AN INVESTIGATION OF K^+-INDUCED RELAXATION OF THE RAT ISOLATED AORTA SUSPENDED IN A K^+-DEFICIENT SOLUTION

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Isolated vascular smooth muscle of various mammalian species when suspended in a K^+-deficient solution respond with a relaxation to addition of extracellular K^+($[K^+]_o$). These effects are suggested to result from a hyperpolarisation mediated by K^+, which is carried into the cytosol by the Na, K-ATPase (Webb et al., 1981). This study was planned to investigate the possible roles of certain calcium and potassium channels in the K^+-induced relaxation of the rat isolated aorta, suspended in K^+-free medium.

Aortic rings from male Sprague-Dawley rats (300-350 g) were suspended in a K^+-free physiological salt solution (composition in mM : NaCl 117 ; $CaCl_2$ 5.0; $NaH_2PO_4$1.0; $MgSO_4.7H_2O$ 1.2 ; glucose 11.5 ; $NaHCO_3$ 25). A tension of 2 g was applied to each tissue. A functional endothelium was indicated by the relaxant effect of acetylcholine (2 µM) following a noradrenaline (20 nM) evoked contraction. Cumulative concentration-response curves to KCl were constructed 10 min after exposure to apamin, BRL 34915, diltiazem, nitrendipine, ouabain or ethanol (0.1 % : control). In a separate study, concentration-response curves to $CaCl_2$ were determined 10 min after exposure to nitrendipine or ethanol (0.1 % : control). The concentrations of KCl producing a decrease in tension of 0.5 g ($EC_{R0.5}$), maximal relaxation (E_{Rmax}) and contraction to 50 mM KCl ($E_{C\ max}$: from plateau phase : see results) were calculated for each preparation and reported as means ± s.e. mean.

The K^+ concentration-response curve ($[K^+]_o$ = 0.1 - 50 mM) exhibited three distinct phases ; an initial relaxation occuring for $[K^+]_o$ 0.1 -> 2.0 mM followed by a plateau ($[K^+]_o$ 1.0 -> 5.0 mM), then a contraction ($[K^+]_o$ 4.0 mM -> 50 mM) during which base-line tension was not only recovered, but surmounted. In fact, whereas the magnitude of the relaxant phase increased with the extracellular calcium concentration, the contractile response was virtually independent of the concentration of this cation ($[Ca^{2+}]_o$ = 5.0 mM : E_{Rmax} = 1.43 ± 0.11 g, E_{Cmax} = 1.61 ± 0.09 g, $EC_{R\ 0.5}$ = 0.49 ± 0.08 mM, n = 6 ; $[Ca^{2+}]_o$ = 2.5 mM : E_{Rmax} = 0.73 ± 0.11 g, E_{Cmax} = 1.31 ± 0.12 g, $EC_{R\ 0.5}$ = 0.41 ± 0.05 mM, n = 8 ; $[Ca^{2+}]_o$ = 1.25 mM : E_{Rmax} = 0.49 ± 0.09 g, E_{Cmax} = 1.70 ± 0.13 g, n = 13). The calcium channel blockers, nitrendipine (10 nM) and diltiazem (1 µM) blocked the latter effect, without affecting the relaxant activity of K^+. It should be noted that in rings suspended in $[K^+]_o$-free solution, Ca^{2+} (0.8 - 40 mM) produced contractile responses, which were not modified by nitrendipine (10 nM). Ouabain (0.1 and 0.3 mM) displaced the K^+ concentration-relaxant curve, in the presence of nitrendipine (10 nM), to the right of the control, without changing the maximum. The $EC_{R\ 0.5}$ values were 0.46 ± 0.07 mM for control, 1.83 ± 0.26 and 3.3 ± 0.39 mM for rings exposed for 10 min to 0.1 and 0.3 mM ouabain, respectively (n = 6/ group). However, 1 mM ouabain significantly reduced the $E_{R\ max}$ to K^+ (control : 1.19 ± 0.13 g ; after ouabain 0.46 ± 0.15 g, n = 6, P < 0.05). Apamin (100 nM), a K^+ channel blocker, and BRL 34915 (1 - 10 µM), a purported K^+ channel activator (Buckingham et al., 1986), failed to modify the K^+-induced relaxant activity. However, the onset of the contractile phase to K^+ was delayed by BRL 34915 (10 µM), but not changed by apamin (100 nM).

In conclusion, the ouabain inhibition of the K^+-induced relaxation of rat isolated aorta in a K^+-free medium supports the involvement of Na, K-ATPase. Furthermore, the magnitude of the relaxation is related to the basal tone of the tissue, which is a direct function of the extracellular calcium concentration. Interestingly, the pathways used by Ca^{2+} to enter the cytosol do not appear to be voltage-operated calcium channels. Finally, the apamin- and BRL 34915-sensitive K^+ channels do not mediate these K^+-induced relaxant responses.

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THE EFFECTS OF BRL 24924 AND METOCLOPRAMIDE ON MYOELECTRIC ACTIVITY OF THE RAT SMALL INTESTINE

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In recent years, substituted benzamides structurally related to metoclopramide (Mcp; Maxolon: Beecham Pharmaceuticals) have been synthesised which retain the beneficial actions of Mcp (i.e. stimulation of gastric motility by increasing enteric cholinergic activity; McClelland & Sanger, 1982) but minimise the side effects associated with dopamine antagonism. One very potent example, BRL 24924 [(±)-(endo)-4-amino-5-chloro-2-methoxy-N-(1-azabicyclo[3.3.1]non-4-yl)benzamide HCl] is now compared with Mcp for ability to stimulate myoelectric activity of rat intestine. BRL 24924 and Mcp were compared at the subcutaneous (s.c.) doses which have previously been shown to increase gastric motility in conscious rats (Cooper et al, 1986).

Male CFY rats were implanted (at least 2 weeks previously) with silver monopolar electrodes (0.2mm diameter; 0.5mm length) onto the serosal surface of the small intestine, 20cm from the ligament of Trietz and referenced to adjacent peritoneal muscle (Hutton & Wingate, 1981). Myoelectric activity was recorded onto magnetic tape (Ampex/Racal), after differential amplification (HDX 82; Oxford Medical) and later analysed by counting the spike activity superimposed on slow wave activity after filtering at 2.5-40Hz (Francis et al, 1987). Recordings were made after an overnight fast or approximately 1h after feeding following an overnight fast. "Fasted" myoelectric recordings were characterised by 3 recycling phases: quiescence (slow waves only), irregular then regular spiking activity (spike bursts on most slow waves), whereas "fed" recordings displayed almost continuous irregular-type activity (previously described by Ruckebusch & Fioramanti, 1975). Saline control (which had little or no effect) was administered s.c. approximately 0.5h before BRL 24924 or Mcp and recording was continued for approximately 1h after drug administration. Drug effects were expressed as geometric mean changes (and ranges) from saline control baselines.

Mcp, 1mg kg^{-1} (n=5). 5mg kg^{-1} (n=4) or 10 mg kg^{-1} (n=3), did not consistently affect the total number of analysed spikes in fasted rats although Mcp 5mg kg^{-1} tended to increase periods of quiescence [by 90 (5-166)%].

BRL 24924, 0.1mg kg^{-1} (n=3), 0.5mg kg^{-1} (n=5) or 2mg kg^{-1} (n=3), decreased quiescence in all fasted animals by 35 (12-50)%, 41 (19-58)%; $P<0.05$, Student's t-test and 21 (11-27)% respectively. Since BRL 24924 did not consistently affect the total number of analysed spikes, the decrease in quiescent periods may reflect a change in the proportions of irregular and regular spiking activities. In addition BRL 24924, 0.5mg kg^{-1} increased the total number of analysed irregular-type spikes in 5 of 5 fed rats [by 139 (42-240)%; $P<0.01$].

Thus in contrast to Mcp, BRL 24924 consistently affected the myoelectric activity of the rat small intestine, probably by increasing irregular spiking activity, at doses which have previously been shown to stimulate gastric motility.

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SPLEEN CELL RESPONSES TO EMULSIFIED PERFLUOROCARBONS IN VITRO

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Recent interest has focussed on tissue uptake of perfluorocarbon (PFC) emulsion particles, particularly into cells of the reticuloendothelial system (Lowe & Bollands, 1985). In earlier work we observed a decrease in spleen weight in mice injected intravenously with the proprietary emulsion, Fluosol-DA 20% (F-DA; Green Cross, Japan) (Bollands & Lowe, 1986). This may be attributable to cell depletion caused by some cytotoxic component of F-DA but the mechanism(s) involved have not been identified. To clarify this we have studied the direct effects of F-DA on murine splenocytes in culture.

Female NIH mice, 2-3 months old, were used. Animals were anesthetized with ether and then killed by cervical dislocation; spleens were removed aseptically. Tissues were homogenized and single cell suspensions in RPMI medium supplemented with sterile fetal calf serum and glutamic acid (Mosier, 1981) were prepared. Suspensions were enumerated by fluorescein diacetate staining (FDS) and adjusted to give a working concentration of 4×10^6 cells.ml^{-1}. 1.0 ml of this cell suspension was then added to either 1.0 ml of medium (controls) or 1.0 ml of medium containing either 2%, 5% or 10% sterile F-DA emulsion; cultures were then incubated at 37°C under 5% CO_2: air atmosphere for 24h. Cells were subsequently harvested and viability tested by FDS.

Cells showed a decrease in adherence and a tendency to aggregate when cultured with all concentrations of F-DA tested. Mean (± s.e.m.) viability in control cultures after 24 hr was 72 ± 3% but this showed a progressive decrease as the concentration of F-DA added to the culture medium was increased. Cell viabilities following exposure to different F-DA concentrations were:

Treatment	Percentage viability
Controls	72 ± 3
F-DA 2%	64 ± 2
F-DA 5%	53 ± 6*
F-DA 10%	48 ± 6**

Values are mean ± s.e.m.; n = 3-9
*P < 0.05; **P < 0.01 compared to mean control value

These results show that F-DA can have dose-dependent, cytotoxic effects on murine splenocytes in vitro. Previous work has shown emulsified perfluorotributylamine (FC-43) to be selectively toxic to mouse macrophages in culture (Bucala et al., 1983) and it is therefore possible that the decrease in cell viability observed in the present experiments reflected specific toxic effects on splenic macrophages. This possibility is currently being studied using fluorescent-labelled monoclonal antibody techniques.

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EFFECTS OF PAPAVERINE AND ISOBUTYLMETHYL XANTHINE (IBMX) ON NORMAL AND CHEMICALLY SKINNED GUINEA-PIG TRACHEALIS

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The mechanism underlying the bronchodilator effect of the methylxanthines is not understood. Various mechanisms, e.g., adenosine antagonism and inhibition of cyclic nucleotide phosphodiesterases (PDE) have been proposed and at different times rejected (see Persson, 1985). The object of this study was to examine the possibility of a relationship between cyclic AMP levels and relaxation in airway smooth muscle using two drugs known to inhibit PDE. Thus, we have compared the effects of papaverine and IBMX on tension responses and cyclic AMP levels in normal and chemically-skinned guinea-pig trachealis.

Rings of guinea-pig trachea were set up for recording of isometric tension in Krebs-Henseleit solution containing flurbiprofen (1μM) at 37°C and bubbled with 5% CO_2 in oxygen. Tissues were contracted with histamine (10μM) and the relaxant effects of papaverine and IBMX examined against this background contraction. Cyclic AMP was measured in tissues rapidly frozen in liquid nitrogen, either before or at the peak effect of papaverine or IBMX, using a competitive protein binding assay (Amersham). The results are summarized in table 1.

Table 1 Effects of papaverine and IBMX on tension responses and cyclic AMP levels in guinea-pig isolated trachealis (mean values ± s.e. mean; n=4 or 5)

Drug treatment	Concentration	% Relaxation	Cyclic AMP (pmol mg^{-1})
Papaverine	Vehicle (control)	0	0.18 ± 0.002
	1μM	35 ± 5	0.28 ± 0.03*
	10	90 ± 5	0.37 ± 0.05*
	100	110 ± 3	1.26 ± 0.02*
IBMX	Vehicle (control)	0	0.13 ± 0.04
	1μM	36 ± 4	0.15 ± 0.02
	10	95 ± 6	0.18 ± 0.05
	100	111 ± 2	0.49 ± 0.04*

* $p < 0.001$ Mann-Whitney U-test

Papaverine and IBMX were approximately equipotent in producing concentration-dependent relaxations of the guinea-pig trachealis. Only papaverine, however, caused significant concentration-related elevations of cyclic AMP levels.

Tracheal rings were skinned of their plasma membranes using Triton X 100, according to the method of Sparrow et al (1984). Three contractions were induced (at 60 min intervals) in these preparations by raising the free calcium ion concentration to 20μM. The drug under test was present for 5 min prior to, and throughout, the second calcium-induced contraction.

In the skinned trachealis both cyclic AMP (10μM) and the catalytic sub-unit of its protein kinase (0.1μM) inhibited the calcium-induced contraction,by 60±8% (n=6) and 40±3% (n=5) respectively. The calmodulin antagonist calmidazolium (10μM) caused a 20±7% (n=5) reduction in the calcium-induced contraction. Of the two PDE inhibitors only papaverine (100μM) caused a significant reduction in contraction (31±6%; n=5). IBMX (100μM) was without effect (0±9%; n=4).

These results are consistent with the hypothesis that cyclic AMP-dependent mechanisms have an inhibitory action on the biochemical processes that lead to contraction of the guinea-pig trachealis. The results also suggest that a functional (intact) smooth muscle cell membrane is important for the expression of IBMX-induced relaxation, which is not the case for papaverine. The mechanism underlying the relaxant/inhibitory effects of papaverine remains to be determined.

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NOVEL COMPOSITIONS OF EMULSIFIED PERFLUOROCARBONS FOR BIOLOGICAL USES: EFFECTS OF SURFACTANT AND OIL ADDITIVE CONCENTRATIONS

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In previous papers we have reported the development and preliminary biological assessment of novel compositions of emulsified perfluorocarbons (PFCs) for possible uses related to in vivo oxygen transport (Davis et al., 1986; Bollands et al., 1987). The emulsions contained perfluorodecalin (FDC) and were stabilized against Ostwald ripening by the addition of small quantities of polycyclic, perfluorinated, higher boiling point oils (HBPO) (Davis et al., 1986). We now report the effects of varying either the HBPO additive or surfactant concentrations on FDC emulsion stability.

FDC (ISC Chemicals Ltd., Avonmouth) was emulsified by sonication for 30 mins with either 1.0% or 4.0% Pluronic F-68 in an aqueous phase to give a final 20% (w/v) preparation. Test emulsions also contained either 0.5%, 1.0% or 2.0% of the following HBPO additives to enhance stability (Davis et al., 1986): perfluoroperhydroacenaphthylene (C-12), perfluoroperhydrofluorene (C-13), perfluoroperhydrophenanthrene (C-14) or perfluoroperhydrofluoranthrene (C-16); control emulsions contained no HBPO additives. Stability of emulsions during storage for up to 56 days at 4°C or 37°C was assessed by determination of a stability parameter, Dt/Do, where Do = initial mean particle size and Dt = mean particle size after time t days (Davis et al., 1986). Changes in Dt/Do for the following emulsion formulations containing 4% Pluronic F-68 and stored at 4°C for 38-56 days were:

Oil Additive	HBPO Concentration 0.5%	1.0%	2.0%
Control (no additive)	>3.0	2.14	2.19
C-12	>3.0	2.10	2.20
C-13	>3.0	1.84	1.61
C-14	-	1.49	-
C-16	1.54	1.20	1.15

Values are duplicates of means ($n \geqslant 10$) in each case.

Emulsion stability decreased by upto 25% as the concentration of Pluronic F-68 was reduced to 1.0%.

These results show that stability of FDC emulsions against Ostwald ripening can be enhanced by increasing the concentration of HBPO additives. Moreover, stability against coalescence can be achieved by increasing surfactant concentration. While greatest emulsion stability occurred with 4% Pluronic F-68, we are aware that this is slightly higher than that present in available commercial PFC emulsions, such as Fluosol-DA 20% (F-DA; Green Cross, Japan). Since the formulations described here are intended for biological applications, it may prove to be necessary to reduce the surfactant concentration, albeit with a small reduction in stability, since previous work has implicated Pluronic F-68 and its peroxide contaminants in some of the adverse effects of emulsified PFCs in vivo (McCoy et al., 1984; Lowe & Bollands, 1985).

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THE EFFECT OF ETHANOL ON TONE AND CONTRACTILITY OF RAT ISOLATED ILEUM

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It has been shown that many anaesthetics inhibit intestinal movements and that this effect can be attributed to (a) a direct effect on intestinal smooth muscle, (b) an action on intrinsic nerves of the intestine,(c) an action on the extrinsic nerve supply ro the gut,and (d) release of mediators and other factors(Speden, 1965).
In the present investigation,we have studied the effects of ethanol on the tone and contractility of rat isolated ileum,by analysing its effects on the spontaneous contractions,the contractions produced by periarterial nerve stimulation,and on the contractions produced by acetylcholine (ACh),to see if ethanol modified the cholinergic transmission and reduced the contractions produced by periarterial nerve stimulation.

The results showed that,in low concentrations,ethanol(3.9 mM),reduced the amplitude of the spontaneous contractions by 60±1.5% of the control value(0.6±0.1 g, mean±s.e.,n=6). In intermediate concentrations (39 mM),ethanol produced a biphasic response in the rat ileum,an initial relaxation (0.5±0.1 g),followed by a low amplitude and prolonged contraction(0.3±0.1 g). In high concentrations (390-3900 mM),ethanol produced a marked contraction(3.2±0.3 g) in the muscle.

