Cardiac effects of a series of l-substituted dihydroisoquinoline derivatives

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The apparently contradictory reports of the cardiac effects of papaverine are probably a reflection of the multiple effects of this alkaloid. In addition to being a potent inhibitor of phosphodiesterase (Kukovetz & Poch, 1970), papaverine also inhibits the transmembrane movement of calcium into muscle cells (Schneider, Brooker & Sperelakis, 1975). We have attempted to investigate these actions by comparing the effects of a series of structurally-related isoquinoline derivatives on guinea-pig isolated paired atria.

Guinea-pig paired atria were mounted in McEwen's Solution at 37°C for isometric recording. The rate and force of beating were displayed on a Devices M2 recorder. Cumulative concentration-response curves were performed in all cases. Positive inotropic effects (PIE) were found to be relatively rapid in onset (less than 5 min) whereas negative inotropic effects (NIE) required at least 20 min for maximal development. The results are illustrated in Table 1.

Papaverine produced a characteristic PIE at low concentrations (a 43% increase in the force of beating at 3×10^{-5} M) followed by a NIE at a concentration of 10^{-4} M. There was a concommitant decrease in the rate of beating. The response to M78 (1-(phenylethynyl)-6,7-dimethoxy-3,4-dihydroisoquinoline) had a similar biphasic profile, but there was no significant effect on the rate of beating. M66 (1-(4-aminophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline), whilst it caused a comparable PIE to M78, it did not have any depressant effect on the force of beating. In addition, M66 had a considerable negative chronotropic effect.

M26 (1-(2,6-dichlorophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline) showed a depressant action on both the rate and force of beating, at all concentrations, as indeed did verapamil, the latter being at least ten times more potent. None of the derivatives which have been examined have been found to mimic the actions of isobutylmethylxanthine (IMX), which is considered to exert its PIE, at least, via its ability to inhibit phosphodiesterase (Korth, 1978). The inotropic effects on paired atria strongly resembled those seen previously on electrically-driven left atria (Anderson *et al.*, 1980).

Table 1 Comparison of the actions of Papaverine and derivatives on guinea-pig paired atria with effects of verapamil and isobutylmethylxanthine (IMX). Positive inotropic or chronotropic effects: +, marked; ++ considerable. Negative inotropic or chronotropic effects: —, marked; —— considerable; O, no effect.

Compound	*Concentration*	*Inotropic effect*	*Chronotropic effect*
IMX	10^{-6}–10^{-4} M	++	++
Papaverine	10^{-6}–3×10^{-5} M	+	—
	10^{-4} M	—	—
M78	10^{-6}–10^{-4} M	++	O
	10^{-4} M	—	O
M66	10^{-6}–10^{-4} M	++	——
M26	10^{-6}–10^{-4} M	——	—
Verapamil	10^{-7}–10^{-5} M	——	—

The results indicate that by simple chemical modification of the papaverine molecule, compounds have been produced which show a more selective action on atrial muscle. Interestingly, it would appear that the depressant effect on the force of atrial contraction is mediated via a different mechanism to the depressant effect on the rate of beating.

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Positive inotropism and cyclic nucleotides in mammalian cardiac muscle

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It has been reported that the ratio of cyclic 3',5'-adenosine monophosphate to cyclic 3',5'-guanosine monophosphate ([cyclic AMP]/[cyclic GMP]) is an important determinant of contractile force in frog isolated ventricle (Flitney, Lamb & Singh, 1978). A similar study in mammalian myocardium has, however, not been reported. In this study, therefore, we have compared the effects of isoprenaline and stimulation frequency (interval-force relationship) on cyclic nucleotide levels and developed tension in rabbit myocardium.

Papillary muscles from the right ventricle of NZ white rabbits were suspended in Krebs-Henseleit solution at 32°C and their electrically-evoked contractions recorded by conventional methods. Cyclic AMP and cyclic GMP levels were measured using standard protein binding assay kits (Radiochemical Centre, Amersham).

The results are summarized in Table 1.

Isoprenaline induced concentration-related increases in developed tension and in the levels of both cyclic AMP and cyclic GMP without altering the nucleotide ratio. These effects were inhibited by propranolol (5×10^{-7} M). The concentration of isoprenaline correlates well with the levels of cyclic AMP ($r = 0.99$) and cyclic GMP ($r = 1.0$) in the tissues and the tension developed ($r = 0.99$). In contrast, there was no relationship between the levels of cyclic nucleotides and tension developed at different frequencies of stimulation. To test the importance of elevated tissue cyclic GMP, measurements were made after treatment with sodium nitroprusside (1 mM; $n = 3$). Nitroprusside reduced cyclic AMP levels by approximately 10% whilst increasing cyclic GMP by over 150%. Consequently the [cyclic AMP]/[cyclic GMP] fell from about 26 to approximately 8. Despite such an alteration in the ratio neither positive nor negative inotropism resulted.

These data demonstrate that cyclic AMP in rabbit myocardium is a determinant of isoprenaline-induced positive inotropism whereas cyclic GMP appears to play little part. Unlike amphibian cardiac muscle the [cyclic AMP]/[cyclic GMP] does not correlate well with changes in mammalian cardiac muscle contractility. Furthermore, the mechanism underlying frequency-dependent inotropism is independent of changes in cyclic nucleotides.

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Table 1 Effects of isoprenaline and stimulation frequency on tension responses and cyclic nucleotide levels (mean ± s.e.mean) in rabbit isolated papillary muscles ($n = 3–5$)

Isoprenaline-induced effects (stimulation frequency = 0.4 Hz)				
Isop. concentration (ng/ml)	*tension (mg)*	*C-AMP (pmol/mg)*	*C-GMP (pmol/mg)*	*AMP/GMP*
0	250 ± 39	0.69 ± 0.08	0.036 ± 0.010	19.2
16 ± 2.9	640 ± 58	0.91 ± 0.05	0.053 ± 0.009	17.1
43 ± 6.7	842 ± 190	1.11 ± 0.05	0.058 ± 0.004	19.1
144 ± 16.0	1319 ± 238	1.29 ± 0.02	0.068 ± 0.006	19.0
512	1894 ± 176	1.45 ± 0.08	0.076 ± 0.012	19.1
Frequency-dependent effects				
frequency (Hz)	*tension (mg)*	*C-AMP (pmol/mg)*	*C-GMP (pmol/mg)*	*AMP/GMP*
0.01	214 ± 42	0.72 ± 0.11	0.024 ± 0.008	30.0
0.40	537 ± 64	0.60 ± 0.06	0.021 ± 0.002	28.6
1.0	1229 ± 141	0.90 ± 0.10	0.034 ± 0.004	26.5

The attenuation by adenosine of the positive chronotropic and inotropic responses to noradrenaline and adrenaline in guinea-pig isolated atria

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Adenosine has been demonstrated to attenuate the β-adrenoceptor mediated increase of developed tension in the left ventricle of isolated hearts (Schrader, Gerlach & Baumann, 1979) and in isolated papillary muscle (Endoh & Yamashita, 1980). This suggests that adenosine is a functional β-adrenoceptor antagonist. Such antagonism involves an interaction at some common stage of events following activation of two different drug receptors (Van den Brink, 1973). The present investigation was undertaken to evaluate the activity of adenosine as a functional β-adrenoceptor antagonist by comparing the effects of adenosine on the positive chronotropic and inotropic responses of guinea-pig isolated atria to noradrenaline, adrenaline and $CaCl_2$.

Guinea-pig isolated atria were suspended in Krebs-bicarbonate solution, containing hexobendine (1×10^{-6} M) and gassed with 5% CO_2 in oxygen. Rate responses were obtained from spontaneously beating right atria, and inotropic responses from paced left atria (2 Hz, 0.5 ms, threshold voltage +50%). In experiments involving catecholamines, the atria were treated with phenoxybenzamine (5×10^{-5} M). Cumulative concentration-response curves, before and after the addition of adenosine, were constructed to either noradrenaline and adrenaline or $CaCl_2$. Increases in rate or tension were plotted as a percentage of the maximum possible increase, as previously described for experiments involving functional antagonism (Broadley & Nicholson, 1979). Results presented are the mean of at least four experiments. Shifts in concentration-response curves are expressed as dose-ratios of geometric mean EC_{50}-values.

In the presence of adenosine (1×10^{-7} M), the noradrenaline tension and rate maxima were not significantly ($P > 0.05$) reduced. The concentration-response curves showed no significant ($P > 0.05$) shift to the right. Adenosine (1×10^{-6} M) reduced the tension and rate maxima to noradrenaline to $63.6 \pm 12.1\%$ and $75.1 \pm 4.1\%$, respectively. The concentration-response curves were again not significantly ($P > 0.05$) shifted to the right. Adenosine (1×10^{-5} M) further depressed the tension and rate maxima to noradrenaline to $42.5 \pm 8.3\%$ and $36.4 \pm 7.8\%$, respectively. In this case, the concentration-response curves were significantly ($P < 0.05$) shifted to the right (tension dose-ratio 4.1, rate dose-ratio 4.4). The effect of adenosine on the adrenaline-induced positive chronotropic and inotropic maxima and EC_{50} dose-ratios was, in each instance, effectively the same as that on noradrenaline.

Adenosine (1×10^{-5} M) did not reduce the maximum tension developed in response to $CaCl_2$. The maximum developed rate of beating was significantly ($P < 0.05$) reduced to $62.8 \pm 7.5\%$.

These results suggest that an intracellular consequence of purinoceptor activation inhibits the expression of the responses normally produced by β-adrenoceptor activation in guinea-pig isolated atria. The maximum responses to noradrenaline and adrenaline are reduced before any rightward shift in concentration-response curves. This indicates the absence of any large reserve in the adrenoceptor stimulus-effect chain prior to the step at which inhibition due to activation of purinoceptors occurs. As the maximum responses to noradrenaline and adrenaline were reduced to the same extent, the agonists would seem to have equal efficacies at the β-adrenoceptors in guinea-pig isolated atria.

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Protective effect of α-tocopherol in vascular hypoxia

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The presence of α-tocopherol (Vitamin E) has been shown to protect rabbit heart muscle from the effects of hypoxia (Guarnieri, Ferrari, Visiolo, Caldarera & Nayler, 1978; Nayler, 1970). This study aims to confirm the finding, by using guinea-pig atrium, and to explore possible protective actions of the vitamin on vascular smooth muscle.

Guinea-pig atrial, spirally cut portal vein and aortic preparations were suspended vertically in tissue baths containing Krebs-Henseleit solution at 37°C with resting tensions of between 1–2 g. Tension changes were measured isometrically using a force displacement transducer and recorded on an oscillograph. All tissues were maintained in Krebs-Henseleit solution bubbled with 95% O_2/5% CO_2 for 15 min before the experiment began. Atrial force and rate together with responses to electrical stimulation of portal vein and aorta were measured during alternating periods (20 min) of full oxygenation or hypoxia, induced by using a 95% N_2/5% CO_2 gas mixture.

Hypoxic conditions produced a reversible inhibition of the responses in all of the tissues. Incubation, with α-tocopherol (25 μg/ml–100 μg/ml) afforded the tissues significant protection ($P<0.001$) from inhibition during hypoxia whilst having no effect on the responses during full oxygenation. Inhibitions produced by hypoxia were 70.6 ± 5.0% ($n=13$) for atrial force, 38.2 ± 3.7% ($n=14$) for atrial rate, 38.9 ± 3.9% ($n=13$) for portal vein contractions and 44.2 ± 4.3% ($n=5$) for aortic contractions. The corresponding values obtained during incubation with the concentration of α-tocopherol producing the greatest protection were 24.0 ± 7.0%, 8.0 ± 3.2%, 2.0 ± 0.8% and 5.0 ± 1.7% respectively ($n=5$, standard errors shown).

The protective effect of α-tocopherol persisted when noradrenaline was used, in place of electrical stimulation, to induce vascular contractions. This indicated that the site of action of the vitamin was the vascular smooth muscle and not the sympathetic nerve endings.

The results confirm the finding with rabbit heart and indicate that the functioning of guinea-pig portal vein and aorta can be protected from the inhibitory effects of hypoxia by α-tocopherol's action on smooth muscle.

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Noradrenaline dilates rat uterine arterioles by activating β_1 adrenoceptors

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Previous studies (Koo & O, 1979) indicate that rat uterine arterioles are constricted when phenylephrine is applied topically, and phentolamine competitively blocks this response. Topically applied isoprenaline produces a dilator response which is competitively blocked by propranolol. Stimulation of the hypogastric nerve or topical application of tyramine both constrict the arterioles, presumably because noradrenaline is released and activates the alpha adrenoceptors (Koo, 1980). Noradrenaline is also a β_1 adrenoceptor agonist, but whether it dilates the uterine arterioles has not been investigated. The present studies show that it does.

Ten pentobarbitone-anaesthetized female rats (120 g) at metoestrus were laparotomized. The mesometrium of the uterus was suffused with Krebs solution (pH 7.4, 37°C), transilluminated and observed with a video-microscope. Freshly prepared noradrenaline (with 0.2 mg/ml ascorbic acid) was topically applied to the preparation and the arteriolar diameter change was measured on the video monitor with calipers.

Results show that noradrenaline (6.3×10^{-8} M) induced a half-maximal constrictor response and no dilator response was observed. Phentolamine added

to the suffusing Krebs solution not only blocked competitively the noradrenaline-induced constrictor response, but also unmasked a dilator response at low concentrations of noradrenaline. Increasing phentolamine blockade augmented the dilator response, reaching a maximum with noradrenaline (10^{-8} M) in the presence of phentolamine (10^{-3} M). Phentolamine-unmasked, noradrenaline-induced dilator responses were competitively blocked by the selective β_1 adrenoceptor antagonist metoprolol which was added to the suffusing Krebs solution: no dilator response was observed with metoprolol (10^{-3} M).

These data confirm that noradrenaline constricts uterine arterioles by activating the alpha adrenoceptors. Provided that the alpha adrenoceptors are blocked, noradrenaline can dilate uterine arterioles but does so mainly at low concentrations by activating the β_1 adrenoceptors.

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Antagonism of alpha-adrenoceptor mediated contractions of the isolated saphenous vein of the dog by diltiazem and verapamil

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Alpha-adrenoceptors can be divided on a pharmacological basis into α_1 and α_2-subtypes. In peripheral noradrengergically innervated tissues, α_1-adrenoceptors are present postsynaptically, and α_2-adrenoceptors are present presynaptically on noradrenergic nerves and postsynaptically in vascular smooth muscle (Langer, 1980). In rat the pressor responses to α_2-adrenoceptor agonists but not those to the α_1-adrenoceptor agonists are reduced by calcium antagonists (Van Meel, De Jonge, Wilfert, Kalkman, Timmermans & Van Zwieten, 1981). We now report the effects of the calcium antagonists verapamil and diltiazem on the responses of dog isolated saphenous vein to α-adrenoceptor agonists of varying selectivity for α_1 and α_2-adrenoceptors.

Veins were removed from dogs anaesthetized with Nembutal, cut into spirals, suspended between a tissue holder and a Grass FTO3 transducer, under 3 g tension and superfused with Krebs solution (6 ml/min) at 37°C and aereated with 95% O_2, 5% CO_2. Phenylephrine (PE), a selective α_1-agonist, noradrenaline (NA), a non selective agonist (Starke, Endo & Taube, 1975) and M7, a preferential α_2-adrenoceptor agonist (Drew, 1980) were injected into the perfusion stream. Diltiazem or verapamil (10^{-6}–10^{-4} M), were added to the Krebs solution 30 min before responses were obtained to the agonists. In experiments conducted with M7 prazosin (30 nM) was added to the Krebs solution to block α_1-adrenoceptors.

All three agonists produced contractions of the tissue. The maximum tension developed in response to NA being 7.6 ± 0.45 g, to PE being 8.8 ± 0.66 g, and to M7 6.3 ± 0.47 g. Omission of calcium chloride from the Krebs 10 min before the dose response curve abolished the response to M7, and shifted the dose-response curves to NA and PE to the right depressing the maximum response by approximately 60%.

Verapamil (10^{-5} M and 10^{-4} M) increased the EC_{50} for NA by 5.4 and 8.7 fold respectively with no change in the maximum response. Diltiazem had no significant effect on the NA response in concentrations up to 10^{-4} M. The response to PE was also inhibited by verapamil, the EC_{50} being increased 5.4, 13.9 and 215 fold at 10^{-6}, 10^{-5} and 10^{-4} M respectively with no change in the maximum response. Diltiazem (10^{-5} M and 10^{-4} M) increased the EC_{50} for PE by 3.5 and 8.3 fold respectively without affecting the maximum response. The response to M7 was inhibited by both calcium antagonists. Verapamil reduced the maximum response to M7 by 68.6 ± 5.12% of control at 10^{-6} M, 61.0 ± 10.0% at 10^{-5} M and 58.0 ± 10.3% at 10^{-4} M, the EC_{25} being increased 2.5, 5.4 and 28.1 fold respectively. Diltiazem reduced the maximum response to 74.3 ± 4.7% of control at 10^{-6} M, 51.0 ± 2.1% at 10^{-5} M and 33.1 ± 5.3% at 10^{-4} M, the EC_{25} being increased 1.35, 3.5 and 12.7 fold respectively.

In conclusion, the dog saphenous vein preparation contracts in response to the α-adrenoceptor agonists NA, PE and M7. The response to NA is inhibited by both calcium antagonists. The dose response curve to the α_1-agonists shifted to the right by the calcium antagonists with no significant depression of the maximum response. Conversely the dose response curve of the α_2-agonist M7 was displaced to the right with a marked depression of the maximum response. These results are consistent with the calcium antagonist effects of verapamil and diltiazem. We cannot exclude the possibility that the increased effectiveness of verapamil in blocking the responses to PE and NA could be due to an additional property such as α-adrenoceptor blockade.

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Effect of chlorpromazine on adrenergic neuroeffector transmission in isolated blood vessels

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Phenothiazines have significant haemodynamic effects which may be of clinical importance (see Elkayam & Frishman, 1980). Chlorpromazine (CPZ) has recently been found to be an effective agent in the treatment of acute hypertensive crisis (Danish multicenter study, 1980). The purpose of the present study was to observe the effects of chlorpromazine on peripheral sympathetic neuroeffector transmission in isolated blood vessels from the rabbit. The methods described in detail (Nedergaard, 1977; 1980) were used.

The effect of CPZ on the overflow of tritium evoked by electrical-field stimulation, potassium, nicotine and tyramine was studied on the rabbit isolated pulmonary artery and aorta pre-loaded with (−)-[7-[^{3}H](N)]-noradrenaline ([^{3}H]-NA; New England Nuclear Corporation). In the presence of cocaine (3×10^{-5} M) + corticosterone (4×10^{-5} M) + (−)-propranolol (10^{-7} M), rauwolscine (10^{-8}–10^{-5} M) and phentolamine (10^{-7}–10^{-5} M) considerably enhanced the stimulation-evoked [^{3}H]-overflow from pulmonary artery, but CPZ (10^{-6}–10^{-5} M) and prazosin (10^{-7}–3×10^{-7} M) had little effect. CPZ also caused an enhancement in the absence of cocaine + corticosterone + (−)-propranolol. The enhancing effect of CPZ (3×10^{-6} M) was dependent on the frequency (1–30 Hz) of field-stimulation (300 pulses). It was most marked at 1 Hz and nearly absent at 30 Hz.

The effect of CPZ on the metabolism of [^{3}H]-NA released spontaneously or by electrical-field stimulation (1000 pulses; 3 Hz) was studied on the aorta preloaded with [^{3}H]-NA. CPZ (10^{-6} M) did not change the percentage distribution of the passive [^{3}H]-outflow on [^{3}H]-NA and its [^{3}H]-metabolites. In contrast, CPZ (10^{-6} M) altered the distribution in the stimulation-evoked [^{3}H]-overflow. Thus, [^{3}H]-DOPEG, [^{3}H]-DOMA and [^{3}H]-OMDA were decreased; [^{3}H]-NMN was increased; and [^{3}H]-NA was unchanged.

Pretreatment with CPZ (10^{-5} M) and prazosin (3×10^{-7} M) had little or no effect on the inhibition of stimulation-evoked [^{3}H]-overflow seen with clonidine (10^{-7}–10^{-6} M). On the other hand, rauwolscine (10^{-6} M) and phentolamine (10^{-6} M) antagonized the clonidine-induced inhibition. CPZ (10^{-5} M) failed to increase any further the enhancement of the stimulation-evoked [^{3}H]-overflow seen with rauwolscine (10^{-6} M). In contrast, CPZ (3×10^{-6} M) and cocaine (3×10^{-5} M) potentiated the enhancement caused by 4-aminopyridine (10^{-4} M). CPZ (10^{-6} M) prevented the block of stimulation-evoked [^{3}H]-overflow seen with bretylium (10^{-5} M).

