ASSESSMENT OF BEHAVIOURAL PARAMETERS ASSOCIATED WITH ANXIOGENESIS IN THE COMMON MARMOSET (CALLITHRIX JACCHUS)

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The behavioural repertoire of the common marmoset (Callithrix jacchus) has been described in detail by Stevenson and Poole (1976). From this study several behavioural parameters have been identified which occur in response to a potentially threatening situation such as the presence of a human experimenter. For example, time spent on the cage front in confrontation with the experimenter and the number of "postures" displayed have been shown to be increased and decreased, respectively, by anxiolytic agents in the marmoset human threat model (Costall et al. this meeting).

In the present study we analyse the behavioural response of the common marmoset to an anxiogenic agent. To demonstrate this, marmoset behaviour was assessed by remote video recording in conditions of reduced "anxiety", that is, absence of human threat. FG 7142 (methyl-β-carboline-3-carboxamide) was used to induce anxiogenesis (Dorow et al. 1983).

The behaviour of adult marmosets (n = 6-10), housed in pairs, was assessed over two 15min periods in the animals' home cages adapted for video recording. The initial assessment followed a period of habituation to the camera equipment and was, for the purposes of this study, termed "normal" behaviour. 40 min subsequent to subcutaneous dosing with drug/vehicle or following handling alone (control), a second behavioural assessment was made.

Several behavioural parameters were measured; i) time on or in front of the front perch, ii) time spent out of the nest box, iii) the number of "postures" (detailed by Stevenson and Poole, 1976), iv) the number of jumps and line crossings (arbitrary, central line on video screen) dividing cage into two sections. Body temperatures were also recorded by means of a rectal thermometer.

FG 7142 (5-20mg/kg s.c.) caused approximately 30-65% reductions in time spent at the cage front compared with the behaviour of untreated control animals ($P<0.05$), Mann Whitney U test), while time spent out of the nestbox was reduced by 5mg/kg FG 7142 when comparisons were made with the behaviour of vehicle treated animals ($P<0.05$) (but not with that of untreated controls). Treatment with FG 7142 also caused a decrease in locomotor activity with the number of jumps reduced by 52.4% as compared with the responses of untreated control marmosets ($P<0.05$). The number of line crossings were similarly reduced; for example, treatment with 1mg/kg FG 7142 reduced line crossings by 68.9% (comparison with control animals' $P<0.05$). The number of postures was not significantly altered by FG 7142.

Following administration of FG 7142 (1-10mg/kg s.c.), body temperatures were raised from a normal level of 37.7^{o}C to 38.15-38.18^{o}C ($P<0.05$-$P<0.01$, matched pair t test). This is in contrast to the FG 7142-induced hypothermia reported in mice by Stanford et al. (1987).

This data suggests that the marmoset is a useful animal in which the actions of anxiogenic agents can be assessed, although changes in behaviour in further situations of anxiogenesis should be determined to define more precisely the behavioural measures indicative of anxiogenesis in this species.

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CHARACTERIZATION OF THE PHYSOSTIGMINE STIMULUS IN RATS

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Direct agonists and antagonists at the muscarinic receptors have already been studied using drug discrimination procedures (Overton 1977; Meltzer and Rosecrans 1981; Jung et al. 1987 a,b). Little is known however about the discriminative stimulus properties induced by cholinesterase inhibitors. The present experiments investigated whether low doses of physostigmine could induce a discriminative stimulus in a two-bar food-reinforced task, and intend to further characterize the physostigmine stimulus.

The procedure used in the present experiment has been described elsewhere (Jung et al. 1987b). Briefly, twenty four male Long Evans rats were trained to bar press (FR 10) for food reinforcement on either of two levers. Following injection of physostigmine (0.1 mg/kg SC) 15 min before the session the rats had to press one lever to get a food pellet whereas after administration of saline (1 ml/kg SC) 15 min before the session they had to press the opposite lever. Drugs were injected SC 15 min before testing, or 15 min before physostigmine, except for arecoline (5 min) and THA (30 min).

The physostigmine saline discrimination was acquired by all the rats tested, with a mean number of sessions of 30. After physostigmine treatment, the percent drug choice increased with the dose (ED50 = 0.015 mg/kg; 95% CL : 0.009-0.024). Arecoline (1.5 mg/kg), oxotremorine (0.075 mg/kg), RS 86 (0.5 mg/kg) and THA (2 mg/kg) elicited complete generalization to the physostigmine stimulus. Neostigmine (0.2 mg/kg) and nicotine (0.8 mg/kg) did not produce any significant drug lever selection. Scopolamine hydrobromide (0.07 - 0.12 mg/kg) dose-dependently blocked the stimulus induced by physostigmine whereas scopolamine methylbromide (0.145 - 0.580 mg/kg) did not. Mecamylamine (1 mg/kg) was unable to reverse the cue elicited by physostigmine. Pirenzepine (40 µg ICV; 15 min) significantly antagonized the discriminative effect of 0.1 mg/kg physostigmine.

The present study showed that rats could use 0.1 mg/kg of physostigmine as a discriminative stimulus in a two-bar operant task. This discrimination was of central origin since physostigmine did not generalize to equimolar doses of the quaternary compound neostigmine and was not antagonized by the peripheral drug scopolamine methylbromide. Generalization experiments demonstrated that the physostigmine discrimination was mediated by muscarinic receptors since arecoline, oxotremorine and RS 86, direct muscarinic receptor agonists completely mimicked the physostigmine cue. The involvement of nicotinic receptors was unlikely in light of the lack of generalization by nicotine and of the inability of mecamylamine a nicotinic antagonist, to antagonize the physostigmine cue. Pirenzepine (40 µg), a selective M1 receptor antagonist completely antagonized the cue elecited by physostigmine suggesting that activation of M1 receptors might be a prominent component of the physostigmine cue.

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FURTHER EVIDENCE THAT LIPOXYGENASE PRODUCTS OF ARACHIDONIC ACID PLAY AN ESSENTIAL ROLE IN THE RELEASE OF HISTAMINE FROM MAST CELLS

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Rat mast cells have the ability to biosynthesize prostaglandins from endogenous precursors (Roberts et al, 1979) and respond to histamine liberators by releasing increased quantities of arachidonic acid from the membrane phospholipids (Okano et al., 1985). Earlier workers have suggested that products of arachidonic acid (AA) metabolism generated via the lipoxygenase pathway(s) are essential for the secretion of histamine (Sullivan & Parker, 1979). Recently however it has been indicated that products of AA generated by the cyclo-oxygenase pathway are also essential (Masini et al., 1987).

Indomethacin is a known inhibitor of the cyclo-oxygenase enzyme system while nordihydroguairetic acid (NDGA) is a potent inhibitor of 5-lipoxygenase enzyme system. We have used these agents to investigate if a selective block of one pathway of AA metabolism can affect the mast cell histamine release induced by concanavalin-A.

Suspensions of purified mast cells in 300 µl volumes (approximately 3 x 10^5 cells) were prepared as described earlier (Sharma & Gulati, 1985). Fifty µl of buffer was added to the control aliquots and indomethacin or NDGA (in 50 µl buffer) was added to the other aliquots. After a period of 15 minute incubation at 37°C, 50 µl buffer containing 40 µg of concanavalin-A (plus 4 µg of phosphatidyl serine) was added. All the tubes were then further incubated for 30 minutes and histamine release in the supernatant was measured using the fluorimetric method of Shore et al (1959). In some experiments the concentrations of prostaglandins $F_2\alpha$, E_2, 6-Keto $PGF_1\alpha$ and Thromboxane B_2 were also simultaneously measured in the supernatant using radioimmunoassay techniques.

Percent histamine release (mean ± SEM; n = 6)

Drug concentration (M)	Indomethacin	NDGA
-	33.41 ± 3.73 (Control)	36.63 ± 3.78 (Control)
10^{-8}	39.65 ± 4.36	35.73 ± 3.04
10^{-7}	40.66 ± 4.82	27.42 ± 3.68
10^{-6}	34.53 ± 3.35	22.81*± 1.86
10^{-5}	30.96 ± 4.09	16.65*± 2.92

* $P < 0.01$ compared with control value in the group.

The results show that unlike NDGA indomethacin has no ability to block concanavalin-A induced histamine release from mast cells. On the contrary a small increase in histamine release is observed with indomethacin concentration of 10^{-7} to 10^{-8} M. Incubation of mast cells without concanavalin-A caused 7.29±2.14% (mean ± SEM) release of histamine but this was not affected by NDGA or indomethacin. Indomethacin failed to block the release of histamine even when it produced a complete inhibition of prostaglandin biosynthesis by the mast cells. Neither indomethacin nor NDGA caused any degradation of histamine and did not interfere in the fluorimetric technique used for histamine estimation.

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HISTAMINE AND 5-HYDROXYTRYPTAMINE CONTENT AND RELEASE IN RAT TISSUE MAST CELLS

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Rat mast cells from different anatomical locations have been shown to exhibit heterogeneity with respect to histamine content and release after stimulation with a variety of ligands (Ennis and Pearce, 1980; Ali et al, 1986). Rat peritoneal mast cells additionally contain 5-HT (Cohen et al, 1987), but little data is available concerning the content and release of 5-HT from other rat mast cell types. We report here the histamine and 5-HT content of mesentery and lung isolated mast cells and compare amine release from these cells following challenge with compound 48/80 and the calcium ionophore A23187, with that from peritoneal mast cells.

Mast cells were obtained from female Wistar rats (280-330g). Peritoneal mast cells were collected following lavage and used without further purification (Cohen et al, 1987). Tissue mast cells were isolated after digestion with collagenase (80 U ml^{-1}, 90 min,37°C: Pearce and Ennis, 1980) in Locke's buffer with BSA (1 mg ml^{-1}). Cells were washed three times (200xg,5 min,4°C) with buffer, and aliquots ($1x10^5$ cells ml^{-1}) incubated (15 min,37°C) with compound 48/80 (0.1-100.0 µg ml^{-1}) or A23187 (1.0-600.0 µg ml 1). Histamine (Hi) and 5-HT were measured fluorimetrically (Cohen et al, 1987). Total amine content of the cells was measured after sonication (10 sec,50W). Results (Table 1.) are expressed as a percentage of total (mean ± SEM; number of experiments in brackets), after correcting for spontaneous release.

TABLE 1. HISTAMINE, 5-HYDROXYTRYPTAMINE AND RAT MAST CELLS.

	PERITONEAL	MESENTERY	LUNG
AMINE CONTENT			
Amount 5-HT (pg $cell^{-1}$)	4.18 ± 0.48(9)	6.67 ± 0.83(8)	21.58 ± 1.97(8)
Amount Hi (pg $cell^{-1}$)	15.56 ± 1.34(9)	1.98 ± 0.24(8)	3.11 ± 0.40(8)
Ratio 5-HT : Hi	0.23 ± 0.05(9)	3.46 ± 0.31(8)	7.28 ± 0.58(8)
SECRETION			
Spontaneous 5-HT Release (%)	4.81 ± 0.52(9)	24.75 ± 1.21(6)	26.23 ± 1.91(6)
Spontaneous Hi Release (%)	4.68 ± 0.31(9)	20.00 ± 4.33(6)	22.35 ± 2.15(6)
COMPOUND 48/80			
Maximal 5-HT Release (%)	58.36 ± 3.21(3)	43.24 ± 3.15(4)	19.23 ± 0.33(4)
Maximal Hi Release (%)	77.93 ± 3.88(3)	51.55 ± 3.10(4)	23.83 ± 0.68(4)
5-HT EC_{50} (µg ml^{-1})	2.40	5.80	1.90
Hi EC_{50} (µg ml^{-1})	0.58	0.74	1.50
IONOPHORE A23187			
Maximal 5-HT Release (%)	64.56 ± 7.69(3)	33.55 ± 1.28(3)	18.15 ± 2.11(3)
Maximal Hi Release (%)	81.80 ± 3.07(3)	61.88 ± 2.54(3)	31.27 ± 0.70(3)
5-HT EC_{50} (µg ml^{-1})	120.0	38.0	34.0
Hi EC_{50} (µg ml^{-1})	27.0	4.0	56.0

These results indicate that whilst tissue mast cells contain significantly more 5-HT than histamine, when compared with peritoneal cells, the secretion of the two amines follows an essentially similar pattern to that seen in peritoneal mast cells.

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FURTHER STUDIES ON IgE-MEDIATED EICOSANOID RELEASE FROM HUMAN DISPERSED LUNG CELLS

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Together with preformed mast cell mediators such as histamine, lipid derived autacoids such as prostaglandins (PG) and leukotrienes (LT) are believed to play roles in inflammatory reactions in the lung. We now describe our further studies on the IgE-dependent release of these mediators from human lung.

Human lung was obtained at lobectomy and subjected to proteolytic disaggregation (Holgate *et al.*, 1984). This procedure yielded a cell population containing 34 ± 2.5% macrophages; 29 ± 3% lymphocytes; 24 ± 2.3% monocytes; 7 ± 1% mast cells; 3 ± 1% epithelial cells; 2 ± 0.4% neutrophils and 0.2 ± 0.1% eosinophils (n=17). All cell challenges were performed in a protein free HEPES-buffered physiological salt solution at 37°C. In 5 experiments challenge of passively-sensitized cells with 50 $\mu g\ ml^{-1}$ ϵ-chain specific anti-IgE resulted in the release of histamine (measured by radioenzymatic assay), PGD_2, TXB_2 and sulphidopeptide LTs, the latter being quantified by specific RIA. The maximum release of histamine was 31 ± 0.1% net and occurred 5 min after challenge. In contrast, maximum eicosanoid release occurred 20-45 min after challenge.

The concentration-dependency of IgE-mediated release was studied in cells challenged for 45 min. Net release of all products measured rose to maxima at 75-125 $\mu g\ ml^{-1}$ anti-IgE with net releases of (ng per 10^6 nucleated cells) 2.12 ± 0.63 PGD_2, 0.26 ± 0.02 LTC_4 and 0.19 ± 0.04 LTD_4/LTE_4 at 125 $\mu g\ ml^{-1}$ anti-IgE (n=5). With the exception of TXB_2, there were significant correlations between the release of eicosanoids and histamine. When normalized to histamine release, the following amounts of eicosanoids were released (ng μg histamine^{-1}): PGD_2 17.89, TXB_2 1.44, LTC_4 2.10 and LTD_4/LTE_4 7.51. With A23187 (0.1-5.0μM) the normalized release was: PGD_2 15.74, TXB_2 5.75, LTC_4 2.17 and LTD_4/LTE_4 6.99 ng μg histamine^{-1} .

Preincubation of cells for 20 min with the non-steroidal anti-inflammatory drug flurbiprofen (1-300 nM) prior to 45 min challenge with 50 $\mu g\ ml^{-1}$ anti-IgE significantly inhibited the release of PGD_2 and TXB_2 with IC_{50} values of 1.00 ± 0.09 and 1.4 ± 0.43 nM respectively (n=5) but had no significant effect on histamine or LT release.

Our results indicate that although IgE-dependent challenge of human lung results in a significant release of sulphidopeptide LTs, the amounts are 8.4 - 87.6 fold less than that of the mast cell derived prostanoid PGD_2. These results contrast with the larger release of LTs reported by other workers using different analytical techniques (MacGlashan *et al.*, 1982). The inhibitory potency of flurbiprofen in this system is of note in view of its ability to attenuate the early response to allergen in atopic volunteers (Holgate *et al.*, 1987).

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ICI 192, 605: A POTENT, SELECTIVE THROMBOXANE A_2 RECEPTOR ANTAGONIST ON SMOOTH MUSCLE

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We have previously described the properties of a novel series of 1,3-dioxane thromboxane A_2 (TxA_2) receptor antagonists typified by ICI 185,282 (Byland et al, 1987; Jessup et al, 1987). Since these disclosures several analogues more effective than ICI 185,282 have been identified and characterised, one of which is ICI 192,605 (4(Z)-6-[(2,4,5-cis)2-(2-chlorophenyl)-4-(2-hydroxy phenyl)1,3-dioxan-5-yl]hexenoic acid) (Brewster et al, 1988). We now describe the antagonist potency and selectivity of ICI 192,605 at a variety of smooth muscle receptors in vitro.

