LEUKOTRIENES - A ROLE IN THE PATHOGENESIS OF BYSSINOSIS?

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Byssinosis is an occupational lung disease of the cotton industry. Acute symptoms include chest tightness, shortness of breath and a decrease in ventilatory capacity (McKerrow et al. 1958). Inhalation of cotton dust causes perturbations of the respiratory pattern of guinea pigs (Ellakkani et al. 1984) and this species has been suggested to be a suitable animal model for the study of the disease. Recently, the bronchoconstrictor response of the guinea pig to inhaled cotton dust extract (CDE) was found to be blocked by the cysteinyl-containing leukotriene antagonist FPL 55712 (Johnson & Nicholls, 1987). The present work further investigates this phenomenon by examining the effect of the 5-lipoxygenase inhibitors piriprost (U-60,257; Bach et al. 1985) and azelastine (Zechel et al. 1981) upon the action of CDE.

Constant volume plethysmography was employed to measure specific airways conductance (sGaw) in unanaesthetized female guinea pigs (350-500g). Animals were exposed to an aerosol of an aqueous extract of cotton dust (equivalent to 6g dust/50ml saline) for 6 min and sGaw was measured 1, 2 and 3.5h later. Piriprost (0.5% w/v) was administered by aerosol (3 min exposure) immediately prior to challenge with CDE. Azelastine (0.14mg/kg) was administered orally 6h before exposure to CDE. The results are presented in the Table and it may be observed that following exposure of the animals to CDE there was a significant ($P<0.01$) decrease (bronchoconstriction) in sGaw at 1 and 2h. By 3.5h recovery to within baseline values ($0.54\pm0.14 cmH_2O/s$) had occurred. Administration of either piriprost or azelastine alone had no significant ($P>0.01$) effect on sGaw. However, both agents were able to inhibit the airways response to the inhaled CDE.

Table Effect of piriprost and azelastine on airways response of guinea pigs to inhaled cotton dust extract

	Challenge	sGaw (% change from baseline) at 1	2	3.5h
Group 1*	CDE	-21(±3)	-24(±3)	- 9(±4)
	CDE + piriprost	- 3(±4)	- 9(±5)	- 9(±4)
Group 2*	CDE	-22(±4)	-23(±3)	-10(±3)
	CDE + azelastine	- 3(±3)	- 5(±4)	+ 2(±2)

Values are means ± s.e.mean, n=15. For doses and dosing schedule see text. *In each group, the same set of animals was used for exposure to both CDE and CDE + enzyme inhibitor. There was a 1 week interval between the two exposures.

The data provide additional support for the view that the acute bronchoconstrictor response to CDE is at least partly due to the synthesis and release of bronchoconstrictor leukotrienes.

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ROLE OF THROMBOXANE A_2 IN THE EFFECTS OF ADRENALINE ON THROMBIN AND COLLAGEN-INDUCED PLATELET RESPONSES

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Besides directly activating platelets at high concentrations (1-10μM), adrenaline (adr), at lower concentrations sub-threshold for activation, can potentiate platelet responsiveness to a variety of agonists [O'Brien 1964; Mills & Roberts 1967]. Although endogenous thromboxane A_2 (TxA_2) formation and release of granule constituents are known not to be pre-requisites for the potentiation of agonist-induced platelet aggregation by adr, the role of TxA_2 in the potentiation by adr of agonist-induced secretion itself is unclear. Previous workers in this area [Steen & Holmsen 1985; Bushfield et al 1987] have not addressed this question, although Bushfield et al concluded that the effects of adr were mediated via an increase in 1,2-diacylglycerol (DAG) formation and intracellular calcium, $[Ca^{2+}]i$ levels. We have studied the effect of adr on arachidonate release and 5HT secretion induced by varying concentrations of thrombin and collagen, in the presence and absence of the cyclooxygenase inhibitor, indomethacin, to determine the role of TxA_2 in mediating these platelet responses.

Washed, human platelets prelabelled with [^{3}H]arachidonic acid (AA) or [^{14}C]-5HT were used, adr was added 10 sec before thrombin or collagen and the reaction was terminated 3 min later. In the range 1-100μM , adr induced no detectable aggregation or 5HT secretion, but potentiated platelet aggregation when added with sub-threshold concentrations of thrombin or collagen, which on their own induced no secretion. At 2-4 fold higher concentrations of thrombin and collagen (threshold to sub-maximal for 5HT secretion - (0.03-0.05U/ml thrombin and 0.5-2μg/ml collagen), 5HT secretion and AA/TxB_2 release induced by these agonists were also potentiated by adr (1-10μM) by 30-50%. Collagen (0.5-2μg/ml)-induced phosphorylation of a 45Kd protein (an effect attributed to activation of protein kinase C by endogenously formed DAG) was also potentiated 200-300% by adr. Pre-treatment of platelets with indomethacin (10μM) abolished threshold to sub-maximal thrombin and collagen-induced 5HTsecretion, collagen-induced 45Kd protein phosphorylation, as well as the potentiation of these responses by adr. However, approximately 2-fold and 5-fold higher concentrations of thrombin and collagen respectively were able to induce 'indomethacin-insensitive' secretion and 45Kd protein phosphorylation, which was further potentiated by adr. In indomethacin-treated platelets, collagen (0.5-2μg/ml)-induced AA release and its potentiation by adr were also abolished, suggesting a role for endogenously formed TxA_2. This was confirmed by addition of the TxA_2 mimetic, U46619 (0.3μM), which potentiated collagen-induced AA release in the presence and absence of adr, even though it induced no AA release on its own or in combination with adr alone. The higher agonist (thrombin & collagen) concentrations at which adr potentiates 5HT secretion and 45Kd protein phosphorylation in the presence of indomethacin, compared to that in the absence of indomethacin, suggests that the primary effect of adr in the potentiation of agonist-induced platelet responses is the potentiation of TxA_2 formation. The potentiation of agonist-induced $[Ca^{2+}]i$ rises and DAG formation reported earlier [Bushfield et al 1987] can be explained at low agonist concentrations, as events secondary to, and caused by TxA_2 formation. Our results also implicate endogenous TxA_2 as an amplifying factor in collagen-induced AA release.

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230P

INFLAMMATORY EFFECTS OF INTERLEUKIN-1 AND FMLP IN RABBIT SKIN

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In acute inflammation, the accumulation of polymorphonuclear leucocytes (PMNs) occurs at the same time as increased vascular permeability but there are opposing views as to the importance of PMNs in bringing about vascular changes. Humphrey (1955) found that inflammation associated with Arthus reactions was dependent upon PMNs but Willoughby and Spector (1968) showed that the oedema caused by thermal injury or turpentine-induced pleurisy was not reduced in PMN-depleted animals. More recently, Wedmore and Williams (1981) reported that plasma exudation induced by a number of chemotactic factors is dependent upon circulating PMNs. Interleukin 1 (IL-1) causes the accumulation of both PMNs and mononuclear leucocytes in rabbit joints (Pettipher et. al., 1986) but does not cause joint swelling. We have now compared IL-1 with another chemotactic factor, formyl-methionyl-leucyl-phenylalanine (FMLP), for their relative activities in causing leucocyte accumulation and plasma exudation in rabbit skin.

The effects of highly purified human IL-1 (Genzyme, Suffolk, U.K.), or FMLP on leucocyte accumulation in the dermis, 30 min, 90 min, 4h and 24h after intradermal injection were measured. IL-1 was supplied in a solution of 100 units/ml in 5% foetal calf serum (FCS) with a specific activity of 8 units/pg of protein. Test solutions were diluted with saline and similar solutions of FCS were diluted in the same way to provide appropriate controls. FMLP was dissolved in saline. All test solutions were injected intradermally in the shaved backs of New Zealand White rabbits (2.5 - 3.5 kg) in volumes of 100µl. After killing the animals, skin injection sites were fixed, processed, cut and stained for microscopic examination. Slides were read on a randomised blind basis and the numbers of PMNs were counted in five high-power fields (x1000) arranged vertically through the dermis (Higgs et. al., 1981). Plasma exudation was measured following the intravenous injection of ^{125}I human serum albumin and the detection of radioactivity at skin injection sites (Wedmore and Williams, 1981).

Significant PMN accumulation in the dermis was apparent 30 min after injection of IL-1 (10 units) or FMLP (20ng) but highest numbers of cells were seen after 4h and by 24h the response had subsided to control values. At 4h, IL-1 induced the accumulation of significantly ($p<0.05$) more PMNs (140±12, mean ± s.e. mean, n=7) than FMLP (76 ± 11). FMLP also caused plasma exudation which peaked at 90 min (78 ± 16µl) whereas IL-1 did not cause significant plasma leakage. The vasodilator prostaglandin (PG)E_2 (10ng) had no effect on leucocyte accumulation or plasma exudation but enhanced vascular permeability caused by FMLP (198 ± 40µl). Combinations of IL-1 and PGE_2 caused some leakage but the effect was less than 25% of that caused but combinations of FMLP and PGE_2. These results indicate that leucocyte accumulation is not directly correlated with increased vascular permeability in rabbit skin.

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EFFECT OF A PLATELET-ACTIVATING FACTOR ANTAGONIST AND CYCLOSPORINE A ON PAF-INDUCED BRONCHOCONSTRICTION IN GUINEA-PIGS

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The effects of a PAF antagonist, BN 52021 (Braquet et al., 1987), and cyclosporine A (CsA), either alone or in combination, on PAF- and antigen-induced bronchoconstriction were investigated in control and passively sensitized guinea-pigs, respectively. CsA (25 mg/kg) or BN 52021 (15 mg/kg or 30 mg/kg) or both were given orally either 2 h or twice a day for 48 h prior to challenge with PAF or antigen. Bronchopulmonary responses were recorded according to the Konzett and Rossler's method.

Injection of PAF (60 ng/kg) in the jugular vein induced an important bronchoconstriction accompanied by a decrease in the number of circulating platelets and leukocytes. CsA did not inhibit the effects induced by PAF, nor did the low dose of BN 52021. In contrast, the combination of BN 52021 with CsA markedly reduced (81 % inhibition, $p < 0.01$) the bronchoconstriction induced by PAF. As well, the accompanying thrombocytopenia and leukopenia were inhibited to a similar extent.

The activity of PAF antagonists has previously been shown to be inhibited by β antagonists. Therefore, in some experiments, guinea-pigs received an i.v. injection of the β_1 selective antagonist, atenolol (1 mg/kg) or of the β_2 antagonist, butoxamine (1 mg/kg). Both compounds reduced the inhibitory action of the combination of CsA and BN 52021 on the PAF-induced bronchoconstriction (CsA + BN 52021 ; 81 % inhibition, CsA + BN 52021 and atenolol ; 54 % inhibition, CsA + BN 52021 and butoxamine ; 39 % inhibition).

Passively sensitized animals were challenged by an i.v. injection of 0.75 mg/ml ovalbumine. Oral administration of CsA (12.5 mg/kg, twice a day from 48 h before antigen challenge) significantly reduced (- 68 %, $p < 0.001$) the antigen-induced bronchoconstriction. BN 52021 (15 mg/kg, twice a day) had no effect whereas at 30 mg/kg (twice a day) it produced a 32 % inhibition. The combination of CsA (12.5 mg/kg, twice a day) with an inactive dose of BN 52021 (15 mg/kg, twice a day) gave a similar inhibition as CsA alone (65 %, $p < 0.05$). Surprisingly, the association of CsA with the active dose of BN 52021 (30 mg/kg, twice a day) led to a lower inhibition of antigen-induced bronchoconstriction as compared to that obtained with CsA alone (25 % vs 68 %, respectively).

The reason why CsA potentiates the effect of BN 52021 on PAF-induced bronchoconstriction is presently unexplained. Among various assumptions, the possible inhibition of PAF-induced tumor necrosis factor (TNF) release may account for this phenomenon. Indeed, it has been shown that PAF induces TNF release from various cell systems. In addition, the involvement of the adenylate cyclase complex deserve futher investigations.

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EFFECT OF EP 092 ON WHOLE BLOOD AGGREGATORY RESPONSES *EX VIVO* AND *IN VIVO*

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EP092; (±-endo-(6'-carboxyhex-2'Z-enyl)-6-exo-[1'-[N-(phenylthiocarbamoyl)-hydrazono]-ethyl]-bicyclo [2,2,1]-heptane) is a potent, selective antagonist at the human platelet thromboxane A_2 receptor *in vitro* (Armstrong et al, 1985). However, the potency and duration of action of this compound against platelet responses *in vivo* remains to be fully evaluated. In the present study, we have investigated the ability of EP092 to inhibit collagen-induced whole blood aggregation *ex vivo* (guinea pig, Rhesus monkey) and collagen or thrombin-induced intravascular aggregation *in vivo* (rabbit). Whole blood aggregation *ex vivo* was induced using a range of collagen concentrations (0.03-3, 0 μg ml) in 0.5 ml citrated blood samples, pre-equilibrated to 37°C and stirred at 1000 rpm. Platelet aggregation (change in blood single platelet count) was monitored in sequential 50 μl samples withdrawn prior to and at 1 minute intervals up to 4 minutes following addition of collagen, by rapid micor-centrifugation and single platelet counting (Coulter Thrombocounter). Intravascular aggregation *in vivo* was determined in dial-urethane anaesthetized NZW rabbits (2.5-3.5 kg) using the continuous platelet count monitoring technique of Smith and Freuler, 1973.

EP092, administered to guinea pigs by intravenous (0.03-3.0 mg kg^{-1}) or oral (0.3-10.0 mg kg^{-1}) routes, significantly inhibited collagen-induced platelet aggregation *ex vivo* in dose-related fashion. Intravenously, the effect of EP092 was maximal at 30 minutes after drug administration (ED_{50}: 1.30 mg kg^{-1}), and the duration of action appeared <3 hours. Orally, peak inhibitory activity against collagen was observed 1 hour after dosing (ED_{50}: 1.40 mg kg^{-1}) and the duration of action (>50% inhibition of submaximal collagen responses) was >3 hours following 3 or 10 mg kg^{-1} doses. In the Rhesus monkey, similar results were obtained, (Table 1) with collagen-induced aggregation again being inhibited by orally administered EP092 in dose-related fashion (ED_{50}: 0.9 mg kg^{-1} at 1 hour after dosing).

Table 1 **Effect of EP092 on collagen-induced aggregation of Rhesus monkey whole blood *ex vivo***

EP092 dose (mg kg^{-1} p.o.)	n	Inhibition (mean % + s.e.mean) of collagen aggregation ex vivo at time after oral dose: 1 hour	3 hours
0.3	4	22 ± 8	20 ± 15
1.0	4	52 ± 7*	33 ± 9
3.0	5	60 ± 12*	76 ± 8*
10.0	5	87 ± 7*	73 ± 6*

*Statistical significance: $p<0.05$ relative to vehicle controls (Student's t-test).