Repetitive nerve stimulation (periarterial stimulation),at 1-100 Hz,with 20 V (maximal) and 0.2 ms pulse duration,produced frequency-dependent contractions and/or relaxations,in the rat ileum.At low rates of stimulation(1-10 Hz),only contractions were produced(0.2-0.7 g),whereas at high rates of stimulation(15-25 Hz) a biphasic response,similar to that produced by intermediate concentration of ethanol (39 mM),was produced.At high rates of stimulation (>30 Hz),only relaxations were observed. Repetitive periarterial nerve stimulation,at 20 Hz,in the presence of propranolol(1 μM),guanethidine(10 μM),to block adrenergic influences,methysergide(1 μM),to block 5-HT effect,mepyramine(1 μM) and indomethacin(2.8 μM),to block histamine and prostaglandin effects,respectively, produced only small contractions (0.3-0.5 g),which were reduced by atropine(1 μM),by 71±2.5%,and by ethanol(39 mM), 80±3.4%, respectively.

Ethanol (39 mM) also reduced the contractions produced by ACh (0.005-1.0 μM) and increased the control ACh-induced EC_{50} values (i.e concentration to produce 50% maximum contraction),from 0.55±0.06 μM to 5.28±0.26 μM, thus an increase of about 10-fold was obtained.(means±s.e.,n=6,$P < 0.001$).

Although the mechanism of action of ethanol,at the rat ileum,was not further analysed,it is known that ethanol may block conduction,in skeletal muscle (Gage & Hamill,1981),depolarize the cell membrane,in frog sartorius muscle (Knutsson, 1961),and alter membrane protein or lipid bilayer (Seeman,1972). We conclude that ethanol reduces the spontaneous contractions,the contractions produced by periarterial nerve stimulation and by ACh in the rat isolated ileum.In high concentrations,ethanol,itself,produces a marked contraction in the rat ileum.These results suggest a bimodal action of ethanol,producing greater effect on the nervous structure than on the muscle of rat ileum.

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EFFECTS OF MECAMYLAMINE ON CHOLINERGIC RESPONSES OF THE FROG OPTIC TECTUM IN VITRO

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In the optic tectum of lower vertebrates acetylcholine (ACh) has been proposed to be the optic nerve neurotransmitter acting mainly through nicotinic receptors (Freeman and Norden 1984). Since tubocurarine appeared to be an unreliable blocker of these receptors in vivo (Stevens 1973; Freeman and Norden 1984), we studied electrophysiologically another nicotinic antagonist, namely mecamylamine, as a selective blocker of cholinergic neurotransmission in the frog optic tectum in vitro.

Isolated brain preparations of the frog (Rana temporaria) were superfused with Ringer solution containing test compounds. Synaptic field potentials were recorded with a glass microelectrode from the upper tectal layers following electrical optic nerve stimulation (Berti et al. 1985). Antidromically elicited action potentials were also recorded from the severed end of the optic nerve after intratectal stimulation.

In isolated forebrain preparations (n=12), ACh (5-100 μM) produced a biphasic effect, namely enhancement followed by depression of tectal field potentials (Berti et al. 1985). Mecamylamine (10 μM) antagonized these ACh effects and shifted the ACh dose-response curve to the right while decreasing its maximum. Mecamylamine also modified tectal synaptic responses, by increasing (up to 63%) the amplitude of field potentials produced by low intensity stimulations while slightly reducing (up to 22%) the ones produced by high intensities. The nicotinic receptor agonists carbachol (CCh; 100 μM) and tetramethylammonium (TMA; 100 μM) enhanced the amplitude of antidromically elicited submaximal optic nerve action potentials (up to 56% for CCh and 71% for TMA). In 7 experiments mecamylamine (10 μM) prevented these effects and greatly reduced the amplitude of optic nerve action potentials (up to 53%) throughout the whole range of stimulations. Slices of the optic tectum with the optic nerve representing their main anatomical input were prepared. In these slices (n=9), mecamylamine (10 μM) depressed (up to 39%) synaptic responses throughout the whole range of stimulations and antagonized the potentiating effects of ACh. Mecamylamine (10 μM) produced no effects on glutamate (1 mM) or GABA (0.5 mM) induced responses.

In conclusion, these data show that in tectal slices mecamylamine was a selective blocker of synaptic transmission and ACh evoked responses. The blocking action of mecamylamine had a partly presynaptic origin. The effects observed on isolated forebrain preparations were probably indirect and due to changes in extratectal circuits.

C.B. is a Fellow of Fondazione Levi-Accademia Dei Lincei.

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THE PROTECTIVE EFFECT OF ACYLATION ON THE STABILITY OF EMINASE (APSAC) IN HUMAN PLASMA

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The novel fibrinolytic agent APSAC (anisoylated plasminogen-streptokinase activator complex) is a stoichiometric complex of streptokinase (SK) and human lys-plasminogen with the active site reversibly protected by a p-anisoyl group. In A.M.I. patients, APSAC is an effective thrombolytic agent and circulates, in an active form, in the bloodstream for longer than other thrombolytic agents such as SK, urokinase, tissue plasminogen activator (t-PA) and pro-urokinase (scu-PA) (Been, M. et al., 1986; Verstraete, M. & Collen, D., 1986). Studies, in vitro, were instigated to examine the mechanisms whereby acylation protects against loss of activity.

Stability studies were performed by incubation of equimolar concentrations of APSAC, SK and SK.plasmin in pooled volunteer plasma, pH 7.4 at 37°C. Samples were removed, the euglobulin fraction precipitated and total potential fibrinolytic activity measured by a calibrated fibrin plate assay. APSAC was more stable in human plasma than either SK.plasmin(ogen) formed in situ or pre-formed SK.plasmin and the differences were statistically significant at 15 minutes ($p=0.05$) and thereafter ($p<0.01$). The half-lives of fibrinolytic activity, 120 minutes for APSAC and 15-20 minutes for SK, are of the same order as the clearance half-lives found in patients (Been, M. et al., 1986; Mentzer, R.L. et al., 1986). Some of the loss of activity of SK at early times could be attributed to neutralisation by inhibitors. The effect of endogenous inhibitors on the activity of SK.plasmin(ogen) was investigated by two methods. First, fibrinolytic activity was monitored in α_2-antiplasmin depleted plasma, prepared by plasmin-sepharose adsorption and survival of SK was found to be relatively promoted at 4 and 15 minutes ($p<0.05$). Second, the appearance of inhibitor complexes in normal plasma was monitored by polyacrylamide gel electrophoresis and immunoblotting with rabbit antibodies against SK and α_2-antiplasmin. In this manner the rapid formation (by 4 mins) of complexes of SK-plasmin-α_2-antiplasmin was demonstrated. Corresponding studies performed with α_2-macroglobulin-depleted plasma suggested a slight, late, influence on SK.plasmin but immunoblotting produced equivocal results. In further studies using SDS PAGE and immunoblotting, we have demonstrated that acylation also protects against proteolysis of SK. Fibrinolytically-inactive degradation products (<30,000 daltons) appeared after only 15 minutes incubation of SK in plasma whereas for the SK moiety present in APSAC degradation occurred much more slowly.

The fibrinolytic activity of scu-PA, UK and t-PA after incubation in human plasma at 37°C was also examined. It appears that the stability of APSAC is similar to that of scu-PA whereas SK-plasmin(ogen) resembles UK and t-PA is intermediate in stability.

We conclude that neutralization by inhibitors and proteolytic degradation both contribute to the loss of functional activity in vitro and protection can be afforded by acylation of the SK.plasminogen complex. These studies contribute to our understanding of how a high level of fibrinolytic activity can be achieved by an intravenous bolus injection of APSAC.

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MONENSIN AND NIGERICIN DELAY THE DELIVERY OF THE INFLUENZA VIRUS ENVELOPE PROTEINS TO THE PLASMA MEMBRANE IN MDCK CELLS

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In ion transporting epithelia, functional asymmetry depends on the differential localisation of proteins in the two domains of the plasma membrane, apical and basolateral. Investigations of the mechanisms underlying this asymmetric targetting of plasma membrane proteins have used epithelial cell lines, such as MDCK, infected with enveloped viruses such as influenza and vesicular stomatitis virus (VSV). These viruses bud asymmetrically from MDCK cell monolayers (influenza apically and VSV basolaterally) and the envelope proteins of the viruses are inserted exclusively into the surface from which the virus will bud (Rodriguez-Boulan & Pendergast, 1980).

Carboxylic ionophores, such as monensin and nigericin, interfere with the intracellular processing of proteins, and have been used widely to characterise the transport pathways of newly synthesised proteins (Tartakoff, 1983). Interestingly, monensin has a differential effect on the intracellular transport of the envelope proteins G of VSV and haemagglutinin of influenza, blocking the transport of the former in the Golgi complex (Alonso-Caplen & Compans, 1983) but only delaying the appearance of the latter at the cell surface (Edwardson, 1984). In addition, it has been reported (Alonso-Caplen & Compans, 1983) that nigericin (a potassium-selective ionophore) reduces the yield of influenza virus from infected MDCK cells by about ten-fold, while monensin (a sodium-selective ionophore) has no effect on virus yield. This observation raises the possibility that ionophores of different ion selectivities may have qualitatively different effects on the intracellular transport of newly synthesised proteins.

We have examined the effects of these two ionophores on the intracellular transport and processing of the two envelope proteins of influenza virus, haemagglutinin and neuraminidase, in MDCK cells. Our aims were to ascertain whether both envelope proteins were affected similarly by ionophore treatment of the cells and whether the two ionophores produced the same effects. We found that terminal glycosylation of haemagglutinin in the Golgi complex was interrupted at the same stage by both ionophores, and that both ionophores caused a delay of approximately 1h in the delivery of haemagglutinin and neuraminidase to the plasma membrane. Hence, both envelope proteins are affected similarly by ionophore treatment of the cells, and the two ionophores produce similar effects on processing and transport, despite their different ion selectivities.

Both of the apically directed proteins examined were able to reach the plasma membrane in the presence of ionophore, unlike the basolaterally directed VSV G (Alonso-Caplen & Compans, 1983). It is still not clear whether this differential effect of the ionophores on proteins destined for different surfaces of an epithelial monolayer is revealing a genuine difference between the ways in which two groups of proteins are handled by the cells, or simply the idiosyncrasies of a small set of proteins. In order to answer this question, it will be necessary to establish whether this is a general phenomenon, and also to elucidate the mechanism by which the ionophores produce their effects on protein processing and transport.

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ACTIONS OF SOME ANALOGUES OF BRADYKININ ON RAT PAW OEDEMA

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Bradykinin (BK) is known to have pro-inflammatory effects when injected s.c. In this study, the effects of four BK analogues, B4148,(N-Lys,Lys,2-Hyp,5,8-Thi,7-D Phe BK), B4146(3-Hyp,5,8-Thi, 7-D Phe BK), B4144(5,8-Thi,7-D Phe BK) and B4162 (N-D Arg,3-Hyp, 5,8-Thi,7-D Phe BK) synthesised as potential antagonists were examined in a rat paw model of oedema. It has previously been reported that such analogues show competitive antagonism to BK.

Groups of at least 5 male Sprague Dawley rats (200-250g) were used. Oedematous swelling was measured as dorso-ventral thickness using a simple constant load lever system. Swelling was expressed as percentage increase in paw thickness from pre-injection levels. Test agents (0.1ml) were injected s.c. into the right hind paw. Left hind paws were used as controls and injected with saline. Paw thickness was measured at 5 min intervals for 45 min and at 15 min intervals for a further 45 min.

BK in doses ranging from 10^{-9}M to 10^{-6}M gave a dose-dependent swelling as compared to controls, peaking at 5 to 10 min,($P<0.05$) and thereafter subsiding. When 0.2mg captopril,a kininase inhibitor, was injected s.c. with BK 10^{-9}M or BK 10^{-8}M, both the initial peak swelling and the degree of swelling over the entire time course were enhanced as expressed as area under time course curve.

Each of the four BK analogues was tested at 10^{-7}M in the presence of BK 10^{-9}M with and without captopril. None of the four analogues at this concentration showed any activity in reducing either the BK-induced initial peak swelling or the degree of swelling over the entire time course. When injected alone, compared with control, all four analogues produced significant swelling at 10^{-7}M ($P<0.01$). When injected with captopril, the overall degree of swelling produced by each analogue was significantly increased ($P<0.05$). Initial swelling was increased significantly for B4148 at 5 and 10 min ($P<0.05$), B4144 at 10 min ($P<0.05$) but not at all for B4146 and B4162.

These results may be inconsistent with previous findings by Longridge et al (1985) suggesting that substituting the D-Phe at the Pro-7 position of the BK molecule confers resistance to kininase II.

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MODIFICATION OF THE COTTON GRANULOMA MODEL OF CARTILAGE BREAK-DOWN FOR DIRECT PHARMACOLOGICAL INVESTIGATIONS

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The implantation of articular cartilage together with cotton in the subcutaneous tissues (s.c.) of rodents results in the induction of a granulomatous reaction by the cotton and an accelerated breakdown of the cartilage (De Brito et al, 1986a; 1986b). In the present study we examined the effect of endotoxin challenge on this model.

Freshly collected intact Wistar rat (body weight 150-200 gm) femoral head cartilage was implanted either alone or wrapped with 2 mg sterile cotton into the s.c. tissues of the flanks or into the peritoneal cavity of ether-anaesthetised Balb c inbred mice (20-30 gm). Four days after implantation, groups of 6-8 mice were challenged intraperitoneally (i.p.) with Salmonella typhimurium lipopolysaccharide in sterile pyrogen-free saline (volume 1.0 ml) or saline alone. On day 7 of implantation the mice were sacrificed and the implants were removed. Where cotton was present, this was dissociated from the cartilage and the granulomatous reaction reaction induced was assessed by determining the weight of granulation tissue (dry weight - original weight of cotton) in the implanted cotton. Cartilage proteoglycan was determined by the method of Farndale et al (1982).

	endotoxin per mouse	cartilage proteoglycan (μg GAG) no cotton	with cotton (a)	cotton granulation tissue weight(mg)(b)
s.c. implants	0	600 ± 29	482 ± 42 (20)*	4.3 ± 0.2
	0.1 μg	580 ± 31	486 ± 38 (16)*	3.5 ± 0.5 (-19)
	1.0 μg	610 ± 32	530 ± 43 (13)	4.6 ± 0.4 (2)
i.p. implants	0	560 ± 25	460 ± 32 (18)*	7.5 ± 0.5
	0.1 μg	595 ± 36	465 ± 38 (23)*	9.2 ± 1.5 (23)
	1.0 μg	565 ± 42	380 ± 38 (32)*+	11.7 ± 2.8 (35)+

Each result represents the mean ± SEM of 6-8 mice; figures in brackets indicate %change (a) from corresponding no cotton result, (b) from endotoxin-free saline challenge result; *, + $p<0.05$ significance levels Students T-test, significantly different from corresponding cotton-free result (*) and endotoxin-free saline challenge (+);

The table above summarises the results of this study. Endotoxin challenge of mice bearing s.c. implants of cartilage with or without cotton, produced no significant effect on cartilage proteoglycan loss, or on the intensity of the granulomatous reaction induced by cotton (when present). The implantation of cartilage with cotton in the peritoneal cavity, resulted in an accelerated loss of cartilage proteoglycan similiar in magnitude to that caused by cotton in s.c. tissues. However endotoxin (1.0 μg) challenge of these implants potentiated the granulomatous response induced by cotton and caused a significantly greater loss of proteoglycan from cartilage. This indicates that breakdown of cartilage by endotoxin was due to the release of destructive substances by the cotton granuloma. This model may be useful for determining the destructive potential of putative mediators in chronic inflammatory diseases.

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RELEASE OF SRS-LIKE ACTIVITY FROM GUINEA-PIG ALVEOLAR MACROPHAGES BY COTTON DUST

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Inhalation of cotton dust extracts by rabbits induced the release of $PGF_{2\alpha}$ and thromboxane A_2 in the alveolar spaces of the lung (Mundie et al. 1985). Such effects may be responsible for some of the symptoms of byssinosis in cotton mill workers. However, it is possible that other products of the arachidonic acid cascade may also be liberated to play a similar role. The present investigation has examined the ability of cotton dust and bract particles and their aqueous extracts to release smooth muscle contractor agents of the 5-lipoxygenase pathway (SRS-like activity) from guinea pig alveolar macrophages.

Female guinea pigs (400-500g) were killed with pentobarbitone sodium (100mg/kg,i.p.). Blood was removed from the pulmonary circulation by perfusion via the right ventricle with phosphate-buffered saline containing 0.6mM EDTA at 37°C. The trachea was cannulated and 5x10ml of the EDTA-buffered saline were used to lavage the lung. The alveolar macrophages were separated by centrifuging the lavage fluid at 500xg for 8 min at 4°C. The macrophages were suspended in Tyrode solution containing ℓ-cysteine (0.5μM) to give a concentration of $5x10^6$ cells/ml. The cell suspension was incubated in the presence of either dust and bract particles, their aqueous extracts, or the calcium ionophore A23187 for 20 min. Following sedimentation of the macrophages by centrifuging (500xg, 4°C, 8 min) SRS-like activity was assayed by bracketing on guinea pig isolated ileum in the presence of atropine, mepyramine and methysergide, LTC-4 being used as standard. Specificity of the assay was checked by determining the sensitivity of the responses to the SRS antagonist FPL 55712 (0.1μg/ml; Augstein et al. 1973). The results are presented in Table 1.

Table 1: Release of SRS-like activity from guinea pig alveolar macrophages by cotton dust

Material	CDP		CDE	BP	BE	A23187
Concentration (mg/ml)	10	20	80*	20	80*	$5x10^{-4}$
SRS activity	25	35	26	0	0	30
(ng LTC-4/$5x10^6$ cells)	±2	±3	±3			±1

Results are means ± sem, n=5. CD = cotton dust, B = bract, P = particles, E = extract. *aqueous soluble material from 80mgCDP or DP.

The cotton dust particles and extract and the calcium ionophore A23187 all released SRS-like activity from the macrophages. However the extract was much less active than the particles. Neither the particles nor extract of bracts were able to release this type of material from the cells. The results are consistent with the finding that cotton dust extract but not bract extract produces bronchoconstriction of the guinea pig perfused isolated lung by liberating 5-lipoxygenase products (El-Mahdy and Nicholls, 1986). However, the present data indicate that, in addition to soluble factors in cotton dust, a particle effect may be important.