ICI 192,605 competitively antagonised U-46619 responses of rat thoracic aorta and pig coronary artery preparations. Analysis of this data (Arunlakshana and Schild, 1959) gave pA_2 values (mean ± s.e.) of 8.43±0.05, n=12 (rat) and 9.4±0.1, n=5 (pig) with Schild plot slopes (0.9 and 1.14) not significantly different from unity. The drug (0.01 μM) also inhibited responses of rat aorta to $PGF_{2\alpha}$, PGE_2 and PGD_2 giving concentration ratios (mean ± s.e., $n \geq 4$) of 5.9±0.9, 6.7±0.8 and 9.8±2.1, respectively. The selectivity of ICI 192,605 for the TxA_2 receptor was confirmed by the observation that on rat aorta the drug (10μM), gave concentration ratios (n=4) of 2.8±1.4 and 1.9±0.7 against noradrenaline and 5-HT. ICI 192,605 was also a selective antagonist at pulmonary TxA_2 receptors in vitro. The compound (0.1 μM) inhibited U-46619 contractions of guinea pig trachea and lung parenchyma preparations, giving concentration ratios ($n \geq 4$) of 19.6±1.4 and 16±2.5. This antagonism was selective as the drug (10μM) did not potently modify histamine responses of guinea pig trachea or lung parenchyma (concentration ratios of 2.3±0.4 and 1.35±0.28, $n \geq 4$). ICI 192,605 (0.01μM) also proved to be a potent antagonist of U-46619 contractions of marmoset trachea and lung parenchyma in vitro, giving concentration ratios ($n \geq 4$) of 24.2±2.4 and 20.8±8.2. The antagonist had no significant activity at other prostanoid receptors; thus ICI 192,605 (10μM) did not potently inhibit responses of guinea pig ileum to PGE_2, $PGF_{2\alpha}$, PGD_2, histamine and acetylcholine, neither did it affect $PGF_{2\alpha}$ induced contractions of dog iris.

These results indicate that ICI 192,605 is qualitatively similar to ICI 185,282, and as such is a potent, selective, competitive antagonist which exerts activity at vascular and pulmonary TxA_2 receptors.

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THE EFFECT OF ICI 192, 605, A SELECTIVE THROMBOXANE A_2 RECEPTOR ANTAGONIST, ON PLATELETS

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The in vitro pharmacology and oral activity of the potent, selective thromboxane A_2 receptor antagonist ICI 192,605 (Brewster et al, 1988; Jessup et al, 1988) has been investigated further using platelets from several species.

The methods used for in vitro and ex vivo platelet studies have been described elsewhere (Jessup et al, 1986; Jessup et al, 1987). For studies using marmosets, animals were anaesthetised, blood withdrawn from the abdominal aorta into citrate (3.8%, 1 part to 9 parts whole blood) and platelet rich plasma (p.r.p.) prepared by centrifugation.

In vitro, ICI 192,605 caused concentration dependent shifts to the right of U-46619 aggregation curves in rat, rabbit, guinea pig and human p.r.p. Analysis of this data (Arunlakshana and Schild, 1959) gave mean pA_2 values ($\pm$s.e., $n \geq 4$) of 6.8 ± 0.1, 6.3 ± 0.05, 8.6 ± 0.05 and 8.2 ± 0.1 with Schild plot slopes of 0.74, 0.99, 1.28 and 1.39, respectively. On marmoset platelets in vitro ICI 192,605 (0.01μM) caused significant ($p<0.05$) inhibition of U-46619 responses giving a mean concentration ratio ($\pm$s.e., $n=5$) of 4.2 ± 1.4. However the antagonist, when added in concentrations in excess of 0.02μM, caused insurmountable blockade of U-46619 aggregation. In human platelets ICI 192,605 (0.1, 1μM) inhibited collagen induced aggregation, yielding concentration ratios (mean $\pm$s.e., $n=4$) of 7.1 ± 2.3 and 17.3 ± 1.2; the antagonist (100μM) did not modify the primary phase of adrenaline and ADP induced aggregation (mean concentration ratio $\pm$ s.e. of 1.03 ± 0.2 and 1.18 ± 0.1). ICI 192,605 (10μM) did not affect PGI_2, PGE_1, or PGD_2 inhibition of ADP induced human platelet aggregation, neither did it modify human platelet microsomal thromboxane synthase or cyclooxygenase activity. When dosed orally to rats ICI 192,605 (10,20 and 40 $mg.kg^{-1}$) antagonised U-46619 platelet aggregation ex vivo giving mean ($\pm$s.e., $n \geq 4$) peak concentration ratios of 8.5 ± 2.2, 19.4 ± 7.4 and >200 which occurred after 1 hour. At the highest dose tested significant antagonist activity persisted for 8 hours. When orally dosed to guinea pigs ICI 192,605 (0.02, 0.05 and 0.2 $mg.kg^{-1}$) antagonised U-46619 platelet responses ex vivo; mean peak concentration ratios ($\pm$s.e., $n=4$) of 3.8 ± 0.5, 11.5 ± 1.0 and 47 ± 23 again occurred at 1 hour. In the marmoset ICI 192,605 (0.01 and 0.05 $mg.kg^{-1}$) when given orally, caused insurmountable blockade of platelet TxA_2 receptors at 1 ($n=4$) and 8 ($n=4$) hours. The same doses of drug yielded U-46619 concentration ratios (mean $\pm$ s.e., $n=6$) of 3.2 ± 1.1 and 7.4 ± 2.9 respectively, at 24 hours.

Thus ICI 192,605 is a potent, selective antagonist at platelet thromboxane A_2 receptors which, when dosed orally, has a long duration of action in rodents and primates.

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IS ENDOTHELIUM-DEPENDENT RELAXATION MEDIATED BY ARGININE-DERIVED NITRIC OXIDE?

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In isolated blood vessels, ATP and acetylcholine (ACh) stimulate synthesis and/or release of an endothelium-derived relaxing factor (EDRF) leading to relaxation of the adjacent vascular smooth muscle. EDRF has recently been identified as nitric oxide (Palmer *et al.*, 1987; Ignarro *et al.*, 1987). Biosynthesis of nitric oxide and related NO-containing compounds is not limited to the vascular endothelium but has also been shown in bovine retractor penis, mouse liver, murine adenocarcinoma cells and human macrophages (Martin *et al.*, 1988; Varich *et al.*, 1987; Amber *et al.*, 1988; Iyengar *et al.*, 1987). The latter group showed that nitrite, nitrate and, when the secondary amine morpholine was offered, N-nitrosomorpholine in cultured human macrophages are exclusively derived from the terminal guanidino-nitrogens of L-arginine (ARG). L-canavanine (CAN), a guanidinooxy analogue of ARG, inhibited this biosynthetic pathway. Sturm *et al.* (1988) identified a smooth muscle relaxing factor from polymorphonuclear leukocytes with a pharmacological profile most similar to $NaNO_2$. We tested the hypothesis that ARG is also the physiological precursor of endothelium-derived nitric oxide (EDNO). If so, CAN should inhibit endothelium-dependent vasorelaxation.

Rat thoracic aorta ring preparations were mounted in 25 ml organ baths filled with a modified Krebs-Henseleit bicarbonate solution (37°C) gassed with 95 % O_2/ 5 % CO_2. Rings were eqilibrated at an isometric resting tension of 1.5 g before noradrenaline (NA; 0.1 µM) was added for submaximal stable precontractions. Then ATP, ACh or glyceroltrinitrate (GTN) were added. In part of the experiments, rings were pretreated with CAN (2 mM) or ARG (1 mM) for 2 h. Contractions and relaxations in control experiments were stable.

ATP, ACh and GTN produced dose-dependent relaxation of NA-induced precontractions. ATP- and ACh-induced relaxations were dependent on an intact endothelium. In endothelium-denuded rings, contractions were observed with ATP and ACh. When rings were pretreated with CAN, the dose-response curve for ATP-induced relaxation was shifted towards higher ATP concentrations (from 66.4 to 695 µM). Similar results were obtained with ACh. Contractile responses to ATP and ACh were unaltered by CAN, suggesting that no antipurinergic or anticholinergic effects of CAN were involved. GTN-induced relaxation was unaffected by CAN-pretreatment, excluding any direct inhibitory effect of CAN on vascular smooth muscle relaxant mechanisms. Pretreatment with ARG had no effect. Pretreatment with CAN also increased the maximum force of NA-induced contraction ($+26.3 \pm 8.7$ %) by preventing NA-induced minor EDRF release counteracting NA-induced contraction.

We propose that (1) ARG is the physiological precursor of EDNO and (2) ARG-derived NO is a ubiquitary new autacoid molecule.

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THE EFFECTS OF INDOMETHACIN ON RAT CARTILAGE BREAKDOWN IN VIVO AND IN VITRO: POTENTIATION WITH INTERLEUKIN 1 IN VITRO

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Destruction of articular cartilage can be enhanced by the administration of non-steroidal anti-inflammatory agents (De Brito etal.,1987;Parnham and De Brito,1987).These findings have recently been confirmed by Bottomley etal. (1988) in the mouse air pouch model . The original work was performed in a rat in vivo model, which entailed the subcutaneous unilateral implantation of femoral head cartilages (FHC) .This study utilises a) the in vivo rat model and b) an in vitro system to investigate cartilage breakdown by indomethacin.
In the in vivo model animals were dosed orally with either vehicle (tap water) or indomethacin (3mg/kg) for a period of 2 weeks. The implants were subsequently removed and assessed for proteoglycan contents. The FHC's in the in vitro system were incubated with a)interleukin-1 (IL-1)(100ng/ml) ,b)indomethacin (100uM) and c) IL-1(100ng/ml and indomethacin (100uM).In this system all FHC's plus drugs were incubated in RPMI 1640 medium ,with a supplement of antibiotics and foetal calf serum.

Table 1. In vivo study

	FHC Proteoglycan (Mean±SEM ug GAG)		
	Vehicle treated	Indomethacin	% Loss
Implant alone	372±30	346±18*	7
Implant+cotton	193±22	149±14*	23
Non-implanted FHC	492±21		

Table 2. In vitro study

	N	Proteoglycan Contents (ug GAG)
Cart. alone	6	252±13
Cart.+IL-1	6	215±19
Cart.+Indo.	5	214±28*
Cart. + IL-1 + Indo.	6	152±11*

Cart.=Cartilage; Indo.=Indomethacin ;GAG= Glycosaminoglycan

The above results show that in animals treated with indomethacin (3mg/kg) , there was a 7% loss in proteoglycan contents from the cartilages implanted alone (Table 1). However, this loss was potentiated ($P<0.05$) to 23 % in the cotton wrapped bilateral implants. In table 2 indomethacin and IL-1 individually produced slight loss of proteoglycan from the FHC's but this was not significant, when compared with the controls. Incubation of FHC's in the presence of both indomethacin and IL-1 resulted in significant ($P<0.05$) breakdown of the FHC's .

The present findings suggest that indomethacin will increase the breakdown of cartilage as shown by the in vivo model whilst the in vitro study indicates that this breakdown may occur via an interaction with IL-1. This interaction is presently under further investigation.

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PLATELET-ACTIVATING FACTOR : A POSSIBLE MEDIATOR OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Platelet-activating factor (1-O-octadecyl [or hectadecyl]-2-(R)-acetyl-gylcero-3-phosphorylcholine), (PAF), is a phospholipid mediator released from many cell types including macrophages, polymorphonuclear neutrophils (PMN), vascular endothelium. platelets, eosinophils and basophils (Roubin etal.,1983). The association of PAF with inflammatory cells has led to the suggestion that it may be a mediator of allergic responses and acute inflammation (Vargaftig and Braquet,1987). It is a potent mediator of increased vascular permeability in man and animals (Archer etal.,1983) and in rats, injections of as little as 0.04pmol induces plasma protein extravasation(Pirotzky etal.,1984). Recently, specific PAF antagonists have been developed ,the most potent being BN52021 (Braquet etal.,1987). In this study the role of PAF in the development of the clinical signs of experimental allergic encephalomyelitis (EAE) will be examined in the rat using BN52021.

Rat spinal cords (Hooded Listers ,Roche strain) were excised, washed and homogenised in sterile saline (500mg/ml) and then emulsified with an equal volume of Freund's Complete Adjuvant. Three groups of animals (n=6) were injected intradermally at the base of the tail with 0.1ml of adjuvant. Group 1 was administered (i.v.) with 0.25ml of sterile saline containing 4ug/ml PAF on day 5 post adjuvant treatment whilst groups 2 and 3 were dosed (P.O.) twice daily with BN52021 (10mg/Kg) and vehicle (tap water) respectively on day 1. In order to quantify the development of EAE , a point scoring system was devised. One point was assigned for partial and two points for complete tail droop and similarly one point for partial and two points for complete hind limb paralysis.These clinical signs of EAE were monitored for 22 days post adjuvant injections.

The control (group 3) animals showed initial signs of tail droop on day 14 and achieved a maximum score of 13 on day 16. In contrast, those animals injected with PAF (group 1) showed an earlier onset of clinical signs starting on day 12 and all animals reached a maximum on day 15 with a score of 19. Animals treated with the PAF antagonist (group 2) showed virtually total suppression of the clinical signs and only two animals on day 14 showed the earliest tail signs which persisted for 1 day only.

The above results indicate that administration of PAF on day 5 causes an earlier onset and a more severe form of EAE than was developed in the control group. The rationale in selecting day 5 for PAF treatment is based on histological studies that the first lesions appear in the brain on day 6 after adjuvant injection (Waksman and Adams,1962).The attenuation in the development of clinical signs produced by BN52021 indicates that PAF may play a role in early development of EAE. The involvement of PAF in development of EAE is under further investigation.

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THROMBOXANE RECEPTOR AGONISTS AND ANTAGONISTS: RADIOLIGAND DISPLACEMENT AND PHARMACOLOGICAL ACTIVITY ON HUMAN PLATELETS

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Radio-iodination of the p-hydroxy derivative of ONO 11120 yields a ligand (^{125}I-PTA-OH) with high specific activity useful for studying the properties of thromboxane receptors (Mais et al, 1985; Narumiya et al, 1986). We have used this ligand to determine the equilibrium dissociation constants (K_d) of several potent thromboxane agonists and antagonists for the human platelet thromboxane receptor and correlated these with pharmacological activities for inducing platelet shape change and antagonising U-46619-induced aggregation respectively.

Binding assays were performed on intact human platelets which were incubated with 0.1nM ^{125}I-PTA-OH (75 TBq/mmole, Amersham) and competing ligand at 37°C for 30 min followed by centrifugation separation. Platelet shape change was measured optically and antagonism of platelet aggregation induced by U-46619 determined using a platelet counting method (Lumley & Humphrey, 1981).

Table 1 Comparison of binding affinities and pharmacological activity (mean ± s.e.m.; n = 4) of some thromboxane mimetics and antagonists on human resuspended platelets ([a] K_d = antilog pA_2).

Agonist/Antagonist	Displacement of ^{125}I-PTA-OH: K_d (nM)	Shape change EC_{50} (nM)
EP 171	2.9 ± 0.4	0.065 ± 0.011
EP 031	11 ± 1	0.55 ± 0.08
16-p-chlorophenoxy-w-tetranor-11-deoxy $PGF_{2\alpha}$	23 ± 4	2.7 ± 0.6
STA_2	27 ± 4	1.8 ± 0.4
U-46619	69 ± 14	5.4 ± 0.9
16-p-fluorophenoxy-w-tetranor $PGF_{2\alpha}$	440 ± 32	27 ± 8
		K_d[a] vs U-46619 platelet aggregation
GR32191	4.6 ± 0.7	1.7 ± 0.2
AH23848	8.8 ± 1.0	5.4 ± 1.1

The potency of GR32191 and AH23848 for displacement of radioligand and antagonism of U-46619-induced platelet aggregation correlated well (Table 1) providing confirmation by two independent measures that both drugs interact with the platelet thromboxane receptor. Ranking of agonist activity correlates well with the ranking for radioligand displacement. The low K_d of the most potent thromboxane mimetic EP171 (Jones et al., 1985) may account for its slow onset of action on platelets. For example, if $k_1=1x10^7$ $M^{-1}s^{-1}$ and $k_2=0.015s^{-1}$ (K_d=1.45nM assuming only one enantiomer is active) then at an EP171 concentration of 0.05nM the calculated occupation half-time = 45s. The measured half-time for onset of shape change with EP171 = 38 ± 3s (n=7) and for U-46619 = 9.4 ± 0.4s (n=7). Studies are in progress to ascertain the rate constants for the EP171-thromboxane receptor interaction.

