ACTION OF SECOVERINE ON M_1 AND M_2 RECEPTOR MEDIATED EFFECTS.

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Secoverine, a gastrointestinal spasmolytic agent, has been reported to exhibit selecivity in vivo (Zwagemakers and Claassen, 1981). Despite various studies with secoverine no firm conclusion as to the basis of the selectivity demonstrated has been reached. There is much evidence in support of subdivisions of muscarinic receptors and selective action such as that seen with secoverine may be due to preferential action on one of these receptors.

In order to test this hypothesis secoverine was tested in a variety of isolated organs, generally thought to be predominantly M_2 receptor systems; jejunum, atria, bladder, decorneated iris and trachea. All organs were taken from the rat and the same agonist, carbachol, was employed throughout.
Atropine acted as a reference compound as it is generally reported to be non-selective. In addition a series of experiments were carried out to investigate the effects of secoverine at the M_1, or ganglionic muscarinic receptor, using the pithed rat. The effects of secoverine, pirenzepine, the standard M_1 antagonist, and atropine, again included as a non-selective reference compound, were investigated on the pressor effect of the M_1 selective agonist, McN-A-343 to which cumulative dose-response curves were constructed.

pA_2 values were calculated for each compound in each tissue for the in vitro experiments. The values for atropine ranged from 8.32-9.03 and were found not to differ significantly. The values for secoverine ranged from 7.42-8.41, here a slightly higher affinity for the atria and a slightly lower affinity for the iris and bladder were observed when compared to the jejunum and trachea, non of these trends however reached statistical significance.

In the experiments to investigate secoverine's effects on M_1 receptors McN-A-343 produced dose-related effects on blood pressure observable between 30 µg/kg and 300 µg/kg. Parallel shifts of the dose-response curve to the right were seen with each antagonist, the order of potency was atropine >pirenzepine >secoverine. We were unable to demonstrate a higher affinity of pirenzepine over atropine as has been reported by Hammer and Giachetti.

In conclusion, from these studies it would appear that secoverine exerts antagonistic effects on both M_1 and M_2 receptor mediated events and as such cannot be classified as being selective for one type of muscarinic receptor. The trends seen in the pA_2 values for secoverine in the in vitro studies may indeed be indicative of receptor differences but the selectivity of secoverine demonstrated in vivo cannot be explained by a selective action on muscarinic receptor subtypes.

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NON-COMPETITVE ANTAGONISM EXHIBITED BY PUTATIVE M_1 SELECTIVE MUSCARINIC ANTAGONISTS.

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Dicyclomine exhibits a higher affinity for muscarinic binding sites in the cerebral cortex, in comparison to those in the myocardium (Kenny et al., 1985). Dicyclomine has been shown to be more potent against electrically-induced acid secretion in comparison to acid secretion induced exogenously (Schiavone et al., 1985). These effects have been reported to be due to its higher affinity toward M_1 receptors (Schiavone et al., 1985). However, dicyclomine has also non-specific actions (Downie et al., 1977). The aim of the study was to assess the affinity of dicyclomine and related compounds such as adiphenine and hexahydroadiphenine.

Antagonist affinities were determined using membranes derived from rat cerebral cortex and myocardium, in competition binding studies using ^{3}H-N-methyl scopolamine as the ligand (Kenny et al., 1985). The affinity at muscarinic receptors, in guinea pig tissue, was determined using the method of Arunlakshana and Schild (1959). Carbachol was used as the agonist (Clague et al., 1985).

The affinities (pKi) of the antagonists at cortical sites was: dicyclomine, 7.7; adiphenine, 7.2, and hexhydroadiphenine, 8.4. The affinities were lower at myocardial membranes: dicyclomine, 7.4; adiphenine, 6.3 and hexahydroadiphenine, 7.3. The Hill slopes were not significantly different from unity. The pA_2 values are shown below:

Antagonist	Ileum	Bladder	Trachea	Atria
Dicyclomine	7.3* ± 0.03	7.3* ± 0.05	7.2* ± 0.04	6.8 ± 0.05
Adiphenine	7.0* ± 0.4	6.8* ± 0.4	6.9* ± 0.05	6.5 ± 0.07
Hexahydro-adiphenine	8.0* ± 0.02	7.9* ± 0.06	8.0* ± 0.08	7.3 ± 0.05

Values are mean ± SEM, n = 4-6. * Denotes Schild slope significantly ($p<0.05$) less than unity.

The Schild slopes at smooth muscle receptors did not exceed 0.6 with any of the antagonists. In contrast, those slopes obtained at atrial receptors were not significantly different from unity.

In summary, all the compounds studied were more selective for binding sites in the cortex, in comparison to those in the myocardium. In contrast to data reported for pirenzepine, heterogenous binding was not observed at cortical sites. This group of compounds probably exert other actions at smooth muscle preparations, in addition to muscarinic antagonism because of non-competitive kinetics were observed. The properties of these antagonists limit their use in muscarinic receptor classification.

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DEMONSTRATION OF HEMICHOLINIUM-3 HIGH AFFINITY BINDING SITES IN RAT DIAPHRAGM USING AUTORADIOGRAPHY. EFFECTS OF DEXAMETHASONE.

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Using receptor autoradiography we were able to demonstrate the presence of high affinity [^{3}H] -hemicholinium-3 binding sites in the endplates of the rat diaphragm. Frozen sections taken from the endplate area of the rat diaphragm were incubated in 2.5 nM [^{3}H] -hemicholinium-3 (120 Ci/mmol), rinsed and processed for receptor autoradiography[1]. After an exposure period of 4 weeks the emulsion was developed and fixed. The frozen sections were processed for acetylcholinesterase and counterstained with haematoxylin. Grain counts above the acetylcholinesterase positive parts of the endplates revealed that the average grain density above the endplates (12 gr/100 μm^2) is significantly greater than the average grain density above the muscles (4 gr/100 μm^2) ($P \leqslant 0.005$). Addition of an excess of non-radioactive hemicholinium-3 (1000 nM) reduced the grain density above the endplates almost to the level of that above the muscles (4 gr/100 μm^2). The grain density above the muscles was not affected by the addition of non-radioactive hemicholinium-3.

Hemicholinium-3 is known to inhibit the high affinity choline uptake in the endplates of the rat diaphragm[2]. This inhibition is less in the presence of the glucocorticoid dexamethasone[3]. Using an identical autoradiographic technique to determine the effects of dexamethasone on high affinity binding of [^{3}H] -hemicholinium-3, we found that in the presence of 200 nM dexamethasone the grain density above the endplates was significantly reduced to a level as low as that of the grain density above the muscles ($P \leqslant 0.005$). Grain densities above the muscles were not affected by the addition of dexamethasone to the incubation medium.

We may conclude that high affinity binding sites for hemicholinium-3 as they occur in the CNS[4] are also present in the peripheral cholinergic system. Moreover, dexamethasone, apart from its intrinsic effects on the high affinity choline uptake, reduces the binding of hemicholinium-3 to its high affinity binding sites and therefore interferes with the reduction of high affinity choline uptake by hemicholinium-3.

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COMPARATIVE CSF AND PLASMA PHARMACOKINETICS OF HEPTABARBITAL IN INDIVIDUAL RATS.

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Wide interindividual variability in the response to the same dose of various centrally acting drugs is well established and may be explained by variability in the kinetics or pharmacodynamics of the drug. A new experimental strategy involving CSF withdrawal (Danhof and Levy, 1984) permits a clear distinction to be made between these alternatives in the case of phenobarbital. We now report a related study on heptabarbital which is a particularly convenient model drug as it is not optically active and is probably not converted to active metabolites (Dingemanse and Danhof, in preparation).

We have now made a preliminary study of the kinetics of heptabarbital in plasma and CSF of individual rats during and after an i.v. infusion. Heptabarbital was infused for about 30 min in the left carotid artery of 9 female Sprague-Dawley rats (160-200 g) until deep sleep was achieved. Blood samples (for plasma) were taken at intervals from the right jugular vein of each rat. Simultaneously, small serial CSF samples were taken via an indwelling cannula in the cisterna magna (Sarna et al., 1983). Drug concentrations in plasma and CSF were determined by HPLC assay and elimination half-lives calculated for both compartments by linear regression analysis.

The most striking finding was that the apparent $t_{\frac{1}{2}}$ in CSF (250 min) was consistently greater than in plasma (130 min). The animals showed considerable interindividual variation especially in the CSF values. The mean CSF/plasma concentration ratio rose from 0.24 at the end of the infusion period to 0.38 at 2.5 h. The latter value was close to the fraction of plasma heptabarbital which is present in the free (protein unbound) form.

The study confirmed the value of the CSF monitoring method for studying the kinetics of centrally active drugs in individual rats (Hutson et al., 1985). The CSF compartment is presumably more relevant than plasma to the sites of action of such drugs. Indeed, in the case of heptabarbital it is pharmacokinetically indistinguishable from the site of action (Danhof and Levy, 1984).

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AN INVESTIGATION OF CARBAMAZEPINE NEUROPHARMACOKINETICS IN INDIVIDUAL UNRESTRAINED RATS USING REPEATED SAMPLING OF CSF.

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Repeated CSF withdrawal has previously been used in a pharmacokinetic study of tryptophan. Data such as $t_{\frac{1}{2}}$ values were determined for individual rats (Hutson et al., 1985). The method permits central levels of drugs and their metabolites and behaviour to be concurrently monitored in the same animal. We have now used this approach in an investigation of the anticonvulsant drug carbamazepine.

Individually housed male Sprague-Dawley rats (Charles River, U.K.) weighing 180-200 g were put on a 12 h reversed light-dark cycle (white light off, red light on 06.00 h). After 2-3 weeks, a flexible polyethylene catheter was implanted in the cisterna magna as previously described (Sarna et al., 1983) except that it was implanted into a 3 mm slit made 1 mm more dorsally than before. Three days later the catheter was connected for CSF sampling (30 µl every 0.5 h between 10.00 and 18.00 h). Carbamazepine (35 mg/kg in propylene glycol) was given i.p. immediately after taking the third sample. Motor activity was monitored throughout using infra red sensors. CSF concentrations of the drug and its primary metabolite carbamazepine -10, 11-epoxide were determined by HPLC using a modification of the method of Elyas et al. (1982).

Carbamazepine concentrations rose rapidly and then declined exponentially. Concentrations of the epoxide both rose and fell more slowly. Kinetic parameters as indicated in Table 1 were determined for each rat after a single drug injection.

Table 1. Kinetic data on carbamazepine in rat CSF.

	Carbamazepine	Carbamazepine-10,11-epoxide
Time to peak concn.(h)	0.62 ± 0.28	2.33 ± 0.94
Peak concn. ($\mu mol.l.^{-1}$)	30.0 ± 3.6	12.3 ± 4.2
AUC ($\mu mol.l.^{-1}.h$)	89.2 ± 27.5	109.5 ± 46.8
$t_{\frac{1}{2}}$ (h)	1.57 ± 0.66	4.35 ± 0.86

Results are given as means ± SD for 6 rats. $t_{\frac{1}{2}}$ was calculated from the terminal exponentials for the decline of drug concn. in the CSF.

Carbamazepine injection caused decreased motor activity. For each rat, the time of lowest activity coincided with the peak of drug concentration.

The results illustrate the use of the method in the study of relationships between drug metabolism and behaviour. Numerous other applications are possible. For example, we have also used the method to obtain repeated series of kinetic measurements on animals given daily drug injections.

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ROLE OF THE ENDOTHELIUM IN THE POTASSIUM-INDUCED RELAXATION IN RAT ISOLATED AORTA.

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Inhibitors of the Na^+,K^+ pump inhibit the endothelium-dependent relaxation induced by acetylcholine (De Mey and Vanhoutte, 1980; Rapoport et al., 1985). The purpose of this study was to further investigate the role of the Na^+,K^+ pump in this relaxation. We have therefore examined the role of endothelium in the potassium-induced relaxation due to Na^+,K^+ pump activation (Webb and Bohr, 1978), the magnitude of this relaxation being used as an index of the Na^+,K^+ pump activity.

Pairs of aorta rings were taken from male Wistar rats (250-350 g). One ring was left intact and the other was mechanically rubbed of its endothelial cells. The rings were mounted in 50 ml organ baths filled with a physiological salt solution (PSS) at 37°C gassed with 95% O_2 : 5% CO_2. Tissues were contracted with a depolarizing solution containing 100 mM KCl and challenged with ACh 1 uM which induces a relaxation in intact rings and no relaxation in rubbed rings. The rings were preincubated in a low potassium (0.1 mM) PSS for 15 min before adding noradrenaline to the bath to give a submaximal concentration of 300 nM. Thirty min later, a transient relaxation was induced by raising the concentration of potassium in the bath to 2.1, 3.1 and 5.1 mM. After a period of relaxation, a spontaneous return of tension was observed. This transient relaxation was inhibited by ouabain 300 uM, preincubated 30 min before the addition of noradrenaline to the bath.

The maximum contractile response evoked by noradrenaline 300 nM in a low potassium PSS was not significantly different in the preparations with and without endothelium (2597 ± 189 mg and 2649 ± 189 mg, n = 5, $P > 0.1$, respectively). The presence of endothelium increased significantly the magnitude of the relaxation induced by the addition of 2, 3 and 5 mM of KCl and decreased significantly the spontaneous recontraction tension. For instance, with endothelium, after addition of 2 mM KCl, aortae relaxed to 42.5 ± 3.3 % of noradrenaline contraction and recontracted to 70.4 ± 4.5 % (n=5); without endothelium, these values were respectively 62.6 ± 3.8 % and 100.4 ± 0.5 % (n=5). The difference between intact and denuded preparations were more obvious by the addition of 2 and 3 mM of KCl than by the addition of 5 mM of KCl.

These results show that the presence of the endothelium modulated tension changes evoked by activation of the Na^+,K^+ pump.

