pattern. Maximal elimination rates were enhanced after Aroclor 1254 pretreatment (500 mg/kg). When exposures were done under such conditions that the olefins were metabolized at a constant v_{max} rate, and when these were extended over a period of several hours an increase in ethene oxide (exposure to ethene) or butadiene monoxide (exposure to butadiene) was observed in the gas phase; peak concentrations occured after about one hour. After this time a plateau was reached. This means that in case of exposure to these olefins the epoxides are formed, distributed in the body and, in part, exhaled.

Abteilung für Toxikologie, Pharmakologisches Institut, Obere Zahlbacher Str. 67, D-6500 Mainz

492

ROLE OF EXTRAHEPATIC METABOLISM FOR TISSUE SPECIFIC TOXICITY OF TRANS-4-ACETYLAMINOSTILBENE

A. Pfeifer and H.-G. Neumann

Acute toxic doses of trans-4-acetylaminostilbene (AAS) produce necroses in glandular stomach of rats but not in liver. The epithelial lesions lead to lethal bleedings which can be prohibited by methylcholanthrene (MC) pretreatment. This suggests prerequisite metabolic activation and prompts the question, can local activation explain the tissue specific toxic effect?

(^3H)-labelled AAS (50 mg/kg) was orally administered to female Wistar rats. The pattern of metabolites was determined in urine and bile, and macromolecular binding was measured in several tissues after 24 h. The tissue dose of reactive metabolites was greatest in liver (DNA-binding 80 vs. 15 pmol/mg in gland.stomach, uninduced). MC-pretreatment reduced DNA-binding in extrahepatic tissues (gland.stomach 50%, intestine 39%, kidney 29%), which correlates with changes in phase I and phase II metabolism towards better inactivation. Phenobarbital had only moderate effects. In uninduced animals, gland.stomach is exposed to the greatest conc. of the parent compound (324 vs. 97 pmol/mg total metab. in liver), an effect which can be eliminated by MC-pretreatment due to an increased metabolic rate.

It is concluded that AAS inhibits physiological functions of the stomach before necroses occur. Necroses may be caused by reactive metabolites subsequently, but this effect cannot be correlated with tissue dose. MC-pretreatment influences the overall metabolism, but tissue specific activation of AAS could not be established.

Institut für Pharmakologie und Toxikologie der Universität, Versbacher Str. 9, 8700 Würzburg, FRG

Supported by Deutsche Forschungsgemeinschaft

493

CLEARANCE OF BENZO(A)PYRENE BY ISOLATED RAT LUNG AND LIVER AND DIFFERENCES IN THE OXIDATIVE AND CONJUGATIVE METABOLISM BETWEEN THESE ORGANS

M. Mollière, H. Foth, P. Philipp and G.F. Kahl

Concentrations of benzo(a)pyrene (BP) and of its free and conjugated metabolites in perfusate, tissues of perfused organs and bile were studied in isolated rat lung and liver in a recirculating perfusion system at 1 µM BP by TLC and HPLC techniques using organs from oil and 5,6-benzoflavone (BF) pretreated animals (80 mg/kg for 36 h).

Over a perfusion period of 120 min the total clearance of BP was 9.1 ± 1.3 ml/min in liver compared with 1.7 ± 0.3 ml per min in lung. Per g of organ the clearance was 30% higher in lung (1.7 g wet weight) than in liver (11.8 g wet weight). Under these conditions free plus conjugated dihydrodiols compared with the sum of phenolic metabolites in tissue, perfusate and bile were 1262 to 640 pmol at 30 min and 2696 to 735 pmol at 120 min in lung versus 3669 to 3700 pmol and 4006 to 3441 pmol in liver. The pattern of biliary conjugates of isolated livers changed after pretreatment of animals with BF. Only 5.8 ± 3.3% of total activity were detected as diol glucuronides whereas 25.0 ± 3.6% and 17.5 ± 1.7% were identified as glucuronides and sulfates of phenolic metabolites (control: 37.9% versus 4.6 and 12.1%). No BP was detected in bile either after oil or BF pretreatment. The concentration of unmetabolized BP in the tissue of liver was 2 pmol/g and about 100-fold higher in lung at 30 min, while similarly low concentrations were found in both organs at 120 min of perfusion (1.3 resp. 3.9 pmol/g). In the combined perfusion system the liver decreases the concentration of BP in lung to 1.6 pmol/g already at 30 min. The previously demonstrated (Klaus et al., BBRC 105, 596, 1982) protective effect of the liver for the lung against covalent binding of BP metabolites in the lung is explained by the lack of substrate due to the clearance of BP by the liver.

Pharmakologisches Institut der Universität Mainz,
Obere Zahlbacher Str. 67, D-6500 Mainz

494

MUTAGENESIS, CARCINOGENESIS, AND DNA-REPAIR INDUCED BY ANTHRACYCLINE ANTITUMOR ANTIBIOTICS: STRUCTURE-ACTIVITY RELATIONSHIP

J.Westendorf[1], H.Marquardt[1,2], M.B.Ketkar[3], H.Marquardt[1,2]

Previous studies have shown that anthracycline antitumor antibiotics, such as daunomycin (DNM) and adriamycin (ADM), are mutagenic in bacterial and mammalian cells and induce mammary tumors in female Sprague-Dawley rats.
We now investigated the genotoxic properties of a variety of structure-related anthracyclines with potent antitumor activity in some *in vitro* short term tests (mutagenesis in Salmonella typh. and V79 Chinese hamster cells and induction of unscheduled DNA-synthesis (UDS) in primary Wistar rat hepatocytes). Anthracyclines with a daunosamine sugar moiety (DNM, ADM, 4-demethoxy-DNM, 4-demethoxy-ADM and carminomycin) were mutagenic in both assays and induced UDS. In contrast, anthracyclines with N-alkylated sugars (dimethyl-DNM, dimethyl-ADM, dibenzyl-DNM, morpholino-DNM, aclacinomycin A, pyrromycin, musettamycin, marcellomycin and rudolphomycin) were only weakly or not mutagenic. However, these latter compounds were active at inducing UDS. The *in vivo* carcinogenicity of these anthracyclines (mammary-tumor induction in female Sprague-Dawley rats) correlates with their mutagenic activity: In addition to the previously reported carcinogenicity of ADM, DNM and 4-demethoxy-DNM, we now found aclacinomycin A and marcellomycin to be non-carcinogenic.
These results with anthracyclines demonstrate that alkylation of the primary aminogroup of their sugar moieties leads to reduced mutagenic and carcinogenic activity and they suggest that mutagenic/carcinogenic and cytotoxic properties of these antibiotics can be separated.

Supported by Deutsche Forschungsgemeinschaft

Abt. f.Allgemeine Toxikologie der Universität Hamburg[1], Fraunhofer Institut für Toxikologie und Aerosolforschung[2] und Institut für Experimentelle Pathologie der Medizinischen Hochschule Hannover[3]; Grindelallee 117, 2 Hamburg 13

495

EFFECTS OF EPOXIDE-BEARING VALEPOTRIATES ON MOUSE EARLY HEMATOPOIETIC PROGENITOR CELLS AND HUMAN T-LYMPHOCYTES

R. Braun[1], G.E. Hübner[2], H.R. Maurer[2], W. Dittmar[3]

The epoxide-bearing valepotriates valtrate/isovaltrate and dihydrovaltrate, isolated from Valerianaceae roots and used as common sedative drugs, were investigated for their effects on untransformed hematopoietic cells after an alkylating potential and cytotoxicity to tumor cells had been found. The compounds were added to in vitro assays using granulocyte/macrophage (GM-CFC) and erythrocyte (E-CFC) colony forming cells from murine bone marrow early progenitor cells as well as colony forming human PHA-stimulated T-lymphocytes. The ID_{50} for both GM-CFC and T-lymphocytes incubated with valtrate was found to be about 3×10^{-6}M, with dihydrovaltrate about 2×10^{-5}M. On the erythrocyte colonies (E-CFC) both compounds showed an ID_{50} of about 3×10^{-8}M. Valtrate and dihydrovaltrate effects were not reversible by washing the cultures. These in vitro effects could not be observed when mice were treated i.p. with 45 and 65 mg/kg or p.o. with 45 and 1350 mg/kg of the drug. Three days after treatment, colony formation of progenitor cells (CFU-S, GM-CFC, E-CFC) was not significantly different for control and experimental groups. Therefore, the effect of p.o. and i.p. applied valtrate an the ability of the liver to metabolize ^{14}C-methacetin was investigated by measuring the $^{14}CO_2$ exhalation. There was a clear reduction of initial ^{2}exhalation of $^{14}CO_2$ after i.p. 50 mg/kg of valtrate, but no effect was found after p.o. administration up to 1500 mg/kg. Therefore, it can be concluded that the distribution of the drug via circulation is obviously small.

[1]Institut für Arzneimittel (BGA), Berlin; [2]Institut für Pharmazie, FU Berlin; Institut für Pharmakologie und Toxikologie, Lahnberge, Marburg.

496

INDUCTION OF BOTH CYTOSOLIC AND MICROSOMAL EPOXIDE HYDROLASES IN MOUSE LIVER FOLLOWING TREATMENT WITH NAFENOPIN, A PEROXISOME PROLIFERATOR.

F. Waechter, F. Bieri, W. Stäubli, P. Bentley

Epoxide hydrolase activity is found in both the cytosolic and microsomal fractions of mouse liver. The enzymes in the two fractions differ markedly in both structural and catalytic properties. Little is known about the inducibility of the cytosolic hydrolase. However, the microsomal enzyme is known to be induced by many compounds including several hepatocarcinogens. Thus, as part of an investigation into the mechanism of tumour formation by peroxisome proliferators we studied the effect of nafenopin upon cytosolic and microsomal epoxide hydrolase activities. Treatment with 5 daily doses of 100 mg/kg induced cytosolic hydrolase activity (substrate: trans-stilbene oxide) in five different mouse strains (C57BL6, MAGf, DBA2, Balb-C and C3H). Microsomal hydrolase substrate: benzo(a)pyrene 4,5-oxide) was also induced in all strains except C3H and MAGf. Treatment was accompanied by hepatomegaly and proliferation of hepatic peroxisomes. Further investigations using DBA2 mice showed that cytosolic hydrolase activity was maximally induced (2.5-3.0 fold) following 7 doses of 50 mg/kg, whilst microsomal hydrolase required a higher dose for maximal induction. Glutathione S-transferase activity was not affected by nafenopin treatment.