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EFFECT OF ATRIAL NATRIURETIC PEPTIDE (ANP) ON EDRF-INDUCED INHIBITION OF PLATELET AGGREGATION

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Endothelium-derived relaxing factor (EDRF) inhibits platelet aggregation and relaxes vascular smooth muscle by stimulation of soluble guanylate cyclase and elevation of intracellular cyclic GMP. ANP also elevates cytosolic cGMP but by stimulation of particulate guanylate cyclase (Leitman and Murad 1986, Martin et al 1988). Release of EDRF is inhibited by 8-bromo-cyclic GMP from rabbit aorta (Evans et al., 1987) and from cultured endothelial cells by ANP (Busse et al., 1988). In this study the effect of ANP on EDRF release from rabbit aorta is investigated using inhibition of platelet aggregation as a measure of EDRF release.

Platelet rich plasma (PRP) was prepared by standard techniques from human venous blood collected into 3.2% trisodium citrate (9ml blood per 1ml citrate). 1cm lengths of rabbit aorta, with and without endothelium, prepared as previously described (Furlong et al., 1987) and pretreated with indomethacin (10^{-5}M) (with or without ANP) for 60 mins , were incubated for 10min with 1ml aliquots of PRP with or without carbachol (1.4 x 10^{-6}M) to stimulate EDRF release. Superoxide dismutase (60U/ml) was added to the PRP to inhibit the breakdown of EDRF; haemoglobin (10^{-5}M) was used in some experiments to inhibit EDRF activity. Platelet aggregation was measured by impedance aggregometry and was induced immediately upon removal of the aortic segment from the PRP by a previously determined threshold dose of ADP ($5x10^{-7}$M to $5x10^{-6}$M).

In initial control experiments neither ANP (10^{-7}M) nor carbachol ($1.4x10^{-6}$M) when added to PRP in the absence of an aortic segment affected platelet aggregation. In the presence of endothelium-intact aorta, carbachol significantly inhibited ADP induced aggregation by 57$\pm$ 7.0% (Mean $\pm$ s.e.mean n=6 $p<0.01$). This carbachol- induced effect was inhibited by haemoglobin (10^{-5}M) and in endothelium-denuded vessels carbachol itself had no effect on platelet aggregation. Incubation of endothelium-intact aorta with ANP (10^{-7} and 10^{-8}M; n=6 and n=3 respectively) abolished the carbachol induced inhibition of aggregation; 10^{-9}M ANP reduced the effect of carbachol from 57$\pm$7% to 29$\pm$5% (n=3). The results of this study are consistent with either inhibition of EDRF release by ANP or antagonism of its effects by combination or other means.

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SURGICAL PREPARATION OF HUMAN SAPHENOUS VEIN AUTOGRAFTS REDUCES THE RELEASE OF ENDOTHELIUM DERIVED RELAXING FACTOR (EDRF)

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Surgical preparation of human saphenous vein (HSV) for coronary artery bypass grafts has previously been shown to attenuate the release of prostacyclin, a potent inhibitor of platelet aggregation (Angelini et al., 1987). We investigated whether surgical preparation of HSV led to impaired EDRF release, which would also favour platelet aggregation and adhesion, and may contribute to early occlusion of these grafts (Furlong et al., 1987; Sneddon et al., 1988).
EDRF release from HSVs was compared using a cascade bioassay system as previously described (Christie & Lewis, 1987). Endothelium-denuded rings (2-3mm wide) of pig coronary artery (PCA) were mounted for isometric tension recording, and superfused at 2ml/min with warmed (37°C), gassed (95% O_2, 5% CO_2) Holman's solution containing indomethacin (10^{-5} M). A 2 cm length of HSV was used as a donor vessel for EDRF, released in response to bradykinin (10^{-9}-10^{-6}M) or the calcium ionophore A23187 (10^{-6}M). The PCA recipient rings were preconstricted to 95% of maximum with 5-hydroxytryptamine (3 x 10^{-6}M), and the EDRF-induced relaxation expressed as a percentage of preconstricted tone. A23187 and bradykinin alone did not alter the constrictor responses of the PCA recipients.
Control HSVs were obtained as soon as possible after the first skin incision and stored in heparinised whole blood (3mg/l) at room temperature for up to 60min (33±8 min, mean±sem; n=5) before being assayed for EDRF activity.
Similar unprepared HSVs were stored for longer periods at room temperature, in either heparinised whole blood (125±11min, n=5) or heparinised Hartmann's solution (3mg/l) (160±23min, n=7).
Surgically prepared HSVs were also obtained post-operatively after adventitial stripping, side branch ligation, distension with saline at <300mmHg pressure and storage at room temperature either in heparinised whole blood (124±11min, n=10) or heparinised Hartmann's solution (191±12min, n=8). Results are compared using Student's t test for unpaired data.

	CONTROL	HARTMANN'S		BLOOD	
		Unprepared	Prepared	Unprepared	Prepared
Bradykinin(10^{-6}M)	50±6	32±5*	13±2*	24±5*	17±2*
A23187(10^{-6}M)	61±9	42±4	16±2*	26±4*	19±2*

* Significant difference from control ($p<0.05$)

The table shows the EDRF activity of HSVs in response to a maximal dose of bradykinin and A23187. EDRF activity of all veins is reduced from control values after storage in heparinised whole blood or heparinised Hartmann's. Surgical preparation further reduces EDRF activity in both blood and Hartmann's stored veins.
We conclude that routine surgical preparation and storage reduces the ability of human saphenous vein to release EDRF.

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ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) INHIBITS INOSITOL TRISPHOSPHATE (IP_3) FORMATION IN RABBIT AORTA

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The vasodilator properties of EDRF, like the nitrovasodilators, are mediated through stimulation of soluble guanylate cyclase and elevation of intracellular levels of cyclic GMP. We have previously shown that EDRF acting through cyclic GMP inhibits the influx and intracellular release of calcium (Collins et al., 1986). Since changes in calcium fluxes may be secondary to changes in phosphatidylinositol (PI) turnover, in the present study we have investigated the effects of EDRF on levels of inositol phosphates in the rabbit aorta and compared its effects with those of sodium nitroprusside.

New Zealand White rabbits (2-2.5kg) were killed by a blow to the neck. The thoracic aorta was removed, cleaned of connective tissue, placed in Krebs-Ringer Bicarbonate at room temperature, pH 7.3 gassed with 95% O_2, 5% CO_2 and cut into 4cm lengths. Inositol phosphates were measured by the method of Berridge et al. (1983). Briefly, the aorta (with or without endothelium) was incubated at 37°C with myo [2-^{3}H(N)] inositol (25μCi/ml) for 5hrs followed by 20min incubation with 10mM lithium chloride. Tissues were immersed in liquid N_2, weighed and homogenised in 1.5ml 3.6% perchloric acid at 4°C. After centrifugation, the pH of the supernatant was adjusted to 8-9 and the inositol phosphates were separated using anion-exchange chromatography. Interventions were made after the lithium incubation and included: noradrenaline (NA)(10^{-5}M) to stimulate PI hydrolysis, acetylcholine (ACh)(10^{-6}M) to stimulate EDRF release, haemoglobin (Hb)(10^{-5}M) to inhibit EDRF activity and sodium nitroprusside (NP)(10^{-5}M) to stimulate soluble guanylate cyclase in the absence of endothelium. Peak IP_3 levels only are presented as counts/min/mg wet weight and given as mean ± s.e.m.

In endothelium-intact vessels NA significantly increased IP_3 levels from 0.26±0.04 (n=6) to 0.88±0.07 (n=6)($p<0.01$). ACh reduced this NA-stimulated increase to 0.33±0.05 (n=7)($p<0.01$ cf NA). This ACh-induced inhibition was significantly reduced by Hb, IP_3 levels increasing to 0.62±0.07 (n=6)($p<0.01$ cf ACh+NA). In endothelium-denuded vessels NA significantly increased IP_3 levels from 0.23±0.02 (n=6) to 0.75±0.08 (n=6)($p<0.01$). NP significantly reduced this NA stimulated increase to 0.28±0.04 (n=7)($p<0.01$ cf NA).

These results show that EDRF and NP inhibit IP_3 formation in vascular smooth muscle and it is likely that the previously demonstrated inhibition of intracellular calcium release by EDRF and NP (Collins et al., 1986) is a direct consequence of inhibition of IP_3 formation.

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TREATMENT IN VIVO WITH Ph CL 28A ALTERS PROSTAGLANDIN METABOLISM IN RAT ISOLATED LUNG

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The prostaglandin dehydrogenase (PGDH) inhibitor, Ph CL 28A, when infused through the pulmonary circulation of isolated lungs, decreases PGE_2 and $PGF_{2\alpha}$ catabolism in this tissue (Berry et al., 1985; Bakhle & Pankhania, 1987). This inhibitor also increases output of PGs from the lungs from either exogenous or endogenous arachidonic acid (AA) as substrate (Bakhle & Pankhania, 1987). We have recently investigated PG catabolism and synthesis in isolated lungs from rats treated in vivo with this PGDH inhibitor.

Male rats (220-270g) were given a single i.p. injection of CL 28A (10 or 30 mg/kg) dissolved in Na_2CO_3 solution (0.5%, w/v). At 1h and 2h after this treatment, the rats were anaesthetised with pentobarbitone (60mg/kg, i.p.) and the lungs removed. They were perfused as described previously (Bakhle & Pankhania, 1987) with Krebs solution via the pulmonary circulation. After treatment with 10 mg/kg of CL 28A, survival of PGE_2 (500ng) increased ($p < 0.05$) from 11±2% (mean ± se mean) in lungs from untreated rats to 62±6% (1h) and 35±5% (2h; n=3 at each condition), as measured by RIA for PGE_2. At the same times, the $T_{1/2}$ values (time for 50% of injected radioactivity to emerge in lung effluent perfusate) following an injection of ^{14}C-PGE_2 (0.1nCi, 500ng) were 56±7s (1h) and 48±6s (2h), higher than in lungs from untreated rats (32±2s; n=3-5). The higher dose of CL 28A (30mg/kg) also increased PGE_2 survival and $T_{1/2}$ at 1h (80±1%, 84±2s) and at 2h (58±4%, 66±3s, n=3-5).

Output of PGE_2, $PGF_{2\alpha}$, TxB_2 and 6-oxo-$PGF_{1\alpha}$ in effluent perfusate from lungs following either an injection of exogenous AA (10µg) or of the calcium ionophore, A23187, (3µg) was assayed by RIA. The major effect at 1h after treatment with 10mg/kg of CL 28A was an increase in output of 6-oxo-$PGF_{1\alpha}$ after exogenous AA. This rose from 3.1±0.4ng/ml in lungs from untreated rats to 8.5±1.4ng/ml. Treatment with 30mg/kg CL 28A had a similar effect, 6-oxo-$PGF_{1\alpha}$ output rising to 9.0±1.0ng/ml (n=3-5). The other cyclo-oxygenase products were not affected. From endogenous AA (using the calcium ionophore), small increases only in $PGF_{2\alpha}$ and TxB_2 output were observed, with output of 6-oxo-$PGF_{1\alpha}$ being unaffected.

Our experiments show that treatment in vivo with CL 28A does markedly affect PGE_2 catabolism in isolated lung ex vivo but that the effects of CL 28A on the output of cyclo-oxygenase products were much less clearly demonstrable. It would appear that for CL 28A inhibition of PG catabolism is not strongly correlated with effects on PG output under these experimental conditions.

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THE TP-RECEPTOR BLOCKING DRUGS GR32191 AND BM13.177 HAVE A DIFFERENTIAL PROFILE OF ACTION ON VASCULAR SMOOTH MUSCLE

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We have previously shown the anaesthetised guinea-pig to be a sensitive animal model for detecting thromboxane (TP-) receptor agonist activity (Humphrey et al., 1986). A recent study by Terres and colleagues (1987) showed that the TP-receptor blocking drug, BM13.177, induces myocardial ischaemia at rest in patients with stable exertional angina pectoris, an action suggested to occur via TP-receptor stimulation. We have therefore investigated the TP-receptor agonist activities of BM13.177, and the recently described potent TP-receptor blocking drug GR32191 (Lumley et al., 1987) in vivo in the anaesthetised guinea-pig and also upon dog isolated vascular smooth muscle preparations in vitro.

Anaesthetised guinea-pigs were prepared for experimentation as described previously (Humphrey et al, 1986). Isolated vascular smooth muscle preparations from the dog were prepared either as rings or spiral strips and set up in modified Krebs solution (Apperley et al., 1976)for recording changes in isometric tension.

In the anaesthetised guinea-pig GR32191 (1mg/kg i.v.) and BM13.177 (30mg/kg i.v.) produced mean dose-ratios (95% confidence limits) of 103 (50-212, n = 4) and 35 (13-95, n = 4) respectively against vasoconstriction induced by the TxA_2 mimetic, U-46619. However, these doses of GR32191 and BM13.177 themselves were without effect upon blood pressure (BP) and tracheal inflation pressure (TIP). In dog coronary artery ring preparations BM13.177 (3, 30 and 300μM) produced concentration-related contractions (5 ± 2.6, 9 ± 2.7 and 26 ± 7.3% of potassium chloride (KCl, 30mM) induced maximum respectively, mean ± s.e.mean, n = 4). The profile of the drug was not dependent on the presence or absence of vascular endothelium. Under conditions of elevated tone (KCl, 20mM) the contractions to BM13.177 (300μM) appeared to be potentiated. GR32191B (10μM) failed to affect contractions to BM13.177 under all conditions. GR32191 (10μM) itself did not display any contractile activity either in the presence or absence of vascular endothelium or on preparations with normal or elevated tone. In fact higher concentrations of GR32191 (100μM) produced up to 70% relaxation of the KCl-induced tone. In contrast to the dog coronary artery, BM13.177 (3μM - 3mM) produced concentration-related contractions of the dog isolated saphenous vein which were abolished by GR32191 (10μM), the latter being without direct effect upon the tone of the preparation. Compared with U-46619, BM13.177 was only a very weak TP-agonist (equipotent concentration = 250,000) with a maximum response at 3mM of 37 ± 4% (n = 4) of the U-46619-(3μM) induced maximum.

In conclusion, in the present study GR32191 was without any TP-receptor agonist activity in any of the systems examined. BM13.177 displayed some weak TP-partial agonist activity but only on the dog isolated saphenous vein preparation. In addition BM13.177 contracted the dog isolated coronary artery through a TP-receptor-independent mechanism, the nature of which is unknown. Thus it may be that the episode of myocardial ischaemia seen with BM13.177 in man occurred via this mechanism rather than through TP-receptor stimulation. No such effect would be anticipated with GR32191.

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THE PROTECTIVE EFFECT OF ETHAMSYLATE AGAINST INDOMETHACIN-INDUCED DUODENAL ULCERATION IN RATS

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Ethamsylate (diethylamonnium 2,5,-dihydroxybenzenesulphonate, Dicynene®) has been shown to reduce capillary blood loss in menorrhagia (Harris & Campbell, 1976), and during surgery (Arora & Manford, 1979) and to reduce serous ooze at skin graft donor sites (Richmond & Sutherland, 1986). The haemostatic effects are related to increased vascular wall resistance (Huguet *et al.*, 1969) and increased platelet adhesiveness (Vinazzer, 1980). Any drug which limits capillary blood loss may be of use in the treatment of gastrointestinal ulceration, where reduction of capillary leakage may reduce the extent of ulceration. This study examines the effects of ethamsylate on drug-induced duodenal ulceration in rats.

Male Sprague Dawley rats (150-200 g) received ethamsylate (250 mg/kg) or vehicle control p.o. daily for 4 days. On the final two days of treatment the animals also received either vehicle control or indomethacin (40 mg/kg) p.o., the total dose volume was always 5 ml/kg. On the fifth day the proximal 10 cm of duodenum was removed for assessment of duodenal damage using an adaptation of the method of Ezer & Szporny (1975). This technique involves inflating the isolated duodenum with normal saline and recording the pressure of intestinal wall rupture. The pressure of rupture ie related to the extent of ulceration (ibid.) Data analysis was performed using 1-tailed non-parametric statistics.

The results, Table 1, show that ethamsylate alone did not affect duodenal tensile strength, whilst indomethacin produced a significant decrease ($p < 0.007$). The indomethacin-induced decrease was prevented by the concurrent administration of ethamsylate, the values for the group treated with indomethacin + ethamsylate being significantly higher than those for the group treated with indomethacin alone ($p < 0.03$) and not significantly different from the vehicle control.