CPZ (10^{-6} M) markedly inhibited the [^{3}H]-

overflow from aorta evoked by either nicotine (10^{-4} M) or tyramine (3×10^{-7} M). In the absence of cocaine + corticosterone + (−)-propranolol, CPZ (10^{-5} M) enhanced the [^{3}H]-overflow evoked by K^+ (60 mM).

The ability of CPZ (10^{-8}–10^{-4} M), desmethylimipramine (3×10^{-9}–10^{-5} M), cocaine (3×10^{-8}–3×10^{-4} M) and prazosin (10^{-7}–10^{-5} M) to reduce the accumulation of [^{3}H]-NA (10^{-8} M) by aorta was examined. Aortae were treated with pargyline (5×10^{-4} M) and U-0521 (3′, 4′-dihydroxy-2-methylpropiophenone; 10^{-4} M) and catechol-*O*-methyltransferase, respectively. The rank order of inhibitory potency (ID_{50}) was: desmethylimipramine > CPZ > cocaine > phentolamine. Prazosin did not reduce the accumulation.

CPZ (3×10^{-9}–10^{-5} M), prazosin (3×10^{-10}–10^{-7} M), phentolamine (10^{-8}–3×10^{-5} M) and rauwolscine (3×10^{-9}–3×10^{-5} M) decreased the contractions of pulmonary artery evoked by field-stimulation (150 pulses; 3 Hz). The rank order of inhibitory potency (ID_{50}) was prazosin > CPZ > phentolamine > rauwolscine. Rauwolscine at the lower concentrations (3×10^{-9}–10^{-6} M) enhanced the neurogenic response moderately.

CPZ (10^{-8}–10^{-6} M) and phentolamine (10^{-8}–10^{-6} M) antagonized the contractions of aorta evoked by exogenous (−)-noradrenaline (10^{-9}–3×10^{-4} M) and phenylephrine (10^{-9}–3×10^{-3} M).

It is concluded that CPZ is a rather potent inhibitor of post-synaptic α_1-adrenoceptors and of uptake-1. Furthermore, CPZ is a weak inhibitor of presynaptic α_2-adrenoceptors on adrenergic nerve terminals.

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Dopamine receptors mediating relaxation of the human basilar artery *in vitro*

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Cerebral arterial spasm (CAS) resulting from subarachnoid haemorrhage is often associated with mortality (Allcock & Drake, 1963). There are conflicting reports regarding the role of dopamine in the aetiology and therapeutic management of CAS. Wurtman & Zervas (1974) have suggested that dopamine produces CAS whereas Boullin, Adams, Mohan, Green, Hunt, DuBoulay & Rogers (1977) have indicated dopamine and dopamine-like compounds for the therapy of CAS. The human basilar arterial strip has recently been demonstrated to be a useful model for investigating receptor mechanisms for agents (e.g. 5-hydroxytryptamine) believed to be involved in CAS (Forster & Whalley, 1980). This study investigates the effect of a selection of well-established dopamine agonists, haloperidol and sulpiride, in an attempt to characterize the dopamine receptor on the human basilar artery.

Human basilar arteries were obtained post mortem and set up as described previously (Forster, Whalley, Mohan & Dutton, 1980). Propranolol (10^{-6} M) and indomethacin (2.8×10^{-6} M) were included in the bathing fluid. Dopamine (10^{-7}–10^{-4} M) was observed to produce contraction, relaxation or both when applied to the tissue under resting tension (1.5 g) or $PGF_{2\alpha}$ (approx. EC_{70}–EC_{80}) induced tone. From preliminary studies using either phentolamine or phenoxybenzamine to effect α-adrenoceptor blockade all subsequent experiments were performed utilizing preparations which had been incubated with phenoxybenzamine (5×10^{-6} M) for 30 min prior to the commencement of the experiment. Tone was induced by $PGF_{2\alpha}$ (EC_{70}–EC_{80}) in all experiments.

Dopamine (5×10^{-9}–10^{-4} M) produced concentration-dependent relaxations a maximum relaxation being 64 ± 8% of the $PGF_{2\alpha}$ responses. Concentration-dependent relaxation responses were also produced by 5,6-ADTN(2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene), 6,7-ADTN,

N,N-diethyldopamine N,N-dipropyldopamine, apomorphine and SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine). Dopamine, 6,7-ADTN, apomorphine and SKF 38393 were full agonists although SKF 38393 was almost 100 times less potent than dopamine. 6,7-ADTN was equipotent with dopamine and apomorphine was 10 times less potent than dopamine. 5,6-ADTN, N,N-diethyldopamine and N,N-dipropyldopamine were relatively inactive at the concentrations used, maximum relaxations being 25%, 34% and 36% that of the maximum relaxation obtained with dopamine respectively. By comparing the geometric mean of individual EC_{50} values with that of dopamine in the same preparation a rank order of potency for dopamine and the agonists was obtained as follows:- 6,7-ADTN > dopamine > N,N-diethyldopamine > 5,6-ADTN > N,N-dipropyldopamine >> apomorphine >> SKF 38393.

Haloperidol (1.5×10^{-7}–1.5×10^{-5} M) caused a concentration-dependent shift to the right of the concentration effect curve to dopamine. There was no significant change in the maximum relaxation obtained suggesting competitive antagonism. Arunlakshana-Schild plots gave a pA_2 value of 6.85 (95% confidence limits being 6.08 and 7.92) and a slope of −0.92 (95% confidence limits being −0.65 and −1.19) Sulpiride ($2.6 \times 10^{-6} \rightarrow 1.3 \times 10^{-4}$ M) did not significantly affect relaxation concentration-effect curves to dopamine on the human basilar artery.

These results are similar to those outlined by Drew & Hilditch (1980) for the rabbit splenic artery and the dog mesenteric artery and are consistent with specific dopamine receptors being present on the human basilar artery. However, in contrast the above workers reported SKF 38393 to be inactive in the rabbit splenic whereas in this study SKF 38393 was seen to be a full agonist, an observation reported recently by Brodde, Meyer, Schemuth & Freistühler (1981). The studies of Brodde *et al.* (1981) demonstrated apomorphine to be inactive at producing relaxations of the rabbit vasculature an effect which is inconsistent with this present study. It is implied from this study that selective dopamine receptor agonists may be potentially useful in the treatment of CAS.

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A comparison of the effects of PGI_2 and captopril on systemic and renal haemodynamics in conscious dogs

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The angiotensin I converting enzyme inhibitor captopril produces a sustained fall in systemic blood pressure which is related to the activity of the renin-angiotensin system. It has been suggested that part of the hypotensive effect may be mediated by increased production of PGI_2 (Mullane & Moncada, 1980) (Swartz, Williams, Hollenberg, Levine, Dluhy & Moore, 1980). To further investigate this hypothesis we compared the effects of a continuous intravenous

infusion of PGI_2 on systemic and renal haemodynamics in conscious dogs with those seen following Captopril administration.

Seven dogs were surgically prepared with carotid arterial loops and indwelling venous catheters several weeks before the studies. Dextrose solution (5%) containing inulin and PAH was infused intravenously at a rate of 4 ml/min to establish a modest water diuresis and permit measurement of glomerular filtration rate and renal plasma flow. A catheter was inserted into the bladder for timed collections of urine.

During the first 60 min of the PGI_2 infusion ($15\,ng\,kg^{-1}\,min^{-1}$), sodium excretion, renal plasma flow and glomerular filtration rate showed no significant change whereas systemic blood pressure fell by 11 ± 1.8 mmHg. Infusion of the PGI_2 for longer periods resulted in a progressive increase in sodium excretion whereas the systemic blood pressure returned to control levels. Captopril (5 mg/kg) alone caused a small, immediate decrease in systemic blood pressure which was sustained for 2 h, however within 60 min of administration, there were marked increases in renal plasma flow and sodium excretion, but not in glomerular filtration rate.

The different time courses and patterns of action of captopril and PGI_2 indicate different mechanisms of action and it is unlikely that circulating PGI_2 is mediating any of the hypotensive effect of captopril. There is still a possibility that captopril increases intrarenal PGI_2 production, however it is difficult to envisage how this could contribute to the hypotensive action of captopril.

The gifts of PGI_2 from Schering Akteingesellschraft, Berlin, Berghamen and Captopril from Squibb (U.K.) Ltd are gratefully acknowledged. This work was supported by grants from M.R.C. and the British Heart Foundation.

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The effects of calcium antagonists on pulmonary haemodynamics

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In the present investigation we have examined the effect of verapamil and bepridil, a novel calcium antagonist (Vogel, Crampton & Sperelakis, 1979) on the pulmonary circulation of anaesthetized dogs and on guinea-pig isolated perfused and ventilated lungs.

Anaesthetized dogs (pentobarbitone, 30 mg/kg, i.v.) were prepared according to the method of Pace (1978) except the chest was not closed. During the course of the experiments, both stellate ganglia were excised. In five dogs, bepridil (2.5 mg/kg, i.v.) produced immediate increases in pulmonary arterial pressure (PAP) (55 ± 18 mean ± s.e.mean, range 16–98%) and in pulmonary flow (PF) (30.5 ± 9, range 0–50%) whilst pulmonary venous pressure (PVP) was unaffected. The changes in PF were more transient (< 5 min) than those in PAP so that 5 min after administration pulmonary vascular resistance (PVR) was increased by 52 ± 15%. This dose of bepridil also caused a transient fall in mean arterial blood pressure (MAP) of 42 ± 6% (from control of 98 ± 8 mmHg) and a more sustained decrease in heart rate (HR) (mean decrease 21 ± 3% from control of 122 ± 11 beats/min). Verapamil at a dose (0.5 mg/kg, i.v.) which produced a comparable fall in MAP (41 ± 7%), increased PAP by 34 ± 9% but left PVP unaffected. In four of the five dogs, verapamil increased PF by 5–28%. The fifth dog showed a fall of 6%, so that overall, 5 min after verapamil administration PVR was increased by 44 ± 17%. With ganglia intact, bilateral carotid occlusion for 30 s raised HR by 12 ± 7% and PVR by 21 ± 7%, but was ineffective after bilateral stellate ganglionectomy. Moreover, bepridil (2.5 mg/kg, i.v.) now raised PAP and PVR by 13 ± 7 and 12.5 ± 4% respectively, a statistically significant smaller response ($P < 0.05$) than before ganglionectomy. Only two of five dogs survived administration of verapamil (0.5 mg/kg, i.v.) after stellate ganglionectomy and these exhibited rises in PVR of 8 and 12%. Bilateral stellate ganglionectomy had no significant effect

($P > 0.05$) on control valves of PVR.

Guinea-pig isolated, perfused and ventilated lungs were prepared according to the method of Olson, Orbeck, Graven & Zachman (1977) except that lungs were removed from the thorax and perfused at constant flow. Addition of bepridil in bolus doses of 0.3, 1.0 and 3 mg to the perfusate, raised perfusion pressure in a dose dependent manner, by $14 \pm 5\%$. $42 \pm 4\%$ ($P < 0.05$) and $90 \pm 13\%$ ($P < 0.05$) respectively ($n = 5$ preparations for each dose) and verapamil (150, 300 μg) increased perfusion pressure by $16 \pm 3\%$ and $36 \pm 9\%$ ($P < 0.05$) ($n = 5$ preparations for each dose), from an initial mean perfusion pressure of 9.5 ± 0.7 mmHg. These raised values did not return to control levels for the duration of the experiment (1–1.5 h).

The present findings indicate that in anaesthetized dogs the rise in pulmonary vascular resistance produced by bepridil and verapamil was mainly due to a sympathetic reflex. The residual response obtained after pulmonary sympathectomy may be a direct effect, as demonstrated by the pressor responses observed in isolated perfused lungs.

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The haemodynamic effects of labetalol and sodium nitroprusside during minaxolone or halothane anaesthesia in the dog

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Labetalol and sodium nitroprusside are used clinically to produce controlled hypotension during surgery (Cope & Crawford, 1979). In the present study we have investigated the haemodynamic effects of these drugs in the dog during maintenance of anaesthesia with halothane or minaxolone, a short-acting water-soluble steroid anaesthetic (Davis, Dodds, Dolamore, Gardner, Sawyer, Twissell & Vallance, 1979).

Experiments were performed in adult beagle dogs with chronically implanted recording devices (Twissell & Dodds, 1979) so that the drug-anaesthetic combinations could be studied in each animal. Recordings of aortic pressure, cardiac output, left ventricular pressure and the electrocardiogram were obtained in each dog on four occasions at 2–3 day intervals. After a standardised procedure of induction with minaxolone (2.5 mg/kg iv), and orotracheal intubation, labetalol (2.5 mg/kg iv) or sodium nitroprusside ($10\ \mu g\ kg^{-1}\ min^{-1}$ iv for 10 min) was given during controlled ventilation with N_2O (67%): O_2 (33%), with anaesthesia maintained either by continuous infusion of minaxolone (0.1 mg/kg iv) or by halothane (1.0 vol %).

Labetalol reduced mean aortic pressure significantly during maintenance of anaesthesia with both minaxolone and halothane (Table 1), and the hypotensive responses were accompanied by large reductions in systemic vascular resistance. Similar responses were obtained with sodium nitroprusside, however in contrast to labetalol, nitroprusside caused marked increases in heart rate. Recovery from these effects of nitroprusside infusion occurred more rapidly than from labetalol injection. During minaxolone anaesthesia a rebound vasoconstriction developed in recovery from nitroprusside, systemic vascular resistance increasing $474\ dyn\ s^{-1}\ cm^{-2}$ above control 10 min after the end of infusion. Although this increase was not statistically significant ($P < 0.05$), it was very similar in nature to that reported by Pagani, Vatner & Braunwald (1978) in conscious dogs after cessation of nitroprusside infusion.

Labetalol may therefore be preferred to nitroprusside for the production of controlled hypotension during anaesthesia, as the combined α, β-adrenoceptor blockade induces systemic vasodilatation without concomitant tachycardia.

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Table 1 Systemic haemodynamic effects of labetalol and sodium nitroprusside in dogs during maintenance of anaesthesia with minaxolone or halothane

Drug	*Labetalol*				*Sodium nitroprusside*			
	Minaxolone		*Halothane*		*Minaxolone*		*Halothane*	
Anaesthetic	*Control*	Δ 10′	*Control*	Δ 10′	*Control*	Δ 10′	*Control*	Δ 10′
HR	138±15	−16±14	105±15	+7±12	116±13	+61±8*	117±26	+50±15*
BP	81±5	−28±4*	67±4	−21±3*	77±7	−41±9*	67±7	−31±5*
CO	175±17	−5±17	149±8	+18±12	150±17	−14±16	148±9	−11±12
SV	1.35±0.21	+0.05±0.09	1.52±0.20	−0.01±0.10	1.39±0.30	−0.62±0.28	1.47±0.31	−0.61±0.17
SVR	3303±437	−1038±291*	3010±133	−1128±225*	3669±203	−1737±133*	3206±338	−1302±378*
DPDT	2100±235	−480±183	1590±200	−280±83*	1738±209	−150±92	1563±63	−150±94

HR = Heart rate (beats/min); BP = Mean aortic BP (mm Hg); CO = Cardiac output (ml kg^{-1} min^{-1}); SV = Stroke Volume (ml/kg); SVR = Systemic vascular resistance (dyn s^{-1} cm^{-2}); DPDT = Left ventricular dP/dt max. (mm Hg/s)
Values are mean ± s.e.mean ($n = 4$ or 5) after 20 min maintenance anaesthesia, immediately before drug administration (control), and changes from control 10 min after labetalol injection or at the end of nitroprusside infusion (Δ 10′).
*Significant change from control ($P < 0.05$, paired t test).

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Effects of centrally-administered α-adrenoceptor antagonists on baroreflex bradycardia in the unanaesthetized rat

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The baroreflex arc contains α-adrenoceptors, and their activation facilitates the baroreflex (Haeusler, 1975). These adrenoceptors receive an input from catecholaminergic (CA) neurones, since the baroreflex is facilitated by α-methyldopa which acts via CA neurones (Haeusler, 1975). However, the role of these α-adrenoceptors in the normal reflex is unclear, as functional impairment of the CA neurones does not affect the baroreflex (Kobinger & Pichler, 1974). We have therefore investigated the effects of blocking central α-adrenoceptors on baroreflex bradycardia (BRB) in unanaesthetized rats.

Male Wistar rats (230–280 g) were used. During ether anaesthesia a tail artery (for blood pressure) and a tail vein (for infusions) were cannulated; electrocardiogram electrodes (for heart period) and a colon thermocouple (for core temperature) were positioned. One hour after recovery, rats received 10 μl of the appropriate vehicle intracerebroventricularly (i.c.v.) via a cannula implanted 4–9 days previously. BRB was determined 5 min later by intravenous infusion of phenylephrine at 3–50 μg $kg^{-1}min^{-1}$ Little & Redfern, 1981). The drug was injected (10 μl, i.c.v.) 0.5 h later, and BRB was re-determined. Correct positioning of the cannula was checked after each experiment by injection of ink (10 μl, i.c.v.). Regression lines of heart period (HP) against mean arterial pressure (MAP) were constructed. Comparisons of HP were made at 125 mmHg (i.e. HP_{125}).

Three antagonists were used, with different selectivities for α_1- and α_2-adrenoceptors. Phentolamine

(50 μg, i.c.v.; $n = 5$) caused a slight increase in MAP but did not affect the HP_{125} (vehicle: 142.6 ± 4.9 ms; drug: 145.3 ± 5.9 ms, NS) or the slope (vehicle: 1.100 ± 0.176 ms/mmHg; drug: 1.390 ± 0.189 ms/mmHg, NS). Piperoxan (50 μg, i.c.v.; $n = 7$) did not affect MAP, nor HP_{125} (vehicle: 151.8 ± 7.3 ms; drug: 155.1 ± 4.2 ms, NS) nor slope (vehicle: 0.971 ± 0.114 ms/mmHg; drug: 0.983 ± 0.130 ms/mmHg, NS). Prazosin (0.1 μg, i.c.v.; $n = 5$) affected neither MAP nor HP_{125} (vehicle: 159.8 ± 8.4 ms; drug: 172.8 ± 8.6 ms, NS) nor slope (vehicle: 0.921 ± 0.041 ms/mmHg; drug: 1.327 ± 0.157 ms/mmHg, NS). Larger doses of prazosin (1 and 10 μg) had no effect on the HP-MAP relationship but lowered MAP and antagonized the pressor response to intravenous phenylephrine, suggesting leakage into the periphery.

The lack of effect of these drugs is not due to inadequate sensitivity of the method, since cholinergic antagonists have marked effects in this system (unpublished observations). We have previously noted that pretreatment with i.c.v. 6-hydroxydopamine does not affect BRB (Little, Redfern & Stoner, 1981). In anaesthetized dogs phentolamine given intracisternally inhibits BRB (Kobinger & Walland, 1973). This may be a species variation, as a recent study in anaesthetized cats supports our negative findings (Connor, Drew & Finch, 1981). These discrepancies might also be due to the effects of anaesthesia, which impairs cardiovascular reflexes (Korner, 1971).

Thus in the unanaesthetized rat, baroreflex bradycardia functions apparently normally without central α-adrenoceptors. This supports previous suggestions (Kobinger & Pichler, 1974; Haeusler, 1975) that central CA systems are not essential to the baroreflex.

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The responses to pressor agents in man are affected by the sensitivity of the baroreceptor reflex

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The rise in blood pressure produced by injections vasoconstrictor agents is often interpreted as an indirect reflection of vascular reactivity. It is known, however, that as the blood pressure rises the baroreceptor reflexes are promptly engaged and in some degree modify the response. The magnitude of this interaction and the possible effect this may have on the interpretation placed on the pressor response has not been quantified.