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PRELIMINARY STUDY OF AMINOGLUTETHIMIDE IN AN ARTHRITIC MODEL IN RATS

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Aminoglutethimide (AG), a non-steroidal competitive inhibitor of several cytochrome P-450 dependent systems, is used in the therapy of oestrogen-dependent breast cancers in postmenopausal women.

It has recently been shown that AG inhibits adenosine diphosphate (ADP)-induced platelet aggregation in man (Rao & Nicholls, 1986). This taken together with the reports that institution of therapy with AG results in a decrease of the bone pain suffered by patients with the metastatic disease (Harris et al. 1983) and that the drug is also effective in relieving the bone pain in men suffering from advanced prostatic cancer (Rostom et al. 1982) suggest that AG may have an effect on the arachidonic acid cascade.

We report here the results of a preliminary study of AG in an arthritic model in rats. Female Sprague-Dawley rats (150g at the start of the experiments) were used. Adjuvant-induced arthritis was produced by a single intradermal injection into the base of the tail of 100µg heat-killed Mycobactorium butyricum in 100µl complete Freud's adjuvant on day 1 (Pearson 1956). Animals were treated with either AG (n=12) 50mg/kg (increasing to 100mg/kg once the arthritis had developed; day 11) or vehicle (n=11, 0.1% w/v carboxymethylcellulose/0.1% v/v Tween 80, 1ml/100g) daily, orally, throughout the three week period of the experiment. The onset and severity of the arthritis was assessed daily, each limb being given an arthritic score (0=no arthritis; 5=severe) for the duration of the experiment. The results are shown in Table 1.

Table 1: Mean arthritic score (± S.E.M.) for control and AG treated animals.

	Day				
Treatment	11	14	16	18	20
Vehicle	0.50	7.27	8.64	9.27	9.27
n=11	±0.32	±2.0	±2.2	±2.33	±2.4
AG	0.79	3.67	4.75	7.88	7.46
n=12	±0.54	±1.5	±1.47	±2.0	±1.95

Statistical analysis of the results (3-way analysis of variance) showed AG to have no significant ($P>0.05$) effect on the arthritis compared with the control group.

Although the arthritic score does not appear to have been significantly altered by AG treatment, it is of interest to note that, compared with the controls, the AG-treated group appeared to suffer less discomfort and pain as a result of the arthritis. In addition, the AG group was significantly heavier than the control group on day 20 (231.5±4.7g AG group; 215.8±5g control group; $P<0.05$). These mild but beneficial effects of AG in the arthritic animals would appear to be worthy of further investigation.

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THE INFLUENCE OF SOME LABORATORY ANAESTHETICS ON RENAL HAEMODYNAMICS: SIGNIFICANCE IN PHARMACOKINETIC STUDIES

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We have previously reported that urethane (U) anaesthesia in comparison to pentobarbitone (P) and 'Hypnorm/Hypnovel' (H) anaesthesia produces an alteration in the pharmacokinetic handling of para-aminohippuric acid (PAH) (Gumbleton et al. 1986). U anaesthesia in comparison to P anaesthesia has also been reported to significantly alter the pharmacokinetics of carboxyfluorescein (Woolfrey et al. 1985) and thiamine (Pipkin et al. 1982), compounds whose elimination, like that of PAH, is highly dependent on renal clearance.

With the aim of examining the basis to these reported U-induced reductions in renal clearance, this present study in male Wistar rats (250±16g) has investigated the extent to which laboratory anaesthetics can alter renal blood flow (R.B.F.) and glomerular filtration rate (G.F.R.). For this three anaesthetic regimens were selected:- fentanyl + fluanisone (0.26+8.3mg/kg i.p. 'Hypnorm') given in combination with midazolam (4.16mg/kg i.p. 'Hypnovel'), urethane (1.75g/kg i.p.) and pentobarbitone sodium (67mg/kg i.p.).

R.B.F. was assessed by the total body clearance and extraction ratio across the kidney of PAH. G.F.R. was assessed by the total body clearance of inulin. Given that these compounds are totally eliminated by renal mechanisms and that their volumes of distribution were not significantly ($P>0.05$) different between any of the anaesthetic regimens examined, the total body clearances were taken as renal clearance. Measures of R.B.F. and G.F.R., under P anaesthesia, observed in this study are in close agreement with previously reported values of 6.20 and 0.97mls/min/100g bodyweight, respectively in rats anaesthetised with P 40mg/kg i.v., R.B.F. and G.F.R. measurements of 8.28 and 1.30mls/min/100g body weight being obtained in unanaesthetised animals (Walker et al. 1983). ^{3}H-labelled PAH (10.8×10^{-3}µmole; 5.41µCi)/250g and ^{14}C-labelled inulin (41.2×10^{-3}µmole; 0.47µCi)/250g were injected via the left jugular vein. Blood samples (100µl) were obtained via the carotid artery with intravenous normal saline replacement. At termination aortic artery and renal vein blood samples were taken to assess PAH extraction. Blood samples were solubilised and decolourised prior to liquid scintillation counting. Results from each animal were analysed according to two-compartment models.

Table 1. Anaesthetics and Renal haemodynamics

Anaesthetic Regimens	PAH Clearance (mls/min)	PAH Extraction Ratio	R.B.F. (mls/min)	Inulin G.F.R. (mls/min)	Inulin G.F.R. R.B.F.
Urethane	6.58**	0.79	4.76**	2.00**	0.42**
	±0.69	±0.04	±0.25	±0.17	±0.05
Pentobarbitone	12.85	0.84	10.80	3.38	0.31
	±0.97	±0.05	±0.54	±0.22	±0.03
Hypnorm/Hypnovel	11.52	0.84	9.51	3.16	0.33
	±1.55	±0.06	±0.92	±0.60	±0.05

*P<0.05, **P<0.01 Significantly different to all other treatments. One-way analysis of variance and Duncan's test. Mean ± S.D. (n=5).

The results demonstrate that U anaesthesia in comparison to both P and H anaesthesia causes a significant ($P<0.01$) reduction in R.B.F. and G.F.R. To conclude, U anaesthesia produces significant alterations in renal haemodynamics and is the probable cause of reported U-induced reductions in the clearance of various renally eliminated compounds. The H anaesthetic combination may provide a superior alternative to U anaesthesia if the use of P is contra-indicated.

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THE EFFECT OF GLYCEROL-INDUCED ACUTE RENAL FAILURE ON THE HEPATIC UPTAKE OF SODIUM CROMOGLYCATE IN THE ISOLATED PERFUSED RAT LIVER

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The hepatic uptake of sodium cromoglycate (SCG) has been investigated in livers taken from control rats and those with induced renal failure.

Acute renal failure (ARF) was induced in male Sprague-Dawley rats by withdrawal of drinking water for 24 hours followed by injection, in divided doses, into the hind limb musculature of 10 $ml.kg^{-1}$ of 50% (v/v) glycerol in 0.9% (w/v) saline. Free access to drinking water was permitted following injection.

Hepatic uptake was studied employing an isolated single-pass perfused liver system similar to that described by Ockner et al (1983). This system permits analysis of steady state uptake kinetics over a range of concentrations. Some experiments (Series 1) involved the addition of known fixed amounts of serum albumin (0.2mM) to the perfusate (150ml). Uptake of [^{3}H]-SCG was investigated over a range of perfusate concentrations (0.067-0.67mM) resulting in an SCG-albumin ratio of 0.33-3.33. In other experiments (Series 2) the molar ratio of SCG to albumin remained fixed at 1:1 while concentrations of both were varied together over the same range.

During each perfusion, samples of perfusate were collected over the entire perfusion period. The uptake concentration data was subjected to analysis by non-linear least squares regression techniques using the computer program 'BMDPAR', (BMDP-83).

In both series uptake appeared to be saturable and saturation of 'Series 2' occured at lower substrate concentrations and at uptake rates below those attained in 'Series 1' (see Table 1).

Table 1. Maximum uptake (Umax) and concentration at 50% Umax (UC50) values from control and ARF induced livers.

	Umax ($\mu g.g^{-1}$. liver).	UC50 (mM).
Control Rats.		
Ser. 1.	230 ± 80	1.28 ± 0.56
Ser. 2.	65 ± 22*	0.21 ± 0.10*
ARF Rats.		
Ser. 1.	108 ± 41+	0.62 ± 0.53@
Ser. 2.	25 ± 7*£	0.08 ± 0.13

Values are mean ± S.D. (n = 5).
* p <0.01 non-paired Student's t-test within the control and ARF groups.
+ p <0.05 non-paired Student's t-test between the groups for Series 1.
@ p <0.01 non-paired Student's t-test between the groups for Series 1.
£ p <0.01 non-paired Student's t-test between the groups for Series 2.

The results may reflect saturation of an earlier event in the uptake process which may be the transfer of SCG from albumin at the surface of the hepatocyte (Ockner et al., 1983). In addition, glycerol induced ARF reduced both the maximum uptake (Umax) and the concentration at 50% Umax values (UC50) compared to controls.

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STUDIES ON THE EFFECTS OF OMEPRAZOLE ON THYROID FUNCTION IN THE RAT

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Toxicological studies on Omeprazole, a K^+,H^+-ATPase inhibitor, in rats (400-1200 μmol/kg/day) have shown changes in thyroid hormone metabolism with no pathological changes in the thyroid gland (Ekmann et al, 1985). As well as a reported effect on liver 5'-deiodinase activity (Ekmann et al, 1985) Omeprazole could have direct effects on the thyroid gland in view of the K^+,H^+-ATPase immunoreactivity in thyroid tissue (Saccomani et al, 1979). In order to fully evaluate the mechanism of the changes produced by Omeprazole we have studied its effects on a number of thyroid parameters.

SK&F Wistar rats were dosed with Omeprazole (414-500 mg/kg p.o.) for 7-14 days. Following administration of Omeprazole at 500 mg/kg p.o. for 14 days there were reductions (ranging between 20-52%) in plasma TT_3 and TT_4 concentrations on days 1, 3, 7 and 14 both 6 h and 24 h following drug compared to controls. Serum TSH concentrations were increased in male rats after 14 days (24 h post dose) where a 60% increase against controls was observed. Despite these changes in hormone levels the accumulation of ^{125}I by the rat thyroid gland and its organification in vivo as measured by the perchlorate-discharge test (Atterwill et al, 1986) were unaltered by Omeprazole. In vitro studies with cultured porcine thyrocytes revealed that Omeprazole did inhibit TSH-stimulated ^{125}I organification by these cells at high concentrations (IC_{50} = 300 μM) but was only a weak inhibitor compared with Methimazole or PTU (IC_{50} = 2.8 μM and 1.5 μM respectively). In agreement with the results of Ekmann et al (1985) treatment of male rats with the same dose of (414 mg/kg) Omeprazole for 7 days caused a 55% decrease in liver 5'-deiodinase activity (Jones et al, 1986) (control = 147 ± 9; treated = 66 ± 7 pg T_3/mg protein/10 min; $P<0.05$, n=6), but female liver enzyme activity was unaltered. T_4 clearance as measured following the elimination of ^{125}I-T_4 (10 μCi/kg) was, however, unaltered following one dose of 500 mg/kg Omeprazole. In conclusion, therefore, these data support the findings of Ekmann et al (1985) where Omeprazole appears to inhibit peripheral 5'-deiodinase activity and decrease plasma T_3 concentrations. However, in view of the concomitant reductions in circulating T_4 it may also be a weak, and direct inhibitor of thyroidal hormone biosynthesis as supported by its in vitro action in cultured thyrocytes. Despite these actions no effect on radioiodide disposition in the rat thyroid gland in vivo could be detected indicating that the small rises in serum TSH are probably not sufficient to markedly affect this aspect of thyroid follicular cell function.

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OPPOSITE EFFECTS OF 5-HT_1 AND 5-HT_2 AGONISTS ON SUPRAHYOID MUSCLE TWITCHING

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Suprahyoid muscle twitching in urethane-anaesthetised rats is enhanced by serotonin (5HT) - releasers and the agonist MK 212, an effect mediated via central 5HT receptors (Bieger et al., 1972; Clineschmidt et al., 1977). In this study we have investigated the pharmacological characteristics of the 5HT receptors involved in this response.

Nialamide-pretreated (50mg/kg i.p. 18h prior), male, Wistar rats were anaesthetised with urethane (1.2-1.6g/kg i.p.), the suprahyoid muscles exposed, a thread tied around the muscle and connected to a 0-100g range isotonic transducer. Traces were displayed on pen recorders (Devices). A low spontaneous rate of twitching (0.2-2/min) was markedly enhanced in frequency and amplitude for more than 30 min by p-chloroamphetamine (0.2-1mg/kg i.v.) and fenfluramine (0.5-2mg/kg i.v.). There was an initial 2-5 min duration reduction in amplitude after fenfluramine, associated with increased frequency of twitching.

MK212 (0.1-1mg/kg i.v.) quipazine (0.5-1mg/kg i.v.) m-trifluoromethyl phenyl piperazine (TMPP, 0.5-2mg/kg i.v.)and meta chlorophenyl piperazine (MCPP, 0.2-1mg/kg i.v.) induced twitching of similar frequency although the initial reduction of amplitude was more marked with MK212, TMPP and MCPP such that little increase in twitch amplitude occurred on its recovery, and in some preparations the pre-dose twitch amplitude was not regained. Ritanserin (1-2mg/kg i.v.) and metergoline (0.2-1mg/kg i.v.) antagonised the effects of MK212, quipazine, fenfluramine or MCPP, both in terms of twitch frequency and amplitude suggesting an involvement of $5HT_2$ receptors.

The $5HT_{1A}$ receptor agonists 8-OH-DPAT (0.1-1mg/kg i.v) and ipsapirone (TVXQ 7821, 0.05-0.5mg/kg i.v.) inhibited spontaneous twitching and twitching induced by amphetamine (1mg/kg i.v.) or fenfluramine. These effects were associated with increased respiratory rate. RU24969, an agonist which preferentially interacts with $5HT_{1B}$ receptors, also inhibited spontaneous twitching and that induced by amphetamine or fenfluramine but at relatively high doses (0.5-2mg/kg i.v.) and this was associated with decreased respiratory rate. Little antagonism of the effects of any of these agonists was observed after metergoline (0.2-0.5mg/kg i.v.) or $\pm$ propranolol (0.2-2mg/kg i.v.), although the doses of antagonists were limited by other pharmacological effects of the drugs alone.

Thus, $5HT_2$ and $5HT_1$ receptor agonists have opposite effects on suprahyoid muscle twitching. The involvement of $5HT_2$ receptors in the enhancement of twitching is indicated by the blockade by ritanserin. The nature of the receptors involved in the $5HT_1$ agonist inhibitory effect remains to be established.

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EFFECT OF THE OPIOID ANTAGONIST 16-METHYL-CYPRENORPHINE (M8008) ON OPIOID-INDUCED CHANGES IN URINE OUTPUT IN THE WATER LOADED RAT

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Opioid receptor agonists produce marked effects on urine output in the rat (Leander, 1983). Kappa-selective agonists increase urine output, μ-selective agonists decrease urine output, whereas non-selective agonists exert both antidiuretic and diuretic effects. 16-Methyl-cyprenorphine (M8008) is an opioid antagonist with high affinity for μ and δ opioid receptors, but low affinity for the κ opioid receptor (Smith, 1985). In the present study, the effect of the opioid antagonist has been investigated on opioid-induced changes in urine output in the water-loaded rat.

Rats (male PVG hooded, 100-130g) were starved overnight and then water-loaded (25ml/kg p.o.) immediately prior to s.c. injection of opioids, alone or mixed with M8008. Urine output was recorded hourly for 5 hours after injection. The μ-selective agonist fentanyl (0.03-0.3mg/kg s.c.) produced a marked antidiuretic effect, which was antagonised by M8008 in a dose-dependent manner (dose-ratios obtained were: 2.6, 5.7 and 16.5 for M8008 at 0.25, 1 and 4mg/kg s.c., respectively). Similarly, the μ-selective agonists morphine, buprenorphine and profadol produced large antidiuretic effects, which were blocked by co-administration of M8008 at 1mg/kg s.c., (Table 1).

Table 1

Drug	Dose mg/kg	Mean urine output (ml±s.e.) at 1hr Control	Drug alone	Drug + M8008
Fentanyl	0.3	3.1±0.2	0.0±0.0	2.3+0.4
Morphine	10	2.4±0.5	0.0±0.0	2.4±0.3
Buprenorphine	0.05	2.9±0.2	0.1±0.1	2.8±0.2
Profadol	20	2.9±0.2	0.1±0.1	2.6±0.2
Butorphanol	5	3.0±0.2	0.9±0.3	2.9±0.2
EKC	1.5	2.7±0.2	0.4±0.2	1.0±0.1*
U50488H	10	3.1±0.1	1.5±0.2	1.1±0.2**

M8008 at 1mg/kg except, *=M8008 at 4 mg/kg, **=M8008 at 16 mg/kg

The opioid agonists U50488H (1.1-10mg/kg), EKC (1.5-6mg/kg) and butorphanol (5mg/kg) produced an initial antidiuresis, followed by a diuretic effect. The diuretic actions of these three agonists were not antagonised by M8008 in the dose range 1-16mg/kg s.c. The antidiuretic effect of butorphanol was completely antagonised by M8008 at 1mg/kg, whereas the antidiuretic effect of EKC was only partially reversed and that of U50488H was not antagonised by M8008 at 4 and 16mg/kg s.c., respectively (Table 1).

In summary, the opioid antagonist M8008 antagonises the antidiuretic effect of μ-selective agonists in the water-loaded rat, without antagonising the κ receptor-mediated diuretic effect. The reason for the inability of M8008 to reverse the antidiuretic effects of U50488H and EKC is not known.

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INFLUENCE OF NEOMYCIN PRETREATMENT ON THE DISPOSITION OF PARACETAMOL IN THE MOUSE: A POSSIBLE ROLE FOR THE INTESTINAL MICROFLORA?

