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AN EXAMINATION OF INTERACTIONS BETWEEN SUBSTANCE P AND OTHER AGONISTS IN AIRWAY SMOOTH MUSCLE

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A large number of compounds found in the airways are capable of contracting bronchial smooth muscle. Many of these, e.g. neutrotransmitters, play a role in the normal physiological function of the lung whereas others e.g. inflammatory mediators, are released primarily in disease states such as asthma. In asthma, therefore, the bronchoconstrictor response results from the interactive effects of a multiplicity of agents, the combined effect of which may not be predicted from a knowledge of individual agents. The neuropeptide, substance P, has been demonstrated in the innervation of the airways (Nilsson et al, 1977; Hakonson et al, 1983). In addition to a direct effect, substance P has been shown to potentiate the action of histamine in guinea-pig lung parenchyma (Webber & Foreman, 1984). We have, therefore, tested the ability of substance P to potentiate the contractile effects of methacholine, histamine, 5-hydroxytryptamine (5-HT), prostaglandin D_2 (PGD_2), leukotriene C_4 (LTC_4) and adenosine in the rat and guinea pig trachea and human bronchus. Furthermore, we have examained the possibility that combinations may be synergistic because the individual agents have a different mechanism of action on the lung musculature.

Guinea-pig and rat trachea and human bronchus (3-5 mm diameter) were cut spirally, suspended in Krebs solution at 37°C and contractions measured isometrically. Substance P and other agonists in concentrations calculated to produce contractons of 20-70% of maximum were administered either alone or in combination. Responses larger than those predicted by addition of the individual effects were interpreted as synergism.

No synergism between substance P and other agents was observed in the rat trachea or human bronchus. In the guinea-pig trachea, substance P and histamine together produced a response 68% greater than that predicted by addition. The calcium dependency of the effects of substance P and histamine were examined in the guinea-pig trachea. Calcium removal caused an initial potentiation of substance P responses by 70% which declined over the next 100 min. Responses to histamine declined at the same rate but no initial potentiation was seen.

The observation that synergism between substance P and histamaine occurred only in the guinea-pig trachea illustrates species differences between the actions of contractile agents. Furthermore, substance P and histamine appeared to have a different dependency on extracellular calcium.

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BOMBESIN IS A POTENT ANTINOCICEPTIVE AGENT IN MOUSE WRITHING AND TAIL FLICK TESTS

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The nature of antinociception produced by nonopioid peptides has been studied by several workers (e.g. calcitonin - Braga et al, 1978; neurotensin - Clineschmidt et al, 1979). Bombesin has previously been shown to have an antinociceptive action, when given into the periaqueductal gray of rats, in hot plate and tail flick tests (Pert et al, 1980). In the present work, we have extended the antinociceptive profile of bombesin by examining this tetradecapeptide in the acetic acid writhing test after i.c.v., i.th. and i.p. injection to mice. We also obtained dose-response curves for bombesin-induced scratching in mice by the same three routes so that we could compare antinociceptive and scratching data. Additionally, we ran bombesin after i.c.v. administration in the mouse tail flick test. All studies were conducted with the observer unaware of compound and dose.

Male ICR mice (25-30 g; n=9) received bombesin or distilled water i.p., i.c.v. (Pedigo et al, 1975) or i.th. (Hylden and Wilcox, 1980). Five min later, acetic acid (0.6%; 10 ml/kg) was injected i.p. and, after a further 5 min, writhing was counted for 5 min. Potential antagonists were given 5 min before bombesin (0.10 µg, i.c.v.).

In separate experiments, bombesin-induced scratching was quantitated by observing each mouse (n=4) for 5 sec every 20 sec for 15 min and calculating the percent of maximum number of grooming episodes.

Antinociceptive A 50 values for bombesin were 0.004 (0.0008-0.016) µg i.th., 0.014 (0.012-0.16) µg i.c.v. and 19 (5.4-67) µg i.p. Bombesin A 50 values for scratching were 0.010 (0.003-0.030) µg i.c.v., 0.019 (0.010-0.30) µg i.th. and 68 (32-147) µg i.p. Bombesin is thus extremely potent as an antinociceptive agent and scratch inducer in mice after both i.c.v. and i.th. administration. That scratching provides a counter-irritant effect to acetic acid-induced writhing is a possibility.

Naloxone (3 mg/kg, s.c.) and the proposed bombesin receptor antagonists, [D-Arg^{1}, D-Trp^{7}, Leu^{11}]-substance P (2 and 10 µg, i.c.v.) and [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}]-substance P (5 and 10 µg, i.c.v.), did not markedly influence antinociception associated with bombesin (0.10 µg, i.c.v.).

Bombesin was potent as an antinociceptive agent in the mouse tail flick test when water at 55°C was used as the noxious stimulus (Cowan et al, 1985). The A 50 value for bombesin at the time of peak effect (10 min) was 0.009 (0.003-0.03) µg i.c.v.; the corresponding value for morphine (at 20 min) was 0.21 (0.076-0.60) µg i.c.v. In this procedure, the bombesin log dose-response relationship was curvilinear with the antinociceptive effect peaking at 0.30 µg and decreasing at higher, non-toxic doses (1 and 3 µg). When mice (n=7) were pretreated with naloxone (3 mg/kg, s.c. at -5 min), the antinociception associated with 0.10 µg of bombesin was reduced from 73% to 26%. This result was replicated (again under blind conditions) on a second occasion.

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A STUDY OF BRADYKININ BINDING TO RAT JEJUNAL EPITHELIAL CELL MEMBRANES

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The activity of the renin-angiotensin and the kallikrein-kinin systems are inter-related as the production of angiotensin II and the destruction of bradykinin are brought about by the same enzyme. Further, changes in jejunal epithelial transport have been observed in response to both peptides (Davies et al 1972; Cuthbert & Margolius 1982). The present work reports on the binding of [^{3}H]-bradykinin and the analogue [^{125}I] -tyr8 bradykinin to rat jejunal epithelial membranes.

Membranes were prepared from 250 g male Wistar rats as described by Manning et al (1982). The final pellet was resuspended in 10 volumes 5 mM TES buffer pH 6.8 containing 1 mM o-phenanthroline. The binding assay contained membranes (100 µg protein) ligand (either [^{125}I]-tyr8 bradykinin S.A. 2200 Ci per mmol or [^{3}H]-bradykinin S.A. 56.9 Ci per mmol) and buffer containing 25 mM TES, 0.2% bovine serum albumin, 1 mM o-phenanthroline, 1 mM dithiothreitol, 0.1 mM Bacitracin and 1 µM SQ20881, pH 6.8. Specific binding was determined by the addition of 1 µM tyr8-bradykinin or phe8-bradykinin. After incubation at 4 °C for 90 min free and bound ligand were separated by filtration through Whatman GF/B filters (presoaked in 0.1% polyethyleneamine). Results are expressed as fmol bound per mg protein.

Both ligands bound to a high affinity saturable site. The kinetics of binding (determined from computer fit analysis to a Law of Mass Action curve) were similar for both ligands. Thus for [^{125}I]-tyr8 bradykinin Kd was 0.69 ± 0.31 nM and Bmax was 332.8 ± 72 fmol per mg and for [^{3}H]-bradykinin Kd was 0.82 ± 0.14 nM and Bmax 337.0 ± 30.9 fmol per mg. The pharmacological profile examined by the ability of analogues to displace the binding of 1 nM ligand showed that both ligands labelled sites which showed specificity for bradykinin and related kinins. Angiotensin (IC_{50} > 1 µM) captopril (IC_{50} > 1 mM) and SQ20881 (IC_{50} > 100 µM) were all relatively inactive at displacing the binding of both ligands, while bradykinin, kallidin and met-lys-bradykinin were all potent displacers of binding. However, the activity and the rank order of potency of the kinins was dependent on the ligand used. Thus with [^{3}H]-bradykinin the order of potency was bradykinin (IC_{50} 0.2 nM) kallidin (0.28 nM) met lys bradykinin (0.80 nM) and tyr8 bradykinin (3.90 nM). When [^{125}I]-tyr8 bradykinin was the ligand bradykinin displaced with an IC_{50} 1.2 nM, tyr8 bradykinin 2.0 nM and kallidin 3.3 nM.

The results suggest that [^{3}H]-bradykinin is a better ligand to use for the definition of bradykinin binding sites to rat jejunal membranes, although it is of lower specific activity. The natural kinins are potent displacers of the binding with an order of potency similar to their response. The reduced activity of the analogue tyr8 bradykinin at this site is in keeping with its reduced biological activity.

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A NOVEL ANGIOTENSIN II ANALOGUE WITH HIGH AFFINITY FOR EPITHELIAL ^{125}I-ANGIOTENSIN II BINDING SITES

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Specific high affinity ^{125}I-AII binding sites have been identified in rat renal cortex and intestinal epithelial membranes (Cox et al, 1984; 1985). While many of the binding characteristics were similar in these two tissues the maximal binding capacity in intestinal membranes was 4-7% of that measured in renal cortex membranes. Since intestinal epithelia are notoriously rich in protease activity the comparatively low levels of intestinal specific ^{125}I-AII binding may be a result of rapid ^{125}I-AII hydrolysis together with that of the binding site. To minimise the extent of ligand hydrolysis an enzyme resistant, photoactivatable AII antagonist (γ-N-(4-azido-2-nitrophenyl propanoic acid[1])-ile^8 AII ([ANPPA^1-ile^8]-AII) has been synthesised and its affinity for both renal and intestinal ^{125}I-AII binding sites tested.

The N-hydroxysuccinimide ester of N-(4-azido-2-nitrobenzene)-β-alanine was coupled with [des asp^1-ile^8]-AII in dimethylformamide at room temperature and gave [ANPPA^1-ile^8]-AII which was purified by reverse phase HPLC. Rat kidney cortex basolateral and brushborder membranes were prepared according to Heidrich et al. (1972) and crude intestinal epithelial membranes according to Manning et al. (1982). Specific ^{125}I-AII binding was assessed as described previously (Cox et al. 1984).

In displacement studies performed under dark conditions (ANPPA^{1}ile^8]-AII displaced ^{125}I-AII specific binding with an IC_{50} value of 10 nM in renal cortex and 5 nM in intestinal membranes. In studies where membranes were to be irradiated, initial preincubations were conducted in the dark for 5 min at 22 °C to achieve equilibrium with [ANPPA^1-ile^8]-AII. Membranes were then irradiated in plastic cuvettes (with increasing peptide concentrations) 22.0 cm from a high energy UV light source for varying time intervals at 2-4°C. Incubations were diluted and washed by centrifugation before resuspending in hypotonic buffer pH 7.6 ready for ^{125}I-AII binding assays. Following a 3 min. irradiation with increasing concentrations of [ANPPA^1-ile^8]-AII specific ^{125}I-AII binding exhibited dose dependent reductions in both membrane preparations. IC_{50} values of 10 nM and 1 nM were obtained in renal and intestinal membranes respectively. At 10^{-6} M [ANPPA^1-ile^8]-AII reduced specific ^{125}I-AII binding irreversibly by 60-75% whereas 10^{-9}-10^{-5} M AII did not bind irreversibly to either renal or intestinal ^{125}I-AII binding sites after photolysis. The binding sites remaining after photolysis exhibited the same affinity for AII as nonirradiated preparations.

These preliminary studies demonstrate the potential of a photoactivatable enzyme resistant AII analogue for labelling renal and intestinal epithelial ^{125}I-AII binding sites.

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LACK OF ROLE OF ANGIOTENSIN-II IN ORTHOSTATIC BLOOD PRESSURE CONTROL

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The role of angiotensin-II in orthostatic blood pressure control is uncertain. The converting enzyme inhibitor captopril is an effective long-term antihypertensive agent in man (Case et al, 1982) and does not normally produce orthostatic hypotension (Cody et al, 1981). However acutely, captopril can produce excessive supine hypotension without compensatory tachycardia, and occasionally orthostatic hypotension particularly in high-renin patients (Fagard et al, 1980). In a conscious normotensive dog model of orthostatic hypotension (Baum et al, 1981b) captopril was acutely hypotensive after sodium depletion but did not compromise orthostatic blood pressure control (Baum et al, 1981a). Conversely, converting enzyme inhibition did interfere with sympathetic reflex vasoconstriction in anaesthetised cats subjected to lower body negative pressure (Adigun et al, 1981). The present study was designed to assess the role of angiotensin-II in resting and orthostatic blood pressure control in hypertensive dogs by examining the acute effects of captopril before and after a period of sodium depletion.

A group of 8 male beagles with established hypertension induced by bilateral cellophane perinephritis was used. Mean arterial blood pressure (MBP , diastolic plus 1/3 pulse pressure) was measured by acute percutaneous catheterisation of carotid artery loops with the animals unsedated and resting in canvas slings. Captopril, 2.5 mg/kg, was administered orally in a hard gelatin capsule during normal sodium intake (approximately 100 mEq/day) and again after at least 10 days administration of a low-sodium diet (< 14 mEq sodium/day). Blood pressure and heart rate responses to intravenous injections of angiotensin-I (AI) and to 2 min of lift to an upright standing position were measured before and for up to 6 h after captopril administration.

On normal sodium intake resting MBP was 135 $\pm$ 3 mmHg (mean $\pm$ s.e.mean) and lift produced increases in both MBP and heart rate. Despite inhibiting the AI pressor response for the whole of the 6 h measurement period by a maximum of 94%, captopril produced negligible effects on MBP or heart rate either in the resting position or during lift. Following sodium depletion resting MBP fell to 120 $\pm$ 4 mmHg although the lift response was unchanged. Captopril now reduced MBP by a maximum of 34 $\pm$ 9 mmHg ($P < 0.01$, paired t test) at 90 min after dose and increased heart rate slightly but not significantly (+ 8 $\pm$ 5 beats/min, $P > 0.05$). Pressor responses to AI were inhibited as before and again lift responses were unaffected by captopril administration.

Thus, angiotensin-II appears to have no functional role in the blood pressure control of sodium-replete hypertensive dogs. After sodium depletion however, angiotensin-II seems to be involved in maintaining blood pressure at rest but not following postural change.

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USE OF TACHYKININ ANTAGONISTS AGAINST ELEDOISIN-EVOKED NEUROTRANSMITTER RELEASE

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We have previously compared the agonist sensitivities of tachykinin receptors on the longitudinal smooth muscle of the guinea-pig ileum with those in the myenteric plexus evoking ACh release (Fosbraey et al, 1984). The former show a rank order of potency of tachykinin agonists compatible with a population of predominantly P-type receptors (Lee et al, 1982) while the latter would be classified as E-type in view of the much higher potencies of eledoisin and kassinin than of substance P and physalaemin.

