THE EFFECTS OF BW B385C UPON THE CARDIOVASCULAR RESPONSES TO AUTONOMIC NERVOUS STIMULATION

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BW B385C is a novel ACE-inhibitor which also possesses β-adrenoceptor blocking properties (Allan et al, 1987). It would therefore be expected to attenuate β-adrenoceptor mediated cardiovascular responses to autonomic nervous stimulation. We have therefore carried out a series of studies in vivo to investigate the effects of this agent upon the cardiovascular responses to stimulation of the sympathetic and parasympathetic components of the autonomic nervous system.

Male Wistar rats (250g, n=6 per group) were anaesthetised by inhalation (isoflurane/O_2), tracheotomised, pithed and immediately pump-ventilated. Arterial/venous cannulae were then implanted and the animals paralysed with 1mgkg^{-1} i.v. dimethyl-tubocurarine. Electrical stimulation (via pithing rod) was applied to discrete areas of the spinal cord (C7-T1 or T7-9) to achieve selective stimulation of the preganglionic sympathetic nerve fibres to either the heart or the vasculature, in order to increase heart rate (HR-mediated by β-adrenoceptors) or blood pressure (BP-mediated by α-adrenoceptors) respectively. Frequency-related (0.3 / 1 / 3 Hz) HR increases were significantly attenuated by 1 mgkg^{-1} i.v. BW B385C (42 ± 9 / 112 ± 11 / 132 ± 8 to 12 ± 4 / 28 ± 4 / 55 ± 7 btmin^{-1}, $p<0.05$), however, the BP increases (14 ± 3 / 39 ± 6 / 71 ± 5 mmHg) were unaffected. At 10 mgkg^{-1} i.v. BW B385C, the HR increases were further attenuated, but the BP increases remained unaffected. In these animals, BW B385C also significantly attenuated the HR and BP increases due to i.v. isoprenaline and angiotensin I, respectively.

Beagle dogs of either sex (9-12 kg, n=5 per group) were anaesthetised by i.v. injection (α-chloralose/pentobarbitone), tracheotomised and pump-ventilated prior to right thoracotomy. Arterial/venous cannulae were implanted and the descending cardiac sympathetic nerve trunks arising from the right stellate ganglion were sectioned and placed on a bipolar electrode. Electrical stimulation of these post-ganglionic sympathetic nerve fibres produced a frequency-related (1 / 2 Hz) increase in HR (mediated by β-adrenoceptors). Cumulative BW B385C (up to 0.3 mgkg^{-1} i.v.) significantly attenuated these HR increases (51 ± 5 / 90 ± 5 to 11 ± 2 / 40 ± 7 btmin^{-1}, $p<0.05$ at 0.3 mgkg^{-1}). In addition, BW B385C significantly inhibited plasma ACE-activity (34 ± 6 to 5 ± 1 U.l^{-1}, $p<0.05$ at 0.3 mgkg^{-1}).

Cats of either sex (3kg, n=4 per group) were anaesthetised by i.v. injection (α-chloralose/pentobarbitone), tracheotomised and pump-ventilated. Arterial/venous cannulae were implanted and the descending right cervical vagus nerve trunk was sectioned and placed on a bipolar electrode. Electrical stimulation of these preganglionic parasympathetic nerve fibres produced a frequency-related (1 / 3 / 10 Hz) reduction in HR (mediated by cholinergic receptors). Cumulative BW B385C (up to 30 mgkg^{-1} i.v.) had no effect upon these HR reductions (30 ± 2 / 105 ± 13 / 160 ± 8 btmin^{-1}), but did cause a significant inhibition of plasma ACE-activity (91 ± 6 to 8 ± 2 U.l^{-1}, $p<0.05$ at 0.3 mgkg^{-1}).

It is concluded from these studies that BW B385C does not affect the response of the cardiovascular system to either parasympathetic or sympathetic nervous stimulation except when the latter is mediated through a β-adrenoceptor.

Allan et al (1987) Brit. J. Pharmacol. 92 (Suppl.) 571P.

A CONSCIOUS RAT MODEL FOR HEART FAILURE INDUCED BY MYOCARDIAL INFARCTION (MI); EFFECTS OF LONG-TERM CAPTOPRIL AND DOBUTAMINE

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MI is widely used to produce congestive heart failure in rats. We investigated the acute and long-term effects of MI on cardiac function in conscious animals. For the acute study rats were prepared for coronary artery ligation by placing a loose ligature around the left descending coronary artery, which was passed to the neck. Rats were equipped with an electromagnetic flowprobe on the ascending aorta for measurement of cardiac output (CO). Animals were allowed 5-6 days for recovery. Catheters were placed in the abdominal aorta and thoracic vena cava to measure arterial (MAP) and central venous pressure (CVP) and in the abdominal vena cava for infusions. After 1-2 days recovery, animals were connected to the measurement equipment, baseline hemodynamics were obtained and the heart was maximally stimulated by a volume loading (12 ml Ringer's solution in 1 min). Two days later, animals were reconnected to the measuring equipment. During continuous registration of hemodynamics, the ligature was tightened in the conscious animal. This resulted in an immediate increase in CVP from 1.9±0.7 to 3.5±1.0 cm H_2O and a decrease in CO from 89±4 to 70±5 ml/min, resulting from a decrease in stroke volume (SV), while MAP and heart rate (HR) were not affected. Twenty-four hr later, the volume infusion protocol was repeated. Whereas before MI no differences were found beween the groups, 24 hr after MI cardiac function was significantly depressed during volume loading in infarcted rats compared to sham animals (MI: 93±7, sham: 134±3 ml/min). Stimulated CO was linearly related to infarct size ($r=-0.958$, $p<0.0001$) (ie. inversely related).
Long-term effects of MI were studied 5 weeks after MI at baseline conditions and during maximal stimulation by volume loading. In contrast to the acute situation, baseline hemodynamics were not significantly different from sham animals. Stimulated cardiac output was depressed in relation to infarct size but infarct sizes of 20% or less seem to be hemodynamically compensated.
These results indicate that acute and long-term hemodynamic effects of MI can be studied in conscious rats. Heart failure as evidenced from a depression of cardiac function, was found to be related to the size of the MI.

In parallel, we investigated the effects of 14 days treatment with two different agents. The angiotensin I converting enzyme inhibitor (ACEI) captopril (CAP) was administered at rates of 100 or 500 µ/kg.hr, using osmotic minipumps. Besides a decrease of MAP (control, 100, 500 µg/kg.hr: 91±8, 87±5, 74±7 mm Hg) and total peripheral resistance, no effects were seen on baseline hemodynamic parameters, while effects on stimulated values were pronounced. CAP dose-dependently restored the MI induced depression of the cardiac function from 103±8 to 123±7 at 100 and to 138±11 ml/min at 500 µg/kg.hr. Since HR was not changed, this was due to an increase in SV.
Dobutamine (DOB) 1 mg/kg ip, twice daily did not change resting values of MAP and HR but increased baseline CO to 101±5 ml/min (control 73±7 ml/min). Similarly, maximally stimulated CO was increased as a result of DOB treatment.
These data suggest that in the presented conscious rat model effects of different types of therapeutic agents can be studied. The ACEI CAP decreased afterload to the heart and improved cardiac function dose dependently, whereas the positive inotropic agent DOB had no effect on afterload to the heart but stimulated CO even at baseline conditions.

INTERACTIONS BETWEEN THE TACHYKININS AND CALCITONIN GENE-RELATED PEPTIDE (CGRP) ON THE MICROVASCULATURE

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Substance P (SP) and CGRP are often co-localized in sensory C-fibres (Gibbons et al., 1985). CGRP, as a consequence of its vasodilator activity, potentiates oedema formation induced by SP and by the structurally related neurokinins A and B (NKA and NKB) in rat skin (Brain & Williams, 1985; Gamse & Saria, 1985). SP activates mast cells due to its basic N-terminus, which NKA and NKB lack (Devillier et al., 1986). We have compared interactions between SP and CGRP with those of the neurokinins and CGRP by measuring oedema and blood flow responses in rat skin.

Oedema formation was measured in response to intradermal (id) injections of agents in the dorsal skin of Sprague Dawley rats, by the extravascular accumulation of intravenously-injected ^{125}I-albumin over a 30 min period. The ability of tachykinins to stimulate mast cell degranulation in rat skin was investigated by comparing oedema in untreated rats with that in rats pretreated with mast cell amine antagonists. Pretreatment with mepyramine (6mg/kg, ip) and methysergide (6mg/kp ip), which inhibited oedema induced by the mast cell degranulating agent compound 48/80 (1µg/site 71.8±3.7% reduction, mean±sem, n=9), significantly inhibited oedema induced by substance P (100pmol/site), both in the presence and absence of CGRP, 20pmol, (SP 41.5±6.2% reduction, n=7, $p<0.01$, paired t-test; SP+CGRP 31.5±6.4% reduction, n=13, $p<0.01$). In contrast, pretreatment had no significant effect on oedema induced by the neurokinins (NKA 9.9±9.0% reduction; NKA+CGRP 11.9±1.2% reduction; NKB 16.2±6.4% reduction, NKB+CGRP 3.1±4% increase, n=9). These results indicate that oedema induced by SP is partially dependent on mast cell amines.

The effect of the tachykinins on the prolonged vasodilator activity of CGRP (20pmol, id) was investigated in the amine antagonist-pretreated rats using a laser Doppler blood flow meter. The co-injection of SP, but not of NKA or NKB, with CGRP caused a loss of the vasodilator activity of CGRP ($p<0.01$). Results (% increase in blood flow at test sites compared with vehicle-injected sites at 30 min after ids) are: CGRP 196±8.7% increase, SP+CGRP 55.2±24.5%, NKA+CGRP 154.7±23.3%, NKB+CGRP 173.4±13.1%, n=6). The loss of CGRP activity only at SP sites suggests an involvement of mast cells, which is independent of mast cell amines. It is suggested that mast cell-derived proteases degrade CGRP (Brain & Williams, 1988).

The results show that SP can act through mechanisms which are distinct from those of the neurokinins. Two opposing interactions between SP and CGRP were observed. Firstly, CGRP acted to potentiate oedema induced by SP and secondly, SP caused an attenuation of the vasodilator activity of CGRP.

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REGIONAL HAEMODYNAMIC RESPONSES TO CALCITONIN GENE-RELATED PEPTIDE OR ATRIAL NATRIURETIC PEPTIDE IN CONSCIOUS RATS

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The possibility that regional haemodynamics may be influenced selectively by particular peptides could be of substantial therapeutic importance. In this context we have compared the cardiovascular responses to i.v. infusions of rat calcitonin gene-related peptide (CGRP; 0.6 nmol h^{-1}) and α-rat atrial natriuretic peptide (ANP; 3.7 nmol h^{-1}) matched for their effects on mean blood pressure (MBP). Male, Wistar rats (400-500 g) were anaesthetised (sodium methohexitone 60 mg kg i.p.) and pulsed Doppler probes (Haywood et al., 1981) were implanted around left renal (R) and superior mesenteric (M) arteries and around the distal abdominal aorta (H; to represent hindquarters). After at least 7 days, animals were briefly re-anaesthetized (sodium methohexitone, 60 mg/kg i.p.) and had i.v. and intra-arterial catheters implanted. The experiment was begun the following day; continuous recordings were made of MBP, instantaneous heart rate (HR) and mean R, M and H Doppler shift (DS) signals. Regional vascular resistance (VR) changes were derived from MBP and mean R, M and HDS signals (Haywood et al., 1981). Following a baseline period of 30 min, CGRP or ANP were infused (in random order) for 1 h; recordings were continued for 1 h after stopping the infusions. The table summarizes the data obtained after 60 min infusion.

Table: Cardiovascular changes with CGRP or ANP infusion in Wistar rats (n=9); values are mean (s.e.m.).

			% DS			% VR		
	MBP (mmHg)	HR (b min^{-1})	R	M	H	R	M	H
CGRP	−15*	+66*	+4	−20*	+22*	−16*	+9*	−28*
	(3)	(18)	(4)	(3)	(4)	(4)	(5)	(3)
ANP	−17*	+8^{+}	−27*$^{+}$	−32*$^{+}$	−37*$^{+}$	+22*$^{+}$	+27*$^{+}$	+42*$^{+}$
	(3)	(10)	(5)	(4)	(5)	(11)	(7)	(12)

*$P<0.05$ for differences from baseline (Friedman's test); $^{+}$for differences between response to CGRP and ANP (Wilcoxon rank sum test).

In the present experiment the hypotensive response to infusion of CGRP was associated with R and H vasodilatation and M vasoconstriction, whereas infusion of ANP, which caused MBP changes similar to those seen with CGRP, was associated with R, M and H vasoconstrictions. These results are consistent with a reduction in cardiac output during ANP infusion (Lappe et al., 1985). The increase in MVR during CGRP infusion is notable since in vitro this neuropeptide is a potent mesenteric vasodilator (Marshall et al., 1986).

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ENDOTHELIUM-DEPENDENT RELAXING EFFECT OF APOMORPHINE IN THE HUMAN UTERINE ARTERY

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In several human blood vessels the endothelium-dependent relaxation caused by a variety of agents has been demonstrated (Barnes et al., 1986; Luscher et al., 1987). This relaxation in other species has been ascribed to the release of prostaglandin-like products and endothelium-derived relaxing factor (EDRF) (Gerritsen, 1987). The purpose of this work was to study the role of the endothelium in the response of the human uterine artery to apomorphine, a dopamine (DA) receptor agonist.

Uterine artery strips were obtained from patients undergoing uterus resection by miomatosis. Removal of endothelial cells from half of the segments by gentle abrasion was confirmed histologically and by the absence of relaxation with acethylcoline. Arterial strips were suspended in an organ bath containing oxygenated Krebs-Henseleit solution and was pretreated with 70 μM phentolamine before contracted by 3 μM $PGF_{2\alpha}$. Relaxation dose-response curves were obtained for apomorphine (0.2 - 49.0 μM) alone or after pretreatment with DA_1 and DA_2 receptor antagonists, acetylsalicylic acid or methylene blue. Binding studies were performed on microsomes isolated by the method described by Kutsky and Goodman (1978). Bound ^{3}H-apomorphine was determined by liquid scintillation counting of radioactivity trapped on Whatman GF/B filters after rapid vacuum filtration.