Table 1 The effects of ethamsylate and indomethacin on the pressure of rupture of isolated duodenum

TREATMENT	NUMBER OF SAMPLES	MEDIAN PRESSURE OF RUPTURE (mmHg)	INTERQUARTILE RANGE (mmHg)	DIFFERENCE FROM VEHICLE CONTROL
Control + Control	24	210-215	180-235	-
Etham. + Control	13	190	40-250	NS
Control + Indometh.	27	165	35-250	$p < 0.007$
Etham. + Indometh.	29	210	25-250	NS

These results indicate that indomethacin causes a significant degree of duodenal ulceration in rats. Ethamsylate alone had no effect on the tensile strength of the duodenal wall but significantly reduced the ulcerogenic effect of indomethacin to a level not significantly different from vehicle control, thus showing that ethamsylate offers some protection against indomethacin-induced duodenal ulceration.

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THE LACK OF INFLUENCE OF THE ENDOTHELIUM ON RESPONSES TO 5-HT AND OXYGEN IN THE HUMAN UMBILICAL CORD BLOOD VESSELS

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With the use of a bioassay technique, Van de Voorde et al (1987) have shown that histamine and ATP but not acetylcholine(Ach) release substance(s) from human umbilical arteries and veins with an intact endothelium which causes relaxation in endothelium denuded pre-contracted rat aortic rings. This prompted us to seek a possible influence of umbilical vessel endothelium on its own smooth muscle. However, when umbilical preparations were set up for isometric tension recording and precontracted with 5HT, histamine produced no relaxation. We have therefore examined the influence of the endothelium on the contractile responses of the smooth muscle of the umbilical vessels to both 5HT and oxygen.

Umbilical cords collected after normal or caesarian deliveries were placed in deoxygenated Krebs solution at 4°C and used within three hours. Longitudinal strips(1-1.5cm) of artery and vein and also rings(2-3mm) of artery were used. Some strips and rings had the endothelium removed by rubbing gently with the roughened end of a pair of forceps. Strips and rings were mounted under 1 and 1.5g.wt. tension respectively. The Krebs solution was gassed with either 95%O_2 5%CO_2 (Van de Voorde et al 1987) or with 2.5%O_2 8%CO_2 balance N_2 which mimics physiological levels(McGrath et al 1987). In strips of both artery and vein cumulative concentration response curves(CRCs) to 5HT were obtained. 5HT(30nM) was used to examine relaxation induced by cumulative addition of ATP, acetylcholine or histamine. Contraction to increasing oxygen tension was also examined in rings and strips of artery only. Oxygen contracts only the arterial preparation and methods were followed as in McGrath et al (1986). Histological examination(silver staining) was also carried out on the vessels.

In 95%O_2 /5%CO_2 strips of artery and vein contract in a concentration dependent manner to 5HT with similar sensitivities, pD_2 values 7.38$\pm$0.12/7.22$\pm$0.15 respectively and maximum responses of 1.84$\pm$0.2/2.13$\pm$0.3. There was no significant difference between rubbed and unrubbed preparations of either artery or vein. Neither ATP, Ach nor histamine produced any relaxation in either arteries or veins, with histamine causing a further contraction. However, the endothelial-independent agent sodium nitroprusside(SN) produced relaxation in both arteries and veins, being more potent in the arteries. Rings and strips of artery, (endothelium intact or rubbed), contracted to increasing oxygen tensions reaching maximum at (282$\pm$8mmHg). There was no significant difference in either sensitivity or maximum response to oxygen between intact or rubbed strips or rings. Histological evidence showed that endothelial cells were present in unrubbed tissues but not in rubbed tissues.

These results show that there is histologically intact endothelium present in isolated blood vessels from the umbilical cord. The results from Van de Voorde (1987) suggest that a relaxatory subtance can be released whose effect on other vessels is not blocked by indomethacin but is blocked by methylene blue. Our results suggest that if this substance is released by histamine and ATP then it has no effect on the tone of the underlying smooth muscle. It is possible therefore that the endothelium of umbilical blood vessels can release an EDRF substance but which is not able to induce relaxation of the adjacent smooth muscle. Since SN can cause relaxation the cyclic guanosine phosphate pathway (the final common pathway of action of both EDRF and SN) is functional and is not responsible for the lack of effect of the endothelium-dependent agents histamine and ATP. It is possible that the mechanism coupling EDRF with the smooth muscle is not functional. Physiologically the results show that the endothelium is not involved in the modulation of umbilical vascular tone by O_2 via cyclooxygenase products (McGrath et al 1986) and that damage to the endotheium may not produce as much disruption of vascular tone control as in other blood vessels.

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REGIONAL HAEMODYNAMIC EFFECTS OF ENDOTHELIUM-DEPENDENT VASODILATORS

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The roles of endothelium-derived relaxing factor (EDRF) and prostacyclin in the control of regional organ bood flow and haemodynamics were studied using the radioactive labelled microsphere technique in rats. Agonists of EDRF and prostacyclin release (acetylcholine and bradykinin) were compared with sodium nitroprusside for their regional vasodilator actions.

Male Wistar rats (280-330g) anaesthetized with sodium pentobarbital were used. The right femoral artery was cannulated for the measurement of systemic blood pressure and the left jugular vein for the administration of drugs. A polythene cannula was introduced via the right common carotid artery into the left ventricle. The animals were respired with air. A stabilization period of 15 min was allowed after the operative procedures and arterial blood samples were taken for measurement of pH and blood gasses. The animals were then vagotomised. Test drugs were administered and during a stable depressor response of 75 - 100 mmHg, 60,000 - 80,000 ^{113}Sn labelled microspheres 15 $\pm$ 3μm diameter, suspended by ultrasonication in 0.3 ml 0.9% saline containing 0.01% Tween 80, were injected into the left ventricle over a period of 20 seconds. Concurrently, blood was withdrawn from the left femoral artery at a rate of 0.5 ml/min during the introduction of the microspheres and for 70 sec afterwards. The animals were then killed with an air embolism and the tissues dissected out, weighed and placed in vials for counting.

The depressor effects of nitroprusside and acetylcholine were heavily dependant upon cardiac effects. Thus both agents reduced cardiac output to such an extent that the effects on total peripheral resistance (TPR) were masked. Bradykinin, however, had little effect on the heart but decreased TPR. Indomethacin (4mg/kg) had little effect on the vasodilator properties of bradykinin in any of the tissues studied with the exception of the skin and the brain where the vasodilation was reduced. Acetylcholine was a particularly potent vasodilator in the kidneys, stomach, pancreas/mesentery, epididymedes and skeletal muscle, but due to the lowering of cardiac output this did not automatically lead to increased blood flow. However, in areas where acetylcholine increased vascular resistance (heart and testes) this did result in a reduction of perfusion. Bradykinin reduced vascular resistance in a number of tissues including liver, epididymedes,skeletal muscle, fat, brain and the whole of the gastrointestinal tract. In those tissues where vascular resistance was reduced by more than 60% there was a resultant increase in blood flow.

In conclusion, the data show that, with the possible exception of the brain and the skin, the vasodilator actions of bradykinin can adequately be transduced (presumably by EDRF) in the absence of prostacyclin synthesis. Additionally, these results indicate that the vasculature of the stomach, pancreas/mesentery, epididymedes and skeletal muscle are equally sensitive to both acetylcholine and bradykinin, whereas the kidneys showed selectivity towards acetylcholine and the intestines towards bradykinin. This may indicate differential receptor populations or multiple EDRFs.

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ANTI-ATHEROSCLEROTIC POTENTIAL OF NITRIC OXIDE (EDRF) VIA INHIBITION OF PLATELET MITOGEN RELEASE

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Prostacyclin (PGI_2) inhibits platelet aggregation and subsequent release of mitogens such as platelet derived growth factor (PDGF) (Willis et al, 1986). Nitric oxide (NO) which has recently been identified as an endothelium-derived relaxing factor (EDRF)(Palmer et al, 1987), inhibits platelet aggregation (Radomski et al, 1987). We now report that NO inhibits mitogen release from stimulated human platelets.

Platelet-rich plasma (PRP) was prepared from citrated (0.38%) venous blood by centrifuging (800 g, 8 min) and aggregated by addition of collagen (1.2-2.5ug/ml) in a Payton Aggregometer (37°C,1000 rpm, 5 min). NO solution was prepared by injecting 2.5ml of NO gas at atmospheric pressure into 40 ml of helium deoxygenated water held in a rubber sealed Wheaton flask. Aliquots of the NO solution were withdrawn with a Hamilton microsyringe and added to PRP 2 min before addition of collagen. PGI_2 dissolved in tris buffer (pH 8.4) was added in a similar manner. PRP from aggregation studies was centrifuged twice at 13,000rpm to prepare platelet-free plasma. This plasma was clotted by the addition of 1U/ml thrombin followed by heating for 5 min at 37°C. The clot was then expressed and the resulting serum filtered with sterile 0.2 micron Acrodisc 13 filters. The mitogenic activity of this serum was assayed on mouse fibroblasts (3T3 cells). These cells were plated out in microtiter plates and growth restricted for 24 to 48 hours by addition of minimal heat-inactivated fetal calf serum (0.25%). Serum samples were heat-inactivated (56°C,30 min) and diluted to 2.5 and 5% before addition to 3T3 cells. After 24 hours, incorporation of ^{3}H-thymidine (3HTdR) was assayed subsequent to separation of cells and supernatent using a cell harvester and glass-fibre filter paper.

Serum from collagen-aggregated platelets increased 3HTdR incorporation over unstimulated controls by 50 to 100% ($p< 0.05$) dependent upon assay conditions. This increase in mitogenic activity was blocked by doses of PGI_2 (3-10ng/ml,$p<0.025$-0.001) and NO (10-20μl/ml,$p<0.05$-0.001) that prevented platelet aggregation.

Inhibition of mitogen release may have implications in atherosclerotic diseases where mitogen-induced proliferation of the cells of the vasculature leads to pathogenic stenosis of blood vessels. Thus NO (EDRF) might have an important anti-atherosclerotic property.

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THE IRON CHELATORS DESFERRIOXAMINE AND 1,2-DIMETHYL-3-HYDROXYPYRIDONE INHIBIT HUMAN PLATELET CYCLOOXYGENASE AND LIPOXYGENASE ACTIVITY

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A recent study has demonstrated that the iron chelator desferrioxamine (DFO) and 1,2-dimethyl-3-hydroxypyridone (L1) inhibit prostanoid synthesis in vascular smooth muscle through removal of iron associated with cyclooxygenase (COX; Jeremy *et al.*, 1988). Since certain prostanoids are proinflammatory, it was suggested that iron chelators may prove beneficial in the treatment of inflammatory and other disease. Since platelets and their release products have also been linked to the aetiology of inflammatory and atherosclerotic disease, we investigated the effect of DFO and L1 on human platelet aggregation, thromboxane A_2 (TXA_2) synthesis and platelet lipoxygenase (LOX) activity.

Platelet rich plasma (PRP) was prepared from human subjects and aggregation studies carried out in a dual channel aggregometer. Aggregation was elicited with adrenaline, collagen and ADP. Iron chelators were added to PRP 3 min prior to the addition of aggregating agents. Concomitant TXA_2 release was assessed as previously described (Mikhailidis *et al.*, 1983). In separate experiments, 300 µl PRP was centrifuged, the resultant pellet washed in saline, frozen to -70 °C, thawed and sonicated, and incubated at 30 °C for 1 h in Krebs Ringer bicarbonate buffer containing varying concentrations of iron chelators. An aliquot of supernatant was taken for estimation of TXB_2 by radioimmunoassay (TXA_2 synthesising capacity: TXSC; Jeremy *et al.*, 1988). In separate experiments, platelet pellets from 300 µl PRP were incubated with 100 nCi ^{14}C arachidonic acid (^{14}C-AA) in the presence of varying concentrations of iron chelators at 37 °C for 30 min. Incubates were extracted with ethylacetate and LOX products by thin layer chromatography. Zones corresponding to hydroxyeicosatetraenoic acid (HETE) and hydroperoxyeicosatetraenoic acid (HPETE) were removed and counted for radioactivity, and percentage changes in LOX activity calculated.

Both DFO and L1 inhibited platelet aggregation and concomitant TXA_2 release (ADP-, collegen- and adrenaline-elicited) in concentration-dependent manners (mean IC_{50}'s: DFO, 9×10^{-4} mol.l^{-1}; L1, 3×10^{-4} mol.l^{-1}). Platelet TXSC was inhibited at similar concentrations by DFO (IC_{50}: 8.8×10^{-4} mol.l^{-1}). LOX activity was also inhibited by DFO (IC_{50}: 1.2×10^{-3} mol.l^{-1}) and L1 (4.6×10^{-4} mol.l^{-1}). The presence of 300 µM Fe^{3+} completely reversed the inhibitory action of DFO and L1 on TXSC and LOX activity.

The present study indicates that DFO and L1 inhibit both COX and LOX activity of platelets through removal of iron (both enzymes contain haem). Notwithstanding the potential use of these iron chelators as antithrombotic drugs, the *per se* inhibition of COX and LOX by iron chelators may also be of therapeutic value in the treatment of inflammatory disease and atherosclerotic disease *via* several systems - (1) inhibition of proinflammatory prostanoid synthesis; (2) inhibition of lipid peroxidation; (3) inhibition of toxic free radical generation by COX and LOX.

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INVOLVEMENT OF PROSTAGLANDINS IN UTERINE INHIBITORY RESPONSES TO ADRENOCEPTOR AGONISTS IN PREGNANT AND POST-PARTUM RATS

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The relaxation induced by β-adrenoceptor agonists in the rat uterus throughout the oestrous cycle is enhanced following cyclooxygenase inhibition (Boyle & Ohia, 1985a). This indicates the involvement of intramurally generated prostaglandins in adrenoceptor agonist responses (Boyle & Ohia, 1985b). We have now extended this study to investigate the possible role of prostaglandins in the uterine responses in late pregnancy and early post-partum to adrenoceptor agonists.

20 Day pregnant and 1 day post-partum Wistar rats (200-280 g) were used. The uterine horns were cut along the mesenteric border to obtain strip preparations. In some experiments the endometrial layer, a major source of prostaglandins (Campos et al., 1980), was removed. Both intact and endometrium-free strips were set up for isometric recording and uterine tone was induced with sub-maximal (60-70%) doses of acetylcholine (ACH, 10^{-6}M) and the inhibitory responses to adrenoceptor agonists measured as % reductions of the standard ACH contraction (Boyle & Ohia, 1985a,b). The cyclo-oxygenase inhibitor, flurbiprofen (FBF, 10^{-5}M) was added to the Tyrode's solution at least 30 min before addition of ACH. FBF had no effect on the response to ACH.

Adrenaline (ADR, 10^{-9}M - 10^{-6}M) and salbutamol (10^{-9}M - 3×10^{-7}M) each produced concentration-dependent inhibition of ACH in uteri from 20 day pregnant and 1 day post-partum rats. FBF significantly a) displaced the ADR and SAL concentration-response curves to the left ($p < 0.05$) and b) enhanced the maximum degree of inhibition ($p < 0.05$) in preparations with an intact endometrium.

Removal of the endometrium produced a significant decrease ($p < 0.05$) in the pD_2 values and inhibitory responses to ADR and SAL in 1 day post-partum rats but not in 20 day pregnant animals. In endometrium-free preparations, FBF did not alter either the pD_2 values or the degree of inhibition produced by ADR and SAL.

These results indicate that intra-murally generated prostaglandins are involved in the responses to adrenoceptor agonists in the uterus from late pregnant and early post-partum rats and support the view that the endometrium is the major source of prostaglandins in the rat uterus.

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THE ROLE OF PROSTACYCLIN IN HYPOXIC VASOCONSTRICTION IN THE SHEEP ISOLATED CORONARY ARTERY

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In the sheep isolated coronary artery, hypoxia causes a contraction if the endothelium is present but relaxation if the endothelium is absent (Kwan et al, 1988). The mechanism(s) underlying these effects of hypoxia are not known but one possibility is that hypoxia may modify the release of vasoactive materials from the coronary artery. The aim of this study is to investigate the role, if any, of a reduced synthesis of prostacyclin in the development of the hypoxic contraction.

Rings of sheep circumflex coronary artery were suspended under their optional resting tension in Krebs solution and isometric force measured. The solution was aerated either with 95% O_2:5% CO_2 giving PO_2 = 605±5 mm Hg (standard Krebs) or with 95% N_2:5% CO_2 giving PO_2 = 45±2 mm Hg (hypoxia). Bath fluid was collected at 30 min intervals and immediately frozen after addition of indomethacin 5 μM. Aliquots of the sample were assayed for 6-keto-$PGF_{1\alpha}$ by radioimmunoassay.