In view of these receptor differences, and with the increasing use of substance P antagonists in investigating the role of tachykinins in neurotransmission, it is of some interest to quantify any receptor or tissue selectivity shown by such antagonists. Tabulated below are results from experiments with 3 substance P antagonists, of which the first and last have been compared extensively by ourselves in a number of smooth muscle preparations (Bailey et al, 1983, 1985) where in most they show roughly comparable pA_2 values (range 5.5-6.5). The second antagonist listed (Spantide) is a close analogue of the first which has been claimed to show certain advantages. In the present experiments ACh output could be estimated indirectly from mechanical activity in the preparation which under these conditions is largely ACh dependent, or more directly by [^{3}H]-ACh output which in the table is normalised with respect to the effect of 10^{-8}M eledoisin in each matched set of experiments.

ANTAGONIST	EFFECT ON [^{3}H]-ACh OUTPUT				pA_2 estimate
	ELE $[10^{-8}]$	ELE + ANTAG $[10^{-8}]+[10^{-5}]$	ELE + ANTAG $[10^{-7}]+[10^{-5}]$	ANTAG $[10^{-5}]$	(95% limits)
(D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11})SP	1.00 (n=8)	0.76 * (0.04)	1.07 (0.20)	0.29 $^+$ (0.10)	5.3 (5.1-5.5)
(D-Arg^1,D-Trp^{7,9},Leu^{11})SP	1.00 (n=3)	0.78* (0.04)	1.27 (0.43)	0.23$^+$ (0.09)	5.2 (4.9-5.1)
(D-Pro^4,D-Trp^{7,9,10})SP$_{4-11}$	1.00 (n=8)	0.88 (0.12)	2.34 (0.39)	0.49$^+$ (0.17)	<4.8

{(S.E.M.). sig. diff. ($P<0.05$) cf unity*, cf zero$^+$ }

It can be seen that the first two antagonists at 10 μM produce antagonism of eledoisin-evoked [^{3}H]-ACh release that was fully surmountable when the agonist concentration was increased tenfold, and on this basis pA_2 estimates could be made. However, the third antagonist was of markedly lower potency, in fact, in 3 out of 8 experiments it showed no significant antagonism so the pA_2 estimate is likely to be optimistic.

It is also evident from the table that at 10 μM all three antagonists showed considerable stimulatory activity. The mechanical estimate of ACh release largely corroborated both partial agonist and antagonism estimates.

Thus the antagonists demonstrate tissue selectivity, with the octapeptide showing markedly low activity on these neuronal receptors as compared to the smooth muscle receptors in this and several other preparations (although the guinea-pig bladder smooth muscle receptors are also remarkable for being resistant to this antagonist; Bailey et al, 1983,1985; Featherstone et al, 1985). Furthermore, partial agonist actions of these analogues are very marked on this neuronal system limiting the concentrations that may usefully be used.

The consequences of these findings are discussed in relation to the potential uses of the antagonists in the analysis of the role of tachykinins in neurotransmission.

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INTESTINAL KALLIKREIN AND CARCINOGENESIS IN RAT COLON: EFFECT OF DIET AND APROTININ

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Aprotinin, a proteinase inhibitor, has been reported to prevent the growth of tumour cells in vitro and in vivo (Fritz & Wunderer, 1983). Kallikrein-like proteinases are present in the mammalian gut (Frankish & Zeitlin, 1980), can stimulate mitotic activity in the gastrointestinal tract (Pisano, 1975) and have been isolated from rat tumours (Back & Steger, 1976). In this study, the relationship between kallikrein and carcinogenesis has been examined with particular reference to the effects of diet and kallikrein inhibition.

Male Swiss albino rats received either no drug or a daily s.c. injection of aprotinin (Trasylol, Bayer UK, 20,000 KIU/kg) or vehicle. One group received a high fat diet, the other groups received normal rat diet (Oxoid Ltd). Carcinogenesis was induced, 4 weeks after establishing each treatment regimen, by 12 weekly s.c. injections of azoxymethane (10mg/kg). Intestinal tissue kallikrein-like amidase (TKLA) was measured using a tissue kallikrein-selective tripeptide substrate (S-2266, Kabi Ltd) in the presence of excess soybean trypsin inhibitor to block interfering proteinases.

Table 1 The effect of diet and proteinase inhibition on mean tissue levels of TKLA (nMoles pNA.min^{-1}.g^{-1}) and on tumour induction in rat large bowel. Brackets indicate standard deviations.

Diet		Normal	Normal	Normal	High Fat
Treatment		-	Aprotinin	Saline	-
Number		6-12	6-11	4-5	4-10
	Wks.				
Caecum	4	176 (53)	136 (34)	174 (39)	118 (34)
	24	115 (61)	119 (21)	207 (51)	37 (23)
Dist.Colon	4	55 (10)	34 (21)	-	33 (13)
	24	34 (11)	19 (3)	23 (12)	23 (6)
Rectum	4	43 (23)	10 (4)	-	31 (7)
	24	22 (15)	19 (5)	23 (14)	20 (7)
Tumour Incidence	24	11/12	7/11	2/5	3/10

In healthy animals, the mean TKLA level fell progressively from caecum to rectum. The incidence of tumours was highest in the upper distal colon and was unexpectedly reduced ($P < 0.01$) in the group on the high fat diet when compared with that on a normal diet (Table 1). The high fat group also showed a significant reduction in TKLA in the upper distal colon (by 34%, $P < 0.05$). Aprotinin treatment had no significant effect on tumour incidence ($P > 0.05$). However, although aprotinin treatment caused marked reductions in distal colonic TKLA at 2 weeks (not shown) and 4 weeks (Table 1, $P < 0.05$), by week 24 levels did not differ significantly from those of the untreated animals.

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EFFECT OF UK-14,304 ON NERVE-INDUCED RESPONSES IN RAT GASTRIC FUNDUS

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Previously we reported that the selective α_2-adrenoceptor agonist UK-14,304 (Cambridge, 1981) inhibited cholinergic excitatory and non-adrenergic non-cholinergic (NANC) inhibitory responses in rat gastric fundus (Dettmar et al, 1984). The present experiments further examine these effects.

Strips of rat gastric fundus were prepared by the method of Vane (1957) and suspended in Krebs medium containing propranolol (2 μM) and guanethidine (5 μM). Atropine (2 μM) was also present in experiments investigating NANC nerve-induced responses. Tone was induced by the addition of $BaCl_2$ (0.1-2 mM) to the bathing fluid. Electrical field stimulation (EFS) was carried out via silver/silver chloride ring and hook electrodes (1 ms pulse width; 0.2-20 Hz; supramaximal voltage).

In low tone preparations, EFS produced frequency-dependent cholinergic nerve-induced contractions with a maximum contractile effect at 10 Hz. UK-14,304 (0.01-1 μM) produced a dose-related inhibition of these contractions with a maximum inhibitory effect occurring at frequencies less than 1 Hz. The UK-14,304 -induced inhibition was partially antagonized by idazoxan (0.03-0.3 μM) which returned the responses to around 80% of their control values. Prazosin (0.1 μM) had no effect on the inhibition induced by UK-14,304, nor did it produce any further reversal of the effect of UK-14,304 after idazoxan. Idazoxan alone (0.03-1 μM) had no effect on the cholinergic nerve-induced responses even when guanethidine was omitted from the Krebs solution.

In raised tone preparations, EFS produced frequency-dependent NANC nerve-induced relaxations, with the greatest relaxations occurring at 1 Hz. UK-14,304 (0.03-1 μM) produced a dose-related inhibition of these relaxations, with the maximum inhibitory effect occurring at frequencies below 1 Hz. Reversal of UK-14,304-induced inhibition by idazoxan (0.03-1 μM) was less marked than with cholinergic nerve-induced responses, with the responses returning to less than 70% of the control values. A similar reversal was shown with rauwolscine (0.03-1 μM). Prazosin (0.03-1 μM) had no effect on UK-14,304-induced inhibition, either alone or after idazoxan. Idazoxan alone (0.03-1 μM) had no effect on the NANC nerve-induced response.

In conclusion, UK-14,304 inhibits both cholinergic excitatory and NANC inhibitory nerve-induced responses in the rat gastric fundus by an α_2-adrenoceptor-mediated effect. Inhibition of both responses is most marked at low frequencies of stimulation. α_2-adrenoceptor agonists may thus affect the inhibitory as well as the excitatory component of gut reflexes. This may be important, as the inhibitory nerves are involved in the control of processes such as receptive relaxation in the stomach and the descending inhibition of peristalsis (Gillespie, 1982).

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HETEROGENEITY OF SOLUBLE SPLENIC α_2-ADRENOCEPTORS

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Work from a number of laboratories has indicated possible heterogeneity of α_2-adrenoceptors (Cheung et al., 1982; Feller & Bylund, 1984; Dickinson et al., 1985). It is presently unclear whether this relates to subtype, species or tissue differences. We have examined the pharmacological characteristics of α_2-adrenoceptors from human and rabbit spleen in order to determine whether differences observed in membrane preparations are maintained in solution.

A purified plasma membrane preparation was obtained from human and rabbit spleen (Dickinson et al., 1984). α_2-adrenoceptors were solubilised by incubating membranes with a digitonin containing buffer (1% digitonin, 50mM Tris, 0.5mM EDTA, 100mM NaCl, pH 7.5) for 1 hour at 4°C using a digitonin:protein ratio of 5:1. The pharmacological profile of the soluble α_2-adrenoceptors was examined using (^{3}H)-Yohimbine. Incubations were carried out in 0.5ml and bound and free ligand was separated by gel filtration through Sephadex G-50. K_i values are the means of 2-5 determinations.

	HUMAN		RABBIT	
	Membranes	Soluble	Membranes	Soluble
B_{max}	456 fmol/mg protein	885 fmol/ml	1020 fmol/mg protein	949 fmol/ml
K_D (nM)	1.6	2.3	7.3	7.9
K_i (nM)				
Yohimbine	2.3	1.2	10.3	6.9
WY 26392	4.0	4.4	49.0	75.0
Idazoxan	9.5	3.3	42.0	15.0
Phentolamine	9.9	19.3	18.6	43.0
Prazosin	1110.0	2865.0	5344.0	3655.0

The K_D of (^{3}H)-Yohimbine for the α_2-adrenoceptor was unchanged upon solubilisation. Moreover, the difference in affinity of (^{3}H)-Yohimbine between human and rabbit spleen was maintained. There may be small differences in the absolute potency of antagonists upon solubilisation due to possible conformational changes in the α_2-adrenoceptor. However, the relative potency of a compound for human and rabbit adrenoceptors is generally maintained. The α_2-selective antagonists idazoxan and WY 26392 exhibit marked selectivity for human α_2-adrenoceptors in both membrane and soluble preparations. These findings suggest that possible α_2-adrenoceptor heterogeneity derives from the structural characteristics of the receptor per se rather than its membrane environment. Whether these heterogeneities result from absolute conformational differences (Nahorski et al., 1985) or from differences in receptor structure remains to be determined.

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THE EFFECT OF COLD AND RESTRAINT ON ACTIVATION OF PRE- AND POST SYNAPTIC α-ADRENOCEPTORS IN THE RAT

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A combination of cold stress and restraint can produce a number of physiological changes including ulceration in the gastric mucosa of rats (Senay & Levine, 1967; Seyle, 1950), and activation of dopamine metabolism (Dunn & File, 1983). It has also been suggested that in arteries from cold-restrained rats, acetylcholine can inhibit the response to nerve stimulation to a greater extent than in non-stressed rats (Merrigan & Lias, 1981). Little is known, however, about the direct effect of cold restraint on alpha-adrenoceptors. The purpose of the present study was to examine the effect of cold stress and restraint on the activation of both $alpha_1$-and $alpha_2$-adrenoceptors in the thoracic aorta and vas deferens of the rat.

All animals used were taken from the same stock population of rats (Charles River males, 100-120g). Unrestrained rats were maintained in standard cages at room temperature and allowed free access to food and water except for a three hour period. Cold-restrained rats were placed in restraint cages to prevent movement and placed in a moderately cold room (4°C) for three hours. Thoracic aortae and vasa deferentia were removed from both groups of animals after this period; the aortae were prepared as spiral strips and the vasa deferentia were set up using a modification of the method of Drew (1977). Vasoconstrictor responses to stimulation of $alpha_1$-adrenoceptors of the thoracic aorta by methoxamine, noradrenaline and phenylephrine were obtained. Inhibition of electrically induced twitch responses of the vas deferens were obtained using clonidine, B-HT 933 and UK 14304. Agonist EC_{50} values were calculated in ng/ml and are shown in Table 1.

Table 1. The effect of cold stress and restraint on activation of pre-and post-synaptic alpha-adrenoceptors in the rat

	EC_{50} (SEM) FREE	STRESSED
RAT AORTA		
noradrenaline	0.23 (0.04)	3.70 (1.00)**
phenylephrine	0.73 (0.06)	2.32 (0.21)***
methoxamine	11.00 (3.67)	166.3 (25.25)***
RAT VAS DEFERENS		
clonidine	0.67 (0.14)	1.57 (0.20)**
B-HT 933	7.04 (3.99)	77.22 (17.76)**
UK 14304	0.69 (0.17)	4.29 (0.57)***

** $p < 0.01$ *** $p < 0.001$ n=4-6

The results indicate a decrease in sensitivity of both $alpha_1$-and $alpha_2$-adrenoceptors in cold stressed animals as seen by a significant increase in EC_{50} values over those obtained from 'free' rats. The results of the present study suggest a reduction in sensitivity of both $alpha_1$-and $alpha_2$-adrenoceptors in 'stressed' rats. It is possible that this reduction may be due to increased levels of circulating catecholamines that may occur under conditions of stress, producing desensitization of these receptors to their agonists. We thank Miss D Beattie for the production of stressed animals.

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THE EFFECT OF AGE ON ACTIVATION OF PRE- AND POSTSYNAPTIC α-ADRENOCEPTORS IN THE RAT

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Several reports suggest that alpha-adrenoceptor mediated responses may be decreased with age (eg. Docherty & Hyland, 1984), whereas others suggest no change (eg, Wyse et al, 1977). The present study was designed to examine the effect of increasing age on the responsiveness of post-synaptic $alpha_1$ and pre-synaptic $alpha_2$-adrenoceptors of the rat to selective agonists and antagonists using the thoracic aorta and vas deferens.

Aortae were prepared as spiral strips and vasa deferentia were prepared by a modification of the method of Drew (1977). The selective $alpha_1$-adrenoceptor agonist methoxamine and antagonist indoramin were used to assess the effects of age on the postsynaptic $alpha_1$-adrenoceptors of the thoracic aorta. The selective $alpha_2$-adrenoceptor agonist clonidine and antagonist Wy 26392 (Lattimer et al, 1984) were used to assess age-related changes in responses of the presynaptic $alpha_2$-adrenoceptors of the vas deferens.

Agonist EC_{50} values (ng/ml) were calculated for each type of receptor for rats of 5, 10, 20 and 50 weeks of age, together with antagonist pA_2 values and slopes. The data are shown in Table 1.