Apomorphine relaxed the vascular strips (EC_{50} = 23 μM, maximal relaxation = 62%). The endothelium removal strongly decreased the apomorphine relaxing effect (maximal relaxation = 24%). SCH 23 390 (0.1 and 3.5 μM) decreased such effect. Sulpiride (1.0 μM), propranolol (2.8 μM), cimetidine (10.0 μM) and atropine (1.0 μM) did not affect the relaxation. Acetilsalicylic acid (2.0 and 20 μM) partially inhibited endothelium mediated apomorphine relaxation, whereas methylene blue (10.0 μM) abolished such effect. ^{3}H-apomorphine binding to the membranes of vascular microsomes was specific, saturable (B_{max} = 333.7 fmol/mg protein), of high affinity (K_D = 19.71 nM), to a single class of binding sites (Hill coefficient = 1.002), fast (K_1 = 0.033 nM^{-1}, min^{-1}) and quickly dissociable (K_{-1} = 0.128 min^{-1}). It is preferably displaceable by DA_1 antagonists (rank order of potency: SCH 23 390 > (+) butaclamol > domperidone > metoclopramide > (-) butaclamol > spiroperidol > haloperidol ≫ sulpiride) and agonists (apomorphine > A - 6,7 DTN > dopamine > isoprenaline > serotonin ≫ (±) noradrenaline). After endothelium removal there was a strongly reduction in the number of the ^{3}H-apomorphine binding sites.

In conclusion in the endothelium of the human uterine artery there exist dopamine receptors of the DA_1 subtype which induce a relaxing effect through the EDRF and a cyclo-oxygenase product.

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EFFECT OF PRELOAD AND AGONIST-INDUCED TONE ON ACETYLCHOLINE-INDUCED RELAXATIONS OF RABBIT BLOOD VESSELS: INFLUENCE OF ENDOTHELIUM

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Endothelium-derived relaxing factor (EDRF) is released spontaneously from the vascular endothelium and further release may be provoked by acetylcholine (ACh) and certain other vasodilators (Martin et al., 1986). It is well known that the magnitude of noradrenaline (NA)-induced contractions of blood vessels in vitro varies with tissue preload (Miller and Vanhoutte, 1985). However it is unclear how tissue preload and agonist-induced tone modify endothelium- dependent relaxation.

In this study we have examined the optimal pretension-response (P-R) conditions required for ACh-induced relaxation of NA responses. Ring preparations of 3 rabbit vessels, aorta (A), mesenteric (M) and left renal (R) arteries, were mounted in organ baths containing Krebs' solution gassed with 95% O_2, 5% CO_2 at 37°C. Isometric contractions were elicited by NA (0.01, 0.1, 1 or 10 µM) and relaxations induced by a maximum concentration of ACh (1 µM) at each preload setting. Tissues were allowed 15 min equilibration following preload adjustments before contractions to NA were evoked. In all arteries P-R curves were bell-shaped with maximum responses at preloads of 10-12 g, 4-6 g and 4,5 g for A, M and R, respectively. The maximum responses occurred at the same pretension for each concentration of NA examined. In vessels where the endothelium had been removed by rubbing, concentration-response curves to NA were shifted to the left and the maximum response increased in each vessel. The greatest change was seen in M (NA EC_{50} 360 nM reduced to 92 nM). Smaller but comparable changes occurred in A (EC_{50} 43 to 31 nM) and R (EC_{50} 48 to 26 nM). Maximal relaxation was produced by 1 µM ACh in each vessel, at each concentration of NA. In percentage terms, the endothelium-dependent responses to ACh were not influenced by the tissue preload but were markedly dependent on the concentration of NA used to elicit the contraction. Almost complete relaxation was obtained against 0.01 and 0.1 µM NA, whereas with 1 and 10 µM NA the extent of the ACh-induced relaxation was vessel dependent. In A, ACh-induced relaxations against 1 and 10 µM NA were 66 ± 3 and 44 ± 3%, the equivalent values in M being 81 ± 6 and 56 ± 7% and in R 47 ± 2 and 22 ± 4% respectively ($n \geq 6$ in each case). Relaxations induced by the endothelium-independent vasodilator forskolin (1 µM) were less sensitive to, but similarly influenced by, the concentration of NA used to induce contraction in A, M and R, either with or without endothelium.

In conclusion these results support the view that ACh-induced release of EDRF is vessel dependent with, in these experiments, release being relatively more important from M than from A or R. Furthermore, in all 3 vessels studied, ACh-induced relaxations were independent of tissue preload but were markedly dependent upon the concentration of NA used to induce tone. Since forskolin, like ACh, was less effective against high concentrations of NA, a physiological-type antagonism is probably involved in this phenomenon.

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Key words: Vascular smooth muscle, Preload, NA-induced tone, Acetylcholine-induced relaxation, Endothelium.

PROSTANOID RECEPTORS MEDIATING CONTRACTION OF RABBIT RENAL ARTERY

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Prostanoids, particularly thromboxane A_2 (TxA_2) and the prostaglandin (PG) endoperoxides, PGG_2 and PGH_2 are potent vasoconstrictor agents, an effect mediated by TP-receptors (see Coleman et al., 1981). In contrast, PGE_2 is a vasodilator agent, an action believed to be mediated via EP_2-receptors (Coleman et al., 1987a). In preparations devoid of inhibitory EP-receptors, such as the rabbit and rat aorta, PGE_2 can exhibit weak constrictor activity, but this is mediated by TP-receptors. However, we have recently shown that PGE_2 exhibits potent vasoconstrictor activity on the rabbit renal artery (RbRA), and in the present study we have investigated the nature of the receptors involved.

Left renal arteries were removed from male New Zealand White rabbits, killed with an overdose of sodium pentobarbitone. Eight rings were prepared from each artery and mounted as described by Bradley et al. (1985) in organ baths in Krebs solution maintained at 37°C, containing indomethacin (2.8μM) and bubbled with O_2/CO_2 (95%/5%). A resting tension of 300mg was applied.

Prostanoids caused concentration-related contractions of the RbRA preparation. The TxA_2-mimetic, U-46619 had an EC_{50} of 132nM (95% C.L. 101-172, n=41) and PGE_2 was of similar potency (equipotent concentration, EPC = 0.85, 0.04-15, n=4). In contrast, $PGF_{2\alpha}$, PGD_2 and PGI_2 were less potent (EPC = 11, >85 and >1000 respectively). The TP-receptor blocking drug, GR32191 (10μM) caused a 101 (20-506, n=4)-fold rightward shift of concentration-effect curves to U-46619, but against PGE_2 and phenylephrine, it caused shifts of only 2.0 and 1.2 fold respectively. All of the subsequent experiments were conducted in the presence of GR32191 (10μM). Under these conditions, PGE_2 had an EC_{50} of 72 (54-96, n=35)nM and $PGF_{2\alpha}$ was 41 (22-77, n=5)-fold weaker. The high potency of PGE_2 in contracting this preparation is consistent with the involvement of EP-receptors, and therefore the EP-receptor selective agonists, sulprostone (EP_1/EP_3) and AY23626 (EP_2/EP_3) (Coleman et al., 1987b) were tested. Both agonists caused concentration-related contractions of RbRA, with EPC values of 0.3 (0.1-1.1, n=7) and 8 (4-17, n=5) respectively. Whilst these data suggest that the response is mediated by EP_3-receptors, they do not rule out the possible contribution of a minor population of EP_1-receptors. However, the EP_1-receptor-blocking drug, SC-19220 (300μM) was without effect against responses to PGE_2, sulprostone and AY23626.

These results are therefore consistent with the presence of EP_3-receptors mediating contraction of RbRA. The RbRA preparation represents the first example of EP_3-receptors mediating constriction of vascular smooth muscle.

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ENDOTHELIN IS A POTENT CONSTRICTOR OF ISOLATED HUMAN RESISTANCE ARTERIES

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The endothelium is an important modulator of vascular smooth muscle function (Furchgott, 1983). Endothelin is a novel 21 residue peptide showing structural homology to some peptide neurotoxins. Endothelin has been isolated from vascular endothelium and has been reported to possess potent vasoconstrictor actions both in vivo and in vitro in several species (Yangisawa et al, 1988). We have, therefore, investigated the effect of endothelin on isolated human omental resistance arteries.

Omental arteries (internal diameter 245±47 μm; n=7) were obtained from tissue resected at surgery and mounted in a myograph (Mulvany & Halpern, 1977) to allow measurement of isometric tension. The myograph contained 10 ml physiological saline (PSS) maintained at 37°C and aerated with 95% O_2, 5% CO_2. Following equilibration, arteries were set to an internal circumference of 0.9 L100, where L100 is the internal circumference corresponding to a passive wall tension which would be produced by 100 mmHg distending pressure (calculated by Laplace's relationship).

Vessel viability was assessed by exposing arteries to a sequence of PSS containing 125 mM K (KPSS), noradrenaline 10 μm (NA) and KPSS containing NA. Arteries not generating force equivalent to 90 mmHg in response to KPSS were considered non-viable and not used for further studies. EC_{50} data are presented as geometric means (95% Confidence limits) and maximum tension (max) as means ± s.e. mean.

Cumulative addition of endothelin (10 pM - 100 nM) caused a concentration dependent contraction (EC_{50} = 2.89 (0.75 - 11.1) nM, max = 1.92±0.21 N/m) compared with a max of 1.68±0.15 N/m in response to KPSS and 1.07±0.17 N/m to NA. Following washout of endothelin recovery of tone to basal levels was slow in comparison to recovery from KPSS and NA induced tone. Subsequent responses to endothelin displayed marked tachyphylaxis. Endothelin induced contraction was blocked by pre-incubation with nifedipine (100 nM), a dihydropyridine calcium channel antagonist and was markedly attenuated in the absence of extracellular calcium.

These results indicate that endothelin is a potent vasoconstrictor of isolated human resistance arteries and that this effect probably involves the influx of extracellular calcium through voltage dependent calcium channels.

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RELEASE OF EDRF BY ENDOTHELIN IN THE RAT ISOLATED PERFUSED MESENTERY

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A potent vasoconstrictor peptide, endothelin (ET), has been isolated from cultures of porcine aortic endothelial cells (Yanagisawa et al., 1988). We report here that synthetic endothelin releases endothelium-derived relaxing factor (EDRF) in the rat isolated perfused mesentery.

The mesenteric bed was prepared by the method of McGregor (1965) and perfused at a constant flow of 5ml/min with oxygenated (95%$_2$-5%CO_2) Krebs' solution at 37°C containing indomethacin (5 μM) and albumin (5mg/ml). Vasoconstriction was induced by an infusion of methoxamine (50 μM) to give a rise in perfusion pressure from 15-22 mmHg to 80-120 mmHg. After observing the effects of injected drugs the endothelial cells were removed by an infusion of sodium deoxycholate (1 mg/ml for 30 s) and injections of the test drugs were repeated.

Acetylcholine (ACh, 5.5-550 pmol) induced endothelium-dependent vasodilatations whereas sodium nitroprusside (NaNP, 0.038-3.8 nmol) still caused vasodilatation after endothelial cell removal as described by Warner et al. (1988). ET in the dose range of 1-10 pmol induced endothelium-dependent vasodilatations (8 out of 16 experiments); at higher doses ET provoked sustained increases in perfusion pressure (n=16). Vasopressin (VP, 1-30 pmol) did not induce release of EDRF but caused sustained increases in the perfusion pressure (n=4). In preparations where no methoxamine was used, ET (1-100 pmol) induced dose-related vasoconstrictions which were substantially enhanced by the removal of the endothelial cells (n=8). Vasopressin (1pmol-1nmol) also induced vasoconstrictions which were again augmented by the removal of the endothelial cells (n=5). However, the vasoconstriction induced by noradrenaline (NA, 100pmol-10nmol) was not increased when the endothelium was removed (n=4).

Thus, ET releases EDRF from the rat mesentery as well as eicosanoids from rat and guinea-pig lungs (Antunes et al., 1988). This may be a protective mechanism to abrogate the vasoconstrictor effects of any ET that is released into the circulation. The enhancement of the vasoconstrictor effects of VP and ET, but not of NA may be due to the removal of EDRF release but could also be due to the removal of a diffusion barrier.

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GASTRIC ANTISECRETORY PROSTANOIDS: ACTIONS AT DIFFERENT PROSTANOID RECEPTORS

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Prostaglandin E_2 (PGE_2) and a variety of its analogues are potent inhibitors of gastric acid secretion in both animals and man, and it is believed that these prostanoids mediate their gastric antisecretory effects via EP_3-receptors (Reeves et al., 1988). In the present study, we have determined the agonist potency of some of these compounds on a range of smooth muscle preparations containing other prostanoid receptor types. The analogues tested were 16,16-dimethyl PGE_2 (DME_2), sulprostone, enprostil, misoprostol and rioprostil, and the preparations used were guinea-pig fundus (GPF), cat trachea (CT), dog iris (DI) and rat aorta (RA), which contain EP_1-, EP_2-, FP- and TP-receptors respectively.

The preparations were set up in organ baths containing Krebs solution maintained at 37°C, containing indomethacin (2.8μM) and bubbled with O_2/CO_2 (95%/5%) as described by Coleman (1987). In these experiments we compared the potencies of the analogues with that of PGE_2 on GPF and CT, with that of $PGF_{2\alpha}$ on DI and with that of the selective TP-receptor agonist, U-46619, on RA.

The results of these studies are summarized in the Table.

Agonist	Equipotent Concentration (Standard agonist = 1)			
	GPF (EP_1)	CT (EP_2)	DI (FP)	RA (TP)
PGE_2	1 (EC_{50}=6.3nM)	1 (EC_{50}=20nM)	515 (200-1500)	180 (130-240)
$PGF_{2\alpha}$	33 (23-66)	1000 (300-3200)	1 (EC_{50}=11nM)	140 (57-329)
U-46619	650 (390-1110)	2500 (1240-4900)	131 (52-333)	1 (EC_{50}=5.7nM)
DME_2	0.08 (0.05-0.14)	20 (8-49)	125 (56-282)	127 (72-226)
Sulprost.	2.5 (1.5-4.0)	>7000	177 (70-488)	508 (353-732)
Enprost.	1.8 (1.1-2.9)	[850-1580]	4.4 (1.8-11)	12 (7-22)
Misoprost.	43 (20-92)	3.7 (1.4-9.4)	6500 (1400-31,000)	256 (130-504)
Rioprost.	1200 (160-9080)	3.5 (1.5-8.2)	>1000	>3000

Values are geometric means (95% C.L.) where n>4, or [range] where n=2.

Whilst all of the analogues are potent EP_3-receptor agonists, being at least as potent as PGE_2 on preparations containing this receptor subtype (see Table in Reeves et al., 1988), none of the compounds is wholly selective. Thus, DME_2, sulprostone and enprostil are potent EP_1- receptor agonists, misoprostol and rioprostil are potent at EP_2-receptors, and enprostil has high to moderate potency at FP and TP-receptors. Prostanoids with antiulcer activity have been associated with a range of side-effects including nausea, uterine stimulation and diarrhoea, and it is possible as suggested for enprostil (Clayton et al., 1988), that such side-effects are mediated by receptors different from those which mediate the inhibition of gastric acid secretion.

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MECHANISM OF STIMULATION OF COLONIC SECRETION IN THE RAT BY THE PROSTANOID, ENPROSTIL

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Diarrhoea is a common clinical side effect of prostanoids such as enprostil. Since the colon is important in regulating faecal water excretion (Binder, 1979) we investigated the effect of enprostil on fluid and sodium fluxes in the ascending colon of the anaesthetised rat, and the involvement of thromboxane receptors in this secretory response.