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THE ENTEROHEPATIC CIRCULATION OF PHENOLPHTHALEIN IN THE DOG, A SYSTEMS DYNAMICS APPROACH.

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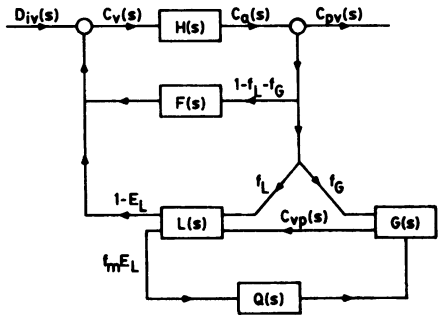
Many drugs are excreted either unchanged or as a conjugate in the bile. In the gut they may be deconjugated and reabsorbed through the gut wall. The free drug is carried back to the liver/bile system by way of the vena porta. This phenomenon is called enterohepatic circulation (EHC) and may give rise to falsely elevated values of area-under-plasma-concentration-curve (AUC), elimination half-lives ($t_{\frac{1}{2},el}$) and mean residence time (MRT) in pharmacokinetic studies. In order to correct these parameters we have to know the amount of drug involved in EHC. We have characterized the EHC of phenolphthalein (Ph) in the Labrador dog. The experimental results were interpreted in terms of systems dynamics pharmacokinetics (Figure 1 and systems dynamics equations).



$D_{iv}(s)$ = Intravenous dosage function
$H(s)$ = Heart-lungs transfer function
$F(s)$ = Rest of the body transfer function
$L(s)$ = Liver transfer function
$Q(s)$ = Transfer function from liver to gut
$G(s)$ = Gut transfer function
f_L = Hepatic arterial flow/cardiac output
f_G = Gut arterial flow/cardiac output
f_m = Fraction involved in EHC
E_L = Liver extraction
$C_{pv}(s)$ = Concentration in periferal vein
$C_{vp}(s)$ = Concentration in portal vein
$C_a(s)$ = Concentration in aorta
$C_v(s)$ = Concentration in venous pool

Figure 1 Systems dynamics block diagram.

Systems dynamics equations.

$$C_{pv}(s) = \frac{D_{iv}(s)}{\dot{V}_B} \cdot \frac{H(s)}{1-H(s).(1-f_L-f_G).F(s)-H(s).\frac{(f_L.L(s)+f_G.G(s).L(s)).(1-E_L)}{1-f_mE_L.L(s).Q(s).G(s)}} \quad (I)$$

$$AUC_{pv} = \frac{D}{\dot{V}_{el}} \cdot \frac{1-E_Lf_m}{1-f_m} \quad (II) \qquad AUC_{vp} = \frac{D}{\dot{V}_{el}} \cdot \frac{1+E_Lf_mf_L/f_G}{1-f_m} \quad (III) \qquad \dot{V}_{el}=(f_L+f_G).E_L.\dot{V}_B \quad (IV)$$

The experimental values of AUC_{pv} and AUC_{vp} (see "systems dynamics equations") are 10.04 and 11.82 mg l/h, respectively. We assume f_L, f_G, the cardiac plasma output $\dot{V}_p$ and the portal plasma flow $\dot{V}_{vp}$ to be 0.07, 0.12, 120 l/h and 17.4 l/h, respectively. These values are dierived from the mongrel dog (Liard *et al.*, 1983); the plasma flows are corrected for the weight and the hamatocrit of our Labrador. From f_L, f_G, $\dot{V}_p$ and the equations II to IV we can calculate $\dot{V}_{el}$=15.05 l/h, E_L=66 % and f_m=0.15. The amount of Ph involved in EHC is 31.0 mg = $(AUC_{vp}-AUC_{pv})\times\dot{V}_{vp}$.

Conclusion. The Ph-molecules reabsorbed into the blood after an EHC, together accounting 21.6 % of the administered dose, all appear in the portal vein (ΔAUC_{vp}= + 24.5 %), but are eliminated for 66 % (E_L) during the first pass. The complementary 34 % ($1-E_L$) give rise to an increase in AUC_{pv} of 5.9 %. It is easily imaginable that the effect of EHC on AUC and MRT can be very pronounced for a drug involved in EHC to an equal degree as is Ph, but less subject to first-pass metabolism.

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IN SEARCH OF A PHARMACOKINETIC MODEL FOR THE ASSOCIATION OF ANTIBIOTICS TO NEUTROPHILS.

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In order to obtain a more profound view on the possibilities and impossibilities of antibiotic drugs in aiding the human complex to extinguish invading bacterial pathogens, it is necessary to study the pharmacokinetics of the interaction between antibiotics and various parts of the body. Since blood is the main mode of drug transport within the body, it deserves the main attention. The present study is centered on the association of the antibiotic erythromycine A to neutrophils. These cells may function as specific carriers to infected areas (Baggiolini & Dewald, 1985); in some cases however they act as a shelter to bacteria against antibiotic activity (Mandell, 1973), depending on the accumulation level of the antibiotic within the neutrophil. There is no question of proliferation within the cell when the bacterium is susceptible to the activity of erythromycine A, since this antibiotic accumulates highly within neutrophils (Prokesch & Hand, 1982; Miller et al, 1984). More detailed study of this accumulation was performed on the basis of a cellular pharmacokinetic model including several modes of association. It is possible to eliminate certain possibilities concerning transport and actual association/dissociation mechanisms by careful evaluation of the results of experiments executed in the following way.

After isolation of the neutrophil fraction of human blood by means of successive dextran sedimentation, Ficoll-Paque and Percoll gradient centrifugation, incubation of intact cells with drugs is carried out upon a silicone oil layer. The essence of the method is that it allows a vigourous separation between associated and unassociated ligands through pelleting of the cells below the silicone oil during centrifugation. Since lysed cells can not be separated from the medium in this manner, a second centrifugation step is used to achieve this (Raghoebar et al, 1986).

The association - relatively slow compared to most other antibiotics (Prokesch & Hand, 1982; Hand & King-Thompson, 1986) - reaches the same level relative to the extracellular concentration , which eliminates the possibility of easily saturable binding sites at this concentration range (up to 150 μM). The 30-fold accumulation and its temperature dependence point towards an active uptake process. Lysed cells - important for a clear distinction between transport mechanism and association/dissociation mechanism - show a temperature dependent association (37 °C > 24 °C > 4 °C). This association to lysed cells is stimulated when transferred from 4 °C to 37 °C. When transferred from 37 °C to 4 °C however, no reduction of association is observed which indicates that the rate constant of association is more temperature dependent than the rate constant of dissociation. Since association is equal for intact and mechanically lysed cells only at 37°C, in contrast to formaldehyde-killed cells (Prokesch & Hand, 1982) and the association to lysed cells is higher at 24 °C and 4 °C than to intact cells, it is clear that the transport mechanism is temperature dependent as well. Association can not occur solely on the outer surface. Further manipulation with physical as well as chemical parameters will be useful to frame a more detailed pharmacokinetic model.

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ADJUVANT INDUCED POLYARTHRITIS AND THE SUBCUTANEOUS MODEL OF SYNOVIUM.

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Adjuvant induced polyarthritis of rats has long been used as a model of rheumatoid arthritis although little is known of the events and factors that contribute to the erosive synovitis of this disease (Billingham, 1983). One reason for this has probably been the difficulty of obtaining synovial tissue from the small joints affected by the disease. We have shown however, that a large and accessible synovial-like structure can develop in subcutaneous (sc) tissues of rats, six days after inflation with air (Edwards et al,1981). Inoculation with adjuvant twelve days earlier, causes changes to this structure that are histologically comparable and coincident with the proliferating synovitis that develops in the peripheral joints of the same animals (de Brito, 1985; de Brito et al 1986). The opportunity therefore to throw more light on the mechanisms of this disease has led us to extend our studies futher. The findings presented here are those of a time course study undertaken to examine the histological changes that occur to an established pseudosynovium following exposure of rats to adjuvant.

Male Wistar rats (150-200 gm) were injected sc in the back with 20 ml of air (day -6) followed every 3-4 days by futher injections of 5-10 ml into the formed cavity. Rats were inoculated intradermally with o.1 ml saline or 0.5 mg dead M. tuberculosis in paraffin oil in the base of the tail. After certain time intervals groups of each were sacrificed. The thickness of the skin overlying the cavity was assessed using callipers and a sample excised for histology. 5µm sections were cut and stained with Martius scarlet blue and phosphotungstic acid haematoxylin. The cavity was examined for an effusion and peripheral joints for signs of inflammation.

Table

Days after adjuvant —>	4	8	10	12	14	18	22	28
Oedema	0	++	+++	+++	++	+	0	0
Fibrin	0	+	++	++	+	0	0	0
Inflammatory cell infiltration	0	+	+++	+++	++	+	+	+
Neovascularisation	0	++	+++	+++	++	++	++	++
Fibroplasia	0	+	+++	+++	+++	++	++	++
Skin thickness	0	±	+++	++	+	-	-	-
Effusion	0	0	±	±	±	0	0	0
Arthritis	0	0	+	++	+++	+++	++	++

0 = no change; + = minimal; ++ = moderate; +++ = marked; - = negative change; ± = present only in some; Changes indicated are relative to air pouches of non-inoculated rats.

The table above summarises our findings. Histological changes to the air pouch lining were observed well before the development of arthritis in these rats . The most striking macroscopic observation was the thickening of the air pouch skin; this occured just prior to the appearance of arthritis but coincident with the early synovial and periosteal hypercellularity reported by other workers (Billingham, 1983). This change however was not maintained as arthritis became severe. The adjuvant-air pouch thus offers the opportunity to assess drug activity at the cellular level of arthritis, preceeding the gross articular changes.

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VITAMIN K1,2,3 EPOXIDE REDUCTASE IN CULTURED B16 MOUSE MELANOMA CELLS; A POSSIBLE EXPLANATION FOR WARFARIN CYTOTOXICITY.

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Oral anticoagulants of the coumarin type (e.g. warfarin and acenocoumarol) are believed to exert on tumor cells an antimetastatic and/or cytotoxic effect (Hilgard, 1984). Recently we have reported a cytotoxic effect of warfarin towards the highly metastatic B16/F10 mouse melanoma subline in vitro (Uitendaal et al, 1985). Warfarin in therapeutic doses is believed to act via inhibition of the enzyme vitamin K1 2,3 epoxide reductase (vit KO reductase; Whitlon et al, 1978). We used an assay, adapted from the one described previously (Thijssen et al, 1986) - but with an endvolume of 100 ul and using only 0.25 to $1x10^6$ cultured B16 cells - to investigate whether B16 cells contain, besides the vitamin K-dependent carboxylase (M.A.G. de Boer-van den Berg, pers. commun.) another enzyme of the vitamin K metabolizing cycle, vit KO reductase. The 100 ul incubation mixture contained besides the cell lysate 10 mM Tris-HCl buffer pH 7.4, 10 mM dithiotreitol (DTT) and 0.1 mM vit KO. After extraction, the mixture was analysed by HPLC as described before (Thijssen et al, 1986). Vit KO reductase capacity is present in B16 cells, amounting to approximately 200-500 pmol/10^6 cells /min at 37°C. In our test system the activity is linear with time up to 1 h. Although there is no proof yet that the activity is one enzyme specific for vit KO, several characteristics suggest that it is an activity similar to the vit KO reductase found in hepatocyte microsomes. As is the case for the hepatocyte reductase (Whitlon et al, 1978), the B16 reductase is fully dependent on DTT as reducing agent and does not accept NADH as a cofactor. Secondly, B16 vit KO reductase is sensitive to inhibition by warfarin, the ID_{50} being in the same order of magnitude as for hepatocytic vit KO reductase (both about 1 uM). In addition, reductase of B16 cells cultured in the presence of warfarin is reduced. Culture for 3 days of 10, 30 and 100 ug/ml warfarin gives a reductase activity of respectively 57%, 45% and 41% of control values. Activity of cells cultured at 300 ug/ml could not be tested since this concentration kills all cells. The activity cannot be recovered by extensive washing of the cells or dilution of cell lysate. This parallels the finding that liver reductase of rats treated with warfarin is irreversibly diminished; apparently the warfarin inactivates or binds irreversibly to the hepatocyte reductase. The demonstration of vit K-dependent carboxylase and, as reported in this study, vit KO reductase suggests a fully operating vitamin K cycle in B16 tumor cells. The function of this metabolic pathway is yet unclear, but the finding that reductase in B16 cells cultured in near-toxic dosages of warfarin is inhibited considerably suggests that the vitamin K cycle may be essential for survival of these cells and that warfarin cytotoxicity in vitro is due to inhibition of this vital pathway.

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COMPARATIVE EFFECTS OF EMULSIFIED PERFLUOROCARBONS ON LYMPHOID TISSUE IN RODENTS.

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Emulsified perfluorocarbons (PFC) have been proposed as oxygen transport fluids and some of their physiological effects have been studied in animals and man (Tremper & Anderson, 1985; Lowe & Bollands, 1985). PFC are retained in lymphoid tissues and can impair reticuloendothelial system clearance function (Castro et al., 1984). Conflicting results have been obtained on the extent to which in vivo use of emulsified PFC can alter normal immunological competence, which depends on the species used and dose administered (Shah et al., 1984; Bollands & Lowe, 1986). To clarify this, we have investigated the effects of low doses of a commercial emulsion, Fluosol-DA 20% (F-DA; Green Cross, Japan), on lymphoid tissues and responses to injected sheep red blood cells (SRBC) in rats and mice.

