## MOLECULAR PHARMACOLOGY OF NEURAL MELANOCORTIN RECEPTORS

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Melanocortins have various physiological actions on the brain. The recent cloning of neural melanocortin (MC) receptors opened new avenues to study the effect of these neuropeptides on the nervous system. We investigated the structure activity relations (SARs) of peptides derived from adrenocorticotrope hormone (ACTH) on cloned MC3, MC4 and MC5 receptors in vitro. Analysis of the effects of various melanocortin peptides on cAMP accumulation in and on binding to cells that expressed either the rat MC3 receptor, the human MC4 receptor or the ovine MC5 receptor demonstrated that different ACTH fragments and analogs could selectively activate or inhibit the MC receptor subtype activities. The SAR of the MC4 receptor resembled that of the induction of excessive grooming behavior by melanocortin peptides. Antagonists that blocked the MC4 receptor were also tested to block a behavioral response induced by α-MSH. α-MSH-induced excessive grooming behavior in rats was inhibited by [Phe-I$^7$]ACTH-(4-10), [D-Arg$^8$]ACTH-(4-10) and [Pro$^{8,10}$,Gly$^9$]ACTH-(4-10), but not by [Ala$^6$]ACTH-(4-10). From these 4 antagonists, only the latter compound did not antagonize the MC4 receptor in vitro. Therefore, we suggest that this behavioral response is mediated by MC4 receptors. ORG2766, an ACTH 4-9 analog that is very potent in an active avoidance task, did not activate, antagonize or bind to the MC receptors. This suggests the presence of still other MC receptors than the MC3, MC4 and MC5 receptors in the brain.
In order to develop more selective MC (ant)agonists, the interaction of melanocortins and receptors at molecular level were investigated. Based upon a 3D-model for MC receptors and the differences in primary structure of the MC receptors, receptormutagenesis was preformed in order to identify the amino acids in the receptors that underly the selectivity that these receptors display for the different melanocortins. Therefore, the MC4 receptor, which is not activated by low doses of γMSH, was genetically modified with sequences that occur in the MC3 receptor, which is activated by γMSH. Furthermore, amino acids that according to our model were important for peptide binding were mutated. Both the binding characteristics as well as the activation of the mutated MC4 receptors by melanocortins were altered.
These studies may be employed for development of novel MC-receptor-specific ligands.

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## ARGININE VASOPRESSIN-INDUCED RESPONSES OF THE HUMAN ISOLATED CORONARY ARTERY

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Arginine vasopressin (AVP) may contribute to cardiovascular homeostasis by causing vasoconstriction and stimulating renal water absorption. In the present study we investigated the effect of the non-peptide receptor antagonists OPC-21268 and SR49059 (both selective for vasopressin $V_1$ receptors: 1,2), and OPC-31260 (selective for $V_2$ receptors: 3), on AVP-induced contractile responses of the human coronary artery (HCA). We obtained right coronary artery segments from 12 organ donors (4F, 8M, 10-54 yrs), whose heart valves were used for homograft transplantation. Contraction was measured isometrically in 15 ml oxygenated organ baths (37 °C), and expressed as a percentage of $K^+$ (100 mM)-induced contraction (all data: mean ± s.e.m.). AVP caused concentration-dependent contraction of the HCA ($pD_2$: 9.19 ± 0.17; $E_{MAX}$:10.2 ± 1.6%; n=12). Removal of the endothelium (as verified by the relaxant response to 1 nM substance P after precontraction with 1 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)) did not result in significantly altered responses to AVP (endothelium intact: $pD_2$: 9.06 ± 0.24; $E_{MAX}$: 10.7 ± 2.0%; endothelium removed: $pD_2$: 9.30 ± 0.32; $E_{MAX}$: 14.8 ± 4.3%; n=8 paired segments). Furthermore, we did not observe relaxation after adding 0.1-1 nM AVP to coronary artery segments (n=3) precontracted (15.4 ± 1.1% of $K^+$, 100 mM) with 0.1 μM $PGF_{2\alpha}$. By contrast, addition of 1 nM AVP induced an additional contractile response (6.7 ± 2.9% of $K^+$, 100 mM). These segments subsequently completely relaxed to 1 nM substance P. In endothelium-intact coronary artery segments, SR49059 (3-30 nM) induced a concentration dependent parallel rightward shift of the concentration response curve (CRC), resulting in a $pA_2$=9.76 ± 0.16 (n=7; slope in Schild analysis: 1.08 ± 0.10). However, only a concentration of 3 μM OPC-21268 caused a small rightward shift of the AVP CRC (approx. $pK_B$=5.6 ± 0.3; n=6). OPC-31260 (0.3-3 μM) induced a parallel rightward shift of the AVP CRC with a $pA_2$=7.31 ± 0.18 (n=7; slope in Schild analysis: 1.07 ± 0.07). We conclude that AVP contracts the HCA with high potency but low $E_{MAX}$, and that the response to AVP is mediated via smooth muscle $V_1$ receptors with higher affinity for SR49059 than for OPC-21268, suggesting that the reported high $V_1$ receptor affinity of OPC-21268 may be confined to rat $V_1$ receptors (1). The $pA_2$ value of OPC-31260 is possibly explained by the previously observed additional affinity for $V_1$ receptors (3). Supported by The Netherlands Heart Foundation, grant no. 89.252.

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## CONTRACTILE RESPONSES TO DIFFERENT $\alpha_1$-ADRENOCEPTOR AGONISTS AND CALCIUM IONS IN HEARTS OF HYPERTENSIVE DIABETIC RATS.

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Hypertension and diabetes mellitus are known to coincide frequently. Both diabetes and hypertension induce cardiac damage, which may lead to altered responses to drugs which increase cardiac contractile force. Several abnormalities in cellular handling of calcium have been suggested to occur in such hearts.
It was the aim of the present study to investigate the profile of the contractile effects of $\alpha_1$-adrenoceptor agonists and of calcium ions in isolated perfused hearts taken from diabetic hypertensive rats.
Spontaneously hypertensive (SHR) and normotensive control (WKY) rats of 12 weeks of age were made diabetic by an injection of streptozotocin (55 mg/kg i.v). 8 Weeks later the inotropic effects of cirazoline, ST587 and extracellular calcium were assessed in paced (6Hz) Langendorff hearts (37°C) and expressed as left ventricular pressure (LVP) and dP/dt max.
The developed left ventricular pressure of control WKY hearts (means ± SEM, n≥22, 85.82 ± 2.91 mmHg) and control SHR hearts (83.67 ± 2.58 mmHg) were significantly ($p<0.05$) higher compared to hearts from both diabetic WKY (51.06 ± 2.51 mmHg) and diabetic SHR (50.72 ± 2.77 mmHg).
Table 1. Max increase in LVP (mmHg), induced by $\alpha_1$-adrenoceptor agonists and calcium ions in isolated hearts, means ± SEM, n= 5-8, *: $p<0.05$ vs control or #: vs WKY, @: $p<0.05$ vs control WKY)

| | cirazoline | ST587 | $Ca^{2+}$ |
|---|---|---|---|
| control WKY | 34.73 ± 1.11 | 29.22 ± 1.86 | 79.60 ± 4.40 |
| diabetic WKY | 51.69 ± 0.63 * | 46.03 ± 1.59 * | 70.77 ± 5.11 |
| control SHR | 33.07 ± 1.86 | 18.17 ± 0.82 # | 73.08 ± 4.25 |
| diabetic SHR | 51.90 ± 1.27 * | 28.16 ± 0.54 *# | 55.99 ± 5.98 @ |

The inotropic responses to both cirazoline and ST587 were increased in hearts from diabetic WKY when compared with those from control WKY. Additional hypertension of the donor animals, however, impaired the inotropic response to ST587 but not that to cirazoline when compared to (diabetic) WKY.
The combination of hypertension and diabetes appeared to decrease the inotropic response to extracellular calcium ions .
The inotropic effect of ST587 is known to require an influx of calcium ions, whereas the response to cirazoline is mediated by an intracellular $Ca^{2+}$ release only. Accordingly, the influence of hypertension and diabetes on the contractile responses to $\alpha_1$-adrenoceptor stimulants and to calcium ions is reflected by substantial changes in calcium handling in such hearts.

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## ANTI-OXIDANT ACTIVITY OF LIPOIC ACID INVOLVES GSH AND VITAMIN C

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Lipoic acid (LA) exerts its therapeutic value in pathologies in which free radicals are involved. Therefore, we study the anti-oxidant mechanisms of LA.

In the protection against lipid peroxidation (LPO), the direct scavenging of free radicals by LA is of minor importance. We determined its indirect anti-oxidant capacity. It was found that in the protection against LPO, dihydrolipoic acid (DHLA) potentiates the protection provided by GSH. It keeps GSH in the reduced, active form. Of the DHLA, consumed during the regeneration proces, only the R-enantiomer could be enzymatically recycled.
During oxidative stress vitamin C is converted into a semi-dehydroascorbate radical and subsequently into dehydroascorbate. DHLA and GSH both regenerate vitamin C. Our experiments show that DHLA regenerates vitamin C much faster.

We conclude that the anti-oxidant effect of lipoic acid in the protection against LPO is due to a cooperation with the anti-oxidants GSH and vitamin C.

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## (S)-UH 301 ANTAGONIZES 5-$HT_{1A}$ RESPONSES INDUCED BY 8-OH-DPAT INJECTION INTO THE MEDIAN AND THE DORSAL RAPHE NUCLEUS IN THE RAT

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5-$HT_{1A}$ receptors are localized presynaptically (median and dorsal raphe nucleus; somatodendritic) and postsynaptically (various brain regions; e.g. the hippocampus). Activation of the presynaptic 5-$HT_{1A}$ receptor leads to decreased serotonergic neurotransmission, while postsynaptic activation by a 5-$HT_{1A}$ agonist mimics part of the natural serotonergic neurotransmission. Systemic application of 5-$HT_{1A}$ receptor agonists, activating pre- as well as postsynaptic 5-$HT_{1A}$ receptors, induces flat body posture (FBP) and lower lip retraction (LLR). In this study only presynaptic 5-$HT_{1A}$ receptors were activated by local application in the median (MR) and dorsal (DR) raphe nucleus.

Local application of the selective 5-$HT_{1A}$ receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) into the DR as well as the MR dose-dependently induced LLR and FBP. 8-OH-DPAT applied into the MR, but not in the DR, also induced hyperlocomotion. The LLR elicited in both raphe nuclei was antagonized by intraperitoneal application of the 5-$HT_{1A}$ receptor antagonist (S)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin ((S)-UH 301; 10 mg/kg). (S)-UH 301 also antagonized hyperlocomotion induced by 8-OH-DPAT in the MR. A dose of 10 μg/rat (S)-UH 301 (intra raphe) could not antagonize the 8-OH-DPAT induced behaviours. These data suggest that the median but not the dorsal raphe nucleus has a disinhibitory role in locomotor activity. It is also concluded, that lower lip retraction is mediated by somatodendritic 5-$HT_{1A}$ autoreceptors in the dorsal as well as the median raphe nucleus, while hyperlocomotion is mediated by somatodendritic 5-$HT_{1A}$ autoreceptors in the median raphe nucleus.

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## PREFRONTAL DOPAMINE IS INVOLVED IN ANXIETY-RELATED BEHAVIOURAL RESPONSES.

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An enhanced dopamine (DA) release in the prefrontal cortex (PFC) of rats has often been observed in response to presentations of mild stressful stimuli. In accordance with such findings, we recently demonstrated pro- and anti-conflict effects of the DA receptor agonist apomorphine (APO) and the DA receptor antagonist *cis*-flupenthixol (FLU), respectively, locally applied in the PFC. Local DA transmission in the PFC thus appears to be relevant for the generation of an affective state (anxiety). In extension of this line of research, we now studied the involvement of prefrontal DA in the behavioural effects of pentylenetetrazole (PTZ). In humans PTZ has anxiogenic properties and in rats the discriminative stimulus properties of PTZ have been suggested as an animal model of human anxiety. After it was found that PTZ, like other anxiogenic drugs, increased DAergic activity in the PFC of rats, we trained rats to discriminate between the effects of PTZ (20 mg/kg, s.c.) and saline in a two-lever drug discrimination procedure. In these animals the effects of bilateral infusions of DAergic drugs in the PFC on the PTZ-induced interoceptive cue were determined. Infusions of FLU antagonized the PTZ cue, whereas infusions of APO produced partial generalization. Taken together, these data strongly indicate a role of prefrontal DA in anxiety.

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## ENDOTHELIAL FUNCTION IN THE RAT HINDLIMB AFTER MYOCARDIAL INFARCTION

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Aberrant vascular responses to acetylcholine (ACh) and nitric oxide synthase inhibitors have been observed in humans with heart failure (HF). This has been interpreted as evidence for endothelial dysfunction in this disease and as possibly contributing to the hemodynamic disturbances seen. This study was therefore undertaken to evaluate minimal vascular resistance and responses to ACh in the isolated rat hindlimb as respective indicators of structural changes in the vascular network and functional changes in the endothelium in this vascular bed following induction of experimental HF. Accordingly, Wistar rats were subjected to either LAD coronary artery ligation or sham surgery. At 7, 21, 35, and 90 days after surgery, the hindlimb was perfused with an isotonic, iso-oncotic Krebs-Henseleit solution after cannulation of the aorta and vena cava. Flow was adjusted to 7.5±0.5ml/min. and resistance was calculated from perfusion pressure and flow. Minimal resistance was determined at maximal vasodilation after two cumulative injections of sodium nitroprusside (SNP) (2*500μg). After a 20mmHg precontraction with phenylephrine (7.5±0.5μM), endothelial vasodilator function was assessed by an ACh dose response curve (0.0001-10μg). Constitutive nitric oxide (NO) release was deduced from the contraction after a 150μg bolus injection of the NO synthase inhibitor, L-nitro arginine (L-NA). Furthermore, a subsequent bolus injection of ACh (10μg) was administered to discern the contribution of NO, as opposed to other endothelium-dependent factors, to the ACh response.

Although minimal resistances (normalized for body weight) at the maximally dilated state increase as the animals age, we see no significant differences between infarcted and sham-operated animals suggesting that no structural abnormalities develop in the hindlimb of this model (9.71±0.30 vs 9.80±0.41 mmHg·min/ml·100g at 7 days (means ±SEM; n=7-8), 14.39±0.60 vs 17.14±1.78 mmHg·min/ml·100g at 90 days (means±SEM; n=7-17), sham vs infarct). Furthermore, the senstivity ($ED_{50}$) and reactivity ($R_{max}$) to ACh is also preserved at all stages after infarction ($ED_{50}$: 2.19±0.42 vs 1.39±0.28($\cdot 10^{-8}$) g at 7 days, 2.07±0.35 vs 1.83±0.40($\cdot 10^{-8}$) g at 90 days, sham vs infarct; $R_{max}$: 77±4 vs 79±3 % relaxation at 7 days, 65±4 vs 62±4 % relaxation at 90 days, sham vs infarcts). The vasoconstrictor effect of L-NA is likewise similar. Notably, we were unable to inhibit the ACh relaxation with L-NA and instead saw an enhanced response in both groups (77±2% before L-NA vs 85±2% after L-NA in shams).

As no differences in vasodilated resistances between shams and infarcts develop, we conclude that no structural vascular changes develop in the hindlimb in the first three months after myocardial infarction in this model of HF. Furthermore, endothelial function as assessed by ACh-stimulated relaxation and constituitive NO production remains preserved. Interestingly, the resistance of the ACh response to L-NA suggests that other endothelium vasodilators, such as endothelium-derived hyperpolarizing factor (EDHF), are major components of ACh-dependent relaxations in the hindlimb resistance bed. Also this mechanism is preserved during HF in this rat model.

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## A NOVEL APPROACH FOR THE CALCULATION OF PLASMA CONCENTRATIONS OF INTRA-ARTERIALLY INFUSED COMPOUNDS IN FOREARM PLETHYSMOGRAPHY

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Forearm blood flow plethysmography is a widely accepted *in vivo* technique for pharmacologic and functional studies in peripheral resistance vessels and veins[1]. The effects of the infused compounds on forearm blood flow (FBF) are usually expressed by means of dose-response relationships. This approach does not take into account the fact that plasma concentrations of the compounds infused are influenced by variations in FBF, nor does it allow a quantitative comparison of the pharmacologic characteristics of different compounds[2].

We here validate a novel equation to estimate the plasma concentrations of intra-arterially infused compounds, using indicator dilution techniques with indocyanine green (ICG) and inulin at different levels of FBF. In six healthy volunteers we infused ICG (0.5 mg/min) and inulin (5 mg/min) into the brachial artery, in the presence of sodium nitroprusside (SNP 10 ng/kg/min; *high FBF*), vehicle (0.9% saline; *intermediate FBF*), and methoxamine (MTX 1000 ng/kg/min; *low FBF*). FBF was measured using automated R-wave triggered venous occlusion plethysmography. Plasma concentrations ($C_{plasma}$ in mg/ml) of the infused indicators ICG and inulin were calculated from the rate of drug infusion (*IR* in mg/min), hematocrit (*Ht*), forearm volume (*V* in ml, minus hand volume), and forearm blood flow (*FBF* in ml/100ml/min), as follows:

$$C_{plasma} = \frac{IR}{(1-Ht)\times FBF\times V}\times 100$$

Plasma concentrations of the indicators, measured in venous blood samples, were compared with the calculated values.

Good correlations were observed between calculated and actual plasma concentrations of both ICG and inulin (calculated/actual ratios at *high*, *intermediate*, and *low FBF* of 1.07±0.09, 1.30±0.20, and 1.78±0.55 for inulin, and of 1.11±0.27, 1.18±0.30, and 1.87±0.76 for ICG, respectively; mean±s.e.mean). Venous plasma concentrations of ICG (>95% protein binding), reached the steady-state within four minutes, independent of FBF, whereas venous plasma concentrations of inulin (negligible protein binding) fitted a two-compartment distribution model, which is dependent of FBF. The equation used apparently provides an appropriate estimate for the total plasma concentrations of intra-arterially infused drugs at the level of the arterioles, although it does not account for the nonspecific loss of infused vasoactive compounds caused for example by plasma protein binding, uptake or degradation mechanisms.

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## MODULATION OF MUCOSAL EXUDATION IN THE MOUSE AIRWAYS BY CAPSAICIN-SENSITIVE NERVES

Theresa L. Buckley & Frans P. Nijkamp

Mucosal exudation is the passage of plasma proteins across the epithelial barrier into the airway lumen. This process could be important in delivering leukocytes and bioactive mediators into the airway lumen under pathophysiological conditions (i.e. during an asthmatic reaction). In this study, mucosal exudation (µl/lung) associated with a pulmonary delayed type-hypersensitivity (DTH) reaction was measured using Evans blue dye as a tracer molecule. The DTH reaction was induced by skin-sensitizing mice with dinitrofluorobenzene (DNFB; 0.5 %). Control mice were treated with the vehicle solution (acetone: olive oil, 1:4). Five days later all mice were challenged intranasally with dinitrobenzene sulphonic acid (DNS). A significant ($P<0.01$) enhancement in mucosal leakage was observed 24 h after the challenge in DNFB-sensitized mice (vehicle group 9.19 ± 1.37µl: DNFB group 20.22 ± 3.29 µl mean ± SEM n=6-8 mice/group). This increase in mucosal exudation was virtually abolished by the neurokinin-1 antagonist RP 67580 ($5.10^{-9}$mol/site) which was administered 5 min before and 1 hr after the challenge (vehicle group 12.54 ± 1.37µl: DNFB group 13.96 ± 2.91µl mean ± SEM n=5-6 mice/group). In contrast, the CGRP antagonist $CGRP_{8-37}$ had no effect on this reaction. Surprisingly, capsaicin pretreatment (14 days before sensitization) markedly enhanced the mucosal leakage response 24 hour after the challenge, however this increase was observed in vehicle- and DNFB- treated mice (vehicle group 46.5 ± 6.5µl: DNFB group 73.68 ± 9.9µl mean ± SEM n=5 mice/group).

