EFFECT OF CROMAKALIM ON CHOLINERGIC NEUROTRANSMISSION IN THE GUINEA-PIG TRACHEA

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In the airways, transmitter release from postganglionic cholinergic nerve terminals is modulated by pre-junctional inhibitory muscarinic autoreceptors (Fryer & Maclagan, 1984). Antagonist potencies at these receptors suggest that they may resemble atrial muscarinic receptors, which are directly coupled to potassium (K^+)-channels. Other studies have shown that drugs which block K^+-channels increase transmitter output from many autonomic nerves (Bowman, 1982). Therefore, drugs which open K^+-channels may reduce transmitter release from parasympathetic nerves innervating airway smooth muscle.

We have examined the effects of the K^+-channel activator cromakalim (BRL 34915) in the isolated, innervated guinea-pig trachea. The trachea was set up with vagi and recurrent laryngeal nerves attached (adapted from Blackman & McCaig, 1983) in oxygenated Kreb's solution containing 5μM indomethacin. Pre-ganglionic supramaximal stimulation of both vagi (30Hz, 0.2ms, 5s) caused an increase in intraluminal pressure (ILP) due to contraction of the trachealis muscle. Cumulative addition of cromakalim (0.1-6.4 μM) caused a slowly-developing, concentration-dependent reduction in response to nerve stimulation. However, the reduction in nerve-induced response never exceeded 65 ± 3% even at the highest dose of cromakalim used (n = 6). The effect of cromakalim was not altered by reducing the stimulation frequency to 5Hz, nor by stimulating at submaximal voltages.

Addition of 2μM cromakalim caused a slight shift to the right of the cumulative dose-response curves to exogenously applied acetylcholine (Ach) or histamine, confirming the results of Allen et al (1986). Slight inhibition by cromakalim (2μM) was also obtained when single doses of Ach (10μM) were applied for short (30s) periods (31 ± 2% reduction; n = 3).

Since the inhibition of nerve-induced responses was much greater than the inhibition of responses produced by applied Ach, these results suggest that this effect of cromakalim may be due, in part, to pre-junctional inhibition of transmitter release. However, the relative importance of the pre- and post-junctional effects is not yet known.

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RENAL HAEMODYNAMIC EFFECTS OF CROMAKALIM, NIFEDIPINE AND PINACIDIL IN THE CONSCIOUS CAT

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The antihypertensive agent cromakalim was shown to increase mean renal blood flow (MRBF) in the anaesthetised cat (Buckingham et al, 1986). More recently this activity was confirmed in the conscious cat using chronically implanted doppler flow probes (Clapham & Buckingham, 1988). This investigation extends these studies and compares, in the conscious cat, the effects of cromakalim on MRBF and plasma renin activity (PRA) with those of nifedipine and pinacidil.

Male cats were prepared for measurement of arterial pressure and MRBF as previously described (Clapham & Buckingham, 1988). On experimental days the cats were set up for continuous recording 1 h prior to and for 5 h after oral dosing with lactose (10 mg/kg), cromakalim (0.015, 0.03 or 0.06 mg/kg), nifedipine (0.15, 0.3 or 0.6 mg/kg) or pinacidil (0.3, 0.6 or 1.2 mg/kg). Blood samples (1 ml) were taken -0.5, 2 & 4 h post dose for assay of PRA by standard radioimmunoassay. Results are given as means ± sem (n=5).

Cromakalim (0.03 mg/kg), nifedipine (0.6 mg/kg) and pinacidil (0.6 mg/kg) elicited similar falls in mean arterial pressure (MAP). Despite the similarity of blood pressure lowering activity, the drugs had differing effects on heart rate (HR), PRA and MRBF:

Table 1. Changes in MAP, HR, MRBF and PRA.

	lactose	cromakalim (0.03mg/kg)	nifedipine (0.6mg/kg)	pinacidil (0.6 mg/kg)
MAP	-9±5	-28±3**	-29±4**	-30±7**
HR	-7±4	+3±6	+24±7**	+18±6*
MRBF	-6±3	+40±7*	+1±10	+1±8
PRA	0.23±0.24	1.46±0.48	2.65±0.38**	2.16±0.72**

Values were measured 2 h post dose. MAP, HR & MRBF are % change from predose. PRA is ngAI/h/ml of plasma. *=$p<0.05$ and **=$p<0.01$ (Dunnett's test for multiple comparisons). Between group predose values were not significantly different.

All doses of cromakalim significantly ($p<0.05$) increased MRBF, the maximum effect occurring 1.5-2 h post dose. After 3 h MRBF subsided towards predose levels despite a maintained reduction in MAP. Little or no change in MRBF occurred following either nifedipine (0.15-0.6 mg/kg) or pinacidil (0.3-1.2 mg/kg).

These findings show that, at submaximal equihypotensive doses, cromakalim causes less reflex stimulation of HR and PRA than nifedipine and pinacidil. This is consistent with the finding that cromakalim, but not pinacidil, potentiates reflex bradycardia to phenylephrine in the anaesthetised cat (Clapham & Cooper, 1988). Furthermore, of the three agents, only cromakalim significantly increased MRBF.

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COMPARISON OF THE EFFECTS OF CROMAKALIM (BRL 34915) AND PINACIDIL IN GUINEA-PIG MODELS OF BRONCHOCONSTRICTION

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The observation that cromakalim was able to relax vascular smooth muscle by activating potassium channels, leading to hyperpolarisation of the plasma membrane, (Hamilton et al, 1986) has resulted in the identification of several other structurally distinct compounds sharing a similar mechanism of action (Weston and Abbott, 1987). One compound in particular, pinacidil, has been extensively evaluated (Cohen, 1986) and was recently compared with cromakalim for its relaxant effects on the vasculature (Cook et al, 1988). Cromakalim relaxes airway as well as vascular smooth muscle (Buckle et al, 1987) and in this communication we describe a comparison of cromakalim with pinacidil in guinea-pig models of bronchoconstriction.

In the anaesthetised guinea-pig preparation of Konzett and Rossler, both cromakalim (10-300μg kg^{-1}) and pinacidil (100-1000μg kg^{-1}), given i.v., produced a dose-related inhibition of the bronchoconstrictor effects of an ED_{60} dose level of 5-HT (3.3-5.2μg kg^{-1} of free base given as the creatinine salt), the maximal effect being on the 5-HT challenge that was given 1 or 6 min after compound administration. The ED_{50} values were 84 (61-114)μg kg^{-1}, n = 17, for cromakalim and 562 (354-903)μg kg^{-1}, n = 5, for pinacidil, indicating some 6- to 7- fold greater potency for cromakalim.

In conscious guinea-pigs, both cromakalim and pinacidil inhibited the bronchoconstriction induced by a 20 s exposure to an aerosol of 5 x 10^{-3}M histamine when administered orally at various times prior to challenge. The time for maximum effect was 1 h for cromakalim and 30 min for pinacidil. At these optimal times, a dose of 2.5mg kg^{-1} of cromakalim and 12.5mg kg^{-1} of pinacidil protected approximately half of the animals from respiratory embarrassment during the 4 min observation period.

Cromakalim (1-20mg kg^{-1}, p.o.) and pinacidil (5-20mg kg^{-1}, p.o.), when administered at 1 h and 30 min before challenge respectively, similarly inhibited antigen-induced dyspnoea in sensitised conscious guinea-pigs exposed to an aerosol of ovalbumin (50mg ml^{-1} for 10 min) (Dawson and Sweatman, 1980). A dose of 5mg kg^{-1} of cromakalim and 15mg kg^{-1} of pinacidil protected approximately half of the animals from dyspnoea during the 10 min observation period.

It is concluded that cromakalim and pinacidil, two compounds capable of activating potassium channels, are able to inhibit bronchoconstriction in animal models believed to be relevant to asthma. Cromakalim has a long plasma half-life in man (ca 24h; Davies et al, 1988), which suggests that it may be of value in the treatment of nocturnal asthma. In contrast, the plasma half-life of pinacidil is only 2h (Eilertsen et al, 1982).

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CROMAKALIM INHIBITS CHOLINERGIC-MEDIATED RESPONSES IN HUMAN ISOLATED BRONCHIOLES BUT NOT IN GUINEA-PIG AIRWAYS

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Cromakalim (BRL34915) is a potassium channel activator (Hamilton, et al, 1986) which has been reported as having potential as a bronchodilator in the treatment of asthma. Thus, cromakalim inhibits spontaneous tone in guinea-pig trachea (Allen, et al, 1986; Buckle, et al, 1987) and prevents bronchospasm in vivo both in the guinea-pig (Buckle, et al, 1987; Arch, et al, 1988) and in man (Baird, et al, 1988). However, Allen, et al (1986) reported that cromakalim had little inhibitory effect against acetylcholine-induced tone in the guinea-pig trachea. In the present study, the effects of cromakalim have been examined against responses induced by cholinergic and other agonists both in vitro and in vivo in the guinea-pig and in human isolated bronchioles.

In guinea-pig tracheal spirals, cromakalim markedly inhibited tone induced by various spasmogens except carbachol (Table).

Table IC_{50}(μM) and intrinsic activity (in parentheses) relative to a isoprenaline (10^{-3}M) maximum relaxation.

	Spontaneous Tone	PGE_2 (10nM)	LTD_4 (3μM)	Histamine (5μM)	5-HT (0.8μM)	Carbachol (0.3μM-Guinea -pig)(5μM-Human)
Guinea-pig Trachea	1.1 (0.89)	0.9 (0.93)	1.2 (0.87)	3.2 (0.77)	0.53 (0.93)	>100 (0.21)
Human Bronchioles	0.35 (0.93)	-	-	0.57 (0.93)	-	1.96 (0.82)

In the guinea-pig Konzett-Rossler preparation cromakalim inhibited both 5-HT-(ID_{50} μgkg^{-1} 84(61-114), n=17) and histamine-($ID_{50}\mu gkg^{-1}$ 123(61-250, n=5) induced bronchospasm whereas up to a dose of $1mgkg^{-1}$ it caused no inhibition of an acetylcholine-induced spasm (n=6). Cromakalim was a more potent inhibitor of spontaneous and histamine-induced tension in human isolated bronchioles than in guinea-pig tracheal spirals. Furthermore, in the human tissue it had a marked effect on carbachol-induced tension (Table).

In conclusion, these results suggest that in the guinea-pig but not in man, cholinergic-induced responses involve a different mechanism from those of other spasmogens. The ability of cromakalim to inhibit tone induced by a variety of spasmogens suggests this compound should be a useful bronchodilator in the treatment of asthma.

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EFFECTS OF 8-PT AND DIPYRIDAMOLE ON BRONCHOCONSTRICTOR AND RELAXANT RESPONSES TO ADENOSINE

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The bronchodilator effects of adenosine upon isolated guinea-pig trachea is well documented (Jones et al. 1980; Darmani & Broadley 1986). Constrictor responses, however, are rarely seen in the trachea, although they have been reported when adenosine is administered to asthmatic patients (Cushley et al. 1983) and in perfused lungs taken from sensitized guinea-pigs (Thorne & Broadley 1988). Both the tracheal relaxation (Darmani & Broadley 1986) and bronchoconstriction in asthmatics (Mann & Holgate 1985) are inhibited by the methylxanthine, theophylline. The present study therefore investigates the effect of 8-phenyltheophylline (8-PT) and the adenosine transport inhibitor, dipyridamole, upon the bronchoconstriction induced by adenosine in perfused lungs, and the responses of the trachea both from sensitized guinea-pigs.

Guinea-pigs (400-550g) were sensitized to ovalbumin (OA) by i.p. injections 14 (5mg in 0.1ml water for injection) and 12 days (10mg) before killing. The lungs were bisected at the bifurcation and each half perfused at 5ml min^{-1} via the airways with warmed (37.5°C) Krebs-bicarbonate solution gassed with 5% CO_2:95%O_2. Perfusion pressure was recorded by means of a pressure transducer at the bronchial cannula. One half served as the control while the other was perfused with antagonist throughout. Agonists were administered as a bolus in the following order; carbachol (10µg), adenosine (300µg) and OA (0.5µg). 8-PT (3.9µM, n=6) did not significantly (P>0.05) affect either the adenosine- (control 12.8±4.5, 8-PT 17.8±7.0mm Hg) or carbachol-induced constriction. However, the response to antigen (OA) challenge was significantly (P<0.05) reduced in the 8-PT-treated halves (control 28.8±6.9; 8-PT 10.8± 4.2mmHg). Dipyridamole (2µM, n=6) virtually abolished the response to adenosine (0.2±0.2mmHg) compared with paired controls (7.0±1.4mmHg).

Since 8-PT failed to antagonize the bronchoconstrictor response to adenosine of the lungs, its effect upon the sensitized trachea was examined firstly to establish antagonism of the relaxant response and secondly in the hope of revealing a constrictor response. Tracheal spirals (3-4cm) were set up under a resting tension of 10mN and after intrinsic tone had developed, a cumulative concentration-response curve to adenosine was established. At the maximum, isoprenaline (5.4µM) was added and changes in tension expressed as % of this relaxation. In the control tissues, a dose-related relaxation occurred. In the presence of 8-PT (3.9µM), adenosine caused an initial contraction of 4.3±2.3% at the 25µM dose and the curve was significantly displaced to the right from an EC_{30} of 177 (97.1-322)µM to 362 (292-451) µM. In the presence of dipyridamole (2µM), there was a significant reduction of the maximum relaxation from 86.8±3.2 to 60.3±3.3% and leftward shift of the curve to an EC_{30} of 3.6 (1.9-6.6)µM. With 8-PT and dipyridamole, the relaxation curve was significantly shifted to the right (EC_{30}, 51.1 (27.8-94.1)µM) compared with dipyridamole alone, but the early contraction again occurred.

The contractile response of the lung to adenosine is probably mediated via an intracellular purinoceptor since it is unaffected by 8-PT and blocked by the transport inhibitor. Although relaxation still predominates in sensitized tracheas, 8-PT reveals a contraction but this does not appear to be removed by concomitant presence of dipyridamole.

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CHARACTERIZATION OF THE MUSCARINIC RECEPTOR SUBTYPE INVOLVED IN CONTRACTION OF BOVINE TRACHEAL SMOOTH MUSCLE

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The cardioselective muscarinic antagonist, AF-DX 116 has been shown to bind to two populations of muscarinic receptor in bovine tracheal smooth muscle (Roffel et al 1987). Approximately 75% of these binding sites are of the cardiac subtype having high affinity for AF-DX 116. We have attempted to characterise the muscarinic receptor mediating contraction of bovine tracheal smooth muscle and to correlate this with receptor sites identified in ligand binding studies.