Female Wistar rats (body wt: 140-160g; n = 37) or female NIH mice (body wt: 17-22g; n = 28) were used. Animals received an intravenous (i.v.) or intraperitoneal (i.p.) injection of either 5 or 10 $ml.kg^{-1}$ b.w. F-DA; control animals were injected with sterile saline (0.9% NaCl). 24h later, rats were injected i.p. with 5×10^8 SRBC suspended in 1.0 ml Hank's balanced saline while mice received an i.p. injection of 0.1 ml Hank's solution containing 5×10^7 SRBC. Animals were sacrificed 7 days later and weights of liver, spleen, thymus and mesenteric lymph nodes (MLN) were recorded. The specific plasma antibody titre to SRBC was measured using a conventional serial-dilution haemagglutination test. Tissue weight changes are summarized as follows:

		Liver	Spleen	Thymus	MLN
Rats					
5 $ml.kg^{-1}$ F-DA	i.v.	Unc.	↑P<0.05	Unc.	↑P<0.05
	i.p.	Unc.	↑P<0.05	Unc.	Unc.
10 $ml.kg^{-1}$ F-DA	i.v.	Unc.	↑P<0.05	Unc.	Unc.
	i.p.	Unc.	↑P<0.05	Unc.	Unc.
Mice					
5 $ml.kg^{-1}$ F-DA	i.v.	↑P<0.005	↓P<0.05	Unc.	↓P<0.05
	i.p.	↑P<0.005	Unc.	Unc.	↓P<0.05
10 $ml.kg^{-1}$ F-DA	i.v.	↑P<0.005	Unc.	Unc.	↓P<0.05
	i.p.	↑P<0.005	Unc.	Unc.	↓P<0.05

↑ = increased, ↓ = decreased, Unc. = unchanged compared to mean control values.

Mean $\log_2$ plasma antibody titre to SRBC was increased by a maximum of 49% in rats ($P<0.001$) and upto 40% in mice ($P<0.01$) injected i.p. with both doses of F-DA; titres were similar to controls in all other cases.

These results show that uptake of F-DA into lymphoid tissue is variable in rodents. However, the increase in antibody production against SRBC in all animals injected i.p. with emulsion was consistent with previous findings in the rat and supports the suggestion that F-DA acts either as an immunopotentiator or 'antigen-trapper' when given via this route (Bollands & Lowe, 1986).

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NOVEL COMPOSITIONS OF EMULSIFIED PERFLUOROCARBONS FOR BIOLOGICAL APPLICATIONS.

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Emulsions of perfluorocarbons (PFC) dispersed in isotonic electrolyte solutions have been tested as oxygen-carrying resuscitation fluids in several species including man (Lowe & Bollands, 1985). The most widely tested emulsion contains perfluorodecalin (FDC) and perfluorotripropylamine (PFTP), emulsified with Pluronic F-68. This is commercially available as Fluosol-DA (F-DA; Naito & Yokoyama, 1978). PFC emulsions are ideal in terms of characteristics that relate to in vivo physiological performance, but are relatively unstable (Riess & Le Blanc, 1982). We report the development and preliminary assessment of novel compositions of more stable emulsified FDC for possible biological use.

FDC (ISC Chemicals Ltd, Avonmouth) was emulsified by sonication for 30 mins with 4% Pluronic F-68 in an aqueous phase to give a final 20% (w/v) preparation. 1.0% of the following polycyclic perfluorinated high boiling point (b.p.) oils were added to enhance stability: perfluoroperhydroacenaphthylene (C-12), perfluoroperhydrofluorene (C-13), perfluoroperhydrophenanthrene (C-14) or perfluoroperhydrofluoranthrene (C-16); control emulsions contained no oil additives. Emulsion stability was assessed by particle size analysis after storage at 0°C or 37°C. Mean stability indices after storage at 0°C for 44 days were:

Oil Additive	B.p. of added oil (°C)	Size (nm)	P.D.	Dt/Do
Control (no additive)	-	551	0.40	2.14
C-12	173-175	531	0.26	2.10
C-13	192-194	434	0.22	1.84
C-14	215-216	325	0.20	1.81
C-16	242-245	293	0.14	1.20

P.D. = polydispersity of emulsion; Dt/Do = stability parameter (where Do = initial mean particle size and Dt = mean after time t days.

Female Wistar rats (140-160 g; n = 8) were given either an intraperitoneal (i.p.) or an intravenous (i.v.) injection via a tail vein of 10 ml. kg^{-1} b.w. FDC emulsion containing 1.0% C-16 oil. No adverse effects were observed although mean liver weight at 7 days after injection was increased up to 16% compared with saline-injected controls ($P<0.01$).

These results show that FDC emulsions can be stabilized by addition of perfluorinated high b.p. oils. The degree of stability attained was directly related to b.p., and hence, molecular weight of added oil. FDC emulsion containing C-16 oil appeared to be biocompatible in rats although the observed increase in liver weight conflicted with previous findings in rats injected with identical doses of F-DA (Bollands & Lowe, 1986). We conclude that FDC emulsions stabilized with high b.p. oils may have value as oxygen-transporting fluids, although further work is in progress to substantiate this claim.

This project was supported by I.S.C. Chemicals Ltd, Avonmouth, Bristol. Emulsions described in this paper are covered by Patent No. 85 04916.

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MODIFICATION BY FORSKOLIN OF STIMULUS SECRETION COUPLING IN CULTURED BOVINE CHROMAFFIN CELLS.

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Intracellular cyclic AMP levels have been implicated in the presynaptic modulation of calcium dependent neurotransmitter release. There is evidence that the facilitation of release by cyclic AMP occurs at two sites: (i) the site of calcium entry; (ii) the site of exocytosis. If interaction at the site of calcium entry is significant, then stimuli which lead to calcium influx via different routes might be differentially effected by changes in intracellular cyclic AMP. We have previously provided evidence that nicotine and high K^+ stimulate catecholamine release from cultured chromaffin cells by allowing calcium entry, in part, through different channels (Adams et al. 1986). Here we compare the effect of pretreatment with forskolin, which elevates intracellular cyclic AMP, on catecholamine release stimulated by nicotine, high K^+ and by the calcium ionophore A23187.

Bovine adrenal medulla chromaffin cells were maintained in primary culture for 4-7 days prior to use. A preincubation of 12 min. preceded the stimulation period of 3 min. (high K^+ and nicotine) and 5 min. (A23187) with forskolin present at 10^{-5} M where appropriate, through both these periods. Endogenous noradrenaline and adrenaline, released into the supernatant during the stimulation period, were estimated by high pressure liquid chromatography with electrochemical detection.

Dose response curves to nicotine and K^+ in the presence and absence of forskolin showed that at levels of stimulation producing a high rate of release, release by high K^+ is increased, while release by nicotine is decreased. No clear effect of forskolin was seen at low rates of release. This is illustrated by the data in the table.

Table 1 Release of noradrenaline as a percent of cell content

	K^+			Nicotine		
	5 mM	30 mM	50 mM	10^{-6}	5.5×10^{-6}	3×10^{-5}
Control	0.56±0.18	4.32±0.41	5.16±0.27	0.62±0.19	7.98±1.04	21.8 ±0.4
Forskolin	0.81±0.22	4.72±0.42	6.41±0.15	2.08±0.30	5.82±0.20	12.74±0.74

Mean ± S.E.M., n = 4

The effect of forskolin on release by A23187 was to increase noradrenaline release at both 1 μM (a minimally effective dose in the absence of forskolin) and at 10 μM. Similar results were seen for adrenaline release.

The calcium ionophore results suggest that elevation of cyclic AMP by forskolin does have an effect at the level of exocytosis. This may account, in part or completely, for the augmented release in response to high K^+. However, the decreased response to high nicotine levels in the presence of forskolin suggests that an effect at the level of calcium entry, negative in this case, is a consequence of elevated cyclic AMP levels. The data support the notion that entry of calcium when stimulated by nicotine is by a different route than when stimulated by high K^+.

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TISSUE GLUCOSE UPTAKE IN DIABETIC MICE DURING CHRONIC ETHANOL TREATMENT (CET) AND WITHDRAWAL.

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We have already shown that CET produces hypoglycaemia without altering plasma insulin levels in diabetogenic C57 or normal LACG mice (Connelly & Taberner, 1984) although, after 48h withdrawal from ethanol, the C57 mice showed increased hyperglycaemia and hyperinsulinaemia. Since the C57 mice are not insulin resistant (Connelly & Taberner, 1983), and plasma insulin has an important role to control glucose uptake into tissues, it was considered of interest to investigate glucose uptake during CET and withdrawal in both normal and diabetic mice.

Adult mice of either sex from inbred colonies were used. The CET schedule was as described previously (Unwin & Taberner, 1980), and glucose uptake into cerebral cortex and hemidiaphragms was measured both *in vivo* and *in vitro* by the methods described earlier (Taberner, 1973). Adipose tissue consisted of slices from epididymal fat pads and was processed as for diaphragm tissue using the method of Stauffacher & Renold (1969). Insulin treatment was a single injection of 5000 μIU/ip. Results were compared using a two-tailed t-test.

The effects of CET and withdrawal on cerebral cortical glucose uptake *in vitro* were similar in C57 and LACG mice of both sexes. During CET, uptake was increased by between 32 and 84% above controls. Absolute values were significantly different ($p < 0.05$) in all 4 groups. After 48h withdrawal the uptake values had returned to control values: between 0.26 and 0.26 μmoles/g tissue/min. No effect was observed in diaphragm tissue during CET, and there was no change in glucose incorporation into glycogen in adipose tissue *in vivo*. The basal rate of incorporation of glucose-derived ^{14}C into diaphragm tissue *in vivo* did not change during CET or withdrawal, but when insulin-stimulated uptake was investigated there was a significant increase in incorporation in diaphragms from C57 mice during CET: from 9.09 ± 1.34 to 15.2 ± 1.38 nCi/mg dry weight of tissue. Values returned to normal after 48h withdrawal.

The precise mechanism by whith ethanol can potentiate the effects of insulin are still unclear, but it would appear from the present studies that brain glucose uptake could be an important site of action. The brain accounts for between 20 and 60% of the total glucose utilization in man, and the effect of ethanol on cerebral uptake could therefore be an important factor in the hypoglycemia associated with ethanol consumption.

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EFFECT OF NORADRENALINE ON NON-ADRENERGIC, NON-CHOLINERGIC INHIBITORY NERVE-INDUCED RESPONSES IN RAT GASTRIC FUNDUS.

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Previously we reported that UK-14,304 inhibited non-adrenergic, non-cholinergic (NANC) nerve-induced responses in rat gastric fundus via pre-junctional α_2-adrenoceptors situated on the NANC nerve terminals (Dettmar et al, 1985). The present experiments were carried out to study the effects of noradrenaline on these inhibitory nerve-induced responses.

Strips of rat gastric fundus were suspended in Kreb's solution containing propranolol (2 μM), atropine (2 μM) and guanethidine (5 μM). Cocaine (3 μM), hydrocortisone (30 μM), EDTA (30 μM) and ascorbic acid (30 μM) were also present. Tone was induced by the addition of barium chloride (0.5-2mM) to the bath. Electrical field stimulation was carried out via silver/silver chloride ring and hook electrodes (0.5Hz, 10 pulses, 1ms pulse width, supramaximal voltage).

The effect of noradrenaline on NANC nerve-induced responses proved difficult to assess as the size of the inhibitory responses is dependent on tone and noradrenaline alone caused a reduction in the barium chloride-induced tone. As postjunctional inhibitory α_1-adrenoceptors are present in this preparation (Dettmar et al, 1984; Verplanken et al, 1984) the effects of noradrenaline were therefore assessed in the presence of prazosin (1 μM) to block any post-junctional α_1-adrenoceptor-mediated reductions in tone. Prazosin reduced, but did not abolish, the relaxant effects of noradrenaline. The remaining relaxant effect appears not to be mediated via α- or ß-adrenoceptors (Dettmar et al, 1986). In the presence of prazosin it was possible to observe a significant reduction in the size of the NANC nerve-induced responses with 1 μM noradrenaline. The inhibition was completely reversed by idazoxan (0.1 μM). Lower concentrations of noradrenaline (0.03-0.3 μM) had no effect on the NANC nerve-induced responses.

In order to investigate the presence of endogenous feedback of noradrenaline on to the prejunctional α_2-adrenoceptors the effects of idazoxan alone (0.03-3 μM) in the absence of guanethidine were tested on inhibitory nerve-induced responses over a wide range of frequencies (1-50Hz, 10s train length). No significant effects of idazoxan were observed.

In conclusion, exogenous noradrenaline can inhibit NANC nerve-induced responses in the rat gastric fundus via prejunctional α_2-adrenoceptors. However the effect is difficult to see due to (a) postjunctional α_1-adrenoceptor-mediated relaxation and (b) a relaxant effect not mediated via α- or ß-adrenoceptors. We were unable to demonstrate any effects of endogenous noradrenaline suggesting that the α_2-adrenoceptors may be inaccessible to neuronally-released noradrenaline but are possibly activated by circulating catecholamines.

JK holds an SERC CASE award in collaboration with Reckitt & Colman plc

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LOCAL AND SYSTEMIC EFFECTS OF DIRECT INFUSION OF S-ACENOCOUMAROL INTO THE TESTIS OF THE CONSCIOUS RAT.

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Coumarin-derived oral anticoagulants inhibit the vitamin K-dependent synthesis of several coagulation factors in the liver. It has been suggested that vitamin K-dependent proteins are also formed in non-hepatic tissues like bone and testis (Vermeer et al, 1984). Despite their clinical use for over 40 years effects of oral anticoagulants on non-hepatic tissues have never been reported. However, the existence of warfarin sensitive vitamin K epoxide reductase activity in non-hepatic tissue was shown recently by Thijssen et al (1985). The pharmacokinetic behavior of the 4-hydroxycoumarins is accountable for the above mentioned discrepancy: their uptake into non-hepatic tissues appears to be poor in comparison with their distribution to the liver (Daemen et al, 1986; Thijssen et al, 1986). For studying the effects of 4-hydroxycoumarins on the function of the testis, higher testicular concentrations are necessary without increasing the liver concentration. Under special conditions, target organ directed drug delivery can increase the concentration in the target organ. These conditions are a low blood flow to the target organ and a high systemic clearance of the infused drug at steady-state conditions (Chen and Gross, 1980). This pharmacokinetic principle was applied to study the effects of S-acenocoumarol (S-AC), a 4-hydroxycoumarin, on the vitamin K-dependent systems in the testis of the rat.