In conclusion, tachykinins appear to be important in mediating leakage across the epithelial barrier, however other neural mediators could be involved in protecting the airways against mucosal exudation.

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## NORMOTENSIVE AND HYPERTENSIVE NITRIC OXIDE (NO) SYNTHASE INHIBITORS ATTENUATE MORPHINE WITHDRAWAL IN RATS

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**Introduction.** NO is produced enzymatically in response to activation of NMDA receptors in the postsynaptic structures of the CNS (1) and stimulates the release of various neurotransmitters (2). It is known that blockade of NMDA receptors (3) or inhibition of NO synthase (NOS) attenuated morphine withdrawal syndrome (4).
In order to examine the role of central NO in morphine withdrawal in rats, we compared the effects of the normotensive 7-nitro indazole (7-NI, central NOS inhibitor) and the hypertensive $N^G$-nitro-L-arginine (L-NOARG, peripheral and central NOS inhibitor).

**Methods.** Drug dependence was induced by implantation of morphine pellets (75 mg, s.c.). The withdrawal was precipitated by naloxone (4 mg/kg, i.p.), 72 h following pellet implantation. Morphine withdrawal syndrome was monitored for 30 min following naloxone administration. 7-NI (6.25-50 mg/kg, i.p.) or L-NOARG (7.5-100 mg/kg, i.p.) were administered 5 or 30 min, prior naloxone.

**Results.** Both NOS inhibitors decreased the severity of naloxone-precipitated withdrawal syndrome. This effect was mainly due to a significant decrease of abstinence signs, such as grooming, teeth-chattering, penile licking, diarrhoea, chewing and wet-dog shakes.

**Conclusion.** We concluded that an attenuation of the withdrawal syndrome, induced by NOS inhibitors is unrelated to the inhibition of peripheral (endothelial) NOS and increased blood pressure. Accordingly, it could be suggested that the antiwithdrawal effect of NOS inhibitors is due to decreased levels of central NO. This further implicates that central NO has a facilitatory effect on the expression of some (but not all) opioid withdrawal signs.

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## FLESINOXAN, A 5-$HT_{1A}$ RECEPTOR AGONIST, INDUCES c-FOS IMMUNOREACTIVITY IN CRH NEURONS IN THE PVN

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Flesinoxan (FLES), a phenylpiperazine derivate, acts as a full 5-$HT_{1A}$ receptor agonist and shows anxiolytic properties in a variety of animal models. However, 5-$HT_{1A}$ agonists, among which FLES enhance also plasma corticosterone (CRT) levels. In order to find out which brain areas are involved in the effects of FLES, we investigated the immunoreactivity of the immediate early gene protein product c-Fos (c-Fos ir) in the brain. Male Wistar rats (Harlan-CPB, The Netherlands), used to handling, received an injection of FLES (3 mg/kg s.c.) or vehicle. One hour later the animals were decapitated, blood was collected, and brains were fixated in paraformaldehyde (4%). Only the animals who had received FLES showed c-Fos ir in the paraventricular nucleus of the hypothalamus (PVN). Hardly any c-Fos ir was found in the dorsal raphe nuclei, suggesting a probable postsynaptic activation of the 5-$HT_{1A}$ receptor. The FLES treated rats also exhibited higher plasma CRT levels than vehicle treated animals, which suggests the involvement of corticotropin releasing hormone (CRH). After a double immunolabeling (c-Fos and CRH), we found that each detected CRH producing neuron in the PVN contained also c-Fos. However, a few c-Fos ir neurons in the PVN showed no CRH ir. So far it is not known whether c-Fos ir neurons in the PVN also possess 5-$HT_{1A}$ receptors or vasopressin, which is also known to be involved in the regulation of adrenal corticosterone release. Nevertheless, a dissociation in the organization of serotonergic mechanisms involved in anxiety is suggested: flesinoxan reduces the anxiety and activates the hypothalamic-pituitary-adrenal axis.

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## PHARMACOKINETIC-PHARMACODYNAMIC MODELLING OF THE EEG EFFECT OF ALFENTANIL IN RATS

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Quantitative EEG monitoring in the laboratory animal, in combination with simultaneous pharmacokinetic-pharmacodynamic modelling, may be a useful tool to characterize the pharmacodynamics of CNS-active drugs. Before using effect parameters derived from the EEG, one has to be sure that the EEG is free of any artifacts that may influence the outcome of these effect measures. It has been shown that in rats, high concentrations of the narcotic analgesic alfentanil may produce convulsive patterns in the EEG, thereby possibly confounding the obtainment of meaningful spectral EEG parameters. The purpose of this study was to investigate the influence of the co-administration of the anticonvulsant drug midazolam on the EEG effect of alfentanil in the rat, within the context of pharmacokinetic-pharmacodynamic modelling.
Chronically instrumented rats received 2000 ug/kg alfentanil over a 20 min infusion, 30 minutes after the start of a Wagner infusion scheme of either midazolam (5.5 mg/kg.hr) or saline during the experiment. Serial blood samples were collected for determination of the pharmacokinetics of alfentanil and the rats were artificially ventilated with air when necessary. The EEG was continuously monitored and analyzed off-line using FFT (equivalent amplitude in the δ-frequency range, 0.5-4.5 Hz).
In the midazolam-infusion group, the effect on the δ-frequency band in the EEG was considerably smaller than in the saline-infusion group. Visual examination of the raw EEG signal, however, revealed that in the saline-infusion group, convulsive patterns occurred in all the rats during the alfentanil infusion, while these patterns were absent in the midazolam infusion group. The convulsive patterns consisted of high-voltage waves with a frequency of approximately 2 Hz, thereby thus confoundingly contributing to the effect parameter value derived from the FFT.
The effect on the δ-frequency band in the midazolam infusion group reached its maximum soon after the start of the infusion and subsequently declined during the remainder of the infusion and after the end of the infusion, reflecting the development of acute tolerance. This tolerance development could be successfully described with a tolerance compartment model, resulting in a half-life of 5 min for the rate of tolerance development. The extent of tolerance development was approximately 60%.
It is concluded that the occurrence of convulsive patterns in the EEG emphasizes the need of a co-administration of an anti-convulsant drug, such as midazolam, in order to study the pharmacokinetic-pharmacodynamic relationship of the EEG effect of alfentanil. With midazolam protection, acute tolerance develops to the EEG effect of alfentanil. Future investigations will be necessary to study the tolerance development during different infusion regimens of alfentanil.

## CONDUCTANCE MESENTERIC ARTERY CHANGES IN EXPERIMENTAL DIABETES

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In previous experiments we demonstrated reduced compliance in the carotid artery of diabetic rats. Only limited data are available on compliance changes in peripheral vascular beds, that contribute to a large extent to total systemic compliance. We therefore studied the functional and structural changes of conductance mesenteric arteries, using an in situ perfused mesenteric preparation.

At the age of 6 to 7 weeks diabetes was induced in Wistar Rp rats by an i.p. injection of 70 mg/kg streptozotocin (n=7) or vehicle alone (n=9). Six weeks later, animals were anaesthetized, the last loop of small intestine was exposed, a short segment of mesenteric artery (diameter 300-600 µm) was dissected free and a catheter was introduced in a side-branch downstream. By infusing Tyrode's albumin (4%) solution, intravascular pressure was increased in steps of 25 mmHg at 2 min intervals. Simultaneously, external diameter of the mesenteric artery was recorded and mean arterial pressure was measured invasively.
In the diabetic animals, body weight (mean ± SD) was lower compared to controls (204 ± 26 vs 297 ± 26 g, p<0.001), while blood glucose levels were severely elevated (22.3 ± 5.8 vs 7.3 ± 0.7 mmol/l, p<0.001). Mean arterial blood pressure was comparable in both groups (96 ± 19 and 104 ± 15 mmHg); also pressures in the mesenteric artery were not different (85 ± 21 and 100 ± 13 mmHg). Baseline mesenteric artery diameters were significantly larger in the diabetes group (439 ± 31 vs 388 ± 53 µm, p<0.05), reflecting an increased flow to the intestine. Vessel wall cross-sectional area was comparable. At all pressure steps between 25 and 100 mmHg, compliance of the mesenteric artery was higher in diabetic animals compared to controls; maximal compliance was observed at 25-50 mmHg and amounted to 2276 ± 500 in diabetic animals and 1284 ± 407 µm³/mmHg/µm in controls (p<0.001). Distensibility was the same in both groups.
We conclude that in experimental diabetes a considerable vasodilation of the mesenteric arteries exists. The increased compliance is likely to be caused by an increased blood flow to the mesenteric vascular bed.

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## VALIDATION OF THREE MODELS TO STUDY THE ROLE OF ENDOTHELIUM IN RESTENOSIS AFTER BALLOON ANGIOPLASTY.

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Recently we demonstrated that arterial renarrowing (restenosis) after angioplasty results from both intimal hyperplasia (IH) and arterial shrinkage (remodeling)[1]. IH involves smooth muscle cell proliferation, migration, and matrix synthesis. The mechanism of arterial remodeling is unknown. This study was designed to investigate the role of the endothelium in restenosis after balloon angioplasty with respect to both intimal hyperplasia and remodeling. Rabbit carotid arteries were non-selectively injured with a balloon catheter (endothelial and smooth muscle cell necrosis), selectively injured with a prolene loop (endothelial cell necrosis only) and injured by hyperdistending with contrast medium. It has been suggested that hyperdistension of the rabbit carotid artery with saline causes medial injury with minimal endothelial denudation[2]. Angiograms were made before, during (balloon and contrast) and after the intervention and at follow-up one day after the intervention. After termination, part of the carotid artery was isolated and used to study acetylcholine-induced, endothelium dependent relaxations in organ bath experiments using the sandwich technique. The remaining part of the artery was pressure fixed in situ and processed for histology. One day after the intervention, both balloon and loop injured arteries did not relax in response to acetylcholine. Hyperdistension with contrast medium had no effect on the maximal response to acetylcholine, but shifted the concentration response curve of acetylcholine half a decade to the right. Histological data at 1 day after intervention showed that loop injury had caused 100% loss of the endothelium and 0% medial necrosis. Balloon injury and contrast hyperdistension had caused approximately 100% and 55% removal of endothelium, respectively, and approximately 80% and 70% medial necrosis. **Conclusion:** The variation in endothelial and smooth muscle cell necrosis in the three injury models suggests that these models may be useful to study the role of endothelium in restenosis after balloon angioplasty.

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## REDUCED NITRIC OXIDE SYNTHESIS IN ALLERGEN-INDUCED BRONCHIAL HYPERREACTIVITY

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Recently we described a new guinea pig model of allergic asthma, characterized by ovalbumin (OA)-induced early (EAR) and late (LAR) asthmatic reactions, early (between EAR and LAR, at 6h after OA-provocation) and late (at 24h, after LAR) bronchial hyperreactivity (BHR) to histamine (His) and methacholine (MeCh), and airway inflammation[1,2]. In the present study, using the perfusion model described by Munakata et al.[3], we compared the in vitro His and MeCh responsiveness of intact tracheae from OA-sensitized guinea pigs, 6h after OA-challenge (OA-6h) with those of nonchallenged control animals. Differential pressure change in the lumen was expressed as %ΔP of the effect of 40mM KCl, applied extraluminally (EL). Subsequently, CRCs with His or MeCh, applied intraluminally (IL) and EL were performed. $E_{max}$ of IL applied His increased from 42.9%±5.2 (control) to 93.3%±8.5 (OA-6h; P<0.01). $pD_2$ (-log $EC_{50}$) values slightly, but significantly increased from 3.18±0.08 to 3.57±0.16 (P<0.05). After EL application, $E_{max}$ values increased from 67.6%±5.4 to 104.9%±6.9 (P<0.01), whereas $pD_2$ values were unchanged (4.76±0.08 and 4.74±0.04, respectively). Qualitatively similar results were obtained with MeCh; $E_{max}$(IL) increased from 75.8%±4.6 to 119.9%±10.9 (OA-6h; P<0.01), $E_{max}$(EL) from 100.7%±6.2 to 158.7%±14.7 (P<0.01) and the IL $pD_2$ values from 3.13±0.10 to 3.47±0.09 (P<0.05). The EL $pD_2$ values were not significantly changed: 4.93±0.14 and 5.19±0.07 for control and OA-6h preparations, respectively. In the presence of the NO synthase inhibitor L-NAME (0.1mM, IL) no further increase of $E_{max}$ values of MeCh and His were observed in the challenged preparations whereas in control preparations significant enhancements were found. The $E_{max}$ of MeCh increased, both IL (from 75.8%±4.6 to 147.5%±20.2; P<0.01) and EL (from 100.7%±6.2 to 176.9%±23.3; P<0.01). With His the $E_{max}$ values increased from 42.9%±5.2 to 74.2%±11.5 (IL; P<0.05) and from 67.6±5.4 to 78.9±8.9 (EL). However, L-NAME did not change the $pD_2$ values, the EL-IL difference ($\Delta pD_2$) remaining at 1.7 to 1.8. The results indicate (1) that reduced NO-synthesis is a major factor in the early BHR evoked by allergen provocation and (2) that the barrier function of the tracheal epithelium may be slightly damaged after the EAR.

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## GENERATION OF ANGIOTENSIN I AND II IN THE ISOLATED RAT HEART

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We previously studied the uptake of renin (R), angiotensinogen (Ao) angiotensin I (AngI) and II (AngII) from plasma in isolated rat hearts[1] by using a modified Langendorff model[2], which allows the separate collection of coronary (CE) and interstitial (ISF) effluent. It was the aim of the present study to investigate angiotensin production during R and AngI infusion in this heart model. R (porcine or rat, concentrations corresponding with a $V_{max}$ of 12 and 17 nmol AngI/min per ml, respectively, n = 8) was infused for 40 min, and 10-min fractions of CE and ISF were collected for the determination of AngI and AngII. AngI (arterial concentration of 6.1 ± 2.4 pmol/ml) was infused during 15 min in the absence (n=5) or presence (n=5) of the ACE inhibitor captopril (0.1 mM), and 1-min fractions of CE and ISF were collected for the determination of AngI and AngII. AngI and AngII were measured by RIA after SepPak extraction and HPLC separation.

Prior to the infusions R, AngI and AngII were below the detection limit in both CE and ISF; Ao in CE was undetectable and in ISF it was 0.5-5 pmol/ml (n=5). *R infusion:* AngI appeared in ISF in concentrations of 3.0 ± 1.6 and 6.9 ± 5.3 fmol/ml (mean and SD of 40 min period) for porcine and rat renin, respectively (difference not significant). Towards the end of the infusion period the AngI levels in ISF tended to fall, suggesting Ao exhaustion. AngI in CE was close to or below the detection limit of the assay. AngII remained below the detection limit of the assay in both CE and ISF. *AngI infusion:* Without captopril, the AngI levels in CE and ISF were 59.9 ± 10.3 and 16.8 ± 4.6 % of arterial AngI, respectively. AngII appeared in both CE and ISF in concentrations that were 7.5 ± 6.5 and 9.5 ± 3.4 % of arterial AngI. With captopril, AngI in CE was not significantly different from the concentrations found in the absence of captopril (53.3 ± 12.5 of arterial AngI), whereas AngI in ISF rose to 33.6 ± 8.8 % of arterial AngI (p < 0.05 vs. without captopril). AngII was still present in CE and ISF, although in much lower concentrations (3.8 ± 3.8 and 1.9 ± 1.7 % of arterial AngI, respectively; p < 0.05 vs. without captopril).

*Conclusion:* AngI is generated in the heart outside the vascular compartment from Ao that is either synthesized locally or taken up from the circulation. This local cardiac generation of AngI appears to depend on renin taken up from the vascular compartment. Our results with captopril do not support the contention that cardiac AngII generation depends on enzymes other than ACE. The finding that, during AngI infusion, AngII levels in CE and ISF are equal raises the possibility that cardiac ACE is not limited to the coronary endothelium and the endocardium.

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## THE EFFECT OF RP67580 ON THE BRADYKININ-INDUCED CALCIUM RISE IN BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS

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In the airways bradykinin (BK) is an important inflammatory mediator, capable of increasing vascular permeability[1]. We investigated the effect of bradykinin on bovine pulmonary artery endothelial cells.

Cells were grown on coverslips, and incubated with the fluorescent probe fura-2. Then the BK-induced calcium response was measured using a fluorimeter with dual excitation at 340 and 380 nm. From the ratio of these signals the calcium concentration was calculated.

The effect of BK and related substances was tested at different concentrations. Both BK and the $B_1$-agonist Des-Arg$^9$-BK induced a concentration-dependent transient rise in calcium, followed by a sustained elevated plateau. The plateau remained elevated for at least 15 minutes. The initial rise in $[Ca^{2+}]_i$ elicited by $10^{-6}$M BK was 523±66 nM (n=6), whereas $10^{-6}$M of the $B_1$-agonist could only induce a rise of 257 nM (n=2). After preincubation with the $B_2$-antagonist D-Arg-[Hyp$^3$,Thi$^{5,8}$,D-Phe$^7$]-BK ($10^{-6}$M, n=4) the response was significantly inhibited, but preincubation with the $B_1$-antagonist Des-Arg$^9$-[Leu$^8$]-BK ($10^{-6}$M, n=3) did not change the BK-induced effect. We conclude that the BK-induced rise in $[Ca^{2+}]_i$ is $B_2$-receptor-mediated.

In addition, we investigated the effect of preincubation with $10^{-6}$ M $NK_1$-antagonist RP67580[2]. This compound inhibited the BK-induced rise in intracellular $Ca^{2+}$ significantly (n=4). Since the negative enantiomer of this compound, RP67581, inhibited the BK-induced calcium rise in a similar way, we concluded that it is unlikely that this inhibition is receptor mediated.

In summary, the BK-induced rise in intracellular $Ca^{2+}$ was characterized as $B_2$-receptor-mediated and can be inhibited by RP67580 and RP67581.