Hypoxia produced a contraction of 10.5±1.6 g cm^{-2}, n=12, which is equivalent to 30±4% of the contraction produced by KCl 40 mM. Reintroduction of standard Krebs was followed by relaxation and thereafter the hypoxic contraction could be replicated at least 2 more times (9.8±2.0, 10.7±2.0 g cm^{-2}). Indomethacin 5 μM also induced a contraction (8.9±1.2 g cm^{-2}). The resting tension was reset prior to the reintroduction of hypoxia. When the hypoxic challenge was repeated in the presence of indomethacin, it still caused a contraction but this was poorly sustained. At 20 min after the introduction of hypoxia (the time of maximal contraction before indomethacin) the hypoxic contraction was reduced to 4.6±3.5 g cm^{-2} (n=12, $P<0.05$).

The resting artery in standard Krebs released 6-keto-$PGF_{1\alpha}$ (basal release in 30 min = 364±37 ng/g, n=6). Introduction of hypoxia reduced 6-keto-$PGF_{1\alpha}$ release to 177±22 ng/g ($P<0.05$). A significant correlation was found between the percentage decrease in 6-keto-$PGF_{1\alpha}$ release and the amplitude of the associated hypoxic contraction. After reintroduction of standard Krebs, the release of 6-keto-$PGF_{1\alpha}$ in 30 min was 264±37 ng/g. Indomethacin 5 μM reduced 6-keto-$PGF_{1\alpha}$ output to a very low level (21±3 ng/g) which was not significantly reduced by repetition of the hypoxic challenge (14±4 ng/g).

These experiments have shown that hypoxia reduced the output of prostacyclin from rings of the sheep coronary artery. Reduced synthesis of prostacyclin may contribute towards the later stages (20-30 min) of the hypoxic contraction. Since part of the hypoxic contraction remains in the presence of indomethacin, hypoxia probably also releases other vasoactive mediators. The contraction induced by indomethacin in this preparation is probably caused by reduced formation of prostacyclin.

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FURTHER OBSERVATION ON THE EFFECT OF Ca^{2+} RE-ADDITION IN THE PRESENCE OF PHENYLEPHRINE AND 5-HT IN RAT AORTIC RINGS

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In experiments using rat aorta, where the agonist was added in the presence of nominally "zero" Ca^{2+}, 5-hydroxytryptamine (5HT) produced smaller responses to the cumulative addition of Ca^{2+} than would be expected from its response when 5HT was added in the presence of each Ca^{2+} concentrations: this did not happen with phenylephrine (PhE). This suggested a difference in 5HT- and α-adrenoceptor response coupling (Dainty *et al.*, 1987). We have further investigated this phenomenon.

Male Wistar rats (270g) were killed by a blow to the head and exsanguination. The thoracic aorta was excised and 3mm ring segments cut. The endothelium was removed by gentle rubbing. Isometric tension was recorded in either 2.5mM Ca^{2+} Krebs' saline or modified Krebs' saline (Ca^{2+} 2.5mM, HPO^{4-} 0.12mM), maintained at 37°C and gassed with 95% O_2:5% CO_2. Ca^{2+} re-addition experiments were carried out as previously described (Dainty *et al.*, 1987). In some experiments 1μM BAY K 8644 was added 10 minutes prior to addition of 3μM 5HT or 0.1μM PhE and cumulative re-addition of $CaCl_2$ (0.01-20mM) three minutes later. In some preparations Ca^{2+} re-addition was carried out after addition of 5HT or PhE in tissues which had been maximally contracted previously with PhE or 5HT respectively. The effect of increasing the time between addition of 5HT in "nominally zero" Ca^{2+} and raising the extracellular Ca^{2+} to 2.5mM was examined. The effect of altering the membrane potential on contraction to 5HT and PhE in 2.5mM Ca^{2+} Krebs' saline was examined. 5HT or PhE was added cumulatively to ring segments partially depolarised by the presence of a subcontractile concentration of KCl (20mM) or hyperpolarised by the presence of the K^+ channel activator BRL 34915 (0.01-10μM) (Southerton *et al.*, 1987). Values shown are the mean ± s.e. mean (n=4-6).

BAY K 8644 caused a significant increase in the response to re-addition of Ca^{2+} in the presence of both 5HT and PhE. This effect was greater for 5HT (at 3mM Ca^{2+}; 56 ± 19 to 1730 ± 287 mg.wt.) than for PhE (at 3mM Ca^{2+}; 1565 ± 200 to 2467 ± 130 mg.wt.) eliminating most of the difference between the agonists. There was no significant difference in the PhE Ca^{2+} re-addition response whether the preparations had been previously exposed to PhE or 5HT. However, the 5HT Ca^{2+} re-addition response was significantly less in tissues which had been previously contracted with 5HT but not PhE. Administration of 2.5mM Ca^{2+} 3 minutes after addition of 5HT in the presence of "nominally zero" Ca^{2+} resulted in a contraction which was similar to that seen to addition of the agonist in 2.5mM Ca^{2+}. However, increasing the length of time that tissues were incubated in "nominally zero" Ca^{2+} in the presence of 5HT prior to addition of 2.5mM Ca^{2+} decreased the subsequent contraction. After nine minutes little contraction to addition of 2.5mM Ca^{2+} was seen. In 2.5mM Ca^{2+} partial depolarisation with KCl potentiated and BRL 34915 inhibitied the contractions to both 5HT and PhE with no detectable difference between the two agonists.

These results suggest that BAY K 8644 has a preferentially greater effect on Ca^{2+} re-addition in the presence of 5HT than PhE, inhibiting or reversing the densensitisation or depression of contraction seen with 5HT Ca^{2+} re-addition. The absence of a differential effect of altering the membrane potential with KCl or BRL 34915 eliminates the possibility that this effect of BAY K 8644 may be due to 5HT- and PhE-induced contractions involving activation of different proportions of voltage-operated calcium channels. The phenomenon which we have previously reported (Dainty *et al.*, 1987) appears to be at least in part dependent on the length of time that the 5HT receptors are activated in "nominally zero" Ca^{2+} and on pre-exposure of the preparations to 5HT.

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BAY K8644 A MODIFIER OF RESPIRATION AND TOTAL CALCIUM UPTAKE ASSOCIATED WITH RAT HEART MITOCHONDRIA

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During the onset of ischaemia impairment of oxidative phosphorylation coupled with the accumulation of high levels of calcium (Ca^{2+}) results in changes of mitochondrial function and loss of intracellular Ca^{2+} homeostasis. Recent studies have demonstrated that Ca^{2+} overload can be reduced by either the prophylactic use of Ca^{2+} antagonists (Leblondel and Allain, 1984) or the promotion of Ca^{2+} from the mitochondrial matrix (Baydoun etal, 1986). Bay K8644 a positive inotropic dihydropyridine has been shown to act as a Ca^{2+} agonist at the plasma membrane, we now report its ability to stimulate respiration and total Ca^{2+} uptake associated with rat heart mitochondria.

Tightly coupled rat heart mitochondria were isolated from female Wistar rats (300-400g) and O_2 consumption was measured polarographically at 37°C using a Clark-type O_2 electrode (Rank Bros. Bottisham, U.K.) coupled to a BBC SE 120 pen recorder according to the method of Baydoun etal., 1986.

Ca^{2+} uptake was followed at 37°C using a Corning Ca^{2+}-specific electrode coupled to a pentracourt PM 10 pH meter and a BBC SE 120 pen recorder. The reaction medium contained 250mM sucrose, 5mM succinate, 2mM K^+ dihydrogen orthophosphate, 5mM Tris-HCl buffer (pH7.4) and 12.5μM $CaCl_2$. Ca^{2+} uptake was initiated by the addition of 2mg mitochondrial protein. When present Bay K8644 (10-40μM) was added prior to the initiation of Ca^{2+} uptake.

Bay K8644 (10-200μM) produced a concentration-dependent stimulation of state 4 respiration (substrate and O_2 in excess, ADP absent), inhibited state 3 respiration (ADP present) and reduced both the ADP : O ratio and the respiratory control index (R.C.I.). The more pronounced effect was on state 4 respiration with 200μM Bay K8644 increasing the rate of O consumption from 33.1 ± 0.7 to 187.0 ± 13.3 ng atoms O_2 min^{-1}mg $protein^{-1}$ (n=5). At the same concentration state 3 respiration was reduced from 247.2 ± 11.7 to 174.4 ± 0.06 ng atoms O_2 min^{-1}mg $protein^{-1}$ (n=5), the ADP : O ratio from 2.75 ± 0.03 to 1.3 ± 0.15 (n=5) and the R.C.I. from 5.3 ± 0.45 to 1.1 ± 0.03 (n=5).

Using the Ca^{2+}-specific electrode Bay K8644 (10-100μM) was found to inhibit mitochondrial Ca^{2+} influx resulting in an IC_{25} value of 52.5 ± 2.8μM (n=5). This inhibitory effect was only significant ($p<0.05$) at concentrations >80μM, with 100μM Bay K8644 reducing the control rate of influx from 264.4 ± 13.8 to 157.4 ± 8.9nmol Ca^{2+} min^{-1}mg $protein^{-1}$ (n=5). Over the concentration range 10-80μM, Bay K8644 produced a concentration-dependent increase in the total amount of Ca^{2+} taken up by mitochondria. The maximum effect was obtained in the presence of 60μM Bay K8644 with the total amount of Ca^{2+} taken up increasing from 248.8 ± 8.4 to 406.9 ± 17.6nmol Ca^{2+}mg $protein^{-1}$ (EC_{25} = 18.9 ± 1.4 μM; n=5).

The data presented indicates the ability of Bay K8644 to control intracellular Ca^{2+} homeostasis by modifying mitochondrial Ca^{2+} transport, resulting from a direct action on the respiratory chain causing either the stimulation or maintenance of the transmembrane proton-Ca^{2+} exchange and not by an ion channel effect.

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BENEFICIAL HAEMODYNAMIC PROFILE OF UK-61,260 IN AN ANAESTHETISED DOG HEART FAILURE MODEL

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UK-61,260 is a novel cardiac stimulant with additional vasodilator properties (Ellis et al). This study investigates the compound's haemodynamic profile in dogs in which specific characteristics of heart failure were reproduced.

Following anaesthesia the dogs (n=5) were surgically prepared for BP recording from the abdominal aorta and determination of cardiac output (CO) by thermodilution. The left carotid artery was then exposed and a catheter advanced into the left coronary artery. Heart failure was produced by the injection of Sephadex G50 microspheres (50 to 100 um diameter), suspended in saline, into the left coronary artery. The total dose of 4 mg was divided into 2 mg aliquots given 20 min apart. Following this procedure the coronary artery catheter was removed and a Millar Mikro-tip pressure transducer advanced into the left ventricle for the measurement of left ventricular pressure (LVP), its derivative dP/dt max and left ventricular end-diastolic pressure (LVEDP). Heart rate (HR) was determined by a ratemeter triggered from the arterial pressure pulse. In addition the following derived parameters were calculated - stroke volume (SV) and systemic vascular resistance (SVR). Tocainide (Tonocard, Astra), 900 mg p.o., was routinely adminsitered 30 min prior to induction of anaesthesia, to suppress the ventricular ectopic activity frequently seen in this model.

This model has elevated filling pressure and SVR, with reduced contractility and CO, characteristic of heart failure.

Consecutive 10 min i.v. infusions of UK-61,260 (0.25-4.0 ug/kg/min) produced dose-related increases in dP/dt max, SV, and CO accompanied by falls in LVEDP and SVR, with no significant increase in HR or marked changes in BP.

Inf. rate (ug/kg/min)	Mean BP (mm Hg)	LVEDP (mm Hg)	dP/dt max (mm Hg/sec)	CO (litres/min)	HR (beats/min)	SVR (BP/CO)
Control	108± 8	17±1	2380±102	2.60±0.36	148±8	43.4±4.3
0.25	108± 9	14±2*	2580±153*	2.68±0.38	149±8	42.0±4.1
0.5	109± 9	11±2**	2860±169*	2.81±0.36*	151±8	40.1±3.5
1.0	109±10	9±2**	3200±261*	3.02±0.41**	153±8	37.4±4.0**
2.0	105±11	7±2***	3400±305*	3.14±0.37**	156±9	34.1±3.2**
4.0	92±10*	6±1***	3450±343*	3.39±0.36**	157±9	27.5±2.6**

Values are mean ± S.E.M.

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ from control by paired t-test

Compared to other drug profiles in this model, this drug-induced cardiac stimulation accompanied by a reduction in cardiac filling pressure and peripheral resistance confirms that UK-61,260 is a positive inotrope with an additional vasodilator action on resistance and capacitance vessels. Such a combined cardiac stimulant/vasodilator profile would be expected to produce considerable therapeutic benefit in patients with congestive heart failure.

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POSSIBLE INVOLVEMENT OF OXYGEN FREE RADICAL REACTIONS IN THE CARDIOVASCULAR RESPONSES TO BURN INJURY OF THE ANAESTHETISED RAT

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Free radical reactions have been implicated in haemorrhage/reperfusion shock (Allan et al, 1986) endotoxin shock (Manson and Hess, 1983) and in causing death after burn injury (Saez et al, 1984). We therefore measured cardiac output (CO) following a full thickness scald burn injury to 22-23% of the body surface area of the anaesthetised rat and tested the effect of administation of the free radical scavengers superoxide dismutase (SOD) and catalase (CAT) or the xanthine oxidose inhibitor, allopurinol.

Female Wistar rats (250-280 g) were anaesthetised with sodium pentobarbitone (Sagatal 60 mg/kg I.P. plus supplementary doses as necessary). The CO before burn injury was 70.1 $\pm$ 7.7 ml/min (mean $\pm$ SEM).

Sixty minutes following burn injury the CO was 34.8 $\pm$ 4.1 ml/min in saline treated rats and 32.5 $\pm$ 3.7 ml/min in SOD/CAT (5 mg/hg/hr) treated rats ($P > 0.05$ by Students' unpaired t-test, n = 6 rats per group). Likewise 60 min after burn injury the CO of saline pre-treated rats was 23.1 $\pm$ 2.7 ml/min and 29.6 $\pm$ 2.5 in allopurinol (50 mg/kg) pretreated animals ($P > 0.05$, n = 6 rats per group).

Similarly the SOD/CAT or allopurinol treatment did not modify the stroke volume, heart rate, mean arterial blood pressure or central venous pressure responses to burn injury.

These data suggest that oxygen free radical returns are not involved in these cardiovascular responses to scald burn injury in the anaesthetised rat.

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LOW-DENSITY LIPOPROTEINS ATTENUATE THE CONTRACTILE RESPONSE IN RABBIT AORTA EVOKED BY NORADRENALINE

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Low-density lipoproteins (LDL), known risk factors in coronary heart disease, are thought to exert their harmful effects by infiltration into the arterial wall where they promote atherosclerotic lesions. Coronary arteries of miniature swine which are atherosclerotic, exhibit a supersensitivity to histamine (Shimikawa et al 1983). In contrast, LDL causes a reduced contractile response in the same arteries suggesting that the lipoprotein is not implicated in the supersensitivity (Davies et al 1987). Here, we investigate the effect of LDL on the contractile responses of various agonists in rings from rabbit aorta.

LDL (density, 1.019-1063 g/ml) was prepared from fresh human plasma by density gradient ultracentrifugation. The contraction of isolated intact or endothelium denuded rings of thoracic aorta from 6-month old New Zealand White rabbits was measured as previously described (Andrews et al 1987). Preservation of the endothelium was assessed by relaxation of precontracted tissues to ACh and histologically by silver staining. Tissues were contracted with graded doses of noradrenaline (NA) or KCl. After washout and equilibration for 30 min, LDL (2 mg protein/ml) were added and the tissues were contracted to graded doses of NA or KCl. A further cycle of washout, equilibration and contraction was performed to determine the reversibility of the effects.

LDL caused a two to tenfold rightward shift in the dose response curves to NA compared with control tissues but the maximum response was unchanged. This effect was reversed by washing and was independent of the presence or absence of endothelium. No change in the sensitivity to KCl was observed.

These results show that LDL decreases the sensitivity of rabbit aorta to NA but not to depolarising concentrations of KCl. A similar decrease in sensitivity was found in atherosclerotic compared with normal aortic rings. (Verbeuren et al 1986), which is consistent with our data. LDL is known to cause an increase in inositol phosphate turnover in cultured smooth muscle cells, whether this pathway is involved in the above effects, requires further study. (Block et al 1988).