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The minor analgesic paracetamol is excreted principally as conjugates with glucuronic acid or sulfate, while a small percentage of the dose is converted by the microsomal mixed-function oxidase system to a reactive intermediate which is in turn detoxified by conjugation with glutathione. This glutathione conjugate is further metabolised and excreted principally as the 3-cysteine and 3-mercapturate derivatives of paracetamol, with 3-thiomethyl paracetamol and its sulfoxide also formed. In view of the importance of the intestinal microflora in the catabolism of the glutathione conjugates of xenobiotics (Bakke & Gustafsson, 1986) the present study has been initiated to investigate its role in the metabolism of paracetamol in the mouse.

Male CD-1 mice were treated with neomycin sulfate (200mg/kg) or an equivalent volume of saline p.o. twice daily for 3 days, followed on the fourth day by 200 mg/kg [ring - ^{14}C] paracetamol (5 µCi/mouse) i.p. The mice were placed in metabolism cages and their 0-8h urine collected in ice-cooled flasks. Urinary excretion of ^{14}C was determined by scintillation counting, and metabolites assayed by radio HPLC.

The results of this study are presented in the Table. The principal effect of neomycin pretreatment upon the fate of paracetamol is the highly significant reduction in the excretion of 3-thiomethyl paracetamol and its sulfoxide and the elimination of other paracetamol metabolites is unaltered.

Influence of neomycin pretreatment upon paracetamol metabolism in mice

		% ^{14}C dose as that metabolite in 0-8h urine	
		Control	Neomycin treated
Paracetamol	- free	5.63 ± 0.61	4.53 ± 1.52
	- glucuronide	61.18 ± 4.04	62.18 ± 5.15
	- sulfate	9.50 ± 0.35	11.05 ± 1.57
	- 3-cysteine	18.25 ± 2.81	21.20 ± 4.26
	- 3-mercapturate	0.19 ± 0.21	0.16 ± 0.12
3-Thiomethyl paracetamol			
	- free	0.14 ± 0.10	0.09 ± 0.12
	- glucuronide	0.86 ± 0.60	0.09 ± 0.17*
	- sulfate - sulfoxide	3.66 ± 1.76	0.50 ± 0.82**
	- total	4.67 ± 2.31	0.67 ± 0.94**

mean ± S.D. n = 4 * $p < 0.05$ ** $p < 0.02$ cf. control

The glutathione conjugate of paracetamol is excreted in the bile and transformed to the various urinary thio adduct excretion products by the combined actions of the intestinal microflora and tissue enzymes. The present findings that neomycin pretreatment has no effect upon the conjugation of paracetamol with glucuronic acid, sulfate or glutathione (via the reactive intermediate) but markedly reduces the elimination of thiomethyl metabolites, indicate an important role for the gastrointestinal microflora in the formation of the latter, most likely at the level of the C-S lyase reaction (Bakke & Gustafsson, 1986).

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12(R)-HYDROXY-5,8,10,14-EICOSATETRAENOIC ACID IS A LYMPHOCYTE CHEMOATTRACTANT

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12(R)-hydroxy-5,8,10,14-eicosatetraenoic acid [12(R)-HETE], the major stereoisomer of 12-HETE present in the lesional scale of patients with the skin disease psoriasis (Woollard, 1986), has been shown to be chemokinetic for human polymorphonuclear leucocytes (Cunningham et al., 1986). Since lymphocytes are consistently found in psoriatic lesions, the chemotactic activity of 12(R)-HETE for human peripheral blood lymphocytes has now been investigated.

Mixed human peripheral blood lymphocytes were obtained by Ficoll-Hypaque density gradient centrifugation, followed by removal of monocytes by adherence to plastic for 1 h and subsequent overnight culture. Non-specific esterase staining showed that the lymphocytes were greater than 99.9% pure. Lymphocyte chemotaxis was assessed by use of a 48-well microchemotaxis chamber and 8 μm polyvinylpyrrolidone-free polycarbonate filters (Harvath et al., 1980). Cell migration was measured after one hour with an AMS 40-10 image analyser. Results have been expressed as mean ± s.e. mean migration index [area of lower surface of filter occupied by cells following stimulation with chemoattractant (mm^2) / area of filter occupied by randomly migrating cells (mm^2)]. The chemotactic response to 12(R)-HETE was measured over the range 5 x 10^{-7}M to 5 x 10^{-5}M and responses also obtained to the lymphocyte chemoattractants, zymosan activated plasma (ZAP, 0.1-25%), casein (0.1-2.0 mg ml^{-1}) and formyl-methionyl-leucyl-phenylalanine (fMLP, 10^{-9}-10^{-6}M).

Lymphocyte migration in response to 12(R)-HETE is shown in Table 1. Dose related chemotaxis was also seen in response to ZAP, casein and fMLP, maximal migration indices being 3.5±0.5 (n=6) for ZAP (5%), 1.8±0.1 (n=5) for casein (1 mg ml^{-1}) and 1.5 (n=2) for fMLP (10^{-7}M).

Table 1.

Concentration of 12(R)-HETE	Migration index ± s.e. mean (n=3)
5 x 10^{-7}M	1.34 ± 0.04
10^{-6}M	1.41 ± 0.08
5 x 10^{-6}M	1.71 ± 0.12
10^{-5}M	2.29 ± 0.06
5 x 10^{-5}M	1.48 ± 0.17

12(R)-HETE is thus chemotactic for human peripheral blood lymphocytes in vitro over the range of 4-400 ng per well tested. Since μg g^{-1} quantities of 12(R)-HETE are found in psoriatic scale (Cunningham et al., 1985; Woollard, 1986), 12(R)-HETE may be, at least in part, responsible for the lymphocyte infiltrate which is a characteristic feature of the psoriatic lesion.

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CHARACTERISATION OF TACHYKININ RECEPTORS IN THE VAS DEFERENS PREPARATION OF THE GUINEA-PIG

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Contractile responses to field stimulation of the vas deferens preparation of several species are potentiated by substance P and related tachykinins (TK's). The receptors involved in the rat preparation have been extensively studied with TK agonists, antagonists and ligand binding studies and, though the locus of action is uncertain, may now be regarded as archetypal NK2 - SP-E receptors (Iversen et al, 1987). In contrast the guinea-pig preparation has been little studied, and though originally classified as SP-P (= NK1) (Iversen et al, 1982) seems not to have been re-examined with the extensive range of ligands now available.

We report here on the activities of a series of agonists recently reported on in the rat preparation (Iversen et al, 1987) with a view to redefining the characteristics of the receptors in the guinea-pig. We have evaluated a range of stimulation parameters (Fox et al, 1987) which suggest that an early phase of the contraction is enhanced, so for the main bioassay have used 5 pulse trains at 20 Hz (1.0 ms and supramaximal voltage) at 7 min intervals. Up to seven analogues were assayed in parallel in a randomised block design using proportional potentiation by SP (1 μM) administered at the beginning and end of blocks as an internal control, against which potentiation by other analogues could be expressed. The locus of tachykinin action appears to be neuronal in that, in contrast to the rat preparation, none of the analogues produced an appreciable rise in base line tension at higher concentrations, and this was also clearly the case with bombesin-related analogues which have many similarities (Fox et al, 1987).

Thresholds for the TK's were < 10 nM and their log dose-response curves were approximately parallel, but it was not feasible to determine maxima which appear to lie at concentrations greater than 10 μM. The TK's differed little in activity, ie, physalaemin $\approx$ SP $\approx$ SP methyl ester $\approx$ neurokinin A $\approx$ neurokinin B $\approx$ kassinin $\approx$ eledoisin; which is not inconsistent with current definitions of NK1 receptors (Iversen et al, 1987). This interpretation is given further credence by comparison of activities of [Glp^6,D-Pro^9]SP(6-11) and [Glp^6,L-Pro^9]SP(6-11) where the L isomer was markedly more potent than the D which seems characteristic for NK1, but not NK2 or NK3, receptors (Iversen et al, 1987). In contradiction, a number of antagonists based on the SP sequence were found to be inactive in this preparation against substance P, which may reflect in some way the low potency of such antagonists on neuronal preparations but, alternatively, may indicate these receptors are not identical with those of guinea-pig ileum smooth muscle or rat parotid gland (=NK1) where the antagonists are active; (see; Bailey et al, 1986, Iversen et al, 1987).

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THE ROLE OF THE ENDOTHELIUM IN THE RESPONSE OF THE IN VITRO RAT AORTA TO BK, AII, 5-HT, PHE, AND ACH: EFFECT OF OXYHAEMOGLOBIN

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The role of the vascular endothelium in modulating the action of endothelium-dependent vasodilators has been well documented (Furchgott and Zawadzki 1980, Furchgott 1983). In addition, the ability of the endothelium to modulate contractile responses has been realised (Martin et al 1986). This study investigates the role of the endothelium in the responses of the rat aorta to a number of spasmogens. Oxyhaemoglobin (Hb) was used as an investigative tool, in view of its guanylate cyclase blocking action, which is believed to be the basis of its EDRF-inhibiting properties (Martin et al 1985).

Aortic rings were prepared from female Wistar rats, as previously described (Whalley and Whalley 1986). Concentration-effect (C-E) curves were constructed to BK, AII, 5-HT, PhE and ACh on intact (I) and de-endothelialised (D) tissues under resting tension, in the presence and absence of Hb (10 μM). The purity of the Hb was checked spectrophotometrically.

Hb had a significant effect on the responses of I tissues, to all the above agents (see table). In all cases, the effects of Hb mimicked the effects of endothelial cell removal, the C-E curves with Hb present being shifted significantly to the left of those for control preparations. These results confirm the role of Hb as an inhibitor of EDRF action. In some cases, an effect of Hb on D preparations was seen, the responses to BK and AII in particular being enhanced in the presence of Hb.

Alone, Hb failed to produce concentration-dependent contractions of I or D tissues under resting tension although occasional, irregular contractions were observed with this compound. When tissues were put under sustained tension, however, Hb produced a concentration-dependent increase in tone of I but not D tissues.

	Intact				De-endothelialised			
	EC50 μM		Max gT pull		EC50 μM		Max gT pull	
	Control	+ Hb	Control	+ Hb	Control	+ Hb	Control	+ Hb
AII	n.d.	n.d.	0.09±0.01	0.73±0.02*	n.d.	n.d.	0.25±0.04	0.78±0.13*
BK	n.d.	n.d.	0.17±0.01	0.84±0.11*	n.d.	n.d.	0.45±0.18	0.77±0.13
5-HT	7.82±2.44	2.47 ±0.51	1.52±0.19	2.00±0.27	2.42±0.34	0.78±0.45+	1.81±0.14	1.48±0.19
ACh	n.d.	n.d.	0	0.84±0.20*	n.d.	n.d.	0.44±0.16	0.78±0.19
PhE	0.15±0.03	0.008±0.001+	1.29±0.10	1.57±0.04	0.020±0.004	0.02±0.01	1.70±0.11	1.22±0.02*

Table: n.d. = Value could not be determined, as a true max not achieved. * P<0.01; + P<0.05; n=4-8.

In conclusion, the endothelium clearly modulates the responses of the in vitro rat aorta, the effects of which can be blocked by Hb. Basal EDRF release, which is believed to be considerable in this tissue (Martin et al 1986) may be solely responsible for the effects seen. The increase in PhE tone seen when Hb is applied to tissues under sustained tension may be indicative of this. Finally, an additional action of Hb may be apparent, as reflected by its action on BK and AII responses on D tissues.

H.W. is an SERC CASE student with ICI plc.

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METABOLISM OF THE PYRONE, MALTOL, DURING INTESTINAL ABSORPTION OF AN IRON-MALTOL COMPLEX IN THE RAT

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Maltol (3-hydroxy-2-methyl-4-pyrone), by forming a neutral 1:3 complex with iron (1:3, maltol:Fe^{3+}), is able to enhance iron absorption across the rat small intestine in vivo (Barrand et al, 1987). Since the characteristics of uptake of ^{59}Fe and of ^{3}H-maltol when given as the complex in vitro appear to be similar, the maltol may not only hold iron in soluble form but may also carry the metal into the mucosa (Barrand et al, 1986). However, if all regulatory uptake processes within the intestine were to be by-passed by this complex, there would be a risk of iron overload. In order to examine such a possibility, an attempt has been made to determine the point at which iron and maltol dissociate and the subsequent fate of the ligand itself.

Immediate dissociation of the complex was observed when ^{59}Fe:^{3}H-maltol was given i.v. to rats in doses up to 500ug Fe/kg body weight. The half-life of disappearance of ^{59}Fe from the plasma was 133 min while that of the ^{3}H-label was only 10 min with most appearing in the urine. When similar doses were given into the intestinal lumen, ^{59}Fe levels in the blood rose slowly, reaching a plateau after 1 h while ^{3}H levels rose within minutes and fell over 1 h.

The nature of this ^{3}H was investigated by subjecting samples of plasma, urine and gut washings to gel filtration on Sephadex G-25 columns. A shift in the radioactive peak towards the void volume indicated that ^{3}H was associated with a molecule somewhat larger than the original maltol (126 Mr). Subsequent chromatography on thin layer cellulose showed the ^{3}H metabolite to be more hydrophilic than maltol. A ^{3}H metabolite with the same characteristics was identified in the medium and in homogenates taken from isolated fragments of rat small intestine previously incubated for 30 min with 0.02 to 1mM Fe:^{3}H-maltol or with ^{3}H-maltol alone. Both sulphate and glucuronide conjugates of maltol have been isolated from the urine of dogs given the pyrone either i.v. or orally (Rennhard, 1971). The identity of the ^{3}H metabolite formed in the rat intestine was explored further by incubating the pooled fractions from the gel column for up to 2 h with or without β-glucuronidase in 0.1M Na acetate at pH 5.5 containing 80uM p-nitrophenyl sulphate to inhibit any sulphatase activity. Subsequent thin layer chromatography showed the gradual disappearance of ^{3}H metabolite and reappearance of the ^{3}H-maltol with increasing time of incubation with the enzyme.

Kinetic constants for uptake/formation of this ^{3}H-maltol conjugate of the order of 0.5mM for Km and 0.2 nmoles $min^{-1}mg^{-1}$protein for Vmax were obtained in isolated intestinal fragments. As yet no detectable metabolism of maltol has been found in intestinal homogenates or microsomes although activity towards other phenols still occurs in these preparations. Since conjugation of maltol takes place on the same part of the molecule that complexes Fe^{3+}, it is possible that such metabolism may be involved with dissociating metal from ligand and so modifying the way in which iron is carried across the intestine.

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THE EFFECT OF LITHIUM ON SUPEROXIDE GENERATION AND INOSITOL PHOSPHATE PRODUCTION IN HUMAN NEUTROPHILS AND HL60 CELLS

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Activation of neutrophils by a variety of stimuli, including the chemotactic peptide formylmethionylleucylphenylalanine (FMLP), is brought about by the generation of 2 second messengers, Ins 1,4,5P_3 and 1,2-diacylglycerol from phospholipase C mediated hydrolysis of PtdIns (4,5)P_2. Ins (1,4,5)P_3 releases Ca^{2+} from intracellular stores whilst 1,2-diacylglycerol activates protein kinase C. Together these bring about a number of neutrophil responses such as superoxide (O_2^-) generation, enzyme release, chemotaxis and phagocytosis. Recently it has been reported that Li^+ over the concentration range 1-10mM can inhibit FMLP stimulated O_2^-generation (1). Li^+ is used as an effective treatment in manic illness and it has been suggested that the mechanism by which Li^+ exerts its effect is via an inhibition of inositol 1-phosphatase and thus an alteration in the inositol lipid cycle (2). In order to investigate the nature of the inhibition of O_2^- release by Li^+ and a possible relationship to inhibition of inositol 1-phosphatase we have studied the effect of Li^+ on FMLP and phorbol myristate acetate (PMA) stimulated O_2^- generation in human neutrophils and on FMLP stimulated inositol phosphate production in differentiated human promyelocytic leukaemia HL60 cells, a convenient model of the neutrophil.

FMLP stimulated O_2^- generation was dose related with half-maximal stimulation occuring at 1.6×10^{-8}M. Pretreatment of neutrophils with LiCl led to a dose related inhibition of FMLP (10^{-7}M) stimulated O_2^- production. Half maximal inhibition occurred at 30mM LiCl. In contrast PMA stimulated superoxide generation, which is believed to occur via direct activation of C-kinase, was not inhibited by pretreatment with LiCl. Concentrations of 50mM LiCl and above appeared to potentiate the PMA response.

In HL60 cells prelabelled for 48 hours with [^{3}H] inositol preincubation with 20mM LiCl increased FMLP stimulated IP_1 levels within 1 minute of stimulation. IP_1 levels reached a plateau at 5 minutes suggesting that no further production of inositol phosphates occurs after this time, possibly as a result of receptor desensitization. Half maximal IP_1 accumulation was observed at about 9mM LiCl. Thus, lithium at millimolar concentrations appears to inhibit inositol-1-phosphatase in HL60 cells leading to accumulation of IP_1 following receptor stimulation.

These data suggest that lithium may inhibit FMLP stimulated superoxide generation by interfering with one of the reactions occurring between receptor activation and C-kinase activation. Whether this occurs via inhibition of inositol 1-phosphatase resulting in an alteration in the inositol lipid cycle requires further investigation.

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TISSUE EXPANDER CAPSULE - AN 'IN VITRO' MODEL FOR THE STUDY OF HUMAN MYOFIBROBLAST CONTRACTILITY

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Tissue expansion is a new technique entailing subcutaneous implantation of a silastic reservoir which is progressively inflated over several weeks by percutaneous injection of saline (Radovan, 1984). This provides a skin flap to match tissue deficiencies. However, during the period of inflation a fibrous capsule composed of collagen, fibroblasts and myofibroblasts develops around the tissue expander (Pasyk et al, 1982). This capsule may limit the rate of expansion and modify the shape, size and texture of the flap (Kissin and Kark, 1984). The contractility of the capsule is attributed to the activity of myofibroblasts and similar tissue from around mammary implants when used in vitro contracted to agonists such as histamine, angiotensin and relaxed with papaverine (Baker et al, 1981). Although papaverine has also been used in one in vivo study (Lee et al, 1985) to antagonise myofibroblast contractility the development of more selective inhibitors requires more details as to the pharmacology of the cell. This communication reports on the pharmacology and histology of human tissue expander myofibroblasts.