We have studied blood pressure responses measured directly from the brachial artery to intravenous injection of phenylephrine and noradrenaline in 11 subjects classified according to the sensitivity of their baroreceptor reflex. This was measured by the slope of the regression of R-R interval on systolic pressure produced by phenylephrine (Bristow *et al.*, 1969). Subjects were divided into 3 groups. One with a sensitive reflex (slope > 20 ms/mmHg) ($n = 3$), another with an insensitive reflex (slope < 10 ms/mmHg) ($n = 4$), and an intermediate

Table 1 Responses of systolic pressure (± s.d.) to phenylephrine and noradrenaline in subjects classified according to the sensitivity of the baroreceptor reflex

	Phenylephrine (μg iv)			*Noradrenaline (μg iv)*		
	20 mmHg	40 mmHg	80 mmHg	1 mmHg	2 mmHg	3 mmHg
High sensitivity $n=3$	9.5 ±2.0	17.5 ±10.1	18.0 ± 6.6	9.8 ±1.8	18.7 ± 9.0	20.7 ± 2.6
Middle sensitivity $n=4$	15.4 ± 4.6	16.7 ± 1.8	23.8 ± 8.9	20.4 ± 7.3	29.1 ± 7.5	36.0 ± 9.2
Low sensitivity $n=4$	21.4* ± 1.9	29.4 ± 8.9	37.2** ± 7.3	21.4 ± 8.4	35.1 ±10.2	46.6 ±11.1

High vs Low sensitivity. $^*P<0.05$, $^{**}P<0.02$.

group (slope 10–20 ms/mmHg) ($n=4$). The pressor responses to 3 doses each of phenylephrine and noradrenaline are given in the table where it can be seen that the response to both pressor agents is smallest in subjects with the most sensitive reflex and the slope of the dose response curves was also shallower in these subjects.

It is concluded therefore, that it is difficult to infer changes in vascular reactivity from changes in pressor responses to infused agents.

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The effect of adenosine on the isolated hemidiaphragm of the rat

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Adenosine is present in the resting skeletal muscle and is increased under a number of conditions (Dobson *et al.*, 1971; Bockman *et al.*, 1975). The production of adenosine in the muscle increases as the oxidative capacity increases in the muscle (Bockman & McKenzie, 1979). Adenosine is also supposed to be a physiological modulator of several biological functions (Fredholm, 1980).

In the present study the effect of adenosine on the isometric contraction of skeletal muscle was examined during direct and indirect electrical stimulation. Experiments were carried out on the isolated hemidiaphragm of the rat. The muscle was stimulated with a frequency of 0.15 Hz. Both tension (Td) and the maximum rate of rise of tension (dT/dt max) were recorded simultaneously.

It was found that the increasing concentrations of adenosine (0.1–0.8 mM) produced insignificant changes in Td and dT/dt max during direct electrical stimulation. In the presence of dipyridamole (26.4 μM) the same concentrations of adenosine produced a dose-dependent increase both in Td and dT/dt max. Dipyridamole by itself was also found to potentiate the isometric contraction. Similarly, frusemide potentiated the effect of adenosine. In the medium with double amount of calcium, adenosine also potentiated both parameters of the isometric contraction, but in a calcium-free medium it produced no effect. Aminophylline did not affect the action of adenosine on the isometric contraction. Similar type of response to adenosine was obtained during indirect stimulation of the muscle: dipyridamole and frusemide potentiated its action on Td and dT/dt max. Adenosine was also found to potentiate the effect of (+)-tubocurarine during indirect electrical stimulation.

It is concluded that adenosine acts on the isolated skeletal muscle in two different ways: it stimulates calcium and possibly cAMP mechanisms during direct electrical stimulation, thus potentiating the isometric contraction, but it also inhibits the acetylcholine release during indirect electrical stimulation, thus depressing the isometric contraction.

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Adenine Dinucleotide effects on the isolated vas deferens

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Several structure-activity studies on adenine derivatives have included the observation that some dinucleotides such as nicotinamide adenine nucleotide (NAD) mimic the effects of adenosine (Dowdle & Maske, 1980; Okada & Kuroda, 1980). The present study was designed to examine a number of dinucleotides and to determine their mechanism of action.

After death, one vas was removed from Wistar rats, adhering connective tissue and blood vessels removed, and semen expressed by gentle pressure. The vas was next bisected transversely and the prostate end transferred to an organ bath and perfused with oxygenated Mg^{2+}-free Krebs solution at 37°C at a perfusion rate of approx. 3 ml/min. Contractions were evoked by parallel platinum wires using supramaximal voltage, 1 ms pulses, 0.1 Hz, and were measured by an isometric transducer and Devices M4 recorder.

Adenosine and adenine containing nucleotides (adenosine monophosphate (AMP), adenosine triphosphate, adenosine β,γ-methylene triphosphate and dinucleotides, such as β-NAD, α-NAD, β-NADP, 3-amino-pyridine adenine dinucleotide, 2′5′- or 3′5′-adenylyladenosine inhibited the electrically-evoked contractions (IC_{50} values between 1 and 10 μM). 6-methyladenosine was moderately potent (IC_{50} 12.4 μM). Nicotinamide and nicotinamide hypoxanthine dinucleotide were inactive. The inhibitory effects of all these compounds were reduced by theophylline (100 μM) or adenosine deaminase (10 u/ml) implying the contribution of adenosine (Stone, 1981). The effects of β-NAD and AMP were not affected by a nucleotidase inhibitor (α, β-methylene ADP) but were increased by inhibition of adenosine deaminase.

After preloading vasa with [^{3}H]-adenosine, all the inhibitory compounds caused the release of [^{3}H]-labelled compounds, whereas morphine did not, although it greatly inhibited twitch height.

In view of the potency and rapidity of onset of dinucleotide inhibition of the vas, and the failure of a nucleotidase inhibitor to potentiate the responses, it seems unlikely that dinucleotide responses are entirely attributable either to any free adenosine content, or to their metabolism to adenosine. It is suggested that the dinucleotides may trigger the release of adenosine which is at least partly responsible for the twitch inhibition.

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A comparison of the effects of methoxyverapamil, verapamil and nifedipine on the twitch responses to single pulse stimulation in the rat bisected vas deferens

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Single pulse stimulation causes a biphasic contraction of the rat vas deferens; the second phase appears to be caused by stimulation of adrenergic nerves, but the origin of the first phase is uncertain (McGrath, 1978). Scott & French (1981) have shown that the first phase can be blocked by nifedipine, but the second phase is resistant. We have investigated the mechanism of these effects.

Vasa deferentia were removed from Wistar rats (320–360 g body weight), bisected, and suspended in Krebs-Henseleit solution ($Na^+ = 144$, $K^+ = 5.8$, $Ca^{2+} = 2.5$, $Mg^{2+} = 1.2$, $HCO_3^- = 25$, $H_2PO_4^- = 1.2$, $SO_4^{2-} = 1.2$, $Cl^- = 128.6$, glucose = 11.1 mM) aerated with 5% CO_2:95% O_2 and maintained at 36–38°C; contractions were recorded isometrically. The bisected vasa were stimulated with a single square wave pulse (1 ms pulse width, 150V) delivered every 5 min through parallel wire electrodes (McGrath, 1978). Twitch contractions were abolished by tetrodotoxin (0.3 μg/ml), by incubating in Ca^{2+}-free Krebs, and (using a tris-buffered solution) by Mn^{2+} (1–3mM) or La^{3+} (1–10 mM).

Noradrenaline (10 μg/ml) caused a tonic contraction that reached its peak after 3–60 s and then decayed to be replaced by rhythmic contractions. In the epididymal half, the tonic contraction was abolished by nifedipine (0.1 μg/ml) or verapamil (5 μg/ml). However, in the prostatic half, part of the tonic contraction was resistant even to the combination of nifedipine (5 μg/ml) and verapamil (30 μg/ml). Methoxamine-induced rhythmic contractions could be completely abolished by nifedipine (5 μg/ml) verapamil (50 μg/ml) or methoxyverapamil (50 μg/ml) (Hay & Wadsworth, 1981a).

Verapamil and methoxyverapamil inhibited both phases of the twitch. With methoxyverapamil (20 μg/ml) the second phase was almost abolished and with methoxyverapamil (30 μg/ml) the first phase was reduced by 53 ± 11% ($n = 6$). The second phase was virtually abolished by lignocaine (100 μg/ml) but higher concentrations were required to inhibit the first phase.

As verapamil and methoxyverapamil possess local anaesthetic activity (Hay & Wadsworth, 1981b) it is possible that inhibition of the first phase of the twitch was due to block of conduction in the nerves. However, since the first phase was resistant to lignocaine, an alternative conclusion is that the calcium antagonist action of methoxyverapamil and verapamil is responsible for this effect. Block of the first phase by nifedipine, which is devoid of local anaesthetic activity, is consistent with this idea (Hay & Wadsworth, 1981b).

Inhibition of the second phase of the twitch by methoxyverapamil or verapamil was probably due to their local anaesthetic activity because the second phase was also blocked by low concentrations of lignocaine but is not affected by nifedipine. The resistance of this phase to inhibition by nifedipine suggests two alternative conclusions. (a) The second phase is dependent on intracellular Ca^{2+} while the first phase is dependent on extracellular Ca^{2+}. This idea is supported by the observation of a resistant component of the noradrenaline contraction in the prostatic half. (b) The second phase utilizes extracellular Ca^{2+} entering through an ionic channel that is resistant to block by the calcium antagonists, possibly analogous to the channel in the nerve terminal membrane which also remains operative in the presence of high concentrations of nifedipine.

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Effects of cholinoceptor blockade on detrusor electromyograms in the cat

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Various explanations have been offered for the hyoscine resistance of contractions of the urinary bladder to parasympathetic nerve stimulation (Taira, 1972). These include involvement of two parasympathetic pathways, a non-cholinergic pathway producing the initial contraction and a cholinergic hyoscine-sensitive pathway responsible for maintenance of the contraction (Henderson & Roepke, 1934). To determine whether there is a dual parasympathetic innervation to the bladders of cats, detrusor evoked electromyograms were studied throughout a 15 s period of sacral ventral root stimulation in anaesthetized animals (sodium pentobarbitone, 36 mg/kg) both before and after hyoscine with the expectation that the later electromyograms and bladder pressure would be attenuated by hyoscine.

Optimal electromyograms, most clearly seen in the 10–40 Hz frequency band, were evoked in the detrusor smooth muscle by electrical stimulation of the sacral ventral roots with a 30 ms burst of six 0.5 ms pulses at a supramaximal voltage (Craggs & Stephenson, 1976). These bursts were applied at repetition rates of between 0.5 and 8 bursts/s for 15 s to produce sustained contractions. Unexpectedly, before hyoscine, the electromyograms waned eventually disappearing despite maintained rises in intravesical pressure when bursts of stimulation were repeated at more than 0.5 bursts/s. After hyoscine (1 mg/kg) the contraction was less well maintained but surprisingly the electromyograms were now more persistent, waning only occurring at faster rates of stimulation (typically $>$ 2 bursts/s).

In bladder smooth muscle the spike action potentials arise from a slow wave of depolarization (Ursillo, 1961). Assuming the electromyogram to be an average signal reflecting the spike activity of a large population of synchronously active cells then an explanation for the waning of the electromyogram is that with increasing frequencies of stimulation, the slow potentials could summate leading to a membrane depolarization sufficient to sustain a maximal muscle contraction in the absence of spike potentials. Intracellular recordings from the isolated colon in response to pelvic nerve stimulation support this explanation (Gillespie, 1962). Assuming acetylcholine to be a transmitter then the electromyographic changes seen after hyoscine could be due to a reduction in the rate of summation of the slow postsynaptic events, faster frequencies of stimulation being required for summation to occur and the electromyogram to wane. However, the contraction was only poorly maintained after hyoscine, a finding not wholly consistent with this explanation.

The effects of hyoscine on parasympathetic ganglia to the bladder require investigation before conclusions can be made as to whether hyoscine resistance in the cat is due to a dual parasympathetic innervation or to other mechanisms, e.g. muscarinic receptors inaccessible to hyoscine (Carpenter & Rand, 1965).

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Modulation of the peristaltic reflex *in vivo* by endogenous opioids

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The hypothesis that endogenous enkephalins modulate gastrointestinal motility is supported by the (−)-naloxone-induced enhancement of peristaltic activity in the isolated ileum of several species (Kromer, Pretzlaff & Scheiblhuber, 1980). However, (−)-naloxone failed to influence the transit of luminal contents in conscious rats (Tavani, Bianchi, Ferretti & Monara, 1980) or mice (Schulz, Wüster & Herz, 1979). Although opiate agonists inhibit peristaltic activity, a preferential action on the induction of the reflex (preparatory phase) has been demonstrated *in vitro* (Fontaine, Reuse & Van Nueten,

1973). Endogenous enkephalins, therefore, may primarily inhibit the induction of peristalsis. Hence, in the present experiments, the effects of opiate antagonists on the intraluminal pressure required to elicit peristalsis have been investigated in isolated and intact preparations of the guinea-pig ileum.

In preliminary experiments, peristalsis was studied *in vitro* by a modified method of Van Nueten, Geivers, Fontaine & Janssen (1973), in which intraluminal pressure was raised by infusion of saline (1.0 ml/min). In the control group ($n=4$), peristalsis was induced at 2.73 ± 0.12 cm H_2O, whilst in the presence of (−)-naloxone (8.25×10^{-7} M, $n=4$), the threshold pressure was significantly reduced to 2.13 ± 0.16 cm H_2O ($P<0.01$, Student's '*t*' test for grouped data). The magnitude of peristaltic contractions, however, were unaffected by (−)-naloxone.

Further experiments were carried out to investigate whether similar effects of opiate antagonists on threshold pressure occur *in vivo*. Guinea-pigs were anaesthetized with ethyl carbamate (1.8 gm/kg, i.p.) and peristalsis was elicited by the infusion of saline (1 ml/min) into the lumen of an ileal segment, as described by Aldunate, Yojay & Mardones (1965). Following intravenous administration of opiate antagonists, the threshold pressure for the induction of peristalsis was consistently and significantly reduced. (−)-Naloxone (0.82 μmol/kg, i.v., $n=5$) reduced the threshold pressure from 3.32 ± 0.52 cm H_2O to 1.46 ± 0.13 cm H_2O ($P<0.02$) and N-methyl nalorphine (2.2 μmol/kg, i.v., $n=5$) from 2.82 ± 0.57 cm H_2O to 1.69 ± 0.32 cm H_2O ($P<0.02$, Student's '*t*' test for paired data). In the presence of the antagonists, the magnitude of contractions and duration of peristaltic activity were usually increased, although these effects tended to be more variable.

Blockade of opioid receptors by (−)-naloxone, therefore, apparently facilitates induction of the peristaltic reflex *in vitro* and *in vivo*. This action may be mediated locally, since the quaternary antagonist N-methyl nalorphine *in vivo* also reduced the threshold pressure. Further support for peripherally mediated effects on peristalsis were obtained with the quaternary opiate agonist, N-methyl morphine. Following intravenous administration, morphine and N-methyl morphine induced a dose-related elevation of threshold intraluminal pressure. The ID_{50} for morphine was 0.25 μmol/kg (95% confidence limits 0.16–0.41, $n=3$, 100% response = 15 cm H_2O) and for N-methyl morphine 10.1 μmol/kg (95% confidence limits 6.5–17.9, $n=3$). Hence morphine was approximately 40 times more potent than its quaternary analogue in this preparation, a potency ratio comparable to that found for inhibition of the electrically stimulated guinea-pig isolated ileum by these compounds (50 times).

In conclusion, opiate antagonists facilitate induction of the peristaltic reflex both *in vitro* and *in vivo*. This effect appears to be locally mediated and suggests an inhibitory role of endogenous opioid peptides in the guinea-pig ileum.

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Differential effects of substance P and related peptides on smooth muscle preparations

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Substance P, some of its C-terminal fragments and some of its homologues (physalaemin and eledoisin) are potent hypotensors in rats anaesthetized with urethane. These peptides act as vasodilators in coronaries (rabbit isolated hearts), inhibit smooth muscle contractions in arteries (noradrenaline-

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induced contractions of the dog carotid artery) and stimulate smooth muscle in veins (rabbit mesenteric vein), intestine (guinea-pig ileum, rat colon, human colon), trachea (guinea-pig).

The effects of these peptides appear to be due to a direct action on smooth muscle mediated by specific receptors in five of eight preparations. Some interactions with endogenous neurotransmitters might be expected in the other three. For example, the stimulation of the guinea-pig ileum by substance P is partially antagonized by atropine while adrenergic antagonists potentiate the actions of substance P in some preparations (rat and human colons).

The order of potency of substance P and various C-terminal fragments were used to determine whether these effects were mediated by single or multiple receptors. Some striking differences in the order of potency have been found between these preparations, thus suggesting the existence of more than one type of receptor for substance P. A cross-desensitization was observed between substance P, various C-terminal fragments (C_{4-11} to C_{7-11}), eledoisin and physalaemin in four of the five preparations in which desensitization experiments could be performed (namely the rat blood pressure, the rabbit isolated heart, the rabbit mesenteric vein and the guinea-pig ileum). In the dog carotid artery, there was no cross-desensitization between substance P and eledoisin but only between substance P, its C-terminal fragments and physalaemin.

The presence and auto-regulation of prolactin receptor in the lung. Its significance

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Surfactant is a complex series of phospholipids lining the alveoli and is essential for lung function. The deficiency of surfactant is the primary cause of respiratory distress syndrome (RDS) in the new born and is the main cause of death in premature infants.

A number of hormones including glucocorticoids, insulin, thyroid hormone oestrogens and prolactin (PRL) have been implicated in the maturation of the lung and surfactant production (Ballard, Gluckman, Brehier, Kinerman, Kaplan, Rudolph & Grumbach, 1978). Low levels of PRL in cord serum are associated with the occurrence of RDS in premature infants (Hamosh & Hamosh, 1977). The rat lung is considered to be a target organ for prolactin since specific prolactin binding site and stimulation of surfactant production by prolactin were shown in foetal lung (Ben-Harari, Amit & Youdim, 1981).

In adult rat lung and liver specific binding sites to PRL are absent, but recently we have shown that administration of exogenous ovine prolactin (oPRL) induces its own receptor in the adult liver (Barkey, Shani, Lahav, Amit & Youdim, 1981). These studies prompted us to examine the effect of exogenous oPRL treatment on PRL binding sites in the adult lung as compared to liver.

Intact adult male rats (Sprague-Dawley 150–200 g) were injected with oPRL in polyvinyl-pyrrolidial (PVP) or PVP alone or saline. Specific binding of [^{125}1]-oPRL in lung and liver was found after daily injection of oPRL (1 mg/kg) for 7, 10 and 14 days. Binding was maximal after 14 days of treatment, linearly related to protein concentration (0.2–0.9 mg protein), equilibrium was approached after 44 h of incubation and binding was greater at 19°C than at 4°C. Specific [^{125}1]-oPRL binding showed saturation of a limited number of binding sites at low physiological concentrations of hormone in lung for each duration of treatment with oPRL (Ka, $4.9–16.8 \times 10^{-9}$ M, $n = 18$). Binding of [^{125}1]-oPRL was shown to be specific in both lung and liver since binding was inhibited by low concentrations of oPRL, ovine growth hormone and human placental lactogen and not by human prolactin, human FSH and human growth hormone.

A positive correlation ($r = 0.90$) existed between the duration of treatment (oPRL 1 mg/kg) and prolactin binding in lung and liver. Over the concentration range 0.125–1.0 mg/kg oPRL injected, binding in lung was unrelated to dose and varied between $48.6 \pm 4.8–42.4 + 5.6\%$ mg protein.

Our results clearly demonstrate that PRL can induce its own receptor in adult lung. This 'up regulation' of specific PRL binding sites in lung correlated very closely with that in liver, a classical target organ for PRL. Thus the reported stimulation of surfactant synthesis in lung by prolactin may be an action via induction of specific PRL binding sites since PRL receptors are obligatory for mediating some, if not all, of the actions of the hormone.

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Defective plasma protein binding in uraemia: extraction of binding inhibitor(s) from normal urine

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Endogenous metabolites, normally excreted in urine, accumulate in renal failure and may cause the binding defect of uraemic plasma by acting as binding inhibitors. These unidentified binding inhibitors are readily extractable from human (Depner & Gulyassy, 1980; Lichtenwalner, Suh, Lorber, Rudnick & Craig, 1981) and rat (Bowmer & Lindup, 1979) uraemic plasma. We have investigated normal human urine as a potential source of such inhibitors.

The putative inhibitors were extracted from acidified human urine by adsorption onto charcoal at 0–4°C and transferred from the charcoal to human albumin (HSA) in solution at pH 7.4 by incubation for 2.5 h at 37°C. The charcoal was removed and the transferability of inhibitors to albumin tested for by measurement of the binding of methyl red (133 μM) and salicylate (140 μM) to HSA (1% W/V) using equilibrium dialysis at 37°C (Bowmer & Lindup, 1979). Acidifed human urine was also treated with Amberlite XAD-2 resin to extract binding inhibitors. These were eluted from the resin with ethanol which was evaporated to dryness and the residue redissolved in buffer prior to testing its inhibitory effect on binding.