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EFFECTS OF RILMENIDINE ON BAROREFLEX SENSITIVITY IN CONSCIOUS DOGS

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Rilmenidine is a novel α_2 agonist which reduces blood pressure in animals (Laubie et al 1986), healthy subjects and hypertensive patients (Dollery et al., 1988). It has been established that the centrally acting α_2 agonists clonidine and azepexole increase baroreflex sensitivity in both animals and man (Kobinger and Walland, 1972, Harron et al., 1985). The present study investigated the effects of oral administration of rilmenidine 1 mg/kg and placebo on baroreflex sensitivity in 6 conscious dogs.

The study was carried out in a temperature controlled laboratory (25°C) at the same time each day. Following an overnight fast, 6 greyhounds - 3 male and 3 female (mean ± SEM weight 30.1 ± 1.3 kg) were restrained in the supine position. Blood pressure (BP) was measured from a cannula placed in the femoral artery connected with a monitoring kit to a pressure transducer. Heart rate (HR) was monitored from a direct writing electrocardiograph. A cannula was placed in a foreleg for administration of drugs. Following the setting up procedure the dog rested quietly for 30 mins, rilmenidine and placebo were then administered orally in random order with 7 days between treatments; HR and BP were recorded at 15, 30, 45, 60, 90 and 120 mins after which baroreflex sensitivity was assessed using increasing doses of glyceryl trinitrate (20-200 μg/kg I.V.) to decrease BP by approximately 50 mmHg and phenylephrine (20-200 μg/kg) to increase BP by 50 mmHg. Comparisons were made between placebo and active drug values using Friedman's non-parametric analysis of variance and Wilcoxon's signed-rank test for matched pairs.

Following placebo no change occurred in systolic and diastolic BP and HR. Rilmenidine reduced ($P<0.05$) systolic BP at 45, 60, 90 and 120 mins compared to placebo; the maximum reduction was at 60 mins (141.5 ± 7.8 (placebo) vs 116.7 ± 7.2 (rilmenidine) mmHg). No change occurred in diastolic BP. HR was reduced ($P<0.05$) 30 mins after rilmenidine administration with the maximum reduction occurring at 60 mins (79.3 ± 5.9 (placebo) vs 54.3 ± 5.5 (rilmenidine) beats/min). Baroreflex sensitivity (RR-interval ms/mmHg systolic blood pressure) calculated from the slope of the regression line of RR-interval vs systolic BP using glyceryl trinitrate to reduce blood pressure was not changed (15.4 ± 4.3 (placebo) vs 31.3 ± 6.7 (rilmenidine)); the intercept of the regression line was not shifted, indicating no resetting of the baroreflex function line. Following phenylephrine to increase blood pressure, baroreflex sensitivity was enhanced ($P<0.05$) (23.1 ± 6.4 (placebo) 46.9 ± 9.0 (rilmenidine)); no change occurred in the intercept. In conclusion oral administration of rilmenidine 1mg/kg reduced ($P<0.05$) HR and BP in conscious dogs. Rilmenidine also enhanced ($P<0.05$) baroreflex sensitivity to increases in BP with phenylephrine but not to decreases with glyceryl trinitrate. This may be due to different baroreceptor combinations being involved in activation/deactivation of the receptors by the vasoactive drugs.

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EFFECT OF ANGIOTENSIN II ON RESPONSES MEDIATED VIA POST-JUNCTIONAL α_1- AND α_2-ADRENOCEPTORS IN ISOLATED RABBIT BLOOD VESSELS

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With angiotensin converting enzyme inhibitors, DeJonge et al., (1982) have shown, *in vivo* , a specific interaction between the renin-angiotensin system (RAS) and post-junctional α_2-adrenoceptors on vascular smooth muscle. In addition, in the rabbit isolated lateral saphenous vein (LSV), angiotensin II (AII) makes responses to BHT-920 more resistant to prazosin, a selective α_1-adrenoceptor antagonist, suggesting an enhancement of α_2-adrenoceptor mediated contractions (Schümman & Lues, 1983). We have therefore attempted a comparison of the influence of AII on responses to noradrenaline (NA), the endogenous ligand, mediated via both post-junctional α_1- and α_2-adrenoceptors, *in vitro*.

3mm segments of LSV or rabbit left renal vein (LRV) were placed in Krebs solution containing 1μM propranolol and 10μM cocaine, gassed with 95% O_2: 5% CO_2 at 37°C, under a resting tension of 0.5g.wt. and 0.2g.wt. respectively. A population of post-junctional α_2-adrenoceptors was isolated in the LSV by receptor protection, using rauwolscine and phenoxybenzamine, as described previously (Daly et al., 1987). The LRV contains a homogeneous population of post-junctional α_1-adrenoceptors (Daly et al., 1988). In the LRV and subsequent to isolation of postjunctional α_2-adrenoceptors in the LSV, two cumulative concentration response curves (CRCs) were obtained to NA. AII was added 15 mins prior to the start of the 2nd CRC to NA in some experiments. AII produced a transient contraction which returned to baseline before the onset of the CRC to NA. In other experiments, saralasin ((sar^1,ala^8)-angiotensin II) was added 10 min prior to the addition of AII. Results (Table 1) are expressed as pD_2 values, the negative logarithm of the concentration of NA which produces 50% of the NA max response.

Table 1

		pD_2 value for NA	
Tissue	Treatment	Pre-treatment	Post-treatment
LSV	time control	7.02 ± 0.07	6.99 ± 0.09
(after α_2 isolation)	A II (5×10^{-8}M)	6.93 ± 0.08	7.23 ± 0.06 *
	Saralasin (10^{-7}M) + A II (5×10^{-8}M)	6.86 ± 0.09	6.86 ± 0.11
LRV	time control	6.01 ± 0.07	6.03 ± 0.08
(α_1)	A II (5×10^{-8}M)	6.22 ± 0.14	6.21 ± 0.08

AII produced a significant leftward shift (* $p < 0.01$, paired t-test (n=6)), and a 15% increase in the maximum response of the CRC to NA, after isolation of a population of post-junctional α_2-adrenoceptors in the LSV. This effect was mediated via specific AII receptors since it could be blocked by the AII receptor antagonist saralasin. In contrast AII had no effect on responses to NA, mediated via post-junctional α_1-adrenoceptors, in the LRV.

These results provide evidence that there is a functional interaction between the RAS and post-junctional α_2- but not α_1-adrenoceptors in these two preparations. This confirms an enhancement of postjunctional α_2-adrenoceptor mediated contractions, produced by AII, with the endogenous ligand NA.

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THE EFFECT OF PROPRANOLOL AND SALBUTAMOL ON PORTAL VENOUS PRESSURE IN A PERFUSED RAT LIVER WITH NORADRENALINE PRETREATMENT

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Isolated perfused rat liver preparations (IPRL) have been used to examine the effects of vasoactive compounds on the hepatic vasculature. However, previous studies have been performed without pretreatment with catecholamines (Bhathal and Grossman, 1985). The present study has examined the effect of propranolol (Prop) and salbutamol (Salb) on the portal venous pressure in a constant flow recirculation IPRL to which noradrenaline (NA) had been previously added.

An IPRL was established as described previously (Burns et al 1988a). The perfusate flow was constant at 10 ml/min and perfusate pressure was measured in the portal vein (Ppv) and hepatic vein (Phv). There was a stabilisation period of 30 min before the addition of any drug. NA was added to give a perfusate concentration of 250nM. Three minutes later Prop was added to give perfusate concentrations of 10 or 100uM or Salb was added to give perfusate concentrations of 12 or 120uM. The effects of these compounds on the portal pressure (mean data (sd)) are shown in the table. In a further series of experiments Salb (1mM, n=6) was given without NA pretreatment.

	n	Pretreatment pressure Ppv (cm water)	Max.% change in Ppv after NA	% decrease from max.Ppv at times after adding NA	
				4 min	10 min
NA alone	8	9.6 (0.7)	+35 (10)	-4 (2)	-10 (3)
NA+Prop 10uM	8	11.2 (1.0)	+39 (13)	-5 (3)**	-7 (5)
NA+Prop 100uM	5	10.9 (1.1)	+29 (13)	-8 (3)**	-10 (4)*
NA+Salb 12uM	4	12.0 (1.5)	+36 (8)	-11 (3)***	-16 (5)
NA+Salb 120uM	6	10.5 (2.4)	+42 (16)	-11 (3)***	-15 (5)

$^{*}p<0.02$ $^{**}p<0.01$ $^{***}p<0.001$ for differences between Ppv after NA alone and NA+Prop or NA+Salb.

There were no significant differences between the pretreatment Ppv values or the maximum hypertensive effect of NA in any of the experiments. In the presence of NA, Prop (100uM) tended to lower the Ppv. Salb (12 and 120uM) also lowered the Ppv following pretreatment. However, Salb (1mM) when given alone without NA pretreatment had no effect on Ppv (mean pretreatment pressure 9.8 (0.7) cm water). No drug altered Phv.

The hypotensive effect of Salb is consistent with the drug being a beta 2 agonist. The hypotensive action of Prop is at variance with its effect in the IPRL when given without NA pretreatment (Burns et al 1988b), but is consistent with data suggesting it may have a vasodilatory effect independent of beta adrenoceptor blockade when injected intravascularly (Shanks 1967).

These data demonstrate that the qualitative and quantitative effects of vasoactive compounds in an IPRL preparation are dependent on the presence or absence of NA. Thus the relative vascular atonicity of such preparations may explain the lack of effect of a number of vasodilator compounds when tested in the absence of NA (Bhathal and Grossman 1985).

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THE EFFECT OF OESTROUS CYCLE ON VASCULAR POSTSYNAPTIC α_1- AND α_2-ADRENOCEPTOR RESPONSIVENESS IN THE PITHED RAT

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Pretreatment of animals with oestrogen causes an increase in the α_1-adrenoceptor mediated constrictor responses of perfused mesenteric arteries. This increase in sensitivity is accompanied by an increase in α_1-adrenoceptor affinity without a change in receptor number (Colucci et al., 1982). The present study examines α_1- and α_2-adrenoceptor mediated pressor responses in the pithed rat during the natural oestrous cycle.

Female Wistar rats (200-250g) were anaesthetized with sodium pentobarbitone (50mg kg^{-1} i.p.), pithed and artifically ventilated with air at 20 ml kg^{-1}. The femoral vein and carotid artery were cannulated for the administration of drugs and the measurement of arterial blood pressure respectively. Responses were obtained to phenylephrine (1, 3, 10µg kg^{-1}) in the presence of propranolol (1mg kg^{-1}) and to UK 14,304 (1, 3, 10µg kg^{-1}) in the presence of propranolol (1mg kg^{-1}) and prazosin (500µg kg^{-1}). The state of oestrous for each animal was determined by histological examination of a vaginal smear.

Table 1 Mean increases in diastolic blood pressure (mmHg) to phenylephrine and UK 14,304 in pithed rats in different states of oestrous

	Phenylephrine		
	1µg kg^{-1}	3µg kg^{-1}	10µg kg^{-1}
Oestrous	17.3 ± 3.8	31.7 ± 7.2	61.2 ± 9.9
Metoestrous	16.6 ± 3.9	33.2 ± 3.7	63.0 ± 7.0
Dioestrous	17.6 ± 4.8	32.0 ± 9.5	64.0 ± 6.4
Proestrous	12.6 ± 2.2	27.4 ± 3.7	53.0 ± 5.3
	UK 14,304		
	1µg kg^{-1}	3µg kg^{-1}	10µg kg^{-1}
Oestrous	8.5 ± 1.5	15.0 ± 2.8	23.5 ± 5.0
Metoestrous	7.5 ± 2.3	14.5 ± 2.3	19.3 ± 5.4
Dioestrous	7.0 ± 1.0	12.0 ± 2.5	19.3 ± 4.5
Proestrous	10.0 ± 1.6	13.3 ± 2.8	25.7 ± 3.7

Pressor responses to phenylephrine were independent of the state of oestrous. Similarly, oestrous state did not influence responses obtained to the α_2-adrenoceptor agonist, UK 14,304 (Table 1).

We therefore conclude that, in contrast to that seen with exogenously administered oestrogen, vascular postsynaptic α-adrenoceptor responsiveness in the rat is not influenced by the natural state of oestrous.

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EVIDENCE THAT SK&F 104078 DOES NOT DIFFERENTIATE BETWEEN PRE- AND POST-JUNCTIONAL α_2-ADRENOCEPTORS

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Various authors have suggested that prejunctional and postjunctional alpha$_2$-adrenoceptors may differ, and the most convincing evidence for this proposal has come from results obtained with SK & F 104078, an antagonist which is reported to have a hundred-fold selectivity for postjunctional over prejunctional alpha$_2$-adrenoceptors in the periphery (Ruffolo et al., 1987). We have now examined the effects of SK & F 104078 at alpha$_2$-adrenoceptors in the pithed rat, rat isolated atrium and human saphenous vein.

In pithed rats, prejunctional effects of the alpha$_2$-adrenoceptor agonist xylazine were assessed as the inhibition of the cardio-acceleration to a single stimulus and postjunctional effects were assessed as the peak pressor response. In rat isolated atrium, prejunctional effects of alpha$_2$-adrenoceptor antagonists were assessed as an EC_{30} (concentration producing 30% potentiation of stimulation-evoked overflow of tritium in tissues pre-incubated with [^{3}H]-NA). In human saphenous vein postjunctional potency of alpha$_2$-adrenoceptor antagonists was taken as the pA_2 obtained against contractions to NA.

In pithed rats, yohimbine (1 mg/kg) produced a 63 fold shift in the prejunctional potency and a 5 fold shift in the postjunctional potency of xylazine, and SK & F 104078 (5 mg/kg) produced 20 fold and 4 fold shifts in the pre- and postjunctional potencies of xylazine, respectively. In rat isolated atrium, prejunctional EC_{30} values of 7.89 (95% confidence limits of 7.75-8.03, -log M) and 6.68 (6.52-6.84) were obtained for yohimbine and SK & F 104078, respectively, so that yohimbine was approximately 16 times more potent than SK & F 104078 prejunctionally. In human saphenous vein, pA_2 values of 7.40 (95% confidence limits of 6.74-8.71, -log M) and 6.33 (5-91-6.92) were obtained for yohimbine and SK & F 104078, respectively, so that yohimbine was approximately 12 times more potent that SK & F 104078 postjunctionally.

Hence, like yohimbine. SK & F 104078 shows apparently selectivity for prejunctional alpha$_2$-adrenoceptors in the pithed rat and, like yohimbine, did not differentiate between pre- and postjunctional alpha$_2$-adrenoceptors in the isolated tissues examined.

In conclusion, we are unable to confirm the reported selectivity of SK & F 104078 for postjunctional alpha$_2$-adrenoceptors. Our results suggest that SK & F 104078 has similar effects to yohimbine at pre- and postjunctional alpha$_2$-adrenoceptors.

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CHRONIC EFFECTS OF ATENOLOL ON MESENTERIC VASCULAR RESPONSES IN DOGS

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We have previously shown that, in the rat, chronic administration of β-adrenoceptor blocking drugs produces changes distinct from the post-synaptic blockade of β-adrenoceptors. (Carr *et al.* 1983, Draper *et al.* 1986) There is some evidence to suggest that atenolol may be acting presynaptically after chronic administration to normotensive and spontaneously hypertensive rats. (Kingsbury *et al.* 1988) These effects are consistent with the time course of the antihypertensive action of the β-blocking drugs. This study investigates the effect of chronic β-adrenoceptor blockade on adrenergic neurotransmission in normotensive dogs.

Atenolol (7.5mg/kg) was administered orally to male Alderley Park beagles (17-20kg) daily for 21 days (n=5). The animals were then anaesthatised with sodium pentobarbitone (30mg/kg i.v.) and maintained with a 1mg/min infusion. A laporotomy was performed and the superior mesenteric artery was cleared to allow the location of a doppler flow probe. Bipolar platinum electrodes were placed around the vessel distal to the probe; a suitable side-branch was cannulated to allow administration of drugs and measurement of mesenteric blood pressure. Following a 30min stabilisation period vasoconstrictor responses to exogenous noradrenaline (1-400ng) and periarterial electrical stimulation were obtained. Control results were obtained from untreated animals (n=6). Resistance was calculated using : $R\,(dyns/sec/cm^5) = \frac{mean\ blood\ pressure \times 1330}{flow\ (mls/sec)}$ from mesenteric blood pressure and flow which were measured directly.