Female Wistar rats (150-200g), were anaesthetized with pentobarbitone, pretreated with indomethacin (20μmol kg^{-1} s.c.) and a 6-8cm segment of ascending colon was prepared in each rat. Isotonic NaCl solution (0.1ml per 100g bodyweight) containing ^{22}Na (125 nCi ml^{-1}) and the test prostanoid was instilled into the colon and left for 60 minutes. The intraluminal contents were collected and net fluid flux was determined gravimetrically. Net sodium flux was determined by flame photometry, ^{22}Na by γ-spectrometry and unidirectional sodium fluxes calculated as described by Matuchansky et al (1972). Some rats were pretreated s.c. 15 minutes before the prostanoid with the selective thromboxane antagonist GR32191.

Table 1 Effects of enprostil on fluid and sodium fluxes in rat colon

Enprostil μmol kg^{-1}	Net fluid flux ml h^{-1}	Sodium fluxes in μEq h^{-1} Net	US	UA
0	+0.00±0.01	-4.8±1.0	33.7±4.1	34.3±1.3
0.03	+0.18±0.14*	+1.9±2.5*	37.3±1.8	35.6±3.5
0.10	+0.22±0.04*	+14.2±2.5*	49.5±2.1*	35.4±1.8
0.30	+0.31±0.02*	+28.7±1.9*	57.7±2.2*	28.9±1.8*
1.0	+0.38±0.04*	+50.3±5.6*	71.3±6.3*	21.0±1.5*

Values are mean ± s.e. mean of 4-6 rats. *$P<0.05$ by unpaired t-test. + = net secretion, - = net absorption. US = unidirectional secretion, UA = unidirectional absorption.

As shown in Table 1, enprostil (0.03-1.0μmol kg^{-1}) caused a dose-related accumulation of fluid and sodium in the colon, resulting from both stimulation of unidirectional secretion of sodium and inhibition of unidirectional absorption. Prostaglandin $F_2\alpha$ (0.3-10μmol kg^{-1}) and the stable thromboxane agonist U46619 (0.01-0.3μmol kg^{-1}) also caused a net secretion of sodium. GR32191 (1mg kg^{-1} s.c.) did not affect basal secretion or the response to $PGF_2\alpha$ (3μmol kg^{-1}) but abolished the effect of U46619 (0.1μmol kg^{-1}). The response to enprostil (0.1μmol kg^{-1}) was markedly reduced by GR32191 1mg kg^{-1} s.c., and abolished by 3mg kg^{-1} s.c. Net Na fluxes in μEq h^{-1} were: Control -8.5±1.6; enprostil +24.9±2.3; enprostil & GR32191 (1) +0.4±3.2; enprostil & GR32191 (3) -8.1±1.7. In conclusion, enprostil stimulates colonic secretion in the rat and this effect is predominantly mediated via thromboxane receptors.

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SUPPRESSION OF NEUTROPHIL ACCUMULATION AND NEUTROPHIL-DEPENDENT OEDEMA BY DEXAMETHASONE IN RABBIT SKIN

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Mediators of increased microvascular permeability can be classified into those acting directly on the endothelium and those whose action depends on a neutrophil/endothelium interaction (Wedmore & Williams, 1981). There is evidence that glucocorticosteroids can exert anti-inflammatory effects by both inhibiting eicosanoid generation and by eicosanoid-independent effects on the microcirculation (Tsurufuji et al, 1979; Foster & McCormick, 1985; Peers & Flower, 1987). The present study was designed to investigate the eicosanoid-independent effects of dexamethasone (Dex) on neutrophil accumulation and neutrophil-dependent oedema. Neutrophils were prepared from donor rabbit blood and labelled with ^{111}In (Rampart & Williams, 1988). The cells were then injected iv. into recipient rabbits together with an oedema marker, ^{125}I-albumin. The 30min accumulation of the labels in skin sites was measured in response to 0.1ml id. injections of three chemoattractants and two directly-acting mediators i.e. complement-activated plasma as a source of C5a des Arg, FMLP (5×10^{-11}moles), LTB_4 (5×10^{-10}moles), PAF (10^{-9}moles) and bradykinin (10^{-10}moles) respectively (doses/site). Responses were subtracted for the low levels in saline injected sites. All mediators were mixed with a fixed potentiating dose of PGE_2 (3×10^{-10}moles) and in such combinations oedema responses were unaffected by indomethacin (5×10^{-9}moles) administered locally at -15min or -4h. In contrast, local Dex. (2×10^{-10}moles) inhibited oedema induced by Bk+PGE_2 with 4h pretreatment i.e. 62±5.4% (n=4, p=0.009) but not with 15min pretreatment i.e. 10.1±6.1% (n=4 ns.). As shown in table 1 local Dex. also suppressed neutrophil-dependent oedema in a time-dependent manner. Neutrophil accumulation was not, however, significantly suppressed. In contrast, Dex. 3mg/kg iv. (4h pretreatment as compared to saline controls) did suppress neutrophil accumulation induced by C5a+PGE_2, together with oedema responses. Responses to FMLP+PGE_2 were similarly suppressed, whereas with LTB_4+PGE_2 only neutrophil accumulation was affected. Dex. iv. also suppressed oedema responses to Bk+PGE_2 and PAF+PGE_2 i.e. 58±6.6% (n=9, p=0.0058) and 75.2±5.9% (n=6, p=0.002) respectively. Results are means±s.e.m for n rabbits; p is paired t-test.

Table 1. Effect of dexamethasone on responses in skin

pretreat	Agent	^{111}In neutro/site		(n)	P	oedema (µl/site)		(n)	P
		control	Dex			control	Dex		
id. -15min	C5a+E_2	1676±430	1599±382	(7)	ns	67.1±9.0	65.1±8.4	(7)	ns
id. -240min	C5a+E_2	1676±430	1294±417	(7)	ns	67.1±9.0	25.3±3.7	(7)	0.001
iv. -240min	C5a+E_2	2239±668	962.7±309	(9)	0.004	46.5±4.7	25.7±3.9	(9)	0.011
iv. -240min	FMLP+E_2	3201±874	1099±325	(9)	0.002	67.1±9.9	36.7±5.3	(9)	0.013
iv. -240min	LTB_4+E_2	3024±843	1880±557	(9)	0.002	58.2±9.8	43.1±6.6	(9)	ns

Thus, Dex. has effects that appear to be independent of cyclo-oxygenase inhibition. Both id. and iv. Dex. suppressed neutrophil-dependent (except for LTB_4) and neutrophil-independent oedema. Systemic, but not local, Dex. suppressed neutrophil accumulation.

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EFFECTS OF FLAVONOIDS ON THE OXIDATIVE MODIFICATION OF LOW DENSITY LIPOPROTEINS BY MACROPHAGES

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The accumulation of lipid by macrophages results in their conversion into foam cells, a characteristic feature of atherosclerotic lesions. In humans this lipid, mainly cholesteryl ester, is believed to be derived from plasma low density lipoproteins (LDL). Macrophages possess few classical LDL receptors, but express scavenger receptors that recognise LDL that has been chemically modified by acetylation (Goldstein *et al.*, 1979) or oxidatively modified by certain cells (Henriksen *et al.*, 1981; Rankin & Leake, 1987) or by high concentrations of cupric or ferrous ions. We have shown previously that mouse peritoneal macrophages cultured in Ham's F10 medium with added Fe^{2+} (3μM) will modify human ^{125}I-labelled LDL (100μg protein/ml) such that it is subsequently taken up up to 20 times as fast as control LDL (incubated in cell-free dishes) by the scavenger receptors on a second set of macrophages. The dependence of this modification process on Fe^{2+} and its inhibition by the general antioxidants butylated hydroxytoluene and vitamin E (100% inhibition at $\leq$ 20μM) suggests that free radical-mediated reactions may be necessary. Macrophages may derive free radicals indirectly as a result of lipoxygenase and cyclooxygenase activity. We have therefore looked at the effect of inhibitors of these enzymes on LDL modification.

Table 1. Inhibition of macrophage-modification of LDL by hypolaetin

Conc^n of hypolaetin(μM)	Uptake by macrophages (μg LDL protein degraded/mg cell protein in 24h±s.e.mean,n=3) Control LDL	Macrophage-modified LDL
0	1.44 ± 0.11	12.8 ± 0.72
1	1.25 ± 0.01	13.1 ± 0.46
3	1.19 ± 0.07	8.89 ± 0.47
10	1.65 ± 0.11	1.96 ± 0.05
30	1.79 ± 0.18	1.65 ± 0.12
100	2.47 ± 0.17	1.49 ± 0.22

We have found that the specific cyclooxygenase inhibitors, aspirin and indomethacin (50μM), have no effect on the modification of LDL by macrophages. In contrast, the lipoxygenase inhibitor nordihydroguaiaretic acid (10μM) totally inhibited modification. Flavonoids, also potent inhibitors of lipoxygenase, were found to inhibit modification: gossypetin (10μM, 100% inhibition), gossypin (10μM,28%), hypolaetin (10μM,97%,Table 1), myricetin (10μM,100%), and 3-hydroxyflavone (10μM,85%). These flavonoids were also capable of inhibiting modification induced by Cu^{2+} (100μM) in the absence of cells. Thus we cannot attribute the inhibition of macrophage modification of LDL solely to an effect on lipoxygenase, and we therefore propose it may reflect the free radical scavenging ability of these compounds. We have also found that the anti-atherosclerotic drug probucol can completely inhibit macrophage modification of LDL as has been shown previously for the modification of LDL by endothelial cells (Parthasarathy *et al.*, 1986). The inhibition of LDL modification in the arterial wall by drugs may be a possible therapy for atherosclerosis.

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"EDRF" RELEASED FROM HUMAN NEUTROPHILS AND PROSTACYCLIN ACT SYNERGISTICALLY TO INHIBIT PLATELET AGGREGATION

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In addition to inducing relaxation of vascular smooth muscle endothelium-derived relaxing factor (EDRF) is a potent inhibitor of platelet aggregation (Furlong et al., 1987). Human neutrophils and monocytes inhibit platelet aggregation by releasing and EDRF-like factor. This inhibitory activity was labile, potentiated by superoxide dismutase, inhibited by haemoglobin and able to elevate cyclic guanosine monophosphate concentrations in platelets (Cynk et al., 1988). We now show that the anti-aggregatory action of neutrophils is potentiated by low concentrations of prostacyclin (PGI_2).

Indomethacin-treated washed human platelets were prepared as described by Cynk et al., 1988. The buffy coat at the plasma-erythrocyte interface was used to prepare neutrophils by density gradient separation on Ficoll-Hypaque. Samples of platelet suspension were preincubated for 4 min in the cuvette of a Payton-dual channel aggregometer, with continuous stirring at 1000 rpm. The appropriate volume of neutrophils and/or PGI_2 was then added directly to the platelet suspension and the incubation continued for a further 60s, before stimulating with thrombin. Typical aggregation traces are shown in Fig 1.

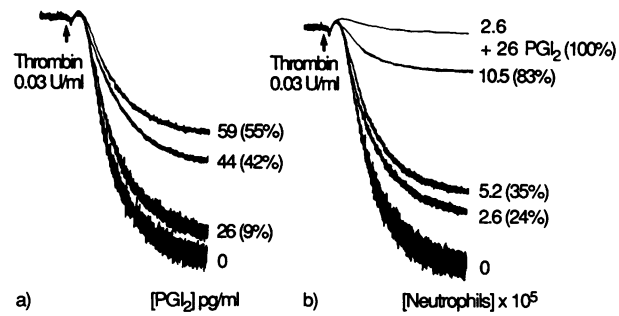

Fig 1. Potentiation of the anti-aggregatory activity of neutrophils by PGI_2 (26 pg ml^{-1}). Values in parenthesis are % inhibition of aggregation.

Fig 1a shows the inhibition of platelet aggregation produced by increasing concentrations of PGI_2. Increasing concentrations of neutrophils also inhibit thrombin-induced platelet aggregation (Fig 1b). In the presence of a minimally inhibiting concentration of PGI_2 (26 pg ml^{-1}) and a low concentration of neutrophils (2.6×10^5) the inhibitory effect is substantially increased showing synergism between PGI_2 and the EDRF-like factor released from neutrophils. This action may be important in influencing neutrophil interactions with endothelial cells.

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EXTRACELLULAR SODIUM INHIBITS HUMAN PLATELET AGGREGATION AND ARACHIDONATE RELEASE

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Recent work from the group of Limbird (Sweatt et al, 1986) has suggested a vital role for influx of extracellular sodium (Na^+) via the Na^+/H^+ exchange mechanism and the resultant increase in pH, in platelet arachidonate release induced by 'weak' agonists, while Siffert & Akkerman (1986) have suggested a role for the Na^+/H^+ exchange mechanism in agonist-induced intracellular calcium ($[Ca^{2+}]i$) elevations. We have checked both hypotheses using human platelets resuspended in Na^+ free buffers, and agents that increase intraplatelet pH, namely the sodium ionophore monensin, ammonium chloride (NH_4Cl) and methylamine (MeA).

Platelets were resuspended in a modified Hepes Tyrode (pH7.4) buffer containing 145mM NaCl ('normal') or 145mM potassium (K^+) chloride or 145mM Nmethylglucamine (NMDG) chloride or 145mM choline (Ch^+) chloride, and pre-loaded with [^{3}H]-arachidonic acid or quin 2 (fluorescent Ca^{2+} probe). All the buffers contained 1mM Ca^{2+}, 0.35% albumin, 0.05U/ml hirudin and 250μM phenidone. In the 'normal' Na^+ containing buffer, all 3 pH elevating agents (10-20μM monensin, 10mM NH_4Cl and 10mM MeA) significantly potentiated ionomycin(4-6μM)- induced $[Ca^{2+}]i$ elevations and arachidonate release (3 fold and 1.5 fold increase respectively). However, these agents had weak potentiatory effects and no effect respectively on thrombin(0.2-2U/ml)-induced $[Ca^{2+}]i$ elevations (0.5 fold increase) and arachidonate release. Monensin also had no effect on collagen (20μg/ml), PAF (0.2μM) or ADP (10μM)-induced arachidonate release, although it had a weak inhibitory effect on agonist-induced platelet aggregation. Varying results were obtained with the Na^+-free buffers, depending on the Na^+-substitute used. In NMDG containing buffer, platelet aggregation induced by ionomycin, thrombin and collagen was severely reduced (50% inhibition) compared with responses in the normal Na^+ buffer. Collagen and thrombin-induced arachidonate release were also reduced; however, ionomycin-induced release was significantly increased. In the K^+ and Ch^+ buffers, aggregation induced by all three agonists, ADP and PAF was significantly enhanced compared with responses in the normal Na^+ buffer. Ionomycin and collagen-induced arachidonate release were also increased in these buffers (1.5 fold increase) while thrombin-induced release was slightly inhibited (20% reduction). Based on the potentiated aggregation responses in the K^+ and Ch^+ buffers, and earlier reports describing inhibitory effects of amines and substituted amines/ammonium ions on aggregation (Kitagawa et al, 1987), we propose that NMDG is a specific inhibitor of platelet aggregation and, therefore, also aggregation-dependent arachidonate release (as seen with weak agonists and collagen). Earlier findings, from the group of Limbird (Sweatt et al, 1986), based on the use of NMDG as the Na^+ substitute, are thus open to re-interpretation. Furthermore, the potentiated aggregation responses with all agonists examined, and the enhanced arachidonate release with ionomycin and collagen in the K^+ and Ch^+buffers, are points supporting the conclusion that, although elevated pH may favour increased $[Ca^{2+}]i$ mobilization and arachidonate release under some conditions (eg with ionomycin), Na^+ itself may be an inhibitor of aggregation and arachidonate release.