Table 1. Effect of age on $alpha_1$-and $alpha_2$-adrenoceptor agonists and antagonists

Tissue	Agonist/Antag.	5 weeks	10 weeks	20 weeks	50 weeks
Aorta	Methoxamine EC_{50}ng/ml(SEM)	11.7(2.67)	48.3(8.3)**	130(18.3)***	135(51.7)**
	Indoramin	7.69	7.54	7.55	7.83
	pA_2(95% limits)	(7.46-7.98)	(7.34-7.77)	(7.26-7.95)	(6.95-8.48)
	slope (SEM)	0.95(0.13)	0.92(0.11)	1.01(0.20)	0.66(0.50)
	r	0.99	0.99	0.97	0.75
Vas Deferens	Clonidine	0.67	0.83	1.11**	2.72***
	EC_{50} ng/ml(SEM)	(0.14)	(0.10)	(0.06)	(0.32)
	Wy 26392	8.39	8.49	7.92	8.43
	pA_2(95% limits)	(7.84-9.63)	(8.18-8.92)	(7.66-8.32)	(8.17-8.74)
	slope (SEM)	1.18(0.46)	1.36(0.24)	1.22(0.26)	1.21(0.16)
	r	0.92	0.98	0.97	0.96

** $p < 0.01$; *** $p < 0.001$ from 5-week values (unpaired t-test) n=4-8

The results demonstrate an age-related decrease in sensitivity of both $alpha_1$- and $alpha_2$-adrenoceptors to their respective agonists as indicated by an increase in EC_{50} values. There was little or no change in the potency of the antagonists. The data obtained would therefore be consistent with a reduction in the number of receptors available for activation as the age of rats increases.

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AGONIST AFFINITY FOR α-ADRENOCEPTORS

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With the subclassification of the α-adrenoceptor into α_1- and α_2-adrenoceptor subtypes, agonist selectivity has been expressed purely in terms of potency with few studies concentrating on determining affinities. In the present study the affinity of a series of α-adrenoceptor agonists for α_1- and α_2-adrenoceptor subtypes has been determined using direct ligand binding studies and also 'in vitro' using the bisected vas deferens (Michel & Whiting, 1981) in an attempt to determine agonist selectivity in terms of affinity.

Ligand binding studies were performed using EDTA washed (Cheung et al, 1982), rat cerebral membranes assayed at 25°C in a 50 mmol.litre^{-1} Tris 0.5 mmol.litre^{-1} EDTA assay buffer (pH 7.4). [^{3}H]-yohimbine and [^{3}H]-prazosin were used to label α_2- and α_1-adrenoceptors respectively. The phasic contracture of the epididymal portion of the vas deferens was used as the index of α_1-adrenoceptor stimulation, whereas inhibition of the Is (Brown et al, 1983) response of the prostatic portion of this tissue was the index of α_2-adrenoceptor stimulation. Agonist affinities at these receptors were determined using the receptor inactivation method of Furchgott (1966).

In ligand binding studies agonists displaced both [^{3}H]-prazosin and [^{3}H]-yohimbine with Hill coefficients lower than unity. To determine agonist affinities in these cases, low affinity agonist binding was resolved using iterative curve fitting techniques or by addition of 5'guanylylimidodiphosphate (0.1 mmol.litre^{-1}) and NaCl (120 mmol.litre^{-1}) to the assay buffer.

A comparison of agonist affinities determined at α_1- and α_2-adrenoceptors using the two methods is shown in Table 1. The pK_I ad pK_A values are respectively the negative logarithms of the K_I (ligand binding) and K_A (vas deferens) values.

Table 1

	α_1		α_2	
	pK_I	pK_A	pK_I	pK_A
xylazine	4.46	4.21	6.17	6.25
clonidine	5.93	5.30	7.41	7.65
guanfacine	5.28	5.15	7.25	7.28
noradrenaline	5.32	5.15	5.27	5.33*
phenylephrine	5.00	4.88	4.47	nd
amidephrine	4.50	4.69	4.58	nd
methoxamine	4.29	4.21	4.89	nd

*Determined in the presence of 3×10^{-7} mol.litre^{-1} prazosin.

While it was not possible to determine agonist pK_A values for all compounds at the α_2-adrenoceptor, a comparison of pK_I and pK_A values obtained at this receptor indicate a good correlation between binding data and pK_A values determined by receptor inactivation. When pK_I values at the α_1- and α_2 adrenoceptor were compared, xylazine, guanfacine and clonidine were selective α_2-adrenoceptor agonists. Noradrenaline, phenylephrine, methoxamine and amidephrine were non-selective α-adrenoceptor agonists.

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DIFFERENCES IN THE SENSITIVITY OF REGIONS OF THE CANINE SAPHENOUS VEIN TO α_1- AND α_2-ADRENOCEPTOR SELECTIVE AGONISTS

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Differences in sensitivity to α-adrenoceptor agonists between veins from the dog have been reported (Shoji et al, 1983). In this study, the sensitivity of two regions of one vessel, the canine saphenous vein (reported to possess both α_1- and α_2-adrenoceptors, Sullivan and Drew, 1980) to the contractile effect of α-adrenoceptor agonists was recorded.

The lateral saphenous vein was removed from beagles (9-16kg) of either sex under pentobarbitone (35 mg/kg) anaesthesia. A section (1.5cm) was cut from the popliteal region (proximal) and from the cranial branch (distal). Helical strips from each were suspended in organ baths (6ml) in Krebs solution (37°C, 5% CO_2 in O_2) with or without catecholamine uptake blockers and propranolol. Isotonic contractions (0.5g load) were recorded to cumulative additions of an α_1-agonist, methoxamine or phenylephrine (in the presence of propranolol) or an α_2-agonist, UK-14304, or B-HT 933. A concentration-response curve was obtained 1h after setting up the tissues; a second curve was constructed following a 45 min wash period. The response to 80mM KCl was then established.

Table 1. Concentration (μM) of α-adrenoceptor agonists giving a response of 30% that to 80mM KCl in the proximal and distal canine saphenous vein

Agonist	Sex	n	Proximal (1)	Distal (1)	Proximal (2)	Distal (2)
Methoxamine	M	7	1*.17±0.237	0.51±0.099	1.08±0.166	1.22±0.243
Methoxamine[a]	F	6	3.93±0.342	2.66±0.448	3.90±0.448	4.11±0.881
Phenylephrine	F	4	0.70±0.109	0.35±0.050	0.95±0.098	0.87±0.049
UK-14304	F	7	0*.010±0.0020	0.006±0.0016	0.012±0.0022	0.008±0.0013
B-HT 933	M	6	2*.02±0.661	0.72±0.138	2*.26±0.468	1.39±0.248
B-HT 933	F	8	2**.00±0.193	0.58±0.118	2**.70±0.651	1.15±0.147
B-HT 933[a]	M	6	2*.01±0.349	0.83±0.073	2*.70±0.404	1.46±0.128

Mean values ± s.e. mean are shown, a- with propranolol ($4x10^{-6}$M), desipramine ($5x10^{-7}$M) and corticosterone ($4x10^{-5}$M). n=number of tissues. *p<0.05, **p<0.01 (Wilcoxon test to compare proximal and distal groups). Curve number shown in ().

Proximal and distal strips contracted by 136.6±8.09 and 106.3±4.47 mm respectively in males (n=18 tissue pairs, 18 dogs) and 145.0±11.74 and 137.1±10.19 mm respectively in females (n=14 tissue pairs, 14 dogs) in the presence of 80mM KCl. Strips from the distal region were more sensitive to α_1-and α_2-agonists than those from the proximal region during curve 1 (Table 1). In curve 2 the difference was less obvious with B-HT 933 and absent with methoxamine. The differences in sensitivity were observed with B-HT 933 but not with methoxamine in the presence of uptake blockers and propranolol. It is thus, unlikely that differences in sensitivity to methoxamine reflect density of α_1-adrenoceptors;however, the differences to B-HT 933 may be due to greater α_2-adrenoceptor density in the distal region of the vessel. In the presence of the uptake blockers and propranolol there was an increase in the ED_{30} for methoxamine but not to B-HT 933; this may be related to α_1-adrenoceptor antagonism by desipramine.

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ADRENOCORTICAL FUNCTION IN RATS DURING ANAESTHESIA WITH ETOMIDATE, METHOHEXITONE OR PROPOFOL

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Ledingham and Watt (1983) suggested that the use of etomidate, to produce sedation in patients with multiple injuries, might be associated with an increase in mortality, possibly from adrenocortical hypofunction. Etomidate has been shown to reduce plasma corticosterone concentration in rats (Preziosi and Vacca, 1982) and in vitro studies have demonstrated inihibition of steroidogenesis (Lambert et al., 1983).

Propofol (2,6-disopropylphenol) ('Diprivan') is a new i.v. anaesthetic (Glen and Hunter, 1984). The present study was performed to compare the effects of propofol on adrenocortical function with those of etomidate and the barbiturate anaesthetic, methohexitone.

Groups of 8 female rats (190-210g) were anaesthetised with an injection of etomidate, methohexitone or propofol. Anaesthesia was maintained with an infusion of the same agent. Sixty minutes after induction of anaesthesia the rats were given 25µg ACTH ('Synacthen') subcutaneously and anaesthesia was maintained for a further 30 minutes. Animals were killed by decapitation and truncal blood collected. Serum corticosterone was measured by radioimmunoassay and results are given in Table 1.

Table 1 Corticosterone levels in anaesthetised rats following saline and Synacthen challenge

Anaesthetic	Maintenance dose mg kg^{-1} min^{-1}	Serum corticosterone µg dl^{-1}	
		saline	Synacthen
Etomidate	0.38 ± 0.003	4.71 ± 3.83 (8)	4.28 ± 0.69 (8)
Methohexitone	1.83 ± 0.04	31.5 ± 17.13*(8)	63.0 ± 26.35*(8)
Propofol	1.02 ± 0.03	6.9 ± 5.05 (6)	56.85 ± 16.99*(8)

Mean values ± S.D.: (n)
* Significant difference from etomidate $P < 0.001$
Student's unpaired 't' test

A reduced corticosterone response to ACTH was observed in rats anaesthetised with etomidate. No such decrease was observed following administration of methohexitone and propofol. The latter two anaesthetics are therefore unlikely to produce adrenocortical hypofunction in a stress situation.

Corticosterone antiserum was generously donated by Dr. David Kime (Sheffield University).

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DISSOCIATION BETWEEN REPERFUSION ARRHYTHMIAS AND INCREASES IN VENTRICULAR α_1 RECEPTOR DENSITY IN THE ANAESTHETISED RAT

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It has been suggested previously (Corr et al, 1981) that the number of alpha-1 receptors in the ischaemic myocardium may double during a 30 minute period of coronary artery occlusion. It has been further argued that this increase in alpha receptor number may lead to enhanced alpha receptor responses and this in turn has been suggested to be a critical factor in the genesis of reperfusion-induced arrhythmias. This hypothesis would account for the reported ability of alpha-blocking agents to reduce reperfusion arrhythmias (Sheridan et al., 1980). However, some investigators have questioned the specificity of these effects (Thandroyen et al., 1983).

In view of this controversy, we have assessed in the in vivo anaesthetized rat (i) whether the density of alpha-1 adrenergic receptors increases during coronary artery occlusion; (ii) whether any change in density can be associated with the onset of reperfusion-induced ventricular fibrillation; and (iii) whether alpha-1 blockade with prazosin modifies the incidence of reperfusion-induced ventricular fibrillation. The incidence of fibrillation upon reperfusion after 3,5,10 and 30 minutes occlusion was 20,75,50 and 8% (n=12 in each group). Alpha-1 receptor density was measured using [^{3}H]-prazosin in non-ischaemic and ischaemic zones of pooled samples obtained after 0, 5 and 30 minutes ischaemia. At least 3 separate Scatchard analyses were performed at each time point. Alpha-1 receptor density was not significantly altered at the time of maximum incidence of reperfusion-induced ventricular fibrillation (5 minutes occlusion) but did significantly increase in both non-ischaemic and ischaemic zones after 30 minutes occlusion when the incidence of fibrillation was very low (8%) (17.0$\pm$2.3 and 18.4$\pm$0.6 fmoles/mg protein in non-ischaemic and ischaemic zones after 30 minutes occlusion compared to 10.7$\pm$0.6 and 12.8$\pm$1.0 fmoles/mg protein in sham-operated control animals; $p<0.05$ in both cases). In addition, prazosin, (at either 0.1 or 1.0 mg/kg body wt IV, 5 minutes prior to coronary occlusion) did not alter significantly the incidence of ventricular fibrillation (75% in controls compared to 58% and 67% in the prazosin-treated groups), ventricular tachycardia (92% in all groups) or total number of premature ventricular complexes (947$\pm$349 in controls compared to 742$\pm$189 and 587$\pm$191 in the prazosin-treated groups) upon reperfusion.

We conclude that ischaemia-induced changes in alpha-1 receptor density do not parallel changes in vulnerability to reperfusion-induced arrhythmias. This dissociation is further indicated by the apparent inability of prazosin to alter the rhythmic consequences of reperfusion. We would therefore suggest that, in the rat, alpha-1 receptor activation and/or changes in alpha-1 receptor density are not involved in the genesis of reperfusion-induced ventricular fibrillation.

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EFFECTS OF 5-HYDROXY-PROPAFENONE ON GUINEA-PIG ATRIAL AND VENTRICULAR MUSCLE FIBRES

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Propafenone is a new antiarrhythmic drug effective in the treatment of both ventricular and supraventricular arrhythmias. Most of its effects can be attributed to a membrane stabilizing (class I) action. Recently, 5-hydroxy-propafnone (5-OH-P) was identified as the main unconjugated metabolite in human plasma (Kretzschmar et al, 1983). The present study was carried out to examine the electrophysiological effects of 5-OH-P on isolated guinea-pig cardiac fibres.

Spontaneously beating right atria and electrically driven left atria and ventricular papillary muscles were set up in a chamber and perfused with Tyrode solution gassed with 95%O_2-5%CO_2. Transmembrane action potentials (APs) were recorded with glass microelectrodes.

In atrial muscle fibres 5-OH-P ,10^{-8}M - $5x10^{-5}$M, produced a dose-dependent decrease in atrial rate, peak contractile force and df/dtmax, reduced atrial excitability and shortened the time to peak tension and time for total contraction. However, it only slightly modified the sinus node recovery time. 5-OH-P also shifted the dose-response curve for chronotropic and inotropic effects of isoproterenol to the right and inhibited the amplitude of the slow contractions elicited by isoproterenol in K (27 mM)-depolarized atrial fibres.

In papillary muscles, 5-OH-P (10^{-7}M - $5x10^{-5}$M) produced a dose-dependent decrease in amplitude and maximum rate of rise (Vmax) of the upstroke and at concentrations higher than 10^{-6}M depolarized the resting membrane potential. At concentrations between 10^{-7}M and 10^{-5}M, 5-OH-P shortened the duration of the AP (APD) at both 50% and 90% level of repolarization. The changes in APD exceeded those in the effective refractory period (ERP). Thus, 5-OH-P prolonged the ERP/APD ratio, even when it did not modify the recovery time (RT) or the ERP/RT ratio. 5-OH-P ,10^{-6}M - $5x10^{-5}$M) also reduced the amplitude and Vmax of the upstroke and shortened the duration of the slow APs induced by isoproterenol in muscles perfused with high K (27 mM) Tyrode solution.