Fresh tracheas from adult cows were rapidly dissected to remove epithelium and connective tissue. The membrane fraction was prepared by homogenisation and differential centrifugation in phosphate buffered saline, pH 7.4, containing 1mM phenylmethyl-sulphonyl fluoride (PBS/PMSF). Antagonist affinity was assessed by displacement of 1nM [^{3}H] N-methylscopolamine from membranes incubated at 30^{o}C for 50 minutes in PBS/PMSF. Data were analysed by non linear least squares regression. For functional studies, smooth muscle strips (0.5mm x 5mm) were suspended under 0.5 - 1gm tension in aerated (95% O_2/5% CO_2) Krebs-Henseleit solution, containing 1uM indomethacin, at 30^{o}C. Cumulative concentration-response curves to methacholine were established and repeated following 30 minutes incubation with antagonist. pA_2 values were derived by Arunlakshana-Schild analysis.

pKi values ± s.e. mean (n=4-6) and pA_2 values ± s.e.mean (n=8-10) are shown in the table. Slopes of the Schild plots did not differ significantly from unity.

Antagonists	pA_2	pKi	pKi(site 1%)	pKi(site 2%)
Atropine	8.98 ± 0.1	8.70 ± 0.09		
Pirenzepine	6.78 ± 0.13	6.18 ± 0.04		
4-DAMP	8.87 ± 0.07	7.80 ± 0.03		
AF-DX 116	6.40 ± 0.14		6.86 ± 0.06	5.42 ± 0.1
			(72 ± 5)	(28 ± 5)
Methoctramine	5.3 ± 0.11		7.29 ± 0.08	5.22 ± 0.08
			(75 ± 2)	(25 ± 2)

Competition binding curves for AF-DX 116 and methoctramine fitted a 2 site model and revealed the presence of a large proportion (ca. 75%) of cardiac type muscarinic binding sites. These data are consistent with other reported binding data in bovine trachea and guinea-pig ileum (Giraldo et al 1987; Michel and Whiting 1987; Roffel et al 1987). Additionally our binding studies with 4-DAMP identify an apparently homogeneous population of cardiac type binding sites.

The binding and functional data for pirenzepine show that the bovine tracheal receptor is not of the high pirenzepine affinity M1 subtype. The other pA_2 values are consistent with those published for the M2 smooth muscle subtype (Eglen and Whiting 1986).

In conclusion the functional muscarinic receptor mediating contraction of the bovine trachealis has been identified as being of the smooth muscle rather than the cardiac subtype. Ligand binding studies identify a high proportion of cardiac type receptors with no currently known functional role.

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INDOMETHACIN-INDUCED GASTRIC MUCOSAL EROSION FORMATION IS PREVENTED BY PUTATIVE PHOSPHOLIPASE A_2 (PLA_2) INHIBITORS

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Evidence from previous studies would suggest that certain products of phospholipid metabolism are involved in the formation of gastric damage due to ethanol (Peskar et al, 1986; Berry et al, 1988) or endotoxin (Wallace et al, 1987). We now describe experiments, with compounds reported to interact with the PLA_2/phospholipid system (Blackwell et al, 1978), on indomethacin-induced gastric mucosal damage in the rat.

Drugs were administered orally in 0.5% methyl cellulose to male Wistar rats (200-250g, n=6; fasted 18h) using the following dose ranges: mepacrine (Mep.), chloroquine (Clq.), chlorpromazine (Clp.) & chloracysine (Cla.), 10-100 mg/kg or dexamethasone (Dex.) 0.05-5 mg/kg. All drugs were dosed 0.5h prior to indomethacin (30 mg/kg s.c.) except Dex. which was pre-dosed by 1h. The animals were killed 4h after dosing with indomethacin and mucosal damage was estimated (on a 0-7 basis) by an independent observer. In a second group of experiments Clq., or Cla. was tested on PLA_2 (Naja naja)-induced 6-keto-$PGF_{1\alpha}$ production in gastric mucosal sections (Melarange & Rashbrook, 1986). In a third group mucosal LTB_4 was measured ex vivo by radioimmunoassay at various times after indomethacin (30 mg/kg s.c.). Results were analysed using Student's 't' test or the Mann-Whitney U test.

Dex. inhibited damage (93%, $p<0.001$ at 5 mg/kg) whereas Mep., Clp. or Cla. were as active but less potent (100 mg/kg produced 96%, 94% & 94% inhibition respectively, $p<0.01$). Clq. at 100 mg/kg produced only 32% ($p>0.05$). Experiments in vitro showed that PLA_2 induced a 4.6-fold increase in 6-keto-$PGF_{1\alpha}$ production (from 9.7±1.0 to 45.1±11.6 ng/section, $p<0.01$). Clq. (100 μM) or Cla. (100 μM) alone failed to alter basal 6-keto-$PGF_{1\alpha}$ production (both $p>0.05$); however at these concentrations Clq. or Cla. in the presence of PLA_2 inhibited the increase in production by 51% ($p<0.05$) and 100% ($p>0.01$) respectively. Control amounts of LTB_4 (104±3.0 pg/section) were not altered by indomethacin, 0.5h (93±7.0 pg/section, $p>0.05$), 2h (97±7.0 pg/section, $p>0.05$) or 4h (89±13.0, $p>0.05$) after dosing.

The results suggest that a correlation exists between inhibition of PLA_2 activity and prevention of damage. Mep., which was found to inhibit PLA_2 (Melarange & Rashbrook, 1986), was more potent than Clq. in this system and also in preventing damage. Similarly, Cla., a chlorpromazine analogue, inhibited both PLA_2 and damage. Chlorpromazine may owe its protective effects, in part, to PLA_2 inhibition but other activities such as α-adrenoceptor and muscarinic receptor blockade should be considered. Results obtained with Dex. would suggest that endogenous lipocortin contributes to the protective effects of this steroid. However, the observation that LTB_4 was not increased during indomethacin-induced damage implicates other agents, such as PAF, that are susceptible to PLA_2 inhibition.

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PLATELET ACTIVATING FACTOR INDUCED CHANGES IN VASCULAR PERMEABILITY: THE EFFECT OF MAST CELL DEPLETION

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Platelet activating factor (PAF) has previously been shown to induce increased vascular permeability in man, guinea-pigs, rabbits and rats[1]. In rats, intradermal injections of as little as 0.04pmol induces increased plasma protein extravasation; guinea-pigs are more sensitive.

We have examined the effect of mast cell depletion with compound 48/80 on increased vascular permeability induced by PAF and other mediators in rat skin. We have also examined the effect of the specific PAF antagonist BN52021 on increased vascular permeability induced by these same mediators.

Outbred male Wistar rats were used throughout this study. The abdominal surface of the animals were shaved and the animals given an intravenous injection of 0.2ml/100g body weight of a 0.5% solution of Evans Blue in saline. Intradermal injections of PAF 1-20ng/site, histamine 1.2-10ug/site, 5HT 15-120ng/site, bradykinin 0.3-2.0ug/site and PGE1 1.3-10ng/site, in 0.1 ml sterile saline on the abdominal surface were given according to a latin square design. 30 min later the animals were killed by cervical dislocation under ether anesthesia. Following the extraction of the Evans Blue dye from each site with formamide, the extent of increased vascular permeability was assessed spectrophoto-metrically. Mast cells were degranulated with a series of daily i.p. injections of compound 48/80, the dose increasing each day from 100 to 1000ug, over a period of one week. Animals dosed with BN52021 were given a single oral dose of 20mg/kg 1 hour prior to skin testing. Skin biopsies from intradermal injection sites were taken from a number of animals for histological examination. Results are expressed as ug Evans Blue/ injection site ± s.e.mean.

All of the mediators used gave dose related increases in vascular permeability over the dose range used. PAF (5-80ng) and PGE1 (1.25-10ng) were the most potent mediators, 5HT (15-240ng) and bradykinin (0.25-2ug) were less effective, histamine (1.25-20ug) was the least potent.

	Mediator					
Treatment	48/80 (1ug)	PAF (40ng)	PGE1 (10ng)	5HT (120ng)	Brady (2.0ug	Histamine (10ug)
Control	8.4±1.2	8.7±1.0	8.0±0.9	7.9±0.6	8.3±1.3	7.7±0.4
48/80	0.6±0.1*	8.9±0.8	7.8±0.7	8.4±1.4	7.9±0.7	7.8±0.6
BN52021	8.0±1.8	0.3±0.1*	7.0±0.9	8.0±0.9	8.1±1.0	8.0±1.1

Table 1. The effect of 48/80 and BN52021 treatment on Evans Blue dye leakage induced by the intradermal injection of the above mediators in 0.1ml saline. Values expressed as ug Evans Blue ± s.e.mean per injection site. Control injections of saline only. * = P<0.001 as compared to control, n=6

Compound 48/80 pretreatment did not affect vascular permeability induced by any of the mediators studied. Mast cell depletion was confirmed in skin sections stained with toluidine blue and by the intradermal injection of compound 48/80 (Table 1). BN52021 treatment totally attenuated the skin response to PAF but did not cause any alteration in the responses to the other mediators (Table 1).

These results demonstrate that the increased vascular permeability induced by intradermal injections of PAF are independent of mast cell derived vasoactive amines and that the "classical" mediators of vascular permeability do not act through a mechanism involving PAF. These results also demonstrate further the specificity of the PAF antagonist BN52021.

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S-ADENOSYL-L-METHIONINE INHIBITS RAT POLYMORPHONUCLEAR LEUKOCYTE PHAGOCYTOSIS AND OXIDATIVE METABOLISM IN VITRO

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S-Adenosyl-l-methionine (SAMe) shows antiinflammatory and analgesic action (Gualano et al 1983). Open and double blind clinical trials have indicated that SAMe stabilised as its sulphate-p-toluenesulphonate derivative has an efficacy comparable to that of ketoprofen and ibuprofen in patients with osteoarthritis (Capretto et al 1985). SAMe is a physiologic molecule involved in transmethylation, aminopropylation and transsulphation reactions. In this study we have investigated the effect of SAMe on the phagocytotic ability and concomitant oxidative metabolism of the rat neutrophil in vitro.

Rat neutrophils were elicited by 12% sodium caseinate injected intraperitoneally and isolated by centrifugation after peritoneal lavage with heparinised Hanks balanced salt solution (HBSS). After washing with HBSS the crude cell preparation was purified by separation on a Ficoll Hypaque gradient. Aliquots of 10^7 neutrophils were suspended in 2ml of HBSS buffered with 50 mM HEPES pH 7.4 and preincubated at 37°C for 15 minutes in the presence or absence of SAMe (10^{-2}- 10^{-6}M). Aliquots of 100 µl were taken and tested for active oxygen species production stimulated by 10^{-6}M FMLP using luminol enhanced chemiluminesence (De Chatelet 1982). Phagocytosis was assessed by a modified version of a fluorometric assay (Oda & Maeda 1986) involving the uptake of 1.1 µm fluorescent latex particles. All results are presented as % control response (controls adjusted to 100%). Statistical analysis was by Student's 't' test.

Table 1 Effect of SAMe on phagocytosis & chemiluminescence

SAMe [M]	% Particle uptake	% Chemiluminescence (mV per 10^5 cells)
10^{-2}	41.6 ± 5.6 ***	25.4 ± 8.4 ***
10^{-3}	66.1 ± 9.1 **	60.3 ± 17.5 *
10^{-4}	96.3 ± 16.3	69.9 ± 5.9 **
10^{-5}	80.4 ± 11.3	110.2 ± 18.1
10^{-6}	90.9 ± 12.3	118.6 ± 23.1

Means ± SEM (n=6) * P< 0.025 ** P< 0.005 *** P< 0.001

The results (Table 1) show that SAMe inhibited both particle uptake and active oxygen species output by rat neutrophils. Experiments with the SAMe metabolites S-Adenosyl-l-homocysteine (SAHc) and methionine show that these compounds did not inhibit phagocytosis but SAHc was more effective than SAMe in inhibiting chemiluminescence at 10^{-3}M. The effect of SAMe and SAHc on neutrophils may be relevant to its antiinflammatory activity.

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INHIBITION OF THE NEUTROPENIA FOLLOWING I.V. ANTIGEN CHALLENGE OF SENSITISED GUINEA-PIGS BY A LTB_4 ANTAGONIST

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Evidence has has been presented elsewhere that suggests the release (Lee et al 1986) and potential involvement of leukotriene (LT)B_4 in the neutropenic response following i.v. antigen challenge of actively sensitised guinea pigs (Walker & Woodhouse, to be published). The present study has shown that a selective LTB_4 antagonist also inhibits the neutropenia following i.v. antigen challenge. LY223980, 5-(3-carboxybenzoyl)-2-((6-(methoxylphenyl)hexyl)oxy) benzeneproprionic acid) has been shown to be selective for LTB_4. This has been accomplished both in vitro, using a filter chemotaxis assay, and in-vivo studying the thoracic accumulation of In-111 tropolonate radiolabelled neutrophils.

In-vitro LY223980 had no significant effect on the in-vitro submaximal neutrophil responses to either PAF, f-met-leu-phe or zymosan activated serum. However the response to 10^{-7} M LTB_4 was inhibited in a dose-related fashion, providing 88% inhibition at 10^{-5} M LY233980.

Similarly, the compound had no effect on submaximal in vivo responses induced by PAF, f-met-leu-phe or the injection of endotoxin (resulting in the systemic activation of C5) at doses of up to 10 mg/kg i.v. However, the thoracic cell accumulation in response to LTB_4 (0.1 µg/kg i.v.), a reflection of peripheral neutropenia (Sweatman et al 1987), was inhibited by 34.1% and 88.4% at 5 and 10 mg/kg i.v. respectively. LY223980 (10 mg/kg i.v.) given immediately before i.v. antigen challenge resulted in a suppression of thoracic radiolabel accumulation of between 40 and 50% over a 20 min observation period. These results provide further evidence for a role for LTB_4 in the immediate neutropenic response which occurs following i.v. antigen challenge.

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EFFECT OF GOSSYPOL ON ACETYLCHOLINE AND SODIUM NITROPRUSSIDE INDUCED VASODILATATION IN THE ISOLATED RAT KIDNEY

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Alheid et al., (1987), have recently described the inhibition of endothelium-dependent relaxation together with the potentiation of endothelium-dependent relaxation following treatment with gossypol. We have recently reported the results of experiments in the isolated perfused rat kidney (Burton et al., 1987) using detergents in an attempt to remove the endothelium. In the present study we have investigated the effects of gossypol on the vasodilatation of acetylcholine (ACh) and sodium nitroprusside (SNP) in order to study the role which endothelium derived relaxing factor (EDRF) may play in the control of renal vascular resistance.