Methods: Via a silastic tube (internal diameter 0.6 mm) protruding into the left testis, S-AC was infused by an osmotic minipump at a constant rate of 1 ug/h. Via a PE-10 catheter in the femoral artery, blood samples of 0.5 ml were taken on the 2nd, 3rd and 4th day. On the 4th day, the animal was euthanized and the liver and both testes removed. A second group of animals received phenobarbital (1 mg/ml) in the drinking water to increase the hepatic clearance of S-AC, thereby increasing the advantage of local over systemic delivery.

Results: In the first group (n=6) of animals S-AC plasma concentrations ranged from 4-8 ng/ml on all three experimental days. In the liver, 520±30 ng/g (means ±SEM) S-AC was found. The left testis contained 1200±200 and the right testis 40±10 ng/g S-AC. The activity of the enzyme reductase in the liver was reduced to 69% of the value observed in the liver of a control series of animals and in the left testis to 29% as compared to the right testis. Plasma coagulation (prothrombin time) was not affected. Phenobarbital reduced the hepatic S-AC concentration to 360±27 ng/g (n=6) and had no effect on the S-AC concentration in the left testis nor on coagulation. Reductase activity was reduced to 65% in the liver and 29% in the left testis.

Conclusions: The application of the pharmacokinetic principles of target organ directed drug delivery facilitates the study of the role of vitamin K-dependent proteins in non-hepatic tissues, such as the testis, without interfering the synthesis of clotting factors in the liver. Chronic local infusions of S-AC in the testis of the rat can be undertaken to resolve the relation of these vitamin K-dependent systems to male fertility.

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RESPONSES OF THE UTERUS TO PROSTAGLANDIN AND OXYTOCIN IN THE DIABETIC PREGNANT RAT.

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An increase in uterine contractility is thought to be involved in the increased incidence of abortion and premature delivery known to occur in diabetics. There is considerable evidence suggesting that prostaglandin (PG) and oxytocin (OX) are potent uterine stimulants and may play a role in the onset of labour (Johnson et al, 1975). This study was therefore conducted to determine any alteration in the sensitivity of the diabetic uterus to these endogenous uterine stimulants and to assess the effect of verapamil, a calcium channel antagonist, in moderating uterine contractility.

Uterine strips from day 21 pregnant rats were isolated and were superfused with Krebs' solution. Dose-response curves were constructed for each agonist in the absence and presence of verapamil (2.036×10^{-7} and 2.036×10^{-6}M). Diabetes was induced by a single intravenous injection of streptozotocin 50 mg kg^{-1} on day 12 of pregnancy.

All the agents tested (OX n = 16, PGE_2 n = 15, $PGF_{2\alpha}$ n = 13, and PGD_2 n = 13) showed a dose-dependent increase in the spontaneous contraction of the uterus and there was no significant difference between the responses of uterine strips from control and diabetic rats to the PGE and D contractile responses. Responses to oxytocin were, however, markedly ($P<0.01$) depressed by diabetes. Verapamil produced a dose related reduction of the responses of both control and diabetic uterine strips to all the agents tested.

These results suggest that an increase in the sensitivity of the uterus to the endogenous uterine oxytocin may not be the underlying cause for abortion and premature labour in the diabetic rat. Other factors such as an increase in the production of PGs reported to occur in diabetics (Waitzman & Rudman, 1978) may be considered. The uterus from the pregnant diabetic rat is more responsive to $PGF_{2\alpha}$. Our results show that verapamil is a relatively potent inhibitor of PG and OX induced uterine contractions suggesting that an increase in Ca^{2+} influx may be the basis for their mechanism of action and that verapamil may be useful in the treatment of premature labour. The reduction in the responses of the diabetic uterus to oxytocin may be responsible for the increased induction-delivery interval which occurs in diabetics.

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EFFECT OF SELECTIVE α_1 AND α_2-ADRENOCEPTOR ANTAGONISTS ON GLUCOSE-INDUCED HYPERINSULINAEMIA IN RATS.

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Insulin release from pancreatic β-islets is modulated by α2-adrenoceptors (Kato and Nakaki, 1983). Stimulation of these receptors decreases the amount of insulin released in response to a glucose challenge (Kato and Nakaki, 1983; Langer et al., 1983). Conversely, the selective α2-adrenoceptor antagonist imiloxan given to fasted rats caused plasma insulin levels to increase (Clague et al., 1984). Idazoxan significantly reduced the hyperglycaemia and potentiated the slow onset hyperinsulinaemia following a s.c. glucose challenge to fasted rats (Roach et al., 1984). We have now examined the effects of the selective α2-antagonist idazoxan and the α1-antagonist prazosin on the fast release of insulin evoked by an intra-arterial (i.a.) glucose challenge. The sulphonylurea glibenclamide was used as the standard hypoglycaemic agent.

Indwelling cannulae were implanted into the abdominal aortae of male Sprague-Dawley rats (weighing 290-405g) at least two days prior to the experiment in order to facilitate the taking of blood samples (for the determination of plasma glucose and insulin levels) and for administration of the glucose challenge (250 mg/kg). Plasma glucose levels were measured using an oxidase analyser (Analox, model GM6). Plasma insulin was measured by radioimmunoassay (Sorin Biomedica, Italy, SB-INSI-5). All experiments were performed in groups (n=6) of normally fed rats since initial experiments showed that insulin responses to an i.a. glucose challenge were greater and more consistent in fed rats rather than in fasted animals. Either distilled water (5ml/kg), prazosin (5mg/kg), idazoxan (10 mg/kg) or glibenclamide (5 mg/kg) were given orally 30 min before i.a. glucose (250 mg/kg). Blood samples (0.6ml) were taken immediately before the drug treatment and before and 2,5,10 and 20 min after the glucose challenge. Blood was replaced with equivalent volumes of heparinised saline.

Prazosin and idazoxan did not alter the baseline plasma glucose and insulin levels whereas glibenclamide decreased significantly plasma glucose levels and elevated plasma insulin. None of the drug treatments affected the magnitude of the hyperglycaemic response to the glucose challenge. The effects of the three compounds and their corresponding controls (dist. water) on insulin levels are shown in Table 1. The glucose-induced insulin response was slightly reduced by prazosin and potentiated by idazoxan and glibenclamide.

	TREATMENT	PLASMA INSULIN LEVELS (μU/ml) BEFORE GLUCOSE CHALLENGE	MAX LEVELS AFTER GLUCOSE CHALLENGE
1.	Prazosin	32 ± 5	107 ± 10
	Dist. water control	36 ± 9	130 ± 15
2.	Idazoxan	25 ± 5	196 ± 12*
	Dist. water control	29 ± 2	112 ± 22
3.	Glibenclamide	46 ± 4*	118 ± 7*
	Dist. water control	19 ± 1	81 ± 8

Table 1. Effects of prazosin, idazoxan and glibenclamide on the glucose-induced increases in plasma insulin levels. * indicates significant difference from controls (P<0.01 unpaired t-test).

These results indicate that glibenclamide and the selective α2-adrenoceptor antagonist idazoxan potentiate the immediate hyperinsulinaemic response to an i.a. glucose challenge. In contrast to the sulphonylurea, idazoxan did not alter basal plasma glucose and insulin levels in fed rats. These data provide further evidence that α2-adrenoceptors modulate the release of insulin.

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ATTENUATION OF 2-DEOXY-D-GLUCOSE HYPERPHAGIA FOLLOWING CHRONIC ADMINISTRATION OF A μ- BUT NOT A κ- OPIOID AGONIST.

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Centrally-applied opioid peptides initiate feeding in satiated animals (Morley et al., 1983). 2-Deoxy-D-glucose (2-DG) has also been shown to increase food intake in rats (Smith and Epstein, 1969), furthermore 2-DG hyperphagia is attenuated in the presence of the opioid receptor antagonist naloxone (Sewell and Jawaharlal, 1980) suggesting that the ingestive response to 2-DG may be mediated at least in part by the release of endogenous opioids. In the present study, we have investigated further the involvement of opioid receptors in 2-DG feeding by examining the appetitive effects of the glucose analogue in animals treated chronically with either the μ-opioid agonist morphine or the κ-agonist Mr 2033.

Subjects were individually housed male Wistar rats in the weight range 350-400g which were allowed free access to a standard powdered rat diet and tap water at all times. Animals were injected twice daily at 08.00 and 20.00 hr with increasing doses (10-50 mg/kg i.p. over 5 days) of morphine or Mr 2033 [(±)-α-5, 9-dimethyl-2-(L-tetra-hydrofurfuryl)-2'-OH-6,7-benzomorphan]. Corresponding control groups were treated twice daily with the distilled water vehicle. At 08.00hr on day 6 animals received injections of either vehicle or 2-DG (500 mg/kg i.p.) to produce four treatment groups as follows: chronic vehicle + vehicle; chronic vehicle + 2-DG; chronic agonist + 2-DG; chronic agonist + vehicle. Feeding jars were weighed after 1,2,4 and 6 hours to enable the calculation of mean group cumulative food intakes g/kg rat weight ± S.E.M. Statistical comparisons were made using the analysis of variance and Dunnett's test.

On the day of experiment the increase in food consumption induced by 2-DG was significantly attenuated in animals treated chronically with morphine whereas animals treated chronically with Mr 2033 displayed the full feeding response to the glucose analogue. Control animals injected chronically with either morphine or Mr 2033 but given vehicle on day 6 ate similar amounts to the corresponding vehicle + vehicle controls throughout the 6h observation period.

It has previously been shown that 2-DG hyperphagia is resistant to antagonism by ICI 174,864 (Jackson and Sewell, 1985), therefore it seems unlikely that the increase in food intake induced by 2-DG is associated with any concomitant release of enkephalins and subsequent activity at δ-receptors. Instead, it is tenable that 2-DG induced feeding is a response to elevated levels of other endogenous opioceptive ligands (eg. β-endorphin or dynorphin), acting at the same receptor as morphine. The results of the current study, however, are not in accordance with a prominent role for dynorphin in 2-DG hyperphagia, as this peptide is the putative endogenous ligand for κ -opioid receptors (Chavkin et al., 1982).

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THE ANALGESIC EFFECT OF BUPRENORPHINE, ETORPHINE AND PETHIDINE IN THE PIG, A RANDOMIZED DOUBLE BLIND CROSS-OVER STUDY.

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The published literature concerning the proper postoperative analgesic treatment of pigs in connection with experimental procedures is only scarce and mainly based on clinical experience from other species. (Flecknell, 1984; Schambye et al., 1985). In order to find a suitable analgesic for the treatment of postoperative pain in pigs the analgesic effect of i.m. administration and buprenorphine (B), etorphine (E) and pethidine (P) has therefore been compared in 8 domestic pigs. For assessment of the analgesic action one thermal (hot plate) and two mechanical (cannulation of ear vein, needle prick) noxious stimuli were employed.

In a pilot experiment on 2 pigs in which methadone was included the maximal effective doses were estimated for each drug. These were 120 µg/kg for B, 3 µg/kg for E and 20 mg/kg for P. Methadone was found unsuitable as an analgesic in the pig, because of unacceptable side effects (respiratory dysfunction, hyperactivity) at effective dose levels.

In the second part of the study B 120 µg/kg, E 3 µg/kg and P 20 mg/kg were compared in a randomized double-blind trial with a balanced cross-over design on 6 pigs. E proved to have the highest and B the lowest maximal analgesic effect which was especially evident in the needle prick test.

The analgesic effect duration-defined as the period which passed from time of medication until the analgesia had decreased to a level corresponding to 50% of the maximum obtainable analgesic score expressed in h (median values (range)) - was for B, E and P: 7.0(7-24), 4.0(3.5-4.5), 2.5(1.5-3.5) in the hot plate test; 7.0 (6.5-24), 5.0(3.5-5.6), 3.5(0.9-6.5) in the cannulation test and 3.5(0-7.0), 3.0 (2.5-5.5) and 1.5(0-2.5) in the needle prick test respectively ($p < 0.05$; Bvs E, Bvs P). Thus B proved to have the longest duration of analgesic effect in all three tests and P the shortest.

In spite of the relatively high dose of B used (120 µg/kg) as compared to that recommended in other species (Flecknell 1984) the pigs showed no adverse symptoms. On the contrary E caused a slightly increased striated muscle tone in 3 of 6 pigs at 3 µg/kg and severe laboured respiration at 10 µg/kg indicating a low margin of safety.

The experimental results indicate that B should be the first drug of choice in the treatment of pain after surgical intervention in the pig due to its long duration of action and lack of side effects.

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ABSENCE OF BENEFICIAL EFFECTS OF NALOXONE AND NALTREXONE IN PORCINE ENDOTOXIC SHOCK.

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Evidence has been presented that opiate receptor blockade is effective in improving cardiovascular functions during endotoxic shock in several species e.g. rat and dog (Holaday & Faden, 1978; Reynolds *et al.*, 1980). In ponies and baboons, however, no significant protection could been observed (Moore *et al.*, 1983; Hinshaw *et al*, 1984). In the present investigation the effect of a rather high dose of two β-endorphin antagonists on the hemodynamic course of endotoxic shock has been evaluated in the anesthetized piglet.

In 28 anesthetized (pentobarbitone and nitrous oxide) and artificially ventilated female piglets (13-17 kg; 10-12 weeks old) the following parameters were recorded: carotid arterial pressure (Pa); portal venous pressure (Pv); central venous pressure; pulmonary arterial pressure (Pap); pulmonary capillary wedge pressure; cardiac output (CO); heart rate (HR); superior mesenteric artery blood flow ($\dot{Q}m$); and total peripheral (Rt), pulmonary (Rp) and mesenteric vascular (Rm) resistances, as previously described (Schrauwen & Houvenaghel, 1985). In the endotoxin control experiments (ENDO-C, N = 17) an LD_{100} dose of endotoxin (LPS E. coli; $O_{111}B_4$;Difco), 0.5 mg/kg, was slowly injected i.v. after a 30 min observation period. In the naloxone (NLX) and naltrexone (NTX) treated groups the antagonists were administered i.v. in a total dose of 10 mg/kg (5 mg/kg 15 min prior to and 5 mg/kg 15 min following endotoxin administration)(ENDO-NLX group, n = 5; ENDO-NTX group, n = 6).