Human urine significantly increased unbound fractions of both test ligands and this inhibitory effect was substantially removed by prior extraction with charcoal at pH 3.0 (Table 1). The extracted inhibitors could be transferred to HSA and they significantly increased the percentage of unbound methyl red ($P<0.01$) from 10.49 ± 0.36 (s.d.) ($n=10$) to 18.75 ± 1.40 ($n=10$) and of salicylate ($P<0.001$) from 25.4 ± 1.28 ($n=10$) to 35.04 ± 1.74 ($n=5$). Extraction with XAD-2 resin also removed inhibitors from human urine and the ethanol eluate of the resin contained binding inhibitors (Table 1).

The urinary inhibitors show similar characteristics to those from plasma i.e. they are stable, extractable at acid pH and are taken up by albumin at pH 7.4. The unidentified binding inhibitor(s) in uraemic plasma (Depner & Gulyassy, 1980) have some of the characteristics of an indole and we have found indole 2- and 3- substituted carboxylic acids to inhibit methyl red binding.

Table 1 Inhibitory effects of human urine on the binding of methyl red and salicylate to HSA

	% Unbound				
			Effect of urine treatment		Effect of ethanol eluate
Ligand	*Control*	*untreated*	*charcoal*	*resin*	
Methyl red	10.49 ± 0.36 (10)	$26.97^{*}\pm1.05$ (10)	$12.08^{\dagger}\pm0.28$ (8)	$13,70^{\dagger}\pm1.79$ (6)	$23.72^{*}\pm0.58$ (6)
Salicylate	25.40 ± 1.28 (10)	$72.34^{*}\pm2.78$ (8)	$31.55^{\dagger}\pm2.49$ (8)	$49.17^{\dagger}\pm3.49$ (3)	$80.14^{*}\pm1.80$ (3)

Results expressed as mean ± s.d.; values in brackets indicate number of urine samples.
* $P<0.001$ relative to control.
† $P<0.001$ relative to effect of untreated urine.

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Effect of prenatal exposure to anticonvulsant agents on maternal and foetal folate concentrations in the rat

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Various reports suggest that the use of antiepileptic drugs in pregnant women provides a considerable risk to the foetus (South, 1972; Smith, 1980; Nakane, Okuma, Takahashi and others, 1980), and it is known that drugs such as phenytoin (Harbison & Becker, 1969) and sodium valproate (Brown, Kao & Fabro, 1980) are teratogenic in some animal models.

It has been suggested that hydantoin teratogenesis may result from interference with folate metabolism (Netzloff, Streiff, Frias & Rennart, 1979) and we considered it of interest to determine whether valproate teratogenicity was associated with disturbances in folate metabolism. We have therefore examined the effects of sodium valproate, phenytoin, phenobarbitone and carbamazepine (at multiples of their anticonvulsant ED_{50}'s; maximum electroshock test) and pyrimethamine (as a known teratogen and inhibitor of dihydrofolate reductase (Sullivan & Takacs, 1972) on maternal and foetal folate concentrations in the rat.

Adult male and virgin female Wistar rats (250–300 g) were housed conventionally using a 12 h light-dark cycle. Food (BP Nutrition PCD diet) and water were given *ad libitum*. Natural mating was employed. Females in proestrous were placed with males, up to four females per male per cage. Females from mating cages were examined the following morning for the presence of sperm in the vagina. Females with positive evidence of mating were admitted to the study. The day of observing a vaginal plug or sperm in the vagina was designated day 0 of pregnancy. Once pregnant, rats were housed individually and allocated to study groups using a random number system. Drugs were suspended in 0.25% Celacol by ball milling. Pregnant rats received one of the following drugs or Celacol 0.25% (0.5 ml/100 g, p.o.) once daily from days 7–15 of pregnancy inclusive (over organogenesis): sodium valproate (656 mg/kg = $1 \times ED_{50}$; Note $LD_{50} = 670$ mg/kg; Jenner, Hagan, Taylor, Cook & Fitzhugh, 1964); phenytoin (250 mg/kg = $10 \times ED_{50}$); phenobarbitone and carbamazepine (40 mg/kg = $4 \times ED_{50}$) and pyrimethamine (5 mg/kg). Animals were killed on day 20 and tissues removed and rapidly frozen for folate analyses. Folates were measured by radioassay using β-lactoglobulin as the binding protein (Rothenberg, Da Costa & Rosenberg, 1972; and Tigner & Roe, 1979).

Sodium valproate and pyrimethamine produced 25% and 91% reabsorptions and 15 and 10 gross malformations respectively, from a total of 137 and 114 implantations in 10 and 11 litters respectively. The spontaneous reabsorption rate in control rats ($n = 272$ foetuses) over 5 experiments was approximately 1%. No gross teratogenicity was seen following treatment with other anticonvulsant drugs. Sodium valproate (SV) significantly decreased both foetal liver folate by 27%; control = 7.46 ± 0.43 μg/g (foetal no. = 107; litter no. = 9), SV = 5.41 ± 0.49 μg/g (103/10), $P < 0.010$, and placental folate by 25%, control = 0.99 ± 0.07 μg/g (placental no. = 107; litter no. = 9), SV = 0.74 ± 0.06 μg/g (137/10), $P < 0.020$, whereas phenytoin, phenobarbitone and carbamazepine had no effect. Pyrimethamine (PYR) significantly decreased placental folate by 62% ($P < 0.010$); control = 1.36 ± 0.16 μg/g (placental no. = 160; litter no. = 13); PYR = 0.65 ± 0.16 μg/g (114/11). Foetal brain and maternal liver folate concentrations were not significantly altered by any anticonvulsant drug treat-

ment. Foetal brain and body weights were significantly decreased ($P < 0.001$) by 25 and 35% respectively after sodium valproate treatment.

Thus, at its anticonvulsant ED_{50} value, sodium valproate but not phenytoin, is both teratogenic and antifolate in this rat model.

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The effect of chronic ethanol treatment on membrane lipids in the mouse

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Ethanol tolerance may be accounted for, in part, by adaptation at the cellular level (Chin & Goldstein, 1977). It is generally accepted that the cell membrane is involved in the action of the drug, and so changes in its constituent lipid and protein provide means by which the adaptation can occur. The importance of changes in lipid composition, by virtue of their effects on membrane fluidity, received support when Littleton & John (1977) showed that there was a reduction in the polyunsaturated fatty acid content of the phospholipids in synaptosomal membranes from ethanol-tolerant mice.

In this study, in order to minimize secondary effects on membrane-lipid composition due to dietary factors, stress and hypothermia, two different methods of administration of ethanol have been used. Male mice of the Charles River CD-1 strain weighing 24.6 g ± 0.28 (s.e.mean) were either injected i.p., twice daily for 7 days, with 20% (w/v) ethanol in saline at 4.5 g ethanol/kg body wt. or exposed to ethanol vapour for 2 weeks at a final concentration of 17 mg/l air. Suitable controls were run with both methods. All animals were allowed free access to food (Charles River diet) and water at all times.

Mice receiving ethanol as a vapour showed no significant change in body weight compared with their controls, but their food consumption was 75% of the control mice. Those receiving ethanol by i.p. injections maintained their initial body weight but, after 7 days, this was significantly less (24.7 g ± 0.4 s.e.mean) than that of controls (27.5 g ± 0.4 s.e.mean); at this time food consumption was 67% of the control level.

Erythrocyte membranes (Chin & Goldstein, 1977) were isolated and the fatty acids obtained from alkaline hydrolysis of the total membrane phospholipids were assayed by gas-chromatography of their methyl esters. Despite the differences in the two courses of drug administration, both ethanol-treated groups showed similar percentage increases in their octadecenoic (18:1) acid content: 7.2% ± 1.8 (s.e.mean) for the injected groups and 7.6% ± 2.4 (s.e.mean) for those exposed to vapour. Other changes in the fatty acid profile of both groups followed a similar pattern, notably an increase in stearic (18:0) acid and a decrease in docosahexaenoic (22:6) acid but these effects were not, in each case, statistically significant. No changes in cholesterol:phospholipid ratios were observed.

Additional tests with the vapour-treated mice also showed a significant 21.0% ± 3.9 (s.e.mean) increase in octadecenoic (18:1) acid from the phospholipids of hepatic plasma membranes. These changes were accompanied by a slight decrease in stearic (18:0) acid content (6.2% ± 1.7 s.e.mean). Again there was no effect on the overall cholesterol:phospholipid ratio.

A number of the observed changes (e.g. increases in octadecenoic (18:1) acid) are inconsistent with the simple view of membrane-lipid adaptation to a fluidizing effect of ethanol. These could represent other metabolic effects of the drug, also suggested by the increase in octadecenoic (18:1) acid content reported in whole liver phospholipids in several strains of mice treated with ethanol by Littleton, Grieve, Griffiths & John (1980). Adaptive and other mechanisms of alteration of membrane lipids may mask each individual effect and vary between membrane types. Preliminary results with synaptic membranes from the vapour-treated mice have not revealed any effects of ethanol on the fatty acid profile of their phospholipid.

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Ethanol preference after chronic ethanol drinking in mice

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Large differences in the preference for drinking ethanol have been observed in many different species and even between strains within species. Various theories have been put forward to account for these differences, notably that ethanol preference is associated with increased ethanol metabolism and reduced brain sensitivity to ethanol (Kakihana, Brown, McClearn & Tabershaw, 1966; Kulkosky & Cornell, 1979).

To test this theory C57Bl 10/ScSn mice were made ethanol tolerant by being given increasing concentrations of ethanol in tap water as their sole drinking fluid up to 20% w/v, and kept at this concentration for a minimum of two weeks (Unwin & Taberner, 1980). Liver alcohol dehydrogenase (AlDH) activities of naive and ethanol tolerant mice were measured by the method of Bonnichsen & Theorell (1951). Preference for ethanol solution (12% w/v) versus water was measured over a period of 48 h with the position of the bottles reversed after 24 h. AlDH activity and preference were also determined in ethanol tolerant mice that had been withdrawn from ethanol and returned to drinking tap water for 12 weeks. Results are shown in Table 1.

The change in AlDH activity confirmed that significant enzyme induction occurs in tolerant mice ($P < 0.001$ using Student's t-test), which reverts to control values in withdrawn mice. However the pre-

Table 1 Ethanol preference and liver AlDH activity in naive, ethanol tolerant and withdrawn mice

	% ethanol consumed mean (12% ethanol/total fluid) ± s.e. mean	*Mean alcohol dehydrogenase activity (nmol ethanol/min)/ mg liver ± s.e. mean*
naive	58 ± 8	0.93 ± 0.02
tolerant	*9 ± 1	*1.12 ± 0.01
withdrawn	*11 ± 3	0.94 ± 0.05

*Significantly different ($P < 0.001$) from control.

ference studies showed the opposite effect to that predicted. The tolerant animals lost their initial preference for ethanol (12% w/v, $P<0.001$ using Student's t-test), this change lasting for 12 weeks after withdrawal.

In conclusion, it appears that although the naive animals' preference for ethanol may be related to AlDH activity, enforced intake of ethanol in some way changes preference (possibly permanently) so that it becomes unrelated to metabolic activity.

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The effect *in vitro* of high extracellular glucose on the axonal transport of glycoprotein, protein and noradrenaline in ligated postganglionic sympathetic nerves of the cat

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Impaired axonal transport of glycoprotein in sensory nerve fibres together with reduced velocities of impulse conduction in the sciatic nerve are the earliest reported neurological disorders in chemical diabetes of the rat (see Jakobsen, Sidenius & Lundbaek, 1980). Sidenius & Jakobsen (1981) have suggested that the cause of imparied glycoprotein transport is elevated blood glucose. The present study was designed to test this hypothesis.

The inferior mesenteric ganglion of the cat together with attached hypogastric nerves was maintained in a twin-compartment culture chamber (Banks, Mayor, Mitchell & Tomlinson, 1971), in which the nerve trunks pass through a silicone grease seal in the dividing barrier, such that the fluid bathing the ganglion may be of a different composition from that bathing the nerve trunks. [^{3}H]-fucose (1.25 μCi/ml) and [^{14}C]-leucine (0.5 μCi/ml) were added to the culture medium in the ganglion compartment to label glycoprotein and protein synthesised in the neurone cell bodies. In experimental preparations the glucose content of the medium in the nerve trunk compartment only was raised to 25 mM. Control preparations were bathed in 5.5 mM glucose on both sides of the barrier. Preparations were incubated for 48 h at 37°C. At the end of this time the nerves were cut into 1 mm segments and homogenised in 0.1 ml 5% perchloric acid.

The accumulations of materials in the 3 mm of nerve trunk proximal to the ligature are expressed after subtraction of the 'background' levels in an equivalent segment of nerve closer to the barrier. Accumulations of radioactivity are expressed as (d/min)/μCi isotope added to the 20 ml fluid bathing the ganglion to take account of variation in the amount of isotope dispensed. All values are means $\pm$ s.e.mean derived from 5 control preparations and 6 preparations in which the nerves were exposed to high glucose.

The accumulation of incorporated leucine (control, 108.5 ± 4.0 high glucose, 97.6 ± 12.1) and of noradrenaline (pmole/nerve: control, 334 ± 31; high glucose, 320 ± 27) were unaffected by elevation of extracellular glucose. However, the accumulation of incorporated fucose in the nerve trunks bathed in 25 mM glucose (257 ± 38) was significantly ($P<0.05$) less than that found in control preparations (367 ± 23). The deficit in accumulated fucose proximal to the constrictions in the nerves exposed to high glucose was balanced by an excess, over control preparations, of 71 ± 12.3 (d/min)/μCi [^{3}H] added found proximal to the barrier. These findings indicate that elevated extracellular glucose causes, within 48 h, an impairment of axonal transport of glycoprotein in noradrenergic neurones without a concomitant effect on transport of protein and noradrenaline. Should such a defect be maintained in hyperglycaemia *in vivo*, then one would expect profound functional lesions of the neurone to ensue.

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The action of 2-phenylisatogen on adenosine nucleotide and phosphate metabolism in mitochondria isolated from guinea-pig liver

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The smooth muscle relaxant properties of substituted isatogens may be related to their ability to interfere with energy producing reactions in mitochondria (Foster, Hooper, Spedding, Sweetman & Weetman, 1978). One of the most potent smooth muscle relaxant drugs in the isatogen series is 2-phenylisatogen, which possesses the additional advantage of being a weak ATP-receptor antagonist. We have therefore selected this molecule for a study of its mode of action on energy metabolism.

The respiratory activity of guinea-pig liver mitochondria was measured using an oxygen electrode, and ATPase activity was estimated by the method of Beechey (1966). Ion transport reactions were measured either by following mitochondrial volume changes spectrophotometrically (phosphate transport), or by enzymic determination of ADP (ADP transport).

2-Phenylisatogen inhibited ADP-stimulated respiration whether succinate (IC_{50} = $2.65 \pm 0.23\,\mu M$; $n = 5$) or glutamate plus malate ($IC_{50} = 5.4 \pm 0.9\,\mu M$; $n = 5$) was used as substrate, indicating that the isatogen was a potent inhibitor of mitochondrial oxidative phosphorylation. In general, inhibitors of ATP synthesis act in one of two ways: (a) by inhibiting one of the enzymes involved in oxidative phosphorylation, or (b) by blocking the uptake into the mitochondrial matrix of a substance essential for the synthesis of ATP. Experiments were designed to test between these two possibilities.

(a) Enzyme inhibition. The inhibition of ADP-stimulated respiration by 2-phenylisatogen was released by uncoupling concentrations of 2,4-dinitrophenol, showing that the isatogen was not interacting with a respiratory chain enzyme. An effect on the phosphorylation system was tested for by measuring mitochondrial ATPase activity, in the presence of 2,4-dinitrophenol, to stimulate the reaction. Control experiments showed that 2-phenylisatogen did not itself stimulate the reaction. The 2,4-dinitrophenol-stimulated ATPase was inhibited by 2-phenylisatogen, but only at high concentrations (IC_{50} = 0.72 ± 0.09 mM; $n = 4$), whereas the energy-transfer inhibitor, oligomycin blocked the reaction at low concentrations (0.5–1.5 μg/ml). These experiments ruled out the possibility of an inhibitory action or uncoupling effect of 2-phenylisatogen on the enzymes that participate in mitochondrial energy production.

(b) Uptake blocking. Since the inhibitory action of 2-phenylisatogen on ADP-stimulated respiration occurred regardless of the substrate used, it was concluded that an action of the compound on a specific respiratory substrate transport system was unlikely. 2-Phenylisatogen, at concentrations up to 0.27 mM, failed to prevent the uptake of phosphate into mitochondria from a medium containing ammonium phosphate. The phosphate-dependent uptake of potassium, in the presence of the ionophore, valinomycin was similarly insensitive to the isatogen. Both reactions were inhibited by p-hydroxymercuribenzoate, a well-established phosphate transport inhibitor, in the range 10–100 μM. The uptake of ADP by mitochondria was inhibited by low concentrations of 2-phenylisatogen (IC_{50} = $2.05 \pm 0.3\,\mu M$; $n = 5$). A similar pattern of activities was found with the ADP transport inhibitor, atractyloside, for example, ADP-stimulated respiration and ADP transport were both inhibited by atractyloside over the range 0.1–1.0 μM.

It is concluded that 2-phenylisatogen inhibits ATP synthesis by preventing the uptake of ADP into the mitochondrial matrix.

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Irreversible inhibition of rat liver monoamine oxidase by histamine and some analogues: protective effects of (−)-mexiletine

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Histamine is not generally considered to be a substrate for mitochondrial monoamine oxidase (MAO). However, Lyles & Shaffer (1979) described a time-dependent irreversible inhibition by histamine of MAO-A and MAO-B in rat kidney mitochondria, which was not reversed by washing. Similar results are now described for histamine and the H_1 receptor agonists 2-pyridylethylamine and 2-thiazolylethylamine (Durant, Ganellin & Parsons, 1975) as inhibitors of rat liver mitochondrial MAO.

Livers from 6 male Sprague-Dawley rats (150–200 g) were homogenized in sucrose (0.25 M), potassium phosphate buffer (0.01 M, pH 7.8) and centrifuged at 800 g for 10 min to remove cell debris. The supernatant was decanted and centrifuged at 12,000 g for 20 min to produce a mitochondrial pellet, which was then resuspended in homonogenization buffer and divided into several portions for storage at −20°C. MAO-A and MAO-B activities were assayed radiochemically by the use of [^{3}H]-5-hydroxytryptamine (5-HT) and [^{14}C]-benzylamine (BZ) as substrates at 1 mM assay concentration. Mitochondrial aliquots were preincubated at 37°C for between 0 and 90 min with 2 mM histamine, pyridylethylamine or thiazolylethylamine, before the addition of 5-HT or BZ to assay for remaining MAO activity. With histamine, MAO-A and MAO-B were inhibited in a time-dependent manner, with little or no inhibition occurring without preincubation, while after 90 min MAO-A was inhibited by about 70% and MAO-B by about 50%. Although they also produced time-dependent inhibition, pyridylethylamine and thiazolylethylamine caused significant inhibition without preincubation.

The reversibility of inhibition was tested by 20-fold dilution of samples which had been preincubated for 60 min with each inhibitor. These mixtures were then centrifuged at 30,000 g, the resulting pellet was resuspended in homogenization buffer, followed by a repetition of the centrifugation and resuspending procedure. No significant reversal of the inhibition by histamine, and only partial reversal of the effects of pyridylethylamine and thiazolylethylamine was obtained by this technique.

(−)-Mexiletine is a reversible competitive MAO inhibitor with a greater selectivity for MAO-A (Callingham, 1977). Consequently, (−)-mexiletine (2×10^{-4} M) was included in some preincubation (60 min) mixtures, with or without histamine and its analogues, before using the washing technique. In samples preincubated with (−)-mexiletine alone and assayed before washing, inhibition of MAO-A (about 85%) was considerably greater than that of MAO-B about (15%). On washing, inhibition was completely reversed. Washing of samples preincubated with both (−)-mexiletine and histamine restored MAO-A and MAO-B activities to control levels. Inhibition of MAO-A activity by combined (−)-mexiletine and thiazolylethylamine was also fully reversible, although MAO-B activity was only partially restored by washing.