Kidneys from male Wistar rats (250-300g) were removed and perfused at constant flow (4.5 $mlmin^{-1}$) with Krebs-Henseleit gassed with 95%O_2/5%CO_2 at 37°C. The basal perfusion pressure was approximately 90 mmHg. The kidneys were preconstricted with an infusion of methoxamine hydrochloride (3 μM) which increased the perfusion pressure by 100-150 mmHg and relaxed by 60-70% by infusions of ACh (0.3 μM) or SNP (1 μM). Following a 10 minute infusion of gossypol (1.5-30 μM), the kidney was perfused with Krebs-Henseleit alone for a further 30 minute period prior to repeating the vasodilator responses to ACh and SNP. Only one dose of gossypol was administered per kidney.

Renal vasodilatation induced by ACh was unaffected by gossypol (1.5 μM) but was reduced significantly at 3 μM by 54.5 ± 5.2% ($P<0.001$, n=6), at 15 μM by 67.1 ± 7.9% ($P<0.001$, n=12), and at 30 μM by 90.5 ± 7.5% ($P<0.001$), n=6). The response to SNP was unaffected at a dose of 1.5 μM and 3 μM, but significantly increased at 15 μM by 31.0 ± 6.8% ($P<0.001$, n=12), and at 30 μM by 57.4 ± 19.2% ($P<0.01$, n=6). Renal vasoconstriction induced by methoxamine was significantly reduced at 15 μM ($P<0.01$) and 30 μM ($P<0.001$) gossypol.

Selective inhibition by gossypol of an endothelium-dependent vasodilator (ACh) without inhibition of endothelium-independent vasodilatation (SNP) is consistent with the presence of EDRF mediated relaxation in the isolated perfused rat kidney. However, the inhibition by gossypol of the vasoconstrictor effect is in contrast to the observations made by Alheid et al., (1987), where no effect on the constrictor agent was seen. The potentiation of SNP induced vasodilatation by gossypol may uncover some competition between EDRF and the nitrovasodilators which as suggested by Alheid et al., (1987) may occur at the level of guanylate cyclase, although the real nature of this competition requires further investigation.

G.A. Burton is an S.E.R.C. student.

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PROSTAGLANDINS AND THE INFLAMMATORY RESPONSES TO CARRAGEENAN AND YEAST IN RAT PAWS

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Carrageenan-induced acute inflammation has been shown to be prostaglandin-dependent. Carrageenan inflammation is reduced by cyclo-oxygenase inhibitors and prostaglandin levels are elevated in inflammatory exudates. Hyperalgesia in response to subplantar injection of yeast in rats is abolished by cyclo-oxygense inhibitors and we have shown an association between the development of hyperalgesia and elevation of paw exudate prostanoids (Carey and Haworth, 1985). We now describe a comparison of the contribution of prostaglandins to inflammatory responses induced by subplantar injection of 1% carrageenan (Viscarin, Marine Colloids) and 20% Brewers Yeast (Boots) in rats using methods previously described (Carey and Haworth, 1985).

Immunoreactive PGE_2 and $6oxoPGF_{1\alpha}$ could not be detected in exudate from carrageenan-inflamed paws at any time up to 4h post-injection ($<0.5ngml^{-1}$ and $<0.3ngml^{-1}$ respectively). However, indomethacin inhibited carrageenan paw oedema to a maximum of 47±4% (mean ± s.e.m., n=6) at $5mgkg^{-1}$ s.c. while injection of indomethacin subplantar with carrageenan produced a lower maximum inhibition of 27±6% (100µg). This contrasts with the inflammatory response to subplantar injection of 20% yeast which induces a similar extent of oedema in control rats. Paw exudate PGE_2 and $6oxoPGF_{1\alpha}$ were elevated to maxima of $6.0\pm1.8ngml^{-1}$ and $2.3\pm0.8ngml^{-1}$ respectively at 4h post-injection while indomethacin at doses to $20mgkg^{-1}$ p.o. had little effect on yeast oedema (maximum inhibition 15±6%).

Subplantar injection of 20% yeast induced hyperalgesia which was maximum at 4h post-injection (83±3%) and was inhibited by 89±11% in rats dosed with indomethacin ($10mgkg^{-1}$ p.o.). In contrast, 1% carrageenan alone did not induce hyperalgesia while subplantar injection of PGE_2 (3nmol) at 2h post-carrageenan injection produced a sustained hyperalgesia similar to that observed with yeast (maximum 72±9%).

These observations suggest differences in localisation of the prostaglandins involved in the inflammatory responses described. Prostaglandins formed in response to carrageenan but not yeast may act locally on precapillary arteriolar smooth muscle receptors causing vasodilatation thus enhancing plasma extravasation (Williams and Peck, 1977). This would explain the lower efficacy of indomethacin when dosed subplantar rather than parenterally and is consistent with the poorer efficacy of topically applied indomethacin which has been previously described (Elliott and Rostron, 1988). Conversely, in response to yeast but not carrageenan, prostaglandins accumulate in the extravascular fluid and sensitise peripheral nociceptors causing hyperalgesia.

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A POSSIBLE RELATIONSHIP BETWEEN THE ADRENERGIC β RECEPTOR AND PROSTACYCLIN SYNTHESIS

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In previous experiments we have observed that propranolol and other adrenergic beta blockers induced changes of the vascular permeability(Toscano Rico & Gião T.Rico 1980) and the blood pressure (Durão & Gião T.Rico 1977)that were inhibited by indomethacin, suggesting an interference of araquidonic acid metabolites. It seemed interesting to see if one of these compounds could be detected when those drugs were introduced directly in the vascular system. As the main metabolite produced in the endothelium is prostacyclin which has a very short half-life, we measured its metabolite, the 6-keto-PGF1alpha (6-Keto).

In all the experiments we used male albino wistar rats (250-300g).The hind quarters were perfused between the aorta and the inferior vena cava with Krebs-Henseleit solution oxygenated with 95%O2+5%CO2 and heated at 37ºC.The perfusion was made by means of a peristaltic pump (2ml/mn).The efluent was collected during successive periods of 10 min., till 30 min.. The drugs were added to the perfusion fluid after collecting the first (basal) sample(except in control group).The drugs used were:dl-; l-; and d- propranolol (dl-;l-; and d-Prop.): 0.33 uM; atenolol (Aten.) 0.37 uM; isoprenaline (Isop.) 1.6 uM; salbutamol (Salb.) 2.1 uM; acetyl salicilic acid (ASA) 990 uM. The 6-Keto-PGF1alfa was assayed by H.P.L.C. (Whorton et al.1979) after deproteinization of the effluent.The results are expressed as mean±s.e.of the mean of the areas under the curve that represents the changes (in % of the initial value) of the production of 6-Keto along the time.

	AREAS	P Contr.		AREAS	P Contr.	P dl-Prop
Controls	-414.76±104.43(7)		dl-Prop+ASA	-2500.00±00.00(3)	<0.001	<0.001
dl-Prop.	+881.69±209.80(6)	<0.0001	dl-Prop+Salb	-614.72±88.86(5)	N.S.	<0.001
l-Prop.	+856.54±183.08(4)	<0.0001	dl-Prop+Isop	-605.97±37.00(3)	N.S.	<0.001
d-Prop.	-318.75± 96.52(5)	N.S.	Salb.	-564.25±109.7(6)	N.S.	<0.001
Atenolol	1063.02±332.79(7)	<0.0001				

The basal production of the 6-Keto decreased along the time thus the area of the curve is negative. The dl-Prop. and the l-Prop. both stimulated the production of the 6-Keto, while the d-Prop. is inactive. The isoprenaline and the salbutamol alone both decrease the basal production of 6-Keto to levels below the control values, and inhibit the response to the dl-propranolol. The acetyl salicilic acid in the Krebs solution completely blocks the response to the dl-propranolol. These results show that the dl-, and l-propranolol both increase the production of a metabolite of prostacyclin in the perfused rat hindquarters. As d-Prop. is inactive and salbutamol inhibits the response to dl-Prop., the increase of the production of 6-Keto seems to be somehow related to the adrenergic beta receptor.

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EVIDENCE FOR THE PGE RECEPTOR SUBTYPE MEDIATING INHIBITION OF ACID SECRETION IN THE RAT

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We have previously reported (Reeves & Stables, 1985) that the inhibitory effects of a number of prostanoids on histamine-induced acid secretion in rat isolated gastric mucosa (RGM) are mediated by prostaglandin E_2-sensitive (EP) receptors (Kennedy et al., 1982). Recently Coleman et al (1987) suggested that three subtypes of EP receptor mediate the effects of prostanoids on smooth muscle preparations, and designated the prostanoid receptor mediating inhibition of electrically-induced twitches of guinea-pig vas deferens (GPVD) as an EP_3 subtype.

We have investigated the EP receptor subtype mediating inhibition of gastric acid secretion by determining the agonist potencies of a range of PGE analogues on RGM, as described by Reeves & Stables (1985). Prostanoids were added to the serosal bathing solution 15min before a submaximal concentration of histamine (10μM) and % inhibition of secretion was calculated with reference to the preceding control response to histamine. Each prostanoid was tested at 3 concentrations, in 4-6 preparations per concentration. Inhibitory EC_{50} values on RGM are shown in Table 1, which also compares equipotent concentration ratios (where PGE_2=1) for the prostanoid on RGM with values obtained on GPVD by the method described by Coleman et al (1987).

Table 1 Prostanoid antisecretory EC_{50} values and equipotent concentrations

Prostanoid	EC_{50} nM (95% C.L.) on RGM	Equipotent concentration ratio RGM	GPVD
PGE_2	88 (55-140)	1	1
Enprostil	1.8 (1.0-3.0)	0.02	0.02
Sulprostone	4.5 (3.2-5.7)	0.05	0.16 (0.12-0.22)
16,16dimethylPGE$_2$	11 (7-18)	0.13	0.12 (0.06-0.24)
Misoprostol	21 (17-33)	0.24	1.0 (0.53-2.0)
Rioprostol	79 (43-160)	0.90	1.1 (0.27-4.7)
AY23626	4600 (3300-7100)	52.2	7.7 (4.8-1.2)

The rank order of potency for the prostanoids on RGM is very similar to that on GPVD, defined as an EP_3-receptor containing tissue. In addition the antisecretory potency of the EP_1/EP_3 selective prostanoid, sulprostone (Coleman et al., 1988) was not altered (EC_{50} 6.5 (4.9-11nM) in the presence of the EP_1-receptor antagonist, SC19220 (300μM). These data suggest that inhibition of acid secretion by these prostanoids in the rat gastric mucosa is mediated by EP_3 receptors.

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FURTHER EVIDENCE FOR THE EXISTENCE OF NEURONAL PROSTANOID RECEPTORS WHICH ENHANCE NEUROTRANSMISSION IN THE GUINEA-PIG ILEUM

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We have previously presented evidence suggesting that the guinea-pig isolated ileum (GPI) contains "excitatory" neuronal prostanoid receptors distinct from the EP_1-receptor which mediates smooth muscle contraction in this preparation (Poll et al. 1988). In the present study, we have attempted to further characterise the neuronal prostanoid receptor(s) in the GPI by examining the ability of various natural and synthetic prostanoids to augment cholinergically-mediated contractions obtained in response to field- stimulation.

Strips of longitudinal muscle with the myenteric plexus intact were prepared from segments of proximal GPI as described by Paton and Zar (1968), mounted in organ baths and field-stimulated as previously reported (Poll et al. 1988). All experiments were performed in the presence of indomethacin (3μM) and the EP_1-receptor blocking drug, AH6809 (10μM; Coleman et al. 1985).

PGE_1 (0.1-100nM), PGE_2 (0.3-100nM), PGI_2 (1-300nM), $PGF_{2\alpha}$ (10-10,000nM), PGD_2 (100-30,000nM) and iloprost (0.1-30nM) all caused concentration-related potentiation of field-stimulated contractions of the GPI preparation. In contrast, the stable thromboxane A_2 mimetic, U46619 was inactive at concentrations up to 30μM. EC_{50} values for potentiation by the various prostanoids are shown in the Table, along with the respective equipotent concentration ratios (ECR), expressed relative to PGE_2, the most potent naturally-occurring prostanoid.

Prostanoid	EC_{50}* nM	ECR**	n
PGE_2	3.9±0.2	1	21
PGE_1	3.2±0.6	0.7 (0.5-1.1)	4
PGI_2	4.5±0.5	1.1 (0.9-1.3)	11
$PGF_{2\alpha}$***	718±248	111 (40-314)	4
PGD_2	6080±1240	1473 (774-2802)	4
U46619	>10,000	n.d.	4
Iloprost	1.5±0.5	0.3 (0.1-0.8)	4

* EC_{50} values expressed as mean ± standard error of the mean.
** ECR values are geometric means ± 95% confidence limits.
*** Agonist curves not parallel to PGE_2 curve (ECR value valid for EC_{50} only).

From the above data, the following rank order of agonist potency was established: Iloprost > PGE_1 > PGE_2 > PGI_2 > $PGF_{2\alpha}$ > PGD_2 >> U46619=0. The high potency of PGE_1 and PGE_2 is consistent with the GPI containing excitatory neuronal receptors of the EP sub-type. In addition, the relatively high potencies of PGI_2 and the stable PGI_2 analogue, iloprost may also be indicative of the presence of neuronal IP receptors in the preparation. In summary, the data suggest that receptors of the EP and IP sub-types may be responsible for enhancing cholinergic neurotransmission in the electrically-stimulated GPI.

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THE ACTIONS OF PLATELET-ACTIVATING FACTOR ON RAT ISOLATED PERFUSED LUNGS

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Platelet-Activating Factor (PAF-acether), administered via the trachea to guinea-pig lungs, causes bronchoconstriction in conscious animals (Lefort et al 1984) and an increase in the perfusion pressure of isolated airway perfused lungs (Fitzgerald et al 1987). We have examined the effects of PAF-acether on rat lungs perfused either singly through the airways or the pulmonary artery, or double perfused through both of these simultaneously.