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## CHARACTERIZATION OF THE INOTROPC EFFECTS OF ACETYLCHOLINE IN HUMAN ATRIAL AND VENTRICULAR TRABECULAE

X.Y. Du, R.G. Schoemaker, E. Bos, P.R. Saxena

We recently reported that on the human myocardium acetylcholine (Ach) elicited a biphasic atrial response (negative inotropic effect from 0.001-1 $\mu$M, positive inotropic effect ≥1 $\mu$M), while only a positive response was observed in ventricles (Du *et al.*, 1994). We have characterized the receptors mediating the inotropic responses to Ach in right atrial and left ventricular trabeculae, prepared from human non-diseased hearts and mounted in organ baths (37°C, gassed with 95% $O_2$ and 5% $CO_2$ and paced at 1 Hz) for isometric tension measurement. One cumulative concentration response curve was obtained with Ach (0.001-1000 $\mu$M) per preparation, in the absence or presence of antagonists: propranolol (ß), atropine (nonselective muscarinic; M), pirenzepine ($M_1 > M_3 > M_2$), AFDX 116 ($M_2 > M_1 > M_3$), or HHSID ($M_3 \geq M_1 > M_2$) (Doods *et al.*, 1993). Atropine (1 $\mu$M), but not propranolol (1 $\mu$M), antagonized both the positive and negative inotropic effects of Ach in the atrial as well as ventricular tissue (shift approximately 3 and 4 log units, respectively). In the atrial tissue, the negative inotropic effect was most effectively antagonized by AFDX 116. The positive inotropic effect in the atrial tissue was not antagonized by either AFDX 116 or HHSID, but was affected by pirenzepine. In the ventricular tissue, the positive inotropic effect was best

**Table 1. Negative and positive inotropic effects of Ach (maximum % change from baseline) and apparent $pD_2$.**

| *Antagonists* | *µM* | *Atrium* | | | | *Ventricle* | | |
|---|---|---|---|---|---|---|---|---|
| | | *n* | *Negative* | *$pD_2$* | *Positive*[a] | *n* | *Positive* | *$pD_2$* |
| Control | | 16 | 47±12 | 6.8±0.2 | 78±11 | 14 | 33±6 | 5.6±0.4 |
| Pirenzepine | 0.1 | 6 | 73±10 | 7.1±0.2 | 29±15* | 5 | 37±5 | 6.3±0.2 |
| Pirenzepine | 1 | 8 | 61±7 | 6.4±0.2 | 37±11* | 4 | 56±16 | 5.1±0.6 |
| Pirenzepine | 10 | 6 | 58±6 | 6.3±0.2 | 4±3* | 7 | 50±20 | 4.7±1.8 |
| Control | | 16 | 66±5 | 6.8±0.2 | 68±12 | 16 | 46±13 | 5.8±0.2 |
| AFDX 116 | 0.1 | 6 | 67±11 | 6.6±0.1 | 50±23 | 6 | 38±9 | 6.1±0.1 |
| AFDX 116 | 1 | 8 | 57±20 | 5.9±0.2* | 47±37 | 6 | 26±11 | 4.8±0.6 |
| AFDX 116 | 10 | 6 | #* | #* | 111±51 | 7 | 29±12 | 3.9±0.1* |
| Control | | 10 | 70±7 | 7.1±0.2 | 75±30 | 10 | 36±6 | 6.2±0.3 |
| HHSID | 1 | 6 | 69±6 | 6.8±0.1 | 51±31 | 6 | 44±12 | 6.0±0.2 |
| HHSID | 10 | 6 | 31±28* | 6.4±0.4 | 106±57 | 5 | 32±10 | 5.3±0.4 |

[a] *Response at $10^{-3}$ M Ach, calculated as difference from the maximal negative effect ($pD_2$ not calculable). *, Significantly different from values in control experiments run in parallel. #, Only positive inotropic response.*

antagonized by AFDX 116 at 10 $\mu$M (Table 1). These data show that the Ach-induced negative inotropic effect in the atrium and the positive inotropic effect in the ventricle were most effectively blocked by AFDX 116 and, therefore seem to be mediated by the $M_2$ receptor. The positive inotropic effect in atrial tissue (unmodified by AFDX 116, increased by HHSID, and attenuated by pirenzepine) is not mediated by $M_2$ or $M_3$ receptors, but could involve $M_1$ receptor.

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## KINETIC PROFILE OF [$^{123}$I]4-IODODETIMIDE AS A POSSIBLE RADIOLIGAND FOR THE IMAGING OF MUSCARINIC RECEPTORS IN THE HUMAN HEART AND BRAIN.

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Muscarinic receptors may play a key role in the pathophysiology of both cerebral (Huntington's, Parkinson's and Alzheimer's disease) and cardiac (congestive heart failure, sudden cardiac death and myocardial infarction) disorders. In the present study we evaluated the use of [$^{123}$I]4-Iododexetimide as a radioligand for the imaging and subsequent quantification of these receptors in vivo in humans. The kinetics of this newly synthesized compound was studied after intravenous injection of 185 MBq in five healthy volunteers, using SPECT (Single Photon Emission Computerised Tomography). Tomographic images of the heart using a three-headed gammacamera (1 and 4 h post injection (p.i.)) and for the brain using a multi-detector system (Strichman Medical Equipment (3,10,24 h p.i.) were made, reconstructed and subsequently quantified. In order to establish radioactivity in the heart, standardized, concentric, elliptic regions of interest of myocardium (MYO) and left ventricular cavity (C) of all short-axis slices were quantified using the CASPAN program (Cardiac SPECT analysis). Radioactivity in C was used as a reference since it can be quantified by measuring radioactivity in a blood sample and systematic errors in reconstruction are similar to those in MYO. The method proved to be accurate and reproducible, yielding a mean MYO/C ratio of 5.15 ± 0.79 (n=5) at 1 h p.i.. Four hours p.i., myocardial uptake could no longer be quantified because of radioactivity accumulating in the lungs. Radioactivity in different areas in the brain was quantified by using a stereotactic atlas of the brain, where regions were drawn on different areas of the brain on the transverse slice where highest radioactivity was observed in the striatal region. The size as well as the amount of radioactivity in the specific areas was measured at the time points mentioned above. In Table 1 the respective values for mean ± s.e.mean radioactivity expressed as Bq/ml) in the frontal cortex (FRC), striatum (ST), occipital cortex(OC), thalamus (TH), anterior cingulate (AC), posterior cingulate (PC), posterior temporal (PT) and the hemisphere (HS) are presented.

Table 1. Radioactivity expressed as means (*100) ± s.e.mean (n=5) in different areas of the brain at 3, 10 and 24 h p.i..

| | 3h | 10h | 24h |
|---|---|---|---|
| FRC | 47.84±3.77 | 58.25±7.0 | 38.41±2.73 |
| ST | 59.22±4.94 | 67.02±6.56 | 47.82±3.16 |
| OC | 49.36±4.61 | 57.09±3.8 | 38.51±1.21 |
| TH | 36.99±2.69 | 37.23±3.50 | 25.31±0.17 |
| AC | 58.35±5.76 | 66.04±8.57 | 49.37±4.50 |
| PC | 51.81±3.01 | 58.78±5.68 | 37.79±1.78 |
| PT | 52.19±5.07 | 61.61±5.11 | 43.27±3.16 |
| HS | 47.56±3.44 | 54.28±4.59 | 38.68±1.45 |

From these in vivo studies we conclude that it is feasible to image and subsequently quantify muscarinic receptors in vivo in the human heart (1h p.i.) and brain (10h p.i.).

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**Title:** Volume Regulation of the *In Vitro* Blood-Brain Barrier during Hyposmolar Stress by Osmo-reactive Amino Acids.

**Introduction:** Endothelial cells of the blood-brain barrier have the need to regulate their volume under the continuously changing intra- and extracellular environment (due to metabolic activity and nutritive fluctuations) in order to maintain their functional properties as a barrier. One of the mechanisms by which the cells can regulate their volume, besides by changing their ion concentration, is by uptake or release of osmolytes. Osmolytes are organic substances which, ideally, change in cytosolic concentration in concert with the osmolarity of the cell exterior without changing the cell membrane potential, enzyme activities or other cell processes. The amino acid taurine meets the requirements for a regulatory osmolyte almost perfectly. In this study we investigate the effect of a hyposmotic stress on the *in vitro* model of a blood-brain barrier by means of amino acid efflux measurements (eg., taurine) in the extracellular fluid (eg., the culture medium).

**Methods:** The endothelial cells from bovine cerebral capillaries were isolated and cultured to confluent monolayers. The culture medium (MEM) was supplemented with 10% fetal calf serum, which contained the amino acid taurine. The concentrations of the amino acids in the culture medium were determined by means of HPLC with fluorometric detection, both before and after incubation with a hyposmotic culture medium. The osmolarity of the culture medium was decreased from ±300 mOsm to ±230 mOsm by lowering the NaCl concentration. After the experiment the cells were checked for their integrity by means of tryphan blue exclusion.

**Results:** Exposure of the endothelial monolayer to a hyposmotic medium increased the extracellular concentration of several osmoreactive, as well as neuroactive, amino acids drastically. Extracellular concentrations of aspartic acid, glutamic acid, glycine and taurine increased several fold while the cells stayed intact, as determined by tryphan blue exclusion.

**Discussion:** In our experiments we have investigated the influence of a stressor (hyposmotic shock) on the properties of (the *in vitro* model of) the blood-brain barrier. In our opinion, the ability of the endothelial cells to regulate their volume is essential for maintaining the integrity of the blood-brain barrier. The data obtained in the experiment show that the volume regulatory response of the brain endothelial cells is, at least partially, dependent on the efflux of taurine. By determining the mechanism by which the volume regulation is controlled, we hope to have a tool with which we can modulate the permeability of the blood-brain barrier under stress conditions resulting in, for instance, vasogenic edema.

## CORTICOSTEROID RESISTANCE IN PATIENTS WITH PERENNIAL RHINITIS

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Most of the patients with perennial rhinitis will respond to treatment with corticosteroids, however, some patients respond poorly to these drugs or need such high doses that side-effects become severe and symptoms do not improve.
To investigate the differences in response of these sensitive and resistant patients to glucocorticosteroids we have performed binding studies with dexamethasone to determine peripheral blood mononuclear glucocorticoid receptor numbers.
During a double blind placebo-controlled cross-over study 200 $\mu$g of the glucocorticosteroid Fluticasone Propionate Aquous Nasal Spray was administered twice daily for two weeks to 22 patients allergic to house dust mite. In response to allergen provocation (100, 1000, 10000 Bu/ml) and during the 9.5 hours after this challenge symptoms were scored.
Six (out of 22) patients showed no significant improvement in symptom score and were defined as resistant. The number of the peripheral blood mononuclear cell glucocorticoid receptors and the affinity of dexamethasone for the receptors were not significantly different between the sensitive and resistant patients. No differences were observed in the levels of leukotriene $B_4$ produced by monocytes *in vitro*, Interleukin-2 soluble receptor released by lymphocytes *in vitro* and cortisol levels in plasma in both groups.
Clinical glucocorticoid resistance in rhinitis patients allergic to house dust mite is probably not due to abnormalities in corticosteroid receptor characteristics.

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## OPIOID BLOCKADE ATTENUATES ACQUISITION AND EXPRESSION OF COCAINE-INDUCED PLACE PREFERENCE CONDITIONING IN RATS

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It is well established that dopaminergic systems in the mesocorticolimbic areas of the brain are primarily implicated in the reinforcing effects of cocaine. Recent studies have suggested an involvement of endogenous opioid systems in experimental cocaine addiction. One aspect of this involvement may be the modulation of the incentive motivational properties of cocaine by endogenous opioids.

In the present study the involvement of endogenous opioid systems in the incentive motivational properties of cocaine was investigated by assessing the effect of graded doses of naloxone (NLX) on the acquisition and expression of cocaine's motivational effects using the conditioned place preference paradigm. Treatment with doses of NLX that did not induce place aversion (0.01 - 1.0 $mg.kg^{-1}$, SC) dose-dependently attenuated place preference induced by cocaine (10 or 20 $mg.kg^{-1}$, IP). This effect of NLX was present when administered during acquisition of cocaine-induced place preference and when administered before expression of cocaine's motivational effects. These findings support the notion that the (conditioned) incentive motivational properties of cocaine are modulated through activation of opioid systems by endogenous opioid peptides. Furthermore, it is suggested that an interaction between endogenous opioid systems and dopaminergic systems in the brain might be of importance in the incentive motivational facilitation of experimental cocaine addiction.

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## MYOCARDIAL INFARCT SIZE LIMITATION BY TRANSIENT RENAL ISCHEMIA IN RATS IS TEMPERATURE DEPENDENT

B.C.G. Gho, *R.G. Schoemaker, P.D. Verdouw.

Ischemic preconditioning is usually acquired by transient ischemic stress, preceding the infarction in the same area. It has also been shown that "virgin" myocardium can be "preconditioned" by preconditioning the adjacent myocardium. In a previeous study, our group has shown that this "remote" myocardial protection can even be obtained by transient renal artery occlusion, however, data were scattered. One of the factors that could contribute to this scatter could be the body temperature, which was not controlled during the experiments. Therefore, we studied whether temperature modulates the cardioprotective effect of transient renal ischemia.

Anesthetized rats were randomly assigned to two study groups. In one group the body temperature was kept between 35°C and 36°C (A2), and in the other the body temperature was kept between 30°C and 31°C (A3). In both groups, the animals underwent Transient Renal Artery Occlusion (TRO) by a 15 min occlusion of the left renal artery and reperfusion of 10 min before a 1 hour Left Anterior Descending (LAD) coronary artery occlusion followed by 3 hours of LAD reperfusion (A2-TRO, n=7 and A3-TRO, n=9). Control animals underwent an abdominal sham operation 25 min before the 60 min LAD occlusion (A2-Con, n=6 and A3-Con, n=6). The Area at Risk (AR), (defined as the non-perfused area after reocclusion of the LAD), was determined after perfusion with Trypan Blue and was expressed as percentage of the Left Ventricle dry weight ($AR_{\%\ of\ LV}$). Myocardial Infarct Area was determined by staining the vital myocardium with Nitro-Blue-Tetrazolium and was expressed as percentage of the Area at Risk dry weight ($IA_{\%\ of\ AR}$). In A2-Con and A2-TRO the $AR_{\%\ of\ LV}$ were not different (28 $\pm$ 5% and 32 $\pm$ 8%, respectively) neither was the developed $IA_{\%\ of\ AR}$ in A2-Con and A2-TRO (72 $\pm$ 4% and 69 $\pm$ 3%, respectively). In A3-Con and A3-TRO the $AR_{\%\ of\ LV}$ were not different (39 $\pm$ 4% and 35 $\pm$ 3%, respectively), whereas the developed $IA_{\%\ of\ AR}$ was significant limited in A3-TRO (49 $\pm$ 6% compared to 69 $\pm$ 3% in A3-Con, $p = 0.02$). The $IA_{\%\ of\ AR}$ in A2-Con and A3-Con were not different, indicating that the lower bodytemperature itself did not limit $IA_{\%\ of\ AR}$.

We conclude that the cardioprotective effect of transient renal artery occlusion in rats can only be obtained at a lower bodytemperature. These data supports the hypothesis that the mechanism leading to cardiac protection by transient renal artery occlusion suggests involvement of a circulating factor with temperature dependent activity.

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## DIFFERENCES IN BEHAVIORAL RESPONSES TO DEXAMPHETAMINE AND ETHANOL BETWEEN HIGH AND LOW RESPONDERS.

M.A. Gingras and A.R. Cools

The aminergic make-up of the ventral striatum differ between high (HR) and low responders (LR) to novelty (Can.J. of Pharmacol. 71, 335, 1993). Accordingly, it can be expected that responses to drugs which affect the aminergic transmission in the ventral striatum will differ between HR and LR. In this study we investigated two drugs known to exert their effects in the ventral striatum: dexamphetamine and ethanol. First, we replicated and extended the study of Piazza et al., (1991) who found that dexamphetamine (1.5 mg/kg/ip) produces a greater locomotor response in HR than in LR. First a dose response curve of dexamphetamine (0.5-2.0 mg/kg/ip) was established, using automated locomotor cages (36x25 cm). Second, a dose response curve of dexamphetamine (0.5-2.0 mg/kg/sc) was made, using a novel openfield (160x160 cm). Finally, differences in ethanol intake and preference were determined, using a free choice drinking paradigm. HR and LR rats were selected according to the procedure described in Cools et al., (1993).
Analysis of locomotor responses to dexamphetamine revealed that (1.5 mg/kg/ip) produced a higher locomotor activity in HR than in LR, whereas the effect of 0.5, 1.5 and 2.0 mg dexamphetamine was only slightly higher in HR than in LR. The openfield study shows that HR develop stereotyped locomotor responses at lower doses (0.5 and 1.0 mg/kg) than LR (2.0 mg/kg).
In the ethanol study, animals were maintained on alternate day presentation of ethanol and water; ethanol solutions were given in increasing steps of 1%. HR showed significantly lower preference and intake than LR. Animals were maintained on 10% ethanol to determine preference stability; individual-specific differences remained stable during the entire period. In conclusion HR differ from LR both in locomotor and stereotyped responses to dexamphetamine and in ethanol pereference and intake. It is hypothesized that differences in the aminergic make-up of the ventral striatum partly contribute to the noted differences.

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## DISCRIMINATIVE STIMULUS PROPERTIES OF ALPRAZOLAM: NO EVIDENCE FOR AN ANTI-DEPRESSIVE MODE OF ACTION

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The benzodiazepine (BZD) alprazolam is used as an anti-anxiety agent and the only BZD prescribed in panic disorder. Alprazolam may also have anti-depressive properties. This could mean that alprazolam has a different mode of action than other BZD's. Drug-discrimination procedures are used extensively to investigate the mechanism of action of psychoactive drugs. Wettstein and Gauthier[1] trained rats to discriminate alprazolam (1.0 mg/kg i.p.) and diazepam (3.0 mg/kg i.p.) from saline. They reported partial generalizations with anti-depressants (imipramine, fluoxetine, amitriptyline and cericlamine) and buspirone to alprazolam but not diazepam. These results strengthens the idea that alprazolam has a somewhat different mechanism of action than other BZD's. It is possible however that the specificity of the alprazolam simulus is less than that of the diazepam stimulus (because, for instance, of low training dose).
We trained rats to discriminate alprazolam (2.0 mg/kg p.o.) from water and tested BZD's, the beta-carboline abecarnil, the 5-$HT_{1A}$ agonist buspirone and the specific 5-HT reuptake blocker fluvoxamine. Chlordiazepoxide and midazolam substituted completely for alprazolam, at doses not disrupting response rates. Abecarnil partially substituted and disrupted response rates substantially. Abecarnil is somewhat specific for the $BZD_1$ receptor while alprazolam does not discriminate between the two BZD receptor types. Buspirone and fluvoxamine did not generalize to alprazolam, even at doses that had a strong disruptive effect on response rates.
It is concluded that alprazolam has stimulus properties that are the same as those of other BZD's and has no anti-depressive qualities.

1 J.G. Wettstein and B. Gauthier, Behav. Pharmacol., 3 (1992) 229.

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## IS (S)-UH301 A SILENT 5-$HT_{1A}$ RECEPTOR ANTAGONIST?