Fibrous capsule samples were obtained during the removal of tissue expanders and were prepared for (a) electron microscopy (1mm cubes), and (b) in vitro studies (36.9±2mm strips, weighing 287±53mg, n = 26) and arranged for superfusion. After equilibration (1hr) the following drugs were examined: $BaCl_2$ (1-8mg), mepyramine (0.1-8mg), 5-HT (100-800µg) and papaverine (10-50µM).

In all the preparations tested $BaCl_2$ (1-8mg) produced neither a contraction or a relaxation of the strips. Mepyramine (0.1-8mg) contracted 24 out of 26 preparations in a dose dependent reversible manner. 5-HT produced variable responses in the preparations examined (n = 18) 13 of which were insensitive whilst 5 contracted, but tachyphylaxis occurred. Papaverine (10-50µM) antagonised the magnitude of mepyramine responses in the strips tested (n = 6) but had no effect on baseline tension. Electron microscopy of the strips examined revealed the presence of myofibroblasts and absence of smooth muscle cells.

Since tissue expander capsules were not affected by $BaCl_2$ this suggests (a) absence of smooth muscle cells and (b) myofibroblasts are insensitive to $BaCl_2$ a finding previously reported for croton oil induced myofibroblasts (Gabbiani et al, 1972). The variable sensitivity of this preparation to 5-HT differs from other reports which describe contractile properties of myofibroblasts to 5-HT (Gabbiani et al, 1972). Since human myofibroblasts contract to mepyramine and are antagonised by papaverine this indicates a functional similarity to rat testicular capsule myofibroblasts (Lal and Naylor, 1986). Electron microscopy shows a possible correlation between the presence of myofibrolasts and in vitro sensitivity to mepyramine. Therefore tissue expander capsule may be used as a suitable in vitro model for investigation of human myofibroblast sensitivities.

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LACK OF SPECIFIC EFFECT OF BENZODIAZEPINES ON GABA-INDUCED CONTRACTIONS IN GUINEA-PIG ILEUM

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Application of γ-aminobutyric acid (GABA) to ileum elicits excitatory and inhibitory effects (Krantis et al, 1980). Recently Luzzi et al (1986) reported that diazepam at low concentrations enhanced the excitatory responses of the longitudinal muscle of the ileum to GABA and thus postulated the existence of GABA-coupled benzodiazepine (BDZ) receptors in the myenteric plexus. We were interested to repeat their studies and extend them using other BDZs.

Longitudinal muscle-myenteric plexus preparations were obtained from guinea-pig mid-ileum and suspended in Krebs' solution at 37°C (gassed with 95% O_2, 5% CO_2). Changes in muscle tension were monitored. GABA (5 μM - 5 mM), administered to the preparation at 20 min intervals, produced transient, concentration-related increases in tension, of rapid onset, which were not subject to tachyphylaxis. These contractions were occasionally followed by small relaxations. The contractions were abolished by atropine (0.3 μM) or tetrodotoxin (0.1 μM) and significantly reduced by bicuculline (10 μM, $n \geq 8$ in each experiment).

Initial observations revealed that, whilst maximal contractions in response to a high concentration of GABA (1 mM) were reproducible, submaximal contractions elicited by lower concentrations of GABA increased significantly in magnitude upon successive applications of GABA. For instance, from first to fifth exposures to GABA (5 μM), the submaximal contractions increased from 0.6 ± 0.1g to 1.3 ± 0.1g (n = 18), responses to subsequent exposures being comparable with this latter contraction in control experiments. Subsequent experiments were thus performed following adequate initial exposure of the tissue to GABA.

Effects of four BDZs were examined on GABA-induced contractions. Each tissue was used to study the influence of one BDZ on responses to one concentration of GABA only. Chlordiazepoxide HCl, flurazepam HCl and midazolam maleate were dissolved in distilled water and diazepam was dissolved in 0.1 N HCl and diluted at least 1:1000 in Krebs' solution (to pH 7.4) before addition to the organ bath. The BDZs were administered 1 or 20 min before administration of GABA and the magnitude of the GABA response was compared with that of the immediately preceding control response. The BDZs (1 nM - 0.1 μM) produced no direct effect on the tissue and did not significantly affect the subsequent submaximal or maximal responses to any concentration of GABA tested (n = 36 for each BDZ).

Increasing concentrations (1 μM - 0.1 mM) of all BDZs produced significant reductions in responses to all concentrations of GABA, contractions being abolished by 0.1 mM BDZ after 20 min incubation (n = 18). The BDZ-induced inhibition could be reversed by washing. Submaximal contractile responses to acetylcholine (40 nM), histamine (0.1 μM), K^+ (10 mM), Ca^{2+} (2-4 mM) and field stimulation (supramax voltage, 0.1 Hz, 0.2 ms) were likewise reduced or abolished by these higher BDZ concentrations ($n \geq 6$ for each stimulant).

Thus, our studies using four BDZs in aqueous solution provide no support for a specific BDZ-GABA interaction in the guinea-pig ileum although we confirm the previously reported (Hullihan et al 1983) depressant activity of BDZs on a range of stimulants.

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5-HYDROXYTRYPTAMINE RECEPTORS IN HUMAN UMBILICAL ARTERY: FUNCTIONAL AND BINDING STUDIES

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5-Hydroxytryptamine (5-HT) is a potent agonist in the human umbilical artery (HUA): at the physiological oxygen tension (pO_2=15mmHg) the contraction is mediated via 5-HT_2 receptors and at higher pO_2 (120mmHg) contraction is via both "5-HT_1-like" and 5-HT_2 receptors (MacLennan & McGrath, 1986).

Lysergic acid diethylamide (LSD) is a non-selective 5-HT ligand (Peroutka & Snyder 1979) which labels both 5-HT_1 & 5-HT_2 sites in the rat cortex, 5-HT_2 sites only on human platelets and a "5-HT_1-like" site in guinea-pig ileum. We used [^{125}I]LSD as a radioligand to study the binding characterisation of the HUA. The arteries were grossly cleaned of fatty adventitious tissue and then homogenised in 20ml of ice cold 0.25 M sucrose. Membranes were prepared from an initial centrifugation at 15000 gav for 10 min at 4°C and the supernatant recentrifuged at 60000 gav for 30 min at 4°C. The assays were performed essentially as described by Engel et al (1984) using 2μM methysergide to define non-specific binding.

Affinity (pA_2) values for the antagonists ketanserin, methysergide and phentolamine against 5-HT-mediated contraction at pO_2=15mmHg were estimated as described previously (McGrath et al, 1985). Functional antagonism by buspirone ($1x10^{-7}$M) and (±)pindolol ($3x10^{-6}$M) against the two 5-HT receptors were assessed against the contractile response to 5-carboxamidotryptamine (5-CT) at high pO_2: low concentrations of 5-CT ($1x10^{-10}$ - $3x10^{-8}$M) contract the HUA via "5-HT_1-like" receptors and higher concentrations ($3x10^{-8}$ - $1x10^{-5}$M) at 5-HT_2 receptors (MacLennan & McGrath, 1986). Under these conditions neither buspirone nor pindolol antagonised either phase of the response to 5-CT.

[^{125}I]LSD was found to bind to a saturable population of high affinity sites (Kd 0.4nM), which was characteristic of the 5-HT_2 site showing high affinity for the 5-HT_2 antagonists and low affinity for the 5-HT_{1A} ligand buspirone and the 5-HT_{1B} ligand pindolol.

Antagonist	Agonist	pA_2	n	pKi	n
Ketanserin	5-HT	8.92 (8.70-9.14)	6	8.88 ± 0.06	4
Methysergide	5-HT	8.52 (8.32-8.72)	5	8.35 ± 0.10	3
Phentolamine	5-HT	6.37 (5.88-6.86)	6	6.25 ± 0.05	4
Buspirone	5-CT	<7.00	5	6.05 ± 0.11	3
Pindolol	5-CT	<5.52	5	<5.00	3

Values are the mean (95% confidence limits) or mean + s.e.mean

The affinity values obtained from binding studies for the 3 effective antagonists were in good agreement with the pA_2 values for functional antagonism of receptors that we have previously defined as 5-HT_2. We have, so far, no positive evidence from the binding studies for the "5-HT_1-like" receptor which mediates contraction under high pO_2 conditions. The methods employed here might therefore be useful for further characterization and study of human vascular 5-HT_2 receptors.

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EFFECTS OF TACHYKININS ON THE SECRETION OF GROWTH HORMONE AND PROLACTIN

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Substance P (SP) has been reported to cause an increased in vitro secretion of prolactin but not growth hormone, gonadotropins or thyrotropin from anterior pituitaries of ovariectomised rats (Vijayan and McCann, 1979; 1980). However, in vivo results with intravenous SP administration in male rats show a rapid increase in growth hormone secretion (Kato et al, 1976).

We have investigated the effects of several tachykinins (that are relatively selective for receptor subtypes NK-1,-2,-3; Iversen et al., 1987) on secretion of prolactin and growth hormone from male pituitary tissue in vitro. Anterior pituitary glands from Wistar rats were sliced into 500μm prisms and incubated for 1 hour at 37°C in Krebs bicarbonate medium pH7.4 containing 0.1% BSA, 2g/l glucose and 30 mg/l bacitracin. Tissue was then continuously superfused at 0.5 ml/min and 2 min fractions were collected. Hormones were measured by double antibody radioimmunoassay. After 90 min baseline, drugs were added.

[Met-OMe^{11}]-SP (0.01-10μM) caused no significant change in either prolactin or growth hormone secretion. [Met-OH^{11}]-SP was also inactive. Neurokinin B caused a marginal (27 ± 7%, n = 3) increase in prolactin secretion only at 10μM, with no apparent effect on growth hormone. Neurokinin A however, caused a concentration-dependent increase in growth hormone output (0.1-10μM) with a peak increase of 162 ± 34%, (n = 5, $p < 0.05$, Mann Whitney U-test) at 10μM. Secretion declined steadily after the initial sharp peak. Only a minor effect (+ 29 ± 8%, n = 3) was apparent on prolactin secretion, at the same concentration.

The results are consistent with the idea that a tachykinin receptor, selective for neurokinin A (NK-2) regulates secretion from male somatotrophes. It also appears that there may be sex/steroid-dependent differences in tachykinin action in the anterior pituitary.

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PHENOTYPIC ASSOCIATION BETWEEN THE EFFECTS OF MPTP TREATMENT AND DEBRISOQUINE HYDROXYLATION IN THE RAT

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MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a potent and selective neurotoxin at dopaminergic neurones of the substantia nigra in primates but not in the rat, producing classical parkinsonian signs. Polymorphic 4-hydroxylation of debrisoquine is reported to be partially or totally defective in many patients with Parkinson's disease compared to normal control subjects[1]. Most laboratory rat strains demonstrate high debrisoquine 4-hydroxylation activity, with the exception of the females of the DA (Dark Agouti) strain. We have therefore compared the effects of MPTP treatment in female DA rats (phenotypically poor metabolisers) and in female Lewis strain rats, which are phenotypically extensive metabolisers of debrisoquine.

Female DA or Lewis rats (200 g) received MPTP (10 mg/kg or 30 mg/kg) or saline i.p. once daily for 5 days. An additional group of each strain received a single injection of 60 mg/kg i.p. MPTP. Behavioural effects of MPTP were studied during this period of treatment and for 17 days after the last dose. Animals were then killed, the brains removed and the striatum isolated. The brain tissue was homogenised in 40 vol 0.1M perchloric acid, 0.4 mM sodium metabisulphite and centrifuged to remove the protein. Tissue content of dopamine, noradrenaline and 5-HT was determined by reverse-phase HPLC utilising electrochemical detection.

Debrisoquine 4-hydroxylation activity was previously determined in each rat following oral administration of debrisoquine sulphate (5 mg/kg). All Lewis rats proved to be extensive metabolisers of debrisoquine and all DA rats were poor metabolisers.

	Noradrenaline	Dopamine	5-HT
Dark Agouti			
Saline	0.69 ± 0.34	51.1 ± 3.8	3.58 ± 0.33
MPTP (10 mg/kg)	48.59 ± 1.96	46.0 ± 2.9	< 0.25
Lewis			
Saline	0.80 ± 0.19	49.0 ± 1.7	3.09 ± 0.15
MPTP (10 mg/kg)	15.10 ± 2.44	41.2 ± 3.0	1.92 ± 0.18

The table above summarizes the effects of MPTP (10 mg/kg) on striatal monoamine levels expressed as nmol/g wet wt; mean ± sem, n = 5 animals per group. Two-way ANOVA identified a significant effect of MPTP on all three monoamines ($p < 0.05$, F-test). A significant interaction between rat strain and MPTP treatment was observed for noradrenaline and 5-HT but not for dopamine. Following MPTP treatment at 30 or 60 mg/kg none of the DA strain rats survived to the end of the observation period whereas only 3/5 Lewis rats receiving 60 mg/kg died within this time, indicating significantly higher toxicity of MPTP in the DA strain of rats ($p < 0.01$, chi-square test).

We conclude that MPTP treatment induces significant changes in rat striatal monoamine levels. In the case of dopamine the decrease is small and is not associated with the strain of rat. For noradrenaline and 5-HT, however, significant interactions were observed, demonstrating greater effects in the DA than in the Lewis rats. Further studies may indicate whether the enzyme responsible for 4-hydroxylation of debrisoquine plays an important role in the metabolic activation of MPTP.

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STEREOSELECTIVE MODULATION OF THE $GABA_A$ RECEPTOR BY PREGNANE STEROIDS

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It has been proposed that some endogenous steroid hormones and their metabolites may act as modulators of the $GABA_A$ receptor-channel complex (Simmonds et al, 1984; Majewska et al, 1986; Callachan et al, 1987). One such steroid, the progesterone metabolite 5β-pregnan-3α-ol-20-one, potentiates responses to GABA recorded from slices of rat cuneate nucleus (Turner, 1986), and enhances the amplitude and duration of chloride currents activated by GABA on bovine chromaffin cells (Callachan et al, 1987). The present study concerns the stereoselectivity of this effect determined by the use of electrophysiological and radioligand binding techniques.

Transmembrane currents activated by locally applied GABA (100 μM) were recorded under voltage-clamp from bovine adrenomedullary chromaffin cells maintained in cell culture for 1 to 7 days. The binding of the $GABA_A$ receptor ligand [^{3}H] muscimol to a crude preparation of synaptosomal membranes derived from pig cortex (Kirkness & Turner, 1986) was performed by a rapid filtration assay.

Over the concentration range 30-100 nM, both 5β-pregnan-3α-ol-20-one and 5α-pregnan-3α-ol-20-one dose dependently enhanced the amplitude of membrane currents elicited by GABA (Table 1). GABA-induced currents were little affected by either 5β-pregnan-3β-ol-20-one or 5α-pregnan-3β-ol-20-one at 30 nM in the medium, but a modest degree of potentiation was observed when these compounds were applied at 1-10 μM (Table 1).

Table 1: Potentiation of GABA-evoked currents by pregnane steroids

Steroid	Concentration (uM)	Amplitude of GABA-evoked current as a percentage of control (mean ± S.E.; n)
5β-Pregnan-3α-ol-20-one	0.03	197.8 ± 14.4 (5)
	0.10	243.4 ± 26.5 (5)
5α-Pregnan-3α-ol-20-one	0.03	207.8 ± 15.3 (7)
	0.10	289.9 ± 36.4 (6)
5β-Pregnan-3β-ol-20-one	1.00	108.8 ± 7.2 (4)
	10.00	119.0 ± 6.3 (4)
5α-Pregnan-3β-ol-20-one	1.00	102.7 ± 2.4 (4)
	10.00	110.6 ± 8.7 (3)

A similar stereoselectivity was observed in the ligand binding studies since only the 3α-ol-pregnane isomers were capable of stimulating the binding of [^{3}H] muscimol substantially. In triplicate replicates, 5β-pregnan-3α-ol-20-one and its 5α-isomer maximally enhanced binding by approximately 55% at 100 μM, whereas the 3β-ol isomers produced only a 10-15% stimulation at the same concentration. Scatchard analysis of the binding data, with [^{3}H] muscimol concentrations ranging from 1 to 100 nM, suggested that the 3α-ol-pregnane isomers (each at 3 μM) had little effect on the affinity of muscimol binding, but increased by 30% the apparent total number of high affinity binding sites in the membrane.

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ANTAGONISTS OF BRADYKININ-INDUCED CHLORIDE SECRETION AND CONCOMITANT MORPHOLOGICAL CHANGE

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Recently, certain synthetic bradykinin (BK) analogs have been shown to have antagonistic effects on BK-induced smooth muscle contraction and changes in blood pressure (Vavrek and Stewart, 1985; Regoli et al., 1986). Epithelial chloride secretion in intestine and trachea (Cuthbert and Margolius, 1982; Manning et al., 1982; Leikauf et al., 1985) and bicarbonate secretion in gallbladder (Baird and Margolius, 1987) are also responses to BK, and we have begun to characterize the effects of these new antagonists on BK-induced chloride secretion in the rat colon.

Segments of rat colonic mucosa were mounted in a Ussing chamber (Cuthbert and Margolius, 1982). A BK analog (1×10^{-6} M) was added to the tissue bath on the serosal side followed 60 sec later by BK (1×10^{-8} M). Changes in short circuit current (SCC), known to be due to enhanced net chloride secretion, were recorded and compared to controls which received only BK (Table 1).