In conclusion, histamine, pyridylethylamine and thiazolylethylamine produce a time-dependent, apparently irreversible inhibition of MAO-A and MAO-B in rat liver. The ability of (−)-mexiletine to prevent, or largely reduce, this irreversible action presumably by protecting the active-site of the enzyme (particularly MAO-A), may indicate that histamine and its analogues (or their metabolites?) also produce their effects by binding directly to this part of the enzyme.

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Studies on the antinociceptive effects of clonidine and 4-hydroxyclonidine in the rat

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Clonidine is a potent antinociceptive agent in the rodent (Paalzow, 1974; Fielding, Wikler, Hynes, Szewczack, Novick & Lal, 1978). The present study was carried out to determine whether this effect is due to (a) an action of the drug on a central or peripheral site, and (b) an action of α_1- or α_2-adrenoceptors. To investigate the site of action of clonidine induced antinociception the potencies of clonidine and 4-hydroxyclonidine, its less lipophilic metabolite, were compared after intracerebroventricular (icv) (Popick, 1976) and subcutaneous (sc) administration in the rat. Nociceptive pressure thresholds were determined in the hindpaws of weanling rats (AH hooded, male, 50–70 g) using an 'analgesymeter' (Ugo Basile). All antinociceptive testing was carried out blind. Testing was carried out at 10 or 20 min after icv administration and 30 min after sc administration as previous experiments had shown these to be the times of peak effect. The antinociceptive potency of clonidine was only 3 × less when given sc than when given icv. The ED50 values (95% confidence limits) were 0.15 (0.05 to 0.46) and 0.047 (0.017 to 0.12) mg/kg respectively. In contrast 4-hydroxyclonidine, which does not readily pass the blood brain barrier, was inactive at 8.1 mg/kg sc, a dose some 18 × greater than the icv ED50, 0.46 (0.15 to 1.2) mg/kg. These results indicate that the antinociceptive actions of clonidine and 4-hydroxyclonidine are mediated centrally rather than peripherally.

Spaulding *et al.*, (1979), suggested that the antinociceptive action of clonidine was due to an action at the spinal level. In the paw pressure test in the rat (AH hooded, male 280–330 g), we have also shown an antinociceptive effect for clonidine (150 μg/kg) intrathecally. However, after icv administration to the weanling rat, increases in nociceptive pressure thresholds were observed with a dose of only 25 μg/kg, a dose which was ineffective after intrathecal administration. Hence, although a spinal component may contribute to the antinociceptive effect of clonidine when given parenterally it is likely that there is also an important supraspinal site of action.

To determine whether the antinociceptive effect of clondine in the rat was mediated via α_1- or α_2-adrenoceptors the order of potency of some drugs which have varying selectivity as agonists for α_1 and α_2-adrenoceptors were determined following icv administration. In these tests clonidine was more potent than xylazine and naphazoline while the selective α_1 agonist phenylephrine was inactive at 500 μg/kg icv. This agrees with the order of potency for these agonists at presynaptic α_2-adrenoceptors in the isolated rat heart preparation (Drew, 1976). Furthermore, yohimbine (0.67 to 6 mg/kg po), a selective α_2-adrenoceptor antagonist, produced a dose-related antagonism of the clonidine induced antinociceptive effect. In contrast, the selective α_1-adrenoceptor antagonist, prazosin (0.15 mg/kg icv), had no effect on the antinociceptive effects of clonidine. These preliminary results suggest that clonidine antinociception in the paw pressure test in the rat is mediated by receptors similar to the presynaptic α_2-adrenoceptors.

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A rapid [^{133}Xe] clearance technique to measure blood flow changes in rat skin grafts

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Lewis, Peck, Williams & Young (1976) used a [^{133}Xe] clearance technique to study blood flow changes in skin auto- and allografts based on the method of Sjersen (1969). It was established that intradermally injected [^{133}Xe]-saline would produce mono-exponential or bi-exponential washout curves depending on the vascular integrity of the grafted tissue. Clearance constants derived from the washout curves provide a measure of blood flow changes which correlate with the presence of vasoactive mediators in homogenates of the grafts (Lewis & Mangham, 1978). However, this technique requires continuous monitoring of the washout of radioactivity for 30–40 min, thus limiting the number of observations that can be made in each experiment. Furthermore, derivation of the clearance constants proved time-consuming. In the present study an attempt has been made to simplify the technique to provide rapid and reliable measurements.

In the previous experiments outbred rabbits were used as inbred rabbits were not available. In the present experiments outbred rats were used initially in order to complement the work in rabbits. However, some variability was encountered and in subsequent experiments male inbred rats of WAG (AgB2) and DA (AgB4) strains were used.

The rats weighing 150–200 g were anaesthetized with Hypnorm (Janssen) 0.25 ml/kg (0.315 mg/ml fentamyl citrate and 10 mg/ml fluanisone) i.m. Full thickness skin grafts (2 cm × 2 cm) were removed from the abdomen and transplanted onto dorsal graft beds of the recipient. Each rat received two isografts or allografts. [^{133}Xe]-saline (0.01 ml) was injected intradermally into skin grafts or normal skin of conscious rats, the washout of radioactivity measured every 40 s for 30–45 min and clearance constants derived as previously described (Lewis *et al.*, 1976).

The same data have been treated in another way. Instead of deriving the faster component of the clearance curve by curve peeling, the clearance was monitored only at 40 s (initial count) and at 6 min after injection of [^{133}Xe]-saline. The clearance at 6 min was then calculated as a percentage of the initial count. When these values were plotted together with K_1 values calculated by the original method, against days after grafting, the correlation coefficient between the two curves was $r = 0.982$. Therefore, the data obtained from the simplified technique provided a good estimate of clearance. As examples of the application of this technique clearance curves have been obtained from rats receiving isografts or allografts, as well as allografted rats treated with an immunosuppressive agent – cyclosporin-A (Sandoz) 40 mg/kg day, intra-muscularly. These results will be demonstrated.

Thus, this simplified [^{133}Xe] clearance technique is valuable in the study of wound healing, skin allograft rejection and also in routine screening of potential immunosuppressives.

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BW12C: a new anti-sickling agent

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Sickle cell disease is a genetic disorder in which the more usual haemoglobin A (HbA) is replaced by sickle haemoglobin (HbS). Deoxygenated HbS has a low solubility compared with HbA and can form intracellular polymers. The consequent distortion of the erythrocyte gives rise to the characteristic sickling phenomenon which leads to the symptoms of the disease. One approach to the therapy of sickle cell disease might be to increase the proportion of soluble oxygenated sickle haemoglobin in regions of low oxygen tension so that the polymerization of the deoxygenated form is inhibited (Dean & Schechter, 1978). This would result in a left-shift of the oxygen dissociation curve (o.d.c.) of sickle blood.

Samples of adult human blood (HbA) were incubated with or without BW12C (5-(2-formyl-3-hydroxyphenoxy) pentanoic acid) (0–9 mM) for 30 min at 37°C under an atmosphere of humidified air/5% CO_2. The o.d.c. was then measured using the 'Hem-O-Scan' apparatus (Aminco) which plots the % oxygenation of the blood as a function of the partial pressure of oxygen. In the absence of BW12C, the curve was sigmoid ($P_{50} \simeq 26$ mmHg) but in the presence of BW12C there was a concentration-dependent left-shift of the o.d.c. and at the highest concentration, the curve appeared hyperbolic with a P_{50} of < 5 mmHg. The shape of the curves suggests a tight-binding of BW12C to haemoglobin and a stable complex could be demonstrated using isoelectric focussing methods by the slight acid shift ($\simeq 0.2$ pH units) of the isoelectric point compared with unmodified haemoglobin (pI = 6.95). The left-shift of the o.d.c. and the modification of the isoelectric point were similarly demonstrated in sickle blood, and in blood from several species including the rat, cat, dog and marmoset.

In other experiments, sickle blood was incubated for 2 h at 37°C under a humidified atmosphere with, or without BW12C (0–9 mM). Samples of cells were fixed in buffered formal saline and scored for cell type as the proportion of 'normal', 'sickle' or 'bizarre' forms (Beddell, Kneen & White, 1979). In aerated controls, the normal form predominated (> 90%), but under a reduced oxygen tension ($\simeq 30$ mmHg) the proportion fell substantially. This fall was prevented by the presence of BW12C, and there was a concentration-dependent rise in the proportion of normal cells. At the highest concentration the appearance of the partially deoxygenated cells was similar to that of aerated controls, demonstrating a complete inhibition of the sickling phenomenon.

Thus, BW12C has been shown to left-shift the o.d.c. of blood from several species, including human sickle blood in which, under conditions of partial deoxygenation, the compound inhibits the sickling phenomenon.

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Phospholipid mediators of human platelet activation

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Studies *in vitro* indicate that 'primary' platelet aggregation is mediated solely by an exogenous agonist, whereas 'secondary' platelet aggregation is initiated by an exogenous agonist and propagated by endogenous mediators secreted or produced by the activated platelets. These endogenous mediators of 'secondary' aggregation include the arachidonate metabolites ($TxA_2 > PGG_2 = PGH_2$), and ADP secreted from platelet dense granules (Holmsen, 1977). Since stimulation of platelets is associated with profound changes in lipid metabolism, it has

been suggested that phospholipids such as Phosphatidic acid or Lyso-phosphatidic acid (LPA) (Gerrard, Kindom, Peterson, Peller, Krantz & White, 1979) and 1-0-Alkyl-2-0-acetyl-sn-glyceryl-3-phosphorylcholine (AGEPC) also known as Platelet-activating factor (PAF or PAF-acether) and as Anti-hypertensive polar renomedullary lipid (APRL) (Vargaftig, Chignard & Benveniste, 1981) may serve as the third endogenous mediator of platelet activation. To examine the possibility that these putative phospholipid mediators exert their effects via a common mechanism, we have investigated the actions and interactions of LPA and PAF-acether on human platelets.

Platelet aggregation was measured photometrically in 0.15 or 0.5 ml samples of human citrated platelet-rich plasma (PRP). Release of platelet 5HT (a dense granule marker) and adenine (a cytoplasmic marker) were monitored by isotope pre-labelling techniques, and release of β-thromboglobulin (βTG, an alpha granule marker) and formation of TxB_2 were measured by radio-immunoassay (MacIntyre, 1979).

Decanoyl LPA ($< 300\,\mu M$) did not affect platelet function, but low concentrations of Oleoyl (O) LPA and Palmitoyl (P) LPA induced reversible aggregation in the absence of 5HT release ('primary' aggregation). The minimal active concentration (MAC) in PRP from different donors ranged from $1-30\,\mu M$. PAF-acether exerted a similar effect but was much more potent (range of MAC, 10–80 nM). Higher concentrations of OLPA or PLPA ($< 300\,\mu M$) and of PAF-acether ($< 0.5\,\mu M$) induced irreversible aggregation, associated with formation of TxB_2 and the selective release of 5HT and βTG ('secondary' aggregation). PGE_1 ($1\,\mu M$) inhibited both 'primary' and 'secondary' aggregation induced by LPA or PAF-acether, but indomethacin ($30\,\mu M$) only blocked 'secondary' aggregation. Addition of PLPA ($30\,\mu M$) or PAF-acether (80 nM) to PRP for > 2 min at 37°C rendered platelets unresponsive (refractory) to a second addition of the same agonist, although responses to ADP ($0.3-1\,\mu M$), U46619 ($0.1-0.3\,\mu M$) 5HT ($30-100\,\mu M$) and Vasopressin (0.01–1 U/ml) were not suppressed. PLPA-refractory platelets responded normally to PAF-acether, and PAF-acether-refractory platelets responded normally to PLPA.

These results indicate that both LPA and PAF-acether can directly activate human platelets. Since no cross-denensitization was observed between these phospholipid agonists, their effects are presumably mediated by combination with different receptor sites in/on the platelet. Hence, there are at least two classes of phospholipid that may function independently as endogenous mediators of human platelet activation.

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The effect of covalent attachment of dextran to the anti-leukaemic agent L-asparaginase on anaphylactic reactions to the enzyme *in vivo* and *in vitro*

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The routine use of L-asparaginase in the treatment of acute lymphoblastic leukaemias is hampered by the development of hypersensitivity reactions. In an attempt to reduce the antigenicity of this enzyme, asparaginase from *Erwinia carotovora* was covalently bound to dextrans of increasing molecular weight (10,000, T10; 40,000, T40; 70,000, T70; daltons) as previously described (Elliott, Foster & Wileman, 1981).

Four guinea-pigs, previously sensitized to the nat-

Table 1 Contraction of sensitized guinea-pig ileum to antigen challenge

First Challenging agent	*Mean Response ± s.e.mean*	*Second Challenging agent*	*Mean Response ± s.e.mean*	*Number of Samples*
Asparaginase – T10 conjugate	49.2 ± 10.8	Native asparaginase	2.1 ± 1.5	8
Asparaginase – T40 conjugate	22.8 ± 4.2	Native asparaginase	20.7 ± 3.8	8
Asparaginse – T70 conjugate	2.8 ± 0.9	Native asparaginase	53.9 ± 5.8	8

The mean responses were calculated from the response to antigenic challenge expressed as a percentage of the maximal response obtainable with histamine in each case.

ive enzyme, were anaesthetized and the trachea of each animal cannulated, and connected to a pneumotachograph. When a steady respiratory rate was established the T70 conjugate (containing 1 mg of asparaginase) was injected into the femoral vein and the respiratory rate monitored for 3 minutes. Five min later the native enzyme (1 mg) was injected into the femoral vein and the animals similarly monitored. In contrast to the T70 conjugate which was well tolerated in all animals, the native enzyme caused an immediate and marked increase in respiratory rate accompanied by respiratory distress resulting in the death of the animals within a few minutes.

Using the Dale-Schultz reaction the size of the contraction of the guinea-pig ileum was found to be inversely proportional to the molecular weight of the challenging conjugate and the response to a second challenge using the native enzyme was directly proportional to the molecular weight of the conjugate used in the first challenge (Table 1).

This study shows that covalent conjugation of asparaginase to dextran can markedly reduce the anaphylactic response that follows injection of the antigen into sensitized guinea-pigs. Furthermore, this lowered antigenicity would appear to be a function of the molecular weight of the dextran attached to the enzyme. In view of this reduced antigenicity, it would appear, therefore, that the asparaginase-T70 dextran conjugate, reported here, should have therapeutic potential.

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Phosphatidylinositol metabolism in GH_3 pituitary tumour cells stimulated by TRH

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Many hormones and neurotransmitters produce their effects by increasing the concentration of free cytosolic Ca^{2+} in the cell (Putney, 1979). These same agents also increase the breakdown and turnover of phosphatidylinositol (PI). Michell (1975) has proposed that the breakdown of PI precedes and, in fact, regulates the entry of calcium into the cell cytoplasm. In order to study this phenomenon in detail, a rat pituitary tumour cell line (GH_3) has been adopted as a model system; these cells contain TRH receptors which enhance the release of prolactin in a Ca^{2+}-dependent manner (for review, see Martin & Tashjian, 1977).

GH_3 cells were grown in monolayer culture at 37°C, under 95% air/5% CO_2 in Minimal Essential Medium (Earle's Salts) supplemented with foetal calf serum (2.5%), horse serum (15%), benzyl penicillin (100 iu/ml) and streptomycin (100 μg/ml). After 30 min pre-incubation at 37°C with 10 μCi [^{32}P]-phosphate per dish, the cells ($\sim 5 \times 10^6$/dish) were exposed to TRH (1 μM). At various times thereafter the reaction was terminated and phospholipids were

extracted and separated on thin-layer chromatograms. Within 10 min of TRH addition, incorporation of [^{32}P]-phosphate into PI and phosphatidic acid (PA) was markedly enhanced (7- and 4-fold respectively above controls). Incorporation into other identifiable phospholipids was unaffected by TRH at this time. At 30 min the effect appeared to be maximal (10- and 5-fold increased incorporation into PI and PA respectively versus controls) and by 2 h the TRH-treated cells showed a rate of [^{32}P]-phosphate incorporation into these phospholipids approaching control values, indicating that the response had desensitized. At incubation times beyond 10 min there was a slight (1.5–2.5 fold), TRH-induced increase in incorporation into many other phospholipids; this occurred later than the effects on PI and PA, and coincided with a concentration-dependent enhancement of [^{32}P]-phosphate uptake into acid-soluble compartments within the cell. Further experiments indicated that the effects on PI and PA were concentration-dependent, a half-maximal response being elicited by around 3 nM TRH. In an effort to distinguish between changes in turnover and net accumulation/degradation of phospholipid, the cells were labelled to equilibrium with [^{32}P]-phosphate prior to stimulation by TRH; preliminary results indicate that there was a specific and rapid 4-fold increase in cellular PA levels in the presence of TRH.

These results provide further support for the view (Michell, 1975) that a Ca^{2+}-dependent cell response (in this case prolactin release) will be accompanied by a 'Pi-effect', and indicate that GH_3 cells may be an appropriate model system for investigating these phenomena. Experiments are underway to determine whether the accumulation of PA, a known calcium ionophore (Serhan, Anderson, Goodman, Dunham & Weissman, 1981), mediates the TRH-induced release of prolactin from these cells.

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Differences in cyclosporin-A sensitivities of tumour-promoting and non-promoting phorbol ester-induced human lymphocyte mitogenesis

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We have previously reported to the Society (Gordon & Nouri, 1981) that human lymphocyte mitogenesis induced by phorbol-12-myristate-13-acetate (PMA) was more resistant to inhibition by the immunosuppressive drug cyclosporin-A than was observed for other mitogenic stimuli such as phytohaemagglutinin (PHA). In contrast, PMA-induced proliferation was sensitive to inhibition by glucocorticosteroids such as dexamethasone whereas maximally PHA-stimulated cells were resistant.

The tumour-promoting and pro-inflammatory *in vivo* activities of the phorbol-12,13-diesters are generally correlated, whereas the naturally-occurring 12-deoxy-phorbol-13,20-diesters are potent inflammatory agents but lack co-carcinogenic properties (Hecker, 1971). In the present study, we found that two such non-promoting compounds, 12-deoxyphorbol-13-phenylacetate-20-acetate (12-DPPAA) and 12-deoxy-phorbol-13-angelate-20-acetate (12DPAA), were also effective human lymphocyte mitogens. PMA (1–1000 ng/ml) increased tritiated-thymidine (^{3}H-TdR) incorporation by human peripheral blood mononuclear cells (Gordon & Nouri, 1981b) in dose-related manner up to 50 ± 12-fold ($n = 7$ donors) compared with unstimulated cells, and the concentration required to produce 40% of the maximum response was 17 ± 7 ng/ml. The

equieffective concentrations of 12DPPAA and 12DPAA were 564 ± 245 and 693 ± 153 ng/ml respectively. Further, the maximum responses achieved using the non-promoters were significantly lower than that of PMA (69 ± 10 and 62 ± 10% respectively, both $P < 0.02$ compared with PMA maximum, Student's paired t-test). However, their corresponding 13-monoesters 12DPPA and 12DPA, which are potent inflammatory agents (Williams, Westwick, Williamson & Evans, 1981), but whose tumour-promoting activities have not been assessed, produced the same maximum level of [^{3}H]-TdR incorporation as PMA and were equieffective with PMA at only slightly higher concentrations (45 ± 19 and 81 ± 40 ng/ml respectively). Glucocorticosteroids are known to inhibit both the tumour-promoting and pro-inflammatory effects of phorbol esters (Slaga, Fischer, Viaje, Berry, Bracken, Le Clerc & Miller, 1978). Dexamethasone (1–1000 ng base/ml) inhibited the effects of all the phorbol esters (1 μg/ml) in a dose-related manner. The concentrations required to produce 50% inhibition (IC50) of the responses to 12DPPAA and 12DPAA were dexamethasone (4.4 and 4.8 ng/ml respectively), whereas the corresponding values for PMA, 12DPPA and 12DPA were 16.2, 11.5 and 41.0 ng/ml respectively ($n = 3$ donors). In agreement with our earlier observations, PHA (1 μg/ml)-induced responses were not significantly inhibited by dexamethasone (1 μg/ml) in the same experiments. Chloroquine diphosphate and p-bromophenacyl bromide were found to inhibit PMA-induced responses (IC50 values 1.6 and 6.0 μM respectively) but were also effective inhibitors of PHA-stimulated cells (IC50 values 2.2 and 14.5 μM respectively).