Ciba-Geigy Limited
CH-4002 Basel, Switzerland

497

MUTAGENICITY TESTING OF CALAMUS DRUGS AND ITS INGREDIENT ß-ASARONE

W. Göggelmann and O. Schimmer

Plants are a major source of naturally occuring mutagens and carcinogens. The rhizomes of Acorus calamus are used as flavor additives and in human phytotherapy as a drug for stomach disorders. ß-Asarone is a major component of the essential oil of calamus. When it was found that ß-asarone caused intestinal tumors in rats, the FDA prohibited the use of calamus as a flavoring agent in soft drinks in the USA. The composition of the calamus oil and its ß-asarone content differ in plants from East Europe, North America and Asia. The carcinogenic studies in USA were carried out with plants of Indian Acorus, which contain the highest concentration of ß-asarone.
In the present study the mutagenicity of ß-asarone and calamus drugs was tested by the method of Ames using Salmonella strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98 with and without S9 mix as metabolic activating system. Mutagenicity tests were performed with S9 from rats after pretreatment with Clophen A50, phenobarbital and methylcholanthrene and with S9 from untreated animals. ß-Asarone was found to be mutagenic in TA 100 only with addition of S9 mix and the number of induced revertants increased with protein concentration. Negative results were obtained in TA 1535, TA 1537, TA 1538 and TA 98 with and without S9 mix. A commercial calamus drug used in Germany was a weak mutagen in TA 100 only in the presence of a high protein content of the S9 mix.

Abteilung für Toxikologie, GSF München, 8042 Neuherberg und Institut für Botanik und Pharmazeutigsche Biologie der Universität Erlangen-Nürnberg, 8520 Erlangen

498

ENTERAL ABSORPTION AND RETENTION OF CADMIUM IN RATS FED Cd AS THE SULFIDE OR CHLORIDE AT TWO LEVELS DURING 90 DAYS

B.Mangler, K.Häberle, G. Fischer, H.G. Classen

The exposure of man to Cd has nearly reached the provisional tolerable weekly oral intake of 400-500 µg total Cd proposed by FAO/WHO regardless of its chemical form.Since kidneys of cattle or pigs are highly contaminated with Cd it has been recommended to consume these products only once all 2 to 3 weeks (Ernährungsber.1980, German Soc. Nutr.). Since Cd is known to be bound to metallothionein in these tissues it seemed of interest to compare the enteral availability of Cd given as the sulfide or chloride.- Female SD-rats weighing c.97 g were kept under controlled conditions with demin. water ad lib. during 90 days. The controls (n=4) received a standard powdery diet (c.0.2 ppm Cd); 4 other groups received this diet enriched with 5 ppm Cd as CdS (n=4) or $CdCl_2$ (n=4), resp.with 50 ppm Cd as CdS (n=4) or $CdCl_2$(n=6). Tissue samples were prepared according to COLLET (Dtsch.Lebensm.rdsch.71,249,1975) and KINRADE (Anal.Chem.46,1894,1974);Cd was determined by the AAS-technique, recovery-rate being 99.8±3.2%.- No overt signs of toxicity were observed in any of the groups. The following Cd-residues (µg/g dry-weight; means ±SD) were measured:

	Left Kidney	Right Kidney	Liver
Controls	0.8 ± 0.5	0.8 ± 0.6	≤ 0.1
5 ppm CdS	2.6 ± 0.8	1.7 ± 0.5	0.5 ± 0.2
5 ppm $CdCl_2$	12.7 ± 1.0	12.8 ± 1.3	3.2 ± 0.2
50 ppm CdS	17.1 ± 1.6	17.7 ± 2.0	4.1 ± 0.3
50 ppm $CdCl_2$	101.0 ± 22.2	108.0 ± 30.0	28.1 ± 6.6

After exposure to CdS instead of $CdCl_2$,Cd-residues are by about 80% lower at both concentrations.Therefore it seems worthwhile to study the availability of Cd in contaminated kidneys or livers of livestock.

Supported by: Min.f.Ern.,Landw.,Umwelt, Forsten,Bad.Wttbg.

Inst.of Food Technology,Pharmacology and Toxicology of Nutrition, Univ. of Hohenheim, D-7000 Stuttgart 70
POB 700 562

499

COMPARISON OF VIABILITY PARAMETERS IN CADMIUM POISONED HEPATOCYTES

L.Müller

Cell membrane permeability of isolated hepatocytes from 18h starved rats was determined by staining with Trypan Blue (TB), extracellular LDH-activity, NADH-penetration, nitrophenylphosphat (NPP)-dephosphorylation and -after treatment with 10 ug/ml digitonin- by the ANS (1-anilino-8-naphthalene-sulfonic acid)-fluorescence. Samples were withdrawn from combined suspensions of vital (10% TB-stainability) and entirely permeable cells. All parameters correlated linearly with TB-stainability (R ≡0.98). However, incubation of vital hepatocytes with 50 uM Cd revealed differences in the validity of the parameters to determine Cd-induced membrane deterioration. The LDH-test was least sensitive. NPP-dephosphorilation was counteracted by NPP-uptake. On the other hand, NADH-penetration and ANS-fluorescence corresponded well with TB-stainability during the total incubation time of 60 min, but they showed higher variations in control- and Cd-incubates.
-In combined suspensions (vital + TB-permeable) lipid peroxidation (LPO) due to CCl_4 increased with the proportion of permeable cells, whereas LPO due to 50 uM Cd decreased. There was, however, an increase in LPO if vital cells only are incubated with Cd. The latter response was prevented by the antioxidant (+)-cyanidanol-3 without any effect on the Cd-induced increase in TB-stainability.
Our results suggest, that TB-staining is a sensitive method to reveal Cd-induced impairment of structural integrity of hepatocytes whereas, generally, an estimation of membrane permeability from Cd-LPO and vice versa seems not to be allowed.

Institute of Toxicology, University of Düsseldorf, Moorenstr. 5, 4000 Düsseldorf.

500

TESTICULAR RESPONSE OF RATS TO CHRONIC ORAL LOADING OF CADMIUM

J.Abel, B.Hilscher*, W.Hilscher*, F.K.Ohnesorge

Three month old Wistar rats received 0, 10 and 100 mg Cd^{++} (as $CdCl_2$) for 13, 52 and 180 days with the drinking water. At the times indicated 10 animals per group were sacrified and their testes were examined morphologically and morhometrically. There was a dose-and time-dependent enlargment of the nuclei as well as the reactions zones of the thiaminepyrophosphatase in the pachytene spermatocytes.

In addition 10 males treated with 0 and 100 mg Cd/l for 6 month were mated at the first with one untreated and after 14 days with 3 untreated females. The rates of stillborn and deadborn pups as well as those of resorption/dam were increased by treatment with Cd, compared to the controls, whereas no differences in the number of implantations were observed (see table).

Exp. 1	n/p	impl.	alive	l/a	i/a	r/a
Contr.	9/9	99	76	8,44	11,00	1,11
Cd	7/10	69	47	6,71	9,81	2,14
Exp. 2						
Contr.	22/27	246	218	9,91	11,18	o,45
Cd	22/30	231	147	6,68	10,50	o,77

P/n=numb. of pregnants per group; impl.=numb. of implanattions sites; alive=numb. of living youngs; l/a=aver. of living youngs/dam; i/a=aver. of implantations/dam; r/a=aver. of resorptions/dam.
In summary: chronic low oral cadmium loading caused a toxic action on the spermatogenesis. It seems likely, that these damages are responsibel for the impairment of reproduction, observed at the high Cd dosage.

Institute of Toxicology, University of Düsseldorf
*Department of Experiment.Pathology of MIUH, University of Düsseldorf

501

NEUROTOXIC EFFECTS OF THALLIUM (Tl)
AS STUDIED BY THE HIPPOCAMPUS SLICE TECHNIQUE

M.J.Csicsaky, H.Wiegand

In a local population accidentally exposed to Tl, sleep disturbances were found to be the only symptoms correlating positively with the degree of exposure. Subsequent laboratory investigations showed amplitude depression of flash evoked potentials and of the background EEG in rats exposed to 5 mg/kg Tl by i.p. injection. The peripheral nervous system was also affected (reduced paralysis time and increased occurrence of MEPPs in the nerve-muscle preparation).
In a pilot study carried out on slice preparations from the guinea pig hippocampus, the toxic effects of Tl could be reproduced at doses between .o5 and .2 mMol Tl applied for 1 hour. The Schaffer collaterals of the CA1/2 regions were used for stimulation with double pulses. Averaged field potentials were recorded from the soma and the apical dendrite of CA1 pyramidal cells.
Results: The steepness and the amplitude of the population spike were reduced in 8 out of 11 animals; in 4 out of the 8, the reduction was preceeded by a short increase at the beginning of the application. In 9 out of 11 cases, the latency of the population spike was prolonged after Tl, but in 3 out of the 9, there was a temporary decrease shortly after the start of the application. These symptoms could not be mimicked by a stepwise reduction of the stimulus intensity. Just like in the nerve-muscle preparation, the toxic effects of Tl were milder in a high calcium medium.

Medizinisches Institut für Umwelthygiene
Gurlittstrasse 53, D-4ooo Düsseldorf 1

502

Effects of subchronic treatment with 2,3-dimercapto-propane-1-sulfonate (DMPS) on the excretion and organ distribution of methylmercury in rats.

M.Storp, S.G. Schäfer, and N.Weger

DMPS is an effective drug in mercury poisoning. Because of the long half life of methylmercury (MeHg) the effects of DMPS on the decorporation of ^{203}Hg-MeHg with urine and feces as well as organ distribution in rats were studied.

After i.p. administration of 1 mg MeHg/kg body wt. feces and urine were collected separately for 28 days. In controls 48.2 + 10.9% of the dose, in DMPS treated rats 82.4 + 18.2% were excreted within the experimental period. DMPS treatment shortened the half life from 30.4 days in controls to 12.5 days in the treated group. After administration of DMPS the excretion pattern of ^{203}Hg was changed. In control animals 41.6 + 9.7% of the dose were excreted and with urine 6.7 + 1.6%. DMPS enhanced urinary excretion of ^{203}Hg by a factor of 5.6 to 37.3 + 11.8% of the dose, whereas fecal excretion was influenced only slightly (45.2 + 8.4% of the dose).
The determination of the distribution of ^{203}Hg-MeHg in the organs showed that ^{203}Hg concentrations in all tissues were decreased significantly to about 30 or 15 percent of the control values with the exception of the skin.

Institut für Pharmakologie und Toxikologie der LMU
Nussbaumstrasse 26, D-8000 München 2, FRG

503

SUBCHRONIC INHALATION STUDIES WITH CHROMATES.
EFFECTS ON WISTAR RATS

H. Kuhnen, W. Kördel and U. Glaser

Within a basic study concerning the inhalative effects of hexavalent chromium compounds on wistar rats we present the results of immunologic examinations and tests on alveolar macrophages.

After continous whole body exposure with an aerosol of 50 µg Cr^{6+}/m^3 as sodium dichromate (particle size for deposition in alveolar region, below 0.5 µm) for three months, the number of alveolar macrophages was reduced. In addition the macrophages were smaller and showed a higher rate of mitosis and phagocytosis.

The serum γ-globulins were already elevated after three months of exposure with an aerosol concentration of 25 µg Cr^{6+}/m^3. At this concentration no effects could be detected in leucocytes number or differential blood picture.