Table 1 Effect of BK analogs on BK-stimulated SCC

BK analog	ΔSCC (μA/0.6 cm²) BK	BK + BK analog
Lys-Lys-[Hyp³, Thi⁵,⁸, D-Phe⁷]-BK	58.7 ± 8.6	20.9± 4.6 (n=8, p<0.002)
D-Arg-[Hyp³, Thi⁵,⁸, D-Phe⁷]-BK	59.9 ± 10.2	31.4±11.1 (n=7, NS)
[Thi⁵,⁸, D-Phe⁷]-BK	31.4 ± 8.5	26.2± 9.5 (n=9, NS)
[Hyp³, Thi⁵,⁸, D-Phe⁷]-BK	40.6 ± 7.0	53.6±15.6 (n=5, NS)

Thi=thienylalanine; values are means ± SEM.

BK also induces a rapid change in the morphology of the colonic mucosa concurrent with chloride secretion (Baron et al., 1986). We have asked whether this response can be antagonized with the BK analogs. Higher concentrations of BK and the BK analog available were used (3×10^{-8} M and 3×10^{-6} M respectively) and the tissues fixed in the Ussing chamber 10 min after addition of BK by adding glutaraldehyde to each bath. Tissues were prepared for morphological evaluation, and the results show that attenuation of BK-induced changes in chloride secretion by at least one of the analogs is accompanied by attenuation of the concurrent morphological change (Table 2).

Table 2 Parallel effects of a BK analog on SCC and morphology

	ΔSCC (μA/0.6 cm²)	Mean epithelial thickness(μM)
BK	86.4 ± 11.2 (n=8)	206.5 ± 10.0
D-Arg-[Hyp³, Thi⁵,⁸, D-Phe⁷]-BK	*27.7 ± 9.9 (n=8)	*266.0 ± 16.5
Control (no additions)	-	*291.0 ± 7.5

*p<0.01 compared to BK

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INHIBITION OF CRF STIMULATED ACTH RELEASE FROM RAT ANTERIOR PITUITARY CELLS BY MEPACRINE

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Rat anterior pituitary cells grown in primary culture provide a useful model for studying the release of adrenocorticotropic hormone (ACTH) in vitro.

Briefly the method for preparing the cell culture was as follows. Following the digestion of rat anterior pituitary with pancreatin and collagenase, cells were centrifuged and resuspended in Dulbeccos Modified Eagles Medium (DMEM) containing 20% charcoal stripped sera, 1% non-essential amino acids (Gibco), 4mM L-glutamine, 100 U penicillin/ml and 10µg gentamycin/ml, at a density of approximately 10^5 cells/ml. The cell suspension was added at 200µl/well to 96 well culture plates prior to incubation at 37°C in 95% air 5% CO_2 in a humidified incubator. Cells were incubated for 3 to 5 days by which time they had adhered to the culture plate and grown to confluence.

Prior to use for secretion studies, the cells were washed 3 times with DMEM containing no sera but with added aprotinin (250KIU/ml) and ascorbate (10^{-4}M). All further incubations were performed in this medium at 37°C. Following a 1 hour preincubation period, 10uM rat corticotropin releasing factor (CRF) (Sigma) produced a time related increase in ACTH secretion over basal levels as measured by RIA. Maximum stimulation of 100% over basal ($n = 8$ $p<0.005$) was found following 2 hours exposure to CRF. The effect of CRF on ACTH secretion after 2 hours exposure was dose related, significant stimulation being present with 10^{-10}M CRF (37% over basal. $n = 8$; $p<0.05$).

The ability of glucocorticoids to inhibit ACTH secretion in vitro is well documented. In this in vitro system prednisolone and hydrocortisone both inhibit the secretion of ACTH stimulated by 10^{-8}M CRF in a dose related manner with IC_{50} values of 10^{-7}M and 1.2×10^{-5} M respectively.

Mepacrine has been shown to be an inhibitor of the enzyme phospholipase A_2 (PLA_2) (Vigo et al, 1980). Mepacrine was also found to inhibit the ACTH secretory response to CRF (10^{-8}M) in a dose dependent manner with an IC_{50} of 3×10^{-5}M.

Glucocorticoids have been reported to exert their effects on ACTH release both by an inhibition of synthesis of ACTH and also by inhibiting release of ACTH (Buckingham 1982). It has previously been suggested that the enzyme PLA_2 may be involved in the release of ACTH since melittin, an activator of PLA_2, stimulates ACTH secretion (Heisler et al, 1982; Axelrod and Reisine, 1984). We now present evidence in favour of this hypothesis with the observation that mepacrine, an inhibitor of PLA_2 activity, inhibits ACTH secretion from anterior pituitary cells.

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POSSIBLE INVOLVEMENT OF ARACHIDONIC ACID METABOLITES IN THE REGULATION OF CORTICOTROPHIN SECRETION

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Observations that various arachidonic acid metabolites influence the secretion of corticotrophin (ACTH) by the rat adenohypophysis in vivo (Hedge, 1977) and in vitro (Vlaskovska, Hertting & Knepel, 1984) have raised the possibility that eicosanoids are involved in the intracellular mechanisms effecting the release of the pituitary hormone. Accordingly, the effects of drugs which modify the generation of these substances on the resting and hypothalamic extract (HE)-induced secretion of ACTH by pituitary cells in vitro are being studied.

Anterior pituitary glands were removed post-mortem from adult male Sprague-Dawley rats, some of which had been adrenalectomized, under pentobarbitone sodium anaesthesia, 7-10 days previously. The cells were dispersed by collagenase (1 mg/ml) and mechanical agitation, washed and suspended in oxygenated (95% O_2 / 5% CO_2) Earle's balanced salts medium, pH 7.4. Aliquots of the cell suspension were pre-incubated for 30 - 120 min at 37°C. After centrifugation and resuspension, the cells were incubated for a further 15 - 60 min in the presence of appropriate secretagogues. The ACTH released into the incubation medium was determined by radioimmunoassay. Where appropriate, antagonists were present in the medium during the pre-incubation period.

Hypothalamic extracts (0.125 - 0.25 HE/ml) and phospholipase A_2 (62.5 - 250 U/ml) stimulated, in a dose-dependent manner, the secretion of immunoreactive ACTH by pituitary cells from intact rats and, at the lowest concentrations, both produced a two-fold increase in ACTH release after 60 min contact with the tissue. Neither mepacrine (10^{-3}M), an inhibitor of phospholipase A_2, nor indomethacin (10^{-6} - 10^{-4}M) influenced the resting secretion of ACTH. However, mepacrine (10^{-3}M) reduced, by approximately 80%, the secretory response to a submaximal dose of hypothalamic extracts (0.25 HE/ml) although indomethacin (10^{-6} - 10^{-4}M) was ineffective in this respect. Hypothalamic extracts (0.05 - 0.4 HE/ml) also stimulated ($P < 0.001$) the secretion of ACTH by cells derived from adrenalectomized rats and, in these cells, a concentration of only 0.05 HE/ml was required to initiate, within 60 min, a two-fold increase in ACTH release. The actions of hypothalamic extracts were mimicked by arachidonic acid (10^{-3}M) which produced a 3 fold increase in corticotrophin release. The responses to both secretagogues were time-dependent and apparent within 15 min ($P < 0.05$) of the onset of stimulation. Addition of dexamethasone (10^{-9} - 10^{-5}M) to the medium 2.0h before stimulation with hypothalamic extracts (0.1 HE/ml) reduced markedly ($P < 0.01$) the secretory responses of cells from adrenalectomized rats. Maximal inhibition (75%) was attained with concentrations of the steroid of 10^{-7}M and above.

The results indicate that products released by phospholipase A_2 facilitate the release of ACTH and that these compounds, which do not appear to be generated by the cyclo-oxygenase pathway, may play an important role in effecting the neurochemically stimulated release of the pituitary hormone.

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THE DISPOSITION OF 3-[^{3}H]PRIMAQUINE IN THE ANAESTHETISED RAT

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Primaquine (PQ) has been used in the treatment of the tissue stages of human malaria for over 30 years. Despite extensive clinical use, little is known about the mechanism(s) responsible for either its toxic and antimalarial actions. Current theory (Howells, 1985) suggests that PQ undergoes hepatic metabolism to form free radicals that mediate the cytotoxic effects of the drug. In the present study the disposition of quinoline 3-[^{3}H]PQ has been investigated in order to assess: (a) the hepatic sequestration of PQ, and (b) the capacity of PQ to generate free radicals *in vivo*, as indicated by irreversible binding of 3-[^{3}H]radioactivity to liver proteins (Maggs *et al*, 1983).

Male Wistar rats (mean weight 465g) were anaesthetised with sodium pentobarbitone (60mg kg^{-1}). Quinoline 3-[^{3}H]PQ diphosphate in saline (equivalent to 1.56, 3.13 and 6.25mg kg^{-1} PQ base) containing a 2.2μCi radiolabelled tracer was infused via the jugular vein. Serial blood (500μl) and hourly bile samples were collected over five hours in order to determine 3-[^{3}H]radioactive content. At the conclusion of the experiment various soft organs were removed and the tissue distribution, the sub-cellular localisation and extent of irreversible binding of the radiolabel to liver proteins was determined.

There were no significant differences in the plasma pharmacokinetics and tissue disposition of radiolabelled drug at the three doses used in this study. Radiolabelled PQ was found to accumulate in soft tissues, principally the liver (24.4 ± 0.6%, $\bar{x}$ ± S.E.M.) and the kidneys (10.5 ± 0.5%). Of the radioactivity that accumulated in the liver the largest percentage dose/sub-cellular fraction was located in the lipid rich 10,000g pellet (12.9 ± 1.1%) followed by the 10,000g and 105,000g supernatants (6.7 ± 0.3% and 5.4 ± 0.4% respectively). However, the microsomal fraction contained the highest concentration of radioactivity when expressed per mg. protein. Determination of the extent of irreversible binding of radioactivity to liver proteins indicated that one tenth of the radiolabel in each fraction was irreversibly bound to protein and that there was an apparent dose-dependent increase in binding.

It would appear from these results that PQ is avidly taken up into the liver where it undergoes transformation to species that have the capacity to irreversibly bind to proteins *in vivo*.

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STEREOSELECTIVE FORMATION OF THE CARBOXYLIC ACID METABOLITE OF PRIMAQUINE BY THE ISOLATED PERFUSED RAT LIVER

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Primaquine (PQ) contains an asymmetric carbon atom in the alkylamino side chain and can, therefore exist as either the (+) or (-) enantiomer. Recent work in the isolated perfused rat liver preparation suggests a stereoselective difference in the rate of elimination of PQ at a dose of 2.5mg of each enantiomer. A carboxylic acid (PQm) has been identified as the major plasma metabolite in man (Mihaly et al. 1984). This study aims to investigate further the stereoselective disposition of PQ with particular reference to the formation and elimination of PQm.

Male Wistar rats (200-250g) were anaesthetized with sodium pentobarbitone (60mg kg^{-1}). Isolated livers were perfused in a constant flow (15ml min^{-1}) recirculating system at 37°C. The disposition of PQ was studied over 4 hours following a bolus dose of (+) PQ diphosphate (n = 6, 2.0mg) or (-) PQ diphosphate (n = 6, 2.0mg) administered as an aqueous solution (0.2ml) to the perfusate reservoir. Samples of perfusate were removed from the reservoir pre-dose and at 5, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes post-dose with the volume removed replaced by fresh perfusate. Bile was collected at hourly intervals. Perfusate plasma was removed following centrifugation (1,000g; 1 min). All samples were stored at -20°C until analysis of PQ and PQm by HPLC using methods modified from Ward et al. (1985). Statistical analysis was by Student's non-paired t-test ($P < 0.05$).

Clearance of (-) PQ (8.8 ± 2.9ml min^{-1}; mean ± SD) was significantly greater than (+) PQ (5.5 ± 1.5ml min^{-1}). Volume of distribution of (-) PQ (606 ± 182ml) was significantly less than (+) PQ (930 ± 171ml). Terminal half-life (54 ± 29 min) and area under the curve, $AUC_{0-\infty}$, (254 ± 96μg ml^{-1} min) for (-) PQ were significantly less than the corresponding values (123 ± 33 min and 387 ± 108μg ml^{-1} min) for (+) PQ. Significantly greater peak plasma concentrations of PQm were achieved following (-) PQ (0.61 ± 0.26μg ml^{-1}) compared with (+) PQ (0.19 ± 0.09μg ml^{-1}). PQ and PQm were excreted into bile. While there was no significant difference in the amount excreted as either (+) PQ (1.1 ± 0.3% dose) or (-) PQ (1.5 ± 0.8% dose) the quantity of PQm recovered was significantly greater after (-) PQ (1.2 ± 0.6%) than (+) PQ (0.3 ± 0.1%). However, there were no differences in the biliary clearances of either (+) or (-) PQ (0.06 ± 0.02ml min^{-1} and 0.14 ± 0.10ml min^{-1}) or the respective carboxylic acids (0.24 ± 0.13ml min^{-1} and 0.29 ± 0.04ml min^{-1}).

These data suggest that the hepatic clearance of PQ to its carboxylic acid metabolite is stereoselective. The importance of this observation depends on the relative pharmacological activity of PQ and PQm.

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EFFECTS OF GENTAMICIN ON CALCIUM TRANSPORT IN RAT RENAL BRUSH BORDER MEMBRANE VESICLES

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The aminoglycoside (AG) are widely used in the treatment of gram negative infections. Nephrotoxicity is a dose-limiting feature of gentamicin (G) therapy. We have previously reported on gentamicin-induced nephrotoxicity in cystic fibrosis patients (Godson et al., 1986). The renal pathogenesis of the AG can be attributed to their selective accumulation within renal proximal tubular cells. Reabsorption via the brush border membrane is thought to constitute the dominant route of tubular accumulation (Wiliams et al., 1986). Because the initial event in the renal tubular reabsorption of AG involves binding to the brush border membrane the use of brush border membrane vesicles (BBMV) may provide useful models for studying in vitro the mechanisms of gentamicin-induced nephrotoxicity.

BBMV were prepared by a divalent cation precipitation technique from renal cortex obtained from male Wistar rats (Biber et al., 1981). Purity of the membrane preparations was assessed by marker enzyme assays. Vesicle morphology was examined by electron microscopy. Intravesicular volume was determined using equilibrated Na-dependent D-glucose uptake (Aronson & Sacktor, 1975). The effects of gentamicin on calcium transport by the vesicles were studied using ^{45}Ca tracer and a rapid filtration technique. Calcium efflux was examined after vesicles had been preloaded with ^{45}Ca at 4°C for 60 minutes.

The marker enzymes for BBMV, γ glutamyl transpeptidase and alkaline phosphatase were enriched from crude homogenate 12.7 ± 0.4 and 8.7 ± 0.2 fold respectively. Contamination with basolateral membranes was low as indexed by the relative specific activity of 0.64 ± 0.07 for Na-K-ATP'ase. Contamination with mitochondria and lysosomes was minimal as indexed by relative specific activities of succinate dehydrogenase (0.5 ± 0.02) and β-N-Acetylglucosaminidase (0.11±0.01). Electron microscopy revealed a high yield of sealed vesicles orientated right side out. Gentamicin in the concentration range 25-200 μg/ml reduced the initial rate of calcium uptake by the BBMV in a dose-dependent manner. The V max was significantly ($p<0.01$) reduced from the control value of 9.32±1.09 (nmol mg^{-1} 20 sec^{-1}) to 2.58±0.50 at a gentamicin concentration of 200 μg/ml. The Km of uptake was not affected by gentamicin. Gentamicin did not alter ^{45}Ca efflux from the vesicles.

The isolation procedure used yields pure and viable BBMV suitable for in vitro investigations of gentamicin interactions with brush border components. The inhibitory action of gentamicin on calcium uptake but not efflux suggests a relatively specific interaction of gentamicin with membrane components. Preliminary results from calcium binding experiments indicate that gentamicin decreases calcium binding to a high affinity binding site in the BBMV.

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ELECTRICAL AND MECHANICAL EFFECTS OF ADENOSINE AND ATP IN GUINEA PIG ISOLATED TRACHEALIS

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Adenosine and adenosine 5'-triphosphate (ATP) both relax guinea-pig isolated trachealis (Coleman, 1976) though it has been suggested that the relaxant effects of ATP are attributable to its metabolite, adenosine (Farmer & Farrar, 1976; Christie & Satchell, 1980). We have examined the mechanical and electrical effects of adenosine and ATP in order to (a) elucidate their mechanisms of action, and (b) assess their ability to mimic the non-adrenergic, non-cholinergic (NANC) inhibitory neurotransmitter.

In tissue bath experiments employing isometric tension recording, adenosine (0.01 - 5 mM) and ATP (0.0001 - 5 mM) each caused concentration-dependent suppression of spontaneous tone. The cumulative log concentration-effect curve of ATP was diphasic. The foot of this curve originated at a lower threshold concentration (0.1 - 1 μM) than that of adenosine and had a shallow slope. A point of inflexion occurred at an ATP concentration of 0.1 mM so that the slope of the upper portion of the ATP curve approximated to that of the linear portion of the curve for adenosine. When assessed at a response equivalent to 50% of the aminophylline maximum, ATP and adenosine were equipotent. Tetraethylammonium (8 mM), 4-aminopyridine (5 mM) and procaine (5 mM) which are K^+ channel inhibitors each suppressed the foot of the log concentration effect curve of ATP but did not depress the upper, steeper part of the ATP curve or any part of that of adenosine.

Simultaneous recording of membrane potential and tension changes (Dixon & Small, 1983) showed that, below 10 mM, ATP caused no electrical change in association with the concentration dependent relaxation. ATP (10 mM) caused a small (7 ± 0.7 mV; mean ± s.e.m; n = 6) transient, hyperpolarisation and abolished slow wave activity. Adenosine caused concentration-dependent relaxation which was accompanied by a concentration-dependent increase in slow wave amplitude but a decrease in slow-wave frequency. No changes in resting membrane potential occurred.

The failure of the purines to markedly change resting membrane potential confirms observations made in the trachealis of other species (Ito & Takeda, 1982; Cameron et al, 1983) and suggests that K^+ channel opening is not a prominent feature of purine action in this tissue. This suggestion is supported by the poor ability of K^+ channel inhibitors to antagonise purines. The NANC inhibitory transmitter does not alter resting membrane potential in this tissue but may, if present in high concentration, abolish slow waves (Boyle et al, 1987). Accordingly ATP was found to better mimic the NANC transmitter than adenosine, though the mimicry was rendered imperfect by the small, transient hyperpolarisation induced by ATP (10 mM).