When given by intravenous infusion to anaesthetised rabbits, EP092 was also effective against intravascular aggregatory responses induced by i.v. bolus doses of collagen (30 μg kg^{-1}) and thrombin (10 units kg^{-1}). In this system, EP092 (1 mg kg^{-1} min^{-1} i.v. infusion for 10 minutes) partially inhibited the response to both agonists, peak activity immediately following drug infusion being 46±8% inhibition of the collagen responses ($n=5$; $p<0.02$) and 35±7% inhibition of thrombin induced aggregation ($n=4$; $p<0.05$).

These studies demonstrate that EP092, administered by intravenous or oral routes, possesses significant inhibitory activity against platelet aggregatory responses in whole blood measured *ex vivo* and *in vivo*. Moreover, whilst reinforcing the recognised central role of prostaglandin H_2 and thromboxane A_2 in collagen-induced platelet aggregation across the species, the data also further support the possibility of a thromboxane A_2-dependent component in the rabbit intravascular platelet response to thrombin as previously suggested (Honey et al, 1986).

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INHIBITION OF COUGH AND BRONCHOCONSTRICTION IN THE GUINEA-PIG BY OPIATES: EVIDENCE FOR A PERIPHERAL ACTION

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Cough suppression by opiates is generally believed to be due to inhibition of a "cough-centre" in the CNS. Opiate receptors have, however, been localized to the peripheral nervous system and the possibility of a peripherally mediated antitussive effect has been suggested (Parsons et al 1986). In the guinea-pig, citric acid (CA) produces cough and reflex bronchoconstriction (Bc), mediated through capsaicin-sensitive C-fiber afferents (Forsberg & Karlsson 1986). We have now studied the effects and sites of action of opiates on CA induced cough and Bc in guinea-pigs.

Unanaesthetized guinea-pigs (275-400 g), pretreated with opiates or saline, were exposed to an ultrasonically generated (Omron NEU 10) aerosol of CA (0.39 M). The number of coughs during the first 3 min of exposure was counted and the time to onset of Bc (development of a slow laboured breathing with exaggerated abdominal movements) recorded. Statistical differences were calculated by use of Student's t-test for unpaired observations. Mean ± SEM values are reported (n=8 in each group).

Codeine (Co, 1-10 $mg \cdot kg^{-1}$, i.p.) and morphine (Mo, 1-10 $mg \cdot kg^{-1}$, i.m.) dose-dependently inhibited CA induced coughing and Bc. At max doses (30 min after administration), cough was inhibited by 79.6±8.7% ($P<0.05$) and 87.3±8.4% ($P<0.01$) by Co and Mo, respectively, and the time to onset of Bc was significantly prolonged ($P<0.01$) by both drugs. Nebulized (5 min) Co (10-30 $mg \cdot ml^{-1}$) and Mo (10-30 $mg \cdot ml^{-1}$) also significantly inhibited both cough and Bc and the maximum dose inhaled was calculated to be about 0.4 $mg \cdot kg^{-1}$. Max effects were obtained 5 min after exposure. The very similar potencies and time courses for Co and Mo indicate that Co may act without conversion to Mo.

Naloxone (100 $\mu g \cdot kg^{-1}$, i.m.), without affecting CA induced responses, completely inhibited the antitussive ($P<0.05$) and partly the antibronchoconstrictor ($P<0.01$) effects of Co. In contrast to naloxone, nalorphine (30 $mg \cdot kg^{-1}$, i.m.) inhibited only partly Co's antitussive (by 58.0±25.9%, P=NS) and antibronchoconstrictor (by 43.4±36.6%, P=NS) effects. Nalorphine significantly ($P<0.001$) inhibited CA induced cough and Bc. The low potency of nalorphine compared with that of naloxone to antagonize Co as well as nalorphine's own inhibitory actions appear to suggest the involvement of opiate kappa-receptors in the inhibition of CA induced cough and Bc. In levallorphan methyl iodide (a quaternary opiate antagonist; 10 $mg \cdot kg^{-1}$, i.m., n=4) treated guinea-pigs, inhaled Co (30 $mg \cdot ml^{-1}$) was completely without effects.

In addition to well known antitussive effects of opiates, these agents were found to attenuate a reflex Bc. It is concluded that in the guinea-pig, opiates may have peripheral antitussive and antibronchoconstrictor effects since 1) inhaled opiates had a rapid onset of action 2) the equieffective inhaled dose was much smaller (20 times) than the systemically administered and 3) an opiate antagonist, with a limited penetration into the CNS, antagonized completely Co.

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EFFECTS OF NIFEDIPINE AND α-ADRENOCEPTOR ANTAGONISTS ON RESPONSES OF RAT VAS DEFERENS TO TWIN PULSE FIELD STIMULATION

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Desheathed rat vasa deferentia were suspended under 0.5 g resting tension in 10 ml tissue baths containing Krebs solution gassed with 5% CO_2 in O_2 at 37°C. Tension responses at 5 min intervals to challenges of twin pulse field stimulation (TPFS - 0.8 msec width, 40 V via parallel platinum electrodes) were digitised at a sampling frequency of 100 Hz and stored on floppy disc under the control of a BBC microcomputer (Marshall & Sparks, 1981).

When the inter-pulse interval of TPFS is 3 s, the response to each pulse of the pair is different (Marshall & Spriggs, 1984): the first shows the characteristic two components of a large first peak at 280 msec followed by a second, smaller peak, 650 msec after the stimulus. Nifedipine selectively inhibits the first component of the response (French & Scott, 1981). The second response attains a greater first peak height with a diminished second phase, compatible with NA causing feedback inhibition on presynaptic alpha-2-adrenoceptors.

Using an inter-pulse interval of 3 s, nifedipine induced a dose-related inhibition of the first phases of the first and second responses to TPFS (90.2% + 6.7% and 95.5% + 3.6% respectively at 10 uM, n = 8) and revealed a small residual component in the second response peaking at 600 msec. In the presence of nifedipine (10 uM) the selective alpha-2-adrenoceptor antagonists yohimbine or imiloxan (10 nM - 10 uM) produced similar effects on the nifedipine modified responses: at 10 nM & 100 nM, each drug inhibited the first response with a small potentiation of the second response: at 1 uM further inhibition of the first response was accompanied by inhibition of the second response: at 10 uM both responses were further inhibited and became of similar magnitude. The selective alpha-1-adrenoceptor antagonists WB4101 or prazosin (1 nM - 1 uM) produced comparable effects in combination with nifedipine which differed from those of yohimbine and imiloxan in that only inhibition of both responses occurred.

These results demonstrate that the residual component remaining in the second response to TPFS following nifedipine treatment is susceptible to alpha antagonists, implicating a noradrenergic mechanism. Although low doses of the alpha-2-antagonists produce some potentiation of this residual component suggesting that autoinhibition of noradrenaline release occurs, it may not be the sole factor regulating the noradrenergic component of the response. The inhibitory effect of the alpha-2-antagonists at higher doses may be attributable to alpha-1-adrenoceptor blocking activity.

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ON THE ANTAGONIST PROFILE OF THE ADENOSINE RECEPTOR IN THE FROG NEUROMUSCULAR JUNCTION

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Inhibition of ^{3}H-adenosine analogues binding to brain membranes by xanthines showed that the antagonist profile of the A_1 adenosine receptor is: DPCPX(Ki=0.3nM)>XAC (1.3nM)≥PACPX(2.5nM)>XCC(58nM)≥DPX(70nM)≥8-PT(76nM)>PGDMX(11000nM)(Ukena et al., 1986a;Daly et al.,1987;Lohse et al.,1987). Inhibition of adenosine analogues-induced stimulation of adenylate cyclase and/or cyclic AMP formation showed that the antagonist profile for the A_2 adenosine receptor is: XCC(Ki=34nM)≥XAC(Ki=49nM) > DPCPX(IC50=330nM)≥PACPX(IC50=600nM)>DPX(IC50=2200nM)≥8-PT(IC50=3700)>PGDMX(Ki=9600 nM)(Noronha-Blob et al.,1986;Ukena et al.,1986b;Daly et al.,1987;Lohse et al.,1987). In the present work we studied the antagonist profile of the receptor that mediates adenosine-induced inhibition of neuromuscular transmission, by using these adenosine receptor antagonists.

The experiments were carried out at room temperature (22-25°C) on the innervated sartorius muscle of the frog. Nerve-evoked (0.2Hz) twitch responses were recorded isometricaly at a resting tension of 50 mN. The bathing solution (pH 7.0) contained (mM): NaCl 117; KCl 2.5 ; NaH_2PO_4 1; Na_2HPO_4 1; $CaCl_2$ 1.8; $MgCl_2$ 1.2.

In Table 1 are shown the pA_2 and the Ki values for the antagonists, obtained from the antagonist-induced shifts to the right of the concentration-response curves for the inhibitory effect of 2-chloroadenosine on neuromuscular transmission.

Table 1 Potencies of xanthine derivatives.

	XAC(n=11)	DPCPX(3)	8-PT(13)	DPX(10)	XCC(3)	PACPX(6)**	PGDMX(2)**
pA_2	7.63	7.48	6.70	6.53	5.88	5.64	4.65
slope*	1.05	1.01	0.94	0.96	1.02	—	—
Ki(nM)	23	33	200	295	1318	2291	22387

*slope of regression line of Schild plot. **Ki value was estimated using one concentration of antagonist in an abbreviated Schild analysis, where a slope of unity was assumed. DPCPX:1,3-dipropyl-8-cyclopentylxanthine;DPX:1,3-diethyl-8-phenyl-xanthine; PACPX:1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine; PGDMX:1-propargyl-3,7-dimethylxanthine;8-PT:8-phenyltheophylline; XAC:1,3-dipropyl-8-(p-$H_2N(CH_2)_2$-$NHCOCH_2$-OC_6H_4)-xanthine; XCC:1,3-dipropyl-8-(p-$HOCOCH_2$-OC_6H_4)xanthine.

Comparing the relative potencies of these compounds with those described for A_1 or A_2 adenosine receptors, it is evident that a different antagonist profile emerges. Whether this antagonist profile results from species differences (cf. Ukena et al., 1986a), functional vs biochemical or binding studies, or is a characteristic of the adenosine receptor that mediates inhibition of neurotransmitter release (A_3 adenosine receptor - Ribeiro & Sebastião, 1986), needs further investigation.

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EFFECT OF MEPYRAMINE ON THE CONTRACTIONS PRODUCED BY ATRACURIUM AND OTHER MUSCLE RELAXANTS IN RAT ISOLATED ILEUM

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Muscle relaxants,e.g.,atracurium,can elicited histamine release in one of the following ways:(a) via a direct effect on mast cells,(b) by an IgE-dependent mechanism(Baldo & Fisher,1983),and (c) via activation of complement componenets (Wali,Bradshaw,Suer,Lowdell,Tugwell & Makinde,1987).

In the present investigation,the effects of three muscle relaxants,atracurium, vecuronium and gallamine,on tone and contractility of rat isolated ileum were studied and the results were compared with those obtained with histamine and 5-hydroxytryptamine(5-HT) in the same preparation.The histamine,H_1 blocker,mepyramine,and 5-HT antagonist,methysergide,were added to characterize the latter contractile responses of rat ileum.

Histamine (0.5-500 μM), 5-HT (0.5-500 μM), atracurium (0.5-500 μM),vecuronium (0.2-200 μM) and gallamine (0.1-7.0 μM) all produced concentration-dependent contractions in the rat isolated ileum. In contrast,mepyramine (0.1-10 μM) relaxed the ileum, a mean maximum relaxation of 1.2±0.1 g was obtained by 10 μM of mepyramine (mean±S.E.,n=8). The mean IC_{50} values (i.e.,concentration to produce 50% maximum relaxation) of mepyramine-induced relaxation was 0.8±0.1 μM(mean±S.E.,n=8). Methysergide (1 μM) had little effect on the contractions produced by histamine and by muscle relaxants.However,methysergide markedly (by 64±3%) reduced the 5-HT induced contractions in rat ileum. On the other hand, mepyramine (0.6 μM) reduced the contractions produced by histamine, 5-HT and muscle relaxants,atracurium, vecuronium and gallamine.The results are shown in Table 1.

Table 1. Effect of mepyramine (0.6 μM) on the contractions produced by histamine, 5-HT,atracurium,vecuronium and gallamine in the rat isolated ileum.

	Tension (Max.) (g)	EC_{50} (C) (μM)	EC_{50} (M) (μM)	EC_{50} ratio(M/C)*
Histamine	1.9±0.2	60±4.3	93±3.8	1.0:1.53
5-HT	3.2±0.5	28±1.1	41±2.1	1.0:1.46
Atracurium	2.4±0.1	32±2.1	56±2.5	1.0:1.76
Vecuronium	0.6±0.1	20±2.2	30±1.2	1.0:1.50
Gallamine	3.7±0.3	0.5±0.1	1.3±0.2	1.0:2.60

*: C, control, M: mepyramine, the results were significant ($P<0.001$) with respect to control values (means±S.E.,n=8).

It can be seen that mepyramine increased the mean EC_{50} values (i.e.,concentration to produce 50% maximum contraction) of atracurium,vecuronium and gallamine-induced contractions, as well as those of histamine and 5-HT,by 50-76%. However,a substantial part of contractile responses by muscle relaxants remained unblocked by mepyramine in this preparation,suggesting that a mechanism other than histamine release may be involved,e.g.,mobilization of intracellular calcium, in the contraction produced by the muscle relaxants (Johnson,Mahmoud & Mrozinski,1978;Pearce,Ennis, Truneh & White,1981).

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EFFECT OF ETHANOL ON NEUROMUSCULAR TRANSMISSION IN THE RAT ISOLATED PHRENIC NERVE-DIAPHRAGM PREPARATION

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Ethanol causes both in vivo and in vitro changes in neuromuscular function (for a review ,see Goldstein,1983).Ethanol increases the amplitude and duration of the miniature endplate potential(mepp)(Gage,McBurney & Schneider,1975),whereas it depresses muscular contractility (Gage,1965). Recently,it has been reported that ethanol decreased the time course of single twitch,tetanic and post-tetanic facilitation in the rat,possibly via a postsynaptic inhibitory mechanism (Borges, Feria,Diaz,Rodriguez-Mendez & Boada,1986).