In vitro experiments with suspensions of spleen cells showed a reduced humoral antibody production against injected sheep erythrocytes depending on the chromate concentration rats had received. The antibody formation by spleen cells was decreased by 30 % in rats exposed to 25 µg Cr^{6+}/m^3 and by 60 % in rats forced to inhalate 50 µg Cr^{6+}/m^3. After two months of regeneration in filtered air, this inhibition effect was moderated but still not back to the level of control values. Serum changes in antibody formation were not pronounced.

This study illustrates the sensitivity of immunologic systems even to low chromium contaminated air.

Fraunhofer-Institut für Toxikologie und Aerosolforschung,
D-5948 Schmallenberg-Grafschaft

504

EFFECT OF MANGANESE IN DRINKING WATER ON FREE POLYSOMAL PROTEIN SYNTHESIS OF IMMATURE RAT BRAIN.

S. Magour, H. Mäser, and I. Steffen

Manganese (Mn) is present in human nutrition and is considered of minor toxicity. However, chronic exposure to high levels of Mn produce neurological disorders similar in many aspects to Parkinson's disease. Recently, it has been reported that Mn in drinking water (1 mg/ml) impaires the learning ability and memory consolidation of immature rats. Moreover, there is increasing evidence implicating cerebral protein and nucleic acids in learning and memory. It is therefore possible that Mn also affects cerebral protein and/or nucleic acid synthesis. We determined in vitro the free polysomal protein synthesis and tRNA contents of immature rat brain (3 weeks old) after 1, 2, 3, and 4 weeks of daily consumption of 54 µg Mn/ml of drinking water. We observed up to 35% inhibition of protein synthesis during the first 3 weeks which returned toward the control level by week 4. Cross-incubation experiments with polysomes and pH 5 enzyme fractions indicated that the observed inhibiton of protein synthesis is due to alteration of the pH 5 fraction. Furthermore, cerebral tRNA content was reduced up to 20% during the first 3 weeks and also returned to the control level after 4 weeks. At the same time no marked changes in aminoacyl transferase levels were observed. Our data suggest that the retardation in learning and memory of Mn-treated immature rats may partly be due to alteration of cerebral RNA and protein synthesis. It is concluded that tolerance to the observed inhibition of polysomal protein synthesis develops after 3 weeks of daily intake of Mn and therefore this dose level may not be considered hazardous.

Dept. of Toxicology, GSF, D - 8042 Neuherberg

505

INTERACTION OF 4-DIMETHYLAMINOPHENOL (DMAP), CYTOCHROM C AND ISOLATED RAT LIVER MITOCHONDRIA

R. ELBERS, E. HEINDL

The extent of DMAP toxicity seems to be determined by the rate of intracellular oxidative activation in relation to the rates of inactivation processes. Changes in the endogenous metabolism of perfused rat livers intoxicated by DMAP (e.g. inhibition of respiration, ketogenesis, and ATP-synthesis) point to a participation of the mitochondrial compartment in the mechanism of cell injury (Elbers et al., Biochem. Pharmac. 29, 1747 (1980)). Cytochrom C (cyt C), occuring at micromolar concentrations in the cytoplasm, is easily reduced by DMAP and reoxidized by the mitochondrial cytochrom oxidase and is therefore capable to catalyse DMAP oxidation.

To evaluate the effects of DMAP and cyt C on rat liver mitochondria we measured the activity of the Krebs-cycle by monitoring the release of $^{14}CO_2$ from mitochondria metabolising 2-^{14}C-pyruvate. Without added cyt C, DMAP 100 µM, 60 min. incub. time, inhibited $^{14}CO_2$ production less than 20%. After cyt C addition, the degree of inhibition was dependent on the concentrations of both DMAP and cyt C. At 100 µM DMAP, 100 µM cyt C, the $^{14}CO_2$ release ceased within 10 min. Similarly, the binding (B) of ^{14}C(ring)-DMAP to mitochondrial proteins was influenced by cyt C (at 60 min. 50 µM DMAP: cyt C = 0 µM B = 5%; cyt C = 20 µM B = 70%) indicating the rate of electron transfer from DMAP to cytochrom oxidase and oxygen via cyt C to be responsible for DMAP toxicity. At high DMAP/cyt C concentrations, where $^{14}CO_2$ release was inhibited and ^{14}C-DMAP was bound, a marked inhibition of mitochondrial electron transfer together with a loss of respiratory control and coupling was observed. An entirely different effect was noticed at low DMAP concentrations (1-10 µM) where DMAP, oxidized by the mechanism described above, was reduced presumedly by the uptake of electrons at the substrate end of the respiratory chain, allowing electrons to bypass a rotenon or antimycin A block. In this catalytic cycle one mole of DMAP was able to transfer more than 50 moles of electrons.

Inst. für Pharmakologie und Toxikologie, Med. Fak. der LMU München, Nussbaumstr. 26, D-8000 München 2, F.R.G.

506

ASSESSMENT OF BIOAVAILABILITY - PHARMACODYNAMIC VERSUS PHARMACOKINETIC APPROACH

U. Gundert-Remy, R. Hildebrandt, H. Möller[a], O. Schmidlin and E. Weber

Using clonidine (C) as a model substance, we compared bioavailability data as calculated from urinary excretion rates of unchanged drug (kinetics) and from different pharmacologic responses (dynamics). The dynamic parameters were mean arterial pressure (RR, lying, MAP), sedation (visual analog scale, SED) and salivary flow rate stimulated with citric acid (SAV). C was estimated by GC-MS[1]. Relative bioavailability was estimated by cumulative amounts excreted in the urine and area under the effect-time-curve. In a separate study dose-response-curves were constructed after 100, 200 and 300 ug of C in solution were given to 6 healthy subjects and a linear dose-effect-relationship was established. In vivo dissolution could therefore be calculated by means of deconvolution[2] without preceeding transformation of the dynamic data. The drug was given as a 250 ug solution (standard) and two sustained release forms, A and B, both containing 250 ug C, to 8 healthy male subjects in a randomized placebo controlled complete cross over trial. Mean relative bioavailability of formulation A was 87.7 % (urinary excretion), 88.5% (MAP), 81.2% (SED) and 97.2% (SAV). The figures of formulation B were 89.8% (urinary excretion), 89.6% (MAP), 83.6% (SED), 96.9% (SAV). Mean in vivo dissolution curves of C from solid dosage froms were nearly identical comparing the results obtained with kinetic and with dynamic data.
In conclusion: dynamic data can be used to establish bioavailability data of C in the dosage range studied.

[1] Edlund et al. J. Chromatogr. 187, 161 (1980)
[2] Langenbucher Intern. Journ. Pharmaceutics (in press)

Abteilung Klinische Pharmakologie, Medizinische Universitätsklinik, Bergheimerstr. 58, 6900 Heidelberg
[a] ZL Deutscher Apotheker e.V., 6236 Eschborn

507

THE EFFECTS OF SEVERAL ENDOGENOUS BINDING INHIBITORS ON SPECIFIC DRUG BINDING SITES OF HUMAN SERUM ALBUMIN. A POSSIBLE MECHANISM FOR DISEASE INDUCED VARIATIONS OF THE PLASMA BINDING OF DRUGS.

E. Kiem, K.J. Fehske, and W.E. Müller

A variety of evidence indicates that the plasma binding of drugs can be altered profoundly by several diseases, especially concerning liver and kidneys. The mechanism is not yet completely understood, but it is generally assumed that several endogenous binding inhibitors, whose elimination is impaired (fatty acids, bilirubin) play an important role. On the other hand, the actual number of drug binding sites of human serum albumin, the most important drug binding protein in human blood, is rather small and only three ligand binding sites are mainly involved in the binding of most drugs to human serum albumin (the indole and benzodiazpine site, the digitoxin site, the warfarin-azapropazone binding area). If each of these sites is affected by endogenous binding inhibitors in a characteristic way, it seems possible to explain the quite different effects of disease states on the plasma binding of drugs and it might become possible to predict the effects of diseases on drug binding in human blood. Accordingly, we investigated the effects of several endogenous binding inhibitors (fatty acids, cholesterol, bilirubin, urea, uric acid, cholic acid, creatinine) on the binding of marker ligands specific for one of the three drug binding sites (diazepam, flurbiprofen, digitoxin, warfarine, azapropazone) to cristalline human serum albumin or to human plasma. Although we found a variety of effects of the binding inhibitors for human serum albumin, their presence in pathological concentrations in human plasma changed the binding of the marker ligands much less. It seems, that fatty acids alter the drug binding to all three sites in human plasma, while the effect of bilirubin is restricted to the warfarin-azapropazone binding area.

Pharmakologisches Institut der Universität Mainz, Obere Zahlbacher Str. 67, D-6500 Mainz

508

CYCLOBARBITAL AND AMINOPYRINE ELIMINATION AS INDICES OF HEPATIC DRUG METABOLISM IN MEDICAL PATIENTS

U. Breyer-Pfaff, M. Weber, H. Seyfert and E.-H. Egberts

In continuation of earlier work (Breyer-Pfaff et al., Eur J Clin Pharmacol 15: 433, 1979), the potential of cyclobarbital as an indicator of hepatic drug metabolizing capacity was investigated in 60 medical patients 44 of whom suffered from liver function disturbances. They received a single dose of 200 mg cyclobarbital-calcium (one tablet of Phanodorm^{R}) in the evening and during the following two days five blood samples were drawn for estimation of cyclobarbital plasma levels by thin-layer chromatography. Binding to plasma proteins was determined by equilibrium dialysis with the aid of ^{14}C-labeled cyclobarbital. Prior to the cyclobarbital test, the course of $^{14}CO_2$ exhalation from an intravenous tracer dose of ^{14}C-aminopyrine was studied.

In comparison with a group of healthy volunteers, patients with viral hepatitis, mild to moderate alcoholic liver damage or liver cirrhosis exhibited significantly increased half-life and decreased clearance values of cyclobarbital. In obese patients with fatty liver the mean half-life was increased, while the clearance did not differ from normal. A significant correlation between cyclobarbital half-life in plasma and $^{14}CO_2$ half-life in breath was present in the patient group as a whole and in single subgroups, but not in cirrhotics. A weak correlation between cyclobarbital clearance and $^{14}CO_2$ elimination rate was detected in the total group.

The cyclobarbital fraction bound to plasma of normal subjects amounted to 41.7 ± SD 1.6 % and equalled that in solutions of human serum albumin at the same concentration. In patients a close correlation existed between binding calculated on the basis of plasma albumin and that found experimentally except in two cases with extremely high bilirubin concentrations.