The hemodynamic effects of endotoxin administration in the ENDO-C group have previously been described (Schrauwen & Houvenaghel, 1985), and are only briefly summarized. Pa gradually decreased, Pv and Pap showed a sustained increase, CO gradually decreased and HR increased, whereas $\dot{Q}m$ displayed an initial transient decrease followed by a return to near control value. Rt and Rm revealed an initial and transient increase followed by a sustained decrease, whereas Rp displayed a biphasic increase. Except for HR all changes were significant (Mann-Whitney U test). Mean survival time amounted to 107±13 min (range 5 to 210 min).

In the ENDO-NLX and ENDO-NTX groups opiate antagonists significantly aggravated the endotoxin induced decrease in Pa, CO and Rm, resulting in the death of 4 out of the 11 piglets within 26±10 min (range 5 to 45 min) following endotoxin. At the end of the 210 min observation period 4 piglets were still alive, presenting higher values for Pa during the second phase of shock as compared to ENDO-C animals.

The results reveal no consistent improvement in cardiovascular functions in porcine endotoxic shock by the opiate antagonists in the dosage used. Possibly their morphinomimetic actions, already evident upon injection prior to endotoxin, predominate so that the normal cardiovascular homeostatic mechanisms are attenuated.

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HORMONAL, OVARIAN AND BEHAVIOURAL MODIFICATIONS INDUCED BY RAM TEASING AT THE BEGINNING OF THE BREEDING SEASON IN THE TEXEL EWE.

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Just before the breeding season (September 13), a vasectomized active ram is introduced into a group of six Texel ewes in anoestrus. Blood is sampled every 6 hours for LH, FSH and P4 measurement, and the ovaries are regularly observed by endoscopy.

Table 1 Days of LH discharges and of oestrous behaviour
(Asterisks indicate LH peaks followed by ovulation)

ewe	discharge of LH			oestrus
18	3.5*	7.8*	25.3*	25.0
86	1.8*	7.5*	24.5*	24.0
91	1.0*	18.8*		18.5
115	2.0	6.8*	22.5*	22.3
211	1.5*	6.0*	22.5*	22.3
239	1.0	4.5*	18.0*	17.8

Discharges of LH of preovulatory appearence were observed 1.8 ±.9 days after the introduction of the ram. They were not always followed by ovulation ; this happened only in four cases. One of these ewes then showed a cycle of normal length while the three others which ovulated presented short cycles characterized by a low but significant increase in progesterone secretion. The ewes which did not ovulate also had short cycles of similar length, 4.4 ±.9 days, but without increased progesterone production.

The second discharge of LH always induced an ovulation and a normal luteal function. The length of these cycles was of 16.1 ±1.5 days. The pattern of FSH secretion (Figure 1) was characterized by a rhythm of about 6 days previously reported (Bister, Paquay, 1983).

Oestrous behaviour (Table 1) is only noted after a priming of the brain by progesterone for at least 8 to 10 days. This "ram effect" induced therefore a synchronization of the oestrus but this was relatively low : 22 ±5 days after the introduction of the ram.

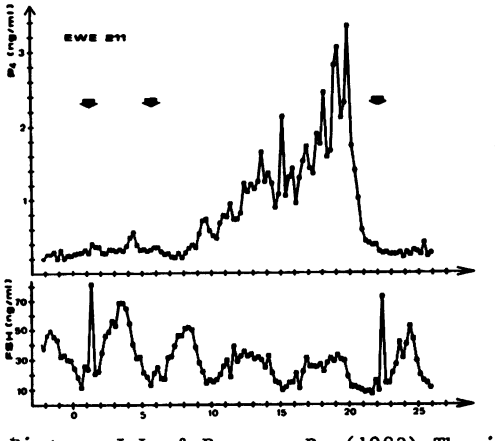

Figure 1 Amount of FSH and progesterone in jugular plasma of a ewe

Day 0 is the day of the introduction of the ram. The arrows indicate LH discharges.

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INHIBITORY EFFECT OF U50488H ON SUCKLING-EVOKED OXYTOCIN RELEASE IS BLOCKED BY PROPRANOLOL.

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A number of different opioid peptides may be involved in the regulation of oxytocin secretion and we have shown that there are at least two possible sites of action, the posterior pituitary (1) and spinal cord (2) and, since different opioid peptides may act preferentially at different subtypes of the opiate receptor, we have continued our investigations by studying the effects of receptor-selective opiate agonists. We now report that the κ-selective agonist U50488H blocks the milk-ejection reflex, but that a β-adrenergic mechanism mediates this inhibitory effect on the release of oxytocin.

Experiments were carried out on lactating Wistar rats separated from all but one of their pups overnight and lightly anaesthetised with urethane (1.0g/kg i.p.). All surgical procedures, which included saphenous vein, intrathecal and mammary gland cannulations were carried out under halothane (0.5-2.0% in O_2/NO_2). Three hours after induction of anaesthesia ten hungry pups were placed on the nipples and the reflex release of oxytocin was detected both by increased intramammary pressure and pup behaviour.

Milk-ejection responses usually commenced within 30 min and continued at regular intervals with a mean of 5.8±0.3 min for a period of several hours. After intrathecal administration of U50488H (10μg) the interval to the subsequent response was 27.7±3.3 min (n=7) which was significantly (t-test $P<0.001$) different from that occurring after 0.9% saline injection (mean = 7.7±3.0 min, n=6). Administration of a higher dose of U50488H (50μg) had a similar inhibitory effect (21.9±4.5 min, n=8, $P<0.001$). These inhibitions were not accompanied by any significant change in mammary gland sensitivity. U50488H was also effective after intraventricular injection of 10μg (31.9±5.8 min, n=8, $P<0.001$) and intraperitoneal injection of 1mg/kg (46.1±7.8 min, n=6). Following injection of the β-adrenergic antagonist propranolol (1mg/kg, i.p) however intrathecal injection of U50488H was ineffective at blocking milk-ejection and neither 10μg or 50μg caused significant inhibition (i.e. $P>0.05$); the mean postdrug interval was respectively 10.1±17.8 min (n=6) and 11.3±1.8 (n=5). Similarly, propranolol significantly reduced inhibition of milk-ejection by intraperitoneal U50488H from 46.1±7.8 (n=6) to 13.0±5.0 (n=7, $P<0.01$) min.

In summary these experiments have shown that the κ-agonist U50488H inhibits the release of oxytocin and suggests there may be at least two sites of action: One within the spinal cord, operating through a β-adrenergic mechanism, and one at a supraspinal site.

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PHAGOCYTIC CELL DEFENCE IS DEPRESSED BY STRESS IN CALVES.

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Pasteurella haemolytica is the most important infectious agent associated with the bovine respiratory disease complex. Its presence in the pulmonary system often results in severe pneumonia and death. Most investigators consider bovine respiratory disease to be caused by the interaction of the animal with environmental factors, such as transportation, cold, crowding, viral infection and other forms of mental and physical stress. However, the exact mechanisms how these stress factors could influence host defense mechanisms are unknown. In our study, it was investigated whether stress affected phagocytic cell function in calves.

Studies were performed on six months old, clinically healthy calves. Six animals underwent 2 h stress on a conveyorbelt, after which venous blood was collected and broncho-alveolar lavage was performed. Polymorphonuclear leucocytes (PMN) were isolated from the blood and alveolar macrophages (aMϕ) from the lavage liquid. Phagocytic cells isolated from nine non-stressed animals served as controls. Phagocytosis was studied using ^{3}H-thymidine-labeled bacteria, superoxide production was assayed by the reduction of ferricytochrome c and chemotaxis was determined using the under-agarose technique (Henricks et al., 1984).

No differences in phagocytic capacity between aMϕ isolated from stressed and from control animals were observed (55±4 vs 60±4% uptake). Also, the amounts of superoxide generated by control aMϕ and aMϕ obtained from stressed animals did not differ (62±16 vs 67±16 nmol superoxide/5x10^{6} cells/10 min). In addition, PMN isolated from venous blood were tested for several phagocytic cell functions. The uptake of bacteria did not differ between PMN isolated from stressed and from control animals (51±6 vs 53±3% uptake). However, the amount of superoxide generated by PMN isolated from stressed animals was significantly diminished as compared to PMN obained from control animals (51±6 vs 89±15 nmol superoxide/ 5x10^{6} cells/10 min; $p<0.05$). The PMN obtained from stressed animals migrated equally well as PMN isolated from control animals (0.94±0.04 vs 0.92±0.07 mm migration towards attractant).

The decreased metabolic activity of PMN after a stress period could be caused by increased cyclic-AMP levels in these phagocytic cells due to endocrine changes like enhanced levels of catecholamines in the blood stream of stressed animals. In fact, a decreased metabolic activity of phagocytic cells can be observed after β-adrenergic stimulation (Henricks et al., 1986). The stress situation did not alter the metabolic and phagocytic responsiveness of the aMϕ. Possibly the changed levels of endocrine factors in the circulation do not reach the aMϕ present in the bronchoalveolar space. The diminished capacity to produce reactive oxygen species by PMN from stressed animals could be one of the factors for an increased susceptibility for infections in calves after stress situations.

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EXCRETORY EFFECTS OF TORASEMIDE AND METABOLITES, AND FUROSEMIDE AFTER UNILATERAL RENAL APPLICATION OR I.V. INJECTION IN THE RAT.

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Effects of torasemide, furosemide and two metabolites of torasemide, C3534 and C3535, upon urine flow ($\dot{V}$), Na^+ ($U_{Na}.\dot{V}$), K^+ ($U_K.\dot{V}$) and Cl^- ($U_{Cl}.\dot{V}$) excretion rates from the left and the right kidney were studied during rapid sequential urine collections in anesthetised rats prepared as for micropuncture. One hundred twenty rats were treated with 200 ul of isotonic saline containing torasemide (10^{-6} to 10^{-2} M), furosemide (5.10^{-5} to 5.10^{-3} M), C3534 or C3535 (10^{-4} or 10^{-3} M). The test solution was applied upon the left renal capsule (A.L.R.C.) or i.v. injected (I.V.). Eight rats were time-control. Cumulated (15, 30 and 60 min) and maximal (highest excretion rate in a timed collection) net effects for the left and the right kidney were calculated.

In contrast to C3534 (10^{-3} M) which induced only minor effects, water and electrolyte excretion significantly increased with torasemide, furosemide or C3535 at appropriate concentrations. Comparison between torasemide (T), furosemide (F) and C3535 showed the following salient results for the left kidney :

	T	F	C3535	T	F	C3535
	$\dot{V}$			$U_{Na}.\dot{V}$		
cumulated						
A.L.R.C. 60 min	10^{-2} M ~ 5.10^{-3} M		< 10^{-3} M	10^{-2} M ~ 5.10^{-3} M		< 10^{-3} M
I.V. 60 min	10^{-3} M ~ 5.10^{-3} M		≪ 10^{-3} M	10^{-3} M ~ 5.10^{-3} M		≪ 10^{-3} M
maximal						
A.L.R.C.	10^{-2} M ≪ 5.10^{-3} M		< 10^{-3} M	10^{-2} M ≪ 5.10^{-3} M		< 10^{-3} M
I.V.	10^{-3} M < 5.10^{-3} M		~ 10^{-3} M	10^{-3} M < 5.10^{-3} M		~ 10^{-3} M

No significant differences between left and right kidney were found.

In another 11 rats, following torasemide or furosemide renal application after decapsulation of the left kidney, cumulated and maximal net effects were markedly enhanced after torasemide but not after furosemide application.

Our results show that torasemide is more potent than furosemide. However, in contrast to furosemide, torasemide's action is impaired by the renal capsule. Torasemide's metabolite C3535, but not C3534, induces highly potent diuretic effects. The order of potency of the various substances tested in the rat under our experimental conditions is the following :

C3535 > torasemide > furosemide ≫ C3534.

EFFECT OF ALKYLXANTHINES ON GENTAMICIN-INDUCED ACUTE RENAL FAILURE.

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We have previously shown that the adenosine antagonist 8-phenyltheophylline (8PT), ameliorated the severity of ischaemic acute renal failure (ARF) produced by i.m. glycerol injection in the rat (Bowmer et al,1985). In the present study we have investigated the protective effect of a range of alkylxanthines with different potencies as adenosine antagonists in a nephrotoxic model of ARF produced by gentamicin (de Rougemont et al,1981).

Rats received daily s.c. injections of gentamicin (200 mg kg^{-1}) for 3 days and were deprived of drinking water for 24h following the first injection. During the same period groups of animals were treated i.p. with equi-molar doses of an alkylxanthine; 8PT (10mg kg^{-1}), theophylline (T) (7mg kg^{-1}), enprofylline (E) (7.6mg kg^{-1}) or vehicle (1.0ml kg^{-1} of 50%v/v polyethylene glycol in 0.1M NaOH) twice daily. A further group of gentamicin-injected rats received no treatment. Blood samples were taken from the tail vein immediately before the first gentamicin injection and 3, 5 and 7 days after the last gentamicin injection. In separate experiments with conscious rats, the effect on heart rate of an infusion of adenosine (1 mg kg^{-1} min^{-1}) was determined before and after i.p. administration of T (7 mg kg^{-1}), E (7.6 mg kg^{-1}) or vehicle (1.0 ml kg^{-1}).