L. Groenink, J. van der Gugten, P.M. Verdouw and B. Olivier.

Serotonin (5-HT) $_{1A}$ agonists such as flesinoxan and buspirone have been reported to possess anxiolytic and antidepressant effects in humans. Apart from these behavioural effects, these drugs also affect several neuroendocrine systems. Studying the mechanism of action of 5-$HT_{1A}$ agonists has been hampered because of the lack of selective and silent 5-$HT_{1A}$ receptor antagonists. Recently, a putative 'silent' 5-$HT_{1A}$ antagonist has been introduced, (S)-UH301 ((S)-5-fluoro-8-hydroxy-2-(di-n-propylamino)tetralin)[1]. We tried to antagonize the endocrine and behavioural changes induced by the selective 5-$HT_{1A}$ receptor agonist flesinoxan with the putative 5-$HT_{1A}$ receptor antagonist (S)-UH301. The interaction study of (S)-UH301 (3 and 10 mg/kg s.c.) with flesinoxan (3 mg/kg s.c.) showed no antagonistic effects of (S)-UH301 on flesinoxan-induced corticosterone secretion. In fact, like flesinoxan (1 and 3 mg/kg s.c.), (S)-UH301 (3 and 10 mg/kg s.c.) dose-dependently increased plasma corticosterone levels itself. Unlike flesinoxan, (S)-UH301 did not induce hyperglycemia, lower lip retraction and flat body posture. Moreover, flesinoxan-induced hyperglycemia and behavioural changes were effectively antagonized by (S)-UH301, showing potent 5-$HT_{1A}$ receptor antagonistic effects of (S)-UH301. Therefore we conclude that (S)-UH301 is a potent 5-$HT_{1A}$ receptor antagonist and that the (S)-UH301-induced corticosterone secretion is mediated by a non 5-$HT_{1A}$ receptor mechanism.

[1] S.-E. Hillver et al. J. Med. Chem. 33 (1990) 1541.

## CHOLINERGIC DRUG EFFECTS ON A DELAYED CONDITIONAL DISCRIMINATION TASK IN THE RAT

A.H.J. Herremans, T.H. Hijzen, J.L. Slangen, B. Olivier.

We investigated effects of cholinergic drugs on Working Memory (WM) in a Delayed Conditional Discrimination (DCD) task that precludes use of mediating behavior. In the DCD task either a tone or a light, is presented randomly, followed by a Retention Interval (RI) of 0, 1, 5, 10 or 20 seconds during which the stimuli is not present. At the end of that RI first the left lever is protruded into the cage. After depressing it one time two levers are presented simultaneously. Depending on the stimulus presented on that trial rats are rewarded after pressing either the left or right lever. Accuracy of choice is measured after every RI to assess WM function. It is assumed that the demand on WM increases with longer RI's. Therefore, when WM is affected by a drug, its effect increases with the length of the RI. This allows a separation of effects of a pharmacon on WM from effects on less specific aspects of the task like motivation or attention, by examining a drug delay interaction. The centrally acting cholinergic antagonist scopolamine (0.025 - 0.10 mg/kg, I.P.) and the peripherally acting cholinergic antagonist methyl-scopolamine (0.01 - 0.10 mg/kg) dose dependently impaired discriminability independent of delay, indicating that neither drug specifically affects WM. Drugs that enhance cholinergic transmission neither improved discriminability, nor attenuated scopolamine induced impairments. Effects of scopolamine seemed dependent on baseline performance, because in a post hoc analysis scopolamine was found to impair discriminability in a delay dependent manner in animals that performed at a high level in pretest sessions. Methyl-scopolamine impaired performance independently of delay in these animals. We suggest that a ceiling effect at short delays produced this drug/delay interaction of scopolamine in the best performing rats. Results support the idea that cholinergic drugs mainly act on attention processes.

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## CROSS-TALK BETWEEN METHACHOLINE-INDUCED PHOSPHOINOSITIDE METABOLISM AND SUBSEQUENT INCREASE IN INTRACELLULAR CALCIUM AND CYCLIC AMP-DEPENDENT MECHANISMS IN ISOLATED AIRWAY SMOOTH MUSCLE CELLS

B.H. Hoiting, H. Meurs, M. Schuiling, C.R.S. Elzinga, J. Zaagsma.

Phosphoinositide turnover and subsequent increase in free intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) are involved in methacholine (MeCh)-induced airway smooth muscle contraction. Inhibition of these events by enhanced cAMP production could play a role in the functional antagonism of this contraction by ß-adrenoceptor agonists. Therefore, we studied the effects of the cAMP stimulants isoprenaline (Iso) and forskolin (Fors), as well as the cAMP analogue, 8-Br-cAMP, on the inositol phosphates (IP) production and changes in $[Ca^{2+}]_i$ induced by MeCh in isolated, enzymatically dispersed bovine tracheal smooth muscle (BTSM) cells. Iso (1 $\mu$M), Fors (10 $\mu$M) and 8-Br-cAMP (1 mM) did not significantly affect the IP production induced by MeCh (100 nM - 1 mM). MeCh induced a fast (max. in 0.5-2 sec) transient increase in $[Ca^{2+}]_i$, followed by a plateau phase lasting several minutes. Addition of 5 mM EGTA abolished the plateau phase, indicating that this phase depends on $Ca^{2+}$ influx. Five min preincubation with Iso (1 nM - 100 $\mu$M) and Fors (10 nM - 100 $\mu$M) caused a concentration-dependent and sustained inhibition of 1 $\mu$M and 100 $\mu$M MeCh-induced $Ca^{2+}$-transient and plateau phase that was dependent on the concentration of MeCh used. In contrast to the preincubation study, Iso added during the plateau phase induced by MeCh resulted in a short-lasting inhibition of $[Ca^{2+}]_i$ that was maximal at 30 sec and almost completely reversed within 2 min. At 2 min after administration, no significant inhibitory effect of Iso (10 nM - 100 $\mu$M) was found on the plateau phase induced by 100 $\mu$M MeCh, while in the presence of 1 $\mu$M MeCh the inhibition of $Ca^{2+}$ influx was significantly smaller than that observed in the preincubation study. By contrast, Fors (10 nM - 100 $\mu$M) added during the plateau phase induced by MeCh (1 and 100 $\mu$M) caused a concentration-dependent and sustained decrease in $[Ca^{2+}]_i$ over a period of at least 2 min, that was similar to the preincubation study. 8-Br-cAMP (1 mM) added prior to addition of the contractile agonist caused a significant inhibitory effect on the $Ca^{2+}$-transient and plateau phase induced by 1 $\mu$M MeCh but not by 100 $\mu$M MeCh. A similar inhibition of the plateau phase was observed when 8-Br-cAMP was added after the contractile agonist. The results strongly indicate that cAMP-dependent inhibition of MeCh-induced $Ca^{2+}$ mobilization is not primarily caused by attenuation of IP production, suggesting that cAMP-induced PKA activation is effective at a different level in the $[Ca^{2+}]_i$ homeostasis. The differential effects of Iso on MeCh-induced $Ca^{2+}$ influx when added before or after administration of the contractile agonist suggest that muscarinic receptor stimulation may cause a reduced ß-adrenoceptor function and that cross-talk between the different signal transduction systems involved in contraction and relaxation strongly depends on the order of exposition to the stimuli.

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## IN-VIVO BUCCAL DELIVERY OF MACROMOLECULAR DRUGS

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To avoid the obstacles observed in peroral administration of peptide and protein drugs, the buccal mucosa has been investigated as an alternative, potential site for delivery of such macromolecular drugs.
The aim of this study was to investigate buccal delivery of a hydrophilic model macromolecular compound, FITC labelled dextran ($M_w$= 4400 Da; FD4) in pigs. Just as humans, pigs have a non-keratinized buccal epithelium (unlike rodents) which is therefore a good model for studying buccal administration.
A random cross-over study was performed in six female pigs: I. an intravenous bolus injection of FD4, II. 4 hours application of a buccal delivery device with FD4, III. 4 hours application of a buccal delivery device with FD4 and an absorption enhancer, sodium glycodeoxycholic acid (a bile salt; GDC). The buccal delivery device consisted of a hill top chamber and an adhesive backing (Orahesive®). The choice and concentration of the enhancer resulted from earlier in-vitro studies. A specific and sensitive size exclusion HPLC system was used to measure the plasma concentration of FD4.
After intravenous injection of FD4 the following pharmacokinetic parameters were found: clearance Cl= 122 ± 26 ml/min, volume of distribution $V_d$= 12.2 ± 4.8 l and half-life $t_{½}$= 67.0 ± 11.7 min, . After buccal administration the half life of FD4 did not change significantly. This indicates that, after removal of the delivery device, the buccal mucosa did not act as a depot for FD4. The bioavailability after buccal administration for 4 hours was 1.8 ± 0.5 % and could be increased by coadministration of GDC to 11.9 ± 2.7 %. The enhancement effect in-vivo was about 300 times lower than in the in-vitro situation, when excised porcine buccal mucosa was used. From the cumulative AUC-time profile, the steady state flux across the buccal mucosa could be calculated. GDC decreased the time necessary to reach steady state, indicating that GDC shortened the transport pathway across the buccal epithelium and/or increased the diffusivity in the transport domain.
In conclusion, a macromolecular compound can be delivered to the systemic circulation by buccal administration and the uptake substantially increased by coadministration of bile salts. These results offer perspectives for buccal delivery of peptide and protein drugs.

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## THE POTENTIATED STARTLE RESPONSE: AN ANIMAL MODEL WITH PREDICTIVE VALIDITY FOR ANXIETY.

R.J.E. Joordens, T.H. Hijzen and B. Olivier

The startle response can be potentiated by presenting the startle-eliciting stimulus in the presence of a visual stimulus previously paired with an electric shock[1]. Potentiation of the startle response is considered to be a measure of anxiety[2].
To investigate the predictive validity of the potentiated startle response, alprazolam (0, 1, 2 and 3 mg/kg i.p.), amitriptyline (0, 2.5, 5 and 10 mg/kg i.p.), carbamazepine (0, 5, 10 and 20 mg/kg i.p.), chlordiazepoxide (0, 2.5, 5 and 10 mg/kg i.p.), clozapine (3, 10 and 30 mg/kg p.o.) and fluvoxamine (0, 5, 10 and 20 mg/kg p.o.) were administered in rats.
The anxiolytic compounds alprazolam and chlordiazepoxide attenuated startle potentiation linearly with increasing doses. The clinically non-anxiolytic drugs amitriptyline, carbamazepine, clozapine and fluvoxamine did not produce dose dependent effects on startle potentiation.
The present results indicate that the potentiated startle response has substantial predictive validity for the anxiolytic properties of drugs.

1 J.S. Brown et al. J. Exp. Psychol., 41 (1951) 317.
2 M. Davis et al. Behav. Brain Res., 58 (1993) 175.

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## INFLUENCE OF POTASSIUM CHANNEL MODULATORS IN MESENTERIC ARTERIES FROM SPONTANEOUSLY HYPERTENSIVE RATS

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In spontaneously hypertensive rats (SHR) the membrane potential in the vascular smooth muscle is known to be different from that in normotensive Wistar-Kyoto (WKY) rats (Hermsmeyer, 1976). In this study we investigated compounds that interfere with intracellular potassium homeostasis in the mesenteric arteries of Wistar rats, WKY rats and SHR. We evaluated the influence of glibenclamide (GLI), an ATP-sensitive $K^+$-channel blocker on the methacholine- (MCh) (which may release the endothelium-derived hyperpolarizing factor (EDHF)) and on the lemakalim- (LEM) (an ATP-sensitive $K^+$-channel opener) mediated responses.
The experiments were performed in rat isolated mesenteric arteries using an isometric wire myograph (Mulvany & Halpern, 1977). The mesenteric arteries were incubated for 1 hour with 0, 0.1, 1 and 3 μM GLI, respectively. After precontraction with 1 μM phenylephrine cumulative concentration-response curves for MCh and LEM were constructed (n=4-7).
In the mesenteric arteries of the Wistar rats the $pD_2$-value (means ± SEM 7.51 ± 0.12) and the maximal relaxation (Emax) (98.97 ± 0.58%) for MCh were not influenced by GLI. The $pD_2$-values for LEM were significantly ($p<0.05$) and concentration-dependently decreased by GLI (6.55 ± 0.14, 5.80 ± 0.19, 5.44 ± 0.19 and 5.33 ± 0.18 for 0, 0.1, 1 and 3 μM GLI, respectively). The Emax-value for LEM (99.12 ± 0.40%) was not influenced by GLI.
In the WKY preparations the $pD_2$- (7.66 ± 0.08) and Emax-values (100.0 ± 0.0%) for MCh did not differ from those obtained in SHR vessels ($pD_2$: 7.56 ± 0.04, Emax: 95.73 ± 2.59%). The preparations obtained from the WKY ($pD_2$ of 6.35 ± 0.12) were more sensitive to LEM than those taken from the SHR ($pD_2$ of 5.55 ± 0.18). In the mesenteric arteries from the WKY GLI caused a significant and concentration-dependent decrease in $pD_2$ (6.35 ± 0.12, 5.90 ± 0.11, 5.27 ± 0.14 and 5.19 ± 0.06 for 0, 0.1, 1 and 3 μM GLI, respectively) and in Emax (99.11 ± 0.39%, 95.87 ± 0.71% and 55.46 ± 6.83% for 0, 1 and 3 μM GLI, respectively). Only a high dose of 3 μM GLI caused a decrease in Emax (83.16 ± 7.94% and 48.00 ± 5.00% for 0 and 3 μM GLI, respectively) in mesenteric arteries obtained from the SHR. The $pD_2$ for LEM remained uninfluenced by GLI.
It is concluded that the methacholine-mediated responses are neither influenced by glibenclamide nor by the hypertensive state. However, elevated blood pressure of the donor animals decreases the sensitivity to ATP-sensitive $K^+$-channel openers in isolated resistance vessels.

Hermsmeyer,K. (1976) Circ.Res. 38, 362-367.
Mulvany,M.J. & Halpern,W. (1977) Circ.Res. 41, 19-26.

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## REDUCED CORONARY VASODILATION IN INFARCTED RAT HEARTS IS DUE TO ALTERED MECHANISM OF VASODILATION RATHER THAN TO STRUCTURAL CHANGES

E.A.J. Kalkman, M.Y. Bilgin, P.R. Saxena, R.G. Schoemaker

Although infarcted hearts commonly showed reduced responsiveness to inotropic stimulation, data about coronary vascular responses to vasodilators are less consistent[1,2]. Part of this inconsistency may be attributed to a lack of information about this phenomenon as being caused by structural changes or alterations in vasodilator mechanisms within the coronary vascular bed. In addition, it is not known whether long-term treatment that alters structural changes could also affect cardiac responsiveness. To address these questions, Wistar rats were subjected to ligation of the left anterior descending coronary artery to induce myocardial infarction. One group received aspirin (25 mg/kg, i.p.) from 2 days before infarction until the end of the protocol. After three weeks, hearts were isolated and perfused according to Langendorff, and coronary flow and developed left ventricular pressure (LVP) were measured. Dose-response curves were obtained for isoproterenol, adenosine, and nitroprusside. Furthermore, perivascular collagen deposition and medial thickness were measured in coronary resistance arteries in the spared myocardium. Maximal LVP increase in response to isoproterenol was reduced in infarcted hearts (64 ± 8 *vs* 106 ± 12 mmHg in sham hearts). In infarcted hearts, maximal increase in coronary flow was reduced with nitroprusside (8.5 ± 1.0 *vs* 13.0 ± 1.2 ml/min in sham hearts), but not with adenosine (10.8 ± 1.5 *vs* 12.4 ± 1.6 ml/min in sham hearts). None of these maximal responses was altered by aspirin treatment. Resistance arteries in spared, septal tissue of infarcted hearts had more perivascular collagen deposition than vessels in corresponding areas of sham hearts (0.97 ± 0.09 and 0.62 ± 0.09 perivascular collagen to lumen area ratio, respectively), which was prevented by aspirin treatment (0.71 ± 0.05). In contrast, medial thickness was not altered by infarction, or aspirin treatment. The observation that in infarcted hearts the response to nitroprusside, but not to adenosine, was reduced, suggests that alterations in vasodilator mechanisms rather than structural mechanical changes within the vascular bed were responsible for a reduced coronary vascular responsiveness. Since infarction as well as aspirin treatment affected perivascular collagen deposition without altering maximal vasodilatory response, we conclude that perivascular fibrosis is not a major determinant of reduced coronary vascular reserve.

[1] R. Karam et al., Circulation, 81 (1990), 238.
[2] H. Drexler et al., Circulation, 86 (1992), 255.

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## GENERAL CHARACTERISTICS AND VASOCONSTRICTOR AND -DILATOR RESPONSES IN ISOLATED RESISTANCE VESSELS FROM OBESE AND LEAN ZUCKER RATS.

K.L. Kam, M. Pfaffendorf and P.A. van Zwieten.

The recently introduced obese (OZR) and lean (LZR) Zucker rat have been proposed as a potentially valuable animal model to study the syndrome of obesity-hypertension-insulin resistance-hyperlipoproteinaemia. The animals were fed *ad libitum* and used at an age of 22 weeks. Basal parameters (n≥7 ± s.e.mean) like the systolic (179.8±3.2 versus 152.1±2.7), diastolic (113.3±2.3 versus 106.5±1.7) and mean arterial (135.5±2.3 versus 121.7±2.0) blood pressures (mmHg) of the OZR were moderately but significantly ($p<0.05$) higher than in the LZR. However, heart rates (beats/min) was significantly lower in the OZR (329±5) compared to the LZR (346±7). Other general parameters were also increased for the OZR compared to the LZR: body weight (587.0±6.8 versus 407.2±5.2 g), plasma glucose (11.0±0.8 versus 6.2±0.2 mM), plasma insulin (59.7±4.0 versus 15.6±2.5 mIU/l), cholesterol (4.28±0.41 versus 1.78±0.06 mM), HDL (2.83±0.39 versus 1.12±0.06 mM), LDL (0.93±0.17 versus 0.41±0.08 mM), triglycerides (1.18±0.17 versus 0.54±0.09 mM), urinary albumin (3.0±0.1 versus 2.1±0.1 scale 0-4) and urinary glucose (1.6±0.6 mM versus normal). However, no differences were found for the morphological characteristics of the small arteries isolated from the OZR and LZR: the tunica media thickness and tunica-media-thickness-to-lumen-diameter-ratio were the same (14.47±1.67 versus 14.58±1.83 µm and 5.71±0.58 versus 5.64±0.65 %, n=7).
Mesenteric small arteries were prepared and normalised to their individual optimal lumen diameter (± 260 µm for both groups) in an isometric wire-myograph. Cumulative concentration-response curves for the contractile responses to methoxamine, noradrenaline, calcium chloride and potassium chloride showed no differences when constructed for the small arteries isolated from OZR and LZR. Neither the relaxation to sodium nitroprusside (endothelium-independent) nor the relaxation to methacholine (endothelium-dependent) showed differences when assessed on the isolated vessels from OZR and LZR. The vasodilator action of the potassium channel opener cromakalim and the antagonistic effect on this relaxation by the potassium channel blocker glibenclamide were not affected when comparing the OZR and LZR, respectively. Nifedipine was used to study the influx of $Ca^{2+}$-ions via the membrane potential-dependent calcium channels, which was the same in preparations isolated from OZR and LZR.
In conclusion, functional pharmacological responses to both vasoconstrictor and vasodilator agents in isolated resistance vessels appear not to be influenced by the syndrome of obesity-hypertension-insulin resistance-hyperlipoproteinaemia, as found in obese Zucker rats.

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## CHOLINERGIC RECEPTOR-MEDIATED RESPONSES IN THE ARTERIOLAR AND VENOUS VASCULAR BEDS OF THE HUMAN FOREARM

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In arterioles acetylcholine (ACh) is a well known vasodilator. However, in veins a wide variation in responses to ACh has been reported.