Cyclosporin-A (0.01–10.0 μg/ml) inhibited the mitogenic effects of the non-promoters 12DPPAA and 12DPAA (both at 1 μg/ml) in dose-related manner, with IC50 values of 0.30 and 0.14 μg/ml respectively ($n = 3$ donors) which were only slightly higher than the IC50 value of 0.07 μg/ml found for PHA-induced responses. In contrast, their corresponding 13-monoesters 12DPPA and 12DPA were as resistant as the tumour-promoter PMA to cyclosporin-A, which only inhibited their effects at the higher concentrations (1 and 10 μg/ml) with IC50 values 1.43, 1.15 and 1.95 μg/ml respectively in the same experiments.

Resolution of the mechanisms underlying the anomalous sensitivities of the tumour-promoting and non-promoting phorbol esters to inhibition by cyclosporin-A should aid elucidation of the precise mode of action of this novel immunosuppressive drug.

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pA_2 determination of muscarinic and H_2-receptor antagonists on gastric acid secretion *in vitro*

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The use of atropine-like drugs as antisecretory anti-ulcer agents is limited by their effects on muscarinic receptors in tissues other than the gastric mucosa. The muscarinic antagonist pirenzepine is able to distinguish between receptor sub-types in tissue-binding studies (Hammer, Berrie, Birdsall, Burgen & Hulme 1980) and may have a selective action *in vivo* on acid secretion compared with muscle contractility (Matsuo & Seki 1979). In this *in vitro* study we have

investigated the activity of pirenzepine (P) against acid secretion and the contraction of gastrointestinal smooth muscle and have included for comparison atropine (A) and the H_2-antagonist metiamide (M).

Using the rat isolated gastric mucosa (Main & Pearce 1978), pairs of control and test mucosae from a single stomach were randomly allocated to treatment groups. In test mucosae, the antagonist (P, 10^{-7} to 10^{-5} M; A, 10^{-8} to 10^{-6} M; M, 10^{-6} to 3×10^{-5} M) was present from 65 minutes. Low and high doses of agonist (methacholine or histamine), chosen to produce similar responses in the absence and presence of antagonist, were added at 120 and 210 min (30 min contact). The acid secretory response was calculated as the difference in output (μmol cm^{-2} h^{-1}) between the peak of the response and the preceding basal rate. A dose-ratio (DR) was obtained for each pair of mucosae. A Schild plot was constructed using individual DRs greater than 4 and the best line fitted by least squares regression. The antagonist potency of P was also investigated on the rat fundus strip and guinea pig ileum. In both tissues, sequential responses to methacholine were obtained before and following 30 min equilibration with P (10^{-6} to 10^{-4} M). Doses producing a 50% maximal response were used to calculate DR's.

In all assay systems increasing concentrations of antagonist produced a parallel shift to the right of the agonist log-dose response curve. Further, as the derived Schild plots had slopes close to unity the data are consistent with competitive antagonism over the concentration ranges investigated. The pA_2 values obtained were: 8.40 (slope = 0.88, $n = 14$) for A, 6.80 (0.89, 7) for P and 6.49 (0.81, 13) for M against acid secretion, and 6.91 (0.82, 14) and 6.90 (0.88, 15) for P on the rat fundus and guinea-pig ileum.

The pA_2 estimates for P obtained in the three tissues were closely similar to each other and to values previously reported for guinea-pig ileum (Barlow, Caulfield, Kitchen, Roberts & Stubley 1981). This observation suggests that the muscarinic receptors mediating gastric acid secretion are not distinguishable from those mediating contraction of gastro-intestinal smooth muscle.

Our finding that the pA_2 for atropine is lower than the standard estimate in other tissues, and the observation (Angus & Black, 1979) that in the mouse whole stomach pA_2 values for both atropine and H_2-antagonists are also consistently lower, raises the possibility that our estimate for pirenzepine on acid secretion may also be low. However, this seems unlikely since the pA_2 for metiamide against histamine in this preparation was not lower than in other tissues.

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A comparison of the effects of indomethacin and methylxanthines on basal and stimulated gastric secretion *in vitro*

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Indomethacin potentiates the acid secretory response to dibutyryl cyclic AMP (dbcAMP), both *in vivo* (Main & Whittle, 1975) and *in vitro* (Main & Melarange, 1978). The mechanism by which indomethacin causes this potentiation is not clear. However, since theophylline, a methylxanthine phosphodiesterase inhibitor, also potentiates the response to dbcAMP it is possible that indomethacin acts by inhibition of cyclic nucleotide phosphodiesterases.

The present study used the rat isolated gastric mucosal preparation, (Main & Pearce, 1978), to avoid any changes in mucosal blood flow or nervous and hormonal influences. The actions of theophylline, isobutyl methylxanthine (IMX) and indomethacin on basal and stimulated gastric acid secretion were investigated using paired test and control preparations. Responses to cumulative doses of dbcAMP (2.5×10^{-5}, 10^{-4} and 2×10^{-4} M) were obtained. The test preparations were pretreated for 1 h with one of the potentiating drugs. Responses to dbcAMP (2.5×10^{-5} M and 10^{-4}) were significantly potentiated by theophylline 10^{-3} M, and in a dose related manner by indomethacin (3×10^{-5} M; 3×10^{-6} M). There was no significant increase in the near maximal response to dbcAMP (2×10^{-4} M). (For indomethacin 3×10^{-6} M response, in μmol cm^{-2} h^{-1}, for increasing concentrations of dbcAMP were 0.79 ± 0.39, 3.44 ± 0.80, 6.49 ± 1.70, in control mucosae, and 1.69 ± 0.58, 6.97 ± 1.14, 8.52 ± 0.37, in the corresponding test preparations, $n = 6$). IMX (10^{-5} M) significantly potentiated only the lowest concentration of dbcAMP while IMX (10^{-6} M) showed no effect on any. In concentrations which potentiated dbcAMP, both methylxanthines caused a significant increase in basal acid secretion, whereas indomethacin had no effect. IMX (10^{-4} M or higher) caused near maximal stimulations. The effect of these drugs on responses to histamine (5×10^{-5} M) were also investigated since phosphodiesterase inhibitors would be expected to potentiate the action of this secretagogue. Histamine was potentiated by IMX (10^{-5} M) and theophylline (10^{-3} M). However, indomethacin (3×10^{-5} M) did not significantly alter the secretory response to histamine.

If indomethacin potentiated dbcAMP by inhibiting cyclo-oxygenase and thus preventing the formation of inhibitory products then treatment with PGE_2 might be expected to reverse this potentiation. However, since pretreatment with PGE_2 (2×10^{-6} M) caused a significant reduction not only of the potentiated response in the presence of indomethacin (3×10^{-5} M) but also of dbcAMP (10^{-4} M) alone, this provides no evidence in favour of the hypothesis. Although, like methylxanthines, indomethacin does potentiate dbcAMP it is unlikely to be acting as a phosphodiesterase inhibitor since it did not potentiate the response to histamine or alter basal secretion.

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The effects of deglycyrrhizinated liquorice and cimetidine (alone and in combination) on resting gastric mucosal blood flow and acid secretion in Man

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Peptic ulcer disease is often associated with high acid output, and as early as 1910 Schwartz (Schwartz, 1910) propounded that 'no acid, no ulcer' and at the same time concluded that peptic ulcer disease was due to an imbalance in the protective and damaging mechanisms. We investigated this idea by studying two agents (deglycyrrhizinated liquorice (DGL) and cimetidine) both of which have been shown to heal peptic ulcers in Man (Morgan, McAdam, Pacsoo, Walker & Simmons, 1978), although the exact mechanisms by which they exert this effect are not entirely understood – cimetidine reduces acid secretion and gastric mucosal blood flow (GMBF) (Fielding, Knight & McIsaac, 1978) while DGL increases basal GMBF in dogs, without a concurrent increase in acid secretion (Johnston & McIsaac, 1981).

Cimetidine and DGL have been tested alone and in combination on basal acid secretion and GMBF in nineteen normal male volunteers (aged 18–28) after an overnight fast. Gastric secretion was collected via a nasogastric tube for 13 consecutive 20 min periods by continuous aspiration. H^+ concentration was measured by titration to pH 7.0 with 0.1 N NaOH,

and GMBF by the neutral red clearance (NRC) technique (Fielding, Knight & McIsaac, 1978). The volunteers were divided into three groups. Group 1 ($n=7$) received 1 g of DGL orally (suspended in 20 ml of distilled water) during period 10, during which time the aspiration pump was turned off. Group 2 ($n=7$) received cimetidine intravenously – a bolus (100 $\mu g\,kg^{-1}$) followed by 100 $\mu g\,kg^{-1}\,h^{-1}$ infusion at the start of period 6 and, as control, 20 ml of distilled water during period 10, with the pump switched off. Group 3 ($n=5$) received DGL as for Group 1, and cimetidine as in group 2. NRC and acid secretion were compared before and after the administration of drugs using the Mann Whitney U Test.

DGL alone was found to increase NRC from 18.2 ± 3.7 ($\pm$s.e.mean) ml/min to 53.1 ± 14.43 ml/min ($P<0.02$), but had no significant effect on acid secretion (41.6 ± 5.44 μmol/min to 34.7 ± 25.89 μmol/min, $P>0.2$). By contrast, cimetidine had no effect on NRC; 21.1 ± 2.08 ml/min pre-treatment, 14.8 ± 1.25 post-treatment ($P>0.2$), but was seen to decrease acid output from 59.7 ± 7.66 μmol/min to 24.2 ± 3.36 μmol/min ($P<0.02$). In Group 3 (DGL in combination with cimetidine), there was still a significant increase in NRC during the time which DGL was in contact with the gastric mucosa: 20.3 ± 3.2 ml/min periods 2–5; 15.5 ± 2.09 ml/min periods 6–9; and 36.8 ± 4.54 ml/min during period 10 ($P<0.02$), without a concurrent change in acid secretion (70 ± 23.6 μmol/min periods 2–5; 37.8 ± 12.2 μmol/min periods 6–9; and 50.8 ± 33.51 μmol/min during period 10; $P>0.2$).

We conclude that in Man, DGL and cimetidine act in different ways – the former increases resting GMBF and the latter reduces acid secretion. Furthermore, cimetidine did not antagonize the effect of DGL on GMBF in a dose which inhibits acid secretion by about 50%. This latter observation may have clinical relevance.

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Comparison of the acute effects of aspirin and paracetamol (acetaminophen USA) on canine gastric mucosa during shock

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(Introduced by B.P. CURWAIN)

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Ingestion of analgesic-antipyretic compounds is associated with chronic peptic ulceration (Piper *et al.*, 1981). The ulcerogenic effects of aspirin compared to paracetamol have recently been reported (Ivey & Settree, 1976; Ivey *et al.*, 1978) though quantiative comparison is lacking.

In six exteriorized double-chambered canine stomachs (Moody & Durbin, 1965) aspirin (20 mM) was added to once chamber and paracetamol (20 mM) to the other. Both sides contained HCl (100 mM) and NaCl (54 mM), control values were obtained during the 1 h prior to addition of the analgesics. Shock was induced by haemorrhage of 300–400 ml of blood, 1 h after addition of analgesic. During the recovery period the analgesics were removed from the bathing solution and the blood reinfused.

The effects of aspirin and paracetamol on potential difference (mv), ion flux (μEq/min) and mucosal blood flow (ml/min) are shown in Table 1. The percentage of mucosa eroded by the aspirin-shock was 14.34 ± 1.72 (mean $\pm$ s.e.mean) which showed a linear correlation with the ratio of H^+ back diffusion/mucosal blood flow ($r=0.86$ $P<0.02$). Although aspirin significantly increased the rate of H^+ back diffusion this did not precipitate barrier-breakage (Na^+ efflux) until the blood flow through the mucosa was reduced during the shock period. At the end of the recovery period the mucosal blood

Table 1 Effect of aspirin and paracetamol on the canine gastric mucosa bathed in 100 mM HCl and 54 mM NaCl. Each value is the mean ± s.e. of 6 observations (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, compared to acid, using a paired *t* test). PD is transmucosal potential difference. MBF is mucosal blood flow measured using γ-labelled microspheres.

	Acid	*Aspirin*	*Shock*	*Recovery*	*Acid*	*Paracetamol*	*Shock*	*Recovery*
PD	66.7 ± 2.1	45.8 ± 3.9	36.3 ± 3.5	37.7 ± 4.3	66.7 ± 2.4	66.0 ± 3.0	61.0 ± 4.2	64.5 ± 3.3
H^+	−1.0 ± 0.9	−4.16 ± 1.26*	−8.8 ± 1.17***	−12.1 ± 3.0**	−1.7 ± 1.17	−0.91 ± 1.83	−1.51 ± 1.35	−0.96 ± 0.81
Na^+	2.7 ± 1.34	4.72 ± 1.41	7.45 ± 1.08**	10.06 ± 2.14**	1.76 ± 0.67	2.00 ± 0.40	1.59 ± 0.04	2.53 ± 0.93
K^+	0.10 ± 0.04	0.16 ± 0.08	0.50 ± 0.07***	0.40 ± 0.08**	0.09 ± 0.03	0.09 ± 0.02	0.096 ± 0.03	0.077 ± 0.03
Cl^-	−0.46 ± 1.12	−1.36 ± 1.8	−8.15 ± 2.42*	−4.96 ± 2.42	0.97 ± 0.67	−1.80 ± 1.26	−2.97 ± 1.74	−3.51 ± 2.26
MBF	2.34 ± 0.57	2.60 ± 0.57	1.20 ± 0.11	2.53 ± 0.43	1.99 ± 0.57	2.04 ± 0.99	0.37 ± 0.09*	0.94 ± 0.32

flow of the injured side returned to a greater level than that of the paracetamol side. Paracetamol had no significant effect on any parameter measured, including gross appearance.

We conclude:

(1) the barrier-breaking effects of aspirin depend on the rate of H^+ back-diffusion and mucosal blood flow
(2) an adequate blood flow will prevent damage to the gastric mucosa, even in the presence of an increased rate of H^+ back-diffusion
(3) paracetamol is without effect on the gastric mucosal barrier.

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Phosphodiesterase inhibition and gastric acid secretion in the rat isolated stomach

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Phosphodiesterase inhibitors can stimulate acid secretion in the guinea-pig isolated stomach and rabbit isolated mucosa (Holton & Spencer, 1976; Fromm *et al.*, 1975). More recently Berglindh & Sachs (1980) have shown a potentiation of gastric secretagogue action by isobutylmethylxanthine (IBMX) in rabbit isolated gastric glands. We have investigated the effect of IBMX on basal and histamine-stimulated acid secretion in the rat isolated stomach.

The isolated stomach was set up as described previ-

ously (Bunce & Parsons, 1976). Results are expressed as mean increase in acid output over basal ± s.e.mean.

IBMX stimulated acid output in a concentration dependent manner over 5×10^{-6} M to 10^{-3} M. A maximum acid response of 32.1 ± 2.4 mol $\times 10^{-8}$ min^{-1} ($n = 5$) was obtained at 10^{-4} M.

In a separate series of experiments two 2-point dose-response curves were constructed in each preparation. One group of stomachs received IBMX alone, a second group were preincubated with metiamide (10^{-3} M) after the construction of the first curve. In the group receiving IBMX alone first and second acid responses to IBMX (10^{-5} M) were 11.2 ± 1.4 and 5.5 ± 1.4 mol $\times 10^{-8}$ min^{-1} ($n = 5$) respectively, and to IBMX (10^{-4} M) were 23.6 ± 2.8 and 16.4 ± 4.6 mol $\times 10^{-8}$ min^{-1} ($n = 5$). Thus, the rat isolated stomach exhibited fade to IBMX. In the metiamide group first and second responses to IBMX (10^{-5} M) were 18.2 ± 6.0 and 10.7 ± 4.6 mol $\times 10^{-8}$ min^{-1} ($n = 5$) and to IBMX (10^{-4} M) were 32.7 ± 7.8 and 21.9 ± 6.9 mol $\times 10^{-8}$ min^{-1} ($n = 5$). Unpaired t-tests showed no significant differences in response to IBMX alone in both groups, or to IBMX alone and IBMX + metiamide (10^{-3} M).

Previous studies on the effects of IBMX on secretagogue-stimulated secretion have used concentrations which alone stimulated acid secretion (Soll, 1978). We have determined a subthreshold concentration of IBMX to enable analysis of results without a previous stimulation of acid output. IBMX (10^{-8} M) produced no significant increase in acid output over basal over four hours.

Responses were obtained to a submaximal concentration of histamine (5×10^{-5} M) alone, and in the presence of IBMX (10^{-8} M) in the same preparation, having first established that two such doses of histamine gave responses that were not significantly different. IBMX (10^{-8} M) produced a significant potentiation of the response to histamine ($P < 0.05$, paired t-test).

The failure of metiamide to inhibit IBMX stimulated secretion is in contrast with results obtained in canine isolated parietal cells where cimetidine inhibited the effects of IBMX (Soll, 1980), and suggests that H_2-receptors are not involved in the IBMX response in the rat isolated stomach. A subthreshold dose of IBMX potentiated the action of histamine. Histamine has been shown to stimulate cAMP formation in canine isolated parietal cells (Scholes, 1976), therefore, in the rat isolated stomach, inhibition of the metabolism of generated cyclic nucleotides would result in their increased levels, and this in turn would produce greater responses than those produced by the same dose of histamine in the absence of IBMX.

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The inhibition of gastric acid secretion by the histamine H_2 agonist dimaprit in the conscious pylorus ligated rat

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Dimaprit (S-[3-(N,N-dimethylamino)propyl]isothiourea) has been reported to be a highly specific histamine H_2 receptor agonist (Parsons, Owen, Ganellin & Durant, 1977). This conclusion was derived from *in vitro* studies on rat uterus and guinea-pig atrium and ileum and *in vivo* measurement of gastric acid secretion in the rat, dog and cat. The data obtained from these and later secretory studies (Peterson & Grossman, 1978; Bunce & Parsons,

Table 1 The effect of dimaprit on basal and histamine stimulated acid secretion in the conscious pylorus-ligated rat

Treatment	Acid Secretion basal ($n=5$)	Acid Secretion histamine stimulated ($n=3-5$)
Control	177 ± 74.8	466 ± 59.6
Dimaprit (4.3 μmol/kg)	292 ± 38.5**	477 ± 106
Dimaprit (12.8 μmol/kg)	298 ± 25.0**	481 ± 154
Dimaprit (42.7 μmol/kg)	243 ± 55.4	347 ± 104
Dimaprit (128.0 μmol/kg)	173 ± 53.1*	236 ± 93.3*†

Results reported are mean values ± s.e.mean of total μequiv HCl excreted.
**Significantly different ($P<0.01$) from the control value.
*Significantly different ($P<0.01$) from 4.3 and 12.8 μmol/kg.
†Significantly different ($P<0.05$) from the control value.

1978) involved intravenous infusion of dimaprit. In addition, the results reported for these later studies did suggest a possible inhibitory effect of the H_2 agonist at higher doses. The present work was undertaken to examine the secretory activity of dimaprit following subcutaneous administration over a relatively wide concentration range.

Acid secretion was studied in male, Sprague Dawley rats (120–150 g) using the method of Shay (1945) with 6–8 rats employed per treatment. Following a 48 h fast, the pylorus was ligated under ether anaesthesia, and dimaprit was administered subcutaneously. Where histamine stimulated secretion was being investigated, histamine (20 mg/kg, s.c.) was dosed at the start of the experiment and after 1 h. The rats were sacrificed 3 h subsequent to dimaprit administration and the pH and volume of the centrifuged stomach contents were determined. Total acid output, expressed as μequivalents of HCl, was compared between treatments using a one-way analysis of variance and a Dunnett's test for significance.

The results shown in Table 1 indicate that dimaprit displays a significant biphasic effect on both basal and histamine stimulated acid secretion. Further preliminary work using isolated gastric mucosa preparations (Main & Pearce, 1978) and a [^{3}H]-QNB receptor binding assay (Yamamura & Snyder, 1974) has suggested the possible involvement of muscarinic receptors. Conclusions derived from the data will be discussed.

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Histamine release and Ca^{2+}-ATPase activities of peritoneal and pleural mast cells

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Mast cells are to be found throughout the body, either free in suspension (peritoneal and pleural cavity) or within tissues such as the lung and mesentery. It has been suggested that mesenteric mast cells obtained by tissue digestion show a different response to some histamine-releasing agents than do peritoneal mast cells (Ennis & Pearce, 1980). Before comparing the properties of free and lung-derived mast cells, it was decided to compare peritoneal and pleural mast cells with respect to histamine release and Ca^{2+}-ATPase activity.