Changes in mesenteric pressure, flow and resistance to exogenous noradrenaline were not significantly different in control and drug treated animals. While there was some indication of a reduction following atenolol pretreatment in the response to electrical stimulation in terms of flow, this did not reach statistical significance. There was some evidence of a reduction in the mesenteric pressure response to electrical stimulation following atenolol pretreatment. However, when this data was used to calculate resistance a significant decrease in the mesenteric resistance response to electrical stimulation was observed. ($p<0.05$)

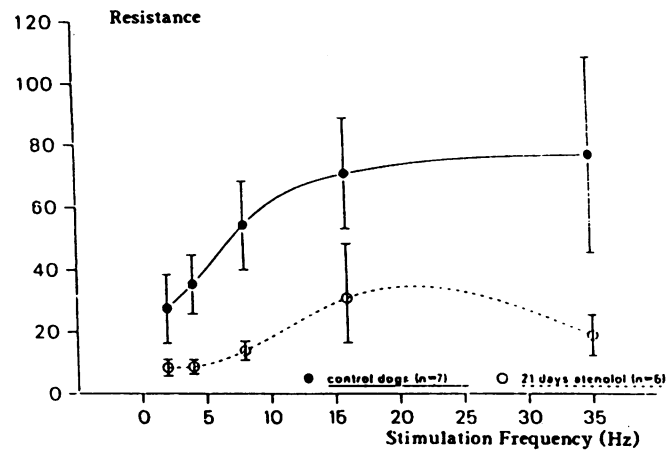

Fig 1: Effect of 21 days atenolol p.o. on resistance response to electrical stimulation

In conclusion, it seems likely that as neither mesenteric flow or pressure was kept constant during the experiment, animals were varying one or both of these parameters in response to stimulation. Only by using both these responses to calculate the response in terms of resistance did the overall trend become apparent. As chronic atenolol treatment produced no change in the response to exogenous noradrenaline but reduced the response to electrical stimulation, it is possible that, in accordance with our previous work in rats, it may be having some presynaptic effect.

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SGD 1/85 (PERBUFYLLINE) IS A COMPETITIVE ANTAGONIST ON 5-HT_2 AND α_1-ADRENOCEPTORS OF THE RAT

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Sgd 1/85 (7-{4-[4-(4-fluorobenzoyl)-piperidinyl] butyl}-3,7-dihydro-1, 3-dimethyl-1H-purine-2,6-dione) has been examined for antagonist activity on rat tissues in vitro.

Tissues were taken from male Wistar rats (250-300g) and suspended in McEwen's solution maintained at 37°C and gassed with 95% O_2, 5% CO_2. The mesenteric vascular bed (MVB) was perfused at 2ml/min and the endothelium removed by a 30s exposure to sodium deoxycholate (1mg/ml in 0.9% w/v NaCl) (Byfield et al 1986). 5HT or phenylephrine (PE) were employed as agonists and doses causing 65mmHg rise in pressure were determined (ED_{65}). For 5HT, the effect of Sgd 1/85 was measured on a second dose response curve relative to the first control, whereas with PE single curves were compared from control and pretreated tissues. Aortic rings (thoracic: rubbed free of endothelium) were dosed cumulatively and dose ratios determined from adjacent rings. Pairs of vas deferens (stimulated at 0.1Hz, 1ms, maximal voltage) were used and the concentration of azepexole that reduced twitch responses by 50% were determined on control and treated contralateral preparations. All dose ratios were subjected to Schild analysis (Tallarida & Jacob, 1979).

TISSUE	AGONIST	ANTAGONIST	MEAN pA_2(limits)	MEAN Slope(limits)	n
MVB	PE(α_1)	Sgd 1/85	10.6(8.6-12.6)	0.81(0.35-1.28)	10
(30min)	5HT($5HT_2$)		8.9 (8.81-8.99)	1.01(0.7401.28)	14
	PE	Prazosin	8.9 (8.62-9.18)	1.05(0.62-1.48)	6
	5HT		approx. 5		3
	PE	ICI 169 369	approx. 6		3
	5HT		8.2 (7.15-9.25)	0.92(0.46-1.38)	12
AORTA	PE	Sgd 1/85	8.4 (8.33-8.47)	0.95(0.70-1.19)	9
(30min)	5HT		approx. 8		
	PE	Prazosin	10.2(9.94-10.46)	0.90(0.54-1.22)	9
VAS DEF	AZE (α_2)	Sgd 1/85	6.4 (5.37-7.73)	1.1 (0.57-1.63)	6
(15min)		*Yohimbine	8.0 (7.96-8.04)	0.76(0.56-0.96)	15

AZE = Azepexole. MVB = Mesenteric vascular bed.
ICI 169 369 $5HT_2$ antagonist (BLackburn, et al, 1987).
n = number of dose ratios obtained on different tissues.
The limits are the fiducial limits, p=0.05.
* Signifies antagonism not competitive.

Thus Sgd 1/85 acts as a competitive antagonist at 5-HT_2 and α_1-adrenoceptors, with at least 100-fold less activity at α_2-adrenoceptors.

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THE EFFECTS OF POCA AND TGDA ON THE ISCHAEMIA-INDUCED INCREASED IN α_1-ADRENOCEPTOR DENSITY IN THE RAT LEFT VENTRICLE

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The ischaemia-induced changes in myocardial receptor traffic which may be proarrhythmic have been well-documented. Heathers et al (1987) showed that 30 min hypoxia in canine myocytes produced a 3-fold increase in α_1-adrenoceptor and sarcolemmal long-chain acylcarnitine densities, both of which were inhibited by the carnitine palmitoyl transferase 1 (CPT1) inhibitor POCA (sodium 2-[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate). In the present study we have compared the abilities of POCA and another CPT1 inhibitor, 2-tetradecylglycidic acid (TGDA), to inhibit ischaemia-induced increases in α_1-adrenoceptor binding in the rat left ventricle (LV) *in vivo*.

Pentobarbitone-anaesthetised male Sprague-Dawley rats (250 - 350g) were respired with room air and the heart exposed via a left lateral thoracotomy. A ligature was placed round the left anterior descending coronary artery (LAD) and drug was administered 15 min prior to LAD occlusion. Following 30 min occlusion the potential ischaemic zone of the LV was excised and α_1-adrenoceptor binding was performed with [^{3}H]-prazosin essentially as described by Williams et al (1981). Control data was obtained from saturation experiments on pools of 3 samples, while drug data are from individual animals, the results are shown in Table 1.

Drug	Route	N	α_1-adrenoceptor density (fmol/mg protein)	
Control-A	-	4x3	8.65 ± 1.04	
Ischaemia-B	-	4x3	16.30 ± 1.87	**A
POCA	ip	7	11.76 ± 2.67	nsA,B
POCA	iv	11	7.18 ± 1.40	nsA,**B
TGDA	ip	9	9.27 ± 2.76	nsA,B
TGDA	iv	10	2.47 ± 0.71	***A,B

Table 1 - The effects of POCA and TGDA (500µg.kg^{-1}) on the ischaemia-induced increase in α_1-adrenoceptor binding in rat LV. Apparent receptor density is calculated at 0.1nM [^{3}H]-prazosin. **P<0.01, ***P<0.001, ns not significant, Student's two-tailed t-test.

Accumulation of long-chain acylcarnitines in the sarcolemma with resultant alteration of sarcolemmal fluidity is the likely mechanism for the increased α_1-adrenoceptor density in the ischaemic LV by a shift in equilibrium between operating and cryptic forms of the α_1-adrenoceptors within the sarcolemma available to the radioligand. We observed a doubling of the apparent LV α_1-adrenoceptor density following ischaemia with no change in the affinity of the adrenoceptor for the ligand. The effect was inhibited by both POCA and TGDA (both drugs being more active by the iv route). TGDA (iv) significantly reduced the receptor density to below control value. However, the calculated affinity values of TGDA and POCA for the α_1-adrenoceptor were 7.3 and <5.0 respectively. The results indicate that both CPT1 inhibitor drugs are active *in vivo* to inhibit the ischaemia-induced alteration in myocardial receptor dynamics. TGDA appears to possess additional activity in either masking the α_1-adrenoceptor by directly binding or indirectly by internalisation thereby producing an apparent decrease in density.

The functional relevance of these ischaemia-induced α_1-adrenoceptor density changes is the subject of further evaluation.

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EFFECTS OF TIME UPON STIMULUS-INDUCED ARRHYTHMIAS AND INFARCT SIZE IN A CHRONIC CANINE MODEL

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Recent evidence suggests that inducibility of arrhythmias by programmed electrical stimulation (PES) is determined largely by infarct size (Wilber et al (1985). Since the natural history of myocardial infarction is one of shrinkage and scar formation, the present study investigated the relationship between these parameters in a group of greyhounds prepared for chronic electrophysiological studies and followed over a 4 week period.

Adult greyhounds of both sexes were anaesthetised using I.V. sodium methohexitone (18 mg/kg), intubated and ventilated (rate 18 per min, tidal volume 13 ml/kg) (Palmer Ideal Pump) with room air and halothane (1.5%). Following thoracotomy the fourth or fifth left rib was resected to expose the heart. The anterior descending branch of the left coronary artery was ligated in a two-stage procedure below its second branch, (Harris 1950). Myocardial pacing wires (Medtronic 6400) were sutured into the heart in the centre of the area supplied by the occluded artery and in an area supplied by an adjacent branch of the anterior descending artery (interelectrode distance 1.0-1.5 cm). Both pacing wires were brought out through skin distant from the wound, the chest was closed in layers and the dog allowed to recover. Streptomycin (250 mg) and procaine penicillin (300,000 iu) were given intramuscularly for three days. Infarct size was assessed by slicing the heart and staining with triphenyl tetrazolium chloride (1%). A total of 119 dogs were studied. Of these, 87 (73.1%) were alive and ambulant 24 hours after surgery. Acute ischaemic ventricular fibrillation occurred in 20 of the 32 which died. Other deaths were due to irreversible bradycardia and asystole (3), anaesthesia (4), haemorrhage (2) and cardiac failure (1). Two animals initially recovered from the operation but were later found dead. Of the 87 dogs which survived the initial 24 hour period, 6 subsequently had to be destroyed because of severe wound infections (3) vomiting and dehydration (2) or surgical problems (1). Another fifteen dogs died suddenly and unexpectedly during the first postoperative week. A ruptured left ventricle was diagnosed at post-mortem in one but no cause was apparent in any of the remaining 11 which were the subject of autopsy (mean infarct size 11.1 ± 1.5% of left ventricular (LV) mass). There were no deaths after the first week. Of the 66 animals surviving for 7 days, 61 (92.4%) exhibited an arrhythmia (sustained or non-sustained ventricular tachycardia or ventricular fibrillation) when stimulated (up to 3 extra stimula of twice diastolic threshold). The corresponding figures at 2, 3 and 4 weeks were 66.7%, 64.3% and 55.6% ($p=0.002$, Chi-square analysis). Mean infarct size for the animals which died in response to PES at 1 week was 7.0 ± 0.5% of LV mass. Respective values for 2 and 3 wks were 5.8±0.6% and 4.6±0.8%, ($p=0.001$, ANOVA). Infarcts could not be identified macroscopically after the third week.

These results suggest a time-dependent decrease in inducibility of arrhythmias by PES which may be related to reductions in infarct size and may be of importance for studies with antiarrhythmic drugs.

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REDUCTION IN MYOCARDIAL INFARCT SIZE BY CORONARY REPERFUSION WITH t-PA

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Thrombolytic therapy has been heralded as a major breakthrough in the treatment of myocardial infarction (Rentrop, 1985). The prompt restoration of coronary blood flow to the ischaemic area has been perceived as an essential prerequisite for the conservation of myocardial function. In this study, we demonstrate the beneficial effects of reperfusion induced by recombinant double chain tissue plasminogen activator (Wellcome Biotech. Ltd., BW t-PA) in reducing infarct size in an anaesthetised canine model of coronary thrombosis.

Anaesthetised beagle dogs of either sex (n = 20, 8.9-14.3 kg) instrumented for blood pressure, coronary perfusion pressure, blood gas monitoring and lead II electrocardiogram were used. A thrombus was induced in the left anterior descending coronary artery (LAD) by a copper coil placed there under fluoroscopic control. Blood samples were taken at 30 minute intervals for measurement of blood gas status, lactate, total creatine kinase (enzyme kit-method Boehringer Mannheim) and fibrinogen (Clauss method).

The clot was allowed to age for 90 minutes before infusing BW t-PA (1×10^6 i.u. kg^{-1}) over 60 mins (n = 10) or vehicle (0.85% saline, 0.01% Tween) over 30 minutes (n = 10). Restoration of LAD flow was assessed angiographically every 5 minutes during the t-PA infusion. After two hours of reperfusion (from the end of t-PA infusion), the area of myocardium at risk and infarcted was assessed and measured as described previously (Hughes et al., 1987).

BW t-PA restored main LAD patency within 40 ± 4.5 mins. The effects of thrombolysis with BW t-PA on areas at risk (R), infarct size (I) (as % of the left ventricle) and I/R ratio are shown in the table below. Also, the effects of reperfusion on total CK release are shown.

	R	I	I/R	Peak CK
BW t-PA (90-150 min occ)	32.6±1.9	14.8±4.3 *	41.8±10.9 *	10428±3730 *
CONTROL (4 hour occ)	30.9±1.9	23.7±2.4 *	75.0±10.0 *	4296±1063 *

* Significant difference in infarct size I/R ratio and peak CK by Mann Witney U - test ($P<0.05$)

A transient decrease in plasma fibrinogen (1.96±0.17g l^{-1} to 0.58±0.11g l^{-1}) was noted in the BW t-PA treated animals, however, this returned to normal at the end of the t-PA infusion.

These data show that coronary thrombolysis with BW t-PA restores blood flow to the ischaemic myocardium, salvages myocardial tissue and thus reduces infarct size in an anaesthetised beagle dog model of coronary thrombosis.

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ANTAGONISM OF THE CARDIOVASCULAR EFFECTS OF PGD_2 BY BW A868C IN THE RAT IN VIVO

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BW A868C [3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin] is a novel and selective antagonist of the prostaglandin DP-receptor through which PGD_2 can mediate vascular smooth muscle relaxation in vitro (Giles et al., 1988). In this study, the effects of BW A868C following intravenous or oral administration, on the cardiovascular effects of PGD_2, as well as those of prostacyclin and the hydantoin prostaglandin BW 245C, which can act at vascular PGD_2 receptor sites (Whittle et al., 1983), have been investigated in the rat.

In pentobarbitone-anaesthetised rats (200-300g), systemic arterial blood pressure (BP) was recorded from a carotid cannula. Intravenous infusion of BW A868C (1-10 $\mu g\ kg^{-1}\ min^{-1}$) in doses that had no direct action on BP, dose-dependently reduced the fall in BP induced by PGD_2 (1-40 $\mu g\ kg^{-1}$ i.v.). Thus, the fall in BP with near-maximal doses of PGD_2 (20 $\mu g\ kg^{-1}$ i.v.) of 22 ± 2 mmHg (n = 24) was significantly (P<0.001) reduced to 9 ± 2 mmHg (n = 8) and abolished (n = 4), with doses of BW A868C (1 and 5 $\mu g\ kg^{-1}\ min^{-1}$ i.v.) respectively. Bolus injection of BW A868C (100 $\mu g\ kg^{-1}$ i.v.) similarly antagonised the vasodepressor responses to PGD_2 (13-fold rightwards parallel shift of the dose-response curve; P<0.001). Likewise, BW A868C (10 $\mu g\ kg^{-1}\ min^{-1}$) caused a 59-fold shift (P<0.001) of the dose-response curve to BW 245C (0.25-10 $\mu g\ kg^{-1}$ i.v.). However, higher doses of BW A868C (20-100 $\mu g\ kg^{-1}\ min^{-1}$) caused no further shift of the dose-response curve, suggesting BW 245C also acts on a vascular receptor which is not of the DP-type. The vasodepressor responses to prostacyclin (0.05-0.5 $\mu g\ kg^{-1}$ i.v.) were unaffected by BW A868C (1-100 $\mu g\ kg^{-1}\ min^{-1}$ i.v.).