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WEAK INHIBITION OF PROTEIN KINASE C AND NON-SPECIFIC EFFECTS MAKE SPHINGOSINE AN UNSUITABLE TOOL IN PLATELET STUDIES

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The calcium (Ca^{2+}), and phospholipid-dependent protein kinase, protein kinase C (PrKC) is now recognized to play an important role in signal transduction in various cell types including platelets (Nishizuka, 1984). Recently, Hannun et al (1986) have described inhibitory effects of long-chain bases such as sphingosine (S) on PrKC activity in vitro, as well as in intact neutrophils and platelets. In the present study, we have examined the effects of S on agonist-induced human platelet activation and assessed its usefulness as a tool in the elucidation of the role of PrKC in platelet signal transduction.

All experiments were performed using washed, human platelets resuspended in a modified Hepes-Tyrode (pH7.4) buffer containing 1mM Ca^{2+}, and pre-loaded with [^{14}C]-5HT (dense-granule marker), [^{3}H]-arachidonic acid (AA) (to monitor AA release due to phospholipase activation), quin 2 (to monitor intracellular Ca^{2+} elevations) or [^{32}P]-phosphate (to measure changes in protein phosphorylation, a marker of PrKC activation), in response to agonist stimulation. Varying concentrations of S (5-100μM) were incubated with platelets for 1 min before agonist addition, and reactions terminated after set time intervals.

At concentrations previously reported to inhibit PrKC activation in platelets (10-25μM), S had no significant effect on thrombin (0.04-0.2U/ml), phorbol 12-myristate 13-acetate (PMA, 5-16nM) or 1,2-dioctanoylglycerol (diC_8, 30-60μM)-induced 45Kd protein phosphorylation (P) in washed, indomethacin-treated platelets, in our hands. Apart from some inhibition (20-30%) of low-dose thrombin (0.03-0.05U/ml)-induced secretion, S also had no effect on PMA or diC_8-induced 5HT secretion. However S effectively inhibited platelet aggregation induced by a range of agonists, including thrombin, PMA, diC_8, ADP and low-dose ionomycin (25-100nM), and this explains the inhibition of aggregation-dependent secretion with thrombin. As the drug is, at best, a weak inhibitor of PrKC (10-20% inhibition of 45KD P at very low thrombin and PMA concentrations) and, as ADP and ionomycin induced no significant 45Kd P in our assays, we conclude that the inhibition of aggregation is unrelated to any effects on PrKC, but probably due to an effect at the level of the platelet membrane. Membrane leakiness, as monitored using release of [^{3}H]-adenine metabolites or quin 2 quenching in pre-loaded platelets by Mn^{2+}, as indices, was greatly enhanced by S (10-25uM) with concentrations 25μM causing almost total leakiness. The increased membrane leakiness caused an apparent potentiation of agonist (thrombin & ionomycin)-induced intracellular Ca^{2+} elevations; however, despite this, thrombin-induced arachidonate release from membrane phospholipids was severely reduced, underlining the inhibitory effects at the membrane level.

In conclusion, sphingosine, at reasonable concentrations, is not only not an effective protein kinase C inhibitor, but has some undesirable side-effects such as inhibiting platelet aggregation and causing membrane leakiness, that make it unsuitable as a tool for evaluation of the role of PrKC in platelet activation.

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EFFECTS OF SK&F 94836, AN INHIBITOR OF "LOW Km" CYCLIC NUCLEOTIDE PHOSPODIESTERASE, IN HUMAN PLATELETS

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SK&F 94836 is a potent and selective inhibitor of "low Km", type III, phosphodiesterase (PDE) (Reeves et al, 1987) that has a long duration of action in vivo and is being investigated as a therapy for congestive cardiac failure. It appears that phosphodiesterase type III is important in regulating cAMP levels in human platelets (see e.g. Simpson et al 1988) and that compounds of this class may be expected to exhibit functional antagonism of platelet responses to a range of agonists. We report here effects of SK&F 94836 on platelet PDE activity, on cAMP levels and $[Ca^{2+}]_i$ in washed platelets loaded with quin2, and on aggregation in platelet-rich plasma (PRP); the platelets were pre-treated with 100μM aspirin to eliminate effects of platelet-derived TxA_2.

The three main PDE isoenzymes were separated from platelet homogenates by DEAE-ion exchange chromatography. SK&F 94836 selectively inhibited the "low Km" PDE with an IC_{50} of 0.8μM at 1.0μM cAMP. There was no effect on other PDE activities at SK&F 94836 concentrations up to 100μM. SK&F 94836 elevated cAMP, measured by radio-immunoassay, in the washed platelets, but only at high concentrations. The threshold measurable effect was at 10μM and with 100μM of the compound, cAMP was increased only by 95 ± 14%. However, the inhibitory effects on stimulus-evoked $[Ca^{2+}]_i$ rises were seen at lower drug concentrations. The IC_{50} for responses to 10μM ADP and 10μM U44069 were 3.8μM and 1.0μM respectively. In PRP, SK&F 94836 inhibited aggregation evoked by 10μM ADP, 5μg/ml collagen, and 10μM U44069 with IC_{50}'s of 15.9 ± 4μM, 11 ± 3μM and 0.8 ± 0.1μM respectively.

Evidently SK&F 94836 is an effective inhibitor of platelet activation. However, the measured elevation of cAMP produced by this compound was modest and not statistically significant at concentrations that significantly inhibited elevation of $[Ca^{2+}]_i$ and aggregation. This suggests either another mode of action for the inhibitory effects or an elevation of cAMP in some relevant, small compartment. Of the agonists examined, the thromboxane-mimetic was the most susceptible to inhibition by SK&F 94836, as was found for a related compound SK&F 94120 (Simpson et al, 1988). This susceptibility does not reflect unexpected TxA_2 receptor antagonism of SK&F 94120 and 94836 judged by their lack of effect, at concentrations up to 100μM, on the binding of ^{125}I-PTA (Mais et al, 1985) to washed human platelets. These results suggest that the observed ex vivo effects of SK&F 94836 administered to human subjects will depend on the agonist chosen, and that testing with stable thromboxane mimetics may give the most sensitive index of drug activity.

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CROMAKALIM AND THE RAT UTERUS IN VIVO

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Cromakalim, a putative potassium (K) channel opener, is a potent relaxant of isolated uterus of the term pregnant rat (Hollingsworth et al., 1987, 1988). The aim of this study was to determine if this action extended to the rat uterus in vivo, the selectivity of cromakalim relative to cardiovascular actions and whether the effects on the uterus were maintained with repeated treatment.

Non-pregnant or day 18 pregnant rats were anaesthetised with tribromoethanol, bilaterally ovariectomised, a jugular vein cannulated and an intrauterine microballoon inserted (Hollingsworth & Downing, 1988). Pregnant rats received a twice daily injection of oestradiol benzoate (0.5 ug kg^{-1}). A separate group of non-pregnant rats were equipped with a carotid artery cannula instead of an intrauterine microballoon. The following day, blood pressure and heart rate (non-pregnant only) or uterine contractions were measured before (0.5 to 10 h) and after (4 to 48 h) either a single i.v. injection (0.1 or 1 mg kg^{-1}) or four injections (1 mg kg^{-1}) of cromakalim repeated at 12 h intervals.

In non-pregnant rats, cromakalim produced an immediate and complete inhibition of uterine contractions. The duration of the inhibition was dose-dependent as contractions started to reappear 15 min after cromakalim (0.1 mg kg^{-1}) and 90 min after cromakalim (1 mg kg^{-1}). Frequency of contractions was inhibited selectively compared with amplitude. Cromakalim (0.1 or 1 mg kg^{-1}) produced falls in blood pressure of 34.1 ± 3.8 % (mean ± s. e. m., n = 5) and 58.4 ± 3.7 % (n = 7) respectively at 15 min after injection with a partial return to control values by 4 h. Heart rate was not significantly changed.

When cromakalim (1 mg kg^{-1}) was repeated at 12 h intervals in non-pregnant rats, a peak inhibition of 70 - 80 % was seen on each occasion. However, the durations of the effects were markedly less for the second to fourth doses compared to the first. The half-times for recovery from the effect, derived from mean data, were 12 h (1st dose), 2.2 h (2nd), 4.9 h (3rd) and 2.7 h (4th; n = 10). This lack of repeatability of effect of cromakalim (1 mg kg^{-1}) was even more evident in pregnant rats. The first dose produced a marked inhibition of integral (78.3 ± 4.4 %, n = 6) and frequency (88.9 ± 2.0 %, n = 6) of uterine contractions which was sustained for approximately 9 h. The second dose produced a smaller peak inhibition of integral (57.3 ± 11.6 %) and frequency (49.2 ± 10.5 %) and shorter duration of effect of approximately 3 h. The subsequent two doses had no significant effect. The time of onset of delivery was unaffected (43.4 ± 2.2 h after first injection in controls, n = 6; 43.3 ± 2.1 h, n = 5 in cromakalim treated animals).

Cromakalim produced prolonged inhibition of uterine contractions in both non-pregnant and pregnant rats after a single i.v. injection. This was accompanied by significant vasodepression. However, the uterine effect was not maintained with repeated injections. Further studies are necessary to determine the mechanism of this tolerance to a K-channel opener.

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STUDIES ON THE MODE OF ACTION OF MINOXIDIL SULPHATE AND DIAZOXIDE: A COMPARISON WITH CROMAKALIM

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Minoxidil sulphate (Mx) relaxes rabbit isolated mesenteric artery precontracted with noradrenaline, an action which is inhibited by tetraethylammonium (Meisheri & Cipkus, 1987; Meisheri et al., 1988). In addition, Mx stimulates ^{42}K efflux from rabbit mesenteric artery (Meisheri et al., 1988) and increases an outward K^+-current in rabbit portal vein (Wilde and Hume, 1987). The antihypertensive agent diazoxide (Dz) hyperpolarizes mouse pancreatic β-cells and increases ^{86}Rb efflux from rat perfused pancreatic islets (Trube et al., 1987). In the present study the mechanisms of action of Mx and Dz have been further investigated using rat blood vessels and the results compared with those obtained using the K^+-channel opening agent cromakalim (CK) (Hamilton et al., 1986).

Endothelium-free aortic strips or whole portal veins were isolated from male Sprague-Dawley rats (300-500 g). Where applicable, tissues were mounted for isometric tension recordings at 37°C, pH 7.4.

Intracellular microelectrode experiments showed that in portal vein, Mx (100 μM) and Dz (1 mM) abolished spontaneous electrical spiking and increased the membrane potential by 6.6 ± 1 mV, and 16 ± 2.4 mV, respectively (mean ± s.e.mean, n = 5). In the tissue bath CK (0.01-3 μM), Mx (0.0-20 μM) and Dz (0.1-100 μM) inhibited the spontaneous phasic tension waves of portal vein (IC_{50} values, 84 nM, 630 nM and 7.1 μM, respectively; n = 4), and in the same tissue produced concentration-dependent increases in ^{42}K efflux. CK (10 μM), Mx (100 μM) and Dz (1 mM) increased the mean control ^{42}K-efflux rate coefficient 2.4, 1.3 and 1.3 fold, respectively ($p < 0.01$, n = 5).

In rat aorta CK (0.01-1μM), Mx (0.01-3μM) and Dz (0.1-100μM) produced complete inhibition of KCl (20 mM)-induced contractions (IC_{50} values, 68.5nM, 20.5nM and 11μM, respectively, n = 6). In contrast, CK and Mx were essentially without effect on responses to KCl (80 mM), whereas, Dz (100 μM) relaxed a KCl-induced contraction by 75 ± 2% (mean ± s.e.mean, n = 6). CK, Mx and Dz at concentrations 10 times those which fully inhibited 20mM KCl-induced tone, had no significant effect on either cGMP or cAMP levels in rat aorta ($p > 0.05$, n = 5) whereas sodium nitroprusside (1 μM) and forskolin (1 μM) used as standards, produced significant increases in cGMP and cAMP concentrations, respectively ($P < 0.05$, n = 5).

It is concluded that the vasorelaxant effects of Mx result primarily from the opening of membrane K^+-channels. The inhibitory effects of Dz are partly associated with K^+-channel opening and partly with an unknown mechanism which does not involve the production of either cAMP or cGMP.

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CONCENTRATION DEPENDENT INHIBITION BY ADENOSINE ANALOGUES OF ATP RELEASE FROM THE ISOLATED MOUSE VAS DEFERENS.

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Adenosine 5'-triphosphate is proposed as one of the transmitters released from sympathetic post-ganglionic nerves (Burnstock, 1986). It may be assayed at very low concentrations using the ATP specific luciferin-luciferase assay (Ludin & Thore, 1975). Kirkpatrick & Burnstock (1987) have recently demonstrated stimulation induced overflow of ATP from the guinea-pig vas deferens, which is TTX sensitive and not produced by contraction alone, suggesting a neural origin.

We have used an improved version of the ATP assay to examine quantitatively the modulation of ATP release from the field stimulated, isoleted mouse vas deferens, by agonists at prejunctional purinoceptors.

Male MF1 mice were killed by cervical dislocation and both vasa deferentia rapidly dissected. After incubation in Krebs solution for 1 hour at 37 C they were suspended under 0.5g tension in 0.2ml organ bath and continuously superfused with Krebs solution at 1ml/min. Bath temperature was 27 C to reduce loss of ATP by degradation. Contractions were elicited by supra-maximal field stimulation through paralled platinum electrodes (0.5msec pulses at 5Hz) and recorded isometrically. Aliquots of superfusate were collected for 1 minute before and each of 4 minutes during and after stimulation with between 50 and 200 pulses. They were assayed inmediately for ATP. Preparations were stimulated 6 times, with drug present on the 3rd, 4th and 5th stimulus. Drug effects were expressed as percentage of the control responses and concentration effect curves analysed by an iterative computer program ('Allfit'). Only one drug concentration was used for each preparation and at least 3 preparations were used at each concentration.