In the present investigation,the effect of ethanol on neuromuscular transmission in the rat isolated phrenic nerve-diaphragm preparation was studied by analysing its effect on indirectly-elicited twitch,tetanic and post-tetanic twitch tensions and on the phenomenon of post-tetanic potentiation(PTP),which is believed to be of a presynaptic origin(Hutter,1952).

The phrenic nerve was stimulated,and occasionally the diaphragm,at 0.2-100 Hz,with 0.1-0.5 ms pulse duration and 5-10 V(supramaximal).The contractile responses were recorded isometrically and the post-tetanic twitch response (0.2 Hz) was elicited 5 s after a tetanic response (elicited at 50 Hz for 20 s duration).

The results showed that ethanol (0.39-390 mM) reduced the indirectly-elicited twitch tension in a concentration-dependent manner.A mean maximum twitch tension of 2.5±0.3 g was obtained (mean±SEM,n=8).The mean IC_{50} values(concentration to produce 50% maximum inhibition) of ethanol-induced inhibition of twitch tension was 47.7±2.2 mM ethanol.The onset of inhibition was 20 s and a complete block of twitch tension occurred in 4 min.Ethanol also reduced the directly-elicited twitch tension by 17±0.5% of control tension(3.0±0.6 g).A mean maximum tetanus of 5.2±0.4 g was obtained at 50 Hz stimulation,and this was reduced in ethanol by 23±2.1% of the control value. The PTP values in control,ethanol and during recovery period were 33±2.5%, 75±4.2% and 50±1.9%,respectively.There was no tetanic fade in the presence of ethanol,but the peak tetanic tension was reduced by 20-50%,depending on the concentration of ethanol used.

It was concluded that ethanol reduced both twitch and tetanic responses and increased the PTP values,indicating that ethanol produced neuromuscular blockade in the rat diaphragm preparation,possibly via a mixture of pre-and postsynaptic mechanisms.These results support those reported by Gage (1965) in that ethanol produced a greater reduction in the twitch than in the tetanic tension.In addition,the results agree with those reported by Borges,et.al.(1986),in that ethanol decreased the contractile responses at the rat neuromuscular junction.

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METHYSERGIDE CONTRACTS GUINEA-PIG ILEUM VIA HISTAMINE H_1 RECEPTORS

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As well as being a 5-hydroxytryptamine ($5\text{-}HT_2$) receptor antagonist, methysergide, also contracts some isolated vascular smooth muscle preparations by acting as a partial agonist at $5HT_1$-like receptors (Apperley et al 1980). We have now found that, methysergide also contracts the guinea-pig isolated ileum and have investigated the mechanism(s) involved.

Dunkin-Hartley guinea-pigs (200-300g) were humanely killed and whole ileal segments dissected out 10cm from the caecum and mounted in organ baths containing a modified gassed Krebs-Henseleit solution at 32°C. Changes in tension were measured isometrically. Methysergide ($1x10^{-7}$-$1x10^{-5}$M) caused a reproducible concentration-related contraction of the whole guinea-pig ileum. The maximum contraction obtained with methysergide was approximately 30% of the maximum response obtained with histamine ($1x10^{-8}$-$1x10^{-5}$M). The methysergide concentration-effect curve (EC_{50} 1.48μm±0.183, n=8) was not shifted by a 30 minute pretreatment with metergoline ($1x10^{-6}$M), tetrodotoxin ($1x10^{-7}$M), atropine ($1x10^{-6}$M) or ranitidine ($1x10^{-5}$M). Conversely, ketanserin ($1x10^{-8}$-$1x10^{-6}$M) or the H_1 receptor blocker, mepyramine ($1x10^{-9}$-$1x10^{-7}$M) caused a concentration related unsurmountable antagonism of the methysergide response, causing both a displacement to the right of the methysergide concentration-effect curve, as well as a suppression of the maximum response (pD'_2 values; ketanserin 7.4, mepyramine 8.6). As well as its agonist action, methysergide ($1x10^{-5}$-$1x10^{-4}$M, 30min pretreatment) also caused an unsurmountable antagonism of contractions to both histamine ($1x10^{-8}$-$1x10^{-5}$M) and carbachol ($1x10^{-9}$-$1x10^{-6}$M) with pD'_2 values of 4.1 and 5.0 respectively.

Methysergide is generally considered to have a very specific action at 5HT receptors (e.g. see Apperley et al 1976). However, these data provide evidence for the view that methysergide contracts the guinea-pig ileum by activation of histamine H_1 receptors. Whether this is direct i.e. methysergide is acting as a partial agonist or indirect i.e. methysergide releases histamine, still remains to be determined. At high concentrations methysergide also exerts a non-selective spasmolytic effect, the nature of which also remains to be established.

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IDENTIFICATION OF BINDING SITES FOR [^{125}I] SCH 23982, A SELECTIVE D-1 DOPAMINE RECEPTOR LIGAND IN HUMAN RENAL CORTEX

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SCH 23390 is a potent and selective dopamine D-1 receptor antagonist (Billard et al., 1984). [^{125}I] SCH 23982 is an iodinated analogue of SCH 23390 and has been reported to be a selective ligand for D-1 receptors in rat striatum (Sidhu & Kebabian, 1985). Dopamine receptors linked to adenylate cyclase have also been described in the kidney (Kotake et al., 1981). Therefore we have studied [^{125}I] SCH 23982 binding to human renal cortex.

Crude plasma membranes were prepared from human renal cortex (Snavely & Insel, 1982). Kidney was obtained from surgery or less than 36h post mortem. Aliquots were stored at -70°C until used. All assays were carried out in duplicate using the method described by Billard et al. (1984). Non-specific binding was defined using SCH 23390 1μM. Incubations were terminated by washing with a total of 9ml ice cold Tris buffer using rapid vacuum filtration through Whatman GF/C glass fibre filters that had been soaked overnight in 0.3% polyethyleneimine to reduce filter binding. The results were analysed using a non-linear curve fitting program (McPherson, 1985). Saturation studies were consistent with binding to a single population of sites with a Bmax of 10 fmol/mg protein and a Kd of 0.85 nM. The affinity of various drugs to displace [^{125}I] SCH 23982 binding is shown in the Table.

DRUG	Ki (nM)
SCH 23390	0.34
SCH 23388	16,375
(+) butaclamol	11
(-) butaclamol	>10,000
Domperidone	>10,000
Ketanserin	>10,000
Phentolamine	>10,000
Dopamine	2,900
Fenoldopam	64

(means of 2-3 observations)

These studies indicate that SCH 23982 binds to sites in human renal cortical membranes with pharmacological characteristics consistent with binding to a D-1 receptor. The characteristics of this renal D-1 site are similar to those previously described for D-1 receptors defined in rat striatum and human putamen (Billard et al., 1984; O'Boyle & Waddington, 1985).

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ANALYSIS OF THE MUSCARINIC RECEPTOR ON THE RAT AORTIC ENDOTHELIUM

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The muscarinic M_1 and M_2 receptor can be distinguished by pirenzepine with pA_2 values of 8.4 and 6.8 respectively (Eglen and Whiting, 1986). It has been reported from studies using selective antagonists such as 4DAMP (4 diphenylacetoxy-N-methylpiperidine) that the muscarinic receptor present on the rabbit aortic endothelium resembles the M_2 receptor found on nonvascular smooth muscle (Eglen and Whiting, 1985; Choo et al., 1986). However, pA_2 values for pirenzepine obtained in these studies were 7.6 and 6.8 respectively. Choo et al., (1986) also reported that pirenzepine exhibited a pA_2 value of 6.7 at receptors in the rat aortic endothelium although other antagonists were not examined in this preparation.

The aim of the present study was to assess the muscarinic receptor profile on the rat aortic endothelium using the above antagonists and the more selective antagonists himbacine and methoctramine (Anwar-ul et al, 1986 ; Melchiorre et al,1987).

Male Sprague-Dawley rats (290-340g) were killed by decapitation. The descending thoracic aorta was excised and 2-3mm ring segments cut. Care was taken to maintain an intact endothelium and the ring segments were suspended for isometric tension recordings in Krebs' saline maintained at 37°C, gassed with 5% CO_2 in oxygen. Tone (approx 1g) was induced in the preparations using phenylephrine (1 - 10 μM) or, for experiments using methoctramine, U46619 (2.5 - 25 nM). Endothelial dependent relaxations were induced by acetylcholine. Antagonist affinities were estimated by the method of Arunlakshana and Schild (1959) using 3-4 concentrations of each antagonist. All values quoted are mean and 95% confidence intervals derived from 4 to 6 animals.

The responses to acetylcholine, EC_{50} = 89.1nM, were antagonised in a competitive manner by atropine, pirenzepine and 4 DAMP, since the Schild slopes were not significantly different from unity ($p>0.05$). The pA_2 values for these antagonists were 8.72 (8.43-9.01), 7.21 (6.91-7.49) and 8.87 (8.35-9.39) respectively. In contrast, methoctramine and himbacine exhibited Schild slopes less than unity (0.74 (0.50-0.88), and 0.79 (0.62-0.97) respectively). The pA_2 values were 5.87 (5.62-6.12) and 6.92 (6.57-7.23) respectively.

The pA_2 value for atropine was consistent with stimulation of muscarinic receptors. The pA_2 value obtained with pirenzepine was higher than that previously reported at M_2 receptors (6.8), but much less than that previously reported at M_1 receptors (8.4, see Eglen and Whiting, 1986 for review). The value obtained using 4 DAMP was similar to that reported at receptors in smooth muscle (9.0) but dissimilar to that reported for the receptors in the atria (7.9; Eglen and Whiting, 1986). This concurs with the values obtained using methoctramine and himbacine, which were dissimilar to those observed at atrial muscarinic receptors (8.0 and 8.2 respectively) but similar to those observed at smooth muscle receptors (5.9 and 7.1 respectively, Melchiorre et al.,1987; Anwar-ul et al., 1986).

We conclude that the muscarinic receptor present on the rat endothelium is similar to M_2 receptors on non-vascular smooth muscle but dissimilar to M_2 receptors on the atria.

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MUSCARINIC RECEPTOR SUBTYPE INVOLVED IN CONSTRICTION OF THE ISOLATED PIG CORONARY ARTERY

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It has been shown that cholinergic agonists are able to provoke anginal pain with ST-elevation in patients with variant angina and that atropine can abolish this effect (Yasue et al., 1974). In intact anesthetized rats methacholine (MCh) induces coronary constriction (Sakai et al., 1981). Recently, we have demonstrated coronary constriction induced by administered MCh or endogenous acetylcholine released by vagal stimulation in Langendorff-perfused rat hearts (van Charldorp et al., 1987). It was the aim of the present study to investigate the nature of the muscarinic receptor subtype involved in the constriction of pig coronary arteries with regard to the recently proposed subdivision of muscarinic receptors into M_1 (neuronal tissue), M_2 (heart) and M_3 (exocrine glands) (de Jonge et al., 1986). Rings from the left anterior descending coronary artery were used after removal of the endothelium (tyrode solution, Ca^{++}: 1.8 mM, 95% O_2 + 5% CO_2, 37°C). After 3 hours of equilibration a cumulative concentration-response curve of MCh was made. All responses were expressed as a percentage of the preceding maximal response to KCl (118 mM). Pretreatment with antagonists was performed for 2 hours. MCh caused a concentration-dependent coronary constriction. Pretreatment with the α-blocker phentolamine (10^{-5} M) did not affect the concentration-response curve to MCh. All muscarinic antagonists studied (atropine, pirenzepine: M_1-selective; AF-DX 116 = 11-2[(2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one: M_2-selective and 4-DAMP = 4-diphenylacetoxy-N-methylpiperidine), shifted the concentration-response curve of MCh to the right in a competitive manner.

Table 1 pA_2-values ± S.E.M. (n=6-8) for the antagonists studied.

Atropine	9.49 ± 0.01
Pirenzepine	7.31 ± 0.03
AF-DX 116	6.19 ± 0.02
4-DAMP	9.12 ± 0.05

Accordingly, the relative order of potency of the antagonists for the muscarinic receptor inducing coronary constriction was: atropine ≃ 4-DAMP >> pirenzepine >> AF-DX 116. We recently established (de Jonge et al., 1986) the following rank order for the M_1-receptor: atropine ≃ 4-DAMP >> pirenzepine >> AF-DX 116; for the M_2-receptor: atropine >> 4-DAMP > AF-DX 116 > pirenzepine and for the M_3-receptor: atropine ≃ 4-DAMP >> pirenzepine >> AF-DX 116.
The comparison of the relative orders of potency of the antagonists studied indicates that the muscarinic receptor subtype responsible for coronary constriction does not belong to the M_2-subtype. At present we cannot decide whether the muscarinic receptor involved belongs to the M_1- or M_3-subtype, more selective antagonists being required to allow this discrimination.

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SELECTIVITY OF MUSCARINIC ANTAGONISTS IN THE ANAESTHETISED GUINEA-PIG

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There are strong indications for a heterogeneous population of muscarinic receptors in peripheral tissue from functional in vitro studies. A number of antagonists of varying degrees of selectivity for smooth muscle or atria have been described. We have examined 4-DAMP (4-D), methoctramine (M) and pirenzepine (P) in an anaesthetised guinea-pig preparation to determine whether organ selectivity can be seen in vivo.

The model is an adaptation (Alabaster & Keir 1987) of that originally described for the conscious guinea-pig (Amdur & Mead, 1958). The effect of intravenous acetylcholine (ACh) on lung resistance (R_L), dynamic compliance (C_{dyn}) and heart rate (HR) can be measured. We have established ACh dose-response curves for all three parameters and shown that muscarinic antagonists, given intravenously 10 min previously, cause a parallel rightward shift of the curves. This allows calculation of dose ratio (DR). The degree of lung:heart selectivity has been calculated for each antagonist by comparing DR for block of ACh-induced bronchoconstriction with that for ACh-induced bradycardia (the 'L:H ratio'). Dose ratios are given in the table below (mean ± s.e. mean, n=4).

Table 1: Effect of muscarinic antagonists on ACh induced bronchoconstriction and bradycardia

Antagonist	Dose ug/kg	R_L	C_{dyn}	HR	L:H
Atropine	10	12.8±2.7	15.3±2.8	8.2±2.3	1.7
4-DAMP	100	10.0±0.2	9.5±1.8	3.0±1.0	3.3
Methoctramine	1000	1	1	29.0±6.8	<0.03
Pirenzepine	1000	8.4±1.0	10.25±1.5	4.0±0.9	2.3

Atropine, 4-D and P all inhibited ACh bronchoconstrictor responses to a greater degree than ACh-induced bradycardia. However, the selectivity of 4-D for the lung was less than predicted from in vitro data (Gater et al 1987). In addition, 4-D was tenfold less potent than atropine in this system whereas the two are equipotent in vitro indicating possible differences in pharmacokinetic profile in the guinea-pig. P was no more lung-selective than atropine in contrast to a report from studies on the anaesthetised rabbit (Bloom et al, 1987). M, which is about 250-fold atrial-selective in vitro (Melchiorre et al 1987), seems to retain most of this specificity in vivo though it was impractical to exceed the dose reported to give a definitive measure of heart selectivity. These results suggest that this model will detect highly tissue-selective muscarinic antagonists.