In conscious rats previously instrumented (under isoflurane/O_2 anaesthesia) with a femoral arterial cannula for BP and heart rate (HR) measurement and a femoral venous cannula for injection, PGD_2 (1-100 $\mu g\ kg^{-1}$ i.v.) caused only small, inconsistent reductions in BP (<10 mmHg) and widening of the pulse width. However, PGD_2 caused a dose-dependent increase in HR (up to 100 bt min^{-1}) which appeared to be reflex to its vascular effects and of sufficient magnitude to mask any reduction in BP. Pretreatment of these animals with BW A868C (30 and 100 $\mu g\ kg^{-1}$ i.v.) attenuated these small BP changes, and caused a dose-dependent rightwards shift (1.5-fold, NS; 7.5-fold, P<0.01, n = 6, respectively) in the HR dose-response curve. Increasing the dose of BW A868C to 300 $\mu g\ kg^{-1}$ however, caused no further shift (7.8-fold, P<0.01), indicating that high concentrations of PGD_2 may also interact with another vascular receptor. Pretreatment with an oral dose of BW A868C (1 mg kg^{-1} p.o.) also significantly attenuated the HR response to repeated doses of PGD_2 (30 $\mu g\ kg^{-1}$ i.v.) within 30 min of dosing (from 86 ± 5 to 39 ± 11 bt min^{-1}, n = 8). This inhibition was sustained for a further 150 min (P<0.05 by ANOVA).

These studies therefore support the in vitro findings (Giles et al., 1988) with BW A868C and demonstrate that this compound is a selective antagonist of the cardiovascular responses to PGD_2 at the DP-receptor following i.v. and oral administration.

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ACUTE ANTIHYPERTENSIVE EFFECTS OF BW B385C IN THE CONSCIOUS HYPERTENSIVE RAT

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Previous studies with BW A575C, a mixed isomeric form of the novel ACE-inhibitor/β-adrenoceptor blocking agent BW B385C (Allan et al, 1987), have shown this agent to reduce blood pressure (BP) in the acute renovascular hypertensive dog after intravenous administration (Cambridge et al, 1987). Further studies have now been carried out to examine the acute antihypertensive effects of BW B385C in the renovascular hypertensive rat (RHR) and the genetic or spontaneous hypertensive rat (SHR).

Renovascular hypertension was induced in Wistar rats (Charles River) by clipping of the left renal artery using the method described by Morton and Wallace (1983). These RHR presented with elevated plasma renin activity levels (3-fold) and BP when compared with sham-clipped animals. Spontaneous hypertensive rats of the Okamoto type were obtained commercially (Charles River) and presented with similar elevated BP. For these studies, animals of 300-350g body wt. were implanted, under isoflurane/O_2 anaesthesia, with an indwelling arterial cannula (for BP and heart rate (HR) measurement), and a venous cannula. On recovery, each animal was placed in a covered perspex box, connected to a BP transducer for continual recording of BP and HR, and allowed to equilibrate for 60 minutes.

Intravenous administration of vehicle (either saline or dextrose-saline) caused no significant change in BP over a 300 minute study period in both the RHR (n = 8) and SHR (n = 8). However, intravenous administration of BW B385C at 1 $mgkg^{-1}$ caused a significant reduction in BP in both the RHR (193 ± 7/145 ± 6 to 171 ± 8/128 ± 5 mmHg, $p<0.05$ by paired t-test, after 60 minutes, n = 8) and SHR (184 ± 10/129 ± 8 to 161 ± 7/110 ± 9 mmHg, $p<0.05$, after 120 minutes, n = 8). This was further reduced by additional intravenous administration of BW B385C at 5 $mgkg^{-1}$ in the RHR (to 164 ± 8/114 ± 6 mmHg, $p<0.05$) and the SHR (to 144 ± 7/98 ± 6 mmHg, $p<0.05$). In these animals, there were no consistent changes in HR, following vehicle or BW B385C administration.

These results therefore confirm the previous findings that this novel ACE-inhibitor/β-adrenoceptor blocking agent, BW B385C, effectively lowers BP, acutely in a hypertensive animal model (RHR) where the elevated BP is dependent upon the renin-angiotensin system. In addition, these results also demonstrate that BW B385C is equally effective at lowering BP in a hypertensive animal model (SHR) in which the elevated BP is dependent upon factors other than the renin-angiotensin system. Thus, BW B385C exerts an antihypertensive effect in two quite different animal models of hypertension.

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EFFECTS OF SODIUM NITROPRUSSIDE ON RESPONSES OF THE HEART TO NERVE STIMULATION IN ANAESTHETISED AND PITHED RATS

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Field stimulation of isolated atria simultaneously releases acetylcholine (ACh) from parasympathetic nerves and noradrenaline (NA) from sympathetic nerves. Sodium nitroprusside (SNP) increases field stimulation-induced release of NA but decreases release of ACh (Boyle & Pollock, 1988). Since each transmitter acts presynaptically to inhibit release of the other, the dissimilar effects of SNP on release of NA and ACh may be linked. SNP may act on parasympathetic nerves to inhibit ACh release, thereby removing ACh-mediated restraint on NA release and increasing NA overflow. The present study examined this hypothesis by investigating effects of SNP on responses of the rat heart <u>in situ</u> to stimulation of the vagus nerve and to stimulation of the sympathetic cardioaccelerator nerves.

Male Wistar rats (250-300 g) were anaesthetised with pentobarbitone (60 mg kg^{-1}, i.p.). Some were pithed with a teflon-shielded spinal electrode (Gillespie et al, 1970), so that the spinal sympathetic outflows and particularly the cardioaccelerator nerves (C_7-T_1) could be stimulated (60 pulses, 0.03 ms pulse width, 2 Hz, supramaximal voltage). In each experiment one carotid artery was cannulated to record blood pressure (BP) and heart rate (HR). Drugs were administered into the femoral vein. The vagus nerve in the neck was stimulated (100 pulses, 0.1 ms pulse width, 10 Hz; 25 pulses 0.1 ms, 5 Hz; 50 pulses 0.1 ms, 5 Hz, supramaximal voltage) and changes in BP and HR were recorded. Pithed rats received d-tubocurarine (1 $\mu g\ g^{-1}$, i.v.).

In anaesthetised rats, ACh (0.6 $\mu g\ kg^{-1}$) reduced HR (by 274 $\pm$ 25 mean beats min^{-1} $\pm$ s.e. mean). Vagal stimulation (50 pulses) also reduced HR (by 64 $\pm$ 7 mean beats min^{-1} $\pm$ s.e. mean). These responses were abolished by atropine (50 $\mu g\ kg^{-1}$) but were unaffected by SNP (60 $\mu g\ kg^{-1}$). In the presence of prazosin (100 $\mu g\ kg^{-1}$) and yohimbine (100 $\mu g\ kg^{-1}$), the response to vagal stimulation was potentiated (by 30 $\pm$ 5 mean percentage change $\pm$ s.e. mean, $P < 0.01$). SNP (60 $\mu g\ kg^{-1}$) inhibited the response to vagal stimulation in the presence of these α-adrenoceptor blockers (by 25 $\pm$ 5 mean percentage change $\pm$ s.e. mean, $P < 0.01$). The response to ACh was unaffected by prazosin and yohimbine as was the response to ACh in the presence of SNP. SNP (60 $\mu g\ kg^{-1}$) also inhibited vagal stimulation-induced (25 pulses) bradycardia in pithed rats (by 41 $\pm$ 8 mean percentage change $\pm$ s.e. mean, $P < 0.05$) even in the absence of α-adrenoceptor antagonists. The response to ACh (0.6 $\mu g\ kg^{-1}$) was unaffected by SNP. In the pithed rat, stimulation of the cardioaccelerator nerves increased HR (by 35 $\pm$ 4 mean beats min^{-1} $\pm$ s.e. mean) as did noradrenaline (NA, 0.1 $\mu g\ kg^{-1}$) (by 24 $\pm$ 1 mean beats min^{-1} $\pm$ s.e. mean). SNP (60 $\mu g\ kg^{-1}$) did not affect the response to exogenous NA or to cardioaccelerator nerve stimulation.

The results support the hypothesis that SNP inhibits release of ACh from cholinergic nerves and only indirectly potentiates NA release from sympathetic nerves if ACh-mediated neuromodulation of NA release occurs. Vagal stimulation produced complex results suggesting that in the anaesthetised rats a persistent basal sympathetic tone modulates ACh release.

S.J.B. is a University of Glasgow Postgraduate Scholar.

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EFFECTS OF FLOSEQUINAN ON GUINEA-PIG ATRIA AND ON CARDIAC VARIABLES IN THE ANAESTHETISED DOG

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Flosequinan (F), a novel hypotensive vasodilator, (Sim et al 1988) has been shown to relax vascular smooth muscle (Yates 1988) and increase cGMP levels (Allcock et al 1988). It is currently under clinical trial for hypertension and heart failure where studies indicate it dilates both arteries and veins (Cowley et al 1984). Further studies on its effects on cardiac muscle and cardiac variables are presented.

Isolated left atria from reserpinised Dunkin-Hartley guinea-pigs were suspended in Kreb's solution (5mM Ca++) at 34°C and stimulated at 3.0 Hz by square wave pulses of 3 msec at 2.5x threshold voltage. Contractions were recorded via isometric transducers and the signal differentiated to give dF/dt max.

Beagle dogs were anaesthetised with pentobarbitone sodium, 30 mg/kg iv and artificially ventilated. A metal cannula was tied into the left ventricular apex and left ventricular pressure (LVP) measured. LV dP/dt max. was derived by differentiating LVP. Effects of F before and after bilateral vagotomy and propranolol 1 mg/kg iv were studied.

Table 1 Inotropic effects in isolated driven guinea-pig atria

Drug.	Concn. (μM) producing 40% of the maximal force with isoprenaline.		Conc. (μM) causing 40% increase over basal dF/dt max.	
Flosequinan (F)	650	(n=16)	900	(n=4)
Theophylline	640	(n=12)	950	(n=4)
Sulmazole	23	(n=12)	31	(n=4)
Milrinone	22.5	(n= 4)	16.4	(n=4)
Ouabain	0.165	(n= 3)	0.18	(n=3)

Ouabain was the most potent inotrope whereas F and theophylline were weakest (Table 1).
In anaesthetised dogs (3 male; 2 female) F at 0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg iv produced increases in dP/dt max. of 9.8%, 18.6%, 36.4%, 70.4% and 132.9% respectively. There were also dose-related increases in heart rate (0.7-17.8%), decreases in coronary resistance (3.1-52%) and decreases in left ventricular end diastolic pressure (LVEDP) (0.2-2.1mmHg). Following bilateral vagotomy and propranolol 1 mg/kg iv, F, at doses of 0.3, 1.0, 3.0 and 10.0 mg/kg iv gave reduced increases in dP/dt max. of 14.9%, 22.9%, 42.1% and 62.1%. Changes in heart rate (1.1-9.5%) coronary resistance (15.4-31%) and LVEDP (0.13-1.7mmHg) were also reduced.

These results suggest that flosequinan has only weak inotropic activity in vitro. Little inotropic effect was observed at concentrations known to reflect blood levels at hypotensive doses in man (approx. 10μM. Packer et al 1988). The appreciable increases in dP/dt max. and heart rate due to F in anaesthetised dogs were substantially reduced by elimination of reflex activity.

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A COMPARISON OF THE RESPONSES TO VASODILATOR DRUGS IN HUMAN ISOLATED MESENTERIC ARTERIES

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Vasodilator drugs are being used increasingly in the treatment of hypertension, myocardial ischemia and heart failure. These drugs are believed to act on various distinct processes that control contraction, but no comparitive study has yet been made using human isolated arteries.

Mesenteric arteries were obtained fresh from resected bowel specimens. Four arterial ring segments were dissected (length 3-5mm, diameter 1-2mm) and suspended in Krebs solution gassed with 95%O_2 and 5%CO_2, at 37°C, under 1g applied tension and equilibrated for 60mins. The tissues were contracted with 80mM KCl and allowed to reach a plateau phase then the vasodilators were added in a cummulative manner to three of the tissues, the fourth being left as a time control. In addition the inhibition of a 10μM noradrenaline response was examined by the addition of the vasodilator to the bathing medium 15mins before the next dose of noradrenaline.

No relaxation was seen with isoprenaline (< 10^{-4}M), indicating that the human mesenteric artery is devoid of β-adrenoceptors. Acetylcholine (< 10^{-5}M) had no effect or caused an extremely weak relaxation although substance P (10^{-8}-10^{-6}M) gave an endothelium dependant relaxation. Hydralazine (< 10^{-3}M) had no effect, although in vivo it produces a large fall in blood pressure. This suggests that hydralazine either has a specific vasodilator action on a different vascular bed or that it acts indirectly through a metabolite or the release of a mediator. Relaxation of KCl and NA contractions (measured as the EC50 concentrations and maximum relaxation produced) produced by nifedipine (a calcium channel blocker), sodium nitroprusside (SNP) , isosorbide dinitrate and BRL34915 (cromakalim -a potassium channel activator) are given in Table 1.

Table 1 (n=4-10)

	80mM KCl		10^{-5}M NA	
	EC50(uM)	% max inhib	EC50(uM)	% max inhib
SNP	0.15±0.15	87±11	3.2±2.5	85±12
Nifedipine	0.008±0.008	93±10	2.9±2.6	56±16
dinitrate	1.2±0.8	79±21	54±78	64±19
BRL34915	0.39±0.25	62±7	0.12±0.1	75±8

The ratios of the EC50's for KCl to NA are: nifedipine 362, BRL34915 0.3, dinitrate 45 and SNP 21. The high ratio for nifedipine is consistent with selective inhibition of the KCl contraction through blocking the influx of calcium ions when the muscle is depolarised, whereas NA can still mediate a contration by mobilising intracellular calcium ions. BRL34915 has the opposite selectivity profile, consistent with activation of Ca-dependant K channels and therefore causing only weak inhibition of the depolarised muscle. The addition of haemolysate to the bathing medium had no effect on nifedipine or BRL34915 but it attenuated the relaxation to SNP and dinitrate, consistent with an action of the latter two agents through cyclic GMP.

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COMPARISON OF THE PURINERGIC CONTRIBUTION TO SYMPATHETIC VASOCONSTRICTION IN SHR AND WKY RAT ISOLATED TAIL ARTERIES

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The vasoconstrictor response of the rat isolated tail artery to sympathetic nerve stimulation is considerably greater in the spontaneously hypertensive rat (SHR) than in the normotensive Wistar-Kyoto control (WKY). This may be due in part to an increased contribution to the nerve mediated vasoconstriction by adenosine 5'-triphosphate (ATP), which is thought to be a cotransmitter with noradrenaline in the sympathetic nerves innervating this artery (Sneddon & Burnstock, 1984; Vidal et al., 1986). We have tested this proposal by comparing the pharmacological properties of isolated segments of tail artery from SHR and WKY animals.

The systolic blood pressures of 17-20 week old SHR and WKY rats were measured by the tail-cuff method and animals were used for experiments when, on three separate occasions, the values were over 190mmHg for SHR and below 150mmHg for WKY. Ring segments of tail artery, 2-4mm in length, had the endothelium removed by gentle rubbing and were mounted on two parallel steel wires in a 2.5ml organ bath containing Krebs-Henseleit solution bubbled with 95% O_2/ 5% CO_2, maintained at 36.5+0.5°C. Nerve stimulation was induced using square wave pulses (0.2ms pulse width at supramaximal voltage) delivered via two parallel platinum wires, one on either side of the tissue. These responses were abolished by tetrodotoxin ($3x10^{-7}$M) or guanethidine (10^{-5}M) indicating that the responses were mediated by sympathetic nerves.

At all frequencies of stimulation investigated (single pulse - 32Hz) responses of the arteries from SHR were significantly greater than those from WKY. A maximally effective concentration of prazosin, 10^{-6}M, blocked responses from SHR and WKY to approximately the same degree, 70 - 80%. Subsequent desensitisation of P_2-purinoceptors using α,β-methylene ATP ($2.5x10^{-6}$M) practically abolished nerve mediated responses in both SHR and WKY animals.

Concentration-effect curves to exogenously applied noradrenaline (NA), ATP, α,β-methylene ATP and KCl were compared in SHR and WKY tail arteries. In each case the maximum response to the agonist was 3-5 times greater in the SHR than the WKY.

From these results we conclude that ATP does not play an enhanced role in sympathetic vasoconstriction in the tail artery from SHR as compared to WKY, nor is the smooth muscle of the SHR tail artery particularly sensitive to P_2-purinoceptor agonists.

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