In the present study the effects of the cholinergic agonists acetylcholine and methacholine (MCh) were determined simultaneously both in arterial and venous vasculature in the forearm vascular bed of healthy volunteers by means of venous occlusion plethysmography. The vasodilator sodium nitroprusside (SNP) served as an endothelium-independent control agent. The vascular beds were preconstricted by the selective $\alpha_1$-adrenoceptor agonist methoxamine (MTX). Atropine, a non-selective muscarinic receptor antagonist was used to antagonize the dilator effect of MCh.

Overall we observed a weaker relaxant effect of ACh, MCh and SNP in the veins compared to their dilator responses in the arteries. This discrepancy is probably due to a mechanical factor. Arterioles contain a thicker and stronger muscular layer than veins and hence are able to develop a stronger contractile or dilator response. ACh, which is highly sensitive to the hydrolytic inactivation by choline esterases, failed to induce a significant vasodilation in the veins. Atropine blocked the dilator effects of MCh, indicating the involvement of muscarinic receptors. In arteries MCh did not induce a significant stronger vasodilatation than SNP on a molar basis. However, in veins MCh had a weaker relaxant effect ($p<0.05$). This observation may be of physiological significance and therefore deserves more detailed investigations.

The present results well illustrate that the human forearm can serve as a model for simultaneous investigation of vascular responses to vasoactive compounds in resistance vessels as well as in veins.

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## THE ROLE OF THE MUCOSAL MAST CELL IN EARLY VASCULAR PERMEABILITY CHANGES IN A DELAYED-TYPE HYPERSENSITIVITY REACTION IN THE RAT SMALL INTESTINE

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In previous studies it was shown that depletion and stabilization of the mucosal mast cell (MMC) before and at time of challenge were very effective in reducing a delayed-type hypersensitivity (DTH) reaction in the small intestine of the rat, 48 hours after the challenge. The role of the MMC was further investigated in the early component of the intestinal DTH reaction.

Male rats were skin sensitized with dinitrofluorobenzene (DNFB) or vehicle on day 0 and 1. On day 5 the animals were challenged with an intragastric administration of dinitrobenzene sulfonic acid (DNBS). From 0 to 60 minutes after the challenge small intestinal vascular leakage (plasma marker Evans blue dye) and serum levels of rat mast cell protease II (RMCP II, a marker of MMC degranulation) were determined. To investigate the effects of mast cell stabilization on the early events of the DTH reaction, doxantrazole was used (10 mg/kg body weight, intraperitoneally 30 minutes before and at time of challenge). The influence of sensory nerves was studied by means of neonatal capsaicin-induced depletion of sensory neuropeptides (50 mg/kg body weight, subcutaneously).

A significant increase in vascular permeability was found 30 to 60 minutes after DNBS challenge of DNFB-sensitized rats. This was associated with a DTH-specific rise of RMCP II in the serum, indicating MMC activation. In addition, doxantrazole treatment resulted in a significant inhibition of the DTH-induced vascular leakage response and the rise in serum RMCP II 30 to 60 minutes after the challenge. Furthermore, neonatal capsaicin pretreatment abolished the DTH-induced early vascular response as well as MMC activation.

The findings of this study are consistent with an important role of the MMC in the early vascular leakage changes of dinitrobenzene-induced DTH reaction in the small intestine of the rat. In addition, the results with neuropeptide depletion are an indication of sensory nervous control of MMC activation early after the challenge.

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## EFFECT OF DEXAMETHASONE ON THE PRODUCTION OF MEDIATORS OF INFLAMMATION VERSUS GLUCOCORTICOID RECEPTOR CONTENT ON MONONUCLEAR CELLS.

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Most patients with inflammatory bowel disease (IBD) will respond to treatment with glucocorticosteroids but some do not improve even with high doses of oral prednisolone. Whether or not individuals react to corticosteroids may be determined by the functional state of their mononuclear cells (MNC). In order to test this hypothesis we assessed if inhibition by dexamethasone (DEX) of the LPS-stimulated production of $TNF_\alpha$, IL-6, $PGE_2$ and the Ca-ionophore (A23187) enhanced $LTB_4$ release by MNC is determined by the number or affinity of the glucocorticoid receptors (GR) of these cells. MNC were isolated from heparinized peripheral blood from healthy donors and incubated with DEX (conc: $10^{-9}$ to $10^{-5}$ mol/l) for 24 h prior to stimulation with LPS for 24 h or A23187 for 15 min. GR number and affinity of MNC were determined by means of a whole cell DEX binding assay. DEX caused a concentration dependent inhibition of enhanced $TNF_\alpha$, IL-6 and $PGE_2$ production with $IC_{50}$ (and 95 % confidence interval) of: 67 nmol/l (11-398 nmol/l), 144 nmol/l (16-1096) and 138 nmol/l (27-691) respectively. DEX had no effect on $LTB_4$ production. GR-content was 4431±339 sites per cell and Kd was 9.5±0.7 nM. No correlations were found between the inhibition of mediator release and the Kd or receptor number.In conclusion, DEX inhibits the LPS stimulated release of $TNF_\alpha$, IL-6 and $PGE_2$ by MNC. The fact that no correlation was found between the inhibitory effect of DEX and GR content or affinity in MNC in this study, might by explained by the fact that blood was only drawn from healthy volunteers and not from proven non-responders.
Presently, a study is undertaken in which MNC of responders are compared with MNC of non-responders. Preliminary results show a significant difference between receptor characteristics and reaction to DEX between these two groups of patients.

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## MONOCYTE-DEPENDENT AND INDEPENDENT EFFECTS OF SALMETEROL ON IL4 AND IFN-γ PRODUCTION BY HUMAN LYMPHOCYTES

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Asthma is characterized by a chronic inflammatory reaction in the airways. Th2-lymphocytes, as high producers of IL4 and IL5 play an important role in allergic inflammation. In the treatment of asthma $\beta_2$-receptor agonists like salmeterol, seem to be effective by inhibition of airway hyperresponsiveness and the immediate- and late-asthmatic reaction. Given the apparent significance of the Th2-lymphocytes in asthma together with the fact that lymphocytes express $\beta_2$-receptors, we considered it important to determine if the long-acting $\beta_2$-adrenoceptor agonist salmeterol can influence IL4- and IFN-γ-production by human lymphocytes. In the present study, the influence of salmeterol on IL4 and IFNγ production of a total population of human peripheral blood mononuclear cells (PBMC) was examined. This PBMC population is characterized by the presence of lymphocytes and monocytes (approximately 10%). Monocytes (MO) are essential components in the development of specific immunity, through their function as antigen-presenting cells as well as in the nonspecific immunity associated with inflammatory reactions. These cells also express $\beta_2$-receptors. To investigate the possible role of MO in the effects of salmeterol on PBMC cytokine production, purified PBL and MO were used. PBMC or PBL with or without 10% monocytes were incubated ($4*10^4$ cells/200 µl) with varying concentrations ($10^{-11}$, $10^{-9}$, $10^{-5}$M) of salmeterol and subsequently activated with PHA (10 µg/ml). After 96h, supernatants were isolated and IL4 and IFN-γ production was measured by an ELISA. The IL4 production in PBMC and PBL +/- MO is dose dependently inhibited (range:10-40%) at low concentrations of salmeterol ($10^{-11}$-$10^{-9}$M) while this inhibition is reversed into a stimulation at higher concentrations ($10^{-5}$M). In PBMC and PBL this stimulation amounts 5-10%, while this stimulation is enhanced in PBL+MO (14-34%). A U-shaped concentration response curve is also observed for the effect of sameterol on IFN-γ production of purified PBL, the inhibition amounts 36%. In contrast, IFN-γ production of PBMC or PBL+MO are biphasically inhibited. The first phase of inhibition reaches a plateau at $10^{-9}$M (36%) the inhibition at the second phase is increased up to 70%. It can be concluded that very low concentrations of salmeterol ($10^{-11}$,$10^{-9}$M) inhibit IL4 and IFN-γ production by lymphocytes, this is independent of the presence of MO. The second phase of salmeterol induced inhibition of IFN-γ production is dependent on the presence of MO. Clearly, further work is required to determine the mechanisms by which salmeterol affects monocyte dependent effects on cytokine production.

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## INTERMITTENT MORPHINE TREATMENT CAUSES AN ENDURING INCREASE OF NMDA-STIMULATED DOPAMINE AND ACETYLCHOLINE RELEASE IN RAT STRIATUM ASSOCIATED WITH PRESYNAPTIC D2 RECEPTOR DESENSITIZATION

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There is strong evidence that the individual vulnerability to the acquisition and maintenance of drug addiction is enhanced following previous sporadic contact with drugs of abuse, which is thought to be due to drug induced long-lasting changes in central neurotransmission processes. Since drugs of abuse have been shown to share the common effect of activating central dopaminergic neurons, until now attention has been focussed primarily on adaptive changes in dopaminergic neurotransmission induced by repeated treatment with drugs of abuse.

In recent experiments male Wistar rats (150-200g) were s.c. injected with 10 mg/kg morphine once daily for two weeks. Three weeks after morphine withdrawal the NMDA (10µM, 10 min) evoked [$^3$H]-dopamine and[$^{14}$C]-acetylcholine release was studied in superfused striatal slices. The NMDA-evoked release of both neurotransmitters appeared to be enhanced by about 15% in slices of morphine-pretreated rats. Interestingly, whereas in slices of saline-pretreated rats the D2 dopamine receptor antagonist (-)sulpiride enhanced the release of both neurotransmitters by about 35%, such blockade of presynaptic D2 receptors did not facilitate release in slices of morphine-pretreated rats. Moreover, morphine pretreatment caused a shift to the right of the dose-dependent inhibitory effect of the D2 receptor agonist LY171555 on the release of both neurotransmitters. In contrast, the NMDA-evoked release of [$^3$H]noradrenaline from hippocampus slices was not at all affected following morphine pretreatment.
These data suggest that intermittent morphine exposure causes a long-lasting increase of the NMDA receptor-mediated excitatory effect of glutamate on dopaminergic nerve terminals and dopamine innervated postsynaptic neurons in rat striatum due to desensitization of release-inhibitory D2 dopamine receptors, which could play an important role in the enduring behavioral sensitizing effects of drugs of abuse.

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## INTERMITTENT INFUSIONS OF VASOCONSTRICTIVE AGENTS CAUSE HYPOTENSION IN THE DRUG-FREE PERIOD IN SHR.

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Introduction: Following cessation of antihypertensive treatment sometimes periods with blood pressures above those before treatment occur. Upregulation of specific receptors or increased sensitivity to hypertensinogenic mechanisms have been associated with such rebound phenomena. Conversely, we hypothesized that vasoconstrictive agents can cause a decrease in blood pressure after cessation of treatment. To test this hypothesis , spontaneously hypertensive rats (SHR) were subjected to intermittent infusions of pressor agents.

Design and Methods: SHR were instrumented with an arterial catheter for continuous computerized 24-h recording of mean arterial pressure (MAP). Rats were kept on a 12/12 h light/dark cycle. Phenylephrine (PE) 0.6 mg/kg.h, Angiotensin II (AII) 3 microg/kg.h or saline were given on 5 successive days as a 6-h intravenous infusion (0.4 ml/h) starting at different time-points of the day; T1: 3 h before the onset of the dark period and T2: 3 h before the onset of the light period.

Results: In table 1 effects on MAP are given as difference to control in mmHg ± S.E.M.

| | During Infusion | | Following Infusion | |
|---|---|---|---|---|
| Drug | Day 1 | Day 5 | Day 1 | Day 5 |
| PE T1 (n=9) | 26.3 ± 3.5 | -2.3 ± 4.9 | -7.7 ± 4.0 | -13.8 ± 4.2 |
| PE T2 (n=8) | 19.9 ± 1.9 | 20.8 ± 3.6 | -13.0 ± 3.8 | -18.7 ± 5.1 |
| AII T1 (n=7) | 14.4 ± 5.9 | 8.7 ± 7.0 | -2.5 ± 4.4 | -1.8 ± 3.7 |
| AII T2 (n=8) | 20.7 ± 6.2 | 17.0 ± 6.6 | -4.4 ± 5.0 | -0.4 ± 3.9 |

The hypotensive effect after the last infusion of PE lasted more than 24 hours. In a similar protocol using the NO-synthase inhibitor (L-NAME) at an equipotent pressor dose (1.2 mg/kg . h) baseline MAP did not fall.

Conclusion: In SHR, a time-dependent hypotensive effect is seen following intermittent infusions of Phenylephrine and Angiotensin II. The magnitude of the effect seems independent of the size of the blood pressure increase during the infusions. The hypotensive+ effect may be due to the activation of endogenous blood pressure lowering factors.

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## RAT CARDIAC ANGIOTENSIN CONVERTING ENZYME FOLLOWING MYOCARDIAL INFARCTION: QUANTITATION OF ITS mRNA AND LOCALIZATION OF PROTEIN AND mRNA.

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The involvement of the intracardial renin-angiotensin-system (RAS) in heart failure has been suggested. However, quantitative data on the expression of RAS components in this condition are lacking. In the present study we quantified angiotensin-converting-enzyme (ACE) mRNA and localized ACE mRNA and protein in the infarcted rat heart. Wistar rats underwent ligation of the left descending coronary artery, resulting in myocardial infarction (MI), or a sham-operation. At different time points (1-90 days) after surgery (n=3 each) the heart was removed and divided in right ventricle (RV), septum (SE), and left ventricle (LV). Total RNA was isolated and ACE mRNA was quantified by the reverse transcriptase competitive PCR. At 4 and 7 days after MI we found a 3-fold increase of ACE mRNA (n=3; $p \leq 0.05$) in the infarcted LV compared to the LV of the sham group. No increases of the ACE mRNA were found in the non-infarcted hypertrophied septum and right ventricle. In situ hybridization and immunohistochemistry showed increased ACE mRNA and protein density in the border zone of the infarcted area, predominantly in the endothelial cells, lining capillaries. In the non-infarcted myocardium ACE mRNA and protein were confined to endothelial cells of the larger vessels. In addition ACE protein was localized at the endocardial layer. From this data we conclude that the intracardial RAS is involved in the healing of the scar after myocardial infarction in the rat, possibly giving rise to neovascularization and/or myocardial fibrosis. Furthermore, the data suggest that the intarcardial ACE is not necessarily associated with hypertrophy in the rat heart after myocardial infarction.

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## GENERATION OF SUPEROXIDE ANION IN GUINEA-PIG TRACHEA *IN VITRO*

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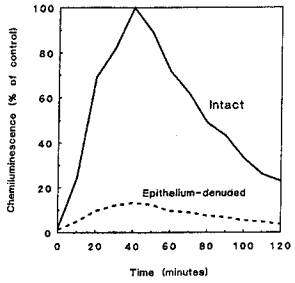
Generation of oxygen radicals in isolated guinea-pig trachea, their interaction with nitric oxide ($NO^{\cdot}$) and the role of epithelium were examined. Tracheal rings generated lucigenin-enhanced chemiluminescence upon exposure to 160 nM phorbol myristate acetate (PMA) or 2.5 $mg.ml^{-1}$ opsonized zymosan. Zymosan induced a higher level of chemiluminescence generated by trachea compared to PMA. The peak chemiluminescence produced by a 2-rings tracheal preparation was 75.8 ± 10.1 mV, almost equal to the peak chemiluminescence produced by $4\times10^4$ alveolar macrophages. Chemiluminescence was decreased by 40% when the preparations were co-incubated with the superoxide dismutase (SOD, 100 or 200 $U.ml^{-1}$). However, catalase (100 $U.ml^{-1}$) had no effect.

Incubation of tracheal rings with 500 μM L-arginine (L-arg) or 100 μM L-$N^G$-nitro-arginine (L-NNA), a $NO^{\cdot}$ synthase inhibitor, had no influence on chemiluminescence production. L-arg demonstrated a slight inhibitory effect only when the rings were simultaneously treated with 10 μM histamine. Histamine was probably responsible for releasing $NO^{\cdot}$ which, in turn, inactivates superoxide ($O_2^{\cdot-}$). Incubation with 1 μM sodium nitroprusside (SNP), a direct donor of $NO^{\cdot}$, significantly decreased the peak production of chemiluminescence by 54%. This might be due to the formation of peroxynitrite ($ONOO^-$) from the combination of $O_2^{\cdot-}$ and $NO^{\cdot}$. However, it is also possible that $NO^{\cdot}$ directly inhibited the radical release from the tracheal tissue.

Removal of tracheal epithelium caused a tremendous drop in the zymosan-induced radical release (see the figure). However, neither isolated guinea-pig tracheal epithelial nor cultured human bronchial epithelial cells generated chemiluminescence when exposed to PMA. Therefore, inflammatory cells or other cell types in the submucosal layer of epithelium could be responsible for release of oxygen species.

The release of $O_2^{\cdot-}$ from submucosal layer of airways may be crucial in the induction of airway hyperresponsiveness as it may inactivate $NO^{\cdot}$ and damage airway cells.

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## A MURINE MODEL TO INVESTIGATE TOLUENE DIISOCYANATE-INDUCED ASTHMA.

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Toluene diisocyanate (TDI), a low molecular weight compound, is known to cause occupational asthma, however, the mechanisms involved in this reaction are unknown. In this study a murine model was developed to investigate TDI-induced asthma.

Mice were sensitized twice on day 0 and day 1 with 1% TDI (sensitized group) or with vehicle control (nonsensitized group) on the shaved abdomen and four paws. On day 8 they were challenged intranasally with 1% TDI and in vitro tracheal reactivity to carbachol was measured isometrically. Twenty four hr after the intranasal challenge with TDI the sensitized group exhibited marked tracheal hyperreactivity (69% increase when compared to the nonsensitized group). Surprisingly, no increase was found in the infiltration of inflammatory cells into the airway lumen as measured in bronchoalveolar lavage. However, the myeloperoxidase activity, which is a marker for neutrophils, was significantly enhanced 24 hr after the challenge in the airway lumen and lung tissue.

To investigate whether this reaction was lymphocyte dependent cells were isolated from spleen and inquinal lymph nodes of sensitized mice. These cells were pooled and $5x10^7$ cells were transferred into the retroorbital plexus of normal recipient mice. One day after the transfer the mice were challenged intranasally with 0.1% TDI. Twenty four hr after the challenge the senstitized group showed a significant elevation in tracheal reacitivity (25% increase when compared to the nonsensitized group).

These results suggest that TDI induces airways hyperreactivity in the mouse. We are currently investigating the mechanisms involved in this reaction and our recent results suggest that the neutrophil and the lymphocyte play an important role.

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## PHOSPHODIESTERASE INHIBITORS REDUCE BRONCHIAL HYPERREACTIVITY AND AIRWAY INFLAMMATION IN CONSCIOUS, UNRESTRAINED GUINEA PIGS.