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NEDOCROMIL SODIUM INHIBITS EARLY AND LATE BRONCHOCONSTRICTION RESPONSES TO OVALBUMIN IN THE GUINEA-PIG

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Bronchial provocation of guinea-pigs sensitized by inhalation of aerosolized ovalbumin induces three phases of airflow limitation; an early reaction (EAR) which peaks at 2 h, a late reaction (LAR) which peaks at 17 h and a second or later late reaction (LLAR) which is observed at 72 h (Hutson *et al*, 1987a). We have previously reported that sodium cromoglycate blocks both the EAR and LAR whereas the β-adrenoceptor agonist salbutamol inhibits only the EAR (Hutson *et al*, 1987b). We now report the effects of a new anti-asthma drug, nedocromil sodium, on these responses.

Male Dunkin Hartley guinea-pigs (500-700 g) were sensitized to ovalbumin (1% inhaled for 3 min weekly for 2 weeks). Groups of 8-16 sensitized animals inhaled nedocromil sodium (1%) or saline (control) for 2 min at 15 min before challenge, 6 h after challenge or 15 min before and 6 h after challenge with antigen. Guinea-pigs were challenged by inhalation of aerosolized ovalbumin (2% for 5 min) under cover of mepyramine (10 mg/kg i.p.). Specific airways conductance (sGaw) was assessed in conscious animals by whole body plethysmography. Bronchoalveolar lavage (BAL) was performed as previously described (Hutson *et al*, 1987a).

Nedocromil sodium inhaled as a single dose before challenge significantly ($p<0.001$) inhibited the EAR by 75% and the LAR by 94% but did not affect the LLAR. Nedocromil sodium also abolished the 14.4 fold increase in neutrophil numbers found in BAL 17 h after challenge. The increases in eosinophils in BAL (4.2 fold at 17 h and 6.9 fold at 72 h) were unaffected by nedocromil sodium given as a single prophylactic dose.

When nedocromil sodium was inhaled at 6 h, i.e. after the completion of the EAR but before the onset of the LAR, the LAR was reduced by 65% ($p<0.001$). No delay of the LAR was seen. Unlike dosage before challenge, nedocromil sodium given at 6 h reduced the LLAR by 78% ($p<0.001$) at 72 h. Analysis of BAL showed nedocromil sodium to have had no significant effect on either the accumulation of neutrophils at 17 h or eosinophils at 17 h and 72 h.

Inhalation of nedocromil sodium at both 15 min before and 6 h after challenge inhibited the EAR by 88%, the LAR by 84% and the LLAR by 86% (all $p<0.001$). This dosage regimen also abolished neutrophil accumulation in BAL at 17 h ($p<0.001$) and significantly ($p<0.05$) reduced the elevation of eosinophils at 72 h but not 17 h.

These results demonstrate that, like sodium cromoglycate (Hutson *et al*, 1987b), nedocromil sodium inhibits both early and late airways responses to allergen challenge. Use of different dosage regimens and parallel assessment of cells in BAL suggests that neutrophil and eosinophil accumulation *per se* are unlikely to be the stimulus for the LAR in this model but we are unable to comment at present on the state of activation of these inflammatory cells.

PAH is a SERC-CASE student with Roussel Laboratories Ltd., Swindon.

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SYSTEMIC AND REGIONAL HAEMODYNAMIC EFFECTS OF DILTIAZEM, NIFEDIPINE AND VERAPAMIL, IN ANAESTHETISED RATS

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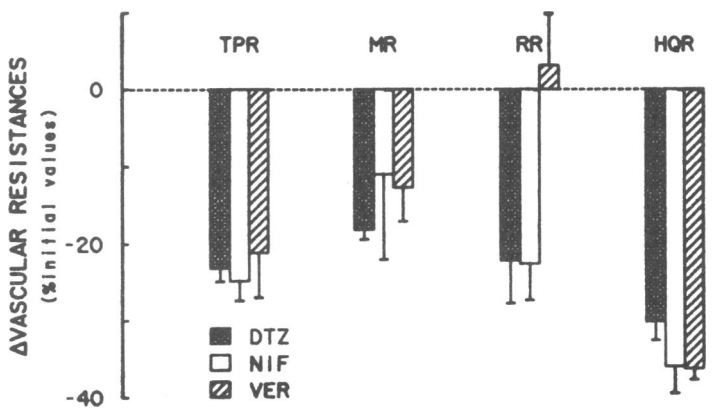
Calcium ions play a pivotal role in the excitation-contraction coupling in vascular smooth muscle. Inhibition of their entry into the cell is accompanied by myorelaxation and thus, calcium antagonists can lower blood pressure by reducing vascular resistance.

The aim of this presentation is to compare the systemic and regional hemodynamic effects of diltiazem, nifedipine and verapamil in anesthetized rats.

Male normotensive Sprague Dawley rats (250-280 g) were anaesthetised with pentobarbitone and prepared for the measurement of the carotid blood pressure and the upper abdominal aorta (cardiac output: CO), mesenteric artery (MF), renal artery (RF) and terminal aorta (hindquarter: HQF) blood flows by means of a pulsed Doppler technique (Hartley et al., 1974). Diltiazem (DTZ: 12.5-100.0 µg/kg/min), nifedipine (NIF: 2.5-10.0 µg/kg/min), verapamil (VER: 12.5-100.0) or saline was infused during a 5 min period.

DTZ, NIF and VER produced dose-related decreases in mean carotid artery blood pressure and heart rate. For a hypotensive effect of 30 mmHg, DTZ and NIF did not significantly modify CO whereas VER lowered it by 10%. MF and RF were virtually unchanged by DTZ and NIF while VER decreased slightly MF and markedly RF. However, the three calcium antagonists increased HQF. Consequently, DTZ, NIF and VER lowered the total peripheral (TPR), mesenteric (MR) and hindquarter (HQR) vascular resistances. Only DTZ and NIF reduced renal (RR) vascular resistances.



In conclusion, the hypotension produced by the studied calcium antagonists was the result of a fall in TPR. In contrast to DTZ and NIF which lowered all of the measured regional vascular resistances, VER did not produce renal vasodilatation and the hemodynamic nature of this failure was a reduction in renal blood flow.

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EFFECTS OF ENALAPRIL ON THE PRESSOR AND CARDIAC OUTPUT RESPONSES TO XYLAZINE IN THE PITHED RAT

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The renin angiotensin system in pithed rats is highly activated (Vollmer et al, 1984). Also, angiotensin converting enzyme inhibitors reduce vascular responses to α-adrenoceptor activation (de Jonge et al, 1982). We have shown that pressor responses to the α_2-agonist xylazine are mediated partly by increases in cardiac output (CO) and also include vasoconstriction in vascular regions (e.g. the superior mesenteric arterial bed) where postjunctional α_2-adrenoceptors have not been found (Hiley & Thomas, 1987). Here we determine the sites of angiotensin II (ANG II) facilitation of the pressor response to xylazine in the pithed rat.

Male Wistar rats (250-300g; Bantin & Kingman) were pithed under halothane anaesthesia. Both femoral arteries were cannulated to allow withdrawal of blood (at 0.5ml/min) during the microsphere injection and continuous monitoring of blood pressure (BP). When BP had stabilised after pithing, the rats were given either 0.5ml saline or 2mg/kg enalapril (Merk, Sharp & Dohme) i.v. For the next 10min the rats received an i.v. infusion of either saline (0.1ml/min) or ANG II (75ng/kg/min; Sigma). They were then given, i.v., 0.5ml saline or 0.5mg xylazine (Bayer) followed by infusion of saline, xylazine (0.1mg/min) or xylazine + ANG II (0.1mg/min + 75ng/kg/min). When a stable response had been obtained, 60000-80000 ^{113}Sn-labelled microspheres (15μm diameter; NEN) were injected through a cannula into the left cardiac ventricle. CO and organ blood flow were determined by the method of McDevitt & Nies (1976). Statistical comparisons were by analysis of variance; n = 8 for each of the 4 groups.

In rats receiving xylazine, CO was greater ($P<0.01$) than in the group given saline alone being, respectively, 15.9±0.6 and 11.9±0.6ml/min/100g body wt. In rats given xylazine and enalapril CO was 17.1±0.5ml/min/100g body wt. Thus enalapril did not affect the CO response to the α_2-agonist but it significantly decreased the pressor response to xylazine ($P<0.01$). In animals given xylazine alone diastolic BP increased by 52±6mmHg compared to 42±2mmHg in those given xylazine + enalapril and -1±1mmHg in saline control animals. After enalapril, infusion of ANG II before and during the administration of xylazine restored the pressor response to 56±5mmHg. Xylazine increased ($P<0.01$) total peripheral resistance (TPR), prior administration of enalapril abolished this change and infusion of ANG II restored the response; TPR values were 4.9±0.3 (control), 6.9±0.6 (xylazine alone), 4.4±0.3 (enalapril + xylazine) and 6.8±0.6mmHg.min.100g body wt/ml (xylazine in the presence of enalapril and ANG II).

Xylazine increased vascular resistance in the kidneys (by 60%), testes (70%), fat (110%), skeletal muscle (88%), spleen (148%), stomach (76%), small intestine (86%), large intestine (64%) and pancreas/mesentery (131%). Enalapril prevented vasoconstriction by xylazine in these vascular beds, such that resistances in the presence of enalapril and xylazine were not significantly different from those in the saline controls, except in the spleen where the resistance was the same after xylazine in the presence or absence of enalapril. Infusion of ANG II after enalapril restored organ vascular resistances in the presence of xylazine to values not significantly different from those in the presence of xylazine alone.

Thus, facilitation by ANG II of the pressor response to xylazine in the pithed rat is due to actions on vascular resistance not cardiac output. Also, vasoconstriction by xylazine in vascular beds where there are no postjunctional α_2-adrenoceptors may be due to renin release caused by renal vasoconstriction.

This work was supported by a grant from The Wellcome Trust.

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THE EFFECTS OF THE NOVEL ANTI-ANGINAL AGENT RANOLAZINE (I.D.) IN A CANINE MODEL OF TRANSIENT MYOCARDIAL ISCHAEMIA

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The novel anti-anginal agent Ranolazine (RS 43285) has been shown to alleviate the biochemical consequences of transient myocardial ischaemia (TMI) in the canine myocardium (Allely et al, 1987). In the present study we investigated the ability of Ranolazine to inhibit ischaemia-induced epicardial S-T segment elevations when administered by the intraduodenal (i.d.) route in a dog model of TMI based on the original model of Szekeres et al (1976).
Pentobarbitone-anaesthetised dogs were prepared as previously described (Allely & Alps, 1987) for the production of TMI episodes. A Walton-Brodie strain guage was sutured across the border ischaemic zone. One min of rapid atrial pacing (50% above resting HR) was followed by 2 min pacing plus occlusion of the left anterior descending coronary artery. S-T segment elevations during the insult (5 time points) and reperfusion (10 time points) phases were expressed as a % of the average of the final two control values and are shown in Figure 1a and b respectively. Control data demonstrates that no changes in epicardial S-T segment elevations occur with increasing numbers of challenges.

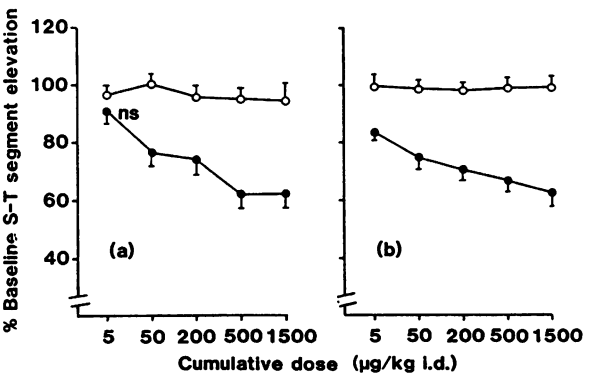

Figure 1. The cumulative i.d. effects of Ranolazine (●;n=3) on S-T segment elevations during the insult (a) and recovery (b) phases of TMI in the dog. Values are mean ± s.e. All points are significantly lower than control (○;n=4) at $P < 0.001$, Student's t-test except as shown n.s.

Ranolazine was as effective by the i.d. route as by the i.v. route in both inhibiting the S-T segment build-up (a) and in promoting the return to the pre-ischaemic baseline (b). Ranolazine also blunted the swings in border ischaemic zone contractile force induced by the insult/reperfusion protocol with no overt effects on general haemodynamics. These effects closely approximated those produced by i.v. administration of Ranolazine (unpublished observations).
The results suggest that Ranolazine is an orally-active anti-anginal agent.

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PROTECTIVE EFFECTS OF THE NOVEL ANTI-ISCHAEMIC AGENT RANOLAZINE (RS-43285) IN PERFUSED RAT HEARTS

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Reperfusion of the heart after a period of ischemia produces tissue damage and reduces cardiac function. Agents that reduce the severity of the injury should be of clinical benefit. We have studied the effects of ranolazine [(+) N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxy-phenoxy) propyl]-1-piperazine acetamide dihydrochloride] a compound that has previously been shown to decrease the harmful effects of ischemia evoked by brief coronary occlusion in dogs (see Allely and Alps 1988).