Table 1 Plasma urea and creatinine in rats 7 days after gentamicin administration

	Plasma urea (mg dl^{-1})	Plasma creatinine (mg dl^{-1})
Untreated	140 ± 23	2.15 ± 0.33
Vehicle treated	122 ± 18	1.84 ± 0.28
8PT treated	64 ± 4 ***	1.21 ± 0.06 **
T treated	85 ± 14 *	1.63 ± 0.22
E treated	79 ± 8 **	1.28 ± 0.08 **

mean ± s.e.mean; *$p<0.05$, **$p<0.01$, ***$p<0.001$ relative to untreated group n = 12.

Plasma urea and creatinine concentrations in untreated rats prior to gentamicin injection were 41 ± 2 and 0.62 ± 0.11 mg dl^{-1} respectively. These were not significantly different from initial levels noted in any other group of treated rats. Gentamicin administration resulted in large increases in urea and creatinine levels in untreated rats (Table 1).

Rats treated with each of the alkylxanthines had significantly lower plasma urea levels 7 days after gentamicin administration than untreated rats. However, only treatment with 8PT and E resulted in significantly lower plasma creatinine levels. The effect of treatment with alkylxanthines on plasma urea and creatinine values 3 and 5 days after the last gentamicin injection showed a similar picture to that noted in Table 1. By contrast to glycerol-induced ARF (Bowmer et al,1985) the vehicle had no protective effect on the development of ARF induced by gentamicin (Table 1). In a previous study we noted that 8PT (10mg kg^{-1} i.p.) antagonised adenosine-induced bradycardia for up to 3h (Bowmer et al,1985) whereas in the present investigation T produced antagonism for up to 1h whilst E and the vehicle were without effect.

The present findings show that all the alkylxanthines tested were effective to some degree in reducing the severity of nephrotoxic ARF produced by gentamicin. This protection does not appear to be related to adenosine antagonism.

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MODIFICATION OF DIURESIS BY PROPRANOLOL IN THE ETHANOL-ANAESTHETISED RAT.

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Isoprenaline and dichlorisoprenaline were shown to produce an antidiuresis and antinatriuresis in conscious water-loaded rats (Botting and Lockett, 1961) whilst Lees (1968) demonstrated that propranolol increased natriuresis without an accompanying diuresis. Banerjee and Hele (1982) reported both increased urine and electrolyte excretion in conscious rats and Davis (1976) obtained similar results in hydrated, ethanol-anaesthetised rats. The aim of the present study is to examine the mechanism by which the diuresis is produced by propranolol in ethanol-anaesthetised rats.

Male rats (Wistar strain) weighing 200-300g, starved for 18-24 hrs. but allowed free access to water, were anaesthetised with 15% ethyl alcohol (5ml/100g) and water loaded orally with a dose equivalent to 2.5% body weight. Drugs were injected via the femoral vein, blood pressure was recorded from the femoral artery and the urine was collected at 10min. intervals for the analysis of Na^+, K^+ and Cl^- ions.

Table 1 Changes in Urine Volume Produced by (±)-Propranolol

Procedure	Control Flow Rate before (±)-propranolol	Maximum Flow Rate after (±)-propranolol	% Change in Urine Volume
Control	1.18±0.05	2.55±0.24	+108.1
Denervation	1.28±0.02	2.06±0.08	+ 80.5
Metyrapone	1.11±0.16	1.80±0.25	+ 81.1
Adrenalectomy	0.93±0.04	0.88±0.06	- 5.4
Demedullation	0.85±0.05	0.63±0.13	- 25.9

(±)-propranolol (see Table 1) and (-)-propranolol at a dose of 0.1mg/kg caused a marked increase in diuresis and natriuresis, whereas (+)-propranolol at the same dose had no effect. Neither (±) nor (-)propranolol caused any decrease in mean arterial pressure, but a small reduction in heart rate was observed. Kidney denervation operations were performed under pentobarbitone anaesthesia (45mg/kg), by application of 20% phenol in glycerol to the renal nerves, and diuretic studies were carried out 7-10 days later. (±) and (-)-propranolol still caused a marked diuresis. In other experiments, aldosterone synthesis was impaired by pretreatment with dexamethasone (200µg/day for 3 days) and the i.p. injection of metyrapone (10mg) one hour before the diuretic study. As with the denervation experiments, (±) and (-)-propranolol produced a marked diuresis and natriuresis. Adrenalectomy or adrenal demedullation carried out under pentobarbitone anaesthesia, abolished the effect of propranolol in diuretic experiments performed 7-10 days later. Adrenal demedullation was shown to decrease the levels of catecholamines in the adrenal glands ($P<0.001$), without significantly decreasing plasma corticosterone levels (measured by the method of Zenker and Bernstein 1958). It is proposed that propranolol is reversing an inhibition of diuresis produced by circulating catecholamines.

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EFFECT OF SUBSTITUTED BENZOATES ON THE ORGANIC ANION TRANSPORT SYSTEM IN ISOLATED DOG KIDNEY MEMBRANE VESICLES.

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The substrate specificity of the renal organic anion transport system was investigated by studying the effect of substituted benzoates on p-amino(^{3}H)-hippurate (PAH) uptake into brush border (BBMV) and basolateral (BLMV) membrane vesicles from dog kidney. BBMV and BLMV were isolated simultaneously from dog renal cortex using a calcium aggregation method in combination with differential and Percoll-gradient centrifugation. The uptake of PAH in the vesicles was studied by a rapid filtration method. Both BBMV and BLMV showed a saturable sodium coupled PAH uptake sensitive to probenecid. BBMV showed also a pH stimulated probenecid sensitive uptake (Russel *et al.*,1986).

Na^+-coupled PAH uptake in BLMV and Na^+- and H^+-coupled PAH uptake in BBMV were evaluated. Transport of 100µM PAH at 15 seconds under all given conditions appeared to be linear with respect to time and concentration. Without inhibition total PAH uptake in BBMV was 210 ± 60 pmol/mg protein and in BLMV 105 ± 30 pmol/mg protein. Probenecid (5mM) reduced PAH uptake by 75% for BBMV and 62% for BLMV. These data were considered as maximum possible inhibition. Relative to this probenecid inhibitable part the inhibitory effect of the benzoates, which were added in a concentration of 5mM, was investigated. The compounds studied were benzoic acid and its analogues which were substituted at the ortho- (o), meta- (m) or para- (p) position by a methyl, methoxy, hydroxy, chloride, nitro or amino group.

In BLMV most of the derivates gave an inhibition of more than 50%, with m- and p-chlorobenzoate as strongest inhibitors (90%) and p-aminobenzoate as weakest inhibitor (38%). BBMV were in general less susceptible showing also m-chlorobenzoate as strongest inhibitor (96%) and p-aminobenzoate as weakest inhibitor (0%). A striking difference was observed between the inhibitory effect of benzoate in both membranes. In BBMV benzoate was one of the best inhibitors while in BLMV it belonged to the less inhibiting substrates. The experiments showed that inhibition of the meta-substituted benzoates was stronger than their corresponding para and ortho analogues and that ortho analogues gave the least inhibition except for amino- and hydroxy-substituted benzoates. H^+-stimulated PAH transport in the BBMV showed the same properties as with the Na^+-stimulated transport but inhibition was less pronounced. The meta- and para-substituted analogues of benzoate inhibited in a fashion predicted by Hammett's theory. Electron withdrawing groups increased the inhibitory effect while electron releasing substituents did less. However, nitro substitutes failed to follow this relation probably because of the strong electron resonance between the substituent and carboxyl group. A lipophylic index ($\log K_o$) for the compounds determined by reversed-phase HPLC correlated with the inhibitory activity of almost all analogues. Also data observed for nitro substitutes fitted this relation. It is noteworthy that our results are in good correspondence with the findings of Ullrich *et al.* (1982) who studied the inhibition of benzoate derivates on the renal lactate transport system.

Our data indicate that an important determinant of the specificity of the PAH transport system in BBMV and BLMV is the electron density at the reaction centre.

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INHIBITION OF HUMAN PLATELET FUNCTIONS BY CYCLANDELATE.

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Hyperactivity of blood platelets is thought to play an important role in the etiology of occlusive vascular disease. Recently it has been demonstrated that the vaso-active compound cyclandelate (Cyclospasmol^{R}, C) interferes with human platelet aggregation (Van den Hoven et al, 1984). This is most likely due to its calcium modulating properties (Van Nueten et al, 1978). The present in vitro study was designed to further define the effects of C on human platelet functions, by investigating thromboxane generation and serotonin release simultaneously with platelet aggregation. Further the activity of C on ristocetin induced platelet aggregation, known to be calcium independent, was determined.

Blood platelet aggregation induced by ADP, collagen, arachidonic acid (AA) and PAF was measured turbidimetrically in platelet rich plasma (PRP) of healthy volunteers. Simultaneously, the release of [^{14}C]-serotonin from preloaded PRP (Mills et al, 1968) was determined and the formation of thromboxane B_2 (TxB_2) was assessed by radioimmunoassay (Cameron et al, 1982).

C, in micromolar concentrations prevented [^{14}C]-serotonin release and aggregation induced by ADP (second wave), collagen and PAF in a dose dependent manner. No effect was noted on [^{14}C]-serotonin uptake. TxB_2 generation produced by ADP, collagen and PAF was also inhibited by C, indicating an effect on the endogenous arachidonic acid pathway. Regression analysis revealed significant regression for the inhibition values of [^{14}C]-serotonin release, TxB_2 generation and platelet aggregation with the agonists used. Further statistical analysis also revealed a significant correlation between these inhibited parameters. However, C failed to inhibit exogenous AA induced aggregation and TxB_2 generation. C was also unable to inhibit ristocetin induced platelet aggregation while indomethacin dose-dependently inhibited aggregation significantly.

The present study demonstrated that C inhibits platelet functions induced by ADP, PAF and collagen. C failed to inhibit platelet activation induced by exogenous AA which suggests that C does not inhibit enzymes involved in AA metabolism, but rather exerts its action upon AA release from platelet membrane phospholipids. That the activity of C was possibly based on calcium modulation, was further strengthened by the fact that C was not able to inhibit ristocetin induced aggregation. The results of these experiments indicate that it is now important to investigate directly the action of C on the calcium handling of the blood platelet during the aggregation process. Confirmation of such an interaction not only will fit with the results of this study, but will also elucidate the underlying mechanism by which C may restore abnormal platelet functions.

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COMPARISON OF THE EFFECTS OF SUBSTANCE P AND NEUROKININ A ON THE RAT STOMACH IN VIVO AND IN VITRO.

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The tachykinins substance P (SP) and neurokinin A (substance K, NKA) coexist in the peripheral nervous system of mammals (Nawa et al., 1984). In the present study the effects of these two tachykinins on the rat stomach were compared in vivo and in vitro.

Rats were anaesthetized with urethane and laparotomized. The abdominal aorta was cannulated for infusion into the coeliac artery. Such an infusion reached the corpus and antrum region of the stomach, but not the fundus. The oesophagus was ligated in the neck, and the stomach was cannulated via the duodenum, filled with 3 ml Tyrode solution, and the cannula connected to a pressure transducer. Infusion of SP or NKA for 5 min led to dose-dependent increases in muscular tone of the stomach, higher doses also caused phasic contractions. NKA was more potent than SP, the threshold dose for NKA lying around 60 pmol min^{-1}, that for SP around 600 pmol min^{-1}. The peak of the phasic contractions obtained by the highest dose of SP used (20 nmol min^{-1}) was 1.1 $\pm$ 0.1 kPa (n = 3), the highest dose of NKA (2 nmol min^{-1}) led to a peak intragastric pressure of 2.1 $\pm$ 0.2 kPa (n = 4). The higher doses of SP also led to decreases in blood pressure by 0.6 - 2.6 kPa, whereas after infusion of NKA hardly any hypotension was observed. Pretreatment of the rats with guanethidine (20 mg kg^{-1} s.c.) increased the effects of both SP and NKA without affecting their relative activities. Therefore, the difference in intrinsic activity cannot be accounted for by the higher potency of SP in producing hypotension which may be followed by a reflex increase in the activity of the sympathetic nervous system. I.v. injection of atropine (1 mg kg^{-1}) did not change the effect of NKA, whereas the contractions evoked by equiactive doses of bethanechol were completely abolished.

For in vitro experiments, circular muscle strips from the gastric corpus were suspended in Krebs solution at 37°C gassed with 95% O_2/ 5% CO_2 for tension recording. Here, the dose-response curves of SP and NKA were parallel, NKA being ten times more potent than SP in causing contraction. However, 10 µM NKA caused only 58 $\pm$ 4 % (n = 7), and 30 µM SP caused only 54 $\pm$ 4 % (n = 6) of the maximal contraction evoked by bethanechol (1 mM).

It is concluded that, in the rat, NKA is more likely than SP to play a physiological role in stomach motility.

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EVALUATION OF A NEW AND POTENT CHOLECYSTOKININ ANTAGONIST ON ENTERIC REFLEXES.

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Cholecystokinin(CCK)-like peptides, particularly CCK octapeptide (CCK-8), are present in neurones of the myenteric plexus (Hutchison et al., 1981) and have been proposed to act as excitatory transmitters in the neural pathways of the peristaltic reflex (Davison & Najafi-Farashah, 1982). We have addressed this question by examining the effect of a new CCK antagonist (Makovec et al., 1985) on enteric reflexes. This antagonist, 4-(3,4-dichloro-benzoylamino),5-dipentylamino,5-oxo-pentanoic acid (CR 1409), was obtained from Rotta (S. Fruttuoso di Monza, Italy). The experiments were made on isolated segments from the small intestine of the guinea-pig suspended in organ baths. Contractions were recorded isotonically.