All these effects are similar to those described with propafenone which confirm that 5-OH-P is an active metabolite which also exhibits membrane stabilizing (class I) actions and suggest that it could be responsible, at least partly, for some of the effects previously attributed to propafenone.

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EFFECTS OF OXODIPINE ON VASCULAR SMOOTH MUSCLE FIBRES

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Oxodipine (OXD), |4-2,3-methylen-dioxyphenyl)-1,4-dihydro-2,6-dimethyl,3-carboxyethyl-5-carboxymethyl-pyridine|, is a new dihydropyridine derivative which chemically is most similar to PY 108-068 and in contrast to nifedipine is not photolabile (Verde et al., 1984). The purpose of this study was to examine the effects of OXD on contractile responses induced by various stimulus on peripheral and cerebral vascular smooth muscles. The effects of OXD on ^{45}Ca movements were also evaluated.

Experiments were undertaken on rat aortic strips and portal vein segments and on rings of rabbit aortae, femoral and basilar arteries. Tissues were set up in organ baths containing Krebs solution (KB) maintanied at 34ºC and gassed with 95%O_2-5%CO_2. ^{45}Ca movements were determined in rat aortic strips placed in Hepes buffer (pH 7.3) gassed with 100% O_2 as previously described (Barrigón et al. 1984).

Concentrations of OXD (M) producing 50% inhibition of the maximal contractile response (IC_{50}) induced by noradrenaline (NA, 10^{-5}M), KCl (80 mM) and 5-hydroxytryptamine (5-HT, 10^{-5}M) in rat and rabbit arteries are shown in table 1.

Table 1. IC_{50} values (M) for inhibition of contractile responses induced by varios stimulus.

species/artery	NA	KCl	5-HT
rat/aortae	$3.0\pm1.0 \times 10^{-6}$	$8.0\pm2.0 \times 10^{-9}$	$> 10^{-6}$
rabbit/aortae	$> 10^{-6}$	$8.5\pm3.0 \times 10^{-9}$	$> 10^{-6}$
rabbit/femoral	$> 10^{-6}$	$8.0\pm1.0 \times 10^{-9}$	$> 10^{-6}$
rabbit/basilar	$> 10^{-6}$	$7.5\pm1.3 \times 10^{-9}$	$5.7\pm2.8 \times 10^{-8}$

OXD also inhibited Ca-induced contractile responses in rat aortic strips incubated in Ca-free high K (80 mM) KS ($IC_{50}=4.5\pm2.5 \times 10^{-9}$M) and suppressed the spontaneous myogenic activity in rat portal veins ($IC_{50}=2.5\pm1.2 \times 10^{-8}$M).

In rat aortic strips OXD (10^{-6}M) significantly reduced NA-induced ^{45}Ca influx but not ^{45}Ca efflux. However, KCl-induced ^{45}Ca influx was dose-dependently reduced by OXD at concentrations (10^{-8}M - 10^{-6}M) lower than those required to antagonize NA actions.

All these results suggest that the effects of OXD on vascular smooth muscle fibres can be related to blockade of Ca entry through channels opened during membrane depolarization or receptor-response coupling.

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EFFECTS OF THYROXINE ON THE FREQUENCY-FORCE RELATIONSHIP OF ISOLATED AND PACED LEFT ATRIA OF GUINEA-PIGS

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Measurements of the frequency-force relationship were made in left atria from control and hyperthyroid guinea-pigs, treated daily for 8 to 12 days prior to sacrifice with either normal saline or thyroxine 100 µg/kg body weight i.p. respectively. Contractile force was measured with the atria suspended in a bath of modified Krebs Henseleit solution of the following composition (mM): NaCl 118, KCl 4.69; $MgSO_4$ 0.45; KH_2PO_4 1.18; $NaHCO_3$ 25; glucose 25 and $CaCl_2$ 2.5 and gassed with 95% O_2 + 5% CO_2 at 37°C. The atria were paced with 10ms electrical pulses at rates from 0.1 to 6 Hz.

Atria from both control and hyperthyroid guinea-pigs showed a decrease in contractile force as the rate of stimulation was increased but this decrease was less pronounced in hyperthyroid atria. At low rates of stimulation (0.1 to 2 Hz) the contractile force was greater in atria from control guinea-pigs but at high rates of stimulation (4 to 6 Hz) the contractile force was greater in hyperthyroid atria. The contractile force observed at 3 Hz was similar in control and hyperthyroid preparations (Figure 1). Thus thyroxine seemed to have a negative inotropic effect at low rates of stimulation and a positive inotropic effect at higher rates. At 5 Hz, which is the normal heart rate of guinea-pigs, thyroxine increased contractile force.

Propranolol hydrochloride (1 µM) depressed the magnitude of contractile force in atria from both control and hyperthyroid guinea-pigs at all the rates studied. In both groups the negative inotropic effect was greater at higher rates of stimulation. Noradrenaline bitartrate (3 µM) produced an increment in contractile force in atria from control and hyperthyroid guinea-pigs. At the higher rates of stimulation the increment was greater in the hyperthyroid preparations than in the controls. Acetylcholine bromide (4 µM) decreased the contractile force in atria from both control and hyperthyroid guinea-pigs. The extent of this inhibitory effect was similar for both groups.

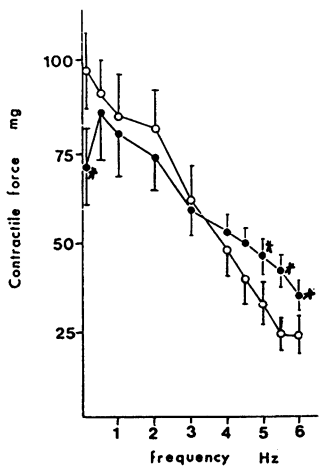

Figure 1. Relationship of frequency to contractile force. Control (open symbol; n=9) and hyperthyroid (closed symbol; n=12). Vertical lines represent s.e. mean value and asterisks indicate a significant difference from the value in the corresponding control preparation($P<0.05$)

EFFECT OF TOPICAL SALBUTAMOL ON EPIDERMAL PROLIFERATION

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The topical application of isoprenaline has been reported to be an effective treatment for psoriasis and to cause a concomitant reduction in the glycogen content of the lesions to normal levels (Das et al, 1978). To investigate whether selective β_2-adrenoceptor agonists might also be effective in psoriasis, we have determined the effect of topical salbutamol on epidermal mitosis in the hairless mouse.

Epidermal proliferation was induced in dorsal skin by cellophane tape-stripping an area of skin 5-7 cm^2 until it just glistened (Clausen & Lindmo, 1976). 4 h prior to killing, the mice were injected with Colcemid (1 mg/kg i.p.) to arrest mitotic cells in metaphase. Samples of normal and tape-stripped dorsal skin were prepared for histological assessment, at least 10 alternate sections from each sample of skin being examined. The number of metaphase-arrested cells in the interfollicular epidermis were counted and expressed as the number of mitotic cells cm^{-1} epidermis. Maximal mitotic counts were obtained 24 h and 48 h after tape-stripping, at these times counts being increased 238 ± 48% and 239 ± 52% respectively (mean ± s.e.mean, n = 6).

The effects of salbutamol on epidermal proliferation were evaluated 24 h after tape-stripping. Salbutamol (50 µg in ethanol) was applied to a 6 mm diameter area of tape-stripped skin at various times prior to sacrifice. Mitotic counts were obtained for samples of normal skin, tape-stripped skin and tape-stripped salbutamol treated skin from each mouse. Maximal inhibition of epidermal mitosis occurred when salbutamol was applied 4 h prior to sacrifice (Table 1). When salbutamol was applied at earlier times, mitosis in untreated samples of normal or tape-stripped skin was also reduced. These results indicate that the topical application of salbutamol might be effective in treating proliferative skin diseases.

Table 1. Effect of salbutamol on the number of metaphases cm^{-1} epidermis (mean ± s.e.mean).

Time of application of salbutamol prior to sacrifice	Normal skin site untreated	Stripped skin site untreated	Stripped skin site treated	n
4h (vehicle only)	4.4 ± 2.3	10.3 ± 5.4	10.6 ± 6.0	6
4h	3.2 ± 0.8	9.1 ± 2.0	1.9 ± 0.5	8
6h	2.3 ± 0.4	5.2 ± 0.5	2.9 ± 0.5	8
10h	2.5 ± 0.6	5.7 ± 0.8	6.0 ± 1.1	8
24h	2.4 ± 0.7	5.8 ± 0.8	6.0 ± 0.9	8

We thank the Psoriasis Association for financial support, Glaxo for supplies of salbutamol and Mr. R. Bone, Histology Dept., St. Mary's Hospital, Portsmouth for the use of facilities.

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NICARDIPINE: BENEFICIAL EFFECT ON CARDIAC FUNCTION AND ENERGY CONSERVATION DURING CORONARY REPERFUSION OF THE RAT WORKING HEART

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It has been shown that calcium plays an important role in the cardiac dysfunction associated with ischaemia and reperfusion of the heart and that some calcium entry blocking agents decrease the injury incurred (Fitzpatrick and Karmazyn, 1984). We have now studied the effect of nicardipine on the malfunction produced by coronary ligation and reperfusion using rat isolated working hearts.

Hearts were perfused via the pulmonary vein with Kreb's solution (Ca^{++} 2.5 mM), gassed with 95% O_2 and Co_2 and heated to 37°C as described previously (Armstrong and Ferrandon, 1985). Perfusion was maintained at a preload of 9 mmHg and an afterload of 59 mmHg. Cardiac function and cellular integrity was assessed from measurements of left ventricular and aortic pressure and their derivatives and from aortic flow together with coronary flow and the lactate dehydrogenase released into it (LDH). A ligature was placed around the origin of the left descending coronary artery incorporating a plastic block of 4 mm width which was removed 15 min later to allow reperfusion of the ventricle. In some experiments nicardipine (1 or 10 nM) was added to the perfusion fluid 10 minutes prior to closing the ligature. Stores of high energy phosphates contained in frozen segments of the ventricle served by the coronary artery were assessed by calculating the energy charge ([ATP] + [0.5 ADP]) / ([ATP] + [ADP] + [AMP]). Cardiac efficiency (%) was assessed using the formula : aortic flow x left ventricular systolic pressure x 0.0000136 / oxygen extracted by the heart. The mean values (± sem) given below are from groups of 11 hearts and the differences between means significant ($p < 0.05$) as assessed using Student's t-test.

Initially, the baseline aortic flow was 45 ± 1 ml/min. After ligation it decreased by 60 ± 5% and after reperfusion it decreased by 95 ± 5%. In parallel heart preparations perfused with nicardipine (10nM) aortic flow was 40 ± 1 ml/min. After ligation it was less by 65 ± 5% and after reperfusion by 63 ± 10%. Adenylate charge was initially 0.72 ± 0.05 but this decreased to 0.52 ± 0.03 after reperfusion without nicardipine and to 0.67 ± 0.03 with nicardipine (10nM). Lactate concentrations in ventricular muscle increased from 19 ± 6 nM/ mg protein to 54 ± 5 nM/ mg protein after reperfusion but in nicardipine-perfused hearts it increased to only 28 ± 4 nM/ mg protein. The LDH released into the coronary effluent was initially 25 ± 4 uU/min and this increased after ligation to 145 ± 31 uU/min. In the presence of nicardipine (10 nM) LDH increased less to 68 ± 9 uU/min. During reperfusion without nicardipine, the LDH release was 203 ± 25 uU/min and with nicardipine (10 nM) it was 97 ± 17 uU/min. Cardiac efficiency decreased from 22 ± 3% by 55 ± 4% after ligation and by 97 ± 1% after reperfusion. In hearts with nicardipine (nM), efficiency was decreased after ligation by 56 ± 5% and on reperfusion it was less by 70 ± 10%. Ventricular arrhythmias were observed in each of 11 hearts reperfused without nicardipine but with nicardipine (10nM) these occurred in only 4 of 11 hearts.

These findings show that nicardipine reduces the severity of the cardiac dysfunction and the reduction in cellular integrity that follows coronary ligation and reperfusion and allows high energy phosphate stores to increase.

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THE USE OF VERAPAMIL TO ANALYSE ALTERATIONS IN Ca^{2+} ACTIVATION OF RAT AORTA DURING AND FOLLOWING AN ACUTE HYPOXIC EPISODE

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Ebgeibe (1982), using isolated rabbit aorta, showed that the depression and recovery of noradrenaline (NA) induced contractions seen with an acute hypoxic episode and subsequent re-oxygenation, was coincident with a reduction and subsequent recovery of Ca^{2+} entry. We have investigated this phenomenon in isolated rat aorta by determining the sensitivity of the various components to verapamil (VER). In these experiments, the responses of the rat aorta have been compared with similar ones produced in the perfused mesenteric bed.

Circular preparations of thoracic aortae and isolated mesenteric beds (RMB) (McGregor, 1965) were removed from male Wistar rats (200-280 g). Aortae were suspended under 3 g resting tension and RMB perfused at ∿4ml/min with Krebs' solution (PSS) maintained at 37 °C, gassed initially with 5% CO_2 in O_2 (pO_2 ∿390 mmHg). Control responses of the aorta and RMB (both ∿90% maximum) were obtained to 1μM NA and perfusion of 50μM NA respectively (normoxic responses). Responses of the aorta and RMB were then elicited during exposure to acute (30 min) hypoxia (hypoxic response) induced by gassing PSS with 5% CO_2 in N_2 (pO_2 ∿78 mmHg). Stimulated tissues were subsequently re-oxygenated by rapidly changing to the 5% CO_2 in O_2 gassing mixture (re-oxygenated response). The effects of exposure to VER (10 nM-10μM) on hypoxic and re-oxygenation responses of both aorta and RMB were then examined.

30 min hypoxia depressed the NA induced contraction to 72.0±2.7% (n=168) and following re-oxygenation the contraction 'returned' to 102.7±3.6% (n=168) of control normoxic responses. Concentration dependent reductions of normoxic, hypoxic and recovery contractions to NA were achieved by VER (10nM-10μM). 10μM VER produced a 50.2±6.1% (n=6), 55.5±6.7% (n=12) and 71.2±4.0% (n=12) reductions of normoxic, hypoxic and recovery contractions respectively. Therefore the verapamil insensitive components of contraction expressed as a percentage of the normoxic contraction were 49.8±6.1% (n=6), 32.0±6.6% (n=12) and 29.6±4.6% (n=12) for normoxic, hypoxic and recovery contractions respectively. Thus it appears that the recovery of contraction on re-oxygenation is dependent entirely on a verapamil sensitive calcium entry. We have previously shown (Downing et al, 1985) that EGTA-resistant contractions (Ca^{2+} release) are unaffected by acute hypoxia, thus the depression of NA contractions by acute hypoxia appears to be due to a decrease in both a VER sensitive and a VER insensitive entry of Ca^{2+}. The perfused mesenteric bed was more sensitive to VER, 10μM producing a 68.3±4.8% (n=6) reduction of normoxic responses. Surprisingly, hypoxia had no effect on the pressor responses to NA, and the inhibitory effect of VER was unchanged (69.5±4.8% n=6 using 10μM VER). There was no detectable change in tension on re-oxygenation nor any change in sensitivity to VER (69.6±2.5% n=6 using 10μM VER). The reasons for the insensitivity of the mesenteric bed to an acute hypoxic episode are unknown.