Cells for histamine release studies were obtained

from Sprague-Dawley rats, groups of which had been previously sensitized to ovalbumin using $Al(OH)_3$ (16 to 23 days) or *B.pertussis* vaccine (21 to 28 days) as adjuvant. Cells were harvested in the buffer medium of Sullivan, Greene & Parker (1975), omitting $CaCl_2$ and heparin. These cells were centrifuged at 50 *g* for 8 min, washed twice and resuspended in buffer medium to which had been added $MgCl_2$ (0.9 mM) and $CaCl_2$ (0.9 mM). Releasing agents dissolved in this medium were present during incubation (37°C; 5 min) in the following concentrations: ovalbumin (1 μg/ml) concanavalin A (25 μg/ml) and compound 48/80 (1 μg/ml). Histamine was assayed spectrofluorometrically using a method based on those of Shore, Burkhalter & Cohn (1959) and May, Lyman, Alberto & Cheng (1970). Released histamine was expressed as a percentage of the total cell content and was corrected for spontaneous release.

Mast cells were purified and their Ca^{2+}-ATPase activity measured according to the methods previously reported (Edgar, Gilbert & Winsey, 1981).

Comparing peritoneal and pleural cells from non-sensitized Sprague-Dawley rats, no statistically significant difference was observed in either Ca^{2+}-ATPase activity or histamine release. Sprague-Dawley rats sensitized to ovalbumin using *B.pertussis* vaccine as adjuvant also showed no significant difference between peritoneal and pleural cell histamine release but peritoneal cell Ca^{2+}-ATPase activity was lower at all Ca^{2+} concentrations used. This was statistically significant using 8 mM Ca^{2+}. Cells obtained from Sprague-Dawley rats sensitized to ovalbumin using $Al(OH)_3$ as adjuvant showed a significantly lower release of histamine from peritoneal as compared to pleural cells in response to compound 48/80. The peritoneal cells also showed lower Ca^{2+}-ATPase activities at all Ca^{2+} concentrations used but the decreases were not statistically significant.

Peritoneal and pleural mast cells obtained from non-sensitized rats have similar characteristics with respect to histamine release and Ca^{2+}-ATPase activity. Cells prepared from sensitized animals, however, show some differences and these may reflect the method of sensitization.

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Inhibition of A23187-induced histamine release from mast cells by ethacrynic acid

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Histamine release from rat isolated mast cells induced by the ionophore A23187 (Foreman, Mongar & Gomperts, 1973) occur by an energy-requiring mechanism (Foreman *et al.*, 1973; Johansen, 1979). The histamine release was inhibited by ethacrynic acid (Chakravarty, 1979). Since ethacrynic acid inhibited glycolysis as well as respiration of Ehrlich ascites tumour cells and slices of kidney (Gordon, 1968; Gordon & Hartog, 1969; Jones & Landon, 1967), the A23187-induced histamine release and the ATP content of ethacrynic acid-treated mast cells were determined in order to study the relation between the effect of ethacrynic acid on cellular energy metabolism and the inhibition of the release of histamine.

Mast cells were isolated as described previously (Johansen, 1979). Histamine was determined by the fluorometric method (Shore, Burkhalter & Cohn, 1959), and the cellular ATP content was determined by a bioluminescence technique as described earlier (Johansen & Chakravarty, 1975).

Ethacrynic acid caused a time-dependent inhibition of histamine release as well as a time-dependent decrease of cellular ATP content. After 10 min exposure of the cells for ethacrynic acid, histamine release was completely blocked, and a steady state ATP level of 35% of the normal ATP level of the mast cells was observed. The steady state ATP level

was 60% of the normal ATP level in presence of glucose – thus being 25% higher than that observed in the absence of glucose. However, almost no effect of glucose on the inhibition of histamine release was observed. Dithiothreitol partly reversed the inhibition of histamine release by ethacrynic acid, and this was associated with only a small increase in cellular ATP content. Furthermore, when the respiratory energy supply of the cells was blocked by antimycin A, dithiothreitol completely reversed the inhibition of histamine release by ethacrynic acid. In these experiments glucose was used as a substrate for the glycolytic energy supply of the cells. This effect of dithiothreitol was associated with a small decrease of the cellular ATP content. In similar experiments, 2-deoxyglucose was used to block the glycolysis of the mast cells (Johansen, 1980). The energy supply in these cells was largely dependent on the respiratory energy production. In contrast to the experiments above with antimycin A, dithiothreitol had almost no effect on the inhibition of histamine release by ethacrynic acid in presence of 2-deoxyglucose.

These observations support the view that ethacrynic acid inhibits A23187-induced histamine release from rat mast cells by a mechanism, which is not related to the cellular energy metabolism. In addition, ethacrynic acid may inhibit the release of histamine by an inhibition of the cellular energy metabolism.

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The role of H_1- and H_2-receptors in histamine-induced rat paw oedema

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We recently reported that intraperitoneal injections of the histamine H_2-antagonist cimetidine, greatly suppressed oedematous paw swelling in rats with adjuvant disease (Al-Haboubi & Zeitlin, 1979). In the present study we have examined the significance of H_2-receptors in the production of histamine-induced rat paw swelling.

Percentage increase in paw volume was measured in male Wistar rats (250–350 g) using a mercury displacement volume meter. Agonists, or agonists plus antagonists were injected in saline (0.1 ml) into the left hindpaw pad, while saline (0.1 ml) or saline plus antagonists were injected into the contralateral paw as control. In some experiments actions of antagonists injected intraperitoneally (i.p.), 1 h prior to the agonists, were also studied. Statistical significance (Mann-Whitney *U*-test, $n > 5$) was taken at $P < 0.05$.

Sub-plantar injection of dimaprit (0.18–18 mM), an H_2-agonist, histamine (0.18–36 mM), or 2-pyridylethylamine (2-PEA, 1.8–72 mM), an H_1-agonist, produced concentration-related paw swelling occurring maximally 30 min after injection. On a molar basis the potency ratios of these drugs were

respectively 1:4:20. In all three cases, swelling was still present more than 5 h after agonist injection.

Cimetidine HCl (1 mg/kg), injected i.p., inhibited the mean histamine (1.8 mM) response at 30 min by 92% while causing no significant reduction of the response to an equiactive concentration of 2-PEA (18 mM). When injected directly into the paw, cimetidine HCl (10.5 mM) caused 80% inhibition of the mean histamine 30 min response, 100% inhibition of the mean 30 min response to dimaprit (1.8 mM) but no significant antagonism of the H_1-agonist, 2-PEA. In the groups in which cimetidine produced inhibition, this inhibition lasted for more than 5 h. When the H_1-antagonist, mepyramine maleate (1 mg/kg) was injected i.p., the mean 30 min response to histamine (1.8 mM) was 50% of that of histamine alone, but the difference did not reach significance. Locally injected mepyramine maleate (18 mM) produced a 75% inhibition of the mean 30 min histamine response which remained for up to 1.5 h, however by 3 h the full histamine response had established itself. The dose of mepyramine reduced the mean 30 min response to 2-PEA by 86% and this response remained blocked for up to 5.5 h.

The permeability of the rat microvasculature can thus be modified via histamine H_2-receptors and, while there is some evidence for the presence of H_1-receptors, the ability of histamine to produce oedema in the rat is largely through H_2-receptor activation.

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Critical examination of the histamine-cimetidine interaction in guinea-pig heart and brain

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Do the histamine H_2-receptors in brain and heart muscle belong to the same class, as judged by their interaction with cimetidine?

Green & Maayani (1977) found that they were unable to use histamine concentrations greater than 1 mM in their studies with the guinea-pig brain histamine-sensitive adenylate cyclase. Consequently, full histamine concentration-effect curves could not be examined in the presence of cimetidine at dose-ratios exceeding 10. As we wished to explore the histamine-cimetidine interaction in that assay at dose-ratios greater than 100, we examined the nature of the depressant action of histamine at high concentrations.

A similar net depressant activity of histamine was observed in right atrial tissues when histamine concentrations in excess of 1 mM were explored. Positive chronotropic responses preceding this effect were themselves associated with an initial transient negative response. Histamine was formulated in these assays as the hydrochloride which led us to examine the pH status of physiological buffers at high histamine concentrations. When histamine hydrochloride (1 mM) was used buffer pH was 7.3 and fell by almost one log unit as the histamine concentration rose to 10 mM. Titration of histamine hydrochloride stock solutions with sodium hydroxide prevented the fall in buffer pH and abolished the transient negative chronotropic response. However, the net depressant effect remained otherwise as it had appeared using non-neutralized histamine solutions. Using neutralized histamine solution, the ED_{50} for histamine control curves was $1.1 \pm 0.1\,\mu M$ (s.e., $n = 24$). Initial fitting of concentration-effect curve data to a general logistic function was unconstrained with respect to the parameters representing slope and asymptote. Where no significant differences across groups were detected, the data was refitted with common slope and asymptote parameters and dose-ratios estimated. Cimetidine displaced these curves in parallel to the right and a Schild regression covering greater than 2 log cycles provided a pK_B estimate of 6.06 ± 0.07 (s.e., 34 d.f.) when the slope which was not significantly different from unity (0.93, 95% c.l. 0.82–1.04) was constrained to unity.

In the cyclase assay the depressant activity leading to bell-shaped concentration-effect curves was also found to be associated with a drop in buffer pH.

However, when this was prevented by neutralization using Tris base, the concentration-effect curve was transformed into one which was biphasic, i.e. higher concentrations of histamine caused further activation of cyclase. Lowering the ATP concentration from 1 mM to 0.25 mM under conditions of neutralization enabled resolution of this response into two clearly defined components separated by more than two log cycles. The lower saturable component whose ED_{50} was 5.1 ± 0.5 μM (s.e., $n = 24$) was resistant to 10^{-7} M mepyramine but was displaced dextrad in parallel by cimetidine. A Schild regression of the dose-ratios (maximum dose-ratio 150) with the slope constrained to unity (1.03, 95% c.l. 0.84–1.22) yielded a pK_B estimate of 6.12 ± 0.09 (s.e., 22 d.f.). There was no significant difference between the pK_B estimate for cimetidine in either assay.

Therefore the H_2-receptors in brain and heart appear to belong to the same class.

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The effects of polyamines, 48/80 and calcium ionophore on histamine release from human dispersed lung and adenoidal mast cells

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The polyamines, polylysine and polyarginine, 48/80 and the calcium ionophore, A23187, are all potent histamine releasing agents in rat peritoneal mast cells (Diament & Patkar, 1975; Ennis, Pearce & Weston, 1980; Coleman, Holgate, Church & Godfrey, 1981). In human basophils, the polyamines and A23187, but not 48/80, induce histamine release (Foreman & Lichtenstein, 1980; Lichtenstein, 1975). We have examined the ability of these secretagogues to release histamine from dispersed mast cells obtained from human lung and adenoids.

Surgical specimens of human lung and adenoidal tissue were chopped finely with scissors, suspended in calcium-free Tyrode solution containing gelatin (1 mg/ml) and DNase (0.01 mg/ml) and the isolated cells separated from tissue fragments by filtration through 60 μm gauze. The cell preparations, containing 1–3% mast cells assessed by microscopy, were washed twice before final suspension in complete Tyrode solution. Cells were incubated with secretagogue for 15 min, centrifuged and the histamine in the supernatant measured spectrofluorimetrically. Cytotoxicity was assessed by measurement of LDH release.

Polylysine (average M.W. 40,000) in concentrations of 1×10^{-8} to 1×10^{-5} M failed to induce significant histamine release from lung cells. The small release of histamine, $4 \pm 2\%$ (±s.e.mean), induced by 1×10^{-5} M was associated with cytotoxicity. In adenoidal cells, 1×10^{-6} and 1×10^{-5} M polylysine released $6 \pm 1\%$ and $13 \pm 2\%$ histamine respectively, but again these were associated with cytotoxicity. Lower concentrations released no histamine. Similar results were found with polyarginine (average M.W. 60,000).

Compound 48/80, over a concentration range of 1–1000 μg/ml, failed to release histamine from either cell preparation except at the highest concentration where a net release of less than 10% was associated with cytotoxicity.

The calcium ionophore, A23187, induced histamine release from both human dispersed lung and adenoidal mast cells in a non-cytotoxic dose-dependent fashion. Maximum net histamine release from lung cells of $35 \pm 8\%$ and from adenoidal cells of $46 \pm 5\%$ occurred with ionophore concentrations of 3×10^{-6} M and 1×10^{-6} M respectively. Increasing the concentration of ionophore to 1×10^{-5} M failed to release further histamine but did cause cytotoxicity. Histamine release from human lung mast cells reached maximum after 15 min incubation with A23187. Preincubation of cells with 10 mM EDTA for 5 min or 1 h, or exposure to A23187 in the absence of calcium, abolished histamine release.

Thus mast cells derived from both human lung and adenoid tissue differ functionally from rat peritoneal mast cells and human basophils in that they do not release histamine in response to polylysine, polyarginine or 48/80. The net amount of histamine released by the calcium ionophore, A23187, was lower than that reported for either rat peritoneal mast cells (Diament & Patkar, 1975) or human basophils (Lichtenstein, 1975) and showed an absolute requirement for calcium.

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A bronchoconstrictor response to β-phenylethylamine of guinea-pig isolated lung

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The indirectly acting sympathomimetic amine β-phenylethylamine (PEA) exerts a biphasic effect on lung parenchymal strips (Broadley & Hawthorn, 1981). An initial relaxation which is abolished by propranolol (10^{-6} M) (unpublished data) and therefore β-adrenoceptor mediated is followed by contraction. Contractions to PEA have been reported in rat aortic strip (Hansen, Greenberg & Mosnaim, 1980) which were thought to be due to direct stimulation of α-adrenoceptors. The aim of this study was to establish if PEA also produces a contraction in lung strip via an α-adrenoceptor mechanism.

Guinea-pig lung strips were prepared by the method of Lulich, Mitchell & Sparrow (1976) and suspended in Krebs-bicarbonate solution at 38°C gassed 5% CO_2 in oxygen in the presence of propranolol (10^{-6} M) and metanephrine (10^{-5} M) to inhibit β-adrenoceptor stimulation and extraneuronal uptake respectively. Isometric contractions were recorded and dose-response curves obtained by cumulative addition. Geometric mean EC_{50} values were calculated ($n > 4$). Contractions were also expressed as a percentage of a maximal response to carbachol (5×10^{-5} M) added at the maximal response.

PEA produced dose-dependent contractions of the parenchymal strips with $79 \pm 8.7\%$ of the carbachol maximum response and an EC_{50} value of 2.3×10^{-4} M. The magnitude of contraction was significantly greater ($P < 0.05$) than that produced by noradrenaline, the maximal response of which was only $42 \pm 6.7\%$. However, noradrenaline was significantly more potent ($P < 0.05$) having an EC_{50} value of 2.6×10^{-7} M.

Pretreatment of the animals with reserpine (0.5 mg/kg i.p. at 24 h) did not significantly affect the maximum PEA contraction of $68 \pm 6.8\%$ ($P > 0.05$), though the EC_{50} value of 1.13×10^{-3} M was significantly greater ($P < 0.05$) indicating that although part of the response may be produced by an indirect effect PEA has a predominant direct effect on the tissue.

The possibility exists that the contractile responses of lung strips arise from contraction of the vascular elements of the tissue. In an attempt to overcome this, PEA was examined in isolated perfused lungs. Guinea-pig lungs were perfused via the trachea at 9–11 ml/min with Krebs-bicarbonate solution at 38°C gassed with 5% CO_2 in oxygen containing propranolol (10^{-6} M). Bronchoconstriction, as measured by a rise in perfusion pressure, was produced by both PEA and noradrenaline. The EC_{50} values for noradrenaline and PEA were 1.6×10^{-6} M and 4.5×10^{-4} M respectively and the maxima as a percentage carbachol maxima were $15 \pm 5.3\%$ and $63 \pm 10.1\%$ respectively.

In lung strip preparations, dose-response curves to PEA were displaced to the right by phentolamine (10^{-5} M and 10^{-6} M) following a 30 min incubation. The mean 50% dose-ratios were 3.2 (10^{-6} M) and 14.4 (10^{-5} M). These values were significantly ($P < 0.05$) lower than the 50% dose-ratios for the displacement of noradrenaline dose-response curves by phentolamine of 112 (10^{-6} M) and 333 (10^{-5} M). This apparent lack of antagonism of PEA by phentolamine and the greater magnitude of PEA contractions suggest that this monoamine induces constriction of guinea-pig lung by a direct effect on a receptor system other than α-adrenoceptors.

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Overflow of [^{3}H]-disaturated phosphatidylcholines from adult rat superfused lung slices: a novel method for the study of lung surfactant secretion

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In vivo experiments suggest that the controls of lung surfactant secretion are distinct from those of surfactant biosynthesis (e.g. Abdellatif & Hollingsworth, 1980; Gilfillan, Harkes & Hollingsworth, 1980). An *in vitro* method has therefore been developed which uses the overflow of [^{3}H]-disaturated phosphatidylcholines (DSPC), a major component of lung surfactant (Van Golde, 1976), from adult rat lung slices as a measure of surfactant secretion.

Lung slices (approx. 0.5 mm thick, total weight approx. 30 mg) from adult male Sprague-Dawley rats were incubated at 37°C in 5 ml Krebs solution containing methyl-[^{3}H]-choline chloride (5 μCi, 77–84 Ci/mmole) for 3 h, based on the method of Epstein & Farrell (1975). A proportion of the choline is incorporated into DSPC. Slices were then transferred to new chambers and superfused with Krebs solution at 0.2 ml/min for 3 h. Fractions were collected every 5 min for 45 min and then every 10 min. The [^{3}H]-DSPC content of each fraction and of the tissue at the end of superfusion was measured by a modification of the method of Mason, Nellenbogen & Clements (1976). The overflow of [^{3}H]-DSPC in each fraction was expressed as a rate coefficient (% of tissue content released per min).

The spontaneous rate of overflow of [^{3}H]-DSPC declined rapidly over the first 30 min and then decreased slowly and approximately linearly up to 3 h. Spontaneous overflow of [^{3}H]-DSPC over 3 h totalled $0.62 \pm 0.10\%$ (mean ± s.e.mean, $n = 7$) of tissue content. Agonist drugs were included in the Krebs solution from 45 to 55 min after commencing superfusion. Drug effects were measured as the maximum increase in rate coefficient after drug addition compared to the immediate pre-drug rate coefficient correcting for the decline in basal overflow in controls.

Potassium chloride (10^{-2} mol/l), added to Krebs solution adjusted to the same osmotic pressure by removing the osmotically equivalent amount of sodium chloride, produced an immediate and transient increase in [^{3}H]-DSPC overflow. Salbutamol (10^{-6} mol/l) tended to induce a more delayed increase in [^{3}H]-DSPC overflow. Propranolol (10^{-7} mol/l), in the Krebs solution throughout the 3 h of superfusion, had no effect on basal or potassium chloride stimulated overflow of [^{3}H]-DSPC ($2P > 0.05$), but inhibited the overflow produced by salbutamol ($2P < 0.01$).

This method enables the magnitude and time course of drug-induced surfactant secretion to be measured. Preliminary results suggest that potassium chloride and a β-adrenoceptor agonist can both evoke secretion and by different mechanisms.

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[^{3}H]-Mepyramine binding to histamine H_1 receptors in guinea-pig airways

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[^{3}H]-Mepyramine has been used by several laboratories to label histamine H_1 receptors in mammalian tissues. However, there appears to be substantial variation in the affinity of this ligand for these sites both in the same tissue in different species or between tissues in a single species (Chang, Tran & Snyder, 1979; Young, 1979). An inherent difficulty with this ligand has been the relatively high degree of non-specific binding found in most tissues, and the consequent difficulty in accurately assessing true receptor specific binding. In the present experiments, we have examined the binding of [^{3}H]-mepyramine binding to guinea-pig airways and demonstrate that under defined conditions, this ligand labels a homogeneous population of histamine H_1 receptors.

Washed membranes of guinea-pig trachea, bronchi and parenchyma were prepared by homogenization, filtration through cheese-cloth and centrifugation. [^{3}H]-Mepyramine binding was performed in Tris-HCl (25 mM) containing 0.9% NaCl, pH 7.8, and specific binding was defined as the binding that could be displaced by (+)-chlorpheniramine (100 nM). Of a number of H_1 antagonists, the latter drug most clearly discriminated between specific and non-specific components of [^{3}H]-mepyramine binding. Specific binding represented 50–60% of the total binding at ^{3}H-ligand concentrations up to 2nM.