In isolated working rat hearts perfused with physiological solution (1.35 mM Ca^{++}), reperfusion after a 15 min period of coronary artery ligation was followed by fibrillation and cardiac failure in 9 of 10 hearts as previously described (Armstrong & Ferrandon 1985). Addition of ranolazine (1 nM) to the perfusion fluid 10 min prior to coronary ligation, reduced the mortality observed on reperfusion to 6 of 10 hearts. The cardiac output of the 4 survivors was 28±2 ml/min. When 10 nM ranolazine was used, the mortality was reduced to 2 of 11 hearts and the cardiac output of the survivors (48±2 ml/min, n=9) was only slightly less than that of controls (55±1 ml/min, n=10). In the presence of ranolazine 10 nM (n=10/group), the left ventricular ATP values was similar to the control value (10.2±1.1 ug/g dry weight) but in 10 hearts without the compound it was significantly less (4.6 ± 0.6 ug/g dry weight, $p < 0.05$ t-test). Tissue lactate, which after reperfusion was elevated from 14.1±2.3 ug/g dry weight (n=10) to 65.3±3.9 ug/g was reduced to 25.5±6.0 ug/g with 10 nM ranolazine and to 19.5±3.7 ug/g with 100 nM of the agent. The effects of ranolazine were also studied in groups of 16 pentobarbitone anaesthetised (50 mg/kg i.p.) open-chest rats (Manning et al. 1984). Reperfusion, after a 5 min period of coronary ligation, resulted in the death of 14 of them. Ranolazine (0.1 mg/kg i.v.) injected 10 min before ligation allowed 6 of the group to survive reperfusion and after 1.0 mg/kg i.v. all of the group survived. The mean systolic pressure of the survivors was 55±10 mmHg and 116 ± 6 mmHg respectively and was 128±10 mmHg in controls. A reduction in the elevated plasma 2-hydroxybutyrate dehydrogenase was obtained 5 min after removal of the ligature in ranolazine dosed animals (controls= 367±30 I.U.; 1.0 mg/kg i.v.= 207±30 I.U., p 0.05 t-test). After oral administration of ranolazine (3, 10 and 30 mg/kg) to rats prior to anaesthesia, 2 of 10 survived, 6 of 10 survived and 9 of 10 survived, respectively. In these animals the corresponding systolic blood pressures were 50±10 mmHg, 65±8 mmHg and 96±5 mmHg and in controls it was 102±4 mmHg. By itself, ranolazine did not reduce the force or rate of ventricular contraction of isolated hearts nor alter the blood pressure <u>in vivo</u>.

Thus, ranolazine protects hearts from the potentially lethal biochemical and functional injury produced by ischemia and reperfusion.

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PHARMACOLOGICAL PROFILE OF RANOLAZINE, A METABOLIC MODULATOR ACTIVE IN ISCHAEMIA

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Ranolazine (RS 43285, Allely & Alps, 1988) alleviates the symptoms of transient myocardial ischaemia in the dog, by changing substrate utilisation without overt haemodynamic effects (Allely et al, 1987). Low concentrations (10-100 nM) protect against the deleterious effects of coronary ligation with reperfusion in the working rat heart, and maintain ATP levels without reducing myocardial work during the preischaemic period (Ferrandon et al., 1988). This suggests that ranolazine would be a novel antianginal agent. We have investigated the general pharmacology of ranolazine.

Ranolazine had negligible affinity (pKi <5) for α_1-, β_1- and β_2-adrenoceptors, D_1, M_1, M_2, $5HT_{1A}$, A_1 and A_2 receptors and low affinity for α_2 (pKi 5.7), D_2 (5.1) and $5HT_2$ (5.5) receptors. Ranolazine was a very weak antagonist of K^+-induced contractions in guinea-pig mesenteric artery (pIC_{50} 3.4) and rat aorta (pIC_{50} <3.5)and of Ca^{2+}-induced contractions in K^+-depolarized taenia preparations from the guinea-pig caecum; ranolazine (10^{-4} M) did not antagonise the augmented sensitivity to Ca^{2+} caused by Bay K 8644 (1-100 nM) or the putative Ca^{2+} channel activator palmitoyl carnitine (10-1000 µM). Responses to phenylephrine in rat aorta were inhibited over the concentration range expected from the α_2-antagonist activity.

Ranolazine had weak inhibitory activity (pIC_{50} 4.5) compared with nicardipine (pIC_{50} 7.5) on electrically-induced Ca^{2+}-dependent action potentials in depolarized guinea-pig papillary muscle; mechanical activity was also inhibited (pIC_{50} 5.5), but at much higher concentrations than with nicardipine (pIC_{50} 8.1). High concentrations (10^{-4} M) slightly reduced the rate of rise of normal action potentials in this preparation and had little effect on action potential duration. Resting potential and hence passive membrane permeabilities to Na^+ and K^+ were also unaffected.

In conclusion, ranolazine has only weak calcium-antagonist effects with selectivity for heart muscle, which are unlikely to contribute to the mechanism of action. As the compound is essentially devoid of haemodynamic effects and has little effect on receptors or isolated tissues, the mode of action appears to be by modulation of metabolism. The pharmacological profile indicates a wide therapeutic window for the antianginal effects.

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EFFECTS OF RILMENIDINE (S 3341) ON THE SYMPATHETIC NERVOUS SYSTEM IN NEUROGENIC HYPERTENSIVE DOGS

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It is well known that alpha-2 adrenoceptor agonists like clonidine induce decrease in plasma catecholamine levels (Lhoste, 1985). However, the mechanism of this effect remains discussed since a direct central effect or an action mediated through baroreflex pathways can be suggested. The present study investigates in a neurogenic model of arterial hypertension following sinoaortic denervation (SAD) (Damase et al, 1987), the effects of Rilmenidine (S 3341), a new alpha-2 adrenoceptor agonist (Laubie et al, 1985) on different indices known to reflect the activity of the sympathetic nervous system.

Rilmenidine (1 mg/kg orally for 15 days) or placebo were administered according to a double blind cross over randomized protocol in 7 SAD hypertensive dogs with a one week wash out interval. Blood pressure (BP) and heart rate (HR) were recorded through an arterial femoral catheter in conscious animals. Plasma catecholamine levels were measured by HPLC and platelet alpha-2 and leukocyte beta adrenoceptors evaluated by (^{3}H) yohimbine and (^{125}I)cyanopindolol binding respectively.

As previously reported (Damase et al, 1987), SAD induced both a sustained rise in blood pressure (systolic : SBP = 205 $\pm$ 7 vs 145 $\pm$ 4 mmHG before SAD, diastolic : DBP = 109 $\pm$ 11 vs 73 $\pm$ 3 mmHg) and heart rate (HR = 159 $\pm$ 9 vs 87 $\pm$ 16 beats/min) and an increase in sympathetic tone : plasma catecholamine levels rose from 1.4 $\pm$ 0.2 pM for noradrenaline (NA) and 0.84 $\pm$ 0.2 pM for adrenaline (A) before SAD to 6.04 $\pm$ 1.4 pM (NA) and to 3.5 $\pm$ 0.8 pM (A) after SAD. The number of alpha-2 as well as beta adrenoceptors on circulating blood cells significantly decreased (Bmax = 133 $\pm$ 11 in SAD vs 198 $\pm$ 12 fmole/mg protein in control dogs for alpha-2 and 19 $\pm$ 4 in SAD vs 48 $\pm$ 2 fmole/mg protein in control dogs for beta) without any variation in affinity.

Rilmenidine induced a significant decrease ($p < 0.001$) in both SBP (114 $\pm$ 5) and DBP (49 $\pm$ 4) and HR (88 $\pm$ 4) when compared with placebo (SBP = 212 $\pm$ 6, DBP = 117 $\pm$ 8, HR = 163 $\pm$ 7) . Plasma catecholamine levels were : 1.8 + 0.3 (NA) and 0.81 $\pm$ 0.3 (A) in rilmenidine-treated dogs and 4.0 $\pm$ 0.6 and 1.9 $\pm$ 0.4 in placebo group ($p < 0.02$ and $p < 0.05$). Treatment with rilmenidine corrected SAD - induced decreased in leukocyte beta adrenoceptor number (Bmax = 57 $\pm$ 3 vs 26 $\pm$ 4). No change in alpha-2 adrenoceptor Bmax was noticed (112 $\pm$ 10 vs 145 $\pm$ 22 after placebo) (cross over ANOVA test).

These results show that this model of neurogenic hypertension is suitable for the study of antihypertensive drugs. Rilmenidine corrected both hypertension and the increase in sympathetic tone elicited by SAD. It is suggested that the changes in beta adrenoceptors are related to the fall in BP rather than to a direct effect of Rilmenidine. Moreover, the present study indicates that the alpha-2 agonist induced-decrease in catecholamine levels is mainly due to a direct central effect without involvement of baroreceptor pathways.

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REVERSAL OF ATP-INDUCED VASODILATATION IN THE ISOLATED PERFUSED RABBIT EAR BY REACTIVE BLUE 2

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Burnstock and Kennedy (1985) suggested that P_2-purinoceptors may be divided into two sub-types which they called P_{2x} and P_{2y}. The proposal was based on the rank order of potency of agonists on different tissues and the selective effects of antagonists or desensitising agents such as αß methylene ATP. In isolated blood vessels ATP may cause contraction by an effect on P_{2x}-purinoceptors on muscle cells and relaxation by an action on P_{2y}-purinoceptors on endothelial cells and the subsequent release of EDRF and PGI_2.

We have investigated the interaction between ATP and the putative P_{2y}-purinoceptor antagonist, reactive blue 2 (Burnstock et al 1986) on pre-capillary resistance vessels in situ. Isolated rabbit ears were perfused at constant flow (2-4 ml/min) through the central ear artery with gassed Krebs Hensleit solution at 35°C and mean perfusion pressure recorded; Noradrenaline (10^{-6}M) was added to the Krebs solution to increase perfusion pressure (mean value from 55 experiments was 180.5 ± 4.0 mmHg). Bolus injections of ATP ($1x10^{-10}$ - $1x10^{-7}$ mol) dilated pre-capillary resistance vessels resulting in dose-dependent decreases in perfusion pressure (Fig.1) of rapid onset and short duration. The ED_{50} was $1.96 \pm 0.27x10^{-9}$ mol (n=36). Reactive blue 2 ($1x10^{-5}$ and $3x10^{-5}$M) caused a reduction in ATP-induced vasodilatation with depression of both slope and maximal response (Fig.1). In 9 out of 14 experiments reactive blue 2 (10^{-4}M) caused a complete reversal of ATP responses and a dose-dependent increase in perfusion pressure was observed (Fig. 1). The vasoconstriction was similar to but less pronounced than the effects of the selective P_{2x} purinoceptor agonist αß methylene ATP ($1x10^{-11}$ - $1x10^{-8}$ mol) in untreated preparations (Fig.1).

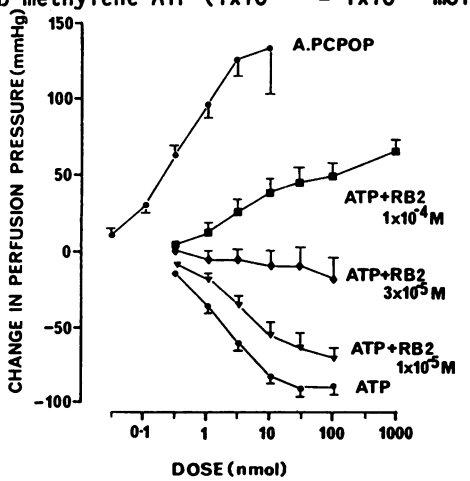

Figure 1.

Effect of ATP on perfusion pressure before and in the presence of reactive blue 2 (RB2) $1x10^{-5}$M (n=14) $3x10^{-5}$M (n=14) and $1x10^{-4}$ (n=9). The effects of αß methylene ATP (A.PCPOP) are also shown (n=18)

These results are consistent with the view that two types of P_2-purinoceptor may be present in blood vessels. The sensitivity of the pre-capillary resistance vessels to ATP and αß methylene ATP suggest that purines may play a part in the local and nervous control of regional blood flows.

Burnstock, G. and Kennedy, C. (1985) Gen. Pharmac. 16, 433-440.
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EFFECT OF αβ METHYLENE ATP ON PRE-CAPILLARY RESISTANCE VESSELS IN VIVO

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Methylene analogues of ATP have been shown to cause vasoconstriction in isolated blood vessels by stimulating P_{2x}-purinoceptors (Burnstock and Kennedy 1985). In contrast, ATP causes vasodilatation mediated by P_{2y}-purinoceptors on endothelial cells with the subsequent release of EDRF and PGI_2. As most previous studies have used isolated blood vessels we have investigated the effects of αβ methylene ATP on pre-capillary resistance vessels in vivo.

Hindquarters, intestinal or left renal vasculatures of pentobarbitone anaesthetised cats were autoperfused at constant flow using peristaltic pumps. Mean levels of perfusion pressure were 72.5 ± 4.3 (n=9), 58.0 ± 3.8 (n=10), 96.3 ± 10.9 (n=6) respectively. Changes in perfusion pressure reflect changes in tone of pre-capillary blood vessels. Guanethidine, 3 mg/kg i.v., was given to inhibit sympathetic nervous activity. Bolus i.a. injections of αβ methylene ATP (0.1 - 100 µg, 1 µg = 2 nmol) caused pronounced but short lasting vaso-constriction of the intestinal vasculature comparable with the effects of 0.03 - 10 µg i.a. noradrenaline (Fig.1A). There was some evidence of desensitisation of P_{2x}-purinoceptors after high doses of αβ methylene ATP making it difficult to determine the true maximal response. The approximate ED_{50} was 7.8 ± 1.2 x 10^{-9} mol (n=7). βγ methylene ATP was less active than αβ methylene ATP (ED_{50} 6.0 ± 1.0 x 10^{-8}, n = 5) and in some experiments a biphasic effect was seen. In contrast, αβ methylene ATP had little effect on hindquarters or renal vascular resistance in comparison to noradrenaline (Fig. 1B). ATP (3 x 10^{-11} - 1 x 10^{-6} mol i.a.) caused vasodilatation in all three vascular beds.

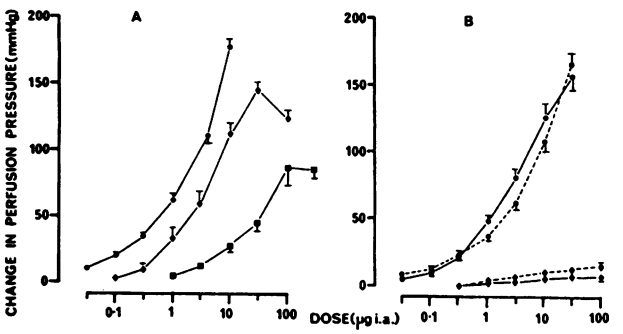

Figure 1.A. Effect of αβ methylene ATP (◆), βγ methylene ATP (■) and noradrenaline (●) on intestinal perfusion pressure. B. Effect of αβ methylene ATP and noradrenaline on hindquarters (—) and renal (---) perfusion pressure.

The results are consistent with the proposal that two types of P_2-purinoceptor may be present in blood vessels although the distribution or functional significance of P_{2x}-purinoceptors shows considerable regional variation. The sensitivity of pre-capillary resistance vessels to both ATP and αβ methylene ATP suggests that purines may play a part in the local and, in some areas, the nervous control of regional blood flow.

Burnstock, G. and Kennedy, C. (1985) Gen. Pharmac. 16, 433 - 440.