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EFFECTS OF C-KINASE ACTIVATORS AND INHIBITORS ON AMYLASE SECRETION BY ISOLATED RAT PAROTID SALIVARY GLANDS

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Although the observation that phorbol esters, which are potent activators of protein kinase C (C-kinase), stimulate amylase secretion by isolated rat parotid glands (Putney *et al.*, 1984) lends support to the notion that C-kinase activation stimulates amylase secretion, it remains to be determined whether C-kinase activation occurs as part of the physiological response of the gland to secretagogues which act upon membrane-bound receptors. We have tested this possibility by investigating the effects of the C-kinase inhibitors H7 and polymyxin B (PMB) on the secretory responses to the phorbol ester PDBu and to two classes of receptor agonists; substance P (SP) and isoprenaline (IPR).

Parotid glands were excised from male Wistar rats and perifused with Krebs Henseleit bicarbonate buffered saline with continuous measurement of amylase release as previously described (Arkle *et al.*, 1987). Glands were preincubated in the presence of either PDBu, H7, PMB or normal buffer for 20 min prior to perifusion for 15 sec with buffer containing either SP, IPR or PDBu. Whereas SP (300pM-100μM), IPR (1nM-100μM) and PDBu (3-100μM) all elicited dose-dependent amylase secretory responses (table 1), α-phorbol (100μM), H7 (50μM) and PMB (1215U/ml) did not alter the basal rate of amylase release. Preincubation with a subthreshold concentration of PDBu potentiated the secretory responses to submaximal but not to maximal concentrations of SP and IPR (table 1), and the responses to SP and PDBu were inhibited by H7 and PMB (table 2).

Table 1. Effect of PDBu on stimulated amylase secretion
Amylase release (i.u./g wet weight). Mean ± SEM. *P<0.05

Addition	30nM-SP	10μM-SP	100nM-IPR	30μM-IPR	10μM-PDBu	100μM-PDBu
none	148±41	1768±414	933±292	15706±1531	1496±95	3296±312
25nM-PDBu	439±83*	1737±412	2572±548*	14809±2187	-	-

Table 2. Effects of H7 and PMB on stimulated amylase secretion
Amylase release (i.u./g wet weight). Mean ± SEM. *P<0.05.

Addition	1μM-SP	100μM-SP	100nM-IPR	30μM-IPR	10μM-PDBu	100μM-PDBu
none	1413±204	2930±272	933±292	15705±1531	1496±95	3296±312
50μM-H7	868±197*	2707±421	1030±121	12855±601	69±26*	1681±209*
1215U/ml PMB	541±116*	1441±310*	929±209	15508±1137	204±8*	-

Our observation that responses to SP and IPR are potentiated by PDBu is consistent with the hypothesis that the processes which link receptor stimulation to amylase secretion include activation of C-kinase. This conclusion is further supported by the inhibition of SP-stimulated secretion by H7 and PMB at concentrations which also inhibit PDBu-stimulated secretion, although our data do not exclude the possibility that these C-kinase inhibitors may exert their effects through inhibition of other enzyme activities. The lack of effect of H7 and PMB on IPR-stimulated secretion suggests the possibility that PDBu may act by different mechanisms to potentiate SP- and IPR-stimulated secretion.

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THE EFFECTS OF PERIPHERALLY ADMINISTERED $ACTH_{1-24}$ AND α-MSH ON NORMAL BODY TEMPERATURE AND DURING FEVER IN THE RABBIT

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The pro-opiomelanocortin derivative α-melanotropin (α-MSH), which is composed of amino acids 1-13 of adrenocorticotropin (ACTH) and ACTH itself, appears to be involved in thermoregulation and control of fever. Levels of immunoreactive α-MSH within the rabbit CNS are reported to be significantly increased in septal extracts of brain from animals made febrile with endogenous pyrogen/interleukin 1 (E.P./IL1) (1). Septal efferents to the region of the hypothalamus (preoptic area) which are known to be important in the central control of temperature have been described in the rat (2). Peripherally administered ACTH reduces fever in response to E.P./IL1 in adrenalectomized rabbits (3) indicating that this is not mediated by corticosteroids and that it may be directly antipyretic. However, little information is available regarding the effects of α-MSH and ACTH on the febrile response to exogenous pyrogens which are thought to act by stimulating the release of E.P./IL1. It has also been shown that α-MSH modulates immune responses (4). In this study the effects of α-MSH and $ACTH_{1-24}$ on normal body temperature and febrile responses of rabbits to polyinosinic:polycytidylic acid (Poly I:C), E.P./IL1 and PGE_2 was investigated.

The body temperature of Dutch rabbits (2.0-2.3 kg), with Collison-type guide cannulae implanted in the third cerebral ventricle, was measured using rectal thermistor probes. ACTH, α-MSH, E.P./IL1 (0.5 ml) and Poly I:C (2.5 μg/kg) were administered via the marginal ear vein. PGE_2 (50 μl) was administered into the third cerebral ventricle.

α-MSH (1-10 μ g/kg) and ACTH (1-5 μg/kg) had no effect on normal body temperature at an ambient temperature of 22 °C. A higher dose of ACTH (10 μg/kg) produced hypothermia at 22 °C. ACTH (1-5 μg/kg) and α-MSH (1-10 μg/kg) induced a dose-dependant hypothermia at 10 °C. ACTH (1-10 μg/kg) produced a dose related hyperthermia at an ambient temperature of 31 °C. The effect of ACTH and α-MSH on the pyrogens were therefore studied at an ambient temperature of 22 °C. A non-hypothermic dose of ACTH (5 μg/kg) attenuated the fever produced by Poly I:C and E.P./IL1. α-MSH (5 μ g/kg) attenuated the fever produced by Poly I:C but did not have any effect on either the E.P./IL1-induced fever or the hyperthermia produced by PGE_2 (500 ng). The increase in body temperature was less with PGE_2 than with Poly I:C or E.P./IL1. A higher dose of α-MSH (10 μg/kg) was required to attenuate the fever produced by E.P./IL1.

These results suggest that both peptides are antipyretic when given peripherally and that they may act to reduce the release and/or actions of the mediators involved in fever. The results also indicate that ACTH and α-MSH can influence body temperature when given systemically, however, it is not known whether this is a centrally mediated effect.

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A STUDY OF THE INVOLVEMENT OF OPIOID RECEPTOR SUB-TYPES IN NORMAL THERMOREGULATION AND DURING FEVER IN THE RABBIT

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The role of endogenous opioid peptides in either normal thermoregulation or during fever is not well delineated. Endogenous opioid ligands are known to interact with more than one opiate receptor sub-type and recently new opioid peptide analogues have become available which are highly selective for one binding site only. Morphine is reported to produce either hypothermia or hyperthermia depending on the site of administration in the CNS and the dose. It has also been reported that morphine stimulates prostaglandin production in homogenates of rabbit brain (1). The rise in body temperature during fever is thought to occur as a result of the actions of prostaglandins on the pre-optic anterior hypothalamus (2) access to which can be gained via the third cerebral ventricle. This implies that opioid-receptors may play a role in the febrile response to pyrogens. In this study we investigated the effects of Naloxone on normal body temperature and during endogenous pyrogen/interleukin 1 (E.P./IL1)-induced fever and the effects of selective agonists on normal body temperature. Tyr-D-Ala-Gly-NMe-Phe-Gly-ol (DAGO), D-Pen^{2}-D-Pen^{5} enkephalin and 3,4-dichloro-N-[2-(1-pyrrolidinyl) cyclohexo] benzeneacetamide (U 50,488) are selective μ, δ and κ-receptor ligands respectively (3,4).

The body temperature of Dutch rabbits (2.0-2.3 kg), with Collison type guide cannulae implanted in the third cerebral ventricle was measured using rectal thermistor probes. Experiments were performed at an ambient temperature of 22-24 °C. All drugs were injected into the third cerebral ventricle (50 µl) except E.P./IL1 (0.5 ml i.v.) and Ketoprofen (3 mg/kg subcutaneously).

Naloxone (100 µg - 400 µg) produced a dose-dependant hypothermia. Doses below 20 µg had no effect on normal body temperature nor did they significantly reduce E.P./IL1-induced fever when administered either 5 minutes prior to, or 15 minutes after E.P./IL1. DAGO (1-10 µg) produced a dose-dependant hypothermia which was attenuated by Naloxone (20 µg). U 50,488 (1-100 µg) had no effect on normal body temperature. D-Pen^{2}-D-Pen^{5} enkephalin (1-10 µg) had no effect on normal body temperature although 100 µg produced a hyperthermia which was not prevented by pre-treatment with Ketoprofen, a potent cyclo-oxygenase inhibitor.

These results indicate that μ-receptors can mediate hypothermia as it is induced by DAGO and that hyperthermia can be produced directly by activation of δ-receptors as it occurs in response to D-Pen^{2}-D-Pen^{5} enkephalin and which does not involve prostaglandins. It is suggested that endogenous opioids may have a minor physiological role in thermoregulation although they are unlikely to be involved in the febrile response to pyrogens.

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CHARACTERIZATION OF THE 5-HT RECEPTOR SUBTYPE MEDIATING ACTH RELEASE IN THE RAT

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Serotonergic (5-HT) neuronal pathways are involved in the release of adrenocorticotropic hormone (ACTH) from the pituitary gland probably through corticotropin releasing hormone (CRH) at the hypothalamic level (Holmes et al., 1982). The 5-HT receptor subtype responsible for this phenomenon has not yet been characterized. We have attempted to do this by investigating the ACTH response to the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT.

Young adult (250-300 g) male Sprague Dawley rats, caged in groups of 5 to 8 under a 12 h light/dark cycle beginning at 7:00 a.m. and fed and hydrated ad lib, were injected s.c. with drugs or vehicle (6 to 8 rats per dose) between 9:00 and 10:00 a.m. after 4 to 5 days of habituation to the environment. They were decapitated 35 to 40 min after the injection. The blood was collected in plastic tubes containing 0.5M EDTA and put on ice until centrifugation. The plasma was then separated from the cellular elements and put into plastic tubes containing an antiproteolytic (Trasylol). ACTH was measured directly from the plasma for each rat by immunoradiometric assay (IRMA)(Bionuclear Services Ltd., Reading, U.K.).

8-OH-DPAT (0.1-3 mg/kg s.c.) dose-dependently increased the plasma ACTH concentration from a baseline value of 28.2 $\pm$ 9.4 pg/ml to a maximum of 220.6 $\pm$ 71.2 pg/ml at a dose of 1mg/kg. The plasma ACTH concentration was similarly dose-dependently increased by buspirone and ipsapirone (2-16 mg/kg) which, like 8-OH-DPAT, have high affinity and selectivity for the 5-HT_{1A} recognition site (Peroutka, 1985).

The ACTH response to a submaximal dose of 8-OH-DPAT (0.3mg/kg) was not altered by the dopamine receptor antagonist, haloperidol (0.2 mg/kg), the β_1 and β_2-adrenoceptor antagonists, betaxolol and ICI 118551 (2.5 mg/kg), or the 5-HT_2 or 5-HT_3 receptor antagonists, ketanserin (1.25-5 mg/kg) and MDL 72222 (1-4 mg/kg). In contrast, the response was antagonised by (+)-pindolol (2-16 mg/kg) which interacts with 5-HT_1 recognition sites and blocks many of the behavioural effects of 8-OH-DPAT (Tricklebank, 1987).

Thus, the results indicate that dopamine receptors, β-adrenoceptors, 5-HT_2 and 5-HT_3 receptors do not play key roles in the induction of ACTH secretion by 8-OH-DPAT and are consistent with the response arising by activation of the 5-HT_{1A} receptor.

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A DUEL EFFECT OF PROPRANOLOL ON DIURESIS IN THE RAT

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We have shown previously that (±)-propranolol and (-)-propranolol at a dose of 0.1mg/kg caused a marked increase in diuresis and natriuresis in the ethanol-anaesthetised rat (Mannion & Davis, 1986). These effects are thought to be brought about through a reversal of the inhibition of diuresis produced by circulating catecholamines. We now report an antidiuretic effect of (±)-propranolol and its reversal by $d(CH_2)_5[D\text{-}Ile^2,Abu^4]AVP$ a specific Vasopressin V_2 receptor antagonist (Manning et al., 1984).

Male rats (Wistar strain) weighing 200-300g, starved for 18-24hrs. but allowed free access to water, were anaesthetised with 15% ethyl alcohol (5ml/100g) and water loaded orally with a dose equivalent to 2.5% body weight. Drugs were injected via the femoral vein, blood pressure was recorded from the femoral artery and the urine was collected at 10 min. intervals for the analysis of Na^+, K^+ and Cl^-ions.

TABLE 1 CHANGES IN URINE VOLUME PRODUCED BY PROPRANOLOL

Treatment	Dose	Flow Rate before Propranolol	Flow Rate after Propranolol
(±)-Propranolol	0.1mg/kg	1.18±0.05	2.55±0.24
(-) "	0.1mg/kg	1.19±0.06	2.12±0.10
(+) "	0.1mg/kg	1.25±0.05	1.30±0.05
(±) "	0.5mg/kg	1.05±0.04	0.17±0.04
(-) "	0.5mg/kg	1.25±0.03	2.16±0.06
(+) "	0.5mg/kg	1.07±0.05	0.26±0.09
(±)-Propranolol & V_2antagonist	0.5mg/kg	1.18±0.08	2.28±0.14

(±)-propranolol (see Table 1) and (-)-propranolol at a dose of 0.1mg/kg caused a marked increase in diuresis and natriuresis whereas (+)-propranolol at the same dose had no effect. At the higher dose of 0.5mg/kg, (-)-propranolol again produced a marked diuresis and natriuresis whereas (±)-propranolol and (+)-propranolol both caused antidiuresis and antinatriuresis.

In the presence of $d(CH_2)_5[D\text{-}Ile^2,Abu^4]AVP$, the antidiuretic effect of 0.5mg/kg (±)-propranolol was abolished and a diuretic effect similar to that produced by (±)-propranolol, 0.1mg/kg, was observed.

We conclude that higher doses of (±)propranolol promotes the release of ADH from the neurohypophysis which acts on the vasopressin V_2 receptors of the kidney to cause an antidiuresis, and that this effect can be abolished with the specific antagonist $d(CH_2)_5[D\text{-}Ile^2,Abu^4]AVP$.

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INACTIVATION OF THE SYMPATHETIC TRANSMITTER IN THE GUINEA-PIG VAS DEFERENS: SUPPORT FOR CO-TRANSMISSION

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In many sympathetically innervated tissues, noradrenergic neurotransmission cannot account for all the observed responses to nerve stimulation (n.s.), such as the excitatory junction potentials (e.j.p.s). These non-noradrenergic responses are believed to be mediated by adenosine 5'-triphosphate (ATP), released together with noradrenaline (NA) as a 'co-transmitter' (Burnstock and Kennedy, 1986). At the autonomic neuroeffector junction (ANJ), ATP satisfies a number of the criteria necessary for a substance to be considered as a neurotransmitter such as storage and release (Sneddon and Westfall, 1984), but surprisingly little is known about its mechanism of inactivation. It is normally degraded enzymatically and in the guinea-pig vas deferens the e.j.p.s which are believed to be mediated by ATP (Sneddon and Westfall, 1984), are prolonged by cooling, suggesting the involvement of a temperature-sensitive enzymatic process (Blakeley and Cunnane, 1982). It was therefore of interest to determine whether cooling also altered junction potentials evoked by exogenous ATP and its stable analogue α,β-methylene ATP.

Vasa deferentia removed from guinea-pigs were pinned out in a 2-ml organ bath perfused with Krebs solution and intracellular recordings made essentially as previously described (Blakeley & Cunnane, 1982). Drugs were applied locally to the surface of the vas from glass micro-pipettes using brief (5-20 ms) pressure pulses.

Local application of ATP (10^{-4} M) resulted in a depolarization that could faithfully mimic the e.j.p. Cooling the tissue from 35^0C to 25^0C did not change the mean resting membrane potential but e.j.p.s were significantly prolonged (rise time and 50% decay time (ms): 52.4 ± 4.8 and 236 ± 20 at 35^0C, 85.4 ± 10.2 and 434 ± 30 at 25^0C, n =36, $P < 0.01$). The responses to exogenously applied ATP were similarly prolonged (rise time and 50% decay time (ms): 243 ± 50 and 663 ± 88 at 35^0C, 713 ± 36 and 1,955 ± 79 at 25^0C, n =31, $P < 0.01$). Local application of α,β-methylene ATP (10^{-6} M) produced a long lasting depolarization (30 - 60 s) which was not affected by cooling, showing that the above changes observed on cooling were not due to a change in passive membrane properties. On no occasion did local application of NA (10^{-4} M) evoke an observable electrical response.

Thus, in contrast to NA, which is removed from the ANJ by re-uptake, the inactivation process for the transmitter mediating the e.j.p. (ATP) may well be enzymatic. This view is supported by the observation that α,β-methylene ATP, which is resistant to enzymatic degradation, produces a much more prolonged electrical response than the labile parent compound, ATP.

In conclusion, since junction potentials evoked by n.s. and locally applied ATP are similarly affected by cooling, it is likely that the actions of endogenous transmitter and exogenous ATP are terminated by the same mechanism. These results are compatible with the view that ATP is the transmitter mediating the e.j.p.s.

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STUDY OF MUSCARINIC RECEPTORS MEDIATING MUCUS SECRETION IN CAT TRACHEA

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Muscarinic receptors have been classed as M_1 and M_2 on the basis of different affinities for the antagonist pirenzepine, PZ (Hammer & Giachetti 1982). The relative selectivity of 4DAMP for the ileum and AFDX116 for the atrium suggest a further subdivision of the M_2 subtype. Airway mucus secretion is stimulated by cholinergic agonists but little is known about the muscarinic receptors underlying the response. We have attempted to characterise these receptors in the cat trachea in vitro using an adaptation of a model developed for the ferret trachea (Robinson, et al 1983).