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Using a new guinea pig model of allergic asthma (Santing et al., Pulm. Pharmacol. 1992; 5: 265-272), the effects of low doses of the phosphodiesterase (PDE) inhibitors rolipram (PDE IV selective), ORG 20241 (dual PDE III/IV inhibitor with some selectivity for the type IV isoenzyme), and of theophylline (non-selective) on allergen-induced early and late phase asthmatic reactions, bronchial hyperreactivity (BHR) to histamine inhalation, and airway inflammation were investigated.
Theophylline (25 mg/kg i.p.) and ORG 20241 (5 mg/kg i.p.) did not affect histamine-induced bronchoconstriction, whereas with rolipram (75 μg/kg i.p.) only a slight reduction of the respons to histamine at 7 h after administration was found.
However, BHR after both the early and the late reaction was significantly reduced by theophylline, rolipram, as well as ORG 20241, while bronchoalveolar lavage studies revealed a selective inhibition of airway inflammation by the PDE-inhibitors. Theophylline significantly reduced the number of eosinophils, neutrophils and macrophages; rolipram the number of neutrophils and lymphocytes, and ORG 20241 the number of eosinophils and macrophages.
None of the compounds at the dosage indicated reduced the early and late reaction when administered i.p. 1 h before allergen exposure to defined dual responding animals.
These results indicate that low doses of these PDE-inhibitors markedly reduce the allergen-induced development of BHR as well as airway inflammation by inhibiting cellular migration. The results also suggest that an orchestrated series of cellular interactions is involved in the development of BHR.
It is concluded that selective type IV PDE-inhibitors may be very useful in the treatment of allergic asthma.
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## BRADYKININ $B_2$ RECEPTORS ARE COUPLED TO BOTH TRANSIENT AND SUSTAINED REDUCTION IN CYCLIC-AMP IN $DDT_1$ MF-2 SMOOTH MUSCLE CELLS

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Stimulation of $DDT_1$ MF-2 cells with bradykinin leads to activation of phospholipase C, the formation of $Ins(1,4,5)P_3$ and a concomitant increase in intracellular $Ca^{2+}$ [ref.1]. This pathway is known to cause contraction of the intact tissue. In contrast, activation of adenylyl cyclase, resulting in an increase in cellular cAMP causing relaxation. Recently we showed that stimulation of $P_{2U}$ purinoceptors leads to simultaneous activation of the PLC pathway and inhibition of adenylyl cyclase activity via an inhibitory G-protein in $DDT_1$ MF-2 cells[2]. In the present study we investigated whether bradykinin also affected intracellular cAMP.

Intracellular cAMP was elevated by activation of adenylyl cyclase with forskolin or by stimulation of $\beta_2$ adrenergic receptors with isoprenaline. Bradykinin evoked a transient reduction in cAMP in forskolin pretreated $DDT_1$ MF-2 cells. This action of bradykinin was concentration dependent ($IC_{50}$ = 36.4 ± 4.9 nM). In contrast, when cells were pretreated with isoprenaline, bradykinin elicited a pronounced and sustained decrease in cellular cAMP ($IC_{50}$ value of 37.5 ± 1.1 nM). The bradykinin $B_2$ receptor agonist D-Arg[Hyp$^3$, Thi$^5$, D-Tic$^7$, Oic$^8$]-BK (HOE 140) completely abolished the bradykinin induced reduction in cAMP in both forskolin and isoprenaline ($IC_{50}$ = 34.0 ± 1.5 nM) pretreated cells. Likewise, the bradykinin induced increase in cytoplasmic $Ca^{2+}$ was abolished by HOE 140. The bradykinin $B_1$ receptor agonist desArg$^9$-bradykinin did not affect isoprenaline induced cAMP, nor did it enhance intracellular $Ca^{2+}$. It is concluded, that both the bradykinin induced reduction in cAMP and the increase in cytoplasmic $Ca^{2+}$ are mediated by bradykinin $B_2$ receptors.
Exposure of cells to the $Ca^{2+}$ ionophore ionomycin, which leads to permeabilization of the plasma membrane and depletion of internal $Ca^{2+}$ stores caused a transient decrease in forskolin induced cAMP and a sustained decline in isoprenaline induced cAMP. These results show, that a rise in cytoplasmic $Ca^{2+}$ mimics the effect of bradykinin on cAMP. This strongly suggests, that the bradykinin induced increase in $Ca^{2+}$ elicits both the transient and the pronounced, sustained decline in prestimulated cAMP levels in $DDT_1$ MF-2 cells. The nature of the response is dependent on the ligand used to elevate cAMP.

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## ROLE OF PREJUNCTIONAL HISTAMINE $H_3$ RECEPTORS IN THE MODULATION OF ELECTRICALLY EVOKED NORADRENALINE RELEASE IN THE PORTAL VEIN OF FREELY MOVING RATS.

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Prejunctional histamine $H_3$ receptors have been suggested to be involved in the modulation of noradrenaline (NA) release from sympathetic nerve endings in the cardiovascular system[1,2]. However, most of the studies on this subject were carried out *in vitro* or in anaesthetized animals. In the present study we investigated the role of prejunctional histamine $H_3$ receptors in the regulation of NA release evoked by electrical stimulation of the nervous plexus (ENS) of the portal vein in freely moving rats.

The portal vein was provided with a bipolar stimulation electrode and a cannula for the sampling of blood. In the abdominal aorta a second cannula was placed for blood pressure and heart rate measurements, and for injection of drugs. To establish the role of prejunctional histamine $H_3$ receptors, the selective agonist *α*-methylhistamine (*α*-MHA) and the selective antagonist *thio*peramide were used.

*α*-MHA (0.01, 0.1 and 1 μmol/kg) produced a significant, dose-dependent inhibition of the electrically evoked NA overflow up to 59.1 ± 3.3% (n = 7) of control (100 ± 2% (n=10)); a higher dose did not inhibit the overflow further. Thioperamide (0.1, 0.5 and 1.0 μmol/kg) antagonized the effect of 1 μmol/kg *α*-MHA dose-dependently, evoked overflow returning to control values (102.4 ± 7.6% (n=6)) at the highest dose. Thioperamide alone had no effect on ENS-induced overflow of NA (99 ± 3.4% (n=4)) and both drugs had no influence on basal noradrenaline levels; blood pressure and heart rate were not influenced either.

This study clearly showed the presence of prejunctional histamine $H_3$ receptors on sympathetic nerve terminals which have a significant inhibitory effect on the electrically evoked noradrenaline overflow in the portal vein of freely moving rats.

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## REGULATION OF HISTAMINE $H_2$ RECEPTOR EXPRESSION

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G-protein coupled receptors show frequently a dynamic regulation of receptor function. For most receptors, agonist stimulation is followed by desensitization, a general process characterized by a decrease in cellular responsiveness despite the conitinuous presence of the stimulus. This process is associated with a rapid uncoupling of the receptor from Gs, which occurs within minutes. Downregulation develops more gradually and results in both reduction in both receptor number and effector stimulation. These processes may become apparent in several pathophysiological conditions, e.g. an allergic reaction or asthmatic attack, where histamine is released in large quantities.

Long-term exposure (24 hrs) of Chinese Hamster Ovary cells (CHO) cells, overexpressing the rat histamine $H_2$ receptor, to histamine resulted in a dose dependent decrease in the number of $H_2$ receptors (max. reduction 50 %). The receptor affinity remained unaltered. This process appeared to be a $H_2$ receptor mediated process as dimaprit induced downregulation and its structural analogues, homo- and nordimaprit, which are devoid of $H_2$ activity, did not. All compounds generating cAMP upon stimulation, such as $H_2$ agonists, forskoline and cholera toxin, induced histamine $H_2$ receptor downregulation, suggesting the possible involvement of cAMP in the process of receptor downregulation. Moreover, long-term incubation of CHO cells expressing the rat histamine $H_2$ receptor with $H_2$ antagonists resulted in $H_2$ receptor upregulation. Posttranslational modification of the receptor by phosphorylation reactions is thought to be one of the major mechanisms of receptor regulation of the G-protein coupled receptors. Currently, we examine the possible role of different kinases in the process of downregulation and determine which structural elements of the receptor are essential for $H_2$ receptor downregulation.

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## EFFECTS OF BQ-123 ON CONTRACTIONS INDUCED BY ENDOTHELIN-1 OR SARAFOTOXIN S6B IN THE HUMAN MIDDLE MENINGEAL ARTERY

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Elevated plasma levels of Endothelin-1 (ET-1) have been observed in patients during a migraine attack[1]. This increase in ET-1 was greatest during the first phase of the attack. Since the meningeal vasculature has been suggested as a likely source of the migraine headache, we investigated the effect of the $ET_A$ receptor antagonist BQ-123 on contractile responses of human middle meningeal artery (HMA) induced by ET-1 or sarafotoxin S6b (Sf6b) (both $10^{-11}$-$3.10^{-7}$ M). HMA segments (2-3 mm) were suspended in an organ bath (15 ml) for isometric tension measurements. For each agonist concentration response curves (CRC) were constructed in parallel in the absence or presence of BQ-123 ($10^{-8}$, $10^{-7}$ and/or $10^{-6}$ M); n=4-5. Both ET-1 and Sf6b contracted HMA in a concentration dependent manner. The maximal response and the potency of ET-1 and Sf6b were: $E_{max}$, 154.2±27.5 vs 78.8±10.2 % of maximal response to KCl (40 mM), respectively; $pEC_{50}$: 9.02±0.14 vs 8.14±0.22, respectively. In the presence of BQ-123 ($10^{-8}$ and $10^{-7}$ M), there was a non-significant shift in the CRC to ET-1. However, in the lower concentration range ($10^{-11}$-$3.10^{-9}$ M), BQ-123 ($10^{-7}$ M) caused a significant rightward shift; the dose ratio, based on only this part of the curve, amounted to 2.92±0.5. It therefore seems that the contractile response to low concentrations of ET-1 are apparently mediated by a $ET_A$ receptor, while at higher concentrations a receptor insensitive to BQ-123 appears to be involved. The CRC to Sf6b was also shifted to the right by BQ-123 ($10^{-7}$ and $10^{-6}$ M). When the CRC to Sf6b in the presence of BQ-123 ($10^{-7}$ M) was extrapolated to the maximum of the control curve, a significant rightward shift was observed; the dose ratio being 27.8±9.5. Since BQ-123 ($10^{-7}$ M) caused a more pronounced shift in the CRC to Sf6b than to ET-1 (lower concentration range), it appears that Sf6b may be acting on a receptor different from those activated by ET-1.

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## MICROCIRCULATORY VASCULAR NETWORK CHANGES IN RATS WITH HEART FAILURE

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Chronic heart failure is associated with a decreased perfusion of peripheral vascular beds, specially skeletal muscle. Little is known about the role of the microcirculation in these changes. We therefore studied structural vacular network changes in the dorsal microcirculatory chamber in the rat. As a model for heart failure we used coronary artery ligated rats. The advantage of this experimental design is the possibility of chronic follow up of the microcirculatory changes.

We measured changes (%) in diameters (μm) and density (intersections/mm² grid) of arterioles and venules in sham-operated and infarcted (MI) rats. Timepoints for measurement were: before, 1, 3 and 5 weeks after the ligation of the coronary artery. Infarct size varied from 40 - 59% in the MI group (sham: infarct size = 0%).

The diameter of arterioles was not significantly altered after MI, although there was a tendency towards vasodilation of the smallest arterioles (A3/A4). Small venular (V3, V4/V5) diameters were reduced significantly 1 and 3 weeks after MI. No significant change was observed in the diameters of larger venules. There was a smaller density in the A3/A4 vessels 1 week after MI. The densities of V3 and V4/V5 vessels, however, were significantly increased. No changes were observed in the densities of larger arterioles and venules.

We suggest a role for angiotensin II (AngII) in the microcirculatory network changes, because the plasma level of AngII is elevated in heart failure. In the past we and others have shown vascular growth stimulating properties of AngII. The results of the present study suggest that AngII has a greater impact at the venular level of the microcirculation and therefore induces venular growth rather than arteriolar growth.

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## CONDITIONAL INVOLVEMENT OF MUSCARINIC $M_1$ RECEPTORS IN VAGALLY MEDIATED CONTRACTION OF GUINEA-PIG BRONCHI

R.E.J. ten Berge, A.F. Roffel and J. Zaagsma

The involvement of facilitatory ganglionic muscarinic $M_1$ receptors in vagally induced bronchoconstriction in guinea-pig airways is controversial. We developed an *in vitro* guinea pig main bronchus preparation in which vagus nerve (VNS, preganglionic) and electrical field stimulation (EFS, postganglionic) were performed alternately, and studied the effects of the $M_1$-selective muscarinic receptor antagonist pirenzepine on these VNS- and EFS-induced twitch contractions under various experimental conditions.

Using identical stimulation parameters for VNS and EFS (8V, 30 Hz, 0.5 ms, 5s every min), the amplitude of the VNS-induced twitch contractions was 30.4 % of the EFS-induced responses, and pirenzepine showed 2.3-fold selectivity ($pIC_{50}$-values 6.45 and 6.09, respectively) to inhibit vagally induced twitch contractions. With the stimulation frequency for EFS lowered to match contraction levels obtained using VNS, pirenzepine was equipotent to inhibit both types of response at $M_3$ receptor-selective concentrations, suggesting that $M_1$ receptors are not involved. By contrast, when the stimulation episode was prolonged until plateau contraction (10 - 20 s), in the presence of the nicotinic antagonist hexamethonium (5 $\mu$M), the $M_2$ receptor antagonist AQ-RA 741 (0.1 $\mu$M) and the ß-adrenoceptor antagonist timolol (1 $\mu$M), and again using matched VNS- and EFS-induced contraction levels, pirenzepine inhibited nerve stimulation-evoked responses in a biphasic manner, yielding $pIC_{50}$-values of 8.01 (indicative of $M_1$ receptor blockade) and 6.42 (indicative of $M_3$ receptor blockade) for the first and second phase, respectively, while postganglionic stimulation showed a purely monophasic inhibition ($pIC_{50}$ = 6.32).

These results show that facilitatory muscarinic $M_1$ receptors are indeed involved in vagally mediated contraction of guinea-pig bronchi, under conditions of elevated neurotransmission and partial nicotinic receptor blockade.

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## DYSFUNCTION OF PREJUNCTIONAL MUSCARINIC $M_2$ RECEPTORS AFTER THE EARLY ALLERGIC REACTION IN THE AIRWAYS OF CONSCIOUS, UNRESTRAINED GUINEA-PIGS

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We studied the function of autoinhibitory muscarinic $M_2$ receptors on vagal nerve endings in the airways of unrestrained, ovalbumin-sensitized guinea-pigs. For that purpose, a small intrapleural balloon to measure airway function and an electrode for vagus nerve stimulation were inserted by surgery. Then, the effects of the $M_2$-selective muscarinic antagonist gallamine were examined on bronchoconstriction induced by vagus nerve stimulation, which was determined as an increase of pleural pressure.

Under control conditions, i.e. before antigen challenge, a significant increase of the vagally induced bronchoconstriction was found with 0.1 mM and, more pronounced, with 1.0 mM gallamine given by inhalation, at medium stimulation frequencies (2-16 Hz), leading to a leftward shift of the frequency-response curve; with 10 mM of this antagonist, a reversal of the leftward shift and a 50 % depression of the vagally induced bronchoconstriction was obtained. However, 6 h after challenge with ovalbumin (i.e. after the early allergic reaction) no increase of nerve stimulation-induced bronchoconstriction by gallamine was found anymore. At that moment, bronchial responsiveness to histamine was enhanced 4.5-fold compared to control. Both after the late allergic response (24 h after challenge; 1.6 -fold histamine hyperresponsiveness) and 4 days after allergen challenge (normal histamine responsiveness) the gallamine-induced potentiation of the bronchoconstriction was restored with 0.1 and 1.0 mM of the antagonist, while with 10 mM gallamine the bronchoconstriction was suppressed, similar to the responses under control conditions.

The results clearly demonstrate that prejunctional $M_2$ receptors control vagally induced bronchoconstriction in conscious guinea-pigs *in vivo*. Furthermore, these autoinhibitory receptors appear to be dysfunctional after the early allergic reaction, but their function has already been restored after the late phase. The dysfunction of autoinhibitory muscarinic $M_2$ receptors might contribute to the strongly enhanced responsiveness to histamine after the early allergic response.

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## INTERMITTENT MORPHINE AND COCAINE TREATMENT INDUCES LONG-TERM CHANGES IN PREPRODYNORPHINE GENE EXPRESSION IN THE CAUDATE PUTAMEN OF THE RAT.

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In addition to the acute euphoric effects, drugs of abuse such as opiates and psychostimulants have been known to induce long-lasting behavioral effects. Thus, intermittent treatment with morphine or cocaine leads to a progressive response to the psychomotor stimulating effects of these drugs (behavioral sensitization) which persists long after discontinuation of the drug treatment. Such a long-lasting sensitization has been suggested to be involved in the acquisition/maintenance and relapse of drug self administration. However, little is known about long-term neuroadaptive processes which may form the neurobiological substrate of these enduring behavioral effects. In this regard, endogenous opioid systems may play an important role since $\kappa$-opioid receptor agonists and $\delta$-opioid receptor antagonists have been reported to block the behavioral effects of cocaine and morphine and, in addition, cocaine treatment has been reported to increase the striatal preprodynorphine (PPD) gene expression. However, it remains unclear whether this involves a long-lasting adaptation and may be generalized to other drugs of abuse such as morphine. Therefore in this study we determined opioid peptide gene expression in caudate putamen and nucleus accumbens in male Wistar rats treated intermittently with daily s.c. injections of morphine (10 mg/kg, 14 days) or cocaine (15 mg/kg, 5 days). In situ hybridization histochemistry was performed employing $^{35}S$ radiolabelled PPD and preproenkephaline riboprobes on transverse sections of animals sacrificed 1, 3 or 21 days after the last drug administration. The results were quantified with IBAS image-analysis system and compared to saline treated controls. Both morphine and cocaine (intermittently administered) induced an increase in PPD gene expression in the caudate putamen lasting till at least 3 weeks after the last administration suggesting that enduring adaptive changes in PPD gene regulation may be involved in the long-lasting behavioral effects of drugs of abuse.

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## HEMODYNAMIC EFFECTS OF $\gamma_2$-MSH ARE NOT (IN)DIRECTLY MEDIATED BY ACTIONS ON PERIPHERAL CARDIOVASCULAR STRUCTURES OR SYMPATHETIC NEUROTRANSMISSION

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The melanotropin $\gamma_2$-MSH is the most potent POMC-derived neuropeptide with respect to its effects on mean arterial pressure (MAP), heart rate (HR), and cerebral blood flow. Intravenous administration of $\gamma_2$-MSH (1.5-100 nmol/kg) to conscious rats causes a rapid and dose-dependent increase in MAP and HR, whereas after intracerebroventricular administration no effects are observed. **Direct** peripheral interactions of $\gamma_2$-MSH with cardiovascular structures were investigated in pithed rats and in the perfused isolated rat heart. In order to demonstrate **indirect facilitatory effects** upon preganglionic and postganglionic sympathetic neurotransmissions the effects of $\gamma_2$-MSH upon MAP and HR were studied in electrically-stimulated and 1,1-dimethyl-4-phenylpiperazine (DMPP)-stimulated pithed rats, resp..

$\gamma_2$-MSH had neither effect on MAP and HR in pithed rats, nor on myocardial contractility and coronary flow in the isolated heart. Therefore, a direct effect on cardiovascular structures is excluded. Whereas the reference compound salbutamol by a facilitatory action increased MAP and HR induced by electrical stimulation, $\gamma_2$-MSH was ineffective. Also DMPP-induced increases in MAP and HR were not altered by pretreatment with $\gamma_2$-MSH. Therefore, no facilitatory actions of $\gamma_2$-MSH on peripheral sympathetic neurotransmission appear to be responsible for the cardiovascular effects of this melanotropin.