Saturation curve analysis by Scatchard plots revealed that [^{3}H]-mepyramine was bound specifically to a single population of sites, and the relative affinities of various agonists and antagonists (Table 1) indicate that these sites possessed properties expected of a histamine H_1 receptor. Na^+ (0.9%) did not influence specific binding, though this monovalent cation significantly reduced non-specific binding.

Histamine H_1 receptors appear to be uniformly distributed in guinea-pig airways (Table 1) and although the K_D value for [^{3}H]-mepyramine was apparently higher in trachea to that found in bronchi or parenchyma, this probably relates to technical difficulties associated with the higher proportion of non-specific binding in trachea rather than to differences in the receptors within the airways. The present experiments suggest that [^{3}H]-mepyramine can label a homogeneous population of histamine H_1 receptors in guinea-pig airways and that the previous report of receptor heterogeneity (Chang *et al.*, 1979) could relate to difficulties in the assessment of specific binding.

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Table 1 Specific [^{3}H]-mepyramine binding to guinea-pig airways. Values shown are mean ± s.e.mean of three separate experiments

	Parenchyma	*Bronchi*	*Trachea*
K_D (nM)	0.7 ± 0.07	0.6 ± 0.08	1.35 ± 0.12
B_{max} fmol/mg protein	44 ± 2.5	38 ± 1.5	52 ± 6.7

	Parenchyma	
Drug	K_i (nM)	n_H (*slope factor*)
Mepyramine	0.56 ± 0.11	0.833 ± 0.068
(+)-Chlorpheniramine	0.85 ± 0.05	1.01 ± 0.06
Triprolidine	2.87 ± 0.022	0.855 ± 0.04
Diphenhydramine	24 ± 3.9	1.14 ± 0.07
(−)-Chlorpheniramine	490 ± 58	0.902 ± 0.09
Histamine	18,000 ± 1,800	0.970 ± 0.005
2-Methylhistamine	13,300 ± 1,140	1.27 ± 0.077
4-Methylhistamine	56,000 ± 2,060	1.068 ± 0.04
Metiamide	10,500 ± 20	1.082 ± 0.008

Possible mediators of browntail moth (Euproctis chrysorrhoea) dermatitis

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There are numerous documented cases of dermatitis caused specifically by the browntail moth and its caterpillar (Tyzzer 1907; Blair 1979; Graham & Hall-Smith 1980). The caterpillars emerge from hibernation in April, varying in numbers from year to year. A peak was reached locally in 1979 resulting in several patients being referred to the dermatology department after contact with caterpillars had caused urticarial wealing, pruritus and maculo-papular rashes of varying severity and distribution.

Inflammatory agents might well be present in caterpillar skin and we have attempted to identify some of them and to locate the structures responsible for their production and transference to human skin.

Anatomical investigation of the caterpillars confirmed the presence of specialised 'nettling' hairs emerging from tubercles on the 5th–12th abdominal segments. Scanning electron microscopy revealed that these hairs (easily distinguishable from tactile hairs common to many lepidopterous larvae) measure 70–100 μm × 4–5 μm and are hollow, barbed, dart-like structures inserted point downwards in cup-like papillae and readily detached. Sections through the tubercles, stained with toluidine blue and eosin and viewed under oil immersion, showed a channel running from each papilla through the cuticle. Mediators of inflammation may be conveyed to the hairs via these ducts which connect with the hypodermis at points where darkly-staining granules, of as yet unknown composition, appear.

Three aqueous extracts were prepared using pooled skin (20 caterpillars/extract) and assayed for histamine and prostaglandins. Similar *n*-butanol extracts were prepared and assayed for 5-hydroxytryptamine. The histamine and 5-hydroxytryptamine contents of lackey moth, a hairy but non-urticating species, were likewise determined using three caterpillars/extract. In addition pooled skin of both types (browntail 17 estimations, lackey 3 estimations) was incubated with [^{14}C]-histidine and, using a modification of the method of Kahlson *et al.* (1963), was assayed for [^{14}C]-histamine. The histamine forming capacity and the histamine and 5-hydroxytryptamine content were all higher in browntail than in lackey caterpillars; mean values, 142 ng/g in 3 hours; 35 μg/g and 0.9 μg/g compared with 2.3 ng/g in 3 hours; 6 μg/g and 0.07 μg/g respectively. The presence of undifferentiated prostaglandins was detected in browntail skin, mean value equivalent to 0.6 μg/g PGE_2.

Whilst each of these mediators could account for a local effect, our results suggest that the minute amounts likely to be introduced into human skin by contact with the hairs would not cause the generalized response invariably seen. Curiously, some individuals manifest skin reactions when merely in the vicinity of caterpillars, yet others are unaffected even by direct contact. The hypothesis of de Jong & Bleumink (1977), that mechanical damage and liberation of enzymes by the nettling hairs initiate non-specific inflammation, fails to explain this completely and we are currently investigating the possibility that an immunological component is involved.

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Pharmacological aspects of the control of leech body wall tension by Retzius cells

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Retzius (R) cells within the leech CNS contain 5-hydroxytryptamine (5-HT) (Rude, Coggeshall & Van Orden, 1969). Their axons run peripherally through the ipsilateral nerves of both the ganglion in which they lie and the two adjacent ganglion (Mason & Leake, 1978). R cell activity induces a delayed and prolonged reduction in basal tension of the body wall

and an increased relaxation rate following evoked contractions. These effects are mimicked by topical application of 5-HT to the denervated body wall (Mason, Sunderland & Leake, 1979). This report presents some evidence that the reduction in tension is mediated by the serotonergic R cells, and describes an additional effect of R cell activity.

The preparation consists of a lateral strip of body wall with the central segment connected to its segmental ganglion by lateral nerves, which pass through a sealed partition in the experimental bath. Intracellular recordings from the R cells (using KCl-filled glass micro-electrodes), extracellular recordings made from the lateral nerves using gold-plated metal electrodes, and isometric tension of the body wall were amplified and recorded using conventional techniques. Permanent records were made on a Gould 4000 pen recorder. Preparations were bathed in normal or magnesium (20 mM) Ringer (Leake, Mason & Sunderland, 1981).

A dose of p-chlorophenylalanine (100 mg), which inhibits 5-HT synthesis (Marsden, 1980) was injected daily for 3 days. Subsequently, in three preparations, action potentials induced in the R cells and recorded from the lateral nerve roots to the body wall strip did not cause a reduction in muscle tension.

A series of putative antagonists (atropine, BOL-148, cyproheptadine, dibenamine, ergometrine, fluphenazine, methysergide, morphine, phentolanine and propranalol) were tested on the R cell effect on tension. Only atropine (5×10^{-6} M) and cyproheptadine (10^{-5} M) proved effective (Leake, Mason & Sunderland, 1981). The same antagonists tested at doses of 10^{-4} M against the reduction in tension induced by topical application of 5-HT were ineffective.

The R cells themselves respond to both 5-HT and dopamine at similar doses, probably at a common receptor (Leake, Sunderland & Walker, 1980). The 5-HT-induced reduction in basal tension cannot be mimicked by dopamine even at doses of 50 × threshold. This implies that the receptor on the muscle at which 5-HT acts is different from the central receptor.

Although activity in the R cell always induces a reduction in tension, in 8 out of 30 preparations this was preceded by a delayed tension increase. The increase was large in 2 preparations in normal Ringer but was much smaller in 6 other preparations in which the ganglion was bathed in magnesium Ringer. This biphasic effect could be mimicked by topical application of 5-HT (5×10^{-6} M) to a denervated body wall bathed in normal Ringer in only one preparation.

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NSAIDs inhibit the late asthmatic response to antigen challenge

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In young people with extrinsic asthma, bronchial provocation with house dust mite allergen, commonly produces bronchospasm with both early and late components (Warner, 1976). The immediate response has been attributed to mast cell degranulation, with the release of spasmogens such as histamine, leucotrienes and other arachidonic acid products. The delayed component is more sustained and the mechanism remains undefined. Recently, considerable attention has been accorded to the newly

characterized leucotrienes which have been presumed to participate in antigen-induced bronchospasm (Morris, Taylor, Piper & Tippins, 1980). Benoxaprofen is a non-steroidal anti-inflammatory drug (NSAID) which inhibits both lipoxygenase and cyclo-oxygenase enzymes. This drug enables the importance of leucotrienes in both the immediate and late reactions to be tested.

Benoxaprofen (600 mg) given 11 h before antigen challenge had no effect on the immediate bronchoconstrictor response, but effectively abolished the late reaction. At this dosage (Nash, Carmichael, Ridolfo & Spradlin, 1980) it is considered that the lipoxygenase (Walker & Dawson, 1979) and cyclo-oxygenase (Cashin, Dawson & Kitchen, 1977) pathways are inhibited. Indomethacin (25 mg) 1 h before challenge also inhibits the late reaction without affecting the immediate bronchoconstrictor response. Neither drug inhibited the late reaction if given after the peak of the immediate bronchospasm (benoxaprofen at 20 min and indomethacin at 1 h). These observations suggest that the late response is associated with arachidonic acid metabolites produced during the immediate response. Mere abolition of the immediate bronchoconstrictor response (inhaled salbutamol 200 μg) was without effect on the delayed response. Disodium chromoglycate (60 mg) 5 min before challenge partially suppressed the immediate response and substantially reduced the delayed reaction; a phenomenon that has usually been attributed to mast cell stabilization (Pepys, Hargreave, Chan & McCarthy, 1968). The other class of drug, which inhibited the delayed response, was corticosteroids (prednisolone, 20 mg) given 80 min prior to challenge. Prednisolone (20 mg) 20 min after challenge also wholly suppressed the delayed response.

It is widely accepted that leucotriene constituents of SRS-A contribute to bronchospasm in antigen provocation and in asthma. The inability of benoxaprofen to modify the immediate phase of response, when leucotrienes can be presumed to be generated (Adams & Lichtenstein, 1977) is not consistent with this hypothesis. Inhibition of the delayed response by both benoxaprofen and indomethacin was an unexpected finding. Such inhibition cannot be attributed to lipoxygenase inhibition, but rather to some property common to both drugs, possibly either inhibition of the cyclo-oxygenase pathway or an effect of these NSAIDs inhibiting the conversion of 12-hydroperoxy-eicosatetraenoic acid into 12-hydroxy-eicosatetraenoic acid (Siegal, McConnell & Cuatrecasas, 1979), the latter being a known chemotactic agent. The cellular basis for the observation remains to be elucidated.

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The effect of adrenalectomy or aminoglutethimide on propranolol-treated DOCA/salt rats

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Buckingham, Hamilton & Robson (1978) reported that, in DOCA/salt hypertensive rats, adrenal catecholamines attenuated the antihypertensive effect of high doses of the β-adrenoceptor antagonist pindolol. It was later suggested by Buckingham & Hamilton (1979) that the failure of acute administration of propranolol to show an antihypertensive response in spontaneously hypertensive rats (SHR)

was also due to catecholamines from the adrenal. In contrast, the evidence of Nijkamp, Van Den Bosch & De Jong (1979) from similar experiments, implicated adrenocortical steroids. In support of the latter we have recently found an antihypertensive effect when spironolactone is administered to DOCA/salt hypertensive rats treated with propranolol (Bold, Dean, Ingham & Spraggs, 1981). This could be due to the ability of spironolactone to inhibit adrenal steroid biosynthesis (Aakvaag, Ødegaard & Sundsfjord, 1978). We have now investigated the effect of bilateral adrenalectomy or treatment with the steroid synthesis inhibitor aminoglutethimide on DOCA/salt hypertensive rats in an attempt to further clarify the role of the adrenal in blocking the action of propranolol.

In 90 g female Wistar rats implanted with DOCA and given 1% NaCl to drink, propranolol (130 mg kg^{-1} day^{-1}, in diet) had no effect on the development of hypertension. After 16 days of this pretreatment bilateral adrenalectomy caused a temporary fall in blood pressure in control and drug treated normotensive and hypertensive animals. This response was greatest in both groups of hypertensive animals and was maintained for a greater period (12 days) in DOCA/salt rats receiving propranolol.

In a second experiment on animals pretreated as described above the blood pressure measured 2 h after aminoglutethimide (100 mg/kg, s.c.) was significantly reduced ($P < 0.01$ paired t test) only in DOCA/salt rats treated with propranolol. Administration of corticosterone (1 mg/kg, s.c.) with the aminoglutethimide and again 1.5 h later blocked this depressor response.

While the results following adrenalectomy confirm the role of the adrenal in the maintenance of DOCA/salt hypertension (De Champlain & Van Ameringen, 1972) they do not clearly show that this gland has a role in masking the action of propranolol. However, the antihypertensive effect observed after aminoglutethimide, reversed by corticosterone, shows the proposal of Nijkamp *et al.* (1979), that adrenocorticosteroids may prevent the antihypertensive effect of propranolol in SHR, to apply to the DOCA/salt hypertensive rat. This also provides further evidence that inhibition of steroid synthesis may be the mechanism for the effect of spironolactone in DOCA/salt rats.

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Effects of dexamethasone on rat uterine contractility *in vitro*

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Several natural and synthetic corticosteroids have been shown to inhibit the contractility of smooth muscle (Mossman & Conrad, 1969; Cheng & Araki, 1978).

Virgin Sprague-Dawley rats (230–300 g) were ovariectomized and injected i.m. with Oestradiol-17β (10 μg/day) for 6 days. Uterine horns were suspended in a modified De Jalons solution at 30°C and gassed with 95% O_2 and 5% CO_2. Contractions were recorded isometrically at 1 g tension. Cumulative log dose-response curves (DRC) to acetylcholine were obtained. The maximum of the DRC was depressed by 12.9 ± 2.8% (mean ± s.e.mean) by dex-

amethasone (10^{-4} M) and by 28.7 ± 3.6% with dexamethasone (2×10^{-4} M, $n = 4$). In cumulative DRCs to oxytocin, these concentrations of dexamethasone reduced the maximum of the DRC by 11.4 ± 3.0% and by 25.5 ± 3.9% respectively. Lower concentrations (2×10^{-6} and 10^{-7} M) had no effect on either of the agonist-induced DRCs.

Uteri of untreated control rats were suspended in a Krebs depolarising solution (Batra & Bengtsson, 1978) at 30°C and gassed as previously. In cumulative DRCs to calcium (0.05 to 1.25 mM), dexamethasone (10^{-4} M and 2×10^{-4} M) lowered the maximum of the DRC by 48.3 ± 4.7% and by 80.1 ± 4.1% respectively. Lower doses (range 10^{-5} to 10^{-12} M) had no effect relative to paired controls.

In similarly depolarised uteri, addition of Ach resulted in a biphasic response, the second phase of which was inhibited by D-600 and EGTA (Batra & Bengtsson, 1978). In this study, the second phase was also depressed by verapamil (20 μg/ml, $n = 3$) and by dexamethasone (10^{-4} M, $n = 8$ and 2×10^{-4} M, $n = 4$).

Thus doses of dexamethasone greater than 10^{-4} M probably cause a decreased membrane permeability to calcium. This result is similar to those of a related study (Cheng & Araki, 1978). No enhancement of the response to calcium was observed with low doses of dexamethasone. The effects of dexamethasone and inhibitory agents on the biphasic response to Ach indicate that dexamethasone inhibited influx of extracellular calcium. At the same concentrations, dexamethasone exerted a depressant effect on DRCs to Ach and oxytocin thus suggesting that this inhibitory effect is at least partially due to inhibition of transmembrane calcium movements.

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A study of factors which influence the irreversible binding of ethynyloestradiol to rat liver protein *in vivo*

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17α-Ethynyloestradiol (EE_2) *in vitro* is oxidized to an electrophilic metabolite which irreversibly binds to proteins (Kappus, Bolt & Remmer, 1973). Therefore, EE2 may function as an immunogenic hapten *in vivo* and anti-EE_2 antibodies have been detected in women taking oral contraceptives (Beaumont, Lemort, Lorenzelli-Edouard, Deplanque & Beaumont, 1979). We have therefore investigated factors which may influence the formation of reactive metabolites from EE_2 *in vivo* in the rat.

Male Wistar rats (250–300 g) were anaesthetized with urethane and their jugular veins and bile ducts cannulated. After intravenous administration of [6,7-^{3}H]17α-ethynyl-oestradiol ([^{3}H]EE_2) (5 μCi; 5 μg, 50 μg or 500 μg/kg) in isotonic saline-ethanol (9:1 v/v) bile was collected for 3 h and hydrolyzed with β-glucuronidase (5,000 Units/100 μl bile) or *H.pomatia* phenolsulphohydrolase (H-1 preparation, 82 Units/100 μl bile). After extraction into ether the de-conjugated radiolabelled material was separated by h.p.l.c. (Breckenridge, Grabowski, Maggs & Park, 1981) and characterized by mass spectrometry. Livers were excised after 3 h and microsomes prepared as described previously (Park & Whittaker, 1978). The irreversible binding of [^{3}H]EE_2 to microsomes and to soluble protein in the 105,000 g supernatant fraction was determined by liquid scintillation spectrometry following exhaustive solvent extraction. The irreversible binding of [^{3}H]EE_2 increased linearly with dose over the range investigated. Three h after administration of [^{3}H]EE_2 (5 μg/kg) 0.27 ± 0.14% (mean ± s.d., $n = 5$) of the dose was irreversibly bound to hepatic microsomes and 0.24 ± 0.16% to soluble protein. During this time, 60 ± 5.7% of the dose was excreted in the bile as water-soluble metabolites.

Hydrolysis of bile with β-glucuronidase released 45 ± 3.5% of the metabolites while hydrolysis with *H.pomatia* phenolsulphohydrolase, which contains β-glucuronidase and sulphohydrolase activity, liberated 61 ± 5.5% of the metabolites for extraction into ether. The major components of the fraction released by phenolsulphohydrolase were EE_2 (14 ± 1.3%), 2-hydroxyethynyloestradiol (2-$OHEE_2$) (15 ± 6.2%) and the 2- and 3-methyl derivatives of 2-$OHEE_2$ (36 ± 3.5%). Previous *in vitro* work indicated that the reactive metabolite of EE_2 was a quinone derived from 2-$OHEE_2$ and that 2-hydroxylation was the predominant route of metabolism (Kappus *et al.*, 1973; Breckenridge *et al.*, 1981). We have found that 2-hydroxylation is also the predominant phase I route of EE_2 metabolism *in vivo*, but the 2-$OHEE_2$ underwent rapid and extensive phase II metabolism involving methylation, glucuronidation and sulphation, preventing oxidation to the reactive metabolite.

Pre-treatment of rats with phenobarbitone (80 mg/kg for 4 days) and β-naphthaflavone (75 mg/kg for 3 days) produced a 94% increase in the concentration of hepatic microsomal cytochrome P-450 (Omura & Sata, 1964) and a 56% increase in cytochrome P-448, respectively. However, neither compound significantly altered the irreversible binding of [^{3}H]EE_2 to rat liver proteins. In both groups of rats the major metabolites were again phase II conjugates of 2-$OHEE_2$.

In conclusion we have found that the irreversible binding of EE_2 to hepatic proteins is a minor route of metabolism *in vivo* in the rat and is not increased by enzyme induction. Phase II biotransformations involving methylation, glucuronidation and sulphation appear to play an important role in limiting reactive metabolite formation.

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A micro-computer system to aid collection and analysis of data from tissue bath experiments

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A micro-computer based system has been developed to aid the collection and detailed analysis of data from tissue bath experiments. The system is built around a PET 3032 micro-computer with 32 K of memory and Computhink 400 K double density floppy discs.

The computer communicates with the tissue bath experiment through a special interface unit which is connected to the parallel input/output port and the two cassette ports. Amplified analogue signals from up to four transducers are fed to a multiplexer. The inputs of the multiplexer are successively sampled by an 8-bit analogue to digital converter (ADC). The ADC is driven to sample at 200 Hz by a programmable timer in the PET and so the input from each of the four transducers is scanned at 50 Hz. Two output lines from the PET are routed through the interface unit where they are optically isolated and then used to trigger stimulators.

Input of digital data to the PET is controlled by a machine code program subroutine which, when called, takes digital data from the parallel port and stores it in the next free location of the block of memory assigned to the appropriate tissue bath. Initiation of periods of stimulation is also carried out by this subroutine. On completion of a stimulation/collection cycle the machine code subroutine returns control to a BASIC program which then stores the data collected on a floppy disc for subsequent retrieval and analysis. The BASIC program also interacts with the user to allow him to determine the interval between periods of collection, whether to store data collected and whether to stimulate a tissue. During the interval between periods of collection the most recent set of data can be displayed graphically on the video display unit.

A suite of BASIC programs has been developed to analyse the data obtained using this system.