The tracheae of male Wistar rats (250-300g), anaesthetised with 1.6mg urethane/g body weight were cannulated. On piercing the diaphragm, artificial ventilation with air was begun at a rate of 60 breaths/min (tidal volume 2 cm^3). The lungs were flushed with 60ml of Greenberg-Bohr Buffer (GBB) (Voelkel et al 1981) via a pulmonary artery cannula. The lungs were excised from the rat and suspended by the tracheal cannula in a moist chamber at 37°C. They were then perfused with GBB containing (0.25% w/v) bovine serum albumin (GBB-BSA) at 37°C, using a Watson Marlow Constant Flow Inducer and gassed with 95% O_2, 5% CO_2. For the arterial and airway perfusions the rate was 5 ml/min and 2 ml/min through the respective cannulae. The perfusion pressure was measured using Bell Howell pressure transducers connected in front of the perfusion cannulae and was recorded on a Devices M_2 recorder.

Test agents were administered via the tracheal cannula for the airway perfusion and via the arterial cannula for other perfusions. Any artifact created by the application of the control (GBB-BSA) was subtracted from the response induced by the PAF-acether or phenylephrine. Whichever method of perfusion was used, arterial responses were measured after 0.5ml and airway responses after 0.76ml infusion of PAF-acether at 0.19 ml/min using a Harvard i.v. syringe pump. Due to desensitisation only one response to PAF-acether was examined per preparation.

PAF-acether (0.01-1.18 nmol) produced dose-dependent increases in arterial perfusion pressure of the arterial (n=6) and double perfused lungs (n=4). The maximal responses were 4.69 ± 0.46 and 3.65 ± 0.32 mmHg respectively, (results expressed as the mean ± s.e. mean). PAF-acether (0.19-2.76 nmol) raised the airway perfusion pressure to a maximum of 1.3 ± 0.11 mmHg in the joint perfusion. Phenylephrine (12.5 nmol, n=4) raised the arterial pressure of the joint perfused lungs to 5.22 ± 0.41 mmHg but did not affect the airway perfusion pressure.

The addition of PAF-acether (13.8 nmol) to the airways induced a rise in the perfusion pressure of 0.3 mmHg. Lyso PAF (0.14 µmol for the airway and double perfusion and 0.10 µmol for the artery perfusion, n=4) produced no response.

Phenylephrine, despite increasing the arterial perfusion pressure of the double perfused lungs, did not affect the airway pressure. In contrast, PAF-acether affected both perfusion pressures and its effect on the airways was greater when applied via the artery than via the trachea. In addition, the double perfused lungs were sensitive to much lower doses of PAF-acether than the airway perfused lungs. Therefore double perfusion is a very useful technique for investigating the actions of PAF-acether and other agents on rat lungs.

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THE RESPONSE OF HUMAN MYOMETRIUM AND UTERINE ARTERY TO PGE_2 IN VITRO

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The control of uterine blood flow is complex and incorporates changes in systemic blood pressure, uterine motility, vascular morphology, neuronal mechanisms and circulating or local hormone systems. Prostaglandins have been shown to be synthesized from the cyclic endoperoxide PGH_2 in arteries from both pregnant and non-pregnant donors (Wallenberg et al., 1981) and may be involved in control of uterine arterial tone. The purpose of this work is to reconcile the uterine artery response to PGE_2 with the myometrial response, in vitro.

Samples of ascending human uterine artery and myometrium (from the anterior wall of the corpus uteri) were obtained from non-pregnant pre-menopausal patients at hysterectomy. The artery was carefully cleared of connective tissue and 5mm segments were suspended in an organ bath under 1g tension in Krebs solution at 37°C. Any relaxant effects of the prostanoids were investigated in the presence of phenylephrine at a concentration that elicited 50% of the maximal response. Myometrial strips were set up as previously described (Massele and Senior 1981) and superfused with Krebs solution at a rate of 2ml min^{-1}. Histological examination of the endometrium was used to determine the stage of the menstrual cycle.

PGE_2 (10^{-6}M-10^{-4}M) evoked only concentration dependent contractions on the uterine artery; no relaxation of preparations contracted by phenylephrine was seen. Responses of the artery were not markedly affected by the stage of the menstrual cycle. On the myometrial strips PGE_2 (2.8×10^{-7}mmol-3.63×10^{-5}mmol) produced a predominantly inhibitory effect which was dose related but this period of inhibition was frequently preceded by a non-dose related increase in myometrial tension.

Study of the PGE_2 response on the myometrium is complicated by the fact that in vitro the predominant effect is one of inhibition of spontaneous activity but in vivo uterine contraction occurs - although inhibition of the non-pregnant uterus, in vivo, was observed by Toppozada et al. (1974) when PGE_2 was instilled directly into the uterine cavity. The results of this study with PGE_2 on uterine artery may indicate that the general discrepancy between myometrial responses in vitro and in vivo are due to a change in blood flow to the uterus. As PGE_2 contracts uterine artery this, in vivo, may represent a decrease in uterine blood flow resulting in a change in oxygen tension in the myometrium with a subsequent increase in myometrial tone (Akerlund, 1979). The increase in uterine activity could further compress the uterine blood vessels and so enhance the ischaemia. Such a mechanism may be involved in menstrual pain.

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A PROFILE OF ENDOTHELIN ON ISOLATED VASCULAR AND OTHER SMOOTH MUSCLE PREPARATIONS

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A potent vasoconstrictor peptide, endothelin (ET), has been isolated from cultures of porcine aortic endothelial cells (Yanagisawa et al., 1988). We have tested synthetic ET on several vascular and other smooth muscle preparations from the rabbit, dog, rat and guinea-pig.

Four spiral strips of smooth muscle denuded of endothelium from the rabbit (mesenteric artery, RbMesA; coeliac artery, RbCA; carotid artery, RbCrA; aorta, RbA; jugular vein, RbJV; mesenteric vein; RbMesV), dog (pulmonary vein, DPV; carotid artery, DCrA) and other smooth muscle preparations (guinea-pig trachea, GPT; guinea-pig ileum, GPI; rat stomach strip, RSS; rabbit duodenum, RbD) were mounted in a cascade (Vane, 1964) and superfused with Krebs' solution (37°C) containing indomethacin (5.6 μM) at 5 ml/min. Agonists such as ET (2.5-75ng), bradykinin (BK, 1-10ng), substance P (SP, 1-10ng) and angiotensin II (AII, 1-10ng) were injected over the tissues. Fig. 1 illustrates the relative sensitivities of the various preparations.

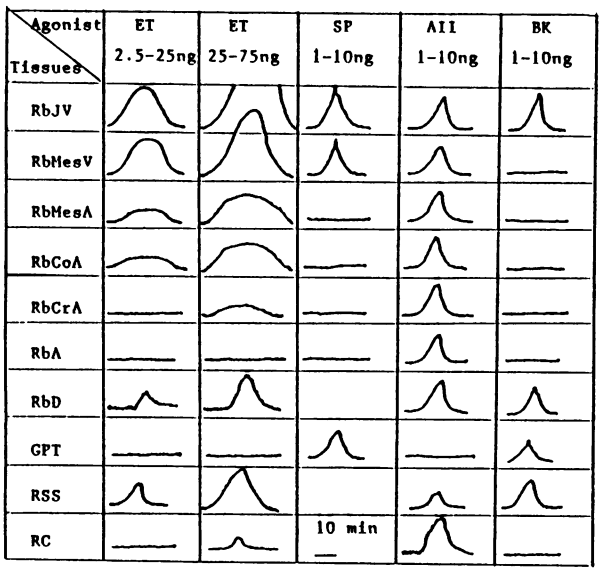

Figure 1
A representation of the contractions of several isolated smooth muscle preparations induced by ET, SP, AII and BK.

Thus, ET is more active on venous than on arterial tissues, making venous tissues the most sensitive ones for detection of ET. The RSS is also useful as a bioassay tissue for ET since the duration of the contractions induced by ET on the RSS are not so prolonged as those seen in vascular tissues.

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INTERACTION OF SELECTED VASODILATING β-BLOCKERS WITH ADRENERGIC RECEPTORS IN HUMAN CARDIOVASCULAR TISSUES

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Increased effectiveness for the treatment of hypertension has been achieved by the development of agents which combine beta-blocking activity with vasodilating properties (Wallin et al., 1987). Because the use of isolated human tissues provides data relevant to human pharmacology of drugs which produce receptor-mediated responses (Docherty et al., 1987), we designed experiments to assess the mode of interaction of new vasodilating beta-blockers (bufurarol, carvedilol, celiprolol, dilevalol, pindolol) with adrenoceptors in human cardiovascular tissues.

The affinity of these compounds for human myocardial beta adrenergic receptors was evaluated in sarcolemmal membrane preparations (125-I-pindolol binding). Interactions with alpha adrenergic receptors were studied in the human mammary artery by evaluating affinity for alpha-1 receptors as labelled by 3H-prazosin and by measuring contractile responses of vascular smooth muscle to norepinephrine. Labetalol, a compound which combines beta and alpha blocking activity in the same molecule, prazosin, an alpha-1 adrenoceptor antagonist and propranolol, a reference beta blocking agent, were used for comparison.

All the drugs examined showed high affinity for beta receptors (Ki values from 10 to 100 nM), except for celiprolol which displayed a biphasic displacement curve (pKi = 6.3 ± 0.1 for beta-1 receptors and pKi = 4.5 ± 0.2 for beta-2 receptors). The compounds differed for their interactions with alpha-1 receptors. Prazosin was the most potent competitive antagonist (pA2 = 9.97 ± 0.26). Labetalol and carvedilol showed a moderate affinity (pKi = 6.2 ± 0.14 and 6.1 ± 0.10, respectively), which was consistent with their ability to inhibit norepinephrine-induced contractions in functional studies (pA2 = 6.93 ± 0.23 and 8.64 ± 0.24, respectively). Dilevalol, bufuralol and pindolol showed weak affinity in binding studies (Ki values were in the umolar range) and poor inhibition of contractile response induced by norepinephrine (pA2 = 5.98, 5.54 and 6.23, respectively). The pA2 value for celiprolol could not be measured for lack of antagonist properties up to 100 uM.

The data indicate that the vasodilating activity of the beta-blockers examined can be ascribed in some instances to an alpha-1 blocking activity (labetalol, carvedilol), whereas for the others alternative mechanisms must be considered.

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INTERACTIONS BETWEEN 5-HYDROXYTRYPTAMINE AND ADRENERGIC AGONISTS ON THE CONTRACTILE RESPONSES OF THE DOG SAPHENOUS VEIN

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The ability of 5-Hydroxytryptamine (5-HT) in low concentrations to potentiate responses to noradrenaline (NA) and other contractile agents has been shown in many arteries. Since the veins are very reactive to 5-HT and there is no report about this potentiating effect in these tissues we decided to examine if 5-HT was able to enhance the responses to NA (an $alpha_1$ - and $alpha_2$-agonist) in the dog saphenous vein, a tissue possessing both types $alpha_1$-and $alpha_2$-adrenoceptors. In preliminary experiments we found that contractions evoked by NA were enhanced by threshold concentrations of 5-HT. To further characterize the potentiation in what concerns the $alpha_1$- and $alpha_2$-mediated responses we also studied the influence of 5-HT on responses to the $alpha_1$-agonists phenylephrine (PHE) and methoxamine (MET) and to the $alpha_2$-agonist UK-14,304 (UK).

Concentration response curves for the different agonists were obtained in vein strips (in the presence of cocaine - 12 μM and propranolol - 0.5 μM, in some experiments Ketanserin 0.1 μM was also present) in the absence and in the presence of 5-HT.

The results showed that 5-HT in a threshold concentration (0.05 μM, a concentration that produced less than 5% of maximum response to NA) caused leftward displacements of the concentration response curves to PHE, MET, NA and UK. As the concentration response curves in the presence of 5-HT converged in the higher concentration range with those in the absence of 5-HT the leftward displacements were estimated at the EC_{30} level. At this level the mean leftward shifts were: 0.291±0.030 (PHE), 0.266±0.039 (MET), 0.224±0.029 (NA) and 0.209±0.043 (UK) log units (mean±s.e.; n=6-8). Ketanserin (a 5-HT2 blocker) inhibited the leftward shifts produced by 5-HT, and the inhibitory effect was dependent on the degree of blockade of the contractile responses to 5-HT.

We conclude that in saphenous vein strips 5-HT enhances both the contractile responses to $alpha_1$- and to $alpha_2$-agonists and that this enhancement is more marked for the lower concentration range of the agonists. The results suggest that this potentiating effect is dependent on the activation of $5\text{-}HT_2$ receptors.

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MODIFICATION BY 1,3-DIPROPYL-8(P-SULFOPHENYL)XANTHINE OF THE RESPIRATORY RESPONSES INDUCED BY COMMON CAROTID OCCLUSION IN RATS

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In rats adenosine and its analogues stimulate spontaneous respiration, an effect antagonized by theophylline and mediated through carotid body chemoreceptors (Monteiro & Ribeiro, 1987); an involvement of endogenous adenosine at this level has been postulated (Ribeiro et al., 1988). Hypoxia, the physiological stimulus for carotid body chemoreceptor activity, as well as ischaemia, are the main stimuli to produce the release of adenosine in blood (e.g. Sollevi, 1986). Occlusions of the common carotid arteries excite carotid body chemoreceptor activity (Alcayaga et al., 1986). In the present work it was investigated the action of the adenosine antagonist, 1,3-dipropyl-8(p-sulfophenyl)xanthine (DPSPX), on the respiratory responses induced by bilateral common carotid occlusions (CCO) in rats. DPSPX was used since in previous work (Ribeiro et al., 1988) antagonized the excitatory effects on respiration of substances that increase the levels of endogenous adenosine.

The animals, anaesthetized with sodium pentobarbitone (60 $mg.Kg^{-1}$, i.p.) and vagotomized, were breathing spontaneously and the airflow ($\dot{V}$), tidal volume (V_T), respiratory frequency (f), BP and heart rate (HR) were continuously recorded throughout the experiments. Both common carotid arteries were dissected approximately 1 cm below the bifurcation and its bilateral arterial lumen was occluded by pulling simultaneously a surgical silk placed around each artery at this level (Chungcharoen et al., 1952). Intracarotid (i.c.) infusions of drugs were made through a catheter introduced via the right external carotid artery just below the bifurcation.