RESPONSIVENESS OF α_1-ADRENOCEPTOR AGONIST INDUCED CONTRACTION IN ENDOTHELIUM-INTACT RINGS OF RAT AORTA IS RELATED TO TURNOVER OF PI

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α_1-Adrenoceptor mediated contraction of rat aorta utilizes both intra- and extracellular calcium (Beckeringh et al., 1984; Chiu et al., 1986) and α-adrenoceptor agonist resistance to calcium entry blockade positively relates to the turnover of phosphatidylinositol (TPI) (Beckeringh et al., 1987; Chiu et al., 1987). With the calcium entry promoter (±)-Bay k 8644 the depressed potency of partial agonists due to spontaneous release of EDRF (Endothelium-Derived-Relaxing-Factor) in endothelium-intact (EI) preparations cam be restored to the level of that in endothelium-denuded preparations (Beckeringh, 1985). In addition, acetylcholine and carbachol (CCh) reverse α-adrenoceptor agonist induced contraction in EI-preparations. The present study examines the relationship between α_1-adrenoceptor agonist-induced contraction in EI preparations, TPI, and the capacity of CCh to relax α_1-adrenoceptor agonist contracted EI-preparations.

Ring segments of rat isolated aorta were suspended in Krebs-Henseleit solution containing 1μM dl-propranolol, 10μM cocaine and 20μM cortisol at 37°C at a resting tension of 8mN. Agonists were added cumulatively to the organ bath. Contractions were expressed as a percentage of the maximal response to 30μM noradrenaline (NA) (E_{max}=100%). At the maximal contractile effect of each agonist CCh was added cumulatively to the organ bath. TPI was measured as described by Minneman and Johnson (1984) using 4 mm segments of rat aorta. Agonists were used at maximal contractile concentrations (100-300μM) and NA induced TPI was considered 100%.

	pD_2 (contraction)	$\%E_{max}$	pD_2-CCh	%Relax	%TPI
AMI	4.92 ± 0.07	33 ± 4*	7.00 ± 0.06	100 ± 3	12 ± 5*
ME	5.74 ± 0.09	96 ± 2	5.82 ± 0.16	75 ± 3*	63 ± 4*
NMN	5.46 ± 0.13	54 ± 2*	6.74 ± 0.09	101 ± 2	24 ± 6*
SKF	5.37 ± 0.12	64 ± 4*	6.15 ± 0.12	99 ± 2	49 ± 5*
SKl	6.96 ± 0.08	31 ± 4*	6.51 ± 0.14	100 ± 2	32 ± 4*

(Mean ± S.E.M., n=5-7; * = significantly different ($p<0.05$) from 100%)

Only methoxamine (ME) displayed full agonism in contractile experiments. All drugs were partial agonists in stimulating TPI. In spite of a much higher TPI than amidephrine (AMI) and normetanephrine (NMN), the maximal response to the aminotetraline SK&F 1-89748A (SKl) was similar to that of AMI. In addition, β-phenylethylamine (β-PEA) (AMI,ME,NMN,SKF(=SK&F 102652)) intrinsic activity correlated well with TPI ($r=0.95$; $p<0.05$).CCh induced full relaxation in rings precontracted by all agonists except ME. The potency of CCh inversely correlated with TPI ($r=0.99$; $p<0.05$). The results of the present study show that activation of TPI counteracts EDRF induced relaxation. EDRF generally seems to interfere with the entry of extracellular calcium; the underlying process, however, appears to differ between β-PEAs and the aminotetraline SKl.

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FURTHER STUDIES ON THE INTERACTIONS BETWEEN AMINE OXIDASE INHIBITORS AND TRYPTAMINE-INDUCED CONTRACTIONS OF RAT AORTA

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In rat aortic smooth muscle, tryptamine (TR) is deaminated by a semicarbazide-sensitive amine oxidase (SSAO) and also by monoamine oxidase (MAO), predominantly MAO-A in this tissue (Lyles & Singh, 1985; Lyles & Taneja, 1987). The latter report showed that the irreversible hydrazide MAO inhibitors (MAOIs) phenelzine and iproniazid, which also inhibit SSAO, can enhance the contractile potency of TR upon rat aorta. Inhibition of MAO appeared to be the major factor involved since the irreversible SSAO-selective inhibitor semicarbazide (SC) alone produced no such effect. We have now studied, either alone or in combination with SC, some other MAOIs lacking activity against SSAO.

Initial cumulative dose response curves (DRCs) for TR were obtained with aortic rings as previously described (Lyles & Taneja, 1987) to determine EC_{50} values designated DRC_1 below. Tissues were then incubated (20 min) with irreversible MAOIs (pargyline or deprenyl) followed after extensive washout, by a repeat DRC (DRC_2) to TR. Some tissues were subsequently incubated (20 min) with SC (10^{-3}M) to inhibit SSAO, before washout and running a third DRC (DRC_3). Use of harmaline, a reversible MAOI, involved incubation of paired tissues (after obtaining DRC_1) with either harmaline or harmaline + SC. These inhibitors were not washed out before obtaining DRC_2. None of the drugs used altered maximal contractile responses to TR in these DRCs.

INHIBITOR	n	TRYPTAMINE EC_{50} (μM, mean ± s.e.m.) DRC_1	DRC_2	DRC_3
Pargyline (10^{-4}M)	8	21.6 ± 2.2	3.4 ± 0.5[a]	1.9 ± 0.2[a]
Deprenyl (10^{-6}M)	6	27.7 ± 2.8	15.8 ± 3.6[b]	not done
Deprenyl (10^{-5}M)	7	28.0 ± 2.6	5.6 ± 0.7[b]	2.5 ± 0.3[b]
Harmaline (10^{-6}M)	7	10.5 ± 2.1	2.3 ± 0.4[b]	
Harmaline (10^{-6}M)+SC (10^{-3}M)	7	9.9 ± 0.8	2.0 ± 0.3[b]	
Harmaline (10^{-5}M)	7	11.9 ± 1.1	6.6 ± 1.1[b]	
Harmaline (10^{-5}M)+SC (10^{-3}M)	7	12.3 ± 1.0	3.4 ± 0.4[b,c]	

$P<0.01$[a] or 0.05[b] vs corresponding preceding column; $P<0.05$[c] vs harmaline alone

10^{-4}M pargyline and 10^{-5}M deprenyl produce substantial inhibition of both MAO-A and B in tissue homogenates, whereas 10^{-6}M deprenyl is relatively selective for MAO-B, and harmaline at the doses used is MAO-A selective. Since significant potentiation (i.e. decreased EC_{50}) of TR contractions was found after each MAOI, their selectivities suggest an important role for inhibition of MAO-A (the major MAO form in rat aorta) in producing this effect, although the data with 10^{-6}M deprenyl may imply some influence of MAO-B also. A further small but significant decrease in EC_{50} was seen after subsequent exposure of pargyline- or deprenyl-treated tissues to SC, suggesting some influence of SSAO also. To support this, corresponding paired tissues treated with these MAOIs but not exposed to SC, showed no change in EC_{50} between DRC_2 and DRC_3. Tissues treated with harmaline (10^{-5}M) + SC were more sensitive to TR than those exposed to harmaline alone, although it is not clear why this effect of SC was not found when combined with 10^{-6}M harmaline.

In conclusion, inhibition of SSAO may contribute to the potentiation of TR-induced contractions of rat aorta by certain amine oxidase inhibitors but its influence appears to be less important and consistent than that produced by inhibition of MAO, and may require prior inhibition of MAO in order to be clearly observed.

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EFFECTS OF THE ACE INHIBITOR CILAZAPRIL AND OTHER ANTIHYPERTENSIVE AGENTS ON EXERCISE TACHYCARDIA AND PRESSOR RESPONSES IN CATS

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It is well established that a number of classes of antihypertensive agents, notably those interfering with sympathetic nervous function, depress the cardiovascular responses to exercise. In the present study the effects of the new antihypertensive angiotensin converting enzyme inhibitor, cilazapril, on exercise induced pressor responses and tachycardia have been evaluated in conscious cats. Mean arterial pressure and heart rate were recorded from cats with exteriorised catheters, exercised in a metal drum for thirty seconds at 20 RPM by the method of Blaber and Burden (1982). Animals had resting heart rates of 196 $\pm$ 10 bpm and blood pressures of 126 $\pm$ 10 mmHg. Exercise induced a tachycardia of 85.8 $\pm$ 12.7 bpm and a pressor response of 38.3 $\pm$ 4.6 mmHg.

Propranolol 10 mg/kg P.O. significantly reduced exercise tachycardia but had no effect on the pressor responses. This effect on exercise has been previously reported in dogs (Staib et al 1983, Adam et al 1973) and man (Leenen et al 1983).

Prazosin 5 mg/kg P.O. had no effect, despite the high dose used, on either the tachycardia or vasopressor responses following exercise. The lack of effect on the pressor response may be due to it being a predominantly α_2-adrenoceptor agonist action produced by circulating catecholamines (Langer and Shepperson, 1982). Phenoxybenzamine (20 mg/kg P.O.) a non-selective α-adrenoceptor antagonist did reduce the exercise pressor response(-37 $\pm$ 3.7 mmHg at three hours). A reduction in exercise tachycardia also occurred after phenoxybenzamine treatment and this effect cannot be explained as the drug is not believed to have β-adrenoceptor antagonist properties.

The ACE inhibitors cilazapril, lisinopril and enalapril were also investigated at 3 mg/kg P.O. and were found to have no effect on either tachycardia or the increase in mean arterial blood pressure following exercise. This finding is in agreement with Becker, Fieber and Schulze (1985) who investigated the effects of ramipril on exercise in dogs and demonstrated that it did not impair exercise performance.

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CAPTOPRIL (CAP) REDUCES THE CONSEQUENCES OF CEREBRAL ISCHAEMIA IN RENOVASCULAR HYPERTENSIVE RATS (RHR)

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As CAP resets the lower limit of cerebral blood flow autoregulation (Barry *et al.*, 1984) - this lower limit being shifted to the right during hypertension (Barry, 1985) - we have investigated whether CAP would protect RHR against cerebral ischemia (ISCH). Systolic arterial pressure (SAP, mmHg) was determined in RHR (clip of 0.2 mm gap on left renal artery) by the tail cuff method following preheating. Rats were injected with CAP (100 mg/kg i.p.) or solvent (0.15 M NaCl) from the 11 th day following clipping onwards and were subjected to four vessel occlusion (15 min, Pulsinelli & Brierley, 1979) on the 24 th day. Motor activity was determined after ISCH in an open field test. Seven days after ISCH animals were perfused transcardially with fixative and sections stained with cresyl violet. Hippocampal (CA 1) and striatal damage was graded on a scale of 0 to 3 with 0 = normal, 1 = a few neurons damaged, 2 = many neurons damaged and 3 = majority of neurons damaged. The striatum was normal in all rats.

	Days after clip	Renovascular hypertension CAP	Renovascular hypertension Solvent	Sham Solvent
n		11	21	18
SAP	10	172±10	160±7	116±4
SAP	23	126±4	190±9	120±3
Mortality upon ISCH (%)				
	24	55	67	22
Motor activity (squares crossed in 6 min)				
n		5	7	14
	25	88±43	141±36	177±23
	26	9±2	65±12	71±10
Grade of CA 1 damage (% hemispheres with grade 0-3 damage)				
3		20	50	54
2		80	50	46

ISCH produced more deaths in RHR than in normotensive rats. CAP normalized blood pressure in RHR but did not significantly lower mortality. In RHR surviving ISCH, CAP attenuated the ISCH-induced hyperactivity and the ISCH-induced hippocampal damage. In conclusion, our results show that CAP does not lower the (increased) mortality in RHR in spite of the fact that it normalizes blood pressure, but that CAP does attenuate the consequences of ISCH in surviving RHR.

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TYPE I ANTIARRHYTHMIC DRUGS BIND STEREOSPECIFICALLY TO A RECEPTOR ASSOCIATED WITH THE CARDIAC SODIUM CHANNEL

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The antiarrhythmic action of type I drugs is thought to be mediated via blockade of the fast sodium channel (Vaughan Williams, 1975). We have recently developed a radioligand assay for a receptor for the type I agents which is associated with the cardiac sodium channel (Sheldon et al., 1987). Binding of type I agents to this receptor is saturable, reversible and occurs at pharmacologically relevant concentrations with similar rank order of potency in vitro and in vivo suggesting that binding is relevant to clinical effect. Most type I drugs structurally consist of an aromatic lipophilic residue connected via an intermediate group to a hydrophilic amine group. These common structural features are illustrated as: **Aromatic-C_1-link-C_2-Amine**, where C_1 and C_2 represent potentially asymmetric carbons. In the present study stereoisomeric pairs of antiarrhythmic drugs with asymmetric carbons at sites C_1 or C_2 were used to determine the steric requirements for drug binding.

The radioligand assay measures the binding of [^{3}H]batrachotoxinin A 20α-benzoate ([^{3}H]BTXB), a sodium channel-specific toxin, to freshly isolated cardiac myocytes (Sheldon et al., 1986). Myocytes were prepared by collagenase dispersion of adult rat ventricles using the method of Kryski et al. (1985). [^{3}H]BTXB binding was determined by incubating aliquots of myocytes with 1.3 μM sea anemone toxin II; 13 nM [^{3}H]BTXB (50ci/mmol) and 0.13 mM tetrodotoxin for 60 min at 37°C in a final volume of 50 μl. The relative affinities of enantiomers were estimated by their ability to inhibit the binding of [^{3}H]BTXB (IC_{50}).

All enantiomers tested inhibited [^{3}H]BTXB binding in a dose-dependent manner with mean Hill numbers ranging from 0.89 - 1.1 suggesting their interaction with a single class of saturable sites. The relative potencies ([IC_{50}(+) isomer]/[IC_{50}(-)isomer]) for C_1 and C_2 pairs are shown in Table 1.

C_1-Pairs	Potency Ratio	C_2-Pairs	Potency Ratio
Quinidine	0.29 ± 0.03*	Mexiletine	2.15 ± 0.41*
Disopyramide	1.11 ± 0.17	Flecainide	1.03 ± 0.01
Cinchonidine	0.63 ± 0.12*	Tocainide	2.97 ± 0.12*
RAC 109	5.33 ± 0.80*		

* Significant difference between (+) and (-) isomers, $p < 0.05$. Number of experiments per pair = 3

Thus the conformation of both asymmetric carbons are important for drug binding. Stereospecific differences in binding were seen in pairs with either C_1 or C_2 asymmetric carbons suggesting the presence of at least two stereospecific domains on the receptor for type I antiarrhythmic drugs.