We hypothesize that the cardiovascular effects of $\gamma_2$-MSH are not mediated via a direct peripheral action, but rather via CNS structures located outside the blood brain barrier.

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## ADHESION OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES TO BRONCHIAL EPITHELIAL CELLS

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Epithelial damage is a feature often observed in the airways of asthmatic patients and can be caused by the interaction of leukocytes with the epithelial layer. This interaction is mediated by adhesion molecules. Bronchial epithelial cells express the adhesion molecule intercellular adhesion molecule-1 (ICAM-1), CD44 en lymphocyte function-associated antigen-3 (LFA-3)[1]. Only the ICAM-1 expression can be up-regulated by stimulation with inflammatory cytokines like interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α)[1]. In this study we determined which adhesion molecules are involved in the adhesion of polymorphonuclear leukocytes (PMNs) to bronchial epithelial cells.

Confluent monolayers of the human bronchial epithelial cell line BEAS-2B or primary cultured human bronchial epithelial cells (HBEC) were incubated with freshly isolated, $^{51}$chromium labeled, human PMNs for 30 min at 37°C. Non-adhered cells were removed by gentle washes. The percentage of adhered PMNs was calculated as cpm adhered cells/cpm added cells x 100%.

Basal adhesion of PMNs to the epithelial cells was approximately 10%. The adhesion of the PMNs to the BEAS-2B cells was increased significantly to 80% upon stimulation with phorbol myristate acetate (PMA; 10 ng/ml) and to 30-40% with platelet-activating factor (PAF; $10^{-7}$ M), TNF-α (20 ng/ml) or formyl-Met-Leu-Phe (fMLP; $10^{-6}$ M). Adhesion of fMLP-stimulated PMNs to HBEC was also increased to 30-40%. It is known that stimulation of PMNs induces an upregulation and/or activation of the $\beta_2$-integrins (LFA-1, Mac-1), the counterstructures of ICAM-1. The increased adhesion in our experiments was absent when the assay was performed at 4°C and could be blocked by addition of inhibiting monoclonal antibodies against CD18 (the common β-chain of LFA-1 and Mac-1). The increased adhesion of PMNs to epithelial cells was also blocked by pre-incubation of the epithelial cells with Fab-fragments of a monoclonal antibody directed against ICAM-1.

In conclusion, adhesion of PMNs to bronchial epithelium is increased by stimulation of the PMNs and is mediated via LFA-1 and/or Mac-1 on the PMNs surface and ICAM-1 on the epithelial cells.

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## PHARMACOKINETICS AND PHARMACODYNAMICS OF INTRAVENOUS APOMORPHINE

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Apomorphine is a potent dopamine agonist, which is used clinically in the management of Parkinson's disease. Because of a very low bioavailability following oral administration, several parental routes of administration have been developed, i.e. subcutaneous, intranasal, rectal and sublingual administration. Despite its wide use, little is known about the pharmacokinetic properties and about the concentration-effect relationship.

Ten patients with idiopathic Parkinson's disease, five men and five women, were selected. Their mean age was 56.2 yrs. (range 37-72), with a mean duration of disease of 8.2 yrs. (range 2-17). The anti-parkinsonian medication was stopped at least 12 hours before starting the study. Domperidone (30 mg t.i.d.) was given to prevent the peripheral adverse effects of apomorphine. The infusion started with a rate of 10 μg/kg/hr. Every twenty minutes the infusion rate was increased wit 10 μg/kg/hr, until a maximum of 100 μg/kg/hr was reached or a lower rate if adverse effects became too severe. Thereafter the infusion rate was decreased every twenty minutes with 10 μg/kg/hr until zero. The blood samples were collected every twenty minutes and analyzed by HPLC with electrochemical detection. Clinical efficacy was assessed by qualitative tremor -and dyskinesia score and a quantitative tapping score. All patients gave their informed consent. The study was approved by the Medical Ethical Committee of the University Hospital Leiden.

The mean $T_{max}$ was 192 minutes range 80-320) with a mean $C_{max}$ of 24.3 ng/ml (range 8.5-60). The mean clearance was 4.5 l/min (range 2.7-6.6). The clearance was not influenced by weight and age, but tended to be higher in male patients compared to the female patients.

The effect were closely related to the plasma concentration of apomorphine. Positive effects on the tremor and dyskinesia score were observed at relatively low concentrations. Side effects (yawning, nausea, vomiting, headache) were only observed at significantly higher concentrations. Between patients a considerable variability was observed. These findings show that controlled delivery of apomorphine may be of value in the management of Parkinson's disease.

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## 13-HYDROXYOCTADECADIENOIC ACID (13-HODE) DECREASES THE PAF-INDUCED CALCIUM RESPONSE AND SUBSEQUENT DEGRANULATION IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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Arachidonic acid-derived metabolites are well-known mediators of the inflammatory response. Less is known about the biological activities of the linoleic acid metabolites 9- and 13-HODE. Previously, chemotactic activity of 13-HODE towards human polymorphonuclear cells (PMN) has been observed. Our present study focussed on the role of 13-HODE in the activation and functioning of PMN involved in the inflammatory process. Activation of PMN with platelet-activating-factor (PAF) is characterized by a transient, biphasic increase of the intracellular $Ca^{2+}$-concentration ($[Ca^{2+}]_i$). The first phase of the PAF-induced $Ca^{2+}$ response is due to release of $Ca^{2+}$ from intracellular stores. Following store depletion an influx of $Ca^{2+}$ through channels present in the plasma membrane is observed. This $Ca^{2+}$ influx is counterbalanced by a simultaneously activated $Ca^{2+}$ extrusion from the cytoplasm, accomplished by activation of $Ca^{2+}$ reuptake into intracellular stores and $Ca^{2+}$ efflux. Recently, we have demonstrated that 13-HODE only slightly inhibited the first phase of the PAF-induced $Ca^{2+}$ increase, but dose-dependently inhibited the second phase of the PAF-induced $Ca^{2+}$ elevation. This indicates that 13-HODE inhibits the $Ca^{2+}$ influx or stimulates the reuptake of $Ca^{2+}$ into intracellular stores. The divalent cation $Ni^{2+}$ (2.5 mM), which is generally used as an inhibitor of $Ca^{2+}$ influx in PMN, also inhibited the second phase of the PAF-induced $Ca^{2+}$ response. However, when PMN were preincubated with $Ni^{2+}$ in the presence of 13-HODE (5 μM) an additive inhibition of the PAF-induced $Ca^{2+}$ response was observed. This result suggest that the inhibitory effect of 13-HODE on the PAF-induced $Ca^{2+}$ response is caused by activation of $Ca^{2+}$ reuptake into intracellular stores. To confirm this interpretation PMN were first stimulated with PAF and subsequently with fMLP in the presence of 13-HODE or $Ni^{2+}$. Restimulation of PMN with fMLP after $[Ca^{2+}]_i$ has returned to basal level, will cause the release of re-stored $Ca^{2+}$. In the presence of 13-HODE, fMLP induced a strong increase of $[Ca^{2+}]_i$. In contrast, when PMNs were incubated with $Ni^{2+}$, stimulation with fMLP subsequent to the first stimulation with PAF, only resulted in a small increase of $[Ca^{2+}]_i$. This indicates that 13-HODE stimulate the $Ca^{2+}$ reuptake into intracellular stores.

Since the PAF-induced increase of $[Ca^{2+}]_i$ is an important trigger for degranulation, we examined the effect of 13-HODE on the PAF-induced degranulation. Degranulation is characterized by upregulation of CD67 and CD11b molecules on the plasma membrane of PMNs which can be measured by flow cytometry. 13-HODE showed intrinsic degranulatory activity. Furthermore, 13-HODE inhibited the PAF-induced expression of CD67 and CD11b.

From these data we conclude that 13-HODE inhibits the PAF-induced $[Ca^{2+}]_i$ increase by activation of $Ca^{2+}$ reuptake into intracellular stores. This inhibitory effect of 13-HODE is reflected in the PAF-induced degranulation of PMNs.

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## CARDIAC CONTRACTILITY AND THE RENIN-ANGIOTENSIN SYSTEM

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In order to investigate the role of the renin-angiotensin system in the regulation of myocardial contractility we studied the hemodynamic and biochemical effects of intracoronary (i.c.) and systemic (i.v.) infusions of the specific renin inhibitor remikiren in open-chest anaesthetized pigs (25-30 kg).

Consecutive 10 min i.v. infusions of remikiren were given at 2, 5, 10 and 20 $mg.min^{-1}$. Mean arterial pressure (MAP), cardiac output (CO), heart rate (HR), systemic vascular resistance (SVR), coronary blood flow (CBF), myocardial oxygen consumption ($MVO_2$) and left ventricular (LV) $dP/dt_{max}$ were not affected by remikiren at 2 and 5 $mg.min^{-1}$, and were lowered at higher doses. At the highest dose, MAP decreased by 48 %, CO by 13 %, HR by 14 %, SVR by 40 %, CBF by 34 %, $MVO_2$ by 28 % and LV $dP/dt_{max}$ by 52 % (mean values; $p < 0.05$ for difference from baseline, n=5). The decrease in $MVO_2$ was accompanied by a decrease in myocardial work (MAP x CO), but the larger decline in work (55%) implies a reduced myocardial efficiency (MAP x CO)/$MVO_2$).

Consecutive 10 min i.c. infusions of remikiren were given at 0.2, 0.5, 1, 2, 5 and 10 $mg.min^{-1}$. MAP, CO, $MVO_2$ and LV $dP/dt_{max}$ were not affected by remikiren at 0.2, 0.5 and 1 $mg.min^{-1}$, and were lowered at higher doses. At the highest dose, MAP decreased by 30 %, CO by 27 %, $MVO_2$ by 45 %, and LV $dP/dt_{max}$ by 40 % (mean values; $p < 0.05$ for difference from baseline, n=6). HR, SVR and CBF did not change at any dose.

Plasma renin activity and angiotensin I (AngI) and AngII were reduced to levels at or below the detection limit at doses of remikiren that were not high enough to exert hemodynamic effects, both after i.v. and i.c. infusion.

Results show that with remikiren i.v. blood pressure is lowered primarily by vasodilatation and with remikiren i.c. by cardiac depression. These haemodynamic effects do not appear to depend solely on a decrease in circulating AngII. Data support the contention that myocardial contractility is increased by renin-dependent AngII formation in the heart.

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## DOES A LOCAL RENIN-ANGIOTENSIN SYSTEM EXIST IN NEONATAL RAT CARDIAC MYOCYTES OR FIBROBLASTS?

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Neonatal rat cardiomyocytes, grown for 1 day in the presence of serum and subsequently cultured for 5 days under serum-free conditions, have been reported to synthesize angiotensin II (AII)[1]. Mechanical stretch of myocytes causes release of AII in the medium and this AII may act as an initial mediator of the stretch-induced hypertrophic response[2]. These data suggest that myocytes can synthesize AII independently from the circulating renin-angiotensin system (RAS). However, it is possible that myocytes have sequestered RAS components from serum during plating. In addition, myocyte cell cultures are always contaminated with some fibroblasts. To investigate AII formation by cardiomyocytes in more detail we measured renin (R), prorenin (PR), angiotensinogen (Ao) in the medium and angiotensin I (AI) and AII in the medium and cell lysate of neonatal rat cardiac myocytes and fibroblasts cultured in the presence or absence of serum. Cells were isolated by enzymatic dissociation of cardiac tissue from 1-3 day old Wistar strain pups and grown for 1 day in Dulbecco's modified Eagle's medium : medium 199 (4:1) containing 5 % horse serum and 5 % fetal calf serum. After 1 day the cells ($1.5\text{-}3 \times 10^6$ cells per dish) were either serum-deprived or maintained in serum-containing medium for 5 days. At the end of the 5-day period medium and cells were collected. PR (after activation to R by plasmin) and R were measured by enzyme-kinetic assay in the presence of excess rat Ao. Ao was measured as the maximum amount of AI generated in the presence of excess R. AI and AII were measured by radioimmunoassay after SepPak extraction and HPLC separation (detection limit for AI and AII in medium 0.2 and 0.1 fmol/ml and in cells 0.3 and 0.2 fmol/$10^6$ cells, respectively). In a separate series of experiments AI-to-AII conversion by ACE was studied, in the absence or presence of 0.5 mM captopril, over a 2-hour period after the addition of AI (1 pmol/ml) to intact myocytes that had been cultured for 5 days.
The levels of RAS components in the medium of myocytes and fibroblasts cultured in the presence of serum were less than or equal to the levels in unconditioned serum-containing medium (R and PR 0.18 ± 0.04 and 1.28 ± 0.02 fmol AI/min per ml, Ao 31 ± 2 pmol/ml, AI and AII 1.3 ± 0.3 and 0.6 ± 0.2 fmol/ml; n=5, mean ± SD). All RAS components were undetectable in unconditioned serum-deprived medium. In the medium of fibroblasts and myocytes cultured in the absence of serum, PR, Ao and AI were < 10 % of the levels in serum-containing medium. These low levels were most likely caused by contamination of the cells with the serum-containing medium of the first 24-hour plating period. R and AII were undetectable. Cellular AI and AII were below the detection limit under all conditions. AI incubated with myocytes was rapidly metabolised ($t_{1/2}$ with and without serum 15 ± 3 and 68 ± 40 min respectively, n=4) with AII being a major metabolite. Captopril significantly ($p < 0.05$) prolonged the half life of AI ($t_{1/2}$ with and without serum 87 ± 33 and 222 ± 237 min respectively, n=4). The results suggest that myocytes or fibroblasts do not synthesize R, PR or Ao in concentrations that are detectable or high enough to result in AI or AII concentrations of physiological relevance. Serum-deprived myocytes are capable of converting AI into AII via ACE, suggesting that ACE might be synthesised by these cells.

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## RECOVERY FROM SCIATIC NERVE CRUSH LESION IN THE RAT CORRELATES WITH NEUROENDOCRINE CHANGES AND INDIVIDUAL BIOLOGICAL CHARACTERISTICS OF RESPONSES TO CHRONIC STRESS.

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Stress induces changes in neuro-endocrine functioning and, on the long run, results in deterioration of the adaptive capacity of the organism. This study focuses on the influence of chronic intermittent stress upon functional recovery from a sciatic nerve crush lesion in rats.

Male Wistar rats underwent a standard sciatic nerve crush. Subsequently footshock stress was applied daily during the period of recovery from the nerve lesion (approximately 27 days). To that end, both CONTROL and STRESS rats were individually placed in a shock box for 30 min daily, where STRESS-, but not CONTROL rats received 3 inescapable, electric footshocks (0.5 s, 1 mA) each session, delivered at random intervals.

Biological indices of chronic stress, such as decreased body weight gain, hypertrophy of the adrenal gland and thymus involution, indicated the achievement of chronic stress in STRESS-rats. Evaluation of the Sciatic Functional index (SF-index) and the return of the foot sole withdrawal reflex revealed significant differences in the rapidity of functional sensorimotor recovery between CONTROL and STRESS-groups at the expense of the latter group. Subsequent statistical analysis of individual SF-index data and values of forementioned stress variables in STRESS-rats indicated that recovery of the walking pattern negatively correlated with adrenal gland ($p<0.01$) and medulla enlargement ($p<0.05$), thymus involution ($p<0.01$), and plasma levels of ACTH ($p<0.01$) and corticosterone at 45 min ($p<0.001$), but not at 0 and 120 min following the final stress session. In CONTROL rats a significant correlation was found for SF-index with adrenal medulla weight ($p<0.05$) only.

The present study demonstrates that chronic stress impedes sensorimotor recovery after sciatic nerve crush lesion. The data correspond with the notion that responses to (chronic) stress are individually determined, and indicate that functional recovery after nerve crush-lesion is related to the adaptive capacity of individual rats. We suggest that chronic stress thus uncovers and magnifies individual differences in adaptive capacity of rats.

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## VASORELAXATION BY PHOSPHODIESTERASE III INHIBITORS IN HUMAN ARTERIES IN VITRO.

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The use of the currentiy available PDE-III-inhibitors, including amrinone, milrinone and enoximone in acute heart failure is associated with marked vasodilation at higher doses. The exact mechanism of these vasodilator properties remains to be elucidated. In the present study, we investigated whether the vasorelaxant effects on human vessels of the PDE III inhibitors used in clinical practice could be blocked by a nitric oxide (NO) synthase blocker, $N^G$-nitro-L-arginine methyl ester (L-NAME), or by the blocker of ATP-sensitive potassium ($K_{ATP}$) channels glibenclamide (Glib).
Small arteries were obtained from 11 healthy female patients (age 18 to 49 years) undergoing reconstructive breast surgery under general anaesthesia. Immediately after removal of a skin segment arteries with a diameter of 415 ± 0.20 $\mu$m (mean ± SEM, n = 116) were dissected and stored maximally one week at 4°C in an organ preservation solution according to the University of Winsconsin (UW)[1]. The experiments were performed with an isometric myograph according to Mulvany and Halpern[2]. At the beginning of each experiment, the preparations were exposed three times for 5 min to an isotonic, high potassium solution (120 mM $K^+$) and once to 10 $\mu$M phenylephrine, at intervals of 15 min. This episode was followed by an equilibration period of 30 min. The drugs, L-NAME (100 $\mu$M) or Glib (1 $\mu$M), were given at the beginning of this 30 min. period and remained present in the bath throughout the experiment. Subsequently, U 46619, a thromboxane $A_2$ mimetic, was administered to all preparations at a concentration of 1 $\mu$M. After stabilization of the contraction fase, cumulative concentration-response curves with L-NAME, Glib or control were constructed for the specific PDE III inhibitors amrinone, milrinone and enoximone as well as for theophylline and dipyridamol. A log concentration-response curve was fitted to the data of each experiment (n = 5-7) by nonlinear regression analysis. There were no significant differences in main vessel characteristics between the groups. The preparations remained stable and responsive in the "UW" solution for at least one week after preparation, when the experiments took place (maximum active force day 1: 3.61 ± 0.46 N/m *vs* day 11: 3.87 ± 1.69 N/m ($P>0.05$). All arteries showed spontaneous rhythmic activity under the described experimental conditions. Addition of L-NAME to the organ bath resulted in significantly higher $pEC_{50}$ (-log of the half maximal concentration) of milrinone (n = 5); 2.59 ± 0.014 compared to controls (n = 7); 3.20 ± 0.014 ($P<0.05$). No significant difference occurred between any other group.
From the presented data we conclude that the vasorelaxant properties of amrinone, enoximone, theophylline and dipyridamol are not dependent on NO release or on an interaction with $K_{ATP}$ channels. However, the effect of milrinone may be partly endothelium-dependent in human vessels *in vitro*.

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## HYPERTHYROID RATS, SUITABLE FOR CARDIOVASCULAR PHARMACOLOGICAL STUDIES.