In the animals with the carotid sinus nerves intact, CCO during periods of 5, 10 and 15 s, performed in the presence of i.c. saline infusions (0.5 $ml.min^{-1}$, for 3 min), induced an increase in respiratory minute volume ($\dot{V}_E$) dependent on the duration of CCO (36±4%, 86±10% and 126±9% increase in $\dot{V}_E$ for 5, 10 and 15 s respectively) associated with an increase in BP without appreciable changes in HR. After bilateral section of the carotid sinus nerves the increases in $\dot{V}_E$ during the brief occlusions (5, 10 and 15 s) almost disappeared, though increases in $\dot{V}_E$ with more prolonged occlusions (>15 s) were observed. DPSPX i.c. infusions (100 nmol. 0.5 $ml.min^{-1}$, for 3 min) reduced the respiratory responses to CCO (42±10%, 30±6% and 39±9% decrease in $\dot{V}_E$ for 5, 10 and 15 s respectively). Enprofylline a xanthine almost devoid of adenosine antagonist properties (Persson, et al., 1986) infused i.c. in the same dose of DPSPX (100 nmol.0.5 $ml.min^{-1}$, for 3 min) did not markedly change the effects of CCO on V_E.

It is concluded that adenosine is involved in the respiratory responses to CCO mediated through carotid body chemoreceptors.

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RP 49356 : A VASORELAXANT AGENT WITH POTASSIUM CHANNEL ACTIVATING PROPERTIES

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Vascular smooth muscle contractility is regulated by plasmalemma ionic permeabilities. Inhibition of inward Ca^{++} current or activation of outward K^+ current can lead to myorelaxation. Cromakalim and pinacidil possess antihypertensive and vasorelaxant properties which are consistent with the activation of K^+ channels (Cook, 1988). The aim of this paper is to describe some pharmacological effects of RP 49356, N-methyl-2-(3-pyridyl)-tetrahydro thiopyran-2-carbothioamide-1-oxyde, a novel K^+ channel activator.

Rat (male Sprague-Dawley, 230-250 g) aortic rings deprived of endothelium were suspended in Tyrode solution and contracted with either 20 or 55 mM KCl. The effects of RP 49356 (1 µM), diltiazem (1 µM) or cromakalim (0.3 µM) were assessed in this preparation pretreated 30 min earlier with either glibenclamide (1 µM) (blocker of an ATP sensitive K^+ channel ; Schmid-Antomarchi *et al.*, 1987) or its solvent. The blood pressure effects of RP 49356 administered p.o. (0.125 - 0.25 mg/kg) or i.v. (10 µg/kg/min during 20 min) were studied, respectively, in conscious SH rats and in normotensive anesthetized rats which had been pretreated with glibenclamide (20 mg/kg i.v.) or its solvent. Papillary muscles obtained from right cardiac ventricles of guinea-pigs were superfused with oxygenated Tyrode solution (37°C) and paced (1 Hz). Conventional microelectrodes were used to record transmembrane action potentials in preparations pretreated with either glibenclamide (30 µM) or its solvent, 30 min before adding RP 49356 (100 µM) or cromakalim (30 µM).

Rat aortic rings deprived of endothelium and contracted with 20 mM K^+ developed approximately 1.5 - 2 g tension. Addition of RP 49356, diltiazem or cromakalim produced almost complete relaxation of this preparation. The effects of RP 49356 and cromakalim but not diltiazem were blocked by glibenclamide. In aortic rings contracted with 75 mM KCl, only diltiazem produced myorelaxation. In spontaneously hypertensive rats, RP 49356 evoked sustained dose-related falls in mean aortic blood pressure (0.25 mg/kg p.o. : -67 ± 4 mmHg from initial value of 185 ± 3 mmHg ; n=6). The hypotension was accompanied by tachycardia. Glibenclamide blocked completely the blood pressure effects of RP 49356. In normotensive anesthetized rats RP 49356 produced hypotension (-28 ± 2 mmHg, initial value = 115 ± 3 ; n=5) which was also prevented by glibenclamide. In the guinea pig papillary muscle, RP 49356 and cromakalim induced a marked (over 60%) shortening of the action potential. Glibenclamide did not modify the baseline profile of the action potential but blocked the effects of RP 49356 and cromakalim.

These results indicate that RP 49356 is a potent vasorelaxant and antihypertensive agent. The shortening of transmembrane action potential produced by RP 49356 in guinea pig papillary muscle appears to be mediated by an increase in a K^+ outward current *via* ATP-sensitive channels as demonstrated by patch-clamp experiments presented at this meeting (Escande *et al.*, 1988). Thus, the K^+ channels activated by RP 49356 appear to have pharmacological properties similar to the pancreatic β-cell or the cardiac ATP-sensitive K^+ channels of which glibenclamide is an antagonist.

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RP 49356 IS A POTENT OPENER OF ATP-MODULATED POTASSIUM CHANNELS IN CARDIAC MYOCYTES

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RP 49356, N-methyl-2-(3-pyridyl)-tetrahydrothiopyran-2-carbothioamide-1-oxide, is a novel pharmacological agent which lowered blood pressure *via* reduction in total peripheral resistance and produced vasorelaxation in K^+ (20 mM) contracted rat aortic rings (Mondot *et al.*, 1988). Since high concentrations of RP 49356 also markedly shortened the action potential duration of guinea-pig papillary muscle preparations, we have investigated its effects on myocardial K^+ currents.

Single myocytes were prepared from guinea-pig cardiac ventricles by an enzymatic method (Mitra & Morad, 1985). Whole-cell and inside-out configurations of the patch-clamp technique (Hamill *et al.*, 1981) were employed to record ionic currents. The effects of RP 49356 (3-300 µM), glibenclamide (0.3-3 µM) or RP 49356 (300 µM) plus glibenclamide (0.3-3 µM) were explored on whole-cell experiments (35°C) in which K^+ currents were isolated by blocking the fast Na^+ and the slow Ca^{++} inward currents with 50 µM TTX and 3 mM $CoCl_2$, respectively. RP 49356 (300 µM) was also studied in inside-out membrane patch experiments (room temperature) with a K^+ concentration of 140 mM at both sides of the membrane.

In whole-cell experiments, RP 49356 evoked a concentration-related time-independent outward current (i_R) in the potential range -80 to +60 mV. The current induced by 300 µM of RP 49356 reversed near the K^+ equilibrium potential which was -85.3 mV under our experimental conditions. This indicates that i_R was mainly carried by K^+ ions. The current-voltage relationship of i_R was linear between -60 and +60 mV ; its slope conductance being 35.4 ± 6.7 pA/mV (n=9) for 300 µM RP 49356. Glibenclamide (0.3-3 µM), a blocker of ATP-modulated K^+ channels (Schmid-Antomarchi *et al.*, 1987), lacked effects on the delayed rectifying and on the background K^+ currents (n=14). However, this sulphonylurea inhibited concentration-dependently the RP 49356 induced K^+ current, i_R. Glibenclamide at 0.3 µM reduced by about 50% (n=5) and, at 3 µM, entirely suppressed the effects of 300 µM RP 49356 (n=3). In inside-out membrane patches, ATP-modulated K^+ channels were identified by their large elementary conductance and their high sensitivity to ATP (3 mM) (Noma, 1983). At a potential of -50 mV, RP 49356 (300 µM) applied at the inner side of the membrane patch reversibly activated ATP-sensitive K^+ channels (n=6).

Our results show that, like cromakalim (Osterrieder, 1988), RP 49356 is a potent K^+ channel opener in heart cells. This investigation demonstrates that the channels activated by RP 49356 are ATP-sensitive and are susceptible to glibenclamide blockade. To our knowledge, such channels have not yet been identified in vascular smooth muscle cells. However, it would appear that the vascular K^+ channel activated by RP 49356 shares some pharmacological characteristics with cardiac ATP-sensitive K^+ channels since the vasorelaxant effects of RP 49356 are also inhibited by glibenclamide (Mondot *et al.*, 1988).

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HAEMODYNAMICS OF PRESSOR RESPONSES TO AZEPEXOLE (B-HT 933) IN ANAESTHETISED RATS

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Single doses of α_2-adrenoceptor agonists, including B-HT 933, decrease blood pressure in anaesthetised rats through stimulation of central α_2-adrenoceptors (Kobinger, 1978). However, in pithed rats they have a pressor effect when given intravenously (Drew & Whiting, 1979). Therefore we set out to determine if B-HT 933 can produce systemic pressor responses by acting on post-junctional α_2-adrenoceptors in anaesthetised rats when more than one dose is given.

Cardiac output (CO), its distribution and vascular resistances were determined by tracer microspheres as described by Nichols & Hiley (1987). Male Wistar rats (250-300g; Bantin & Kingman) were anaesthetised (120mg kg^{-1} i.p. sodium thiobutabarbitone; Inactin, BYK) and both femoral arteries cannulated for withdrawal of blood (0.5ml min^{-1}) during microsphere injection and continuous monitoring of blood pressure. Rats initially received, into the jugular vein, either 1mg kg^{-1} B-HT 933 (Boehringer Ingelheim) or saline (0.2ml). After blood presure had stabilised, the rats given B-HT 933 were given either saline (0.2ml bolus+0.04ml min^{-1} infusion; "B-HT/saline group") or B-HT 933 (0.7mg kg^{-1}+ 0.1mg min^{-1} infusion; "B-HT/B-HT group"); rats given saline at first only received saline ("saline group"). When the responses were stable, 60000-80000 [^{113}Sn]-labelled microspheres (15μm diameter; NEN) were injected through a cannula placed in the left ventricle. In other experiments, a common carotid artery and jugular vein were cannulated for blood pressure monitoring and drug administration; 1mg kg B-HT 933 was given before determining dose-response curves to bolus injections of B-HT 933 (0.18-2.14 mg kg^{-1}).

In the microsphere study, mean arterial pressure (MAP) was 30±6mmHg lower in the B-HT/saline group than in the saline group in which it was 111±5mmHg (n=8 for all groups). CO was reduced as a result of a 17% lower heart rate in the B-HT/saline group but total peripheral resistance (TPR) was the same as in the saline controls. In the B-HT/B-HT group MAP was 43±4mmHg greater than in the B-HT/saline group and this was due to a 31% greater TPR; CO was the same in the two groups. CO distribution was reduced, in the B-HT/saline group relative to the saline group, to the heart (by 38%) and liver (by 34%) but it was increased to the stomach (by 60%) and spleen (by 146%). Distribution of CO to the heart, spleen and stomach was the same in the two B-HT groups but was increased to the lungs (by 43%) and kidneys (25%) in the B-HT/B-HT relative to the B-HT/saline group. Also, the fraction of CO passing to the gastrointestinal tract (GIT) in the B-HT/B-HT group was 30% greater than in the saline group. Organ vascular resistance were the same in the B-HT/saline and saline groups, but in the B-HT/B-HT group resistances were greater in the heart (44%), liver (125%), brain (49%), testes (80%), skin (49%) and GIT (49%) than in the BHT/saline group. In the dose response study, pressor responses to B-HT 933 bolus injections were transient and dose dependent reaching a maximum of 35.5±3.0mmHg at 1.4mg kg^{-1} (n=10). They were inhibited by 0.3mg kg^{-1} yohimbine but not by 50μg kg^{-1} prazosin.

Thus, after first reducing blood pressure by central α_2-adrenoceptor activation, B-HT 933 can induce pressor responses. These share characteristics with postjunctional α_2-adrenoceptor mediated pressor responses observed in pithed rats (Hiley & Thomas, 1987).

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EFFECT OF ENDOTHELIUM DESTRUCTION IN VITRO AND IN SITU ON NORADRENALINE PRESSOR RESPONSES IN RAT SUPERIOR MESENTERIC ARTERIAL BED

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Griffith *et al.* (1988) have proposed that pharmacological constriction of a vascular bed enhances EDRF activity. The aim of this study was to compare the pressor responses to noradrenaline in the isolated Krebs-perfused superior mesenteric bed with those in the *in situ* blood perfused bed before and after destruction of the endothelium with the detergent CHAPS.

Male Wistar rats (250-350g; Bantin & Kingman, Hull) were anaesthetised with 120mg/kg i.p. sodium thiobutabarbitone (Inactin, BYK), heparinised (1000U/kg i.p.) and the superior mesenteric arterial bed prepared for perfusion with Krebs-Henseleit solution (at a rate of 2ml/min) as described by Hiley *et al.* (1986). Dose response curves were constructed for noradrenaline (100ng-30μg; Koch-Light; stock made up in 1mg/ml ascorbate) before and after perfusion for 90s with 0.3% CHAPS (3-[3-cholamidopropyl)-dimethyl-ammonio]-1-propane sulphonate) in distilled water. This treatment has been shown by histology to produce endothelial destruction (Hiley *et al.*, 1987). For *in situ* blood perfusion, weight-matched rats were anaesthetised as above and prepared for perfusion as described by (Hiley *et al.*,1986). The rate of perfusion was 2ml/min and drugs were administered into the extracorporeal circuit. CHAPS was administered as a 0.3% solution in 0.9% NaCl for 150s at 2ml/min in place of blood. In both preparations, functional destruction of the endothelium was confirmed by the inability of 100ng carbachol to oppose the pressor response to noradrenaline (10μg *in vitro*, 1μg *in situ*); opposition of the noradrenaline response by nitroprusside was unaffected.

In both preparations noradrenaline produced dose-related increases in perfusion pressure. However, noradrenaline was significantly ($P< 0.001$) more potent *in vivo* (ED50=623±59ng) than *in vitro* (ED50=1.47±0.09μg), while the maximum pressor response did not differ between the preparations; 123±3mmHg (*in vitro*) compared to 121±8mmHg (*in vivo*). After perfusion of the mesentery, *in vitro*, with CHAPS the ED50 (1.25±0.15μg) and maximum pressor response (109±4mmHg) were unchanged but, *in vivo*, CHAPS treatment significantly decreased the ED50 (to 86.3±13.7ng; $P<0.001$) without changing the maximum response (123±5mmHg). For all comparisons, n=7 *in vivo* and n=6 *in vitro*.

This study shows that noradrenaline is more potent as a vasoconstrictor *in situ*, this is likely to reflect the greater vascular complexity of this preparation which includes the small arterioles present in the gut wall, which are absent in the *in vitro* preparation. The difference between the *in vivo* and *in vitro* preparations after endothelial destruction suggests either that the role of EDRF is more marked in the resistance vessels present in the gut wall than those present in the mesentery or that the effects of EDRF on vascular tone are greater when the more viscous fluid blood is used as the perfusate.

MDR is an MRC Research Student.