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STUDIES ON THE BEHAVIOUR OF PLATELETS DURING ACUTE MYOCARDIAL ISCHAEMIA AND REPERFUSION

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Experimental and post-mortem studies have demonstrated an accumulation of platelets in infarcted myocardium (Leinberger et al, 1979; El-Maraghi & Gentone, 1980). The result of this phenomenon may be to exacerbate tissue injury through the formation of microthrombi and the release of vasoactive substances such as thromboxane A_2. Anti-thrombotic compounds such as aspirin (Coker et al, 1981) have been shown to protect against ischaemia and reperfusion-induced arrhythmias in greyhounds, although it is unclear whether this is due to the prevention of platelet aggregation or to the inhibition of vasoconstriction. This study was performed in an attempt to determine the behaviour of platelets during early myocardial ischaemia and, with the use of the cyclo-oxygenase inhibitor, to define their role in arrhythmogenesis.

Greyhounds were anaesthetised with chloralose and prepared for occlusion of the left anterior descending coronary artery (LAD). Catheters were placed in the coronary sinus (draining the essentially normal myocardium) and in a local coronary vein (draining the area rendered ischaemic by the occlusion) to obtain blood sampling for platelet counts. For the counting 1 ml samples qwere withdrawn at various times prior to and during coronary occlusion and immediately mixed with 0.9 ml 1% EDTA and 0.1 ml 1% formalin (to prevent spontaneous disaggregation). The blood was then diluted with ammonium oxalate (1%) and erythrocytes lysed with saponin (0.0092%) using a Technicon Autocounter (Smith & Duncan, 1981) and counted optically. Treated dogs received Na Meclofenamate in a dose of 2 mg kg^{-1} i.v., 15 min prior to coronary artery occlusion.

In control, untreated dogs which survived the 30 min occlusion period there was a gradual, but continuous, drop in circulating platelet count within the coronary circulation significant by 7 min. This was more marked in the samples obtained from the local coronary vein, reaching a nadir of 68 ± 7% of initial platelet count by 40 min post-occlusion compared to 85 ± 4% of initial count in the coronary sinus. In dogs treated with Meclofenamate there was no effect on basal circulating platelet count (151702 ± 4854 μl^{-1} in controls vs 131588 ± 6932 μl^{-1}),and following occlusion there was no significant change in platelet count in either the coronary sinus or the coronary vein, until 40 min post-occlusion. In both groups count returned to normal within 1-2 min reperfusion. The arrhythmias observed during this 30' occlusion period were similar in both treated and untreated groups (790 ± 215 in controls vs 1113 ± 355 in dogs given Na Meclofenamate). Thus no anti-arrhythmic effect of Na Meclofenamate was observed.

These results have demonstrated that there is a reduction in circulating platelet count in the ischaemic region of the myocardium and this can be delayed, by the administration of an anti-thrombotic compound.

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THE EFFECT OF NIFEDIPINE AND LIDOFLAZINE PRETREATMENT IN EXPERIMENTAL CARDIOPLEGIA

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The guinea-pig heart-lung preparation (HLP) was used as a cardiopulmonary bypass model to study possible additional protective effects of the calcium antagonists nifedipine ($6x10^{-9}$ and 10^{-8} M) and lidoflazine (10^{-6} and $3x10^{-6}$ M) in cardioplegia. After 15 min of equilibration and a 15 min pretreatment period with drug or vehiculum, cardiac arrest was induced by retrograde perfusion with cold ($4^{o}C$) modified St. Thomas Hospital cardioplegic solution (K^{+} 2 mM, Na^{+} 147 mM, Cl^{-} 203 mM, Ca^{++} 2 mM, procaine 1 mM) containing similar concentrations of the calcium antagonist or vehiculum as used during pretreatment. Subsequently, hearts were subjected to 135 min of hypothermic ($22-23^{o}C$) ischaemia. During cardiac arrest and the first 10 min of retrograde reperfusion hearts were allowed to unload via the right ventricle and a needle inserted in the left ventricle. After 10 min of retrograde reperfusion with fresh blood hearts were switched back to the working mode. After 30 min of reperfusion the recovery of functional (LVP, dp/dt_{max} and cardiac output [CO]) and biochemical (ATP and CrP) parameters were monitored and calculated as percentage of normoxic values before and after pretreatment. With respect to the contractility (dp/dt_{max}) no significant improvement of hearts treated with calcium antagonists except for lidoflazine (10^{-6} M) was observed, only when compared with values before pretreatment. Similarly, no significant differences between non-pretreated and pretreated hearts for CO were found except for nifedipine ($6x10^{-9}$ M).

Table 1

	% Recovery		
	LVP	dp/dt_{max}	CO
Control	86.5 ± 4.2	67.6 ± 2.6	78 ± 9.3
Lidoflazine (10^{-6} M)	92.3 ± 5.3	75.4 ± 2.1*	93.5 ± 6.5
Lidoflazine ($3x10^{-6}$ M)	92.5 ± 3.8	75.6 ± 3.4	87.1 ± 7
Nifedipine ($6x10^{-9}$ M)	93.1 ± 1.9	64.5 ± 1.5	98.4 ± 1.2*
Nifedipine (10^{-8} M)	83 ± 3.6	62.4 ± 2.7	84.4 ± 7.3

* significant at $p < 0.05$

Both control hearts and hearts pretreated with a calcium antagonist showed an overshoot of CrP after reperfusion, whereas a decrease of post-cardioplegic tissue ATP levels was found. Treatment with lidoflazine ($3x10^{-6}$ M) revealed a significantly higher recovery of tissue ATP after cardioplegia (92.2 ± 4.4% vs 60.4 ± 9.7% in control hearts).

In conclusion, except for a small but significant improvement for lidoflazine (10^{-6} M) upon dp/dt_{max} and for nifedipine ($6x10^{-9}$ M) upon CO, treatment with calcium antagonists did not affect post-cardioplegic functional parameters. However, for lidoflazine ($3x10^{-6}$ M) a clear improvement of tissue high energy phosphate content was observed.

COMPARISON OF ANTI-ISCHAEMIC EFFECTS OF NIFEDIPINE, LIDOFLAZINE AND MIOFLAZINE UPON GLOBAL ISCHAEMIA IN THE RAT WORKING HEART

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Nifedipine has proved to be effective in reducing the damage due to ischaemia in different heart preparations (Watts et al., 1986). In the present investigation the anti-ischaemic activity of nifedipine (N), lidoflazine (L) and mioflazine (M), respectively, in concentrations which reduced contractile force by 30% were compared. These concentrations amounted to 80 nM, 1 µM, 3 µM for N, L and M. Rat isolated working hearts were perfused with Tyrode solution containing 1.8 mM calcium. After a 15 min period of pretreatment with the drug the atrial filling line was clamped to render the hearts globally ischaemic for 45 min. A 25 min recovery period consisted of 10 min retrograde perfusion, followed by 15 min perfusion in the working mode. The effects of nifedipine, lidoflazine and mioflazine on the recovery of aortic pressure (AoP) and cardiac output (CaO), expressed as the percentage of the initial values, as well as ATP (nmoles/mg dry weight) and $\sim P$, calculated as Σ (creatine phosphate + 2 x ATP + ADP) are shown in the table:

	AoP	CaO	ATP	$\sim P$
Control	23.1 ± 3.8	37.2 ± 6.1	11.68 ± 1.21	38.00 ± 3.58
N	56.5 ± 6.8	56.5 ± 5.8	12.38 ± 1.39	53.00 ± 2.67
L	42.9 ± 5.2	68.4 ± 6.7	14.41 ± 1.68	69.93 ± 5.00
M	63.2 ± 9.6	72.9 ± 8.4	14.67 ± 1.89	63.09 ± 10.95

(Mean ± S.E.M., n=6).

During the first 5 min of reperfusion the coronary perfusate was collected to determine the loss of adenosine, inosine, hypoxanthine, xanthine and uric acid from the tissue. Pretreatment with N and L resulted in a similar slightly decreased loss of adenine nucleotide breakdown products, whereas after pretreatment with M the suppression of the loss of purines and oxypurines was more obvious.
All compounds exerted a similar improvement of the mechanical cardiac functions after ischaemia. However, after pretreatment with L and M a more pronounced recovery of ATP and $\sim P$ of the heart was observed than after pretreatment with N. The results clearly show a potent protective activity upon myocardial ischaemia of L and M, as reflected by the pronounced recovery of the ATP content and the $\sim P$. However, the recovery of mechanical activity was comparable to that caused by N.

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EFFECT OF CHRONIC IN VIVO TREATMENT OF GUINEA-PIGS WITH VARIOUS β-ADRENOCEPTOR BLOCKERS ON β_1 AND β_2-ADRENOCEPTORS

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β-adrenoceptor blockers given in the long-term are widely used for the treatment of various diseases. Abrupt withdrawal of treatment can lead to exaggerated responsiveness of β-adrenoceptors (Alderman et al, 1974). Increased density of β-adrenoceptors in the heart and other tissues has been suggested as the cause of the withdrawal reactions (Aarons & Molinoff, 1982). The aim of the present study is to investigate the effect of chronic treatment of guinea-pigs with various beta blockers on the density and affinity of β_1- and β_2-adrenoceptors. The left ventricle and gastrocnemius muscles were chosen because in the guinea-pig these two tissues contain homogenous populations of β_1- (Hedberg et al, 1980) and β_2-adrenoceptor subtypes (Elfellah et al, 1987) respectively.

Male guinea-pigs each weighing 300-400 gm were divided into groups, each group (7-15 animals) was treated with saline, propranolol (nonselective antagonist, 20 mg/kg), atenolol (β_1-selective antagonist, 10 mg/kg), ICI 118551 (β_2-selective antagonist, 10 mg/kg), or pindolol (β_1-antagonist with a partial β_2-agonist activity, 5 mg/kg). Drugs were given subcutaneously thrice daily for one week. Atenolol was given once daily while pindolol was given orally. The animals were sacrificed and left ventricles and gastrocnemius muscle membranes were prepared and density (B_{max}) and apparent affinity (K_D) of β-adrenoceptors were measured using the radioligand [^{125}I] iodocyanopindolol (ICYP)) as previously described (Elfellah & Reid, 1987).

In the left ventricle neither the density nor the affinity of β-adrenoceptors were altered by any of the β-adrenoceptor blockers used. In skeletal muscle the density and the K_D of β_2-adrenoceptors were increased by pretreatment with propranolol and ICI 118551 but not atenolol. The change in K_D may result from propranolol and ICI 118551 being retained by the tissue. In contrast, pretreatment with pindolol reduced the density of skeletal muscle receptors.

Table 1 Effect of in vivo pretreatment of guinea pigs with β-adrenoceptor blockers on B_{max} (means ± SD).

Pretreatment	Saline	Propranolol	Atenolol	ICI 118551	Pindolol
Left ventricle	27 ± 7	35 ± 6	28 ± 9	33 ± 13	30 ± 8
Gastrocnemius m.	32 ± 8	49 ± 14*	27 ± 7	48 ± 11*	16 ± 5*

* $p < 0.01$

Therefore, in the guinea-pig, β-adrenoceptors in the heart unlike the β_2-adrenocepotors in the skeletal muscle are resistant to up regulation caused by pretreatment with β-adrenoceptor antagonists.

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THE EFFECTS OF HUMAN ATRIAL NATRIURETIC PEPTIDE ON HEPATIC HAEMODYNAMICS IN THE ANAESTHETISED DOG

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Mammalian atrial myocytes contain and release natriuretic peptides with actions on the cardiovascular system. The 28 amino acid atrial natriuretic peptide (ANP) is known to cause systemic hypotension; its regional vascular actions are not completely established (Lappe et al., 1986).

Human ANP was injected into the separately perfused hepatic arterial and portal circulation of the anaesthetised dog to establish its vascular actions at these sites. In one group the hepatic arterial system was cannulated and perfused with arterial blood; continuous measurements were made of hepatic arterial blood flow (HABF) and perfusion pressure (HAPP). Synthetic human α-ANP (Sigma or Nova-Biochem) was injected close arterially over the dose range 0.1 - 20 nmol. At all doses ANP caused a mean increase in HABF reflecting, at constant perfusion pressure, a fall in hepatic arterial vascular resistance. The hepatic arterial vasodilatation was graded with dose. The threshold in all experiments was below 100 pmol ANP and the maximum vasodilator effect achieved at 10 or 20 nmol. Isoprenaline (ISO) was administered intra-arterially to provide a comparison for vasodilator activity. The maximum vasodilator effect (percentage increase in hepatic arterial blood flow, mean $\pm$ s.e.) to ANP (60.7 $\pm$ 11.0%) was not significantly different ($P > 0.5$) than that to ISO (72 $\pm$ 15.8%) in the same preparations. However, the mean molar dose to decrease hepatic arterial vascular resistance to 50% of the maximum effect (ED_{50}, mean $\pm$ s.e.) was significantly greater ($P < 0.01$) for ANP (2.78 $\pm$ 0.87 nmol) than for ISO (0.42 $\pm$ 0.13 nmol); i.e. ANP was less potent than ISO.

In a further series the portal vein was cannulated, perfused at constant flow with blood derived from the mesenteric vein; portal perfusion pressure was continuously recorded as a direct assessment of portal inflow resistance. ANP was injected in doses from 0.1 - 20 nmol; no change in portal pressure was observed at any dose level indicative of an absence of a direct action of the peptide at this site.

Elevated systemic circulating levels of ANP may increase total liver blood flow by hepatic arterial dilatation without any direct change in portal inflow resistance. However ANP may also dilate the mesenteric circulation (Caramelo et al., 1986), increase drainage into the portal vein and further increase total liver perfusion.

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THE ACTION OF HUMAN ATRIAL NATRIURETIC PEPTIDE ON ISOLATED HUMAN RESISTANCE VESSELS

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1-28 human α-atrial natriuretic peptide (ANP) has been shown to relax a variety of large arteries from many species including man (Winquist et al., 1984). However, in a recent study looking at the smaller blood vessels primarily responsible for the control of peripheral vascular resistance in the rat, ANP only relaxed isolated renal arterioles (Aalkjaer et al., 1985) and was without effect on mesenteric, coronary, cerebral or femoral arterioles. We have therefore studied the effect of ANP on isolated human resistance arteries from different sites.