Institut für Toxikologie & Medizinische Klinik, Universität Tübingen, D-7400 Tübingen

509

THE INFLUENCE OF IMIDAZOLE ANTIMYCOTICS ON THE ACTIVITY OF DRUG METABOLISM IN MAN AND ANIMAL

Gerhard Heinemeyer and Gisela Jaeck

Broad spectrum antibiotic treatment is often followed by systemic fungal infection being treated with imidazole antimycotics. Since these compounds have been shown to induce and inhibit drug metabolism in animals, intensive care patients being treated with miconazole (Daktar®) or ketoconazole (Nizoral®) were evaluated for changes in the activity of drug metabolism by determinations of urinary excretion of D-glucaric acid (GA). The basic medication was similar in all patients. Treatment with 1000 - 1500 mg/day of miconazole led to a continous rise of urinary GA excretion over a period of one week with a maximum of 125 ± 95 µMol/24 h. No significant effect was observed during treatment with ketoconazole, presumably as a result of the relatively low dose of 150 mg/day. Patients not being treated with known inducers of drug metabolism excreted 55 ± 35 µMol/24 h GA. The enzyme inducing effect of miconazole was less than found in patients treated with pentobarbital resulting in excretion of 348 ± 155 µMol/24h of GA.
The significance of these findings was evaluated in male Sprague Dawley rats applying the aminopyrine and caffeine breath test. The exhalation rate of $^{14}CO_2$ increased from 0.295 ± 0.06 (% of dose/min) in controls up to 0.382±0.06 and 0.375±0.095 after application of miconazole and ketoconazole (80 mg/kg for 4 days), resp. The caffeine breath test indicated slight induction of cytochrome P-448.
The relatively low response of the breath tests may reflect an interference of induction and inhibition of mixed function oxidase. In addition, the influence of imidazole drugs on further metabolism of $H^{14}COH$ has to be evaluated to demonstrate the specificity of the breath test. The enzyme inducing effect of miconazole should be considered during multiple drug therapy.

Institut für Klinische Pharmakologie, Freie Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45

510

PHARMACOKINETICS OF THE NEW ALCOHOL AVERSIVE DRUG NITREFAZOLE IN ALCOHOLICS WITH AND WITHOUT LIVER DISEASE.

K. Weigand, A. Frei, G. Karlaganis, A. Altorfer, H.D. Brenner

In clinical studies, nitrefazole (N) has been shown to have long acting alcohol aversive effects. Since the drug undergoes extensive hepatic metabolism (including acetylation), and since patients likely to benefit from N may have pre-existing liver disease, we investigated the effect of impaired liver function and the influence of acetylator phenotype on pharmacokinetics of N. To 16 fasting patients (14 men, 2 women, aged 28 to 54 years; 8 slow, 8 fast acetylators), hospitalized for treatment of alcoholism, N was administered in single p.o. doses of either 800 (n=8) or 1200mg (n=8), blood samples collected at appropriate intervals up to 4 days, and N concentration measured by HPLC using a LiChrosorb RP-8 column. 12 patients had normal liver function (NLF), as assessed by aminopyrine breath test (ABT normal 0.68-1.08) and initial bromsulphthalein disappearance rate (K_i normal 9.5-16). In the 4 with impaired liver function (ILF), ABT was 0.40±S.D.0.29%, and K_i 7.4±1.5%/min. N peak plasma concentrations of 4.5±2.0 µg ml^{-1} (800 mg group) and 6.0±1.9 (1200 mg group) in NLF-subjects were reached after 4.0±2.5 hours, with AUC's being 122±41 µg·h·ml^{-1} and 190±73, respectively. T 1/2 was dose-independent, varying from 6 to 35 (15.5±6.1) hours. The only difference in ILF subjects was a slightly increased T 1/2 of 19.3±1.8 hours. The total N plasma clearance was 116±43 and 130±54 ml min^{-1} in slow and rapid acetylators, respectively. Since in 5 patients of the NLF group, there were no changes in ABT or pharmacokinetics of N, repeated after 2 months treatment, it is likely that N neither affects microsomal demethylation nor its own metabolism. Our results therefore indicate that N elimination is relatively independent of liver blood flow and uninfluenced by acetylator phenotype. In addition, if the drug is given on a weekly basis dosage or dosing-interval adjustment in alcoholics with ILF appears unnecessary.

Institut für Klinische Pharmakologie, CH-3010 Bern und Psychiatrische Universitätsklinik, CH-3072 Bern/Ostermundigen

511

EFFECTS OF ETHANOL 7 DAYS AFTER A SINGLE DOSE OF NITREFAZOLE IN MAN.

R. Düsing [1], J. Matthews [2], H. Heyer [2], E. APPEL [3], P. Zimmermann [1], D. Palm [3], G.G. Belz [2].

Nitrefazole (NI, Altimol®) is a long-term inhibitor of low K_m aldehyde dehydrogenase without affecting dopamine-ß-hydroxylase.- In a double blind, placebo-controlled (PL) study three groups (n = 10) of healthy volunteers received 0.5 mg/kg ethanol 7 days after NI (1 x 800 or 1600 mg) or PL. Hemodynamic parameters and plasma catecholamines (CA) were determined before and up to 4 h after ethanol in supine and in head up tilted position (40° and 80°).- Ethanol ingestion produced only small changes in cardiovascular parameters before and during tilt in the PL group; however, definite increases in plasma CA were observed. 1h after ethanol the following statistically significant differences between NI and PL were documented during tilt: increase of heart rate and decrease of diastolic blood pressure. During tilt, only in the NI groups 3 volunteers each collapsed after ethanol ingestion.- A rating scale (LKS) showed a significant deterioration of the parameter 'allgemeines Unwohlsein' 1 h after ethanol when the NI groups were compared to the PL group.
In comparison to PL plasma noradrenaline and adrenaline concentrations were increased 2 h after ethanol in supine and tilted position dependent on the dose of NI.
These results in man confirm the longlasting effects of NI to induce ethanol intolerance as been observed in animal experiments.

1. Klinische Forschung, E. Merck, 6100 Darmstadt
2. Institut für Kardiovaskuläre Therapie, 6200 Wiesbaden
3. Zentrum der Pharmakologie, Universität Frankfurt/M 6000 Frankfurt/M.

512

METABOLISM OF PROSTAGLANDIN E_1 IN MAN

B. Rosenkranz and J.C. Frölich

Metabolism of [17, 18-^{3}H] prostaglandin E_1 was investigated in three healthy male volunteers following its intravenous infusion. The infusion rate was 5.0 ng/kg/min. Blood samples were obtained before and 5, 10, 20, 40, 90 and 180 minutes after the end of the infusion. Urine and feces were collected until 96 and 72 hours after the experiment, respectively. All samples were analyzed for radioactivity. Urine was further chromatographed including two different high pressure liquid chromatography systems and subsequently analyzed by gas chromatography-mass spectrometry. The resulting mass spectra were compared to those of authentic reference compounds.

Radioactivity in plasma rapidly declined during the first 10 minutes after termination of the infusion, and then was eliminated exponentially with a mean half-life of 181 minutes. 12 % of the administered radioactivity were excreted in feces and 88 % in urine from which the following metabolites were identified (the three numbers give the relative amounts of this metabolite obtained from each volunteer): 7α-hydroxy-5,11-diketotetranor-prostane- 1,16-dioic acid (10.4, 20.4 and 30.1 %), 7α-hydroxy- 5,11-diketotetranor- prostanoic acid (8.2, 6.9 and 9.3 %), 5α, 7α-dihydroxy-11-ketotetranor-prostane- 1,16-dioic acid and its δ-lactone (together accounting for 4.1, 2.1 and 3.8 %).

These results demonstrate a large interindividual variation in the rate of prostaglandin metabolism. This has to be considered during assessment of total body prostaglandin synthesis by determination of urinary excretion of their metabolites.

Institut für Klinische Pharmakologie, Auerbachstr. 112, D-7000 Stuttgart 50

513

INFLUENCE OF INTRAVENOUSLY ADMINISTERED ACETYLSALICYLIC ACID (ASA) AND SODIUM SALICYLATE (SA) ON HUMAN RENAL FUNCTION, WITH SPECIAL RESPECT TO LITHIUM CLEARANCE.

I.W. Reimann, E. Golbs

Our earlier investigations showed that indomethacin (50 mg t.i.d.) and diclofenac (50 mg t.i.d.) increased lithium plasma levels (P_{Li}) and decreased lithium clearance (C_{Li}). Contrary to these results orally administered ASA (4.0 g daily for 8 to 10 days) did not influence P_{Li} and C_{Li}. In order to differentiate the acute effects of ASA and its active metabolite SA on renal function and renal lithium excretion six healthy female volunteers (Na^+balance: 150 mmol, Li^+balance: 0.6 - 0.8 mmol/l plasma) were treated with ASA (2.0 g as D, L-lysine-monoacetylsalicylate), equimolar doses of SA (1.776 g) or placebo, given as intravenous bolus (0.5 g ASA and 0.444 g SA, respectively) and subsequent continous infusion during a 3h period in random order, leaving a drug free interval of at least 8 days between the three parts of the study. Plasma levels were between 13.8 ± 5.0 and 22.1 ± 4.2 mg/l for ASA ($\bar{x} \pm s_x$) and between 20.8 ± 9.9 and 82.6 ± 22 mg/l for SA during ASA infusion and between 22.5 ± 10.4 and 108.9 ± 28.8 mg/l for SA during SA infusion. Neither ASA nor SA caused significant changes of urinary volume, the renal clearances of Na, K, H_2O, osmol, uric acid, creatinine, inuline and p-aminohippurate (PAH) or of P_{Li}. The only significant effect (Wilcoxon two tailed match pair signed rank statistic) was a slight reduction of C_{Li} by SA from 37.8 ± 10.6 to 29.4 ± 5.2 ml/min ($p = 0.05$). Since renal prostaglandin synthesis (urinary PGE_2 excretion) was suppressed by 60.6 ± 16 % under ASA but unaffected by SA the C_{Li} decrease cannot be related to cyclooxygenase inhibition.

Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Auerbachstr. 112, 7000 Stuttgart 50.

514

PHARMACOKINETIC AND CLINICAL STUDIES WITH 5-AMINOSALICYLIC ACID, THE ACTIVE METABOLITE OF SULFASALAZINE

U. Klotz, C. Fischer, K.E. Maier

The steady state disposition of 5-aminosalicylic acid (5-AS) administered as suppositories (500 mg tid) has been studied in 5 patients with active ulcerative colitis (UC) and in 7 patients after choledochostomy (single dose of 1 g 5-AS) who received post-operatively a T-tube drained externally. In addition, the long-term therapeutic efficacy of 5-AS (250 mg tid) was investigated in 9 patients with UC and 4 patients with Morbus Crohn (MC). Concentrations of 5-AS, its acetylated (AcAS) and glucuronidated (glu-AcAS) metabolites were measured in plasma (during a dosing interval of 8 hrs), urine and bile (24 h-collections) by a specific and sensitive (limit 0.03 ug/ml) HPLC assay. The single step extraction procedure had to be extended by a sep-pak C-18 cartridge purification step for bile, where glu-AcAS could be also detected. During a dosing interval steady state plasma concentrations (c_{ss}) of 5-AS and Ac-AS fluctuated considerably; the mean c_{ss} averaged (± SD) 0.10 ± 0.07 ug/ml and 0.50 ± 0.20 ug/ml, respectively. Urinary recovery of total AS (5-AS and AcAS) ranged between 141 and 295 mg/day (192 ± 70 mg/day). In the collected bile (85 - 390 ml/day) 5-AS (traces up to 0.2 mg/day), AcAS (1.5 ± 2.2 mg/day) and glu-AcAS (0.3 ± 0.4 mg/day) could be measured. Based on a normal bile flow of 1.2 l/day and related to the low bioavailability of 5-AS-suppositories (about 13 %) approximately 10 % of 5-AS would undergo an enterohepatic recirculation. These kinetic studies would suggest that 5-AS is also available for systemic action. In all 9 patients with previously active UC treatment with 5-AS resulted in a remission and no single relapse occured during the prophylactic treatment period of 16 to 28 months. However, patients with MC did not respond so favourable. Thus, especially patients with UC will benefit from a therapy with 5-AS-suppositories which is so far devoid of any side effects.

Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Auerbachstr. 112, 7000 Stuttgart 50

515

ABNORMAL ESTER-GLUCURONIDATION IN LIVER DISEASE: STUDIES USING THE NEW ANALGESIC ZOMEPIRAC

F. Witassek, R. Preisig

Ether-glucuronidation of various drugs, such as oxazepam, lorazepam and morphine has been shown to remain unchanged despite the presence of advanced cirrhosis. Since the effects of impaired liver function on ester-glucuronidation are unknown, we investigated zomepirac (Z) disposition - a compound undergoing almost complete ester-glucuronidation - in 18 patients with chronic liver disease (CLD) and in 10 healthy volunteers (HV). Severity of CLD, assessed with the galactose elimination capacity (GEC), varied between 6.3±S.D. 1.1 mg $min^{-1}kg^{-1}$ in mild, and 4.5±1.0 in advanced CLD (compared to the normal range of 6.0 to 9.1). Following p.o. administration of 200 mg Z, samples were obtained at regular intervals up to 5h and Z concentrations in plasma and in urine (before and after alkaline hydrolysis) measured by HPLC. The apparent oral clearance of Z in CLD was reduced to 3.0±0.8 ml $min^{-1}kg^{-1}$ and 1.8±0.6 in mild and severe CLD, respectively, compared with 3.7±1.2 in HV ($p<0.001$). In CLD, diminutions of Z clearance were closely correlated ($r=0.83$) with abnormalities of GEC. Furthermore, a similar close relationship was found between fractional Z-glucuronide clearance (computed from urinary Z-glucuronide excretion and corresponding Z plasma levels) and both the apparent oral Z clearance ($r=0.86$) and GEC ($r=0.68$). From these results we conclude that the functioning liver cell mass is the major determinant of Z disposition. In patients with CLD, dosage adjustments may therefore be necessary when administering drugs undergoing ester-glucuronidation.

Department of Clinical Pharmacology, University of Berne, Switzerland.

516

BENOXAPROFEN: AN UNEXPECTED "NEW" DRUG PROBLEM?

P.S. Schönhöfer

The non-steroidal anti-inflammatory drug (NSAID) benoxaprofen was introduced as a new therapeutic approach in the treatment of chronic arthritis due to inhibitory effects both on the lipoxygenase and cyclo-oxygenase pathway. Following reports on serious hepatotoxicity with renal failure in elderly patients the licence of the drug was suspended (A. Goldberg, Lancet II: 396, 1982). Analysis of preclinical and clinical data shows
- that the claims of therapeutic superiority to other NSAID were neither clinically nor theoretically substantiated, since most NSAID inhibit both cyclo-oxygenase and lipoxygenase.
- that in addition to the average spectrum of adverse reactions (ADR) seen in NSAID the drug caused phototoxicity and onycholysis in more that 10% of the patients.
- that the unit dose was selected in the high effective level as compared to other NSAID.
- that the drug had a long half-life time (40 h).

A hepatotoxic and nephrotoxic effect has been described for almost all NSAID at the high dose level. Furthermore, it is wellknown that drugs with long half-life may cause special problems in elderly patients due to reduced metabolic capacity. Therefore, the problems observed with benoxaprofen appear to result from the combination of following factors: long plasma half-life, unit dose at the high effective level, common hepato-renal toxicity of NSAID and use in elderly patients without adequate dose adjustment. This does not represent a new phenomenon, since similar problems were observed with phenylbutazon 25 years ago.

Dardanellenweg 30, D-1000 Berlin 42

517

INTRAINDIVIDUAL COMPARISON OF THREE LOOP DIURETICS IN PATIENTS WITH CONGESTIVE HEART FAILURE (CHF)

B.Krüger, F,Sörgel and R.Koob

It has been shown by Kamp et al.(Drug-Research, 1979)that the dose ratio between furosemide (F) and bumetanide (B) for equivalent diuretic and natriuretic action in healthy volunteers is different from patients with chronic renal insufficiency.The aim of our investigations was to study the dose ratio between the three loop diuretics F, B and piretanide (P) the most recently mrketed loop diuretics.

In nine patients with decompensated CHF (NYHA IV - III) an intraindividual comparison of urine and sodium excretion between F(40mg),P(12mg) and B(1mg) - given intravenously - was performed.

Results:	Urine volume(ml/3h)	Sodium (mval/3h)
F 40mg	915.9 ± 190	82.2 ± 17.8
P 12mg	953.1 ± 245	94.4 ± 25.6
P 6mg	590.9 ± 256	44.6 ± 19.8
B 1mg	743.1 ± 216	63.3 ± 17.4

F/P(12):n.s.,F/P(6):$p<0.05$,F/B:$p<0.05$,P(12)/B: $p<0.05$,P(12)/P(6):$p<0.05$,P(6)/B:$p<0.05$

For P dose linearity seems to be present as 12mg of P exerted twice as much urine and sodium excretion as 6mg of P. 12 mg of P were equivalent to 40mg of F, whereas 1 mg of B had only about. 75% of the effect of 40mg of F or 12mg of P. It seems possible that in patients with CHF a different dose ratio between F and B, but not between F and P can be observed.

Carl-Korth-Institut for Cardiovascular Research
Div. of Clinical Pharmacology
Gebbertstraße 47, D-8520 Erlangen

518

EFFECT OF SALT,DIURESIS,ACETAZOLAMIDE & FUROSEMIDE ON CIS-PLATIN NEPHROTOXICITY IN THE RAT.

H. Heidemann, J. Gerkens, E. Jackson, R. Branch

A limitation in the clinical use of cis-platin (cp) is nephrotoxicity (n-tox). Empiric observation in man have shown that prior hydration decreases n-tox. There is still a controversy, wheather diuretics decrease cp n-tox and if protection is due to increased urine volume. Wistar rats, maintained on either a low or a normal sodium diet, received cp (5mg/kg i.p.) and serum creatinine concentrations (S-Cr) were measured 5 days later. Groups of rats from each dietary group were either controls (c) or pretreated with saline 0.9% (5% of body weight/1h) acetazolamide (a))75 mg/kg i.p.) or furosemide (F) (12.5 mg/kg i.p.). S-Cr (mg/dl) were:

	C	0.9% sal	A	F
Low salt:	6.0±0.3	2.4±0.4 x	2.3±0.3 x	3.2±0.3 x
Norm. salt:	3.0±0.3	1.9±0.2 x	1.7±0.2 x	2.3±0.4

Sodium status was important in the development of n-tox. There was decreasing severity of renal impairment from low salt diet<normal salt diet< low salt diet with acute sodium load<normal salt diet with acute sodium load. S-Cr in a further group of rats on a normal diet who received 4.5% NaCl (5% body Wt) was even lower (0.4±0.02 mg/dl). Increased urine volume alone did not confer protection as rats on a low salt diet who received 5% dextrose (5% body Wt) had S-Cr of 6.6+0.4 on the 5th day. A and F reduced n-tox in rats on a low sodium diet while A reducted n-tox on a normal sodium diet alone. These data suggest that NaCl-ions play a critical role in the degree of cp nephrotoxicity.

Med. Klinik (GHS) 4300 Essen; Dept. Clin. Pharmac. Vanderbilt University, Nashville, Tenn, USA

519

URINARY IRON EXCRETION AFTER SUBCUTANEOUS DESFERRIOXAMIN (DF) INFUSION THERAPY IN TRANSFUSIONAL IRON OVERLOAD

R. Erttmann

Subcutaneous daily infusions of DF in iron overloaded subjects using a lightweight portable infusion pump was introduced by PROPPER et. al. (N Engl J Med 294, 1421, 1976). On this basis iron chelating therapy is possible in outpatients. In this report own experience with 7 regularly transfused thalassemic patients is presented:

1. In 6 patients with transfusional iron overload (at time transfused with 1-2 units of packed erythrocytes per 4 weeks) net iron excretion was reached with DF s.c. 2.5 g per m^2 in 10 hrs.
2. Urinary iron excretion - measured by atomic absorption spectrophotometry - after s.c. as well as i.v. DF-application is closely correlated with iron preload.
3. A dose response relation curve has been established for each patient. Urinary excretion after a 10 hrs. s.c. DF-infusion reaches a plateau at doses higher than 2.5 g per m^2.
4. Side effects of DF were not noticed in our patient group.
5. The main problem for reaching a net iron excretion in our thalassemic children was the motivation to perform the daily infusions by themselves. In general the "compliance" of our patients was not optimal.

Department of Hematology and Oncology
Children's Hospital, University of Hamburg
Martinistr. 52, D-2000 Hamburg 20, GFR

520

STUDIES ON THE DISPOSITION OF VERAPAMIL IN PATIENTS WITH RENAL FAILURE.

M. Schols, J. Mooy, M. van Hooff, M.A. van Baak and K.H. Rahn.

Although verapamil is not rarely used in patients with kidney diseases little is known about the influence of renal function on its disposition. Therefore, the kinetics of verapamil as well as its effect on the P-R interval were studied in 6 patients with end-stage chronic renal failure (creatinine clearance < 5 ml/min). All patients were given in randomized order an i.v. dose of 2,5 mg and an oral dose of 80 mg verapamil. The interval between the 2 doses was at least 1 week. Plasma levels of verapamil and of its metabolite norverapamil were measured by high pressure liquid chromatography. After i.v. injection, terminal phase half-life of verapamil was 227 + 21 min in the patients with renal failure as compared with 184 + 3 min in control subjects with normal kidney function. After the oral dose, a maximum verapamil plasma concentration of 79 + 15 ng/ml was reached after 90 + 15 min in the patients with renal failure. The data of the normal controls were 62 + 8 ng/ml and 66 + 5 min, respectively. Only low plasma levels (<10 ng/ml) of norverapamil were found after i.v. injection of verapamil in either group. However, after oral application norverapamil plasma concentrations up to 55 + 4 ng/ml were measured in patients with renal failure as compared with 75 + 5 ng/ml in normal subjects. Oral and i.v. verapamil caused the P-R interval to increase from 0.18 + 0.01 to 0.20 + 0.01 sec in both groups of subjects studied. This effect lasted for about 4 hours. The study demonstrates that the disposition of verapamil in subjects with normal kidney function and in patients with end-stage renal failure is similar.