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RELAXANT AND β_2-ADRENOCEPTOR BLOCKING ACTIVITIES OF LABETALOL, DILEVALOL, AMOSULALOL AND KF-4317 ON THE RAT ISOLATED AORTA

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Combined α- and β-adrenoceptor antagonists have the potential to cause vasodilation and vasoconstriction by acting as antagonists at α- and β_2-adrenoceptors, respectively. However labetalol, a combined α- and β-adrenoceptor antagonist, is used in the treatment of hypertension and a number of other combined α- and β-adrenoceptor antagonists, including dilevalol (the R'R isomer of labetalol) amosulalol and KF-4317 are being developed for clinical use. Labetalol and dilevalol have been reported to cause vasodilation independent of α_1-adrenoceptor blockade in vivo (Baum et al 1981). There have been no reports of the in vitro effects, other than α_1-adrenoceptor antagonism, of the combined blockers on blood vessels. The present study initially examined the effects of labetalol, dilevalol, amosulalol and KF-4317 alone on the KCl-contracted rat aorta. Secondly, I investigated the effects of ICI 118,551 (a β-adrenoceptor antagonist) on the relaxant responses to labetalol, dilevalol, amosulalol and KF-4317. Finally, I studied the effects of labetalol, dilevalol, amosulalol and KF-4317 on the relaxant responses of the rat aorta to procaterol (a β_2-adrenoceptor agonist).

When the rat aorta has been contracted by the addition of KCl, it is relaxed by procaterol. Labetalol and dilevalol, 10^{-8}-10^{-4}M, have a similar relaxant effect to procaterol. Amosulalol, $\leq 10^{-6}$M, and KF-4317, $\leq 10^{-7}$M, did not relax the rat aorta. Higher concentrations of amosulalol ($\geq 10^{-5}$M) and KF-4317 ($\geq 10^{-6}$M) caused a small relaxation of the KCl contracted rat aorta. The relaxant responses of the KCl contracted rat aorta to labetalol, dilevalol (10^{-6}M), amosulalol and KF-4317 (10^{-4}M) were not altered by the presence of ICI 118,551 at 10^{-6}M. Three successive challenges of the KCl-contracted rat aorta to procaterol produced identical relaxant curves. Labetalol, dilevalol, amosulalol and KF-4317 (all at 10^{-7}-10^{-6}M) produced parallel rightward displacements of the procaterol relaxant curves with no effect on the maximum response. As β_2-adrenoceptor antagonists, dilevalol (pA_2 = 8.3), amosulalol (pA_2 = 7.9) and KF-4317 (pA_2 = 8.4) had similar high potencies and were more potent than labetalol (pA_2 = 7.4).

In conclusion, the relaxations of the KCl-contracted rat aorta to labetalol, dilevalol, amosulalol and KF-4317 are not due to β-adrenoceptor agonism. Labetalol, dilevalol, amosulalol and KF-4317 are potent β_2-adrenoceptor antagonists.

Supported by the Auckland Medical Research Foundation.

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EFFECTS OF FLOSEQUINAN ADMINISTERED INTRAVENOUSLY IN CONSCIOUS CATS AND DOGS

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Flosequinan (7-fluoro-1-methyl-3-methylsulphinyl-4-quinolone) (F) is a novel, clinically effective arteriovenous vasodilator currently undergoing trials in both heart failure and hypertension (Cowley et al 1987, Kessler and Packer 1987). The effects of orally administered F have been described in conscious cats and dogs (Sim et al 1988). Work presented here demonstrates the effect of intravenously administered F in the same species.

Mean blood pressure (BP) and heart rate (HR) were measured at 5 min intervals in conscious renal hypertensive dogs (n=4) and normotensive cats (n=4) via carotid loop puncture for 3h after flosequinan at 1.0, 3.0 or 10.0 mg/kg i.v. Venous blood was taken at 3, 5 and 40 mins after infusion for determination of plasma levels of F and its major metabolite, BTS 53 554 (7-fluoro-1-methyl-3-methylsulphonyl-4-quinolone) (FM). Plasma renin activity (PRA) determinations were carried out on arterial blood taken at pre-dose 15, 45 and 90 min after infusion. A 4 x 4 crossover design was adopted, each animal receiving each dose or saline by the end of four weekly treatments. BP, HR and PRA were expressed as overall means following treatments (Table 1).

Table 1

	Overall means					
	BP mmHg		HR bpm		PRA ng/ml/h	
	Dogs	Cats	Dogs	Cats	Dogs	Cats
Placebo	160	113	63	175	0.66	2.34
F 1 mg/kg i.v.	150	108	64	186+	0.60	2.8
F 3 mg/kg i.v.	140	98**	71	197**	0.46	3.34+
F 10 mg/kg i.v.	115*	86**	85+	180	0.69	5.4**

**p<0.01, *p<0.05, +0.05<p<0.1 (Williams' test or Dunnett's test).

In dogs, F produced dose-related falls in BP associated with increases in HR and little effect on PRA (Table 1). In cats, dose related falls in BP occurred but HR increased at lower doses and was normal at 10 mg/kg. PRA levels, which were higher in the cat, were significantly increased by flosequinan.

Table 2

	Plasma levels at 3, 5 and 40 min after F 10 mg/kg iv					
	Dogs			Cats		
F µg/ml	24 ± 4;	15 ± 0.7;	11 ± 0.4.	36 ± 16;	18 ± 3;	10 ± 0.4.
FM µg/ml	0.3 ± 0.03;	0.4 ± 0.06;	1.2 ± 0.2.	1.2 ± 0.03;	1.2 ± 0.2;	3 ± 0.3

The response to F was not correlated with plasma levels of F since peak hypotensive effects occurred at approximately 60 min in dogs and at 5 to 10 min in cats, whereas higher levels of F occur at 3 min in both species (Table 2).

Flosequinan was an effective hypotensive agent given i.v. in both species but cat and dog responded differently regarding HR which increased with dose in the dog but not the cat and in PRA which was little affected in the dog but showed significant increases in the cat. Similar species differences were seen after oral dosing (Sim et al 1988).

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EFFECT OF FLOSEQUINAN AND OTHER VASORELAXANTS ON RAT AORTIC CONTRACTIONS STIMULATED BY NORADRENALINE AND PHORBOL ESTER

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Flosequinan is a clinically effective, novel arteriovenous dilator currently undergoing clinical trials in both hypertension and heart failure. (Cowley et al 1987, Kessler and Packer 1987). It has been shown to cause increases in cGMP in rat aortic smooth muscle (Allcock et al 1988) and may produce its effects by affecting internally released calcium (Yates 1988). The work presented here shows the effects of flosequinan and other vasorelaxants on noradrenaline (NA) and phorbol dibutyrate ester (PDB) contractions of rat aorta and the effects of methylene blue, a soluble guanylate cyclase inhibitor.

Isolated thoracic aortae from male Charles River Wistar rats weighing 175-200 g were spirally cut and mounted under 1 g tension in normal Krebs for NA contractions or calcium-free Krebs bicarbonate buffer with EGTA 2 mM for PDB contractions, gassed with 95% O_2; 5% CO_2. Contractions were recorded with isotonic transducers on Lectromed recorders.

Table 1

	IC50 against			
	Noradrenaline (NA)		Phorbol dibutyrate (PDB)	
	Control	+Methylene blue	Control	+Methylene blue
Flosequinan (F)	65 μM	105 μM	66 μM	92 μM
Sodium nitroprusside (SNP)	1.64 nM	24 nM	3.3 nM	110 nM
Glyceryl trinitrate (GTN)	6.4 nM	800 nM	36 nM	2700 nM
Atriopeptin II (ANP)	3.15 nM	0.5 nM	2.85 nM	7.2 nM
Nifedipine (N)	190 nM	NT	IA	NT
Diazoxide (D)	18 μM	61 μM	IA	NT-
Cromakalim (C)	620 nM	NT	IA	NT-
8-Br-cyclic GMP (Br-cGMP)	55 μM	NT	9.5 μM	20 μM

IC50: concentration required for 50% relaxation. IA: inactive. NT: not tested.

Relaxation of NA (10nM) or PDB (10μM) contractions by SNP and GTN but not by F, ANP or Br-cGMP were antagonised by methylene blue. N, D and C relaxed NA contractions but had no effect on PDB contractions. The relaxation of NA contractions by D was slightly inhibited by methylene blue (10μM).

Thus both NA and PDB contractions were relaxed by vasorelaxants which increase cGMP (F, SNP, GTN ,ANP), whereas vasorelaxants not thought to involve cGMP such as N and C were ineffective against PDB contractions. SNP and GTN relaxations were antagonised by methylene blue whereas Br-cGMP, F and ANP were not. Morgan and Morgan (1987) have claimed that cGMP can inhibit intra-cellular calcium release and previous results using smooth muscle have suggested that this could occur with F (Yates 1988). The results with PDB confirm and extend the findings of Sybertz et al (1986) in rabbit aortic smooth muscle and suggest that smooth muscle relaxation may be mediated via cGMP inhibition of protein kinase C stimulation.

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DIFFERENTIAL REGULATION OF β_1- AND β_2-ADRENOCEPTORS IN RAT MYOCARDIUM FOLLOWING ISOPRENALINE INFUSION

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A large number of studies have examined down regulation of beta adrenoceptors (AR) in mammalian tissues following chronic agonist infusions in vivo. Studies using the non selective agonist isoprenaline (ISO) suggest relative resistance of the beta-2 AR to desensitisation in tissue containing both beta-1 and beta-2 subtypes (Cohen and Schenck, 1987). We have studied the differential regulation of the beta AR subtypes in rat ventricular myocardium following short infusions of ISO.

Using subcutaneous osmotic minipumps ISO (40 µg/kg/hr) was administered for 16, 24 or 48 hours to groups of AS rats (weight 200-220 g). Control animals received saline or were sham operated. Following infusions rats were sacrified, ventricular membranes prepared using previously described methods (Baker et al, 1980), resuspended in assay buffer (50 mM Tris-HCl pH 7.8 with 0.2 mM Na metabisulphite) and stored at -70°C prior to radioligand binding assay. Membranes were incubated to equilibrium with ^{125}I (-)pindolol (PIN) in a final volume of 250 µl for 40 minutes at room temperature and bound radioactivity separated by rapid vacuum filtration. Non specific binding was defined by 200 µM ISO. Maximal binding capacities (B_{max}) of beta-1 and beta-2 AR were calculated by saturation analyses in the presence or absence of the highly beta-1 selective antagonist CGP 20712A in a concentration (3×10^{-7} M) previously shown to occupy >95% of the beta-1 AR in this tissue. ISO levels measured by HPLC in blood drawn by cardiac puncture at the end of each infusion period, ranged from 10-30 pmol/ml. Noradrenaline and adrenaline levels were significantly elevated at 16 hours returning to control by 48 hours.

At all time points studied beta-1 AR were significantly more resistant to down regulation than beta-2 AR (Table 1). Beta-2 AR had declined to 38% and 75% of control at 24 hours and 48 hours respectively, whereas no change in beta-1 AR was detectable until 48 hours of ISO infusion. The equilibrium dissociation constant (KD) for ^{125}I PIN (30-40 pM) was unaffected by ISO treatment.

These results suggest an increased susceptibility to down regulation of the beta-2 AR in rat myocardium during short periods of agonist treatment in vivo in contradistinction to the relative resistance of this subtype in human heart after chronic exposure to catecholamines during the development of heart failure (Bristow et al, 1984).

TABLE 1 Bmax (fmol/mg protein) of ^{125}I PIN Binding to Ventricular Membranes After ISO Infusions (Mean ± SEM)

	Control (n = 19)	16 hr (n = 7)	24 hr (n = 7)	48 hr (n = 6)
B_1	13.6 ± 0.5	13.4 ± 0.7	13.7 ± 0.7	9.3 ± 0.8*
B_2	6.4 ± 0.3	2.8 ± 0.1*	2.4 ± 0.3*	1.7 ± 0.2*

* significantly different to control ($p < 0.05$)

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COMPARISONS BETWEEN THE EFFECTS OF HYPOXIA UPON NORADRENALINE-INDUCED CONTRACTIONS OF ARTERIES FROM THE RAT AND RABBIT

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Having compared the relaxing effect of hypoxia upon noradrenaline (NA)-induced contractions of a variety of different arteries of the rabbit (Marriott & Marshall, 1988), we have now made direct comparisons between arteries of the rabbit and rat. Cylindrical segments of thoracic aortae, mesenteric, renal and femoral arteries (TA, MA, RA, FA respectively) of rabbits and rats were suspended for tension recording in Krebs' at 37°C, under normoxic conditions (PO_2=103±1mmHg, n=431). Contractions to NA were evoked during normoxia (to 80-90% of maximal) and after 30 min exposure to hypoxia (PO_2=10±3mmHg, n=191) in the presence of Ca^{2+} (2.5mM) or in Ca-free Krebs' (+0.5mM EGTA).

Table 1 Effect of hypoxia (H) and of Ca-free conditions in normoxia (N) and hypoxia, on NA-induced contractions, shown as % of normoxic contraction to NA ±SE.

	Rat (n>5)			Rabbit (n>6)		
		Ca-free			Ca-free	
	(a)H	(b)N	(c)H	(d)H	(e)N	(f)H
TA	74.3±9.7	49.1±1.9	31.0±1.5	33.8±7.2	72.1±1.9	21.4±4.9
MA	101.3±6.5	35.2±3.9	45.3±12.6	47.6±13.6	50.0±5.9	19.7±2.8
RA	52.5±4.5	19.2±3.3	8.3±2.3	47.8±6.2	34.9±3.9	18.4±7.5
FA	9.7±3.3	11.5±2.4	1.8±0.8	5.6±3.0	42.1±3.6	5.3±2.0

Clearly for the rat, as for the rabbit (cf Marriott & Marshall, 1988) there is considerable variation between arteries from different anatomical sites in the extent to which NA-induced contractions are reduced by hypoxia (a, d Table 1). However, while responses of RA and FA were similarly reduced by hypoxia in the two species, the responses of MA and TA of the rabbit were far more attenuated than those of the rat. Further, for the rat as for the rabbit (cf Marriott & Marshall, 1988), the relaxing effect of hypoxia could be partly explained by reduction in the component of NA-induced contraction due to mobilisation of intracellular Ca^{2+} (b,c and e,f Table 1) and partly by reduction in the component which relies on influx of extracellular Ca^{2+}. But for the rat, as for the rabbit, there was no support for the view (see Ebeigbe et al, 1980) that there is a direct correlation between the extent to which the NA-induced contraction of a given artery relies upon influx of extracellular Ca^{2+} and the extent to which that contraction is reduced by hypoxia. Correspondingly, there was no obvious correlation between the extent to which the Ca^{2+} entry blocker verapamil (VER 10µm) could reduce the normoxic contraction to NA and the relaxing effect of hypoxia, eg, VER reduced normoxic contractions of TA and MA of the rat to 53.8±1.8 and 50.9±5.6 of control contractions respectively and those of TA and MA of the rabbit to 88.9±4.7 and 49.7±4.9 of control contractions respectively (cf a,d Table 1).