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FLUFENAMIC ACID PROMOTES THE UPTAKE OF CALCIUM ACROSS THE HUMAN RED BLOOD CELL PLASMA MEMBRANE

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The non-steroidal anti-inflammatory agents (NSAIDs), flufenamic acid and diflunisal have been shown to promote the release of calcium from isolated mitochondria (McNamee et al, 1985). In the intact cell this action would lead to an increase in cytosolic calcium, provided that the accumulated calcium was not removed to the extracellular fluid via a transport system located in the plasma membrane. To investigate this possibility, we have examined the effect of two NSAIDs on the movement of calcium across the plasma membrane of human red blood cells. This model system was chosen for three reasons: (i) the plasma membrane pumps calcium out of the cell against a concentration gradient; (ii) the red blood cell does not possess any calcium accumulating organelles; (iii) the enzymes catalysing oxidative phosphorylation are absent, thus avoiding any complications with the uncoupling action of the NSAIDs.

Human red blood cells were washed three times, aged for 12 hr and loaded with orthophosphate, to ensure that the cells were metabolically depleted. Cell suspensions (5 ml; 10% haematocrit) were incubated at 4°C in a medium containing 2.1 mM 45-calcium chloride (10 nCi/μmol), 50 μM ouabain and flufenamic acid or diflunisal. Calcium transport was initiated by transferring the reaction tubes to a water bath at 37°C. After incubation (0.5-40 min), the cells were washed and haemolysed, and the radioactivity of the deproteinised haemolysate was estimated using a Beckman LS 7500 Scintillation Counter.

Control experiments established the existence of a time-dependent uptake of calcium into the metabolically depleted red blood cells, with maximal uptake (24 ± 0.8 μmol/l cells) occurring at 30 min. A 20 min incubation period was used in all subsequent experiments. In the presence of flufenamic acid (10-140 μM), there was a concentration-dependent stimulation of calcium uptake. Maximal stimulation (313 ± 7.5 μmol/l cells) was obtained at 120 μM flufenamic acid (EC50 = 83.7 ± 0.8 μM). Similar results were obtained with diflunisal; maximal stimulation (185 ± 12.5 μmol/l cells) was found at 120 μM, with an EC50 value of 91 ± 1.5 μM.

NSAIDs are therefore capable of promoting the uptake of calcium ions across the plasma membrane of the human red blood cell, at concentrations within the range of those found in the plasma after administration of the drugs. The cytosolic levels of calcium will be increased as a result of this effect, which could lead to an augmentation of the calcium releasing action of NSAIDs on mitochondria in other cell types.

McNamee, P.M. et al. (1985) Biochem. Soc. Trans., 13, 228.

506P

ENDOTHELIUM-DEPENDENT, CALCIUM-INDUCED RELAXATION OF VASCULAR SMOOTH MUSCLE

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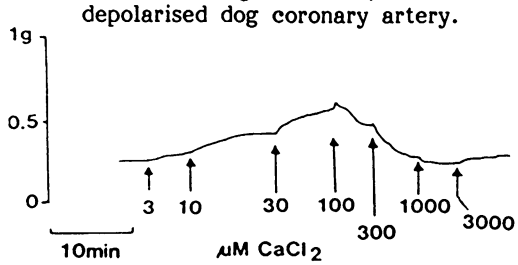
Depolarisation of smooth muscle caused by an elevated extracellular K^+ concentration, opens "voltage-operated channels" (VOC's) through which calcium (Ca^{++}) can enter the muscle cell and cause contraction (e.g. guinea-pig taenia coli, Spedding, 1982). However, I have recently carried out experiments using rings of vascular smooth muscle which suggest that the effect of Ca^{++} may be more complex.

Rings of freshly removed (10 mins post mortem) left anterior descending beagle coronary artery were suspended at a tension of 0.3g in a depolarising, nominally Ca^{++}-free Tyrode solution of the following composition (mmol/l): NaCl 97; KCl 40; $NaHCO_3$ 11.9; NaH_2PO_4 0.4; glucose 5.5. The preparations were maintained at 37°C and gassed with 5% CO_2 in oxygen.

Fig 1 Effect of Ca^{++} on contractile tension in rings of fresh, depolarised dog coronary artery.



Calcium was added cumulatively and changes in contractile tension were measured isometrically. Low concentrations of Ca^{++} (3-100 μM) caused concentrated related increases in contractile tension. However, higher concentrations of Ca^{++} (100 or 300-1000 μM) caused rapid decreases in contractile tension, sometimes to pretreatment levels (Fig 1). The relaxations were abolished when the endothelium was deliberately damaged by the gentle insertion of a stainless steel needle into the lumen of the vascular ring. Ca^{++} (3-3000 μM) then caused only concentration-related contractions.

In separate experiments, histological examination revealed that preparations which were deliberately damaged and only contracted in response to added Ca^{++} (3-3000 μM) contained very few endothelial cells. The endothelium appeared mostly intact in those preparations which were not rubbed and relaxed over the Ca^{++} concentration range 100-1000 μM.

The Ca^{++} relaxations were unaffected by indomethacin ($1x10^{-6}$M) or atropine ($1x10^{-6}$M) ruling out the involvement of cyclo-oxygenase products or an involvement of endogenous acetylcholine, respectively. The relaxations were, however, reversed by methylene blue ($1x10^{-6}$ - $1x10^{-5}$M) and/or haemoglobin ($1x10^{-6}$ - $3x10^{-6}$M) and the reversal was most rapid when the two drugs were used in combination. These results suggest the involvement of endothelium derived relaxing factor (EDRF) (Furchgott et al., 1984) in the Ca^{++}-induced relaxation.

The potency of nitrendipine, a Ca^{++} entry blocker, was assessed against the Ca^{++}-induced changes in tension of rings of depolarised coronary arteries which were either (i) freshly removed and the endothelium left intact or (ii) stored overnight at 4°C in a physiological salt solution and the endothelium then removed. Nitrendipine competitively antagonised Ca^{++} in rings denuded of their endothelium; its pA_2 was 9.52 ± 0.13 (slope = 1.02 ± 0.08; n=4). Analysis was difficult in intact rings but it seemed as though nitrendipine left the relaxant component of the response to Ca^{++} largely unaffected. In conclusion these results show that the endothelium can markedly influence the contractile response to Ca^{++} in rings of depolarised dog coronary artery. Although it is possible that Ca^{++} causes relaxation by releasing an EDRF, the precise mechanism remains to be determined.

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EFFECTS OF INCREASING POTASSIUM ION CONCENTRATION ON STEROID SECRETION FROM THE IN SITU PERFUSED RAT ADRENAL GLAND

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Introduced by P.Moore

The effects of increased potassium ion concentration ($[K^+]$) on adrenocortical function were until recently thought to be confined to aldosterone (aldo), with concentration-related stimulation of synthesis produced between 3.6 & 20 $mmol.l^{-1}$ K^+ (Tait et al, 1980). Recently, however, it has been found that between 30 & 50 $mmol.l^{-1}$ K^+ also stimulates corticosterone synthesis by perifused mouse adrenal cells (Robertson et al, 1984). In order to further investigate this phenomenon we have studied the effects of a wide range (3.9 - 51.4$mmol.l^{-1}$) of $[K^+]$ on both aldo and corticosterone secretion using an intact preparation - the in situ perfused rat adrenal gland.

Male Sprague-Dawley rats (300g-500g) were anaesthetised with urethane ($140mg.100g^{-1}$ i.p.). The left adrenal gland was prepared for perfusion by the method of Sibley et al (1981): the circulation was isolated by ligation of the dorsal aorta above the adrenal arteries and also just below the coeliac artery, through which oxygenated medium was pumped. Effluent was collected from the renal vein which was ligated proximal to its junctions with the kidney and the vena cava. A higher K^+ medium was perfused for ten minutes after a 90 minute control period, samples were then collected for 50 min. The perfusate was modified tissue-culture medium (TC199, Difco Laboratories) plus 0.5% BSA, diluted 4:3 with Krebs-bicarbonate Ringer solutions varying in $[K^+]$ to produce a range of subsequent perfusate $[K^+]$ from 3.9 to 51.4 $mmol\ l^{-1}$. Sodium concentrations were correspondingly decreased. Aldosterone was estimated by radioimmunoassay and corticosterone by gas chromatography (Vinson et al, 1978). Results are expressed as the means of stimulated/basal secretion.

Table One.

$K^+ mmol.l^{-1}$	5.4	8.4	12.6	22.8	42	51.4
ALDOSTERONE	1.75±0.27	2.13±0.38	1.25±0.18	0.72±0.05	0.62±0.17	0.44±0.1
CORTICOSTERONE	0.80±0.17	0.98±0.03	1.04±0.03	0.99±0.05	5.72±1.56	4.21±1.2

Aldosterone secretion (basal=0.26±0.05 $ng.min^{-1}$) was stimulated by moderate increases in $[K^+]$ (up to 8.4$mmol.l^{-1}$) and decreased by higher. Corticosterone (basal=7.9±0.6 $ng.min^{-1}$), in contrast showed no stimulation until 42 $mmol.l^{-1}$ K^+ was reached. The replacement of sodium chloride by choline chloride had no effect on steroid secretion.

These data show contrasting effects of increasing $[K^+]$ on corticosterone and aldosterone secretion by the intact adrenal cortex. The stimulation of corticosterone may be caused by mediator release and/or a direct effect on the steroid-producing cells, dopamine release may be involved in the inhibition of aldosterone secretion (McCarty et al.,1984).

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508P

EFFECTS OF FORSKOLIN ON CARDIAC CONTRACTILE PROTEIN PHOSPHORYLATION

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Forskolin is a specific and potent activator of adenylate cyclase in a variety of tissues (Seamon and Daly, 1981). It produces powerful positive inotropic effects that are associated with large increases in cAMP (Rodger and Shahid, 1984) and a reduction in total twitch duration. Phosphorylation of contractile proteins has been proposed as a mechanism that may partially mediate the positive inotropic, and especially 'relaxant', effects of β-adrenoceptor agonists (England, 1983). The aims of this study were to correlate the inotropic effects of forskolin with changes in several biochemical parameters, including contractile protein phosphorylation.

Hearts from Wistar rats (220-260g) were perfused by the Langendorff technique with modified Krebs-Henseliet HCO_3-buffered medium containing 0.234mMP_i and 11mM glucose and gassed with 5% CO_2 in oxygen. Following a 5 min. preperfusion the hearts were perfused with medium containing 0.5MBq $^{32}p_i$ for 15 min. in a recycling system. After perfusion with 'cold' solution hearts were freeze-clamped and stored at -196° and subsequently analysed for protein phosphorylation (Jeacocke and England, 1980).

Forskolin (10^{-7}M - 1.2 x 10^{-5}M) induced concentration-dependent positive inotropic responses, up to a maximum of 70% ± 9% over predrug tension (45s contact time). These effects were associated, at 45s, with increases in cAMP from 3.35 ± 0.29 to 14.13 ± 2.07 pmol/mg protein and phosphorylase a activity from 7.1 ± 1.1% to 52.7% ± 2.4% (at 1.2 x 10^{-5}M, n = 4). For approximately the same increase in tension, isoprenaline (10^{-7}M), after 30s of perfusion, elevated cAMP to 18.2 ± 0.05 pmol/mg protein (n = 6), whilst maximally activating phosphorylase a to 65.8 ± 6.7% (n = 6). Data for effects on contractile protein phosphorylation are shown in Table 1.

Table 1: Effect of forskolin on contractile protein phosphorylation.

Drug	Concn. (μM)	Time (s)	TN-I	C-protein	P-LC
			mole phosphate per mole protein		
Control			0.21 ± 0.02	0.7 ± 0.09	0.52 ± 0.06
Forskolin	0.1	45	0.08 ± 0.02**	0.5 ± 0.04	0.48 ± 0.04
	0.6		0.12 ± 0.02*	0.8 ± 0.2	0.46 ± 0.06
	3		0.44 ± 0.05*	1.8 ± 0.07***	0.53 ± 0.06
	12		0.83 ± 0.03***	2.9 ± 0.2***	0.49 ± 0.06
Isoprenaline	0.1	30	1.0 ± 0.18***	4.3 ± 0.5***	0.52 ± 0.08

Significantly different from control - *P < 0.05, **P < 0.01, ***P < 0.001.

Clearly forskolin, like isoprenaline augments TN-I and C-protein phosphorylation. However, at low concentrations (0.1 - 0.6μM) forskolin seems to inhibit TN-I.

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BACTERIA-INDUCED HISTAMINE RELEASE FROM HUMAN DISPERSED LUNG AND TONSILLAR MAST CELLS

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Release of mast cell mediators has been suggested as the mechanism by which bacterial infection of the respiratory tract may exacerbate asthma. This is supported by the ability of some species of whole-formalin-killed (WFK) bacteria to release histamine from rat mast cells and human basophils (Jensen et al 1984). This study examines the ability of WFK bacteria to release histamine from human lung and tonsillar mast cells.

Human mast cells were dispersed from fresh macroscopically normal lung or tonsils by digestion with pronase and chymopapain and suspended in HEPES buffered salt solution (HBSS) (Benyon et al, 1984). Bacterial cultures were killed with formalin (1.5%), washed six times and resuspended in saline (Jensen et al 1984). Histamine release was measured by spectrofluorimetric or radiometric assays.

Mast cells were incubated for 30 min at 37°C with WFK bacterial preparations at a final concentration of 10 mg ml^{-1} (wet weight). In 3-18 experiments the histamine release from tonsillar mast cells was P.vulgaris 30.8 ± 4.1%, E.coli 22.9 ± 2.8%, S.epidermis 18.7 ± 5.0%, K.pneumoniae 16.2 ± 2.7%, K.oxytoca 10.1 ± 2.7%, Group B Streptococci 7.4 ± 1.2%, H.influenzae 3.9 ± 1.8% and S.pneumoniae 1.6 ± 1.3%. Similar results were obtained using lung mast cells. The effect of WFK E.coli was concentration related between 0.6 and 10 mg ml^{-1}. In time course experiments, the rate of histamine release induced by WFK E.coli was compared with that of anti-IgE. The initial velocity of histamine release with WFK E.coli was 0.45 ± 0.08% min^{-1} and that of anti-IgE 1.79 ± 0.37% min^{-1} (n=4). Covariant analysis showed these not to be statistically different. Desensitization of mast cells to anti-IgE stimulation abolished the secretory response to anti-IgE but did not alter the response to WFK E.coli. WKF E.coli-induced histamine release was minimal at 4°C and increased with temperature up to 45°C. In contrast IgE-dependent release was optimal at 37°C. Unlike anti-IgE, WKF E.coli-induced release was not significantly inhibited by calcium or glucose deprivation, and only partially inhibited by antimycin A (1mM).