The trachea was cannulated at both ends and mounted larynx end downwards in an organ-bath keeping the lumen air-filled. The upper cannula was connected to a pressure transducer to monitor smooth muscle tone and mucus was collected via a catheter through the lower cannula. Secretion was stimulated by carbachol (CCh, 3×10^{-8} to 10^{-3}M) added for 10 minutes at 80 minute intervals. Samples were weighed and assayed for glycoprotein (GP) content by an Alcian Blue spectrophotometric method. Thus, CCh (10^{-5}M) stimulated secretion of 148 $\pm$ 26 mg mucus with a GP content of 35.7 $\pm$ 7.4 µg (n=8).

An estimate of dissociation constant (K_b) for each antagonist has been made from the degree of rightward shift of a CCh two-point dose-response line. Data from individual experiments have been pooled for this calculation in view of the small number of responses which can be obtained from a single preparation. We have compared these values with the pA_2 for each antagonist on both spontaneously beating atria and tracheal spirals from the guinea-pig using CCh as the agonist.

Table 1: Comparison of muscarinic antagonists on mucus secretion, guinea-pig trachea and guinea-pig atria. (n = 3-4).

	Mucus secretion (pK_b)		Guinea-pig (pA_2)	
	Weight	GP Content	Trachea	Atria
4DAMP	9.1	9.0	9.2	8.2
PZ	7.5	7.4	6.8	6.55
AFDX116	5.9	6.1	6.45	7.45

The low affinity of AFDX116 indicates that the secretory receptor is not of the M_2 atrial type. PZ is a potent M_1 antagonist (pA_2 8 - 8.4), Brown et al 1980)[2] and hence the secretory receptor is unlikely to be an M_1 type. However the affinity of PZ was higher for the mucus response than in either tracheal smooth muscle or atria and resembled more that in the guinea-pig oesophageal muscularis mucosae (Kawikawa et al 1985) and rabbit aortic endothelium (Eglen & Whiting 1985). The differences in antagonist affinities were generally small and definitive classification of the receptor mediating mucus secretion awaits studies with more selective agents.

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A NEW METHOD OF MEASURING ANGIOGENESIS

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Angiogenesis, the formation and growth of new capillary blood vessels, is an important biological process in many physiological (embryonic development, wound healing and endometrial regeneration) and pathological conditions (growth of tumours and rheumatoid synovial hypertrophy). Although much has been learnt about the control of angiogenesis (Folkman, 1982), further development has been hindered by the lack of a reliable and reproducible method for the assay of agents affecting angiogenesis. The new method is based on the subcutaneous implantation of sponges and the subsequent measurement of blood flow through the vascularised sponges. This provides an objective measurement and since, originally, the sponge contained no blood vessels, there is no doubt that development of blood flow represents a neovascularisation.

Sterilised polyester sponges were prepared containing a polythene cannula to facilitate injection and were implanted under the dorsal skin of male rats (140-200 g). Blood flow measurements were used as an indicator of angiogenesis and were made using the ^{133}Xe clearance technique (Lewis, Peck, Williams & Young, 1976; Fan & Lewis, 1982).

In a series of experiments over a period of 26 days after implantation in 15 rats, a clearance of between 10 and 20% occurred during the first 9 days which was partially the result of passive diffusion of the ^{133}Xe from the sponges. After 12 days the clearance was 30% $\pm$ 3%, after 14 days 45% $\pm$ 1.5% and, by 16 days after implantation, the clearance had reached a level between 60% and 70%, which was approaching the level obtained in normal skin. During the subsequent 10 days the clearance remained constant at the level of normal skin.

It has been possible to show that agents which are known to influence angiogenesis are effective in this model. Endothelial cell growth supplement (ECGS) is known to enhance angiogenesis and was found to produce a positive response in the present model. ECGS 100 ug injected into the sponges for 4 consecutive days, from 4 days after implantation, showed enhanced angiogenesis on day 7 and reached the maximum clearance level on day 11, compared with the control group of day 16 or 18.

In contrast, protamine, which is known to inhibit angiogenesis, delayed the onset of vascularisation. After protamine (daily injections of 10 mg for 4 days) the onset of clearance occurred at day 13 compared with day 8/9 and the maximum was not reached until day 20 compared with untreated controls.

The present method offers several advantages over the existing assays of angiogenesis. It provides:
(1) an objective assessment of neovascularisation;
(2) continuous monitoring in the same animal;
(3) reproducibility and (4) the possibility of making localised injections of angiogenic substances or inhibitors.

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CHARACTERISATION OF β-ADRENOCEPTORS IN RABBIT SKELETAL MUSCLE AND REGULATION BY CHRONIC TREATMENT WITH ADRENALINE AND NORADRENALINE

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β-adrenoceptors in skeletal muscle contribute to a variety of muscle activities such as contraction and metabolism. Furthermore these receptors together with the circulating adrenaline have been suggested to regulate plasma potassium especially during and immediately after exercise and stress and were linked to the genesis of ventricular arrhythmias in man (Struthers & Reid, 1984). The aims of the present work are to identify the β-adrenoceptors and their subtypes in the skeletal muscle of the rabbit and to study and compare the effect of treatment of the animals with the circulating hormone adrenaline and the neuro-transmitter noradrenaline. The former has potent β_2-adrenoceptor agonist activity and the latter has relatively selective β_1-adrenoceptor agonist activity.

Rabbits weighing 2.5-3.0 kg were used. Each group was treated with vehicle (0.1% ascorbic acid), adrenaline (0.05 umoles.$kg^{-1}hr^{-1}$) or noradrenaline (0.09 umoles.$kg^{-1}.hr^{-1}$) administered intravenously by osmotic mini-pumps connected to femoral veins. Treatment continued for 10 days. The animals were sacrificed by an overdose of pentobarbitone sodium. Muscle membranes were obtained from gastrocnemius muscles and incubation was carried out with the radioligand (-)-[^{125}I] iodocyanopindolol (ICYP) at 25°C for 2 hours as previously described (Elfellah et al, 1986). B_{max} and K_D were obtained by Scatchard analysis.

Inhibition constants (Ki) in gastrocnemius muscle membranes from 4 control rabbits were determined from competition curves and were 300.3 ± 33.3 pM (mean ± S.E. mean) for the selective β_1-adrenoceptor antagonist metoprolol and 0.16 ± 0.01 pM for the selective β_2-adrenoceptor antagonist ICI 118 551. Hofstee plots for both antagonists were linear indicating that the radioligand ICYP was bound to homogenous population of β_2-adrenoceptors. Table 1 shows that pre-treatment with adrenaline but not noradrenaline significantly reduced B_{max} in the gastrocnemius muscles. The failure of noradrenaline to down-regulate the skeletal muscle β_2-adrenoceptors may result from it being a weak agonist on these receptors. Neither drug altered K_D significantly.

Table 1 Effect of pretreatment of rabbits with adrenaline and noradrenaline on binding characteristics of ICYP in gastrocnemius muscles (mean ± S.E.)

Treatment	(n)	B_{max} (fmoles/mg.protein)	K_D (pM)
Vehicle	(9)	109.0 ± 7.8	7.3 ± 0.9
Adrenaline	(5)	61.6 ± 10.5*	6.3 ± 0.8
Noradrenaline	(6)	102.0 ± 17.9	9.25 ± 2.5

* $p < 0.05$ in comparison to other groups (n) = number of rabbits.

In conclusion β-adrenoceptors in gastrocnemius muscle of the rabbit are exclusively of β_2-subtype and that chronic in vivo treatment with adrenaline but not noradrenaline caused down-regulation of these receptors.

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INTERFERENCE OF A SELECTIVE MESENTERIC VASODILATOR PEPTIDE, UROTENSIN I, WITH ADRENERGIC PRESSOR RESPONSES

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Urotensin I (UI), a corticotropin-releasing neuropeptide isolated from the urophysis of teleost fish, produces a sustained fall in arterial blood pressure when administered in the conscious or anesthetized rat (Lederis & Medakovic, 1974) and a highly selective mesenteric vasodilatation in the anesthetized dog (MacCannell & Lederis, 1977). Recently, it has been determined that UI not only alters Ca-influx but also antagonizes the action or mobilization of intracellular Ca induced by norepinephrine (Itoh & Lederis, 1987). The activation of alpha-1 adrenoceptors liberates intracellular Ca and causes an opening of potential-independent Ca channels that are insensitive to Ca channel blockers, whereas the activation of alpha-2 adrenoceptors causes an opening of receptor-operated Ca channels which are sensitive to Ca channel blockers (Langer & Shepperson, 1982). To further analyze the mechanism of action of this neuropeptide, we examined the interference of UI with vasoconstrictor responses to selective and nonselective alpha adrenoceptor agonists in rat mesenteric arteries in vitro and in autoperfused mesenteric circulation of the rat in vivo.

Helical strips (1.2x12mm) of the rat mesenteric artery were used as previously described (Itoh et al, 1987). The selective alpha-1 adrenoceptor agonist, phenylephrine, the nonselective adrenoceptor agonist, norepinephrine, and the relatively selective alpha-2 adrenoceptor agonist, alpha-methylnorepinephrine, produced similar maximal contractions with respective PD_2 values of 6.19±0.20, 7.13±0.34 and 6.29±0.33. In the presence of increasing concentrations of UI, a marked depression of the responses to all alpha adrenergic agonists was obtained. The calculated IC_{30} values (concentration giving 30% inhibition) for inhibition of the maximal response to phenylephrine, norepinephrine and alpha-methylnorepinephrine were 0.34±0.06, 0.72±0.16 and 1.33±0.15 nM, respectively. UI ($7.5x10^{-10}$M) shifted the concentration-response curve for norepinephrine not only to the right but also downwards. The rightward shift induced by UI was diminished in the presence of prazosin (10^{-8}M), an alpha-1 adrenoceptor antagonist, but was not altered in the presence of yohimbine ($3x10^{-6}$M), an alpha-2 adrenoceptor antagonist. The UI-induced downward shift of the concentration-response curve for norepinephrine was increased in the presence of nifedipine (10^{-8}M), which blocks the opening of receptor-operated Ca channels mediated by the activation of alpha-2 adrenoceptors. In the autoperfused mesenteric circulation of the anesthetized rat, a pronounced selectivity of UI for alpha-1 adrenoceptor-mediated responses was observed. ID_{30} (30% inhibition dose) values of the peptide for the pressor responses to phenylephrine, norepinephrine and alpha-methylnorepinephrine were 0.05±0.01, 0.83±0.20 and >6 nmol/kg, respectively.

These results suggest that UI may chiefly act on the process of alpha-1 adrenoceptor-mediated contractile response and decrease the vascular tone induced by sympathetic nervous system in rat mesenteric circulation.

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RELATIVE ANTAGONIST SELECTIVITY OF BUSPIRONE ON THE CARDIOVASCULAR EFFECTS OF 5-HT_{1A}, DA_2- AND α_2-RECEPTOR AGONISTS

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8-OH-DPAT (DPAT), a purported $5HT_{1A}$-agonist, lowers blood pressure and heart rate by a central mechanism of action (Fozard et al, 1987). Buspirone , a novel anxiolytic agent, has a high affinity for H-DPAT binding and is considered as a partial agonist at $5HT_{1A}$-receptors based on behavioral tests (Clague and Spedding, 1986 ; Fozard et al, 1987). The compound is also a known antagonist at dopamine and α-adrenoceptors. The present study evaluates the relative antagonist effects of buspirone against DPAT, clonidine and quinpirole-induced cardiovascular effects in rats.

Male Sprague-Dawley rats (250 g) were anesthetized with pentobarbitone (55 mg/kg i.p.) and prepared with carotid artery and femoral vein catheters for blood pressure (MAP), heart rate (HR) measurements and drug administration. Separate groups (n=5/7) of rats were treated with either i.v. saline (0.4 ml/kg), buspirone (20, 60 and 600 µg/kg), rauwolscine (R; 200 and 600 µg/kg), S-sulpiride (300 µg/kg) or SCH 23390 (600 µ/kg i.v.). All antagonists were administered by i.v. infusions over 20 min, followed by either DPAT (40 µg/kg i.v. in 2 min), quinpirole (10 µg/kg i.v. in 2 min) or clonidine (1.0 µg i.c.v.). These agonists gave ΔMAP of -23 ± 2, -34 ± 3 and -22 ± 3 mmHg and ΔHR of -46 ± 3, -41 ± 5 and -61 ± 3 b/min, respectively.

Buspirone did not significantly modify resting blood pressure at any dose, but caused a small bradycardia (418 ± 6.6 to 398 ± 8.7 b/min) after 60 µg/kg dose. This effect did not increase with the higher dose. Buspirone (20 µg/kg) antagonised (-34 %) the maximum hypotensive and the bradycardic (-27%) effects of DPAT which were further decreased after buspirone (60 µg/kg; MAP -77%; HR -71%). Antagonist effects of buspirone were observed against the falls in MAP (-52%) or HR (-41 %) evoked by quinpirole, or clonidine (MAP -72%; HR -67 %) at doses of 60 µg/kg or 600 µg/kg, respectively. S-sulpiride (300 µg/kg i.v.) abolished both effects of quinpirole, but at this dose had no effect on DPAT. Rauwolscine at 200 µg/kg antagonised the bradycardic (-54%) and hypotensive effects (-38 %) of clonidine and at 600 µg/kg the MAP and HR effects of DPAT were reduced by 50 % and 54 % respectively, however at this dose R did not antagonise the cardiovascular effects of quinpirole. The DA_1-receptor antagonist SCH 23390 (600 µg/kg) decreased (46 %) the bradycardic effects of DPAT but was inactive against the hypotensive effects of this agonist.

The results show that buspirone is a potent antagonist of DPAT-induced cardiovascular effects in the rat. The rank order of antagonist effects were $5HT_{1A} > DA_2 > \alpha_2$. The antagonist effect of rauwolscine against DPAT may reflect a weak affinity of this compound for $5HT_{1A}$-receptors rather than providing support for a central catecholaminergic link (Fozard et al, 1987).

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SEARCHING FOR PATTERN MATCHES ON DISSIMILAR DRUG MOLECULES

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This poster illustrates a novel and general method of searching for pattern matches of any parameter distributed on two 3-dimensional closed surfaces which approximate to convex hulls. The method has considerable potential use in medicinal chemistry where dissimilar molecules, which apparently bind to a shared set of site points, are frequently studied. Similarities in shape and pattern of the molecular field at the binding faces may explain the binding properties.

Surface patterns of both molecules are mapped gnomonically onto a sphere by an icosahedral tessellation. The pattern presented by a patch of any shape on one sphere is compared with that of a corresponding patch on the other sphere by computing the error function. This function is then used as the objective function for a quasi-Newton minimization. Minimization proceeds from random starting positions for the Euler rotation angles. This minimization can be optimized further by allowing only a small number of iterations of the function to be evaluated. Preliminary end-points along the minimization pathway are therefore distributed in clusters in n-space. Cluster analysis, using a single-linkage agglomerative procedure which is ideally suited to chaining, leads directly to the identification of discrete positions for pattern matching. Cluster centroids are used for a final minimization refinement with high accuracy. The end product is that we have a number of matched patterns corresponding to discrete orientations of the two dissimilar molecules; these can then be ranked in the order of their similarity.

The search procedure will be demonstrated using the two neurotoxins, saxitoxin and tetrodotoxin. Surface parameters studied are: 1. the accessible surface, and 2. the molecular electrostatic potential computed at the accessible surface.

PHARMACOLOGICAL EFFECTS OF HAEM BIOSYNTHETIC INTERMEDIATES IN ISOLATED GASTROINTESTINAL PREPARATIONS

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We have previously reported that the haem biosynthetic intermediates, delta-aminolaevulinic acid (ALA), porphobilinogen (PBG), coproporphyrin I and protoporphyrin IX and also haemin inhibit contractile activity of isolated rabbit jejunal preparations (Cutler et al, 1987). We have also found the duration of inhibitory actions of ALA in rabbit jejunum to be significantly decreased if preparations are pretreated with prazosin to block α_1-adrenergic receptors (Cutler et al, 1985). The present studies assess effects of protoporphyrin (1.1 mM) and haemin (1.1 mM) as well as ALA (3.0 mM) in rabbit jejunal preparations pretreated either with prazosin (10^{-7}M) or with tetrodotoxin (TTX, 0.3 nM) and also re-examine the actions of ALA (0.6 - 6.0 mM), PBG (0.5 - 1.1 mM), protoporphyrin (0.1 - 2.2 mM) and haemin (0.6 - 6.0 mM) in this preparation. Effects of these compounds also have been examined in isolated strips of rabbit gastric fundus because this preparation gives a contractile response to noradrenaline (Khayyal et al, 1976). Isolated preparations of rabbit jejunum and rabbit fundic strip were bathed in oxygenated Ringer-Locke solution at 37°C and contractions of the preparation recorded by isotonic transducer and displayed on a calibrated Washington 400 MD2R oscillograph.

Haemin, ALA, PBG and protoporphyrin all induced concentration-dependent inhibitory effects upon tone and amplitude of contractions in rabbit jejunum, the minimal effective concentrations being 3.0 mM for ALA, 1.1 mM both for PBG and protoporphyrin and 1.5 mM for haemin. Pretreatment with prazosin significantly reduced the inhibitory effects of protoporphyrin.

A concentration-dependent elevation of tone occurred in rabbit fundic strips following administration of ALA (0.1-3.0 mM), protoporphyrin (1.1-2.2 mM) and haemin (1.5 - 6.0 mM), and this resembled effects of noradrenaline. The contractile response was significantly reduced in preparations pretreated either with prazosin or with TTX.

These results indicate a role of α_1-adrenergic receptors in the pharmacological responses of these gastric and jejunal preparations to ALA, protoporphyrin and haemin. Further studies are required to find if effects arise from noradrenaline release or from direct activation of α-receptors, and to ascertain the relevance of these findings to acute porphyria.

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