Thus, our results show that extrapolation from the magnitude of the relaxing effect of hypoxia on a given artery of one species to the corresponding artery of another species should be made with caution. But, arteries of both rat and rabbit would seem to be equally valuable for studies of mechanisms underlying the relaxing effect of hypoxia.

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EFFECTS OF THE COMBINATION OF MEXILETINE AND PROPAFENONE IN GUINEA-PIG PAPILLARY MUSCLES

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Mexiletine (M) and propafenone (P) are two antiarrhythmic drugs which have been classified in groups Ib and Ic, respectively. In the present communication we have studied the electrophysiological interactions of these two drugs at the sodium channel level.

Experiments were performed in guinea-pig papillary muscles excissed from the right ventricle and perfused with Tyrode solution (34º) gassed with $95\%O_2$ - $5\%CO_2$. Maximum upstroke velocity of the action potential (Vmax) was obtained by electronic differentiation and used as indirect estimative index of the magnitude of the fast sodium inward current, INa.

Neither M (10^{-5} M) nor P ($5x10^{-6}$ M) alone modified the characteristics of the ventricular action potentials (APs). However, when papillary muscles were perfused with M plus P the Vmax values were significantly decreased ($P < 0.05$). At 10^{-5} M, M induced a 5.1 ± 2.8% of tonic Vmax block whereas P, $5x10^{-6}$ M, produced one of 9.6 ± 2.3% (n=6). The phasic, frequency-dependent, Vmax block produced by M was 12.5 ± 2.5% whereas that produced by P was 55.4 ± 1.7% (n=6) and this blockade was not reversed when M was added to the perfusate in combination with P. In muscles driven at 1Hz the time- and rate-constants for the onset kinetics of the phasic Vmax block induced by P alone were 5.08 ± 0.49 s and 0.19 ± 0.02 AP^{-1} , respectively, while in the presence of the combination of both drugs were 2.83 ± 0.25 s ($P < 0.01$) and 0.32 ± 0.04 AP^{-1} ($P < 0.05$) respectively. The time constant of recovery of Vmax (τoff) decreased from 22.7 ± 2.2 s in the presence of P alone to 18.2 ± 2.2 s ($P > 0.05$) in the presence of P plus M.

In order to test the possibility that M competes with P for the same receptor site, the magnitude of the slow component of recovery of Vmax was plotted in the form of a Hill plot. The apparent Hill coefficient, nH, was 0.81 in the presence of P alone and 0.64 in the presence of both drugs.

The results demonstrated that the combination of M plus P potentiated the Vmax block induced by each drug. This synergistic combination can be explained by the modulated receptor hypothesis, since M binds to both the activated and the inactivated state, whereas P binds preferentially to the inactivated state of the Na channel.

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ELECTROPHYSIOLOGICAL EFFECTS OF E-3753 IN GUINEA-PIG ATRIAL AND VENTRICULAR MUSCLE FIBRES

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E-3753, |1H-imidazol-2-methanol,α-(3-trifluoromethylphenyl)-α-methyl-1-methyl-0-(3-terbutylaminopropane)-2 ol|, is a new antiarrhythmic drug which has been found to be more potent than quinidine and lidocaine to suppress ventricular arrhythmias (Colombo et al 1987). The present study was undertaken to determine the effects of E-3753 on the electrophysiological properties of ventricular muscle fibres.

Experiments were performed in guinea-pig papillary muscles perfused with Tyrode solution (34ºC). Transmembrane action potentials (APs) were recorded with glass microelectrodes and the maximum upstroke velocity (Vmax) was obtained by electronic differentiation.

In atrial fibres driven at 1 Hz, E-3753 (10^{-7} M - 10^{-4}M), had no effect on resting membrane potential or action potential duration (APD) but at concentrations $\geqslant 10^{-6}$ M produced a dose-dependent decrease of the amplitude and Vmax ($P < 0.05$). Moreover, at concentrations $\geqslant 10^{-5}$M, E-3753 prolonged the effective refractory period (ERP) and thus, increased the ERP/APD ratio ($P < 0.05$). In ventricular muscle fibres E-3753 at concentrations higher than 10^{-6}M produced a decrease of AP amplitude and Vmax which was accompanied by a progressive depolarization of the resting membrane potential. At concentrations $\geqslant 5x10^{-5}$M it shortened the APD and ERP ($P < 0.05$), but the ERP was shortened less than the APD and thus the ERP/APD ratio increased ($P < 0.05$).

E-3753, 10^{-5} M, produced a 6.4 $\pm$ 0.7% inhibition of the Vmax of the first AP of a train of impulses (resting block). E-3753 also produced a phasic Vmax block which increased by decreasing the inter-stimulus interval from 2 s to 330 ms. In muscles driven at 1 Hz the time- and rate-constants for the onset kinetics of phasic Vmax block induced by 10^{-5} M E-3753 were 10.3 $\pm$ 0.6 s and 0.08 $\pm$ 0.007 AP^{-1}, whereas at 3 Hz were 5.6 $\pm$ 0.7 s and 0.05 $\pm$ 0.009 AP^{-1}, respectively. At this concentration E-3753 also prolonged the time constant of recovery of phasic Vmax block to 95.8 $\pm$ 9.1 s.

E-3753 (10^{-5} M - 10^{-4}M) had no effect on the characteristics of the slow APs induced by isoprenaline in muscles perfused with 27 mM KCl Tyrode solution and driven at 0.12 Hz.

These results demonstrated that in guinea-pig atrial and ventricular muscle fibres E-3753 exhibits class Ic antiarrhythmic actions. However, it does not exhibits class IV (Ca antagonist) actions.

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DOPAMINE RECEPTOR BLOCKADE ATTENUATES THE NATRIURETIC AND PHOSPHORETIC EFFECTS OF ATRIAL NATRIURETIC PEPTIDE (ANP) INFUSED IN DOGS

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Dopamine and ANP share similar properties. Both increase diuresis and natriuresis and induce changes in renal hemodynamics. Haloperidol blunts the natriuretic response to ANP in vivo (Marin-Grez et al., 1985) but it is unknown if this depends on hormonal and haemodynamic changes rather than on renal effects. In 6 salt repleted Beagles we compared the urinary, haemodynamic (Swan-Ganz) and hormonal effects of 3h infusions of placebo, domperidone (DOM) (1mg/kg/3h), ANP (1 pmol, 10pmol, 100 pmol/Kg/min - 1h each) and of ANP+DOM. Neither placebo nor DOM alone changed haemodynamic,urinary Na+ and phosphate excretion, glomerular filtration rate (GRF-inulin) and plasma hormonal parameters. Comparing with placebo (pl.),ANP (at the end of the infusion) significantly ($p<0.05$) reduced Cardiac Index (CI) by 17% (pl. 4.9±0.4 L/min/m2), Mean Arterial Pressure (MAP) by 18.5% (pl. 119±5 mmHg), Plasma Renin Activity (PRA) by 75.7% (pl.8.65±1.2 mμCn/ml), Plasma Angiotensin II (AII) by 62.8% (pl.9.28±1.9 pg/ml) and increased GRF by 36.6% (pl. 58.2±3.7 ml/min). ANP+DOM induced the same changes in these parameters as ANP alone.

Table 1.Renal and hormonal effects of ANP and ANP+DOM infusions in 6 dogs (percentual change from baseline values)

	UV	Una+	UPh	FENa+	FEPh	NA	AD	DA
ANP	950 ±306	1731 ±594	373 ±104	2106 ±664	226 ±46	108 ±21	136 ±32	65 ±18
ANP+DOM	446* ±184	885* ±359	233* ±75	1085* ±538	139* ±36	183* ±45	239* ±58	135* ±43

*$p < 0.05$

As shown in Table 1, ANP significantly increased the urinary excretion of Na+ (UNa+), of phosphates(Uph) and of volume(UV) and the fractional excretion of sodium (FENa+) and of phosphates (FEPh); all these effects were significantly attenuated wh n ANP was infused with DOM. For a similar reducing-effect in CI and MAP, whereas ANP did not change catecholamine plasma levels, when ANP was infused with DOM a significant increase in plasma levels of Noradrenaline (NA), Adrenaline (AD) and specially of Dopamine(DA) occurred. These data suggest that the blunting of the diuretic and natriuretic effects of ANP induced by dopamine receptor blockade is due to the inhibition of renal tubular transport reabsorption independently of haemodynamic changes. They also suggest that DOM facilitates the sympathetic outflow in response to the fall in MAP and CI induced by ANP infusion.

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GLOMERULAR FILTRATION RATE IN ANAESTHETISED AND CONSCIOUS RATS: SIGNIFICANCE TO LABORATORY PHARMACOKINETIC STUDIES

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Laboratory pharmacokinetic data are obtained from anaesthetised and chronically-catheterised conscious animals. Deleterious effects of general anaesthesia upon renal haemodynamics have been reported (Walker et al, 1983) and changes in renal blood flow will result in proportional changes in glomerular filtration rate (GFR) at glomerular filtration pressure equilibrium (Deen et al, 1974). Anaesthetic-induced changes in GFR will influence the pharmacokinetics of some renally-eliminated compounds. The aim of this investigation was to compare the differential effects of anaesthetics upon the clearance of carboxy-inulin, as a measure of GFR. The clearance of ^{14}[C]-carboxy-inulin is comparable to that of inulin (Sheikh et al, 1972) and Freestone et al (1986) have reported that GFR can be accurately determined after single i.v. bolus administration of inulin.

^{14}[C]- carboxy-inulin disposition was assessed in male Wistar rats (265±18g), utilising the following anaesthetic treatments: (H) - fentanyl & fluanisone (0.26 & 8.3 mg/kg, Hypnorm) in combination with midazolam (4.16 mg/kg, Hypnovel) given i.p.; (U) - i.p. urethane (1.75 g/kg); (P) - i.p. pentobarbitone (67 mg/kg); (K) - i.p. ketamine (80 mg/kg) in combination with i.p. midazolam (5 mg/kg); (S) - i.v. alphaxalone & alphadolone (9 & 3 mg/kg, Saffan). In the above treatments, carotid artery and jugular vein catheters were surgically-implanted immediately prior to the pharmacokinetic study. A further experimental group was studied, (C) - conscious chronically-catheterised (carotid artery and jugular vein) animals. Carotid blood samples (250µl) were collected at 15, 30, 45, 60, 90, 120, 150 and 180 min after i.v. injection with ^{14}[C]-carboxy-inulin (37 nmol). The samples were solubilised and decolorised prior to liquid scintillation counting.

Table 1. Inulin pharmacokinetics in anaesthetised and conscious rats.

	C	H	P	U	K	S	DMRT
CL_b (ml/min/100g BW)	2.29 ±0.31	1.57 ±0.27	1.72 ±0.21	0.77 ±0.09	1.86 ±0.19	2.03 ±0.24	C S K P H U
V_{ss} (ml/100g BW)	54.9 ±8.7	48.6 ±7.2	46.9 ±7.9	56.7 ±8.2	52.5 ±9.2	45.4 ±6.7	U C K H P S

Results are mean ± s.d (n = 5). DMRT - analysis of variance and Duncan's multiple range test. Groups are ranked left to right in decreasing magnitude and those jointly underlined are not significantly different (P>0.05).

The results demonstrate that GFR (as indicated by CL_b) in the H, P, U and K anaesthetised groups was significantly lower than in the C group. GFR in the U animals was only one third of that in the C animals, whilst in the H, P and K groups, GFR was respectively 32%, 25% and 19% lower than in the C group. GFR between the S and C groups was not signifcantly different . No significant differences in V_{ss} were seen between any of the treatment groups. The differential effects of the anaesthetics on GFR observed in this study demonstrate that the choice of anaesthetic used in experimental pharmacokinetic studies requires careful consideration.

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LYMPHOID TISSUE RESPONSES TO EMULSIFIED PERFLUOROCHEMICALS IN MICE: TIME COURSE EFFECTS RELATIVE TO IMMUNE CHALLENGE

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Lymphoid tissue responses to emulsified perfluorochemicals (PFCs) are variable and depend on emulsion composition and dose, species studied and tissue concerned (Bollands & Lowe, 1986, 1987; Raven et al., 1988). We have now investigated the effects of injecting of low doses of a commercial PFC emulsion, Fluosol-DA 20% (F-DA; Green Cross, Japan), on lymphoid tissues in mice. Emphasis has been placed on the effects of timing and route of F-DA administration relative to immune "challenge" with sheep red blood cells (SRBC).

Female inbred NIH mice (body wts: 16-22g; n = 152) were used. They were immunized with 5 x 10^7 double-washed SRBC in 0.1 ml Hank's saline and injected either intravenously (i.v.) or intraperitoneally (i.p.); the day of immunization = day 0. Groups of mice also received a single 10 ml/kg b.w. injection of F-DA via the same route on day -7, -4, -1, +1, +4 or simultaneously at immunization; control animals received identical doses of saline (0.9% w/v NaCl). Mice were sacrificed on day +7 and weights of liver (LIV), spleen (SPL), thymus (THY) and gut mesenteric lymph nodes (MLN) recorded. Plasma antibody (Ab) titres to SRBC were measured by serial-dilution haemagglutination assay.

Mean liver weight increased consistently to a maximum of 57% ($P<0.001$) in response to injection of F-DA, irrespective of route, whereas no similar pattern of spleen weight increase was observed. In contrast, MLN weight was decreased ($P<0.001$) up to 48% following F-DA injection in almost all groups of mice receiving F-DA; thymus weight was generally unchanged except for significant ($P<0.05$) decreases in mice injected i.p. with F-DA on days -7 and -4. Mean (± S.E.) Ab titres were significantly ($P<0.05$) increased in response to some of the treatments but with no consistent pattern. Changes in mean organ weights and Ab titres with time of F-DA injection relative to SRBC were:

	A. I.V. SRBC and I.V. F-DA						B. I.P. SRBC and I.P. F-DA					
Time	LIV	SPL	THY	MLN	Log_2	Ab TITRE	LIV	SPL	THY	MLN	Log_2	Ab TITRE
-7	↑↑↑	–	↓	↑		↓	↑↑↑	–	–	–		–
-4	↑↑↑	–	↓	↓↓↓		↑↑	↑↑↑	–	–	↓↓↓		–
-1	↑↑↑	–	–	↓↓↓		↑↑↑	↑↑↑			↓↓↓		↑
0	↑↑↑	–	–	–		–	↑↑↑	–	–	↓↓↓		↑↑
+1	↑↑↑	↑	–	–		–	↑↑↑	–	–	↓↓↓		–
+4	↑↑↑	–	–	–		–	↑↑↑	–	–	↓↓↓		–

↑ = increased ($P<0.01$), ↑↑ ($P<0.05$), ↑↑↑ ($P<0.001$); ↓ = decreased ($P < 0.01$);
– = unchanged compared to controls;

These results show that changes in lymphoid tissue weights and plasma Ab titres in mice immunized with SRBC vary according to the time of a previous or subsequent injection of F-DA via the same route. The pattern of liver weight increase in mice was markedly different to that seen in rats injected with F-DA and SRBC (Bollands & Lowe, 1987). This may be due either to compensatory liver hyperplasia following F-DA-induced reticuloendothelial system blockade or hepatomegaly produced by the Pluronic F-68 surfactant (Goodman et al., 1984).