These results illustrate that some species of bacteria are capable of releasing histamine from human lung and tonsillar mast cells. The process of activation is independent of the IgE-receptor. That WFK E.coli-induced histamine release is independent of temperature, calcium and glucose suggests a passive secretory process. These results illustrate a mechanism by which bacterial infection may have a contributary role in asthma.

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510P

POTENTIATION OF THE NANC RESPONSE IN RAT VAS DEFERENS BY α_2-ADRENOCEPTOR AGONISTS

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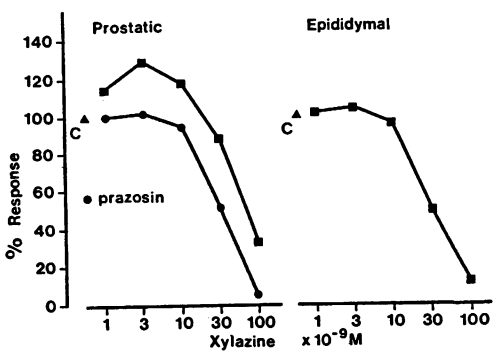
It has been reported previously that the non-adrenergic non-cholinergic (NANC) response in the rat vas deferens can be potentiated by alpha one adrenoceptor agonists at sub contractile concentrations and that this enhancement can be antagonised by alpha one adrenoceptor antagonists (French & Scott 1982). The rat vas deferens is frequently used in the assessment of activity of alpha two agonists and antagonists and it was in this context that a similar enhancement of the NANC response was observed when using the selective alpha two agonist xylazine.

The nature of this potentiation was examined using epididymal or prostatic sections of rat vasa. Tissues were placed between parallel platinum wire electrodes, bathed in Krebs Henseleit solution at 39°C and stimulated at five minute intervals with single shocks (300mA, 1.0mS). When epididymal portions of vasa were used, nifedipine (5×10^{-6}M) was added to the bathing fluid to abolish the NANC contribution to the motor response, this allows the noradrenergic response to be investigated in isolation (French & Scott 1981). Concentration effect curves to xylazine were performed cumulatively.

Concentrations of xylazine from 1×10^{-9}M to 1×10^{-8}M evoked enhanced responses in the prostatic portion of the vasa. These responses were significantly different from pre xylazine controls ($P < 0.05$). However in epididymal portions of the tissue, this range of concentrations did not produce response enhancement. Further increases in the xylazine concentration produced typical alpha two mediated inhibition of response in both sections of the tissue. Xylazine ID_{50} values were calculated and were 7×10^{-8}M and 3×10^{-8} M for prostatic and epididymal portions respectively.

In the presence of prazosin 5×10^{-9}M, the enhanced responses due to xylazine were abolished in prostatic sections of vasa. As the motor response in epididymal sections of vas is noradrenergic prazosin treatment was not attempted. The ID_{50} calculated in the presence of prazosin was 3×10^{-8}M. The preferential alpha two antagonist rauwolscine at a concentration of 5×10^{-8} M did not significantly affect the xylazine enhanced response but did antagonise the xylazine inhibition. Two other preferential alpha two agonists (BHT 920 & BHT 933) produced qualitatively similar effects. Addition of xylazine at high concentration ($>1 \times 10^{-4}$M) did not result in a contractile response suggesting the abscence of excitatory post-junctional alpha two receptors.

These results confirm our previous observation that the NANC response of rat vas is susceptible to post-junctional alpha adrenoceptor mediated facilitation. These post-junctional receptors appear to be highly sensitive and non specific for alpha adrenoceptor agonists as they respond to very low concentrations of preferential alpha one and preferential alpha two agonists. However these receptors are selective in their response to alpha adrenoceptor antagonists, the preferential alpha one antagonist prazosin inhibiting the enhancement elicited by both types of agonist and the alpha two antagonists being ineffective.



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SPECIFICITY OF ISOZYMES OF CYTOCHROME P-450 DETERMINED BY INHIBITORY MONOCLONAL ANTIBODIES

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The cytochrome P-450 dependent mixed function oxidase system is responsible for terminating the activity of a large number of drugs. It also catalyses the conversion of a number of otherwise innocuous drugs and other chemicals to electrophilic intermediates that can cause toxicity or carcinogenicity. There are multiple forms of cytochrome P-450 with unique but overlapping substrate specificities. The major form of cytochrome P-450 catalysing a given oxidation reaction can be determined by purifying the haemoproteins to homogeneity and studying them in reconstituted systems. An alternative is to determine the extent to which monooxygenase activity of the microsomal fraction can be inhibited by specific monoclonal antibodies.

Four monoclonal antibodies have been produced against rabbit cytochrome P-450 form 4 (form 4), the major hydrocarbon-inducible isozyme of cytochrome P-450 in the liver of this species. Three of the antibodies, 105, 107 and 108, were monospecific whereas a fourth antibody, 106, also reacted with form 6, a minor hydrocarbon-inducible isozyme in the rabbit. The effects of the antibodies on the microsomal metabolism of 2-acetylaminofluorene, a model carcinogen, were determined. Antibodies 107 and 108 inhibited both the 1- and N-hydroxylation of the amine (107 inhibited both reactions by 100%, 108 inhibited 1-hydroxylation by 79% and N-hydroxylation by 83%) whilst having no effect on its 7-hydroxylation. Antibody 105 inhibited only the 7-hydroxylation, by 67%, whereas antibody 106 inhibited all three reactions, by 70-74%. These studies on the hydroxylation of 2-acetylaminofluorene revealed three different inhibitory patterns. As the N-hydroxylation of this amine is believed to be the initial step in its conversion to a carcinogenic intermediate, such antibodies should prove invaluable in studies of the biological activity of 2-acetylaminofluorene and related compounds.

An antibody against rat cytochrome P-450 form c, 12/2/3/2, was produced. This antibody inhibited benzo[a]pyrene hydroxylation by microsomal fraction from 3-methylcholanthrene-treated rats by 80%, but had almost no effect with microsomal fraction from control rats (maximum inhibition of 7%). In contrast, phenacetin O-deethylase activity was inhibited with microsomal fraction from both control and 3-methylcholanthrene-treated rats, by 83% and 68%, respectively. Kinetic analysis revealed that the high affinity component of activity was inhibited by 100% in both cases.

These studies illustrate the utility of such antibodies in determining the contribution of a given isozyme of cytochrome P-450 to a particular oxidative reaction. Whereas 80% of benzo[a]pyrene hydroxylase activity of microsomal fraction from 3-methylcholanthrene-treated rats is immunoinhibitable, less than 10% of this activity of microsomal fraction from control animals is catalysed by these isozymes. In contrast, the high affinity component of phenacetin O-deethylase activity in both control and induced rats is catalysed by the same forms of cytochrome P-450. Thus, this activity is presumably catalysed by the constitutive levels of the inducible enzyme in control animals.

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512P

THE ABSORPTION AND DISPOSITION OF TOPICALLY-APPLIED BENZYL ACETATE IN THE RAT

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The simple ester benzyl acetate occurs naturally in the oils of flowers such as hyacinth and gardenia and finds widespread use as a food flavour and as a fragrance. In view of the exposure of human populations to this compound, there is interest in its safety evaluation. We now report on factors influencing the absorption of benzyl acetate through the skin of rats and on the metabolism and disposition of topically-applied benzyl acetate.

The backs of male Fischer 344 rats (200g b.w.) were shaved and [methylene-^{14}C] benzyl acetate applied to the skin (area 6.25-18cm^2) under an occlusive dressing at dose levels of 100-500mg/kg (5μCi/animal). The compound was administered as either the neat substance or as a 50% (v/v) solution in ethanol. Urine and faeces were collected for 72h. After 6h the dressings were removed, the shaven area washed with ethanol and the dressings and washings counted for ^{14}C. Excretion of ^{14}C and urinary metabolites were determined as described by us previously (Caldwell & Chidgey, 1985).

Under the conditions studied, neat benzyl acetate was poorly absorbed from the skin of rats. Following the 6h administration, 33-45% of the dose was recovered from the application site, either on the dressings or in the skin washings. Urinary excretion accounted for 30-45% of the dose, with the majority excreted within 24h, and a small proportion (ca. 1%) of the dose was recovered in the faeces (0-72h). Total recovery of ^{14}C was 79-84%. Less than 2% of the dose was present in the carcasses at the end of the experiments. The extent of absorption of benzyl acetate per cm^2 skin, as assessed by the recovery of its metabolites in urine (0-24h), was dependent upon the dose in mg/cm^2, and rose with increasing dose from 1.4 mg equivalents at a dose of 3.8 mg/cm^2 to 2.9 mg equivalents at a dose of 8.2 mg/cm^2. The absorption of topically-applied benzyl acetate was essentially identical when administered in a 50% ethanolic solution. Urinary metabolites of benzyl acetate were independent of dose and vehicle and comprised the 4 compounds identified in our previous study of the orally administered compound (Caldwell & Chidgey, 1985). Some 95% of urinary ^{14}C was present as hippuric acid, together with much smaller amounts of benzoyl glucuronide, benzoic acid and benzyl mercapturic acid.

These studies have shown that absorption of topically-applied benzyl acetate (per cm^2 skin) is dependent upon the dose per unit area but is unaffected by the use of ethanol as a dose vehicle. Following absorption from the skin benzyl acetate is rapidly eliminated from the body in the form of its metabolites.

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THE EFFECT OF PREGNANCY AND LACTATION ON ALDRIN METABOLISM IN VITRO BY RAT LIVER

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Aldrin a halogenated cyclodiene insecticide is metabolised by the hepatic mixed-function oxidase into an epoxide, Dieldrin, which is relatively resistant to further metabolism. The formation of Dieldrin in rats is markedly increased after enzyme induction by phenobarbitone (PB) pretreatment but not after induction produced by 3-Methylcholanthrene (3MC) indicating that not all the cytochrome P450 (Cyt-P450) isozymes are involved in Aldrin metabolism (Wolfe *et al.*, 1980. In contrast epoxide formation from the carcinogen, benzo(a)pyrene (B(a)P), is increased by both PB and 3MC (Sims *et al.*, 1972). The increase in rat hepatic Cyt-P450 levels that occurs in both sexes, with age, results in male rats showing a 20 fold higher activity in Dieldrin formation than females. (Davies & Keysell, 1983).

In this investigation, Aldrin was metabolised *in vitro* by liver preparations obtained from virgin, pregnant (18 days), lactating (4 days) and either PB or 3MC pretreated Wistar rats. Each incubation mixture was adjusted to give a microsomal concentration of 1mg/ml. Aldrin and Dieldrin were extracted into hexane and assayed directly by gas-liquid chromatography.

Table 1 Kinetics of Aldrin metabolism using female rat-liver

Test animal	Aldrin epoxidation	
	Km µM	Vmax nmol Dieldrin/nm Cyt-P450/min
Virgin control	93.9	0.11
Pregnant (18 days gestation)	126.4*	1.15*
Lactating (4 days post partum)	128.4*	0.28*
Phenobarbitone pretreated	75.7*	1.21*
3-Methylcholanthrene pretreated	83.3	0.09

Results expressed as mean of 3 assays on livers of 4 test animals.
* $p < 0.01$ using students t test to compare test to control animals.

Preliminary results indicated that pregnancy was associated with a substantial increase in Aldrin metabolism, whereas usually drug metabolism is decreased during pregnancy (Cessi, 1952). Kinetic measurements (Table 1) show that pregnancy is associated with a substantial increase in the Vmax for Dieldrin formation. The increase in Km values that occur during pregnancy and lactation may be due to inhibition by endogenous steroid(s) associated with these conditions. The small increase in Vmax seen in lactating rats could be due to steroids and/or a return to the pre-pregnant state.

Pregnancy and possibly lactation may therefore induce the formation of a Cyt-P450 isozyme that is induced by PB and which is either absent or present in low amounts in adult female rats.

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514P

STRAIN DIFFERENCES IN THE HEPATOTOXICITY OF N-METHYLFORMAMIDE IN MICE

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N-Methylformamide (NMF, $OHCNHCH_3$) is an industrial solvent which possesses antitumour activity and is undergoing phase II clinical evaluation for the treatment of cancer. NMF is hepatotoxic and causes periacinar necrosis in the livers of Balb/C mice administered a single dose of 400 mg/kg (Whitby et al, 1984). As part of an investigation into the mechanism of NMF hepatotoxicity, the severity of toxicity has now been evaluated in different strains of mice. Hepatotoxicity was assessed 24 h after a single i.p. dose of 100, 200 or 400 mg/kg NMF by determination of sorbitol dehydrogenase (SDH), L-aspartate aminotransferase and L-alanine aminotransferase in the plasma of Balb/C and CBA/CA mice. Since similar changes in the plasma levels of all three enzymes occurred in response to NMF administration only the values for SDH are shown (Table 1).

Table 1. Activity of SDH (mean ± s.e. mean) in plasma of Balb/C and CBA/CA mice ($n \geq 6$) 24 h after administration of various doses of NMF.

Strain	Control (saline)	Dose of NMF (mg/kg) 100	200	400
Balb/C	41.9±4.07	75.3±8.7*	6206±1068*	7974±1929*
CBA/CA	25.9±2.9	-	178±190	1771±110*

* Within strain differences from control according to Student's t-test, $P<0.001$.

The hepatotoxic dose threshold in the Balb/C mouse (between 100 and 200 mg/kg) was lower than in the CBA/CA mouse (between 200 and 400 mg/kg NMF). This difference in susceptibility to the hepatotoxic action of NMF in Balb/C mice and CBA/CA mice is correlated with the greater degree of irreversible association of radioactivity with hepatic macromolecules in Balb/C compared with CBA/CA mice after injection of ^{14}C methyl-NMF (Gescher et al, 1985). It has been shown recently (Langdon et al, 1985) that the threshold for NMF hepatotoxicity in BDF_1 mice is between 400 and 800 mg/kg. We have studied the amount of label covalently bound to or incorporated into hepatic macromolecules of BDF_1 mice 8 h after a single i.p. dose of 400 mg/kg ^{14}C methyl-NMF using methods described previously (Pearson et al, 1984). Binding in BDF_1 mice (6.16±0.59) was significantly lower ($p<0.05$) than in CBA/CA mice.

These results indicate that the order of susceptibility to NMF induced hepatotoxicity in the three strains of mouse is Balb/C>>CBA/CA>BDF_1 and provides further evidence that the degree of hepatotoxicity is paralleled by the extent to which radioactivity derived from ^{14}C methyl-NMF is irreversibly associated with hepatic macromolecules.