Arteries (median internal diameter 264 μm range 159-729 μm) were dissected from tissue removed at surgery from 8 patients (age range 40-75) and used immediately for studies. Arteries were obtained from omentum (n=3), subcutaneous fat (n=4), kidney (n=4) and skeletal muscle (n=2). Isometric tension was measured using a myograph technique (Mulvany & Halpern, 1977). Vessels were normalised to a resting diameter 0.9 L100 where L100 is the diameter producing the wall tension equivalent to that produced by 100 mm Hg distending pressure calculated by the Laplace relationship. Following 1 hr equilibrium, vessel viability was confirmed by the demonstration of contractile responses to potassium depolarising solution (K 125 mM) and noradrenaline (10^{-5}M). Viable arteries by these criteria were then used for relaxation studies. In order to assess relaxation, tone was induced in the vessels by a depolarising potassium solution. ANP was added cumulatively once a stable tone was achieved. Relaxation was calculated as % reduction in potassium induced tone. The presence of a function endothelium was assessed by addition of acetylcholine (10^{-7}M). Relaxation in response to this agent was taken as indicating a functional endothelium. ANP (10^{10}-10^{-6}M) produced concentration dependent relaxation of renal and skeletal muscle arterioles. Median EC_{50} for ANP was 7 X 10^{-9}M (range 3-27 X 10^{-9}M) in renal arterioles. ANP induced relaxation was not dependent on a functional endothelium. In contrast, ANP was without effect on omental or subcutaneous arterioles.

These results indicate that in contrast to large human arteries (Hughes et al., 1987), there is considerable site variation in the effect of ANP on isolated human resistance arteries and furthermore may indicate an important role for ANP in the regulation of renal vascular resistance in man.

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THE EFFECT OF VERAPAMIL ON CONTRACTION INDUCED IN RABBIT ISOLATED CORONARY ARTERY IN NORMOXIA AND HYPOXIA

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Agonist-induced spasm of large epicardial coronary arteries is thought to be a principal cause of Variant angina (Kalsner, 1982; Yasue et al, 1976). Moreover, Kalsner (1982) indicated that such spasm is not readily reversed by locally produced metabolites or hypoxia resulting from ischaemia. In the present study we have examined the effects of hypoxia upon agonist- and depolarisation-induced contraction of isolated coronary arteries and their sensitivities to the calcium entry blocker, verapamil.

The left anterior descending coronary artery and its larger branches were dissected from hearts excised from New Zealand rabbits (2-2.5kg) under pentobarbitone anaesthesia (45mg/kg i.v.). 3mm cylindrical segments were suspended under a tension of 0.5g in Krebs' maintained at 37°C and gassed with 13%O_2/5%CO_2 in N_2 (normoxia, PO_2=105±5 mmHg) or with 2%O_2/5%CO_2 in N_2 for 30 min (hypoxia, PO_2=27±2 mmHg). Contractions (to 80% of maximal) were elicited by 1μM 5-HT or 30mM KCl. Responses to these same concentrations were then elicited in normoxia and hypoxia (1) after exposure to Ca^{2+}-free Krebs' (+0.5mM EGTA) for 5 min and (2) following incubation with 10μM verapamil (VER) for 30 min.

Hypoxia had no effect on KCl-induced contractions but caused a 53.8±5.7% (n=9) reduction of the contraction to 5-HT. Under Ca^{2+}-free conditions, contractions to KCl were completely abolished and only a transient contraction to 5-HT remained which reached 29.1±6.8% (n=7) and 12.8±0.9% (n=9) of the normoxic control response in normoxia and hypoxia respectively. In the presence of VER (10μM) KCl contractions were also abolished, while 5-HT produced a rapid contraction followed by relaxation to a stable maintained tension. The fast phase reached 60.4±11.9% (n=5) of the normoxic control in normoxia but only 10.6±0.9% (n=4) in hypoxia, while the sustained phase amounted to 16.1±4.9% (n=5) of the normoxic control in normoxia and 8.0±1.2% (n=4) in hypoxia.

These results indicate that in the isolated coronary artery, hypoxia has little effect upon contraction which depends upon Ca^{2+} entry via voltage-operated channels, but can attenuate contractions which are mediated by agonist-induced Ca^{2+} influx and by release of intracellular Ca^{2+}. Thus, in vivo, K^+ released by cardiac muscle cells during hypoxic ischaemia might exacerbate agonist-induced contraction of coronary arteries but this would be expected to be readily reversed by VER.

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FURTHER CHARACTERISATION OF 5-HT_1-LIKE RECEPTORS IN ISOLATED VASCULAR TISSUES WITH ICI 169,369

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We have previously demonstrated that ICI 169,369 possesses high affinity for the 5-HT_2 receptor and low affinity for the receptor mediating contraction to 5-HT in the rat fundic strip (Blackburn et al., 1987). This latter receptor has been classified as 5-HT_1-like (Bradley et al., 1986), however, we have recently demonstrated that there is a dissimilarity between this receptor and that involved in relaxation to 5-HT in the histamine pre-contracted guinea-pig ileum (Growcott et al., 1987).

In an attempt to obtain some meaningful measure of 5-HT_1 affinity with ICI 169,369, we have assessed the effects of the compound, together with ketanserin, methysergide and methiothepin in two proposed 5-HT_1-receptor systems, the canine saphenous vein and basilar artery.

Saphenous vein and basilar artery strips were obtained from dogs weighing 12-14 kg and set up for isotonic recording of length changes. The contractile effects of 5-HT, 5-CT, 8-OH-DPAT and RU24969 were assessed in the saphenous vein, agonist effects of 5-HT were assessed in the basilar artery. Antagonist activity of ICI 169,369 against these agonists was compared to that obtained with ketanserin, methysergide and methiothepin against 5-HT in the saphenous vein. Antagonism of 5-HT with these agents was assessed in the basilar artery. In all cases a 30 min antagonist equilibration period was used.

In the saphenous vein, 5-HT was approximately 10 x less potent than 5-CT (EC_{50} 105 ± 19.5 and 12.5 ± 8.5 nM respectively) whereas 8-OH-DPAT and RU24969 were approximately 300 x less potent than 5-CT (EC_{50} 3.99 ± 0.88 and 2.70 ± 1.03 μM respectively). ICI 169,369, up to 1 x 10^{-6}M, produced no significant antagonism of 5-HT or 5-CT whereas weak, probably non-competitive antagonism of 8-OH-DPAT and RU24969 was observed. Ketanserin, at 1 x 10^{-6}M only, produced non-competitive antagonism of 5-HT. Methysergide (1 x 10^{-7}M - 1 x 10^{-5}M) was a partial agonist. Methiothepin (1 x 10^{-8} - 1 x 10^{-7}M) was a non-competitive antagonist.

In the basilar artery, ICI 169,369 (1 x 10^{-7}M - 5 x 10^{-7}M) produced non-competitive antagonism of 5-HT whereas ketanserin was inactive up to 1 x 10^{-6}M. Methysergide (1 x 10^{-7} - 1 x 10^{-6}M) and methiothepin (1 x 10^{-8}M - 1 x 10-7M), produced non-competitive antagonism of 5-HT.

These results suggest that the 5-HT antagonist, ICI 169,369, has no significant affinity for the 5-HT_1 receptors in the dog saphenous vein. However, low affinity was apparent for the 5-HT receptors in the basilar artery. Could these be atypical 5-HT receptors and resemble those present in the rat fundic strip?

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RU 24969, A 5-HT_1 REFERENCE COMPOUND WHICH BEHAVES AS A POTI 5-HT_2 AGONIST ON BLOOD VESSELS

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The compound RU 24969 (5-methoxy-3-[1,2,3,6-tetrahydro-4-pyridinyl]-1H-indole) shows some specific binding affinity for the subtype 1 of 5-hydroxytryptamine receptors (5-HT_1) (HUNT et al. 1981). Additionnal binding investigations on brain preparations have led to a classification of RU 24969 as a selective $5HT_{1B}$ agonist (SILLS et al. 1984) and this compound is frequently used as such. In order to investigate the effects of RU 24969 on the cardiovascular system we have performed in vitro experiments on rat tail artery. After the animals were stunned, the ventral artery was dissected and placed in bicarbonate buffered physiological solution (mM : NaCl 120.8 ; KCl 5.9 ; $CaCl_2$ 2.5 ; $MgCl_2$ 1.2 ; $NaHCO_3$ 15.5 ; NaH_2PO_4 1.2 ; glucose 11.2 ; pH 7.4). Small rings were mounted in a thermostated (37°C) organ bath between a fixed hook and a force transducer. Cumulative addition of RU 24969 in the bath produced a concentration dependent increase in tension with a threshold concentration at 3×10^{-8}M and a maximal response at 10^{-5}M, both similar to 5HT. However, the maximal developped tension reached only 48.1 % (n=14) of maximal 5HT response. The EC_{50} were 3.19×10^{-7}M and 1.06×10^{-7}M for RU 24969 and 5HT respectively. The concentration response curve to RU 24969 was not affected by the absence or the presence of endothelium (assessed by the acetylcholine relaxing response).

The contractile responses to RU 24 969 were not modified by yohimbine 10^{-7}M ; prazosin 3×10^{-8}M only slightly decreased the responses to the highest doses of the compound. Ketanserine and methysergide produced a concentration dependent inhibition of the maximal response to RU 24969. The reductions of the maximal contractions were of 54.5 % with 10^{-8}M ketanserine (n=8) and 44.1 % with 5×10^{-10}M methysergide (n=7).

These results show that RU 24969 possesses a potent contractile activity on peripheral blood vessels of the rat and that this effect is mediated by an interaction with serotonin receptors of the $5HT_2$ type. No evidence of a $5HT_1$ effect was observed in these experiments.

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ACTIONS OF AGONISTS AT 5-HYDROXYTRYPTAMINE RECEPTORS IN THE HUMAN SAPHENOUS VEIN

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In the human saphenous vein, 5-hydroxytryptamine (5-HT) produces contractions by action at both 5-HT_2-receptors and non-5-HT_2-receptors, which can be classed as 5-HT_1-like receptors (Docherty & Hyland, 1986; Victorzon et al., 1986). We now continue the investigation of 5-HT receptors in the human saphenous vein by examining the actions of a variety of agonists with actions at 5-HT receptors.

Human saphenous veins were obtained from coronary artery bypass grafts of predominantly male patients, were cut spirally into strips and mounted in organ baths for isometric tension recordings.

5-HT contracted the human saphenous vein with a maximum tension of 0.70±0.13 g (n=19) and an EC_{50} of 6.33 (-log M). The 5-HT receptor agonists α-methyl-5-HT (α5-HT) and 5-methoxytryptamine (5-MT) produced similar maximum contractions to those produced by 5-HT (91.0±2.6% and 94.3±2.3% of the maximum contraction to 5-HT, respectively, n=8 each). The 5-HT receptor agonists 5-carboxamidotryptamine (5-CT) and RU 24969 (5-methoxy-3(1,2,5,6-tetrahydro-4-pyridyl)1H indole) produced maximum contractions of 62.3±3.5% (n=11) and 66.0±5.5% (n=7), respectively of the maximum contraction to 5-HT. In contrast, 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin) produced a maximum contraction of 26.1±11.2% (n=5) of that to 5-HT, and TVX Q 7821 (2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-1,2-benzisothiazol-3-(2H) one-1,1-dioxidehydrochloride) failed to contract the human saphenous vein in concentrations of up to 10 μM (n=2).

The 5-HT receptor antagonist metitepin (0.1 μM) produced approximately parallel shifts in the concentration-response curves of all 5-HT receptor agonists tested. In contrast, the 5-HT_2-receptor antagonist ketanserin produced non-parallel shifts in agonist concentration-response curves, shifting mainly the upper part of the concentration-response curves. The ketanserin-sensitive component of the concentration-response curve was large for 5-HT, α5-HT and 5-MT, but relatively small for the other agonists examined.

The rank order of agonist potency at producing contractions of the human saphenous vein was 5-HT > 5-CT = α5-HT > 5-MT = RU 24969 > 8-OH-DPAT, with TVX Q 7821 inactive.

Based on the interaction with the selective 5-HT_2-receptor antagonist ketanserin and the non-selective 5-HT-receptor antagonist metitepin, we can conclude that α5-HT and 5-MT, like 5-HT, interact with both 5-HT_2 and 5-HT_1-like receptors in producing contractions, whereas 5-CT and RU 24969 act mainly at 5-HT_1-like receptors.

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ENDOTHELIUM REMOVAL AUGMENTS REACTIVITY OF CANINE BASILAR ARTERY TO 5-HT AND SELECTIVE 5-HT AGONISTS

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We have previously demonstrated the marked contractile action of 5-hydroxytryptamine (5-HT) and selective 5-HT_1-like agonists such as 5-carboxamidotryptamine (5-CT) and GR 43175 in canine and primate cerebrovascular smooth muscle (Connor et al, 1987). We have now investigated the influence of the cerebrovascular endothelium on the reactivity of canine basilar artery rings to 5-HT, 5-CT, GR 43175, α-methyl 5-HT and the thromboxane-mimetic U46619. Contractile effects were compared in control (untreated) preparations and in preparations which had been perfused with 0.1% Triton X-100 (0.5 ml/min for 1 min) to damage the integrity of the vascular endothelium as described in detail by Connor and Feniuk, 1987.

Unlike control preparations, those perfused with Triton X-100 did not relax in response to the endothelium-dependent vasodilator, substance P (Furchgott, 1983). The contractile response to potassium chloride (30 mM) in control and Triton X-100 perfused basilar arteries was similar (1.68±0.12 g and 1.58±0.10 g respectively, n=20, mean ± S.E.M). Cumulative concentration- response curves to 5-HT, 5-CT, GR 43175, α-methyl 5-HT and U46619 were obtained in control and Triton X-100 perfused preparations; EC_{50} values and maximum response (expressed as % KCl) were measured. Following endothelial damage there was a marked increase in the maximum response to 5-HT, α-methyl 5-HT, GR 43175 and 5-CT but not U46619 (Table 1) although there was little change in EC_{50} values. Neither 5-HT nor 5-CT caused relaxation of control basilar artery rings contracted with prostaglandin $F_{2\alpha}$, U46619, potassium chloride or uridine triphosphate.

	Control		Triton X-100 treated	
	EC_{50} (nM)	max response (% KCl)	EC_{50} (nM)	max response (% KCl)
5-HT	62 (30-126)	67±10	40 (30-54)	130±15
5-CT	4 (3-7)	20±7	5 (4-5)	41±7
GR 43175	94 (19-462)	26±5	107 (19-588)	66±3
α-methyl 5-HT	320 (150-670)	72±3	310 (160-630)	140±16
U46619	4 (2-6)	151±22	3 (2-6)	156±12

Table 1: EC_{50} values and maximum responses produced by agonists in control and Triton X-100 perfused canine basilar artery. Values are mean (95% limits) or mean ± S.E.M, n=4-7.

These results suggest that the cerebrovascular endothelium spontaneously releases a relaxing factor which attenuates the contractile response to 5-HT and selective 5-HT agonists but not that to the thromboxane A_2-mimetic U46619.

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