CR 1409 inhibited contractions of the longitudinal muscle induced by CCK-8 and the related peptide ceruletide and caused a parallel shift of the dose response curves to the right. Analysis of the antagonism with a Schild plot yielded a pA_2 value of about 7.5. CR 1409 (0.2-0.6 μM) did not affect half-maximal contractions in response to bethanechol, dimethylphenylpiperazinium, substance P, histamine or 5-hydroxytryptamine (n = 3-10). Nerve-mediated cholinergic contractions induced by electrical field stimulation (supramaximal voltage, 0.1 ms, 0.05 Hz; n = 10) and nerve-mediated non-cholinergic contractions induced by 40 s trains of stimuli (supramaximal voltage, 0.1 ms, 5 Hz; n = 6) were not changed by CR 1409 (0.6 μM).

Circular muscle contractions caused by ceruletide (tenfold threshold concentrations) were abolished by CR 1409 (0.4 μM) whereas those caused by acetylcholine, substance P, substance K or 5-hydroxytryptamine (tenfold threshold concentrations) were left unchanged by CR 1409 (up to 2 μM). Local distension of gut segments with a balloon led to circular contractions on the oral side of the segments (ascending enteric reflex, Costa et al., 1985). Neither the atropine-sensitive nor the atropine-insensitive component of this reflex contraction was affected by CR 1409 (0.4 μM). The peristaltic reflex recorded according to Bülbring et al. (1958) was left unchanged by CR 1409 (0.2 μM).

These observations show that CR 1409 is a potent and specific antagonist of the contractile effects of CCK on both the longitudinal and circular muscle of the guinea-pig small intestine. However, no evidence for a transmitter role of CCK-like peptides in enteric reflexes was obtained.

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INHIBITION OF MAO IN THE DOG BY MDL 72,394 WITH MINIMAL TYRAMINE POTENTIATION.

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(E)-β-fluoromethylene-m-tyrosine (MDL 72,394) when co-administered with carbidopa (CD) to rats produced neuronally selective inhibition of monoamine oxidase (MAO) with only minimal potentiation of the cardiovascular effects of tyramine (Ty) (Palfreyman et al., 1985). Preliminary experiments in dogs indicated that MDL 72,394 treatment slightly inhibited ^{14}C Ty metabolism in the intestine yet produced substantial inhibition of MAO in the brain (Davies et al., 1984). MDL 72,394 depends for its activation on aromatic L-amino acid decarboxylase, the activity of which is high in the rat but relatively low in dogs and man (Sasahara et al., 1980).

Five mongrel dogs (10-17 kg) of either sex were surgically implanted with abdominal aorta catheters and, after recovery, habituated to blood pressure (BP) and heart rate (HR) recordings while conscious. Control cardiovascular responses to i.v. (10-100 μg/kg) and oral (0.3-10 mg/kg) Ty were established on two occasions and were found to be reproducible. MDL 72,394 (0.5 mg/kg p.o.) was administered daily for 4 days. At 4 and 8 h, respectively, after the last dose, Ty was administered i.v. and orally and BP and HR responses recorded. Subsequently, Ty sensitivity was tested at weekly intervals until a control response was re-established. Dogs were then tested for Ty sensitivity following 4 daily MDL 72,394 (0.1 mg/kg) plus CD (2 mg/kg) treatments. Two weeks later, tranylcypromine (2 mg/kg/day p.o. x 4) treatment commenced and Ty sensitivity was again determined. In all dogs just prior to the administration of Ty, a 5 ml blood sample was taken for measurement of dihydroxyphenylglycol (DHPG) (Brown & Jenner, 1981).

MDL 72,394 alone produced only a moderate (≅4-fold) increase in sensitivity to oral Ty. Tranylcypromine increased the sensitivity >30-fold. Strikingly, MDL 72,394 plus CD treated dogs showed no change in sensitivity to either i.v. or oral Ty. Plasma DHPG decreased substantially following all treatments; control (Mean ± s.e.m. ng/ml): 1.16 ± 0.1; MDL 72,394 alone: 0.43 ± 0.2*; MDL 72,394 plus CD : 0.27 ± 0.1*; tranylcypromine: 0.34 ± 0.1* (*$p < 0.01$); indicating substantial and comparable inhibition of MAO.

Since dogs have a decarboxylase activity similar to man, these results suggest that MDL 72,394 alone will have little propensity to potentiate Ty and that carbidopa co-administration will further reduce this risk.

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THE EFFECTS OF NICORANDIL ON REPERFUSION-INDUCED ARRHYTHMIAS AND TISSUE LACTATE IN THE ISOLATED PERFUSED RAT HEART.

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Nicorandil (SG-75), an anti-anginal drug possessing both coronary and peripheral vasodilator activity (Sakai et al, 1983), has been demonstrated to attenuate myocardial acidosis associated with coronary artery occlusion in dogs (Abiko et al, 1984). In addition a potential antiarrhythmic efficacy has been demonstrated in canine Purkinje fibres in vitro (Imanishi et al, 1984).

We have investigated the efficacy of Nicorandil against reperfusion arrhythmias following coronary artery ligation (CAL) in the intact heart using the model of Lubbe et al (1978), together with the determination of tissue lactate, a close correlate of myocardial acidosis during ischaemia (Ichihara and Abiko, 1982).

Isolated hearts were perfused at a constant pressure of 75cm H_2O with either Krebs-Henseleit (5.9 mM K^+) or Krebs-Henseleit and Nicorandil.[2] Following 15 min of perfusion CAL was performed for 15 min followed by 15 min of reperfusion.

The effects of 1-100 uM Nicorandil on the incidence (%) of Ventricular Tachycardia (VT) and Fibrillation (VF), and on the number (mean ± s.e.mean) of Premature Ventricular Complexes (PVCs) on reperfusion are given in Table 1.

Table 1. Effect of Nicorandil (1-100 uM) on reperfusion arrhythmias in the isolated perfused rat heart.

		n	VT(%)	VF(%)	PVCs(No)
control		19	100	100	20±4
Nicorandil (uM)	1	9	89	89	27±8
	10	9	89	100	26±9
	25	9	100	89	27±13
	50	12	83	92	14±6
	100	9	78	78	9±5

All PVC and incidence values were N.S. from control (Unpaired T-test and χ^2 test respectively).

Ischaemic and non-ischaemic tissue were separated in ligated hearts on the basis of exclusion of Evan's blue stain injected into the perfusion line at the end of the ligation phase.

Control lactate levels were 22±2 and 138±7 uM/g dry weight in non-ischaemic and ischaemic tissue respectively ($P<0.001$, $n=8$), whereas in Nicorandil-treated hearts (50 uM) lactate levels were 30±4 and 126±8 uM/g dry weight respectively ($P<0.001$, $n=6$). Lactate levels between drug and control were not significantly different for either ischaemic or non-ischaemic tissue.

Therefore, in this model, nicorandil did not show a significant reduction in either reperfusion-induced arrhythmias or ischaemic lactate accumulation.

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THE EFFECT OF EDRF ON CONTRACTION OF RAT AORTA BY NORADRENALIN AND INTERACTION WITH AN α_2-ADRENOCEPTOR ANTAGONIST AND A Ca^{++} ACTIVATOR.

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In vitro, release of EDRF is continuous and spontaneous and can be increased by Ach (Furchgott, 1983; Furchgott et al., 1985) or by α_2-adrenoceptor aagonists (Cocks & Angus, 1983). In each case, the contractile effects of various agents on the adjacent vascular smooth muscle is attenuated as shown by the changes in responses caused by destroying (by mechanical rubbing) of the endothelium. Consequently the pressor effects of NA, of if modified by drugs, are both influenced by the endothelium.

We have now examined the α_1-mediated contraction to NA in rat aorta, with and without the endothelium, in the presence of an α_2-antagonist (Wyeth 26703) or in the presence of BAY-K 8644 a Ca^{++} activator to test the validity of the above factors and to see how the relexant effects are influenced.

Male wistar rats 235-280 grammes were stunned and bled. Two thoracic aortic rings 2 - 3 mm in length were mounted under 1.5g tension in Krebs Bicarbonate solution at 37°C bubbled with 95% O_2/5% CO_2 and allowed to equilibrate for at least an hour. A concentration-response curve (cumulative) to NA was first produced to obtain a maximum response for each tissue. Ach concentration-response curves were then obtained at various levels of pre-contraction by varying [NA]. Relaxations to low [Ach] were biphasic; an initial transient followed by a smaller but maintained relaxation (the later plateau taken as response). With pre-contraction of 110%-70% of original control maximums the inhibitory potency of Ach, measured as % loss of tone, decreased as initial tone increased e.g. at 70% contraction the -log IC_{75} was 7.51 ± 0.1 (n = 8) and at 110% was 7 ± 0.1 (n = 8) (mean ± s.e.m.).

After rubbing the intimal surface, compared with unrubbed tissues i) NA CRC shifts to the left. ii) Ach lost its relaxant effect (3 uM) in pre-contracted tissues. iii) Wyeth 26703 (10 nM - 100 nM) produced no potentiation of NA with or without rubbing, causing only attenuation as concentration increased and slightly facilitated the relaxant effect of Ach. iv) BAY-K 8644 (30 nM - 300 nM), in unrubbed tissues potentiated the contraction to NA and reduced the relxant effect of Ach by more than would be expected by the increase in NA-induced tone.

We conclude that contraction to NA is attenuated by spontaneous release of EDRF and that the inhibitory effect of Ach is inversely related to NA-induced tone. There was no evidence for α_2-mediated release of EDRF by NA. BAY-K 8644 does directly affect the vascular contraction to NA but may also indirectly increase it by inhibiting the spontaneous release or vascular effect of EDRF and attenuates the relaxation effect of Ach, presumably by a similar mechanism.

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BLOOD PRESSURE EFFECTS OF DOPAMINE AND BROMOCRIPTINE IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

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The involvement of catecholamines in the regulation of blood pressure in now well established. In contrast to peripheral alpha-receptor stimulation, brain noradrenaline may exert a decrease in blood pressure via medullary mechanisms. The central or systemic administration of dopamine agonists has been described to induce a fall in blood pressure. The site of action of this effect is unknown, however. Both central and peripheral mechanisms have been suggested in this respect. On the other hand, brain dopamine depletion inhibits the development of hypertension in SHR (Van den Buuse et al., 1984,1986). The present study describes the effect of systemic or intracerebroventricular (i.c.v.) administration of dopamine or bromocriptine, a dopamine agonist, on blood pressure of conscious, chronically cannulated SHR. Animals were 12-15 weeks of age, weighing 250-350 g.

Dopamine (0.5 mg/kg i.v.) induced an acute increase in blood pressure with a peak at 2 min after injection of +20 mm Hg. This was followed by a decrease in blood pressure with a maximum at 5-10 min of -15 mm Hg, lasting for more than 30 min. Haloperidol (0.5 mg/kg i.v.) blocked the hypotensive response to dopamine, but the immediate blood pressure increase was unaffected. Phentolamine (1 mg/kg i.v.) completely blocked both effects of dopamine. Propranolol (1 mg/kg i.v.) or domperidone (2 mg/kg i.v.) were ineffective in this respect, although a tendency was found for domperidone to inhibit the blood pressure decrease. After i.c.v. injection dopamine (200 ug, but not 50 ug or 100 ug/kg) induced a decrease in blood pressure. This effect was maximum at 10 min after injection (about -15 mm Hg) and the decrease lasted for 15-20 min.

Bromocriptine injected i.v. (250 ug/kg) or i.p. (250 or 500 ug/kg) induced a marked decrease in blood pressure. The maximum was reached at 15 min after injection (-25 mm Hg) and the effect lasted for more than two hours. Haloperidol (0.5 mg/kg i.p.) completely blocked the effect of bromocriptine. However, the i.c.v. pretreatment with haloperidol (50 ug/kg) did not influence the bromocriptine-induced blood pressure decrease. Domperidone (2 mg/kg i.p.) completely blocked the hypotensive effect of bromocriptine. After i.c.v. injection bromocriptine (50 ug, but not 10 ug/kg) induced a slight decrease in blood pressure with a maximum at 30 min after injection (-8 mm Hg).

These results show that the systemic administration of dopamine or bromocriptine may induce a marked decrease in blood pressure in SHR, an effect involving dopamine receptors. The i.c.v. administration of either compound also decreased blood pressure, but the effect was smaller and relatively high doses were needed. Pretreatment with domperidone blocked the response to bromocriptine. This DA_2 dopamine receptor antagonist does not cross the blood-brain barrier, suggesting a peripheral site of action especially for bromocriptine. It needs further investigation to establish whether leakage to the periphery or interaction with receptors other than dopamine is involved in the effects of i.c.v. administered dopamine agonists. It is concluded that peripheral dopaminergic mechanisms may be involved in the regulation of blood pressure. The present results as yet do not warrant a conclusion about central dopaminergic mechanisms in relation to blood pressure. It is clear, however, that the effects of systemically administered dopamine or bromocriptine on blood pressure are mediated primarily by a peripheral mechanism.

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EFFECTS OF ERGOTAMINE ON CARDIOVASCULAR CATECHOLAMINE RECEPTORS IN THE PITHED RAT.

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Several studies have reported that the pithed rat is a suitable model to study drugs acting at both presynaptic and postsynaptic α-adrenoceptors and presynaptic dopamine receptors in the cardiovascular system(Clapham & Hamilton, 1982; Vila et al, 1985). Ergotamine is a classical ergot alkaloid that could act on different types of receptors. The purpose of the present study has been to evaluate the action of ergotamine on different catecholamine receptors that participate in sympathetic cardiovascular responses in the pithed rat.

Male Sprague-Dawley rats were pithed and prepared for diastolic blood pressure (DBP) and heart rate (HR) measurements as previously described (Moron et al, 1986).