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STRAIN DIFFERENCES IN THE METABOLISM OF N-METHYLFORMAMIDE IN MICE

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N-Methylformamide (NMF, $OHCNHCH_3$) which possesses antineoplastic activity against murine tumours (Gescher et al, 1982) is currently undergoing Phase II clinical evaluation as an antitumour agent. NMF is extensively metabolised in mice, depletes hepatic non-protein thiols and is hepatotoxic (Whitby et al, 1984). The observation that NMF is activated to a covalently bound species implies a role for metabolism in NMF hepatotoxicity (Pearson et al, 1984). As a test of this hypothesis a study was made of the metabolism and covalent binding of NMF in strains of mice which differ in their susceptibility to NMF hepatotoxicity (Gescher et al, 1985).

Following administration of a single i.p. dose of NMF (400 mg/kg) labelled with ^{14}C either in the methyl or formyl moieties, the excretion of radioactivity derived from ^{14}C-NMF in the urine and the breath (as $^{14}CO_2$) of Balb/C and CBA/CA mice was measured for 24 h. In addition the amounts of NMF, methylamine (the major metabolite) and formamide in the urine were determined by GLC or HPLC. The amount of label covalently bound to or incorporated into hepatic macromolecules was determined as previously reported (Pearson et al, 1984). NMF was more extensively metabolised in the Balb/C mouse than in the CBA/CA mouse since the amount of NMF excreted unchanged in the urine over the first 24 h was significantly lower in the Balb/C mouse (10.1 ± 1.5% of dose, n=3) compared with the CBA/CA mouse (26.4 ± 1.9%; Student's t-test, $P<0.001$). There was no difference between the strains in the urinary excretion of the metabolites, methylamine and formamide, nor did they differ in urinary excretion of total metabolites (determined as total radioactivity in urine less the amount of NMF, each expressed as a percentage of the dose). However, Balb/C mice exhaled more label derived from ^{14}C formyl-NMF (51.3 ± 2.8% of dose, n=3) than did CBA/CA mice (40.0 ± 2.6%, $P<0.05$). Total recovery of label (urine + breath + faeces) derived from ^{14}C formyl-NMF was greater at 24 h in CBA/CA mice than in Balb/C mice ($P<0.01$) but in both strains recovery approached 100% of the dose by 72 h. Total recovery of label derived from ^{14}C methyl-NMF did not differ between the two strains at any time but the amount of radioactivity irreversibly associated with hepatic macromolecules 8 h after a single i.p. dose of 400 mg/kg ^{14}C methyl-NMF in Balb/C mice (18.47 ± 0.24 nmoles/mg protein, n=3) was more than twice the amount found in the livers of CBA/CA mice (8.71 ± 0.6 nmoles/mg of protein, $P<0.0001$).

These results clearly indicate strain differences in the metabolism of NMF in mice. The greater degree of metabolism in the Balb/C mouse compared with CBA/CA mouse is paralleled by a slower overall elimination of the drug and an increased association of the methyl moiety with hepatic macromolecules. In view of the greater susceptibility of the Balb/C mouse to NMF-induced hepatotoxicity (Gescher et al, 1985) one might speculate that the amount of reactive metabolite(s) formed determines the severity of the resulting hepatic lesion.

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THE ACTION OF PUTRESCINE ON SUPEROXIDE PRODUCTION FROM STIMULATED POLYMORPHONUCLEAR LEUCOCYTES

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Putrescine, spermidine and spermine accumulate in inflammatory exudates. Putrescine and spermidine were found to have anti-inflammatory activity against animal models (Bird & Lewis 1981, Bird et al. 1983, Oyanagui 1984). The mode of action of the polyamines is unknown. Following an observation by Vanella et al. (1980) using spin-resonance that polyamines react with superoxide we have examined the action of putrescine on superoxide produced from stimulated polymorphonuclearleucocytes (PMNLs). The cells were obtained by peritoneal lavage from male rats or guinea pigs (Best et al. 1985). The cells were suspended in culture medium (EMEM, Flow Labs. Ayrshire) supplemented with 2mM glutamine and 1%/v/v heat inactivated foetal calf serum. The cell suspension was incubated at 37°C for 5 min prior to use. The procedure for measuring superoxide-putrescine interactions from rat cells consisted of dissolving putrescine at various concentrations in EMEM which also contained luminol ($2x10^{-4}$M), Zymosan (100µl) (Sigma Chemical Co, Poole, Dorset) was added to 300 µl portions of the above solution at 37°C. Superoxide production was started when the cells ($5x10^{5}$) and the other reagents were mixed in a luminometer (LKB model 1250) and the output recorded by a potentiometric recorder. When guinea-pigs cells were used a similar procedure was used except that the soluble stimulating agent phorbol myristate acetate was used at a final concentration of 10^{-8}M. Putrescine was omitted from the controls. No loss of cell viability was found during the experiment by the trypan blue exclusion test. With rat cells putrescine was found to inhibit superoxide levels by 36% at 3 mM with total inhibition at 100mM. Similar results were obtained with guinea-pig cells with 20% inhibition at 5mM putrescine and 94% at 50mM. In some experiments with guinea-pig cells (^{14}C) putrescine was used as a tracer and the reaction mixtures were examined for metabolites. Portions of the supernatants were spotted on to 20x5 cm tlc silica plates which were developed in several solvent systems. In addition to unchanged putrescine several metabolites were found to be present. One of these metabolites was identified as γ-aminobutyric acid by the use of standards. Other metabolites were tentatively identified as γ-aminobutyaldehyde and Δ-pyrroline. The aldehyde was identified by its positive reaction with Schiff's reagent and by comparing its chromatographic behaviour with that of γ-aminobutyraldehyde and Δ-pyrroline produced by reacting putrescine with diamine oxidase. The aldehyde is a potent inhibitor of cell division and this property may be important in chronic inflammation.

Using a sensitive chemoluminescence procedure we have demonstrated a scavenging action by putrescine on superoxide output from PMNLs at 3mM. Previously using the relatively insensitive reduction of cytochrome C (Haigh et al. 1984) we could only demonstrate an effect at 100mM.

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RAT TESTICULAR CAPSULE - A TISSUE FOR MYOFIBROBLAST DRUG SENSITIVITY?

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Myofibroblasts are considered to be the cells responsible for the active contractile events which occur in granulation tissue. Many in vitro studies of these cells have been undertaken to determine their pharmacological sensitivities but rather than clarifying their properties this has resulted in conflicting reports. As to their variable sensitivities, this problem may be a simple methodological one due to the diverse nature of the myofibroblast populations studies which have included:- Croton oil induced granulation tissue (Majno et al., 1971; Gabbiani et al., 1972 & Garcia-Valdecasas et al., 1981), wound base tissue (Majno et al., 1971; Gabbiani et al., 1972), human wound tissue (Ryan et al., 1974) and rat blood clot capsule (Majno et al., 1971; Gabbiani et al., 1972). The observations of Gorgas & Böck (1974) showed on the basis of electron microscopical investigations that the rat testicular capsule contained a population of myofibroblasts which prompted the question 'could rat testicular capsule be used as an alternative to the usual myofibroblast models in order to determine the sensitivity of these cells to a range of agonists'. This capsular preparation may enable the myofibroblast sensitivities to be determined.

Rat testicular capsule was prepared as described by Davis & Langford (1969) and used in modified conditions of Bell & McLean (1973). Isometric recordings of the capsule were carried out in both Tyrode and Bretag's solution (1969) from two groups of rats weighing 350 ± 11g and 595 ± 17g respectively.

'Agonists' examined were ACh (3.44×10^{-8}-5.50×10^{-6}M), NA (2.96×10^{-8}-4.74×10^{-6}M), histamine (4.50×10^{-5}-4.50×10^{-4}M), $BaCl_2$ (2.39×10^{-4}-1.92×10^{-6}M), tyramine (3.66×10^{-7}-3.66×10^{-5}M), 5-hydroxytryptamine (2.83×10^{-5}-2.83×10^{-3}M), potassium Chloride (6.70×10^{-4}-5.36×10^{-3}M), mepyramine (1.76×10^{-5}-1.40×10^{-4}M), dimaprit (2.52×10^{-5}-2.02×10^{-4}M), metiamide (2.04×10^{-5}-1.64×10^{-4}M), angiotensin (4.85×10^{-7}M)and hyoscine (1.65×10^{-8}-3.29×10^{-4}M). Final bath concentrations are indicated in the parentheses. All agents were allowed a contact period of 5 minutes and then washed out (over 10 minutes) in a standardised procedure to determine the reversibility of the agonists actions.

In all the preparations tested (n = 32) irrespective of the physiological solution used contractions were produced by:- ACh, NA, histamine, potassium chloride, mepyramine, dimaprit, metiamide and angiotensin. Contractile responses were calculated as the maximum isometric tension produced by the agonist. No attempts were made to correlate the magnitude of the response to its duration, i.e. response area. Tyramine, $BaCl_2$ and 5-HT did not produce either a contraction or relaxation. In all preparations tested there was a poor discrimination between the range of doses of ACh and NA. None of the agonists tested produced a relaxation of the rat testicular capsule. This preparation therefore has many similarities to the myofibroblast population present in granulation tissue with the exception of 5HT to which it is insensitive. This major difference is under further investigation.

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AUTORADIOGRAPHIC LOCALISATION OF [^{3}H]-CLONIDINE AND [^{3}H]-PRAZOSIN IN THE SPINAL CORD OF THE SHEEP

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There have been several reports of autoradiographic localisation of adrenoceptors in the spinal cord of laboratory animals (Unnerstall, Kopajtic & Kuhar, 1984; Dashwood, Fleetwood-Walker, Gilbey, Mitchell & Spyer, 1985), these have indicated that the α_2 sites are localised in laminae I and II of the dorsal horn, whilst the α_1 sites are localised in the ventral horn and the deeper laminae of the dorsal horn, but at a fairly low level.

In this study we have initially investigated the histological structure of the sheep spinal cord, with particular reference to the laminae of the dorsal horn, and found several variations from the structures reported for rat and cat. From this basis we have moved on to the autoradiographic localisation of binding of [^{3}H] clonidine and [^{3}H] prazosin to show α_2 and α_1 binding sites respectively using a method based on that of Herkenham and Pert (1982).

Adult female clun sheep, bodyweight 50-70 kg, were killed with intravenous barbiturates and the spinal cord rapidly dissected out, the cord was cut into sections and placed in ice cold 50 mM Tris buffer pH 7.5 and transported to the laboratory. Frozen sections, 25μm thick, were cut on a cryostat and processed for autoradiography as described by Morris & Livingston (1984). Sections were incubated with 25 nM [^{3}H] clonidine and [^{3}H] clonidine plus 10 μM unlabelled clonidine or 10 μM idazoxan (RX 781094) to demonstrate α_2 binding sites, or 50 nM [^{3}H] prazosin and [^{3}H] prazosin plus 10 μM unlabelled prazosin to demonstrate α_1 binding sites. Sections were exposed to LKB Ultrofilm 3H for up to 50 days.

The sections of spinal cord from cervical, thoracic, lumbar and sacral regions all showed specific clonidine binding located in lamina II and possibly lamina I of the dorsal horn; there was a slight, evenly distributed, localisation in other regions of the grey matter.

The localisation of prazosin binding was less specific, but was again confined mainly to the grey matter with some accumulation, but less specifically than clonidine, over lamina II of the dorsal horn.

These investigations would suggest that α_2 binding sites in the sheep spinal cord are located particularly in lamina II of the dorsal horn and that whilst there is some localisation of α_1 sites in this region, they are much less dense here and more widely distributed throughout the grey matter than the α_2 sites.

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STIMULATION OF FLUID TRANSPORT IN RAT JEJUNUM BY NORADRENALINE - AN ATTEMPT TO CHARACTERISE RECEPTORS

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Levens et al (1979) showed that stimulation of fluid transport by noradrenaline (NA) in rat jejunum in vivo was blocked by phentolamine but not by propranolol. On the basis of in vitro experiments, Cotterell et al (1983) suggested that the effect of NA on rat jejunal fluid transport was mediated by α_1-adrenoceptors. In view of the observations by Cotterell et al (1983) that the selective α_1-adrenoceptor agonists phenylephrine and methoxamine were inactive, it was considered worthwhile to further investigate the role of adrenoceptors in the stimulation of rat jejunal fluid transport by NA.

Fluid transport was measured by a method similar to that described by Wilson and Wiseman (1954). Male Wistar rats (160-200g) were anaesthetised with sodium pentobarbitone and two adjacent 8cm sacs were removed and everted. Fluid transport was measured gravimetrically, the incubation time throughout was 1hr. One sac was used for drug treatment, the other served as a control. Statistical significance of control/test sac differences for fluid absorption were tested using Student's 't'-test for paired sac data; significance was taken at $p<0.05$.

Basal levels of fluid absorption were 0.94 $\pm$0.07g/g wet wt/hr (n=44) there being no significant difference in basal levels between adjacent sacs (adjacent sac difference = 0.01g/g wet wt/hr). NA (.25 to 5 x 10^{-5}M) stimulated fluid absorption, but only the highest concentration used gave reliable, statistically significant increases in fluid absorption (adjacent sac difference with 5×10^{-5}M NA = 0.26 $\pm$0.05 g/g wet wt/hr (n=27)). None of the other agonists tested; (maximum concentration tested shown in parentheses) phenyhephrine (10^{-3}M), isoprenaline (10^{-4}M), B-HT920 (5×10^{-4}M) and UK-14304 (10^{-2}M) were able to stimulate fluid absorption. Only high concentrations of prazosin (10^{-5}M) and propranolol (10^{-3}M) were able to inhibit the stimulant effect of NA (adjacent sac differences = -0.05 $\pm$ 0.05 (n=16) and -0.2 $\pm$ 0.09 (n=8) g/g wet wt/hr respectively; negative values indicate test sac transport less than the control sac transport). When these concentrations of prazosin and propranolol were tested alone, they were found to significantly reduce basal levels of absorption (-0.23 $\pm$0.05 (n=23) and -0.19 $\pm$0.08 (n=8) g/g wet wt/hr respectively). This indicates that the inhibition of NA stimulated absorption was apparently unrelated to adrenoceptor blockade. None of the other antagonists employed, in the concentrations shown, were able to reduce the stimulation of fluid absorption by NA: phentolamine (10^{-3}M); BE2254 (10^{-4}M); corynanthine (10^{-4}M); idazoxan (10^{-5}M); yohimbine (10^{-4}M); timolol (10^{-4}M); haloperidol (10^{-4}M).

The clearly demonstrable increase in fluid absorption produced by NA in the rat jejunum in vitro does not appear to depend on any known adrenoceptor mechanisms. An alternative explanation is that the everted sac technique is unsuitable for the study of adrenoceptor control of fluid absorption in the rat.