- The ATP overflow following stimulation greatly exceeded basal, non-stimulated overflow by a factor of 5.26 ± 3.05 ($n=23$ $p<0.001$) for 50 stimuli.

- The ATP overflow following stimulation was related to the number of stimuli delivered, increasing up to 10.03 times basal for the 200 stimuli, but plateauing thereafter.

- Contractions of similar amplitude to those following 200 stimuli, but induced by a bolus of exogenous phenylepinephrine produced a negligible increase in ATP overflow.

- N^6-cyclohexyladenosine (CHA) produced a concentration dependent inhibition of stimulation induced ATP overflow. Maximum inhibition was 96.4% with EC50 $1.3 \pm 0.45 \times 10^{-8}$M.

- 5'N-ethylcarboxamido adenosine (NECA) also produced concentration dependent inhibition (max 73%, EC50 $1.5 \pm 0.38 \times 10^{-6}$M).

- Dypiridamole (10^{-5}M), which blocks uptake of endogenous adenosine, produced inhibition of stimulation induced ATP overflow, and increased basal overflow.

Nerve stimulation therefore releases ATP in the mouse vas deferens. This is not a product of muscle contraction itself. Stimulation induced overflow increases with increasing number of stimuli, and it is possible to show concentration dependent inhibition of overflow by agonists at pre-junctional purinoceptors, indicating that the technique may be used quantitatively to study pre-junctional modulation of transmitter release.

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RECONSTITUTION OF REGULATED EXOCYTOSIS IN A CELL-FREE SYSTEM

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In cells that secrete in a regulated manner, exocytosis is triggered by intracellular signals, such as a rise in intracellular free calcium concentration. The processes involved in the generation of these signals are now understood, at least in principle. However, the mechanisms underlying later events, such as the final membrane fusion, are still obscure. To enable us to study the molecular basis of this membrane fusion event, we have developed a cell-free system in which pancreatic plasma membranes interact with zymogen granules and trigger the release of the granule contents.

Zymogen granules and a fraction enriched in plasma membranes were prepared from rat pancreas by the methods of Meldolesi *et al.* (1971). These fractions were incubated together at 37°C in a buffer solution containing 280mM sucrose and 5mM MES, pH 6.5. At the end of the incubation, intact granules were pelleted by centrifugation at 900*g* for 10 minutes, and the supernatants assayed for α-amylase (by the method of Rinderknecht *et al.* (1967)) and/or ribonuclease (by a modification to the method of Takahashi (1961)).

In a typical granule preparation, 8% of the amylase was present in the supernatant initially. After incubation in the absence of added membranes a further 27±2% (s.d., n=3) of the granule-associated amylase was released. In the presence of an increasing concentration of plasma membranes, release rose in a dose-dependent manner, to a maximum of 46±2%. Liver plasma membranes, prepared in a similar way, had a protective effect on the granules, reducing release in a dose-dependent manner to only 8±1% at saturating membrane concentrations. The time-course of release is similar in the presence of both types of membranes ($t_{1/2}$ ~ 4 min). Pretreatment of the pancreatic membranes with increasing concentrations of trypsin (1 hr at 4°C) caused a progressive fall in their activity. In a typical experiment, control membranes caused the release of 60% of the total amylase, whereas membranes treated with 20μg/ml trypsin released only 36%. If soybean trypsin inhibitor (100μg/ml) was present during the pretreatment, this effect was abolished. Release in the presence of liver membranes was 19%. It appears then that proteins are involved in the interaction, as would be expected of a specific effect. Calcium-dependence of the interaction was measured using ribonuclease release, as amylase is known to be sensitive to low calcium levels. We first established that the two enzymes were released in parallel. At 100nM calcium, release is 54±7%. This falls to 33±4% at 5μM calcium. Values in the presence of liver membranes are 5±1% and 11±3% respectively. The interaction is therefore not calcium-dependent, at least in this range. It has been shown recently (Cockcroft *et al.* 1987) that a G protein is involved at a late stage of secretion of histamine from mast cells. In our system, GTP enhances amylase release in a dose-dependent manner from 39±5% to 55±4% at the maximum. The EC_{50} of this effect is ~200μM. Its non-hydrolysable analogue, GTPγS, also enhances release from 34±4% to 55±6%, with an EC_{50} of ~20μM. These effects are consistent with the involvement of a G protein. Another hallmark of G proteins is their activation by complexes of aluminium and fluoride ions. We investigated this, and found that aluminium fluoride does indeed stimulate release, the effect being maximal at 50μM aluminium and 10mM fluoride. Values were: control 42±3%, 10mM F^- 42±3%, 10mM F^-+ 50μM Al^{3+} 61±3%, 10mM Cl^-+ 50μM Al^{3+} 45±5%.

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PICROTOXIN ANTAGONIZES RESPONSES TO δ-AMINOLAEVULINIC ACID IN RAT JEJUNUM AND UTERUS

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It has been suggested from in vitro studies that the porphyrin precursor, delta-aminolaevulinic acid (ALA), may precipitate attacks of acute porphyria by inhibiting the stimulated release of GABA from nerve endings (Brennan & Cantrill, 1979; 1981). This effect, occurring at a concentration of 1 μM ALA, is blocked by picrotoxin and bicuculline. Furthermore, ALA has also been found in vivo to act as a partial GABA agonist (Wehner & Marley, 1986). During acute porphyric attacks blood concentrations of ALA are raised to values which range from 10 to 200 μM (Gorchein & Webber, 1987; Shanley et al. 1977).

The present studies assess the effects of pretreatment with picrotoxin (10 μM) on the response to ALA by isolated preparations of rat jejunum and rat uterus. Preparations of rat jejunum were bathed in oxygenated Krebs solution at 37 °C, and preparations of rat uterus were suspended in oxygenated de Jalon's solution. Contractions of the preparations were recorded by an isotonic transducer and displayed on a calibrated Washington 400 MD2R oscillograph.

In rat jejunal preparations, ALA (160-6000 μM) increased the amplitude of contractions and reduced tone. These effects were inversely related to concentrations of ALA ($r=0.8$; $P<0.05$). Pretreatment with picrotoxin (10^{-5} M) significantly inhibited effects of ALA on amplitude of contractions and also on tone ($P<0.05$). In preparations of rat uterus, ALA (320-6000 μM) increased the rate of contractions in inverse relationship to its concentration ($r=-0.9$, $P<0.05$). After pretreatment of preparations with picrotoxin, ALA (320 and 750 μM) no longer altered contraction rate.

In rat jejunum, $GABA_A$ receptors mediate cholinergic contraction along with a non-adrenergic non-cholinergic relaxation (Krantis & Harding, 1987). In rat uterus, $GABA_A$ receptors modulating acetylcholine release have also been identified (Erdo, 1984). The present results indicate that concentrations of ALA which occur in acute porphyric attacks may be sufficiently high to disturb GABA-mediated functions.

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STIMULATION OF BREAST CANCER CELLS BY GROWTH FACTORS: POSSIBLE ROLE IN ANTIOESTROGEN FAILURE

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Tamoxifen is the endocrine therapy of choice for the treatment of hormone-dependent breast cancer. In hormone-dependent cells, this antioestrogen competes with binding of oestrogens to the oestrogen-receptor and inhibits the activation of a cascade of events that would have led to the secretion of growth factors and cell replication (Lippman *et al.*, 1986). Hormone-independent tumour cells constitutively produce stimulating growth factors, thus do not require oestrogen activation, and growth is not inhibited by antioestrogens. The secretion of these factors by hormone-independent cells in heterogeneous tumour may act not only in an autocrine manner but also influence surrounding hormone-dependent cells. We postulate this paracrine stimulation may reverse antioestrogen control of hormone-dependent cells. Initially, epidermal growth factor (EGF) was used as a representative growth factor to investigate if stimulation of the hormone-dependent MCF-7 breast cancer cell line was possible in the presence of inhibitory concentrations of anti-oestrogens. Subsequently an assay using the co-culture of the hormone independent MDA-MB-231 breast cancer cell line with MCF-7 cells was established to examine paracrine stimulated growth.

MCF-7 cells plated (14,000 cells/well in 24 well plates) in phenol red free MEM containing 5% charcoal stripped calf serum (SCS) and subsequently incubated with compounds for 7 days showed a dose related increase in DNA content with both oestradiol (10^{-13}-10^{-8}M) and EGF (10^{-10}-10^{-6}M). The 4-6 fold increase in DNA/well produced by oestradiol (10^{-10}M) could be inhibited back to control in a dose related manner by the antioestrogens 4-hydroxytamoxifen (10^{-10}-10^{-7}M) and ICI 164,384 (10^{-10}-10^{-7}M). However, these antioestrogens, at the same concentrations, were not capable of inhibiting the 2-3 fold increase in DNA/well produced by EGF (10^{-8}M).

MDA-MB-231 cells were grown in phenol red free MEM and 5% SCS on transwell-col. membranes (Costar Corp., Cambridge, Massachusetts, USA). These microporous membranes containing plated MDA-MB-231 cells were combined (for 4 days) with MCF-7 cells (50,000) in 6 well clusters. Growth was maximally increased (80%) in MCF-7 cells grown in the presence of 150,000 MDA-MB-231 cells compared to controls.

The non-steroidal antioestrogens tamoxifen (10^{-10}-10^{-6}M) and 4-hydroxytamoxifen (10^{-11}-10^{-7}M), and the steroidal antioestrogens ICI 164,384 (10^{-10}-10^{-6}M) and RU 39,411 (10^{-11}-10^{-7}M) all inhibited oestradiol (10^{-11}M) stimulated MCF-7 growth in a dose related manner when cultured for 4 days. However, the paracrine stimulation above control (6.8 ± 0.5 µg DNA/well) produced by co-culture with 150,000 MDA-MB-231 cells (12.6 ± 0.4 µg DNA/well) was not inhibited by 10^{-8} (14.7 ± 0.2 µg DNA/well) or 10^{-6}M (15.2 ± 0.6 µg DNA/well) tamoxifen, 10^{-9} (14.7 ± 0.5 µg DNA/well) or 10^{-7}M (14.5 ± 0.2 µg DNA/well) 4-hydroxytamoxifen, 10^{-6}M (12.4 ± 0.5 µg DNA/well) ICI 164,384 or 10^{-7}M (13.9 ± 0.6 µg DNA/well) RU 39,411.

These data suggest stimulatory factors produced by hormone-independent tumour cells can encourage the growth of hormone-dependent cells and this may result in reversal of the inhibitory action of antioestrogens on the growth of oestrogen-dependent cells. We wish to thank Costar Corp. for providing the transwells. This work was funded by grant CA32713.

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IMPAIRMENT OF ANGIOTENSIN II PRESSOR ACTION IN STREPTOZOTOCIN-TREATED RATS IS NOT DEPENDENT ON VASOPRESSIN

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It has previously been shown that Wistar rats treated with streptozotocin (STZ) to induce diabetes mellitus show impaired angiotensin II (AII)-mediated blood pressure (BP) recovery following ganglion blockade and peripheral, V_1-receptor antagonism (Hebden et al., 1987). However, it is possible that chronic elevation of plasma vasopressin (AVP) levels in STZ-treated rats (Van Itallie & Fernstrom, 1982) has an inhibitory effect on renin release (Reid, 1985) which is not reversed by antagonism of acute peripheral, V_1-receptor-mediated actions of AVP. Therefore, AII-mediated BP recovery following ganglion blockade was compared in STZ-treated, AVP-deficient (Brattleboro) rats and in normal (Long Evans) rats. Rats were treated with STZ (60 mg kg^{-1} i.p.) or saline (control, 1 ml kg^{-1}). Twenty eight days later rats were anaesthetised with sodium methohexitone (60 mg kg^{-1} i.p., supplemented) for arterial and venous catheter implantation. Systolic (S) and diastolic (D) BP and heart rate (HR) were recorded on the following day (17-18 h recovery).

Both groups of STZ-treated rats showed a resting systolic hypotension (Long Evans 124±4 mmHg, n=7; Brattleboro 129±2 mmHg, n=8; mean±s.e.m.) compared to controls (Long Evans 137±2 mmHg, n=8; Brattleboro 140±2 mmHg, n=8; $P<0.02$ unpaired t-test), but there was no difference in DBP between control (Long Evans 83±1 mmHg; Brattleboro 87±2 mmHg) and STZ-treated rats (Long Evans 79±4 mmHg; Brattleboro 81±2 mmHg). STZ-treated rats also showed a bradycardia (Long Evans 287±13 beats min^{-1}; Brattleboro 281±9 beats min^{-1}) compared to control rats (Long Evans 343±5 beats min^{-1}; Brattleboro 342±12 beats min^{-1}; $P<0.01$).

The AVP (V_1)-receptor antagonist d(CH_2)$_5$Tyr(Et)DAVP (10 µg kg^{-1}; 10 µg $kg^{-1}h^{-1}$) had no marked effect on BP or HR in any group. Subsequent administration of pentolinium (5 mg kg^{-1}; 5 mg $kg^{-1}h^{-1}$) caused marked falls in SBP and DBP in all groups. In the presence of d(CH_2)$_5$Tyr(Et)DAVP and pentolinium, there was a significant ($P<0.05$, paired t-test) recovery in BP which was greater ($P<0.05$) in control rats (Long Evans 25±4/21±3 mmHg; Brattleboro 34±4/26±4 mmHg) than in STZ-treated rats (Long Evans 12±2/13±1 mmHg; Brattleboro 16±2/11±1 mmHg). This recovery was ablated by the angiotensin converting enzyme inhibitor captopril (2 mg kg^{-1}).

These results show that the impaired AII-mediated BP recovery following ganglion blockade in STZ-treated rats is not likely to be due to chronic changes in AVP-mediated processes.

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DEPLETION OF PROTEIN THIOLS BY REACTIVE METABOLITES IN VITRO

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The hepatotoxicity of a number of compounds is mediated by the production of reactive metabolites, catalyzed by the cytochrome P_{450}-dependent mixed function oxidase system, which bind covalently to microsomal proteins (Guengerich & Liebler, 1985). Binding commonly occurs at protein thiol groups and modification of protein thiol groups occurs in the early stages of metabolism-mediated cytotoxicity (Albano et al., 1985). We have investigated the possibility that depletion of protein thiols in liver microsomal incubations might be a useful, non-isotopic assay for the detection of reactive metabolites.

Liver microsomes were isolated from control or phenobarbitone (PB) or β-naphthoflavone (BNF)-treated male Sprague-Dawley rats. Success of the induction was confirmed by measurement of cytochrome P_{450} and related activity. The microsomes were incubated for 1 hour at 37°C in the presence of cofactors and test chemical, after which time the protein thiol content was measured by the method of Albano et al (1985). The extent of protein thiol depletion for 4 compounds (final concentration 1mM) is presented in the Table and the results represent the mean ± s.e.mean of 4 determinations carried out on microsomes isolated from the pooled livers of 4 control and PB- or BNF-induced animals. The substrates used were paracetamol (P), 4-ipomeanol (4-IPO), 2-methylfuran (2-MF) and cyclophosphamide (CPA). Liver microsomes contained 65±3 nmol protein thiols (as glutathione equivalents)/mg protein; this value represented the maximum depletion attainable.