Ergotamine (3-10 µg/kg) inhibited the increases in DBP induced by electrical stimulation (0.25-6 Hz, supramax voltage,0.5 msec) of the sympathetic outflow without modifying the DBP increases induced by noradrenalina (0.1 to 3µg/kg). Yohimbine (0.3 mg/kg), as well as sulpiride (0.3 mg/kg), partially prevented the inhibitory effect of ergotamine on stimulation-induced pressor responses. Combination of both antagonists abolished attenuation of the stimulation-induced pressor responses by ergotamine (10 µg/kg). Electrical stimulation at the level C7-Th1 produces frequency-dependent increases in HR. Ergotamine (3 µg/kg) only inhibited the HR increases at low frequencies of stimulation (0.25-1 Hz) and ergotamine (10 µg/kg) inhibited them at all the frequencies studied. Pretreatement with yohimbine (0.3 mg/kg) abolished the effect of a low dose of ergotamine (3 µg/kg) but only partially prevented that to 10 µg/kg of ergotamine. Sulpiride (0.3 mg/kg) did not modify the inhibitory effect of ergotamine on stimulation-induced HR increases, results that suggest once more a lack of presynaptic dopamine receptors in the rat heart nerve terminals. Tachycardia elicited by noradrenaline(0.1 to 3 µg/kg) was not reduced by previous administration of ergotamine (3-10 µg/kg). Cumulative ergotamine (1 to 100 µg/kg) administration produced DBP increases that were inhibited to the same extent by yohimbine (0.3 mg/kg) and prazosin (1 mg/kg). When both antagonists were administered together the inhibition of the ergotamine-induced pressor responses was greater. On the other hand ergotamine (3-10 µg/kg) did not modify the DBP responses induced either by methoxamine (10 to 300 µg/kg) or by xylazine (30 to 3000 µg/kg). Results with higher doses of ergotamine (30-100 µg/kg) against pressor responses to both agonists were difficult to evaluate because of the powerful vasoconstrictor effect of the ergot alkaloid at these doses.

These results suggest that ergotamine at the doses used acts on the peripheral sympathetic nervous system of the pithed rat as an agonist of presynaptic dopamine receptors and α_2-adrenoceptors as well as an agonist of α_1 and α_2-postsynaptic adrenoceptors.

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ARE THROMOBOXANE OR PROSTAGLANDIN RECEPTORS INVOLVED IN THE VASCULAR ACTIONS OF LIPOXYGENASE METABOLITES OF ARACHIDONIC ACID?

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The lipoxygenase metabolites of arachidonic acid, 15-hydroperoxyeicosatetraenoic acid (15HPETE) and its hydroxy derivative (15HETE) evoke contractions in a variety of isolated blood vessels (Trachte et al., 1979; Asano and Hidaka 1979; Koide et al., 1981; Van Diest et al., 1986). We recently were able to show that 15HETE and 15HPETE cause relaxations of isolated dog arteries when the tissues are contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or with the thromboxane (TX) - mimetic U46619 but not when they are contracted with noradrenaline, serotonin or K^+ (Van Diest et al., 1986). The contractions caused by PG's and TX most likely are due to activation of specific receptors on the vascular smooth muscle cells (Coleman et al., 1984) and thus our results may indicate a possible antagonism of the lipoxygenase metabolites toward these receptors (Van Diest et al., 1986). The present study was designed to investigate the effects of the TX-receptor antagonist BM13177 on the contractions caused by 15HETE, 15HPETE and $PGF_{2\alpha}$ in dog splenic arteries.

Segments of the arteries were placed in organ chambers filled with Krebs-Ringer solution at 37°C for isometric tension recording. Responses to 15HETE, 15HPETE and $PGF_{2\alpha}$ were obtained either in control solution or in the presence of different concentrations of BM13177 (6.3×10^{-8} to 2×10^{-5} M). The antagonists shifted the dose-response curve to $PGF_{2\alpha}$ to the left without affecting the maximal response. Table 1 illustrates the increased ED_{50}-values obtained with $PGF_{2\alpha}$ in presence of BM13177; in these experiments the pA_2-value for the antagonist averaged 6.8±0.3.

Table 1 : Effect of BM13177 on ED_{50}-values for prostaglandin $F_{2\alpha}$.

BM13177 (-Log M)	0	7.2	6.7	6.2	5.7	5.2	4.7
ED_{50} $PGF_{2\alpha}$ ($\times 10^{-7}$M)	6.0	9.9	12.5	22.2	87	171	1330

Also the contractions caused by 10^{-5} M of 15HETE and 15HPETE were concentration-dependently inhibited by BM13177 as shown in Table 2.

Table 2 : Effect of BM13177 on contractions caused by 10^{-5} M of 15HETE and 15HPETE

BM13177 (-Log M)	0	7.2	6.7	6.2	5.7	5.2	4.7
% of control 15HETE (100% = 13.1±2.6 g)	100	85±6	81±7	56±8	22±6	7±2	5±3
% of control 15HPETE (100% = 4.9± 1.2 g)	100	70±9	51±11	34±4	18±5	13±6	1±0.5

These experiments illustrate that BM13177 antagonizes the responses to $PGF_{2\alpha}$ and those to the lipoxygenase metabolites; they indicate that possibly 15HETE and 15HPETE act on the same (or a similar) receptor as $PGF_{2\alpha}$ and add further evidence in support of the hypothesis that both lipoxygenase compounds may act as endogenous antagonists of PG or TX receptors.

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EFFECT OF ILOPROST (ZK36374) AND PROSTACYCLIN ON IN VITRO HUMAN CEREBRAL ARTERIES.

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Prostacyclin (PGI_2) has been demonstrated to be formed in human cerebral arterial endothelial cells and been suggested to be a physiological cerebral vasodilator (Boullin et al. 1979). It has the capacity to reverse contractions of human cerebral arteries to a variety of agents probably involved in cerebral arterial spasm (CAS) (Paul et al. 1982). The problem with the potential use of PGI_2, for treatment of CAS are its short half life and the fact that high concentrations contract human cerebral arteries in vitro. The carbacyclin derivative, iloprost, is a chemically stable PGI_2-mimetic agent equipotent with PGI_2 in vitro (Schror et al. 1981). This study compares the effect of PGI_2 and iloprost on human cerebral arteries in vitro under resting and sustained tension conditions.

Human cerebral (basilar and vertebral) arteries were obtained 6-18h post mortem. The arteries were cut into spiral strips and placed in 4 ml tissue baths containing Krebs-Henseleit solution at 37°C bubbled with 95% O_2/5% CO_2 at a resting tension of 1-1.5g. After a 90 min equilibration period reactivity of the tissues to KCl was assessed. A concentration-effect (C-E) curve to Phenylephrine (PE) was constructed on each tissue and an approximate EC50 selected. C-E curves were produced to iloprost or PGI_2 on tissues under resting tension or on tissues precontracted with the selected EC50 of PE. These experiments were then repeated in the presence of the thromboxane receptor antagonists AH23848, 10^{-6}M (Humphrey & Lumley, 1984) or EP045, 10^{-5}M (Jones et al, 1982).

Both iloprost and PGI_2, 10^{-10} to 10^{-7}M produced concentration related relaxations of tissues under resting tension or precontracted with PE. Both compounds demonstrated a similar potency in this action. Concentrations of both iloprost and PGI_2 above 10^{-7}M (10^{-6} to 10^{-5}M) produced almost equivalent concentration-related contractions of the tissues. C-E curves to PGI_2 could be repeated and reproduced at 20 min intervals. In contrast, iloprost appeared to produce a long lasting relaxant effect on tissues taking up to 1-1½h to wash off. When C-E curves to iloprost and PGI_2 were repeated in the presence of AH23848 or EP045 under resting or sustained tension, relaxant responses remained the same but the contractile responses seen with high concentrations of each agent were significantly ($P<0.05$) attenuated. The concentrations of both AH23848 and EP045 used produced significant ($P<0.001$) selective antagonism of contractions to U-46619 and also demonstrated partial agonist action on some tissues.

These results are consistent with previous observations that high concentrations of PGI_2 contract human cerebral arteries in vitro (Paul et al. 1982) and that the effecct can be blocked by EP045 (Lye et al. 1983). Iloprost appears to possess an equivalent profile of action to PGI_2 on human cerebral arteries causing relaxation at low and contraction at high concentrations, the latter effect being attenuated by thromboxane receptor antagonists. The major difference between iloprost and PGI_2 in this study appears to be the long lasting relaxant effect of iloprost after washing off.

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ORAL ACTIVITY OF THE PROSTACYCLIN MIMETIC, RS-93427*, DELINEATED IN A NEW HETEROLOGOUS EX-VIVO PLATELET FUNCTION ASSAY.

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We developed a heterologous ex-vivo platelet aggregation assay that allows rapid screening for prostacyclin mimetic compounds with oral activity. In addition to producing data that is highly reproducible, interspecies variability in platelet responsiveness to inducers or inhibitors of platelet aggregation is eliminated (see Mahmud, et al., 1984; Willis et al., 1986).

In initial screening, male Hartley strain guinea-pigs of 250-450 g were anesthetized with ether, a PE90 x 11 cm polyethylene catheter inserted in the carotid artery, and anesthesia maintained with phenobarbital (100 mg, 1 ml/kg in 0.9% NaCl) injected retrogradely down the carotid cannula. Samples (0.5 ml) of blood were withdrawn at regular intervals into 0.1 vol of 3.8% w/v aqueous sodium citrate, and then centrifuged for one minute at 13,000 g to prepare platelet-poor plasma (PPP). A small aliquot (usually 200 µl) was then mixed with an equal volume of citrated human platelet-rich plasma (PRP), using one donor for the entire experiment. Platelet aggregation was routinely induced by ADP (1.6 µg/ml, final concentration); and after aggregation was completed, the platelets were removed by twice sequentially centrifuging for 1 min at 13,000 g to prepare platelet free plasma (PFP) which was clotted with $CaCl_2$ to prepare serum. This was frozen (-20°C) for subsequent estimation of released platelet factor 4 (PF4) and mitogens including platelet-derived growth factor (Willis, et al., 1986). For further evaluation, a similar procedure was used in sedated (30 mg/kg pentobarbitone sodium) chaired male baboons (Papio papio, 10-20 kg). Blood pressure was examined by sphygmomanometry.

In both species, RS-93427 was orally active with onset of activity within 30 min. At 500-1,000 µg/kg, oral activity was prolonged past 3.5 hr. In the baboon, such doses produced increases (30-70%) in heart rate with reductions of 20-60% in diastolic blood pressure. In 6/8 baboons treated orally with RS-93427, there were sporadic increases of ~5-20% in whole blood fibrinolytic activity examined by the method of Moroz and Gilmore (1980).

Both in vitro and ex vivo studies showed that RS-93427 inhibited platelet release of PF4 and mitogens at plasma concentrations that were similar to or even lower than those producing reduction in platelet aggregation. Release of macrophage mitogens is also suppressed (Willis et al., 1986).

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*4-Z-{(3'S,1S,2S,3R,6S)-2-(3'-cyclohexyl-3'-hydroxyprop-1-ynyl)-3-hydroxy-bicyclo [4.2.0]oct-7-ylidene}butyric acid.

RAT GASTRIC MUCOSA BARRIER PROTECTION AND PGE_2 GENERATION AFTER COLLOIDAL BISMUTH SUBCITRATE (DE-NOL).

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Cytoprotection (gastric mucosa barrier protection) has been defined as the ability of pharmacological agents, originally prostaglandins, to prevent gastric injury by a variety of ulcerogenic agents through a mechanism not involving inhibition of acid secretion (Robert et al. 1979). Prostaglandins at non-antisecretory doses stimulate numerous processes that increase gastric mucosa barrier defence (Miller, 1983). The therapeutic efficacy of some anti-ulcer drugs may be mediated by local stimulation of mucosal prostaglandins (Hollander et al, 1984). Colloidal bismuth subcitrate (CBS; DE-NOL) potently inhibits Shay and stress ulcers in rats (Wieriks et al. 1982), and is a well recognized effective treatment of peptic ulcer disease. To further define its gastric protective effects we have examined CBS in comparison with PGE_2, sucralfate and cimetidine on ethanol induced gastric erosions; and, to investigate its mechanism of action, whether CBS administered to rats can stimulate the generation of PGE_2 in biopsies of gastric mucosa.

Fasted Wistar rats received intra gastric treatment, and at 1h thereafter (dose response) or at various times thereafter (time course) 1 ml absolute ethanol was administered (ethanol gastritis studies) or the rats were sacrificed (PGE_2 generation studies). In the ethanol gastritis experiments the rats were sacrificed after a further 1h and mucosal injury was scored on excised stomachs by two observers unaware of the protocol. For the PGE_2 generation experiments the stomachs were excised, dissected along the greater curvature and two 3 mm biopsies removed from standardized places on either side of the washed fundus. To stimulate PGE_2 generation each biopsy was vortexed for 60 s in Tris/HCl buffer according to Whittle (1978). Indomethacin (200 µm) was added and PGE_2 determined by radioimmunoassay in the supernatant.

CBS (30-120 mg/kg) was less potent than PGE_2 (60-240 µg/kg) though 3-4 times more potent than sucralfate (120-480 mg/kg) at inhibiting gastric lesions. Cimetidine (480 mg/kg) inhibited weakly. Complete, partial and no protection against ethanol induced lesions was afforded by CBS at 0.25, 8 and 16h respectively. Basal levels of PGE_2 in distilled water treated rats were 136 $\pm$ 15 pg/mg tissue (s.e. mean, n=9). CBS at 30 mg/kg increased PGE_2 levels at 1h to 170 $\pm$ 18 pg/kg rising dose dependently to 285 $\pm$ 20 pg/mg at 480 mg/kg. Maximum generation of PGE_2 occurred at 0.25h (345 $\pm$ 49 pg/mg), and basal levels returned at 4h after CBS (120 mg/kg).

We conclude that CBS produces potent gastric mucosa protection in rats, having a fast onset and protective effects up to 8h. CBS produced dose dependent local generation of rat gastric mucosa PGE_2. There was a fast onset and basal levels returned at 4h. Since there is a large discrepancy between the duration of the gastric mucosa protective effects and PGE_2 generation after CBS, we suggest that both prostaglandin and non-prostaglandin mediated mechanisms are involved in the action of CBS on gastric mucosa.

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