Dept. of Medicine and Dept. of Pharmacology, University of Limburg, Maastricht (The Netherlands).

521

PHARMACOKINETICS OF MOLSIDOMINE IN HIGH DOSAGE LONG TERM TREATMENT OF MYOCARDIAL ISCHEMIA

D. Oltmanns

Vasodilatating drugs are effective in the therapy of acute and chronic myocardial ischemia by reduction of the preload. Therefore in 5 patients (55±17 years) with acute myocardial infarction the pharmacokinetics of molsidomine in high dosage (4 x 4 mg/die p.o.) were studied over a period of 4 weeks. Measurements of molsidomine plasma concentrations were done at the first and 29th day 0,5, 1, 2, 3, 4 and 5 h after ingestion of the first daily dose (4 mg). The mean maximal plasma levels (Cp max) were about 40 ng·ml^{-1} after t max = 0,8-1,3 h. The elimination half-life in plasma was $T_{1/2}$=1,95 h. 5 h after application of molsidomine the plasma concentrations valued 10-12 ng·ml^{-1}. There were no significant differences in AUC_{∞} (135,5 ±1,22; 1,49±72,7 ng·ml^{-1}·h), duration of presence in the organism T (sys)(3,00±1,22; 3,42± 1,11 h) and total body clearance Cl_{tot} (60,5± 68,1; 33,5±19,0 l·h^{-1}) between both time points. Side effects with regard to blood pressure and heart rate were not observed.
It is concluded that the pharmacokinetic parameters of molsidomine during oral treatment over 4 weeks show a great variability. The pharmacokinetics remain to be unchanged and are in accordance with a nearly unaffected long term efficacy.

Poliklinik für Innere Medizin der
Medizinischen Hochschule Lübeck
Kahlhorststraße 31-35, D-2400 Lübeck

522

PHARMACODYNAMICS AND KINETICS OF HOE 224 (BETA RECEPTOR BLOCKER) IN MAN

P.U. Witte, M.J. Badian, P. Hajdú and V. Malerczyk

HOE 224 (Pacrinol;(-)-p-[3-[(3,4-dimethoxyphenethyl) amino]-2-hydroxypropoxy]-ß-methylcinnamonitrile) is a specific beta$_1$-sympathicolytic agent without intrinsic activity but with marked antihypertensive activity. Under double blind, placebo controlled, conditions 20 and 40 mg HOE 224 p.o. were compared intraindividually with 40 mg penbutolol p.o. (Betapressin$^{(R)}$) in 8 normal subjects. Heart rate, systolic and diastolic blood pressure under different conditions - before, during and after standardized workload on bicycle ergometer - were used as pharmacodynamic variables. The mean maximum percentages of saved beats for penbutolol during steady state of workload were 21% (2 h p.a.), 14% (5,5 h p.a.) and 10% (24 h p.a.), which were statistically significant ($p < 0.05$) when compared to placebo. Only at 2 h after 40 mg HOE 224 there was a statistically significant difference ($p < 0.05$) of 5% saved beats. There was no relevant difference in saved beats after 20 mg HOE 224 and placebo. No relevant differences in blood pressure were seen after any of the treatments.

Serum kinetics of HOE 224 were determined in 6 healthy male volunteers. Dose: 100 mg HOE 224 p.o.. The drug level profile was fitted by a 2-comp-open-model. Mean values for C_{max}, t_{max} and $t_{1/2}$ were: 240 ng/ml, 2.4 h, and 16 h.

Hoechst AG, MA/Klinische Pharmakologie, Postfach 80 03 20, 6230 Frankfurt/M 80

523

THE ELIMINATION OF GUANFACINE, A CENTRAL ACTING ANTIHYPERTENSIVE AGENT, IN NORMAL AND IMPAIRED RENAL FUNCTION AND IN PATIENTS UNDERGOING HAEMODIALYSIS TREATMENT

W. Kirch, E.E. Ohnhaus

As antihypertensive drugs are often used in patients with renal insufficiency, the kinetics of guanfacine were investigated in six subjects with normal renal function (GFR > 90 ml/min), twelve patients with different degrees of impaired renal function (GFR 5-90 ml/min) and in eight patients undergoing haemodialysis treatment (GFR < 5 ml/min). Plasma and urine concentrations of guanfacine were measured by a gas chromatographic method.
Total body clearance of guanfacine did not differ significantly between patients with normal renal function (GFR > 90 ml/min; 360 ml/min) and preuremic patients (GFR < 10 ml/min; 275 ml/min). In normal as well as impaired renal function the elimination rate constant of guanfacine was 0.05 h^{-1}, which corresponds to an elimination half life of 14 h, independent of renal function. Renal clearance of guanfacine fell from 233 ml/min in normals, to 34 ml/min in moderately impaired renal function ($p < 0.05$) and 18 ml/min in preuremic patients ($p < 0.05$). As despite decreasing renal clearance of guanfacine in renal insufficiency, elimination half life of the drug remains constant, increasing non-renal elimination, especially liver metabolism, has to be assumed.
Haemodialysis treatment did not distinctly alter elimination half life of guanfacine, reaching a value of 12 h which did not differ sifnificantly from that in normals. The guanfacine clearance by dialysis was 53 ml/min, which represents about 15% of the total clearance of the drug in normals.
In the present study the blood pressure lowering activity in patients with renal impairment and on haemodialysis was not more pronounced compared to the hypertensives with normal kidney function.

Dept. Internal Medicine, University, School of Medicine, D 4300 Essen, FRG

524

LONG-TERM EFFECTS OF CAPTOPRIL ON VASOPRESSOR AND DEPRESSOR SYSTEMS IN MAN

B. Stanek, K. Silberbauer, H. Sinzinger

Longterm effects of 150 mg/d Captopril were studied in 10 patients with essential hypertension before and during combination with chlorthalidon. Blood pressure and heart rate were assessed and blood samples were drawn for determination of angiotensin converting enzyme (ACE) activity, plasma renin activity (PRA), angiotensin II (AII), aldosterone and a stable prostaglandin E_2 metabolite: bicyclo-PGE_2-m by RIA and for catecholamines (radioenzymatic method) before and 60 min. after captopril (50 mg) before and at monthly intervals up to three months and at three monthly intervals thereafter.
With captopril monotherapy an increase in PRA and bicyclo-PGE_2-m and a decrease in ACE, AII and aldosterone occurred which was further augmented after readministration of captopril. Blood pressure was reduced to 90% ($p < 0,05$) with a positive correlation of the chronic effect to the effect of the first captopril dose ($r = 0,80$, $p < 0,01$) and to pretreatment PRA values ($r = 0,62$, $p < 0,05$). Heart rate and plasma catecholamine levels were unchanged.
After combination with chlorthalidon the increase in PRA and bicyclo-PGE_2-m and the decrease in ACE and AII were sustained, aldosterone was further suppressed. Again these changes were further augmented by readministration of captopril. Blood was reduced to 80% ($p < 0,01$) showing no longer a correlation to the acute blood pressure lowering effect of captopril.

In conclusion the results confirm a sustained effect of captopril on the renin angiotensin and prostaglandin system which is not overruled when a diuretic is added to achieve maximum blood pressure response.

II. Med. Univ.-Klinik und LBI für klin. Endokrinologie, Wien

525

PHARMACOKINETICS OF METHIMAZOLE IN MAN

J.H. Hengstmann, H. Hohn and H.A. Vaupel

The pharmacokinetics of the thyreostatic methimazole are not known in man. With a new HPLC method we were able to measure the drug in human blood and urine.

Pharmacokinetic parameters were established after i.v. injection and oral ingestion of methimazole 40 mg, and the prodrug carbimazole 36.7 mg p.o.

In two euthyroid subjects the total clearance was 200 ml/min, the volume of distribution 48 L. and the biological half-life 150 minutes. In six hyperthyroid patients on maintenance therapy the total clearance was decreased to 90 ml/min, the half-life prolonged to 400 min, the volume of distribution unchanged. Renal elimination was of minor importance in all cases.

These results could be interpreted as inhibition of the metabolic clearance or a metabolic defect in hyperthyroid patients.

The bioavailability of methimazole was complete, carbimazole showed no advantage as regards extent and rate of absorption. Plasma levels during chronic therapy with carbimazole confirmed this.

Medizinische Univ.-Klinik D-5300 Bonn-Venusberg

526

THE INTERACTION OF PRE-INJECTED SUXAMETHONIUM WITH NONDEPOLARIZING MUSCLE RELAXANTS UNDER CLINICAL CONDITIONS.

F.T. Schuh

The influence of a pre-injected dose of suxamethonium (SuM; 0.8 and 1.6 mg/kg) upon the neuromuscular blocking action of pancuronium (PC) and d-tubocurarine (dTC) was studied in 30 patients under neurolept anaesthesia. Dose-response curves of PC and dTC were established by means of mechanomyograms of the hand muscles following electrical stimulation of the ulnar nerve immediately after recovery of the neuromuscular transmission from blockade induced by SuM.- In comparison to controls without SuM (n = 15) the dose-response curves were shifted to the left to a lower dose range indicating an increase in the neuromuscular blocking potency of PC and dTC.- This finding is unexpected with respect to the current opinion of a mutual antagonism of depolarizing and nondepolarizing blockers. However, an explanation can be provided applying a transient-state concept of the muscle relaxant-receptor interaction. According to this concept the initial depolarizing active SuM-receptor complex is regarded to be transformed into a silent inactive form. A fraction of this inactive complex is still persistent when the blockade induced by SuM has disappeared clinically. It will increase the concentration of the inactive complex between nondepolarizing muscle relaxant and receptor and cause a greater neuromuscular blocking effect of PC and dTC.

Supported by Deutsche Forschungsgemeinschaft.

Dept. of Anaesthesiology, Christian-Albrechts University, Hospitalstr. 40, D-2300 Kiel 1

527

MONOAMINE OXIDASE INHIBITION IN HEALTHY VOLUNTEERS BY (-) DEPRENYL (D)

G. Cremer, K.-H. Antonin, P. R. Bieck

Tyramine (TY) is metabolized to a large extent by intestinal MAO-A. Therefore, the clinical use of D, the selective inhibitor of MAO-B should be safe with regard to the hypertensive response ("cheese effect") following TY administration. Recently, a small increase of TY sensitivity with D treatment was described. In order to evaluate further this unexpected finding several pressor amine tests were applied. In 6 healthy volunteers (vols) the extent and duration of MAO inhibition by D in weekly increasing doses (5 - 20 mg p.o. d^{-1} week) was assessed using 3 pressor amines. Tyramine (TY) and phenylephrine (PE) were injected i.v. and norepinephrine (NE) infused in increasing doses until the systolic blood pressure (BP) increased by 30 mm Hg. Tests were repeated at the end of each dosage period and repeatedly after D.

Results: all three pressor amines were potentiated by D treatment. TY and NE sensitivities increased dose-dependently from 1.6 and 1.2fold, resp. (5mg) to 4.1 and 2fold, resp.(20mg). PE sensitivity was highest (3.8fold) with 15 mg. On day 10 after D TY sensitivity was still increased.