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THE EFFECT OF SOME NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON LEUCOCYTE FUNCTION

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Polymorphonuclear leucocytes (PMNLs) were obtained by peritoneal lavage from male Wistar strain rats 4h after injection ip with 10 ml of 1% aqueous oyster glycogen solution. The lavage solution was 30 ml heparinized ice-cold buffered (pH7.4) culture medium HBME (Flow Laboratories, Ayeshire). Chemotaxis and random migration was determined by the agarose plate technique (Nelson et al. 1975) and the modified Boyden chamber assay (Gallin & Quie, 1978) using blind well chambers (Buckley Scientific Ltd. Bucks). Formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) and LTB_4 were used as chemoattractants. PMNLs (3×10^6) were pretreated with the drugs in HBME medium for 30 mins at 37^0C. In some experiments with agarose plates the drugs were incorporated into the gel. Chemotaxis and random migration was determined after 2h incubation in a humid atmosphere at 37^0C. Chemokinesis was determined with agarose plates over a 4h period. Chemoattractant concentrations used were 10^{-6}M for FMLP in the agarose plates and 10^{-8}M in the blind well chambers. LTB_4 was used in agarose plates at a concentration of 10^{-6}M and 10^{-8}M in blind well chambers. Trypan blue exclusion was used to test for cell viability.

Benoxaprofen and fenclofenac were found to be inhibitors of chemotaxis at concentrations of 20-100ug ml^{-1} (benoxaprofen) and 20-90ug ml^{-1} (fenclofenac). These ranges are similar to their therapeutic levels in blood. Both assay systems and both chemoattractants gave similar results. The inhibition of chemotaxis by these drugs compared favourably with that of BW755C. Flurbiprofen (10ug ml^{-1}) and piroxicam (10ug ml^{-1}) also inhibited chemotaxis at the upper end of their therapeutic levels in blood. Indomethacin, levamisole and penicillamine had no inhibitory effects at concentrations in or approaching their therapeutic levels.

Drugs that were effective inhibitors of chemotaxis also inhibited random migration and lowered chemokinetic values. Blind well chambers were used to study the effects of the drugs on monocyte chemotaxis using FMLP (10^{-7}M) as the chemoattractant. Monocytes were isolated from rat blood layered on Hypaque-Ficoll gradients (Ferrante & Thong, 1978). The procedure for determining monocyte chemotaxis was identical to that used for PMNLs except 10^6 cells were used for each determination. Benoxaprofen inhibited monocyte chemotaxis over a concentration range of 12.5-100ug ml^{-1}. Its inhibiting action on monocytes was greater than its effect on PMNLs in agreement with previous findings (Goetzl & Valone, 1982). A similar selective effect with monocytes was found with fenclofenac which inhibited chemotaxis over the range (12.5-100ug ml^{-1}). Flurbiprofen (10ug ml^{-1}) and piroxicam (10ug ml^{-1}M) also inhibited chemotaxis but indomethacin over a therapeutic concentration range of 2.5-20ug ml^{-1} had no effect. Other drugs were not tested. The reference compound BW755C inhibited chemotaxis at concentrations of 12.5-100ug ml^{-1}. Benoxaprofen was established as a inhibitor of leucocyte chemotaxis (Goetzl & Valone, 1982). In our study benoxaprofen and fenclofenac were found to be powerful inhibitors of leucocyte chemotaxis. Flurbiprofen and piroxicam also possessed inhibitory activity.

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DOES LEUKOTRIENE B_4 PLAY A ROLE IN URATE-INDUCED SYNOVITIS IN DOGS?

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Monosodium urate (MSU) crystals are identified in joints of patients with acute and chronic gout; moreover, MSU crystals elicit an acute, painful inflammatory response when injected into the synovial space of the hind knee of dogs (Phelps & McCarty, 1966). The response is characterized by a massive influx of neutrophils into the synovial space. Numerous inflammatory mediators are thought to contribute to this inflammatory response including Hageman factor, the kallikrein-kinin system, complement, histamine and the prostaglandins. Recently, MSU crystals were shown to stimulate the production of arachidonic acid metabolites, including LTB_4 by human neutrophils and platelets (Serhan et al., 1984). Since LTB_4 is a potent chemotactic factor (Ford-Hutchinson et al., 1980), we performed the following studies in an attempt to establish the presence of LTB_4 in the synovial fluid of dogs with urate induced synovitis and to examine the effects of exogenous LTB_4 injected into the synovial space of normal dogs.

Healthy, mongrel dogs of either sex weighing 15-20 kg were adapted to a measuring platform and then the control right hind paw pressure was measured using a physiological integrator-averager (Carlson et al., 1984). Synovitis was induced by injecting 5 mg of MSU crystals (5-15 μ in length) into the synovial space of the right hind knee and hind paw pressure was assessed periodically up to 3 h. Synovial fluid was aspirated 3 h after MSU crystal injection and the leukocyte count was taken. Concentrations of LTB_4, LTC_4 and PGE_2 levels were also assessed by radioimmunoassay using specific antisera. At 3 h hind paw pressure was greatly reduced and the synovial neutrophil count was $1.06 \pm 0.11 \times 10^8$ cells/ml synovial fluid (n = 6). Saline-injected control animals had a synovial neutrophil count of only $1.05 \pm 0.15 \times 10^7$ cells/ml synovial fluid. There was a 20 fold increase in PGE_2 levels in urate injected joints whereas LTB_4 levels were below the level of detection (< 0.125 ng/ml). In contrast, LTC_4 was present in the synovial samples in detectable amounts (1.48 ± 0.02 ng/ml).

In a separate study, LTB_4 (1 μg), PGE_2 (10 μg) or a combination of LTB_4 (1 μg) plus PGE_2 (10 μg) were injected intrasynovially into groups of dogs. None of the treatments influenced hind paw pressure over a 3 h assessment; furthermore, the synovial neutrophil count was similar in these groups of dogs to saline-injected control animals.

Thus, LTB_4 neither appears to play a role in MSU crystal-induced synovitis in dogs nor does it attract neutrophils into the synovial space in the absence or presence of PGE_2. It is possible that the lack of LTB_4-induced effects may be due to its rapid inactivation in the dog's knee joint.

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EFFECT OF INTRA-ARTICULAR YTTRIUM 90 OR CHLORAMBUCIL ON AN EXPERIMENTAL ARTHRITIS

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Chronic synovitis is commonly treated by the intra-articular injection of Yttrium 90 (^{90}Y), but irradiation therapy is not always successful and may cause damage to articular cartilage (Kerschbaumer et al, 1979). An alternative is to inhibit proliferation of diseased synovial tissue with cytotoxic drugs (Hall et al, 1978). We have investigated the relative efficacy of intra-articular ^{90}Y and chlorambucil in treating an experimental arthritis.

An allergic bilateral arthritis was induced in the knee joints of Old English rabbits using the procedure described by Consden et al (1971). Inflammation was assessed by measuring changes in joint diameter and skin temperature over the joint. When the rabbits were killed, the macroscopic appearance of the joint was assessed and sections of the joint prepared so that histological changes in the synovium and articular cartilage could be studied.

Intra-articular injection of ^{90}Y (500 μCi) 1 week after induction of arthritis had an initial irritant effect, but 1 to 5 weeks after injection of ^{90}Y both joint swelling and surface temperature were significantly reduced (Table 1). However, the beneficial effect was not sustained and when rabbits were sacrificed 7 weeks after injection there was no significant difference in the histology of the treated or control arthritic joints. Arthritis of 3 weeks duration was even less responsive to treatment with ^{90}Y. It had been expected that therapy would be less effective in an established arthritis, although Meier-Ruge et al (1976) reported that ^{90}Y (200 μCi) successfully suppressed antigen-induced arthritis of 15 weeks duration.

Intra-articular injection of chlorambucil (1 mg) 1 week after induction of arthritis caused an approx 30% reduction in joint swelling which was sustained for 8 weeks up to the time of sacrifice (Table 1). Chlorambucil also suppressed histological changes in the synovium and articular cartilage. Although to our knowledge the use of chlorambucil in rheumatoid arthritis has been limited to systemic therapy (Amor & Mery, 1980), the results of this study suggest that the intra-articular injection of chlorambucil to control chronic synovitis warrants further investigation.

Table 1. Reduction in diameter (mm) of treated joints compared with contralateral control arthritic joints (mean ± s.e. mean)

Treatment (weeks after induction)	Days after treatment 3	7	21	35	49	n
^{90}Y (1)	+0.2 ± 0.1	0.8 ± 0.3*	0.9 ± 0.2*	0.7 ± 0.2*	0.3 ± 0.2	4
^{90}Y (3)	+0.4 ± 0.1	0.8 ± 0.4*	0.9 ± 0.4*	0.6 ± 0.4	Not done	3
Chlorambucil(1)	0.7 ± 0.1*	1.6 ± 0.3*	1.9 ± 0.3*	1.8 ± 0.4*	1.6 ± 0.4*	4

+ = increase in joint diameter * $P < 0.05$

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FURTHER STUDIES ON THE CARDIOVASCULAR ACTIONS OF THE LEUKOTRIENES IN THE ANAESTHETISED CAT

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We have previously shown that intravenously administered leukotrienes (LTs) are potent pressor agents in the anaesthetised cat, and that LTD_4-induced responses are antagonised to a variable degree by FPL55712 (Feniuk et al, 1983). We now report a more detailed analysis of the haemodynamic actions of LTs in this species and the effects of FPL55712 on LTD_4-induced responses.

Cats of either sex (1.5-2.5kg) were anaesthetised with chloralose (80mg/kg i.v.). A femoral artery and a cephalic vein were cannulated for the measurement of blood pressure (BP) and the administration of drugs respectively. Mean blood pressure (MBP) and heart rate (HR) were derived electronically from BP. An electromagnetic flow probe was placed around the aorta or the superior mesenteric artery and vascular resistance computed on line from the MBP and flow signals using an Apple microcomputer. Dose-effect curves to LTs were constructed cumulatively.

LTD_4 (0.01-10µg/kg i.v.) caused dose-related increases in MBP and increases in total vascular resistance (TVR). Low doses of LTD_4 (0.01-1.0µg/kg) increased aortic flow (AF) by up to 40% without having any significant effect on heart rate. Higher doses (0.3-10µg/kg) decreased AF by up to 80% and caused a small decrease in heart rate (10-20 b.p.m.) arrhythmias were often observed during recovery from response to LTD_4. In those cats where mesenteric flow (MF) was measured, LTD_4 induced dose-related decreases in MF and increases in mesenteric vascular resistance (MVR). LTC_4 (0.01-10µg/kg i.v.) and LTE_4 (0.01-10µg/kg i.v.) had similar effects to those produced by LTD_4 except that higher doses of LTE_4 did not decrease AF. LTC_4 was equipotent with LTD_4 (relative potency 1.3; 95% C.L. 0.6-2.7,n=8), whereas LTE_4 was 8.7 (1.8-41.8,n=8) times less potent .

The leukotriene antagonist FPL55712 (3mg/kg I.V. 1 min prior to agonist, n=6) produced inconsistent and variable shifts to the right of LTD_4 dose-effect curves for MBP, AF and TVR, the dose ratios (DRs) varying between 0.2 and 22. However more consistent antagonism of LTD_4-induced changes in MF and MVR was observed, DRs of 8.0(3.3-19.6) and 3.0(1.5-5.7) respectively being obtained (n=6). This antagonism appeared specific, in that decreases in MF and increases in MVR induced by the thromboxane A_2 mimetic U-46619 were unaffected (n=2, DRs 1.6 and 1.6) by FPL55712 (3mg/kg I.V.).

We have demonstrated that leukotrienes cause vasoconstriction in the cat, and that they can, depending upon dose, either increase or decrease AF. Further work will be necessary to elucidate the mechanisms underlying the changes in AF. The results obtained with FPL55712 must be interpreted with caution, but may indicate the presence of variable proportions of different types of leukotriene receptors in the cardiovascular system of the cat.

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ANTISECRETORY ACTIVITY OF THE PROSTAGLANDIN ENDOPEROXIDE ANALOGUE, U46619, ON THE RAT ISOLATED GASTRIC MUCOSA

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It has previously been shown that the stable thromboxane A_2-mimetic, U46619 (Coleman et al, 1981), inhibits histamine stimulated acid secretion by the rat isolated gastric mucosa (Bunce et al, 1983). The mechanism of this antisecretory effect has been investigated further by studying its modification by indomethacin pre-treatment or the thromboxane receptor antagonist AH23848 (Brittain et al, 1984), and by comparing the effects of U46619 and PGE_2 against different secretory stimuli.

All experiments were carried out in the rat isolated gastric mucosa preparation (Bunce et al, 1983). The serosal surface was bathed with a modified Krebs solution (gassed with 95% O_2/5% CO_2) to which all drugs were added. The mucosal surface was perfused at 0.5ml/min with an unbuffered solution (gassed with 100% O_2) and the pH of the effluent perfusate was continuously monitored. Each drug treatment was tested in at least 4 preparations.

In the absence of indomethacin, U46619 and PGE_2 were equipotent as inhibitors of gastric acid secretion elicited by a submaximal concentration of histamine (15μM). Inhibitory EC_{50} values in μM (95% confidence limits) were: U46619 0.035 (0.008-0.089) and PGE_2 0.044 (0.031-0.071). In the presence of indomethacin (2.7μM) the inhibitory potency of PGE_2, EC_{50} 0.088 (0.054-0.14) μM, was not significantly changed, but that of U46619 was dramatically reduced, EC_{50} 7.2 (4.5-10.0) μM. Methacholine-induced gastric acid secretion (studied in the presence of indomethacin) was unaffected by PGE_2 at concentrations up to 10μM, but was inhibited in a concentration-related manner by U46619, with an EC_{50} of 6.5 (2.2-11.9)μM. In these experiments U46619 also tended to inhibit basal acid secretion whereas PGE_2 did not. Neither U46619 (30μM) nor PGE_2 (10μM) had any significant effect on acid secretion induced by dibutyryl cyclic AMP. The effects of U46619 on basal acid secretion were further investigated in the absence of indomethacin. U46619 (0.1-10μM) produced concentration-related inhibitions of basal secretion reaching a maximum inhibition of 52.0±5.1%. This effect was inhibited by the thromboxane receptor antagonist AH23848 (0.01-0.10μM). A pA_2 of 8.19 (7.80-9.57), derived from these data, was similar to that reported for AH23848 on human platelets and rat aorta (Humphrey & Lumley, 1984). AH23848 (0.1μM) also inhibited the antisecretory effect of U46619 on histamine-induced gastric acid secretion in either indomethacin or non-indomethacin treated mucosae.

In conclusion PGE_2 and U46619 have different profiles of antisecretory action on the rat gastric mucosa. The effects of U46619 are mediated, at least in part, via thromboxane receptors and are reduced by indomethacin pre-treatment. Thus inhibition of acid secretion by U46619 in vitro, in common with its effects on mucosal ulceration and non-parietal secretion in the rat in vivo (Bunce & Clayton, 1985), appears to be mediated via endogenous prostaglandins.

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