Table 1 Protein thiol depletion in liver microsomal incubations

	Protein thiol depletion (nmol glutathione equivalents/mg protein/h)		
	Control	PB	BNF
P	2±1	5±2	5±1^{+}
4-IPO	4±1	3±1^{++}	8±1
2-MF	9±1	15±1^{++++}	8±1^{+++}
CPA	11±0	36±1	5±1

Significantly different from control at $^{+}P<0.05$; $^{++}P<0.02$; $^{+++}P<0.01$; $^{++++}P<0.001$ (Student's unpaired t-test).

The lack of effect of PB treatment on depletion mediated by P and 4-IPO and of BNF treatment on depletion mediated by P did not agree with the reported covalent binding data, whereas the effects of BNF treatment on depletion mediated by 4-IPO, 2-MF and CPA, and of PB-treatment on depletion mediated by 2-MF and CPA mirrored the covalent binding data (see Garle & Fry (1988) for compilation of binding data). These findings suggest that microsomal protein thiol depletion is not a sensitive method for the detection of reactive metabolites.

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DOWN-REGULATION OF β-ADRENOCEPTORS BY ISOPRENALINE IN CULTURED HUMAN LYMPHOBLAST CELLS

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Incubation of human lymphocytes *in vitro* with isoprenaline causes beta-adrenoceptor down-regulation. The extent of down-regulation depends on the concentration of isoprenaline and on the duration of the incubation, tested up to 4h (Sandnes *et al.*, 1987). Studies of longer duration are not feasible due to the limited survival time of lymphocytes *in vitro*. Viral transformation of such cells to replicating lymphoblasts permits desensitisation to be studied over longer time periods under controlled conditions. The beta-adrenoceptor on cultured lymphoblast cells has been characterised by radioligand binding techniques using [^{125}I]-iodocyanopindolol and is qualitatively similar to that of the parent lymphocyte (Elliott, 1987). We have therefore studied the factors controlling the chronic down-regulation of beta-adrenoceptors on human lymphoblast cells cultured *in vitro*.

Stable lymphoblastoid cell lines from five subjects were maintained in RPMI 1640 medium supplemented with 10% foetal calf serum. Confluent cells were incubated with isoprenaline (10^{-8} - 10^{-5} mol/1) for 4h, 24h or 7 days. Ascorbic acid (1 mmol/1) was included to inhibit oxidation of isoprenaline. Control cells were incubated with ascorbic acid alone. Incubations were stopped by centrifugation and the cells disrupted by homogenisation and sonication in hypotonic medium. Nuclear material was removed and the resulting membrane preparation was resuspended in assay medium (Tris 50 mmol/1, $MgCl_2$ 10 mmol/1, pH 7.4). Tissue was incubated with [^{125}I]-iodocyanopindolol at six concentrations (5-100 pmol/1) for 90 min at 37C. Specific binding was defined by 1 *u*mol/1 CGP 12177 and constituted 70% total binding at 20 pmol/1 ligand concentration. The assay was terminated by filtration through Whatman GF-C filters.

Table 1: Down-regulation of lymphoblast beta-adrenoceptor by isoprenaline.

Duration of incubation	Isoprenaline conc. (mol/1) 10^{-8}	10^{-7}	10^{-6}	10^{-5}
4 h	-	49±5 (3)	61±5 (5)	65±2 (3)
24 h	62+3 (3)	75±4 (4)	83±3 (3)	-
7 day	73+3 (4)	81±3 (4)	83±3 (3)	-

Values are % Decrease in B_{max} v Control : Mean ± sem (n)

Down-regulation of lymphoblast beta-adrenoceptors by isoprenaline was concentration dependent for both 4h and 24h incubations ($P<0.05$ ANOVA). Receptor loss also increased with duration of incubation both at 10^{-7} and 10^{-6} mol/1 isoprenaline ($P<0.01$ ANOVA). Repeated experiments within each cell line showed a high degree of individual reproducibility. However significant differences in the extent of down-regulation were observed between different cell lines. The maximum extent of down-regulation observed after 4h incubation in lymphoblasts was similar to that previously reported in lymphocytes (Sandnes *et al.*, 1987).

We conclude that down-regulation of beta-adrenoceptors in cultured human lymphoblast cells by isoprenaline is concentration and time dependent and quantitatively similar to that on lymphocytes in the acute phase. Further studies will investigate other factors which may affect this response and pursue the possible causes of individual differences in sensitivity.

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SCHILD ANALYSIS OF β-ADRENOCEPTOR ANTAGONISTS INHIBITION OF BROWN ADIPOCYTE RESPIRATORY RESPONSES TO NOVEL β-ADRENOCEPTOR AGONISTS

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Previous investigations of in vitro rat brown adipose tissue (BAT) respiration have shown that stimulation of thermogenesis depends mainly on activation of β-adrenoceptors conforming to the β_1-subtype; radioligand studies confirm a predominance of β_1-receptors (ratio $\beta_1:\beta_2$ = 1.5-4.0). These studies involved the use of conventional mixed or selective β_1 and β_2 adrenergic agonists and antagonists. However, Arch et al (1984) have suggested that the brown adipocyte β-adrenoceptor may be "atypical", since the lipolytic responses to several novel β-adrenergic agonists (eg BRL37344) did not conform to the conventional β_1 or β_2 classification. Since lipolysis may not necessarily accurately reflect BAT thermogenesis, the present study was carried out using in vitro measurements of rat brown adipocyte respiration to compare the responses of two novel β-adrenergic agonists (BRL37344 and ICI201651) with a conventional agonist, isoprenaline. Measurements were made with a Clarke-type oxygen electrode, and the effects of three antagonists (propranolol, atenolol and ICI118551) on the responses to all three agonists were tested. Agonist EC_{50} values and antagonist pA_2 values are shown below.

	EC_{50} (μM)	pA_2		
		Propranolol	Atenolol	ICI118551
Isoprenaline	0.005	7.4 (0.19)	5.7 (0.20)	5.9(0.13)
BRL37344	0.003	6.7 (0.03)	5.1 (0.05)	5.4(0.05)
ICI201651	0.010	6.2 (0.02)	5.0 (0.03)	5.4(0.06)

All three agonists produced the same maximal increase in BAT cell respiration, but BRL37344 was the most potent. All three antagonists were less potent inhibitors of BRL37344 and ICI201651 responses than of isoprenaline, requiring 3-15 times higher concentrations of antagonist to produce the equivalent inhibition of the thermogenic response. These results suggest that the interaction of the novel agonists with brown fat β-adrenoceptors differs from that of isoprenaline.

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PHARMACOLOGICAL ANALYSIS OF κ-RECEPTOR AGONIST IN THE GUINEA-PIG ILEUM

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Ethylketocyclazocine (EKC) and U50,488H have been employed widely as k-receptor agonists in the study of opioid receptor systems. However, the quantification of their agonism in terms of affinities and relative efficacies has not been investigated.

In this study, operational model-fitting (Black and Leff, 1983) was used to analyse the effects of irreversible receptor alkylation by β-chlornaltrexamine (β-CNA) (10nM and 30nM, 30 min) on the k-receptor mediated effects of EKC and U50,488H in the isolated, coaxially stimulated guinea-pig ileum.

EKC produced monophasic inhibitory concentration-effect curves which were readily amenable to analysis. In contrast U50,488H produced biphasic curves characterised by a higher potency phase of agonism which was susceptible to antagonism by 16-methylcyprenorphine (RX8008M) (Smith, 1987) and a lower potency phase which was apparently non-opioid in nature. Analysis of the antagonism of U50,488H by RX8008M yielded a pK_B estimate of 7.26. This value is similar to that observed when EKC was employed as the agonist (7.06) (Leff and Dougall, 1988) and is therefore consistent with U50,488H's higher potency phase of agonism being k-receptor mediated.

Operational model-fitting analysis of the k-receptor mediated effects of both agonists indicated that EKC has approximately fifteen-fold higher affinity (pK_A = 8.33) than U50,488H (pK_A = 7.16) and that the two agonists possess similar efficacies (τ = 9.76 and 12.10 respectively).

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CHARACTERISATION OF SOME SUBTYPE-SELECTIVE MUSCARINIC ANTAGONISTS IN FUNCTIONAL AND BINDING ASSAYS

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Pirenzepine has a relatively high affinity for muscarinic receptors of the M_1 subtype. Other drugs have recently become available which are claimed to discriminate further subtypes (Mihm & Wetzel, 1987). We have studied five such drugs, together with atropine and pirenzepine, using functional and binding assays.

Tissues were obtained from 2-4 month old male hooded Lister rats. The functional potency of antagonists was determined by their ability to reduce the potency of bethanechol to contract isolated ileum segments and to reduce the rate of spontaneously beating paired atria, both measured in Krebs-type buffer at 37°C. Antagonist pK_B values were calculated with the equation: $pK_B=\log((dr-1)/[B])$, where [B] is the fixed antagonist concentration, and dr is the dose-ratio. In binding studies, frozen membranes were resuspended in 20mM Na Hepes (100mM NaCl, 0.5mM EDTA) pH 7.4, and incubated at 23°C with antagonist and either ^{3}H-pirenzepine (0.5nM, cerebral cortex) or ^{3}H-N-methylscopolamine (^{3}H-NMS, 0.2-0.3nM, heart and submandibular gland). Incubations were terminated after 90min by centrifugation or filtration. Non-specific binding was defined with 1μM atropine. IC_{50} values were obtained by logistic curve fitting, and those obtained with ^{3}H-NMS were converted to Ki values with the equation $Ki=IC_{50}/([L]/Kd+1)$, where [L] is the fixed ^{3}H-NMS concentration, using Kd values measured in the same experiments (heart: 268±28pM, n=4; gland 136±19pM, n=4). The data are shown in Table 1 below.

Table 1 **Muscarinic antagonist potencies in vitro. Values are mean ± SEM, n>=3**

	Binding pK_i			Functional pK_B	
	Cortex (M_1)	Heart	Gland	Atria	Ileum
Atropine	9.17±0.05	8.99±0.03	9.40±0.02	9.16±0.04	9.28±0.05
Pirenzepine	7.97±0.02	6.42±0.04	6.99±0.03	6.31±0.04	6.73±0.05
4-DAMP	8.85±0.11	8.19±0.04	9.27±0.03	8.20±0.02	8.92±0.05
Hexahydro-siladifenidol	7.88±0.06	6.84±0.09	8.30±0.02	6.76±0.09	8.22±0.04
AF-DX116	6.73±0.06	7.15±0.15	6.48±0.04	7.07±0.03	6.59±0.07
Himbacine	7.06±0.05	8.43±0.07	7.07±0.03	8.13±0.05	7.41±0.09
Methoctramine	7.30±0.04	8.07±0.04	6.56±0.02	8.00±0.02	6.25±0.05

These data (excluding that from atropine and pirenzine) yield the following binding:functional correlations: heart:atria 0.98, gland:ileum 0.97, heart:ileum -0.01, gland:atria 0.12.

Atropine was non-selective and pirenzepine was 10-30-fold selective for the M_1 site. The other five drugs could recognise two subtypes of non-M_1 muscarinic receptor sites, with intermediate affinity for M_1 receptor sites. Methoctramine was the most discriminating of these with about 50-fold selectivity. The good correlation between drug potencies in submandibular gland (binding) and ileum (functional) suggests that the same muscarinic receptor subtype may be involved in both measures.

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EFFECTS OF CALCIUM CHANNEL ANTAGONISTS AND RYANODINE ON ACTION POTENTIALS IN INSECT MUSCLE FIBRES

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The ionic basis of action potentials in muscle fibres from larvae of the moth Plutella xylostella is unknown. A possible role of Ca^{2+} ions in these action potentials and the effects of drugs interfering with Ca^{2+} currents were studied in these experiments.

Action potentials were triggered in these fibres by passing current pulses through an intracellular microelectrode and voltage changes across the muscle cell membrane recorded. Sucrose was present to increase the osmolarity (x 3.2) in order to block contraction and tetraethylammonium (25mM) was present to block potassium currents.

It was found that the inorganic Ca^{2+} antagonists Cd^{2+} (1mM), Co^{2+} (20mM) and La^{3+} (1mM) blocked action potentials in these cells. Reduction in Ca^{2+} concentration from 10mM to 2.5mM caused significant reductions ($P<0.005$, $n=8$) in maximum rate of rise of the action potential (from 13.8 ± 2.5 Vs^{-1} to 5.6 ± 0.7 Vs^{-1}) and amplitude (from 62.4 ± 2.5 mV to 48.9 ± 2.9 mV). Furthermore, action potentials could not be triggered in Ca^{2+} free solution. Tetrodotoxin (2.5μM) had no significant effect on the measured parameters of action potentials. The organic Ca^{2+} antagonists, diltiazem (5μM), nitrendipine (1μM) and verapamil (1μM) had no significant effect on the rate of rise, amplitude and duration of action potentials. Ryanodine (10μM) caused a marked, significant increase ($P<0.005$, $n=5$) in the duration of the action potential (from 49 ± 11 ms to 256 ± 35 ms).

These data show that Ca^{2+} currents are involved in action potentials in these muscles. The channels are not pharmacologically similar to mammalian L-type channels (Nowycky et al. 1985) since they were not inhibited by organic Ca^{2+} antagonists. The data for ryanodine suggest possible slowing of inactivation of the channels at reduced intracellular Ca^{2+} concentrations.

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EVIDENCE FOR A DIHYDROPYRIDINE SENSITIVE AND CONOTOXIN INSENSITIVE VOLTAGE DEPENDENT CALCIUM CHANNEL IN BOVINE CHROMAFFIN CELLS

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Of the three types of neuronal voltage-sensitive calcium channel electrophysiologically defined by Nowyky et al. (1985) (L,N and T), only L channels were dihydropyridine sensitive; recent reports suggest that both L and N channels are both sensitive to ω-conotoxin (Reynolds et al. 1986). We have recently reported that release of noradrenaline from a neurone-like cell in culture, the bovine adrenal chromaffin cell, is stereospecifically sensitive to dihydropyridine calcium channel blockers (Boarder et al. 1987). If the channels conform to the characteristics set out above, then they would also be sensitive to ω-conotoxin.

Primary cultures of bovine adrenal chromaffin cells were prepared by digestion and differential plating as described (Plevin & Boarder, 1988) and cultured on 24 well Primaria plates. Stimulation was for a period of 3 min; dihydropyridine or ω-conotoxin was present where appropriate for 12 min. prior to and during this stimulation period. Noradrenaline release was estimated by high pressure liquid chromatography with electrochemical detection, while Ca^{++} influx was measured by including 0.037 MBq/0.5 ml of $^{45}Ca^{++}$ in the incubation medium. (The conotoxin was simultaneously tested out on release of dopamine from rat brain striatum slices in response to high K^+, and found to inhibit release by greater than 60% at 10 nM).