Conclusion: our results are in contrast to the selective inhibition of MAO-B in vitro. An additional effect at the site of peripheral neuronal BP regulation can be assumed. D is metabolized in vivo to amphetamines which might contribute to the pressor effects seen. The results caution against the proposed immunity to TY during D treatment of patients with Parkinson's disease who are of the older age group and may poorly tolerate even minor BP elevations.

Humanpharmakologisches Institut CIBA-GEIGY GmbH, Ob dem Himmelreich 7, 7400 Tübingen

528

EFFECT OF DIFFERENT MONOAMINE OXIDASE (MAO) INHIBITORS ON HUMAN URINARY TRYPTAMINE EXCRETION

E. Nilsson and Ch. Schick

Tryptamine (T) is present in human brain and belongs to the substances which produce changes in perception and mood. It is the decarboxylation product of tryptophan and an excellent naturally occuring substrate for MAO. During inhibition of MAO increases in the urinary excretion of T occur. This is indicative of inhibition of the enzyme within the kidney. In man it is unknown which of the two forms (type A and B) of the enzyme MAO deaminates T. Substrate specificities differ between species and tissues. In order to distinguish between the specific types of MAO inhibition in man urinary T was measured during subchronic treatment with tranylcypromine (TC) (MAO A+B), (-) deprenyl (D) (MAO B) and CGP 11 305 A [4-(5-methoxy-7-bromobenzofuranyl-2-)piperidine HCl] (MAO A). Healthy volunteers (n=18) received the MAO inhibitors in weekly increasing p.o. doses. 24h urine was collected for 60d and T measured fluorometrically or by GC.

Results:

TC	10 mg	15 mg	20 mg	25 mg
T ratio	2.4	6.4**	7.9**	5.1
D	5 mg	10 mg	15 mg	20mg
T ratio	1.0	1.1	1.3	1.5
CGP 11 305	50 mg	75 mg	100 mg	150mg
T ratio	2.2**	2.2**	2.5**	2.6**

** $p < 0.01$ Dunnett's t-test,
T ratio: avg during treatment/ control avg

Conclusion: T in humans is deaminated mainly by MAO A. The higher increase after the MAO A+B inhibitor TC might probably be caused by the irreversible nature of inhibition compared to a reversible inhibition by CGP 11 305A.

Humanpharmakologisches Institut CIBA-GEIGY GmbH, Ob dem Himmelreich 7, 7400 Tübingen

529

DIHYDROFOLATE-REDUCTASE (DHFR) IN PATIENTS'S SERUM MAY INFLUENCE THE RESULT OF METHOTREXATE (MTX) DETERMINATION

H. Iven

High-dose MTX therapy with folinic acid rescue is part of several cancer treatment schedules. To reduce the risk of MTX toxicity it is mandatory to monitor MTX serum concentrations. Numerous analytical methods have been reported, including fluorometric, immunologic, enzymatic and HPLC assay; the last is considered most specific. While enzymatic and immunologic assays use patient's serum directly, HPLC methods usually require sample cleanup.
Comparing results obtained with HPLC and enzymatic assay (DHFR, bovine liver) I found no differences with the standards (serum, spiked with MTX), however, patient samples drawn ≧ 48 h after MTX infusion always had higher MTX concentrations when determined with HPLC. Prior to HPLC serum proteins were precipitated by keeping a mixture of 0.4 ml serum and 0.2 ml 0.04 n acetic acid 5 min at 96°C. In the resulting supernatant MTX concentrations determined enzymatically equalled those of HPLC analysis.
The following MTX-concentrations ($x10^{-7}$mol/l) were found in serum (direct) and supernatant of two patients after HDMTX-infusions ($\bar{x}$=geometric mean;n = number of infusions)

Patient	48 h		72 h	
D.D.	direct	supernatant	direct	supernatant
$\bar{x}$,n=14	1.8	3.8	0.6	1.5
range	1.6-2.1	2.7-5.6	0.5-0.7	0.9-2.3
A.R.				
$\bar{x}$,n=7	4.4	10.4	1.5	4.8
range	3.4-5.6	8.6-12.7	1.1-2.1	3.9-5.8

Conclusion: Part of MTX in patient serum is strongly bound to a protein, which probably is dihydrofolate-reductase liberated from deteriorating cells. This MTX is inactive with respect to therapeutic and toxic effects. Results obtained with methods using protein precipitation may be misleading as basis for folinic acid rescue.

Institut für Pharmakologie der Medizinischen Hochschule Lübeck, Ratzeburger Allee 160, D-2400 Lübeck

530

THE BENZODIAZEPINE-RADIORECEPTOR ASSAY - ITS USE FOR PHARMACOKINETIC AND CLINICAL STUDIES

R. Dorow* and J. Lund+

We have established a simple, sensitive and rapid technique to use stable benzodiazepine (BD) receptor preparations from bovine brain for a specific radioreceptor assay (RRA). From samples of biological material like blood, urine, CSF the RRA allows in vitro estimation of receptor affinity and concentration of all BD and BD-unrelated compounds which bind to the BD receptor. Several studies in volunteers with short- and long-acting BD, including diazepam (DZP), flurazepam (FZP), flunitrazepam (FNZ), triazolam, oxazepam, temazepam and lormetazepam (LMZ), indicate that binding data and time course of total BD-binding activity (i.e. parent compounds and active metabolites) correspond well with their respective therapeutic dose and pharmacokinetic data from other methods, if active metabolites are taken into account.

In two placebo (PL) controlled double-blind studies (PL vs. 1 and 2 mg LMZ, 2 mg FNZ, 30 mg FZP, 10 mg DZP) long-term and hangover effects at 12 h after drug intake and beyond were determined by EEG-recordings and psychometric tests, and correlated to BD-binding equivalents in plasma as evaluated by RRA. Compared to placebo a distinct increase in the relative power in ß-frequencies (12.5-30 Hz) of the EEG as well as impairment of psychomotor performance could be found at least up to 12 h after FNZ, FZP and DZP. These effects are in good correlation to BD-binding activity in plasma.

Our findings indicate that the RRA is a valuable tool for pharmacokinetic and clinical studies of BD. Plasma levels as determined by this method may reflect better correspondence to some clinical effects of BD than plasma levels of the parent compound alone.

*Schering AG, Berlin(West)FRG, +Ferrosan, Søborg, Denmark

531

COMPARISON OF THE ANTIMYOTONIC EFFECT OF LORCAINIDE, MEXILETINE AND TOCAINIDE

M. Brenner, A. Haass, W. Schmidt and A. Weber

The management of myotonia congenita was very much improved by the orally effective lidocaine derivatives. The symptoms of paramyotonia congenita were prevented by tocainide for the first time. The question arose which of the orally effective local anesthetics, lorcainide, mexiletine or tocainide, is the best antimyotonic drug.

The myotonic disturbance of the skeletal muscle contraction can be induced in animals by clofibrate. Previous investigations had shown that this experimental myotonia of the rat is a suitable model of the myotonia congenita in man. Myotonia was induced by clofibrate (400 mg/kg b.w., i.v.) in female Wistar rats (120 g). The myotonic delayed relaxation of the contractions of the electrically stimulated m. triceps surae was used as a measure of the myotonia. Subsequently, the three drugs were given intravenously in three doses (lorcainide 20; 15; 5 mmol/kg; mexiletine and tocainide 200; 150; 50 mmol/kg). The antimyotonic effect was measured as the reduction of the myotonic delayed relaxation. The plasma and muscle concentrations of the three antimyotonic agents were measured upon termination of the experiments.

In the three doses mexiletine reduced the myotonia slightly more than tocainide. However tocainide seemed to be the more specific antimyotonic drug, because mexiletine had a higher volume of distribution in the muscle tissue than tocainide. Lorcainide had to be given in lower doses due to the cardiotoxic effect and it was essentially less antimyotonic effective. For clinical use mexiletine and tocainide are both promising oral agents for prevention and treatment of myotonia in man. Thus the preference of eather of these two drugs, should be judged by possible side effects, although they are both generally well tolerated.

Neurologische Universitätsklinik und Institut für Pharmakologie und Toxikologie, D-6650 Homburg/Saar.

532

INTRA-INDIVIDUAL VARIATION IN RED-GREEN PERCETION IS RELATED TO RATINGS AT AN ADJECTIVE LIST (EWL-N)IN HEALTHY SUBJECTS

D.Trapp, H.Feller

Nagel's anomaloscope (Nano.) is a valid method to detect clinically significant deutan and protan defects in red-green perception by Rayleigh's equation (Re.)by comparing individual Re.s with those obtained cross-sectionally from normal trichromates. Effects of acute doses of digoxin in red-green perception can be detected by Nano. (Alken et al.,N.S.'sArch.Pharmacol.,313,Suppl.,R234,'80). However, digoxin effects on red-green perception are of minor extent and within the range of cross-sectionally studied normal trichromats. Thus, longitudinal intra-subject comparisons are necessary to detect long-term effects by N.ano. . Its sensitivity threshhold is limited by intra-subject variability.

As the psychological status can affect hue perception, 6 healthy subjects were asked to give daily ratings at an adjective list (EWL-N,Janke-Debus) for 35 days. At 15 d Re was calculated from 10-fold measurements by N.ano.. Both test exhibited characteristic patterns of individual, longitudinal variation. A significant correlation was found between specific subscales (H+I,J+K+L,A+B: general well-being,hostility+anger,concentrated activity) and relative sensitivity to red hue. Computational control of individual Re by the results at the rating scale yields in a reduction of 'noise' in the visual parameters and improves the sensitivity of N.ano. in the detection of drug effects in longitudinal studies.

Zentrum der Pharmakologie,ZPHARM IV,Klinikum der J.W. Goethe-Universität,Theo.-Stern Kai 7, D-6000 Frankfurt 70

533

DIGOXIN - 'REBOUND' IN VARIOUS VISUAL FUNCTIONS ?

R.G.Alken, U.Vitt, I. Peter

Previous studies had shown that single and repeated doses of digoxin impaired color discrimination, affected spectral sensitivity for luminance and hue threshold and increased spectral critical flicker fusion frequency (Alken and Schnabel, Docum.Ophthal.Proc.Ser.:(1982) 33:477-485). 14 d after a 10 d treatment (0.4mg digoxin/d spectral sensitivity was still different from pretreatment status (Alken,ibid.:467-476).

A double-blind placebo-controlled study was done in 12 healthy trichromates. 10x0.6mg/d digoxin (DIGACIN®) was administered orally to 6 subjects. Digoxin plasma concentration,color discrimination (FM-100),Rayleigh equation (N.ano.) and spectral sensitivity for hue and luminance as well as spectral critical flicker fusion frequency was measured repeatedly pre and during digoxin treatment, and 3 weeks after withdrawal in both groups.