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We are reporting on a rat model which was developed for a systematic pharmacological characterization of the cardiovascular system under hyperthyroid conditions.
Hyperthyroidism was induced by feeding Wistar rats with L-thyroxine (T4)-containing chow. For four weeks two groups were fed either with 1 mg T4 / kg chow (low T4) or with 5 mg T4 / kg (high T4). Appropriate control groups were run parallel. Initially, the food intake was similar in all groups (21.2±0.7 g/d, n=8) but from the second week onwards the daily food intake of the high T4 animals increased to 25.8±1.0 g/d, n=8 ($P < 0.05$).
Two weeks after onset of treatment plasma T4 and TSH of all animals were measured by means of RIA's.

Table 1. T4 and TSH plasma concentrations after 2 weeks of treatment with T4.

| | T4 (nmol/l) | TSH (ng/ml) |
|---|---|---|
| control(n=14) | 63.5±1.8 | 0.979±0.13 |
| low T4 (n=6) | 133.3±7.4* | 0.374±0.05* |
| high T4 (n=8) | 279.8±13.9* | 0.315±0.02* |

*P < 0.05

After four weeks of treatment the animals were anaesthetized with pentobarbital 50 mg/kg i.p., connected to an artificial respirator and the blood pressure and the heart rate were measured via a catheter in the left carotid artery.

Table 2. Hemodynamic values, in anaesthetized rats.

| | control n=24 | low T4 n=9 | high T4 n=15 |
|---|---|---|---|
| heart rate (bpm) | 369.2±5.5 | 382.9±6.3 | 444.7±10.1* |
| SBP (mmHg) | 159.3±3.4 | 163.2±5.9 | 169.8±5.8 |
| DBP (mmHg) | 114.5±2.1 | 118.0±4.0 | 110.6±3.6 |
| MAP (mmHg) | 129.5±2.5 | 133.1±4.5 | 126.0±6.0 |
| pulse pressure (mmHg) | 44.75±1.7 | 45.2±2.9 | 59.2±3.0* |

*P < 0.05

Subsequently the hearts were excised for functional or biochemical experiments. The heart to body weight ratio of the high T4 rats proved significantly increased when compared with control animals: 0.41±0.01 versus 0.32±0.01 (n=6, $P < 0.05$).
In conclusion, rats ingesting food containing 5 mg T4/1 kg chow developed a stable condition of hyperthyroidism, associated with biochemical and hemodynamic changes which resemble those found in patients. The model appears to be suitable for cardiovascular pharmacodynamic studies.

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## CALCIUM-DEPENDENT CONTRACTION OF ANGIOTENSIN II IN RABBIT RENAL ARTERY PREPARATIONS

J. Zhang, M. Pfaffendorf & P.A. van Zwieten

In a variety of target tissues, the actions of angiotensin II (AII) have found to be calcium dependent. Our recent studies have shown that AII causes vasoconstriction which is mediated by $AT_1$-receptors in the rabbit isolated renal artery[1]. However, the role of calcium movements triggered by the stimulation of $AT_1$-receptor in rabbit renal artery has so far not been studied.

Isolated renal artery ring preparations of the rabbit were set up as described previously[1] and endothelium-denuded ring preparations were suspended in an organ bath with Krebs solution at 37°C and gassed with 95% $O_2$ and 5% $CO_2$. Isometric force was measured and recorded. After 1 h of equilibration at 2 g of resting tension, the preparation was challenged with a depolarizing potassium solution as a test for viability and then washed and re-equilibrated with Krebs buffer for 1 h. Following protocols were performed: 1) Cumulative concentration-response curves for AII were constructed 1h before and after addition of different drugs. 2) contractions were induced by a single addition of AII ($10^{-7}$ *M* ), drugs were incubated for 1 h and the AII-induced contraction was re-examined after the Krebs solution had been washed and replaced by nominally $Ca^{+2}$-free, EGTA -containing saline (obtained by omitting $Ca^{+2}$ from the Krebs solution and adding 0.5 m*M* of $Na_2EGTA$) for 7 min.

In Krebs solution AII-induced contraction could be suppressed concentration-dependently by calcium antagonists, diltiazem, verapamil, nifedipine and manidipine by maximally 37-58%. The residual responses to AII could almost be completely further suppressed by intracellular calcium release inhibitor W-7[2]and TMB-8[3]. In $Ca^{2+}$-free, EGTA-containing saline, AII ($10^{-7}$ *M*) caused an initial transient contraction (ITC) which could not be affected by calcium antagonists (diltiazem, verapamil and nifedipine) but could be suppressed by W-7 and TMB-8. Losartan ($10^{-7}$ *M*), but not PD123177 ($10^{-6}$ *M*), blocked angiotensin II-induced ITC.

We concluded that AII-induced vasoconstrictor effect in rabbit renal artery are the consequence of both extracellular $Ca^{2+}$ influx and intracellular $Ca^{2+}$ release. Calcium antagonists only partially inhibit AII-induced contraction. Manidipine is more effective and potent among the calcium antagonists. Contractions evoked by intracellular $Ca^{2+}$ release after AII stimulation are mediated by $AT_1$-receptors.

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## EFFECTS OF HYPERTHYROIDISM ON THE PHARMACOLOGICALLY INDUCED CONTRACTION AND RELAXATION OF ISOLATED SMALL ARTERIES

J. Zwaveling, E. A. Winkler Prins, M. Pfaffendorf & P.A. van Zwieten

Hyperthyroidism is known to be associated with several cardiovascular alterations. It may be expected that hyperthyroidism will induce significant changes in the pharmacodynamic behaviour of the cardiovascular system, but this matter has not been studied systematically.

We investigated responses to various compounds in isolated small arteries of chronic hyperthyroid Wistar rats. The rats were made hyperthyroid as described elsewhere (1). Mesenteric small arteries were prepared and normalized to their individual optimal lumen diameter (± 310 µm for both groups) in an isometric wire-myograph. Cumultative concentration-effect curves (CEC) were constructed for methoxamine calcium, potassium-ions, 5-hydroxytryptamine and methacholine, respectively. We also studied the mixed ß1/ß2-adrenoceptoragonist isoproterenol, the ß2-agonist salbutamol and the direct adenylate cyclase activator forskolin. CEC's for vascular relaxation were constructed after precontraction with methoxamine(10 µM).

Hyperthyroidism neither influenced calcium-induced contractions nor the relaxation caused by salbutamol, forskoline and methacholine. Pretreatment of the donor animals with l-thyroxine produced leftward shifts of the CEC for methoxamine (mean $pD_2$ ± SEM, treated vs. control: 6.03 ± 0.07 and 5.81 ± 0.06 respectively; P<0.05, Student unpaired t test), potassium-ions (1.36 ± 0.02 and 1.47 ± 0.03; P<0.05), 5HT (6.58 ± 0.06 and 6.29 ± 0.05; P<0.05) and isoproterenol (6.52 ± 0.08 and 6.16 ± 0.09; P<0.05), as well as a decrease in the absolute maximal response to methoxamine (wall tension (mN): 3.30 ± 0.07 and 4.09 ± 0.06; P<0.05).

The present results indicate that in resistance vessels taken from hyperthyroid animals the sensitivity to alpha-adrenergic and beta-adrenergic effects is increased. The fact that hyperthyroidism did not influence the vascular responses to salbutamol and forskoline indicates that the increased sensitivity to the mixed ß-adrenoceptoragonist isoproterenol is probably limited to a ß1-receptor phenomen. This finding is in accordance with data by O'Donnell and Wanstall (2) who showed that hyperthyroidism enhanced the ß1-agonist noradrenaline in pulmonary arteries and aortic rings taken from hyperthyroid rats.

The increased sensitivity to 5HT and potassium-ions indicates that vascular changes induced by hyperthyroidism are not restricted to supersensitivity to sympathomimetic amines.

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## MODULATION OF THE HYPOTHALAMIC HISTAMINE RELEASE IN VIVO BY THE NEW POTENT AND SELECTIVE HISTAMINE $H_3$-RECEPTOR AGONIST IMMEPIP (VUF4708) AND BY THE $H_3$-RECEPTOR ANTAGONIST CLOBENPROPIT (VUF9153).

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In the present study, the effect of the the new potent and selective histamine $H_3$-receptor agonist immepip (VUF4708) and the histamine $H_3$-receptor antagonist clobenpropit (VUF9153) on *in vivo* neuronal histamine release from the anterior hypothalamic area (AHy) of the rat was studied using microdialysis.

Male Wistar rats (200-250g) were anaesthetized with urethane and placed in a stereotaxic frame. Subsequently, a microdialysis probe was inserted into the AHy. The AHy was perfused with artificial CSF at a constant flow of 1 µl/min. A hundred minutes after implantation samples were collected every twenty minutes and their histamine content was assayed using an HPLC-fluorometric method.

Infusion of the histamine $H_3$-receptor agonist immepip through the dialysis membrane very potently and dose dependently inhibited histamine release. At concentrations of 1 and 10 nM immepip histamine release decreased to 75% and 35% of the basal value respectively. At concentrations of immepip above 10 nM, histamine release decreased below its detection limit.

The histamine $H_3$-receptor antagonist clobenpropit, administered through the dialysis probe, dose dependently increased the histamine release. The maximal increase was 2-fold, observed at a concentration of 10 nM clobenpropit. Intraperitoneal injection of clobenpropit (5-15 mg/kg) caused a sustained increase of histamine release to 200% of its basal value. The effect of clobenpropit was different from the effect induced by the $H_3$-receptor antagonist thioperamide (5mg/kg), which more transiently increased histamine release to 250% of its basal value.

Immepip and clobenpropit, administered through the microdialysis probe, oppositely modulate histamine release from the AHy, probably by their action on $H_3$-receptors located on histaminergic nerve endings. Moreover, clobenpropit modulates histamine release after ip. injection indicating that the compound can penetrate into the brain.

We conclude that both, immepip and clobenpropit are potential tools for studying histamine $H_3$-receptors in the CNS.

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## EFFECT OF RENAL HYDROCHLOROTHIAZIDE HANDLING ON DIURETIC ACTIVITY IN THE ISOLATED PERFUSED RAT KIDNEY

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The diuretic agent hydrochlorothiazide (HCTZ) is known to be secreted via the organic anion carrier system in the proximal tubule of the kidney. Active secretion of organic anions often involves accumulation in the proximal tubule. Accumulation may result in an indirect relationship between perfusate concentration and diuretic response. In the present study renal handling and diuretic effect of HCTZ were investigated in the isolated perfused rat kidney (IPK). HCTZ gave a dose-dependent increase in the fractional excretion of sodium, chloride and potassium, and in urinary flow and pH. HCTZ was subject to saturable tubular secretion following Michaelis-Menten kinetics. Parameters obtained after nonlinear regression analysis were a maximum rate of tubular secretion, $T_M$, of 42 ± 6 µg/min, a Michaelis-Menten constant for tubular secretion, $K_T$, of 38 ± 11 µg/ml, and a fraction of passive tubular reabsorption, $F_{PR}$, of 0.49. HCTZ accumulated extensively in kidney tissue as a result of active cellular uptake and passive diffusion. Active uptake followed Michaelis-Menten kinetics and gave a maximum capacity of renal uptake, $R_M$, of 500 ± 270 µg/g and accumulation constant, $K_A$, of 28 ± 16 µg/ml. To analyze the dose-effect relationship, plots were constructed of the sodium excretion rate *vs* perfusate concentration and HCTZ excretion rate, respectively. The perfusate plot could be described by the sigmoid $E_{max}$ model and resulted in a $E_{max}$ of 6.3 ± 1.0 µeq/min, an $EC_{50}$ of 72 ± 5 µg/ml and a slope factor S of 1.6 ± 0.8. For the response curve in urine, a simplification of the sigmoid $E_{max}$ model had to be used because a maximum effect was not observed. The apparent maximum effect resulting from the perfusate concentration-response curve and the discrepancy with the renal excretion rate-response curve indicated that the diuretic effect of HCTZ is restricted by saturation of secretion into the tubular lumen and extensive tubular reabsorption. In conclusion, the induced diuretic response is limited by the renal handling of HCTZ. The results provide supporting evidence that the renal excretion rate of HCTZ corresponds more closely with the diuretic effect rather than the perfusate concentration. (Supported by the Dutch Kidney Foundation)

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## BENEFICIAL EFFECTS OF αMSH IN THE TREATMENT OF AN EXPERIMENTAL SPINAL CORD INJURY IN THE RAT

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Melanocortins, αMSH and ACTH fragments stimulate postlesion peripheral nerve regeneration and are able to ameliorate neuronal function in experimentally induced demyelinating lesions of the central nervous system. In addition αMSH stimulates axonal outgrowth of the spinal cord in in vivo and in vitro experiments in a dose-dependent way.

In this study the effect of αMSH was investigated on behavioural, electrophysiological (EF) and histomorphological (HM) outcome after spinal cord injury (SCI) in rats. Two groups (n=15) were subjected to midthoracic SCI's (weight drop technique). Under blind conditions for the observers, 75 ug/kg αMSH was administered s.c. every other day during the first 3 post injury weeks. The placebo group received saline. Behavioural assessment was performed weekly during an 8 week follow up period. In the 9th post injury week EF (motor evoked potentials) and HM (spared white matter) examination was performed. The behavioural outcome was small but significantly better in the αMSH treated group (Tarlov motor scores p= 0.037 and video assisted analysis of hindlimb motor function p= 0.018). In addition, a higher number of detectable motor evoked responses was present in the αMSH treated group (p= 0.019). The HM analysis failed to reveal significant differences between control and treatment groups.

These results demonstrate that αMSH enhances spinal cord function after contusion trauma of the rat spinal cord.

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## INHIBITION OF CHOLINE UPTAKE INTO SYNCYTIAL MICROVILLUS MEMBRANE VESICLES OF HUMAN TERM PLACENTA BY CATIONIC DRUGS: COMPETITIVE AND NONCOMPETITIVE INTERACTION

E.M. van der Aa, A.C. Wouterse, J.H.J. Copius Peereboom-Stegeman and F.G.M. Russel

Uptake of the essential quaternary ammonium compound choline by human placental trophoblast across the syncytial microvillus membrane is electrogenic, inhibitable by organic cations and saturable with a $K_m$ of 550 μM[1]. We now have further characterized the specificity and nature of inhibition of choline uptake into syncytial microvillus membrane vesicles (SMMV) of human term placenta. Uptake of 125 and 250 μM [$^3$H]-choline at 10 sec into voltage clamped SMMV was measured in the presence of an outwardly directed 5 mM choline gradient (trans stimulation) and increasing cis-concentrations of inhibitors. Plots of the log concentration inhibitor versus % of control uptake showed a typical sigmoid pattern. The inhibitory constant log$IC_{50}$ was determined by nonlinear regression analysis, using the computer program GraphPad Inplot (4.0). Evaluation of the nature of inhibition (competitive or noncompetitive) was done by Dixon analysis. The inhibitory constant $K_i$ was determined from the Dixon plots by linear regression analysis.

Apparent inhibitory constants of cationic drugs on choline uptake (n=3).

| Compound | $IC_{50}$ (mM) | $K_i$ (mM) | Compound | $IC_{50}$ (mM) | $K_i$ (mM) |
|---|---|---|---|---|---|
| Hemicholinium-3 | 0.05 ± 0.007 | 0.05 | Cimetidine | 3.16 ± 0.30 | 2.19 |
| Mepiperphenidol | 0.83 ± 0.44 | - | Ranitidine | 4.22 ± 0.52 | - |
| Atropine | 1.29 (n=1) | 0.66 | Famotidine | 3.80 (n=1) | - |

The differences in inhibitory potency suggest a certain substrate specificity of the placental choline transporter. Dixon analysis of the inhibition of choline uptake revealed that inhibition by hemicholinium-3 was due to a competitive and by cimetidine and atropine to a noncompetitive interaction. Based on therapeutic plasma concentrations of $H_2$-receptor antagonists, none of these drugs are expected to cause a significant interaction with the placental choline transporter in vivo.

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## CIPROFLOXACIN: PROTECTION AGAINST OR ACCELERATION OF SEPTIC SHOCK?

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Antibiotics are the cornerstone in the pharmacotherapeutic approach of sepsis. However, despite the use of these agents, mortality due to septic shocks remain high. In 1990, sepsis-related mortality in the Netherlands was 3042. It has been suggested that antibiotics may enhance the release of bacterial lipopolysaccharide (LPS) and administration of LPS to normal volunteers produces many of the characteristics of human septic shock.

This study was designated to test the hypothesis of antibiotic-induced acceleration of the septic cascade using rat and rabbit models of septic shock. Characteristic clinical signs in sepsis, in addition to hypotension, are alterations in mental status and development of neurological disorders, probably indicative of cerebral hypoperfusion. Therefore (in the rat) we used Laser Doppler flowmetry for continuous measurement of microvascular blood flow in superficial brain regions. In control rats the cerebral bloodflow (CBF) was adequately maintained until the mean arterial blood pressure (MAP) fell below 40% of the mean control value (using nitroprusside as vasodilator tool). In rats intravenously injected with the lethal dose of live Escherichia coli organisms, both CBF and MAP declined with a similar time-course. This indicates a marked reduction of blood supply to the brain during development of septic shock, partly due to loss of CBF autoregulation, and probably associated with development of cerebral ischemia. Treatment with fluoroquinolone ciprofloxacin prevented against systemic hypotension, hypoperfusion of the brain and mortality in this model. However, ciprofloxacine did not protect against septic shock in rabbits and strikingly, survival time in ciprofloxacin-treated rabbits was further reduced compared to untreated animals.

These data are discussed in terms of differences in LPS-sensitivity between rats and rabbits and may indicate that the antimicrobial therapy itself may negatively affect outcome of clinical sepsis therapy.

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## OPPOSITE EFFECTS OF DEXAMETHASON AND METYRAPONE ON AIRWAY HYPERRESPONSIVENESS AND INFLAMMATION IN A MURINE MODEL FOR ALLERGIC ASTHMA.

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Glucocorticoids are highly effective in the treatment of patients with allergic asthma. The anti-inflammatory actions of these compounds are probably caused through their effect on transcription of mRNA for important mediators of inflammation, such as cytokines. In a previously described mouse model for allergic asthma, we investigated the effect of exogenous applied glucocorticoids as well as the importance of endogenous corticosterone in the development of airway inflammation and hyperresponsiveness. We therefore used the synthetic glucocorticoid dexamethason and metyrapone, a compound that inhibits endogenous corticosterone levels.

Mice were sensitized with ovalbumin and after 4 weeks repeatedly challenged with either ovalbumin or saline aerosols (once a day for 8 days). During the challenge period, the mice were intraperitoneally injected before each aerosol with dexamethason (0.5 mg/kg), metyrapone (30 mg/kg), or vehicle. At 3 hrs after the last aerosol tracheal reactivity was measured in vitro and inflammatory cells in the bronchoalveolar lavage were counted. In vehicle-treated mice, exposition to ovalbumin aerosols induced eosinophil infiltration ($5.5x10^3$ cells) in the bronchoalveolar lavage and an increased airway hyperresponsiveness (with 27%), when compared to saline-challenged controls. Dexamethason treatment largely inhibited airway hyperresponsiveness (with 87%) and completely blocked eosinophil infiltration. In contrast, metyrapone exaggerated both airway hyperresponsiveness (with 13%) and the eosinophil number in the bronchoalveolar lavage ($17.4x10^3$ cells).

Altogether, these results indicate that endogenous glucocorticoids down regulate inflammation and airway hyperresponsiveness in this murine model for allergic asthma.

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