During these experiments the release from chromaffin cells of noradrenaline in response to high K^+ was mostly blocked by 1 μM nitrendipine, consistent with other reports. However, release in response to 65 mM K^+ was not attenuated by ω-conotoxin, e.g. control release 6.1 ± 0.4; 65 mM K^+ 24.3 ± 1.2; 10 nM ω-conotoxin and 65 mM K^+ 21.3 ± 1.0; 100 nM ω-conotoxin and 65 mM K^+ 22.4 ± 0.3. Figures are release as percent of cell content, mean ± SEM, n = 4.

Influx of $^{45}Ca^{++}$ during the stimulation period was recorded as cpm incorporated per well, mean ± SEM, n = 4. Enhancement of calcium influx by 65 mM K^+ was partially reduced by 1 μM nitrendipine (control 685 ± 56; 65 mM K^+ 1799 ± 97; 65 mM K^+ with nitrendipine 1274 ± 40) but was insensitive to 20 nM conotoxin (control 873 ± 49; 65 mM K^+ 2016 ± 44; 65 mM K^+ with conotoxin 2185 ± 78). The small enhancement of calcium influx caused by 25 mM K^+ was potentiated by 1 μM BAY-K-8644. None of this stimulated calcium influx was sensitive to ω-conotoxin.

These results show that both depolarisation stimulated release of noradrenaline and calcium uptake in cultured chromaffin cells are sensitive to dihydropyridine but not to ω-conotoxin. This suggests the presence of a type, or state, of voltage-sensitive calcium channel sensitive to dihydropyridine but not ω-conotoxin.

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DISSOCIATION BETWEEN PGE_1 STIMULATED PHOSPHOINOSITIDE TURNOVER AND CATECHOLAMINE RELEASE IN CULTURED BOVINE CHROMAFFIN CELLS

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Prostaglandins, principally of the E and F series, stimulate catecholamine release from adrenal chromaffin cells in culture through interaction with specific prostanoid receptors (Koyama et al. 1988). The intracellular mechanisms involved in this process are poorly understood. Recently, it has been suggested that prostaglandins stimulate release through increased phosphoinositide (PI) hydrolysis and the subsequent mobilisation of intracellular Ca^{2+} (Yokohama et al. 1988). However, we and others have shown that prostaglandin stimulated release is dependent on external Ca^{2+} and sensitive to Ca^{2+} channel antagonists (Boarder et al. 1988; Koyama et al. 1988). Thus, we examined the characteristics of PGE_1 stimulated catecholamine release and PI turnover in these cells.

Bovine adrenal chromaffin cells were prepared by collagenase digestion, purified by differential plating and cultured as previously described (Plevin & Boarder, 1988). Total labelled inositol phosphates were measured in the presence of 10 mM LiCl following prelabelling with 2 μCi/ml [^{3}H]-myo-inositol for 40 hr. Noradrenaline release was measured using high pressure liquid chromatography with electrochemical detection.

PGE_1, E_2 and $F_{2\alpha}$ stimulated the accumulation of [^{3}H]-inositol phosphates in a dose-dependent manner similar to the catecholamine release. However, the PI response was small compared to that seen with bradykinin stimulation whereas catecholamine release in response to each agonist was similar. In contrast to equivalent release studies, PGE_1 stimulated PI turnover was independent of extracellular calcium. Pre-incubation of cells in the presence of PGE_1 (30 μM) for 2 hr. markedly reduced prostaglandin stimulated catecholamine release whilst the PI response was unaffected (Table 1). Under similar conditions, PI turnover and catecholamine release in response to bradykinin (100 nM) and nicotine (30 μM) were unaffected. The time course of PGE_1 stimulated PI turnover was not altered following down regulation.

Table 1

Preincubation (2 hr)	Release (pmoles)		[^{3}H]-inositol phosphates (D.P.M.)	
	Control	PGE_1	Control	PGE_1
Basal	160 ± 16	162 ± 27	3222 ± 28	3104 ± 214
PGE_1 (10 μM)	575 ± 10	325 ± 10	4989 ± 223	5213 ± 443
PGE_2 (10 μM)	757 ± 30	465 ± 41	7636 ± 277	6951 ± 472
$PGF_{2\alpha}$ (10 μM)	581 ± 30	348 ± 33	5565 ± 222	6169 ± 795
Bradykinin (100 nM)	554 ± 30	443 ± 44	8422 ± 600	9636 ± 891

Each value represents the mean ± SEM of quadruplicate determinations. The control preincubations were with the same dimethylsulphoxide concentration (0.3%) as with the 30 μM PGE_1.

These results show a dissociation between PGE_1 stimulated release and PI turnover in bovine adrenal chromaffin cells and suggest that phosphoinositide hydrolysis is not intimately involved in the release mechanism in response to prostaglandins.

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EFFECTS OF SELECTIVE NK-2 ANTAGONISTS L-659,837 AND L-659,874 ON TACHYKININ-INDUCED INOSITOL PHOSPHOLIPID HYDROLYSIS

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We have previously reported that in slices of hamster urinary bladder (HUB), tachykinin-stimulated inositol phospholipid hydrolysis is mediated via tachykinin NK-2 receptors (Bristow et al, 1987). This is in contrast to the rat parotid gland (RPG) where this effect appears to be mediated via NK-1 receptors (Hanley et al, 1980). Recent data have indicated that the synthetic peptides cyclo(GlnTrpPhe(R)Gly[ANC-2]LeuMet) (L-659,837) and Ac-LeuMetGlnTrpPheGly-NH_2 (L-659,874) are potent antagonists at NK-2 receptors as evidenced by their ability to inhibit eledoisin-induced contractions of the rat vas deferens (McKnight et al, 1988) and to displace binding of [^{125}I]-Bolton Hunter labelled eledoisin to membranes prepared from HUB (Williams et al, 1988). In the present study, we have compared the effects of L-659,837 and L-659,874 on tachykinin-stimulated inositol phospholipid hydrolysis in slices of RPG and HUB.

Slices of HUB or RPG were preincubated in oxygenated Krebs/bicarbonate Ringers (pH 7.4) for 30 min at 37°C. Twenty-five microlitre aliquots of tissue slices (~0.5mg protein) were transferred to tubes containing 2μCi [^{3}H]-inositol and 10mM LiCl in 215μl fresh Ringers, and incubated for 30 min at 37°C. Agonists (10μl) were added and the slices incubated for a further 45 min. Antagonists (10μl) were added 15 min prior to the addition of agonist. Reactions were terminated by the addition of chloroform/methanol and the water soluble [^{3}H]-inositol monophosphates ([^{3}H]-IP) separated by anion exchange chromatography (Berridge et al, 1982). In HUB, eledoisin (0.1μM) induced a submaximal 7-10 fold stimulation of [^{3}H]-IP production over basal levels. In contrast, neither L-659,837 nor L-659,874 at concentrations up to 100μM, stimulated basal [^{3}H]-IP production. However, low concentrations (0.01-100μM) of either L-659,837 or L-659,874 resulted in a dose-dependent inhibition of eledoisin-stimulated [^{3}H]-IP production. IC_{50} values (concentration of antagonist inducing 50% inhibition of response to 0.1μM eledoisin) for L-659,837 and L-659,874 were 0.89μM (0.65; 1.24) and 0.49μM (0.32; 0.76), respectively. Mean IC_{50} values were calculated from 3 determinations each involving separate inhibition curves employing a minimum of four antagonist concentrations. Results were calculated in the logarithms and are given as the antilog of the mean (-SEM; +SEM). In contrast, in RPG, L-659,837 and L-659,874 were devoid of agonist activity and considerably less potent at antagonising the 3-5 fold stimulation of [^{3}H]-IP accumulation induced by 0.01μM substance P, possessing mean IC_{50} values of greater than 100μM. Furthermore, neither L-659,837, nor L-659,874 inhibited the atropine-sensitive carbachol (10μM)-stimulated increase in [^{3}H]-IP accumulation in RPG at doses up to 100μM.

In summary, our results indicate that L-659,837 and L-659,874 are potent antagonists of tachykinin-stimulated inositol phospholipid hydrolysis in HUB whilst being essentially devoid of activity against this response in RPG. These data lend further support to the notion that L-659,837 and L-659,874 are selective antagonists at NK-2 tachykinin receptors.

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CYCLIC AMP-MEDIATED INHIBITION OF HISTAMINE-INDUCED INOSITOL PHOSPHOLIPID HYDROLYSIS IN BOVINE TRACHEAL SMOOTH MUSCLE

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Histamine (HA), which is a potent smooth muscle spasmogen, stimulates the hydrolysis of inositol phospholipids in airway smooth muscle (Hall & Hill, 1988). We have previously shown that β_2-adrenoceptor stimulation can inhibit the accumulation of [^{3}H]-inositol phosphates elicited by HA in slices of bovine tracheal smooth muscle (Hall & Hill, 1988). In this communication we report that this inhibitory effect of β_2-adrenoceptor agonists can be mimicked by two stable analogues of cyclic AMP (cAMP) and by a variety of agents which increase cAMP accumulation in bovine tracheal smooth muscle.

HA-induced accumulation of [^{3}H]-inositol phosphates was measured in slices of bovine tracheal smooth muscle in the presence of 5mM LiCl as described previously (Hall & Hill, 1988). [^{3}H]-cAMP accumulation was measured in [^{3}H]-adenine-labelled tracheal slices essentially as described for brain (Donaldson et al., 1988). When used, phosphodiesterase inhibitors were added 20-30 min prior to agonist administration. Cyclic nucleotide analogues, vasoactive intestinal polypeptide (VIP) or forskolin were added simultaneously with HA.

Inclusion of the non-selective phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine (IBMX, 1mM) in the incubation medium produced a large increase in the accumulation of [^{3}H]-cAMP obtained in the presence (11 $\pm$ 3 fold over basal, HA-response = 2.1 $\pm$ 0.1 fold over basal) or absence (4.8 $\pm$ 1.4 fold over basal) of 0.1 mM HA (n=5). Significant ($p<0.05$, anovar) but smaller increases in cAMP accumulation were also obtained with rolipram (0.1mM, n=4), a selective inhibitor of one of the cAMP selective PDE isozymes (PDE IV, Reeves et al., 1987). SKF91420 (0.1mM, an inhibitor of the other cAMP selective isozyme PDE III) and M&B 22948 (0.1mM, an inhibitor of the cyclic GMP metabolising isozymes) (Reeves et al., 1987), however produced little effect on cAMP accumulation. Both IBMX (1mM) and rolipram (0.1mM) significantly ($p<0.05$) reduced the [^{3}H]-inositol phosphate response to HA (0.1mM) by 81 $\pm$ 6% and 68 $\pm$ 9% respectively (n=4, in each case). SKF 91420 (0.1mM) produced a smaller inhibition (20 $\pm$ 5%, n=4), while M&B 22948 (0.1mM) did not significantly inhibit the HA-stimulated [^{3}H]-inositol phosphate accumulation. The inhibitory effect of salbutamol (1μM) on the inositol phosphate response to HA (Hall & Hill, 1988) was mimicked by VIP (1 μM, 37 $\pm$ 10 % inhibition, n= 4), the cAMP analogues dibutyryl cAMP (1mM, 46 $\pm$ 7% inhibition, n=4) and 8-bromo-cAMP (1mM, 54 $\pm$ 2%, n=4), and forskolin (1μM, 42 $\pm$ 7% inhibition, n=4). In the presence (1st value) or absence (2nd value) of 0.1mM HA, forskolin (17 $\pm$ 6 & 4.2 $\pm$ 0.4 fold), VIP (4 $\pm$ 1 & 3.6 $\pm$ 1.0 fold) and salbutamol (3.9 $\pm$ 0.8 & 3.6 $\pm$ 0.6 fold) all produced significant ($p < 0.05$) increases (over basal levels) in [^{3}H]-cAMP accumulation ($n \geq 3$).

These results show that a variety of agents which raise cAMP levels in bovine tracheal smooth muscle or mimick the action of cAMP can inhibit the accumulation of [^{3}H]-inositol phosphate elicited by HA. This suggests that cAMP mediates this inhibition of inositol phospholipid metabolism.

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SUFOTIDINE, A COMPETITIVE AND LONG ACTING H_2-RECEPTOR ANTAGONIST

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Histamine H_2-receptor antagonists fall broadly into two classes; compounds such as ranitidine that are competitive, fully surmountable antagonists of H_2-receptor mediated responses and unsurmountable antagonists, such as loxtidine and L643,441 (Reeves & Stables, 1987). These unsurmountable H_2-antagonists are long-acting inhibitors of gastric acid secretion whereas competitive antagonists tend to be shorter-acting. We now present data on sufotidine (1-methyl-3-methylsulphonyl-methyl-N-[3-[3-(1-piperidinyl-methyl)phenoxy]propyl]-1H-1,2,4-triazole-5-amine), a competitive H_2-receptor antagonist which has a long duration of action.

The H_2-receptor antagonist activity of sufotidine was studied against histamine-induced chronotropy in the guinea-pig isolated right atrium, and against histamine-induced acid secretion by the rat isolated gastric mucosa. In both preparations, sufotidine caused dose-related parallel displacements of the histamine concentration-response curves with little or no depression of the maximum response. The pA_2 and Schild plot slope values (with 95% confidence limits) for sufotidine were: 6.81 (6.64-7.04) and 1.18 (0.94-1.41) on atrium, and 6.75 (6.53-7.05) and 1.10 (0.90-1.30) on mucosa.

The antisecretory activity of sufotidine was compared with that of ranitidine in the anaesthetised rat perfused stomach preparation and conscious Heidenhain pouch dog; in both preparations gastric acid secretion was elicited by intravenous infusion of histamine. Sufotidine and ranitidine were equipotent inhibitors of acid secretion in the rat with intravenous ED_{50} values in mg/kg (95% confidence limits) of 0.15 (0.10-0.21) and 0.12 (0.08-0.17) respectively. In the dog (n=5) sufotidine was 2-3 times less potent than ranitidine, ED_{50} values following intravenous administration being 0.11 (0.09-0.14) compared to 0.03 (0.02-0.06), and following oral administration 0.27 (0.18-0.37) compared to 0.10 (0.06-0.16). The durations of action of equieffective doses of sufotidine and ranitidine were compared in the dog; as shown in Table 1, sufotidine has a much longer duration of action than ranitidine.