Digoxin exhibited characteristic changes in the spectral sensitivity for both stimuli. 14 d after withdrawal an inverse pattern of changes in spectral sensitivity related to pre-treatment results were obtained, associated by an increase in spectral critical flicker fusion frequency. Both,the cumulative phase and the withdrawal of digoxin treatment was associated by increased color disturbances, however, with opposite shapes in polar plots of the FM-100 test. Confirming results were obtained from another double-blind placebo-controlled study with increasing daily doses over 4 weeks for the rebound in critical flicker fusion frequency (red on white background) after withdrawal. The extent of the rebound was related to digoxin plasma concentration prior to withdrawal.

Zentrum der Pharmakologie,ZPHARM IV, Klinikum der J.W. Goethe-Universität, Theo.Stern Kai 7,D-6000 Frankfurt 70

534

DATA SYSTEM FOR IMPROVEMENT OF INTERPRETATION OF DRUG SERUM CONCENTRATIONS

G. Kreutz, T. Lindner, M. Nitz

Clinical interpretation of quantitative results in drug monitoring requires characterisation of their information properties: pharmacokinetic parameters, pharmacodynamic influences, and methodological restrictions. All of these and their fractional parts are subject to changes by time. Therefore allowance for time in the interpretation of results causes gain of information, improves interpretation and thereby improves therapeutic security.
To comply with the demand for recognition of any former results belonging to each new one a data system of hardware has been combined and software created that can take over this task. It consists of a microcomputer, a double floppy disc drive, and a printer. The programs are written in basic and designed for dialog with the user.
A study for 1 month in 261 patients on digoxin (dg) and 176 patients on digitoxin (dt) revealed relevant information in 63 (24%) of the former and 56 (32%) of the latter ones. Most of them had a test taken less than 4 weeks before the actual test on the same cardiac glycoside (dg: 56=89%, dt: 32=57%) but a substantial part of the patients (dg: 7=11%, dt: 24=43%) had used the other one, respectively.
The system is highly effective in tracing these patients and improving the interpretation of their test results. It causes low costs and is easy to use even for people without special education.

Institut für Klinische Pharmakologie, Klinikum Steglitz der Freien Universität Berlin, Hindenburgdamm 30, D - 1000 Berlin 45

535

ADVERSE REACTION PROFILES OF ANTIDEPRESSANT DRUGS IN A VOLUNTARY REPORTING SYSTEM

H. Ochsenfahrt

The Drug Commission of the German Medical Profession maintains a voluntary reporting system (VRS) for adverse drug reactions (ADR). Recently, some reports on blood dyscrasia directed our attention to the adverse reaction potential of antidepressant drugs, which contribute only 1,2 % to all reports received in the last years. About 300 reports on ADR's refer to 16 antidepressant agents contained in 19 brand preparations. The ADR's of all agents were arranged in groups according to system-organ classes (cf. W.H.W. Inman, Monitoring for Drug Safety, 1980). The percentage for the 6 most common classes was: central and peripheral nervous system disorders: 32 %, skin reactions: 17 %, fever: 10 %, liver damage: 8 %, autonomous nervous system disorders: 8 %, reproductive system disorders: 8 %, blood dyscrasia: 7 %. Other groups like gastrointestinal, cardiac or renal disorders and hypo- or hypertension represent less than 5 %.

Profiles were also constructed for each agent. Most profiles were almost identical except for a relative excess of fever reactions with nomifensin, disorders of the reproductive system with sulpiride, psychosomatic and gastrointestinal disorders with clomipramin and leucopenia with mianserin.

The results were compared with profiles constructed from 5000 reports on antidepressant drugs collected in the WHO collaborative centre in Uppsala/Sweden. Despite of an almost 15-fold difference in the absolute number of reports the profiles were nearly identical but the proportion of severe reactions was somewhat higher in the German reports.

Thus, it is concluded that even with a relatively low number of reports it is possible to identify severe reactions. ADR profiles may give helpful signals, which, of course, deserve further investigation.

Arzneimittelkommission der deutschen Ärzteschaft, Eugen-Langen-Str. 12, D-5000 Köln 51

536

DEVELOPMENT OF A MULTI-CENTRE DRUG MONITORING SYSTEM.

B. Müller-Oerlinghausen, W. Poser, E. Rüther

Although the methodology of new psychotropic drug trials has been much developed during the last decade, an efficient post-marketing monitoring of adverse drug reactions (ADR) in psychiatry did not exist until recently. Therefore, since 1978 a Study Group for Drug Surveillance in Psychiatry (AMÜP) has been established as a task force group of the "Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie"(AGNP). The first phase of this project, which is supported by the Bundesgesundheitsamt Berlin, was designed to test the feasibility of the system in in-patients, and has been finished successfully. AMÜP will now be extended to monitoring of out-patients in private psychiatric practice. 3 psychiatric departments (Berlin, Göttingen, München) collaborated in designing a common protocol and in assessing ADRs in each centre. This system implies 2 different kind of assessments: Intensive Drug Monitoring (IDM) in a limited sample of subjects selected at random, and Organized Spontaneous Reporting (OSR) where ADRs of grade 3 (i.e. ADR results into drug withdrawal) are identified by the drug monitor controlling the patients' charts for drug discontinuation. The protocol including the assessment of laboratory data will be presented in detail.

Psychiatrische Klinik der Freien Universität Berlin, Labor für Klinische Psychopharmakologie, Eschenallee 3, 1000 Berlin 19.

537

INCREASED MORTALITY IN BENZODIAZEPINE DEPENDENT PATIENTS

B.Piesiur-Strehlow and U.Strehlow

The main undesirable side effect of long term benzodiazepine treatment is drug dependence. In the Federal Republic of Germany, an intensive drug surveillance project (AMÜP) was started in 1979 at the university hospitals of München, Berlin and Göttingen (Rüther,E. et al.,Arzneim. Forsch. 30, 1181, (1980)). Within this project, all cases of dependence on legal drugs are recorded. Up to now more than 400 cases of benzodiazepine dependence have been observed in Göttingen, 1/4 abusing benzodiazepines exclusively, and 3/4 combining them with other drugs, mainly alcohol. Since alcoholics carry a greater risk of early death than expected (Nicholls,P. et al. Quart.J.Stud.Alc.35, 841, 1974), we started a study on the mortality of benzodiazepine dependent patients. Up to now 280 patients have been traced (1468 patient years). 35 death have been observed. This is a high excess mortality, since only 7 deaths are expected(life table method). The increase is significant in mixed cases as well as in cases of isolated benzodiazepine abuse. The extra deaths are mainly due to unnatural deaths, especially suicides. An analysis is attempted whether these deaths are caused by the dependence itself or by an underlying disease (i.g. anxiety neurosis or affective psychosis). Further studies might investigate whether abstinence decreases the risk of death.

Psychiatrische Universitätsklinik Göttingen, v.Sieboldstr. 5, 3400 Göttingen

538

COMPUTER ANALYSIS OF EPIDEMIOLOGIC DATA ON ADVERSE DRUG REACTION IN PSYCHIATRIC HOSPITALS.

J. Scherer, E. Rennig, F. Friedl, A. Strauß

All adverse drug reactions (ADRs) are being covered since 1979 at 3 hospitals (Berlin, Göttingen, Munich) within the scope of a drug monitoring project in psychiatry. The design of data acquisition includes recording spontaneously observed ADRs as well as the continuous therapy monitoring of a random sample of patients in intensive drug monitoring. The ADRs are recorded with the aid of a schedule consisting of 16 system areas and 120 items. The following parameters are calculated: 1. The distribution of the relative frequencies of the ADRs and ADR system areas for the individual drugs; 2. The distribution of the relative frequencies of the drugs for the pertinent ADRs and ADR system areas. The data are evaluated on a computer using the program system SEGEWA, which was especially established for this purpose. The problem of the contribution of several drugs to one ADR is taken into consideration. Examples are provided to illustrate the data evaluation procedures, and the problems occurring are discussed.

Psychiatrische Klinik der Universität München, Nußbaumstraße 7, D-8000 München 2, Germany.

539

RESULTS OF A COLLABORATIVE ADVERSE DRUG REACTION (ADR) MONOTORING IN PSYCHIATRIC PATIENTS.

L.G. Schmidt

The feasibility study of a collaborative drug monitoring project (ref. Müller-Oerlinghausen et al., this volume, or in "Multicentre Trials", Eds.: N. Sartorius/H. Helmchen, Mod.Probl. of Pharmacopsychiat. 16, Karger, Basel, 1981) comprises appr. 5,000 hospitalized psychiatric patients. ADRs were assessed by methods described at loc.cit. from May, 1979 to December, 1980. - Selected results will be presented with special attention to the diagnostic classification of patients involved, and to the relationships between sex or age and the occurrence of certain ADRs. Ranked frequencies of specified ADRs are demonstrated with consideration of the particular drug prescribing pattern of each collaborating centre. Data on the "cardiotoxicity" of psychotropic drugs can exemplify the advantages and limitations of the system, as well as inherent pitfalls in interpreting the assessed data. In the future the system aims at estimating the risk of defined ADRs in individual patients.

Psychiatrische Klinik der Freien Universität Berlin, Labor für Klinische Psychopharmakologie, Eschenallee 3, D-1000 Berlin 19, Germany.

540

EFFECTS OF MEASURES DIRECTED TOWARDS TRANSPARENCY OF DRUG MARKETS ON PRICE INCREASES OF CARDIOVASCULAR DRUG PRODUCTS

K. Quiring and D. Schnädelbach

The German transparency commission has been founded by the Federal Government in order to produce pharmacological and therapeutic transparency in drug markets formed by indication areas. The commission has published several transparency lists for cardiovascular indications in which drug products were judged with regard to pharmaceutical quality (judgements on control measures resulting in up to 6 quality symbols) and to therapeutic efficacy. The effects of these judgements on price increases were retrospectively determined.

For lists containing cardiac glycosides and antiarrhythmics, price increases were determined in 1 - 2 years' intervals for pre-publication, publication and post-publication periods. With respect to the numbers of quality symbols, there were homogeneous price increases before publication. During the publication period a positive correlation between numbers of symbols and per cent price increases occurred; this effect was abolished - or even reversed - during the post-publication period.

With respect to efficacy judgements, price increases for antianginal drugs were followed in 6 months' intervals for 3 years. Between the groups of drugs involved (organic nitrates, beta blockers, calcium antagonists, coronary dilators and sedative-containing combinations) no clear-cut differences in price increases were found. Within the group of calcium antagonists however those drugs regarded as therapeutically effective (i. e. nifedipine, perhexiline and verapamil) showed considerably higher price increases than those for which efficacy was considered to be questionable (i. e. etafenone, fendiline and prenylamine).

As far as the effects observed can be attributed to the judgements made by the transparency commission, it seems obvious that they reflect sensible marketing expectations and reactions of the manufacturers. Desirable cost decreasing effects could be expected to occur only if demand would activate price competition on a high quality and efficacy level.

The technical assistance of Ms. B. Hornemann is gratefully acknowledged.

Geschäftsstelle der Transparenzkommission, Bundesgesundheitsamt, Seestr. 10, D-1000 Berlin 65