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IN VITRO CELLULAR RESPONSES TO A NON-IONIC SURFACTANT, PLURONIC F-68

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The non-ionic block co-polymer surfactant, Pluronic F-68 (Poloxamer 188), has been used to emulsify perfluorochemicals (PFCs) for biological uses related to oxygen transport (Lowe, 1987). Commercial grade Pluronic contains low molecular weight impurities and these have been implicated in adverse responses to emulsified PFCs in vivo (McCoy et al., 1984). In contrast, low concentrations of pluronic can enhance nutrient uptake into cultured human lymphocytes (Mizrahi, 1975) suggesting applications for regulating cell growth. In the present work we have examined and compared the effects of Pluronic on growth of fibroblasts and tumour cells in vitro.

Chick embryonic fibroblasts or Syrian Hamster melanoma cells (RRMI 1648) were grown at 37°C under 5% CO_2/95% air for up to 17 days in minimal essential medium containing 15% (v/v) foetal calf serum with antibiotics (de Pomerai & Gali, 1981). Cells were cultured with 0.05-1.0% of either commercial grade Pluronic F-68 (BASF-Wyandotte, U.S.A.) or Pluronic purified by silica-gel column absorption (BDH; 60-120 mesh; Bentley et al., 1988); control cultures contained medium only. Growth was monitored by periodic measurement of cell numbers using a ZBI Coulter Counter (Coulter Electronics, U.K.) after trypsin/collagenase dissociation of replicate cultures.

Low concentrations (0.05-0.1%) of commercial Pluronic stimulated growth of both cell types, whereas high concentrations (1%) were generally inhibitory. However, low concentrations of purified Pluronic inhibited fibroblast growth but strongly stimulated growth of melanoma cells. For example, in fibroblast cultures after 15 days, cell numbers were significantly ($P<0.05$) greater with 0.1% commercial Pluronic than in controls; cell numbers in all other treatments were significantly ($P<0.05$) lower than controls. By contrast, in 7 day melanoma cultures, cell numbers in the presence of 0.05% purified Pluronic were 2 fold greater than in controls ($P<0.01$) and 50% greater ($P<0.05$) than when cultured with 0.1% commercial or purified Pluronic.

These results show that growth of both normal and cancerous cells can be enhanced by low concentrations of commercial Pluronic F-68, but that these cell types differ markedly in their response to the purified fraction. The present findings support previous suggestions that low concentrations of Pluronic can increase nutrient uptake into mammalian cells and thereby increase growth rate (Mizrahi, 1975), but further imply that this effect may result, in part, from impurities present in commercial preparations.

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THE INTRAHEPATIC DISPOSITION OF WARFARIN DURING STEADY-STATE ANTICOAGULATION IN WISTAR RATS

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The S(-) enantiomer of warfarin is a more potent anticoagulant than the R(+) enantiomer in the rat and in man. Yet, in vitro studies show that R(+) and S(-) warfarin are equally effective inhibitors of vitamin K epoxide reductase (Fasco & Principe 1982). To investigate these differences we have measured the whole liver and subcellular distribution of R(+) and S(-) warfarin at steady-state.

Male Wistar rats (200-250g; n=6 in each group) were injected daily with either R(+) warfarin (0.1, 0.4, or 0.8 mg/kg/day; i.p.) or S(-) warfarin (0.1 mg/kg/day; i.p). Tail vein blood samples were taken under light ether anaesthesia for the measurement of prothrombin time (PT) and plasma warfarin concentrations. The criterion for steady-state anticoagulation was a constant PT for at least 4 days. Rats were then killed and their livers perfused, excised and homogenized for the preparation of hepatic microsomes and cytosol. Concentrations of warfarin were measured by normal phase high performance liquid chromatography.

Normal prothrombin times were 17.0+0.7 sec. The lowest dose of R-warfarin (0.1 mg/kg/day) had no significant effect on PT (16.3±0.5 sec). However, PT was prolonged after administration of larger doses of R-warfarin (21.6±1.7 sec, 0.4mg/kg/day; 55.1±9.0 sec, 0.8mg/kg/day), and by S-warfarin (19.7±0.6sec, 0.1mg/kg/day; larger doses of S-warfarin caused severe haemorrhage). The increasing doses of R-warfarin resulted in a significant ($p<0.05$) increase in plasma, hepatic and cytosolic concentrations of the drug. However, there were no significant differences between the microsomal concentrations of warfarin in any of the treatment groups (table).

Table: Concentrations of R(+) and S(-)warfarin

Dose	[dose] (mg/kg/day)	[plasma] (ug/ml)	[whole liver] (ug/g.liver)	[cytosol] (ug/g.*)	[microsomal] (ug/g.*)
R-warfarin	0.1	0.08±0.00	0.8± 0.1	0.5± 0.0	2.8± 0.8
R-warfarin	0.4	0.3± 0.04	1.3± 0.1	2.7± 0.6	3.9± 1.2
R-warfarin	0.8	0.6± 0.05	2.3± 0.4	5.5± 1.2	4.0± 1.2
S-warfarin	0.1	0.2± 0.02	0.9± 0.1	0.9± 0.6	3.9± 0.7

*protein

Thus, in contrast to plasma, liver or cytosol, microsomal concentrations of warfarin did not appear to be related to either the dose or the pharmacological response. These data are consistent with the observation of a saturable binding site in the microsomal fraction of the liver seen after an acute i.v. dose of racemic warfarin (Thijssen & Baars 1987).

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FURTHER EVIDENCE OF TOLERANCE TO THE CNS EFFECTS OF AMINOGLUTETHIMIDE IN MICE

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During the treatment of advanced oestrogen-positive breast cancer in postmenopausal women with aminoglutethimide (AG) a number of CNS side-effects such as ataxia and lethargy are encountered. These effects are self-limiting and subside usually in 2-6 weeks of continuous therapy (Santen 1986). Tolerance to such effects of the drug also develops in mice receiving AG daily (Abusrewill et al. 1986). The present work examines this phenomenon more thoroughly in the latter species.

Groups (n of at least 10) of male albino mice (22-25g) received daily oral doses of either AG (50 or 100mg/kg) or vehicle (0.75% w/v carboxymethylcellulose) for 14 days. Three assessments of CNS-depression were made:- spontaneous locomotor activity measured 20 min after AG (50mg/kg po), oesophageal temperature 1h after AG (100mg/kg po) and period of absence of the righting reflex after AG (250mg/kg ip). Initially, AG (50mg/kg) caused a 64% decrease in locomotor activity. After 14 daily doses at this level, mice had become completely tolerant to this effect. At the first dose, AG (100mg/kg) caused a max. hypothermic response (35.8±0.2°C compared with 37.4±0.2°C for vehicle-treated mice) after 1h. This effect was also abolished by 14 daily doses (100mg/kg) of AG. The sleeping-time response to AG (250mg/kg) was significantly ($P<0.05$) less in AG (100mg/kg)-chronically-pretreated animals compared to their vehicle-treated controls (42±5 and 86±6 min, respectively). AG (100mg/kg) daily for 14 days also significantly ($P<0.05$) altered the pharmacokinetics of a 50mg/kg challenge dose of AG po (Table), the plasma $t_{0.5}$ being reduced and total clearance being increased without alteration in apparent volume of distribution (V_d). As may be observed, these pharmacokinetic parameters of AG in vehicle-pretreated mice were not significantly different from naive animals receiving no form of pretreatment.

Table. Pharmacokinetic parameters†(means±s.e.m.) of AG (50mg/kg po) in mice receiving either no pretreatment, dosing vehicle (po) or AG (100mg/kg po) daily for 14 days.

Pretreatment (n=4)	Plasma $t_{0.5}$ (h)	V_d (ml)	Total clearance (ml/min)
None	3.8±0.3	52.0±2.4	0.16±0.01
Vehicle	3.6±0.2	50.0±1.0	0.16±0.01
AG	2.3±0.1*	55.0±2.4	0.33±0.01*

†Calculated by STRIPE computer program assuming a single compartment (Johnston & Woollard 1983) from plasma level data (0-12h). At each time 4 samples, each one a pooled sample from 4 mice. *$P<0.05$ compared with vehicle group.

In these animals, the pretreatment with AG significantly ($P<0.05$) lowered the brain and plasma levels of the drug 1h after AG (50mg/kg po) compared with controls (26.8±0.7 vs. 31.6±1.2 and 20.9±0.5 ± 25.9±0.5µg/ml respectively).

Thus when mice are repeatedly dosed with AG, a marked degree of tolerance to the drug's CNS effects occurs and this is accompanied by significant dispositional changes of this aromatase inhibitor.

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IN VITRO ANTITHYROID ACTIVITY OF AMINOGLUTETHIMIDE AND THREE RELATED AROMATASE INHIBITORS

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Amongst the several side effects of the aromatase inhibitor aminoglutethimide (AG) is its inhibitory action on the thyroid, although this does not often cause clinically-significant hypothyroidism when the drug is used in breast cancer patients (Hughes & Burley 1970). Three new compounds of analogous structure to AG, 3-(4'-aminophenyl)-3-ethyl pyrrolidine-2,5-dione (WSP1), 3-(4'-aminophenyl)-3-ethyl-1-methyl pyrrolidine-2,5-dione (WSP2) and 3-(4'-aminophenyl)pyrrolidine-2,5-dione (WSP3), exhibit a high degree of selectivity for aromatase (Daly et al. 1986), while possessing lower centrally-mediated side-effects than AG (Ahmad et al. 1987). The present work examines the new compounds for their antithyroid action.

In vitro antithyroid activity was examined using collagenase-separate thyroid cells from the pig. Following 3 days of culture (non-proliferative) in the presence of TSH (100μU/ml), the capacity of the cells to accumulate iodide and for iodide organification (incorporation into thyroglobulin) was assessed using a 5h incubation with $Na^{125}I$. The method has been described in detail by Brown et al. (1986).

Over the range 0.2-20μM, AG exerted a concentration-dependent inhibition of iodide organification. The approximate IC_{50} (1μM) was similar to that found for the classical antithyroid agents, methimazole and propylthiouracil. In this concentration range AG had no effect on ^{125}I uptake by the porcine thyrocytes. The effects of WSP1-3 are presented in the Table.

Table. Effect of WSP1, 2 & 3 on ^{125}I uptake and organification in porcine thyrocytes in culture.

	Concn (μM)	% of Control* WSP1	WSP2	WSP3
a) Iodide uptake	2	85	98	104
	20	96	88	87
	200	77	69	63
b) Iodide organification	2	72	79	77
	20	48	39	38
	200	21	17	17

*Values, expressed as % of control (no drug), are means n=4 (SEM <5% of mean in each case).

At concentrations of 2 and 20μM, none of the WSP compounds significantly ($P>0.05$) affected iodide uptake by the cells. Although a degree of depression was observed at 200μM, it is anticipated that effective aromatase-inhibitory plasma concentrations of these compounds should not be in excess of 40μM. It may be seen that the compounds depressed the organification of iodide in a concentration-dependent manner. The approximate IC_{50} values estimated from the data were 14, 14 and 12μM for WSP1, 2 and 3 respectively. This indicates that they possess a weaker direct inhibition of iodide incorporation into thyroglublin than AG and this represents a further advantage over the latter drug.

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WHARTON'S JELLY: A SUITABLE TISSUE FOR INVESTIGATING MYOFIBROBLAST CONTRACTILITY?

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It has been reported using electron microscopy that Wharton's jelly, the mucous connective tissue which surrounds the umbilical cord, contains "abnormally shaped fibroblasts" (Parry, 1970). These observations when taken in conjunction with the monoclonal antibody studies of Richman et al. (1987), suggests that these "abnormally shaped fibroblasts" are in fact myofibroblasts. This study describes the suitability of this tissue for use as an in vitro model for investigating myofibroblast contractility.

150mm lengths of umbilical cord (n = 18) from full term placentae were removed and were prepared for in vitro use in one of two ways. Firstly, cords were cut into transverse sections, 2mm in thickness (0.54±0.13g) (type a, n = 9). Secondly, the triangular shaped area between the vein and artery was removed ensuring no residual components of either type of blood vessel were present (0.44±0.07g) (type b, n = 9). As control tissues, spiral strips of the artery and vein were prepared (n = 6). Identical specimens were produced for optical microscopic analysis. Tissues were fixed in Susa's fixative and stained using a) Masson's trichome, b) Van Gieson's, c) Heidenhein's haematoxylin and d) MSB.

All tissues were used in a superfusion system previously described (Lal and Naylor, 1985). The agonists administered were potassium chloride (1-8mg), barium chloride (1-8mg) and mepyramine (0.1-4mg). All were administered in bolus doses of <40μl.

The histological analyses indicated that the type b tissue strips used in the in vitro experiments were free from either venous or arterial vascular smooth muscle. Consequently the responsiveness of type b strips could be attributed exclusively to the cellular component of Wharton's jelly.

The venous and arterial tissue both gave dose dependent reversible contractile responses to barium and potassium ions with potassium being the more potent in terms of developing contractile force. In contrast, mepyramine produced a reproducible dose-dependent and reversible relaxatory response. In both the type a and b preparations the response induced by potassium and barium ions were very variable and consistent results could not be achieved. In contrast, meypramine induced dose-dependent reversible and reproducible contractile effects in both the type a and b preparations.

The range of responses to the agonists used indicates that although the myofibroblasts in Wharton's jelly are sensitive to mepyramine, the use of this preparation as a routine model for investigating 'structural' myofibroblasts is possibly not as successful as when using such tissues as the endocervical mucosa (Chander et al. 1988), or placental strips (Cross & Naylor 1988).

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