Table 1 Duration of action of sufotidine (S) and ranitidine (R) in the dog

Drug dose mg/kg	Mean ± s.e. % inhibition of secretion at time (h)					
Intravenous	½	1	2	4	8	12
R 0.2	92 ± 2	85 ± 6	61 ± 8	28 ± 5	-	-
S 0.6	92 ± 4	88 ± 3	84 ± 4	74 ± 10	55 ± 2	23 ± 8
Oral	2	4	8	12	18	24
R 0.3	88 ± 6	56 ± 10	39 ± 7	16 ± 9	-	-
S 1.0	88 ± 5	90 ± 6	63 ± 7	59 ± 7	47 ± 5	15 ± 13

In conclusion, sufotidine is a competitive histamine H_2-antagonist with a long duration of antisecretory action.

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UPTAKE INHIBITORS DO NOT CHANGE THE EFFECT OF IMIDAZOLINE α_2-AGONISTS ON NORADRENALINE RELEASE EVOKED BY SINGLE PULSE STIMULATION

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It has been observed that cocaine reduced or even abolished the inhibition by imidazoline alpha$_2$-adrenoceptor agonists of the electrically evoked release of noradrenaline in isolated tissues (Starke, 1977). The explanation had been that cocaine inhibited the neuronal uptake and increased the biophase concentrations of noradrenaline, that autoinhibition then became near-maximal and that under these conditions an exogenous agonist had little opportunity for further inhibition. These results had to be reinterpreted after the observation by Langer and Dubocovich (1981) that inhibition of reuptake did not reduce the release-inhibiting effect of alpha-methylnoradrenaline. Then, two hypotheses were suggested as possible interpretation: a) the uptake blockers might interact directly with the imidazoline recognition site of presynaptic alpha$_2$-autoreceptors and this selectivity decreases the effect of imidazolines; b) the uptake blockers might act primarily at the uptake mechanism, and this interaction might then disturb in some way the effect of imidazolines but not of phenylethylamines.

The present study was undertaken to compare the role played by neuronal uptake inhibitors on the efficacy of imidazoline alpha$_2$-adrenoceptor agonists under two different conditions: at low and high noradrenaline concentrations in the biophase.

Isolated mouse vasa deferentia were suspended in a 10 ml bath between two platinum electrodes in oxygenated Krebs solution at 37°C. A tension of 500 mg was applied to the tissue which was submitted to field stimulation (85 V, 2 ms, 0.2 Hz). The twitch responses were recorded isometrically. The tissue was stimulated with trains of 7 pulses and the inhibition by alpha$_2$-adrenoceptor agonists (clonidine and UK-14,304; 5-bromo-6--(imidazolin-2-yalamino)-quinoxaline) of twitch responses was measured in the absence and in the presence of uptake inhibitors (40 nmol.l^{-1} desipramine or 12 umol.l^{-1} cocaine). The effects were determined for the first and the last stimulus of each train.

For the last twitch of the train desipramine caused a significant rightward displacement of the concentration-response curve for the inhibitory effect of both alpha$_2$-adrenoceptor agonists (a displacement by 0.26 log units for UK-14,304 and a displacement by 0.27 log units for clonidine-at the IC50 level). A similar effect was caused by cocaine (a displacement by 0.20 log units for UK-14,304 and a displacement by 0.26 log units for clonidine). However, for the first twitch of the train, uptake inhibitors did not cause any displacement of the concentration-response curve for either UK-14,304 or clonidine.

These results suggest that the concentration of noradrenaline in the biophase plays some role in the inhibition by imidazoline alpha$_2$-adrenoceptor agonists of the electrically evoked release of noradrenaline.

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GR50360A, A NOVEL AND HIGHLY SELECTIVE α_2-ADRENOCEPTOR ANTAGONIST

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The pharmacological profile of GR50360A (± 5-fluoro-2,3,3a,9a,-tetrahydro-1H--[1,4]-benzodioxino-[2,3-c]pyrrole hydrochloride), a potent and highly selective α_2-adrenoceptor antagonist is described in this study.

α_2-Adrenoceptor antagonism *in vitro* was measured in the rat field-stimulated prostatic vas deferens mounted in Krebs solution (31°C; 2ms square wave pulses, 0.1 Hz). α_1-Adrenoceptor antagonism was determined against phenylephrine-induced contractions of the rat anococcygeus muscle preparation (Doggrell 1980). Twitch inhibition produced by the α_2-adrenoceptor agonists UK-14304 (Cambridge, 1981) and clonidine on vas deferens was potently and competitively antagonised by GR50360A, idazoxan and yohimbine. However, GR50360A was much weaker than idazoxan and yohimbine at blocking phenylephrine-induced contractions of the rat isolated anococcygeus muscle giving a very high α_2-:α_1-adrenoceptor selectivity ratio (Table 1). There was no significant difference between the α_2-adrenoceptor blocking profiles of GR50360A and its two enantiomers. GR50360A was also specific for α_2-adrenoceptors when evaluated in other functional receptor preparations (Halliday et al., this meeting).

Table 1 **α_2-adrenoceptor antagonism *in vitro* - mean pK_B values ± s.e. (Schild slope)**

Preparation	Agonist	GR50360A	Idazoxan	Yohimbine
Rat vas deferens	UK-14304	7.87±0.05(1.02)	7.73±0.06(1.02)	7.59±0.04(1.08)
Rat vas deferens	Clonidine	7.91±0.07(1.23)*	8.12±0.06(0.98)	7.48±0.07(1.10)
Rat anococcygeus	Phenyleph.	4.45±0.15(1.08)	6.27±0.13(0.90)	6.25±0.08(0.91)
α_2:α_1-Adrenoceptor selectivity		~2750	~50	~20

* = Slope sig. diff. ($p<0.05$) from 1.00

In the conscious rat (Ware RH, male, 70-120g) UK-14304 (2mg/kg s.c.) caused hypothermia and a marked reduction in rotarod performance. The magnitudes of these effects were halved by 30 minute pretreatments with GR50360A, 1.4±0.2mg/kg p.o. or 0.5±0.2mg.kg i.v. (hypothermia) and 1.1±0.2mg/kg p.o. or 1.3±0.1mg/kg i.v. (rotarod). Idazoxan was of similar potency to GR50360A i.v., but 5 and 14 times weaker orally in blocking the hypothermia and sedation respectively. The similarity in the potency of GR50360A by the i.v. and p.o. routes of administration is indicative of excellent oral bioavailability.

Thus GR50360A is a potent and competitive antagonist at α_2-adrenoceptors both *in vitro* and *in vivo*. The high α_2- to α_1-adrenoceptor selectivity makes it a useful pharmacological agent for investigating the clinical properties of this class of antagonist.

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UK-52,046 A NOVEL α_1-ADRENOCEPTOR ANTAGONIST WITH ANTIDYSRHYTHMIC ACTIVITY

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Cardiac alpha$_1$adrenoceptors play a primary role in the pathogenesis of ischaemia induced ventricular arrhythmias and alpha$_1$adrenoceptor antagonists have been shown to be effective in restoring sinus rhythm after such arrhythmias have been induced experimentally (Sheridan et al (1980), Aubry et al (1985)). Clinical studies with existing alpha$_1$adrenoceptor antagonists in the early stages of an ischaemic event have been precluded due to concern about adverse haemodynamic effects. UK-52,046 (4-Amino-6,7-dimethoxy-2-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl) quinoline methanesulphonate) resulted from work seeking an alpha$_1$adrenoceptor antagonist with antidysrhythmic activity associated with minimal effects on heart rate (HR) and blood pressure (BP). UK-52,046 (1-5 μg/kg iv) significantly ($P<0.01$) increased the concentration of adrenaline (5.6±0.61-88.7±8.69 μg/kg/min) required to induce ventricular tachycardia (V.T.) in male rats (n=5/group) anaesthetized with halothane 1% v/v in oxygen. The increases in adrenaline dysrhythmic threshold were not accompanied by significant changes in either HR or BP. In comparison prazosin (6.25-25 μg/kg iv) significantly ($P<0.01$) increased the minimum concentration of adrenaline necessary to evoke V.T. (4.5±0.25-19.5±1.38 μg/kg/min) but significantly ($P<0.01$) lowered BP (16.4±2.71-30.6 ±4.39 mmHg). Similarly UK-52,046 (3.125-100 μg/kg iv) significantly ($P<0.05$- $P<0.001$) increased the concentration of adrenaline (2.1±0.23-32.0±3.4 μg/kg/min) required to induce ventricular ectopic activity in male and female dogs (n=5/group) anaesthetized with halothane 1% v/v in oxygen (3 l/min). In contrast to prazosin UK-52,046 lacked significant effects ($P>0.05$) on HR and BP at these dose levels. Picrotoxin (2-4 mg/kg iv) evoked persistent ventricular arrhythmias in male cats (n=5) anaesthetized with chloralose (80 mg/kg iv) and given decamethonium bromide (0.25 mg/kg followed by 0.5 mg/kg/hr iv) to prevent the clonic convulsions induced by picrotoxin. UK-52,046 (25 and 50 μg/kg iv) promptly restored normal sinus rhythm in the cats with picrotoxin induced ventricular arrhythmias. UK-52,046 (25 μg/kg iv) attenuated malignant ventricular ectopic activity and the induction of ventricular fibrillation (V.F.) when occlusion of the left anterior descending coronary artery was combined with stellate ganglion stimulation in cats (n=6) anaesthetized with chloralose (80 μg/kg iv). UK-52,046 (100 μg/kg iv) produced significant ($P<0.01$) suppression of the early ventricular arrhythmias, reduced both the incidence and duration of V.T. ($P<0.05$) and there was a trend toward reduced mortality due to V.F. during occlusion following left anterior descending coronary artery occlusion in dogs anaesthetised with chloralose (80 mg/kg iv). These effects were not accompanied by significant changes in HR, BP and filling pressure. UK-52,046 (0.05-25 μg/kg iv) attenuated or abolished adrenaline (5 μg/kg iv) induced ventricular arrhythmias in conscious dogs (n=6) recovering from experimental myocardial infarction following insertion of a thrombogenic copper coil (2 mm long 1 mm in diameter) via the left circumflex coronary artery. HR and BP were not modified by UK-52,046, but the anxiety and restlessness induced by adrenaline were reduced.

UK-52,046 may be suitable for the management of ischaemia induced ventricular arrhythmias in patients with coronary artery disease because of its minimal effects on HR and BP at dose levels exerting antidysrhythmic activity.

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THE ROLE OF MAO TYPE B IN NORADRENALINE METABOLISM IN THE HUMAN SAPHENOUS VEIN

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Recent work from this Laboratory (Branco and Osswald, 1988) has shown that the human saphenous vein considerably differs from its canine counterpart in its noradrenaline content and in the way it metabolizes exogenous noradrenaline.

The aim of the present work was to study a) the types of MAO(A and B) present in normal and varicose human veins; b) and their role in the deamination of exogenous noradrenaline.

Normal and varicose veins were obtained at surgery from patients subjected to coronary bypass or stripping operations, respectively. The fragments used were removed from the inferior portion of the lateral saphenous vein and the interval between removal and the experiment was never longer than 1 h. Briefly, the methods used consisted in: a) preparation of homogenates of venous tissue in phosphate buffer pH 7.4 and determination of monoamine oxidase activities of type A and B, using as substrates 5-HT(2 mmol/l) and β-phenylethylamine (PEA-100 µmol/l),as selective substrates of MAO A and B,respectively,as well as tyramine(TYR-2 mmol/l) as a common substrate; b) incubation of venous fragments of about 50 mg with ring labeled 3H-noradrenaline (3H-NA, 0.1µmol/l) for 30 min, with subsequent chromatographic separation and determination of 3H-NA and its metabolites by liquid scintillation counting (for details,see Caramona, 1982; Caramona et al, 1985).

The results of the experiments conducted with homogenates showed that the human vein contains both types of MAO; in the normal vein, the activities of MAO A and B were not dissimilar,but the varicose vein showed a significantly higher proportion of MAO type B activity.

Incubation experiments (done in the presence of pyruvate,fumarate, l-glutamine and l-methionine) showed that normal veins produced higher amounts of DOPEG,OMDA and NMN and that varicose veins had an increased production of DOMA.

Clorgyline (0.1µmol/l),amezinium (10µmol/l) and deprenyl (10µmol/l), selective inhibitors of MAO type A and B,respectively, affected the metabolism of 3H-NA, reducing the formation of OMDA, DOPEG and DOMA and increasing the formation of NMN, in normal and varicose veins.

We conclude that in the human vein,in contrast to what happens in the canine vein(Caramona and Osswald,1985), MAO type B plays an important role in the metabolism of 3H-NA and that the varicose vein differs from the normal vein in the way it handles noradrenaline.

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α-ADRENOCEPTOR-MEDIATED CARDIOVASCULAR EFFECTS OF NORADRENALINE IN THE PARAVENTRICULAR NUCLEUS OF CONSCIOUS RATS

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Vasopressin (AVP)-containing magnocellular cells in the hypothalamic paraventricular nucleus (PVN) are innervated by noradrenergic fibres which originate in the A1 cell group of the caudal ventrolateral medulla (Swanson et al., 1981). Microinjection of noradrenaline (NA) into the PVN of conscious, Long Evans rats produces an increase in blood pressure (BP) which is dependent, in part, on release of AVP into the circulation (Harland et al., 1988). Previous studies suggest that the excitatory effects of NA on hypothalamic AVP neurons are due to stimulation of α-adrenoceptors (Day et al., 1985; Willoughby et al., 1987). In the present study, an attempt was made to characterize the α-adrenoceptor subtype(s) in the PVN which mediate the cardiovascular effects of NA.

Male, Long Evans rats (321 ± 5 g, n = 15, mean ± s.e.m.) were prepared with unilateral guide cannulae for injection into the PVN as described previously (Harland et al., 1988). On the day before the experiment the animals were anaesthetised (sodium methohexitone, 60 mg/kg i.p.) and a catheter placed in the abdominal aorta via the caudal artery. Continuous recordings of systolic and diastolic BP and heart rate (HR) were begun following overnight recovery (17-18 h). The effect of pretreatment with the α_1-adrenoceptor antagonist prazosin (0.7 nmol) or the α_2-adrenoceptor antagonist idazoxan (4 nmol) on the maximum changes in BP and HR produced by PVN microinjection of NA (10 nmol in 0.3 μl) are summarised below.

Table 1 Maximum changes in BP (mmHg) and HR (beats/min) following PVN injection of NA

	n	Δsystolic BP	Δdiastolic BP	ΔHR
control	7	17 ± 2	17 ± 4	-39 ± 10
idazoxan 4 nmol		-13 ± 1*	-8 ± 1*	-41 ± 3
control	8	14 ± 2	17 ± 2	-56 ± 9
prazosin 0.7 nmol		8 ± 4	9 ± 4*	-28 ± 7*

*P <0.05 compared with respective control (paired t-test).

These results suggest that the pressor response produced by microinjection of NA into the PVN of conscious rats is mediated through stimulation of α_2-adrenoceptors. The NA-induced bradycardia persists in the presence of α_2-adrenoceptor blockade and may be due, in part, to stimulation of α_1-adrenoceptors.

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