ALTERATION IN CALMODULIN BINDING: A CONSEQUENCE OF CEREBRAL ISCHAMEMIA

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Ischaemia or hypoxia results in an alteration of a number of biochemical and physiological parameters in brain tissue. The ischaemia-induced reduction in high energy phosphates leads to an efflux of K^+ followed by the opening of voltage-dependent calcium channels and influx of calcium (Siesjo, 1981). The increase in intracellular Ca^{2+}, through its central role in a number of cellular regulatory processes, has been suggested to be responsible for triggering the multitude of bichemical changes that occur in the cell in response to ischaemia (Hass, 1983). This study, in view of the proposed central role of Ca^{2+} in mediating the deleterious effects of ischaemia, examined the effect of periods of ischaemia on the potency of calmodulin (an ubiquitous intracellular calcium binding protein) and the calmodulin antagonists, trifluoperazine and W-7, for calmodulin binding to rat striatal membranes.

Complete brain ischaemia was induced in thirty male Sprague-Dawley rats by the method of Schiu & Nemoto (1981). Depletion of endogenous calmodulin from striatal membranes from ischaemic brains and subsequent calmodulin binding were carried out by the method of Gnegy et al (1980). Each determination represents the mean ± SE of six animals. The results presented in Table 1 show that the potency of cold calmodulin, W-7 and trifluoperazine for inhibiting ^{125}I-calmodulin binding to striatal membranes is altered under periods of ischaemia. Ischaemia for periods of as little as 10 min resulted in a statistically significant increase in the IC_{50} values of calmodulin and trifluoperazine. Maximum effects on calmodulin binding were observed at 20 and 30 min ischaemia.

Table 1 Effect of ischaemia on calmodulin binding

Period of Ischaemia	CALMODULIN BINDING (IC_{50}) W-7 ($\mu mol.litre^{-1}$)	TRIFLUOPERAZINE ($\mu mol.litre^{-1}$)	CALMODULIN ($nmol.litre^{-1}$)
0	149.0 ± 6.0	101.4 ± 6.8	23.2 ± 0.9
10	167.2 ± 29.0	137.2 ± 5.0***	85.6 ± 9.4***
20	187.1 ± 14.7*	149.1 ± 19.0***	85.5 ± 2.2***
30	213.4 ± 1.2***	142.5 ± 7.5***	83.9 ± 6.18***
60	164.5 ± 12.0	109.4 ± 10.1	54.3 ± 4.6**

Statistical significance relative to control animals:

*$p < 0.05$; **$p < 0.02$; ***$p < 0.01$

The finding that brain ischaemia results in a significant shift to the right in the inhibition curves for calmodulin, trifluoperazine and W-7 provides a further example of an ischaemia-induced alteration in a major cellular regulatory process that may contribute to the eventual death of the cell.

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NICARDIPINE INHIBITS CARDIOVASCULAR STIMULATION BY BAYER K 8644

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Within the chemical class of dihydropyridines, some compounds inhibit calcium influx through membrane slow channels while others promote calcium entry to produce their principal activity (see Towart, 1985). We have now studied the interaction of the calcium entry blocking agent nicardipine (for the structure see van Zwieten, 1985) with the calcium entry facilitator Bayer k 8644 (Schramm et al., 1983) using cardiovascular tissues of the rat.

Isolated working hearts were perfused via the pulmonary vein with Kreb's solution (Ca^{++} 1.0 mM) heated to 37°C and gassed with 95% O_2 and CO_2 (Armstrong and Ferrandon, 1985). Left ventricular contractility was assessed from measurements of the rate of pressure rise within the ventricle (+dp/dt). Addition of Bayer (10 nM) to the perfusing solution increased contractility from 3000 ± 127 mmHg/sec (n= 6) to a maximum of 4117 ± 231 mmHg/sec (n= 6) within 15-20 minutes. Nicardipine (1 nM), by itself, did not alter baseline contractility but when added to the perfusion fluid 15 minutes beforehand markedly inhibited Bayer's effect so that contractility increased from 2720 ± 139 mmHg/sec (n= 5) to a maximum of only 3100 ± 78 mmHg/sec (n= 5).
Spirally cut aortic strips that were partially depolarized by adding 15 mM K^+ to the Kreb's solution (Ca^{++} 1.0 mM) bathing the tissues were contracted by the Bayer compound. The responses were expressed as a percentage of the maximum tension produced by 40 mM K^+. Cumulatively increasing concentrations of the facilitator (1 nM-100 nM) produced progressively increasing levels of developed tension from a baseline value of 120 ± 20 mg (n= 10) to a maximum of 420 ± 31 mg (n= 10). When nicardipine (1 nM) was added, the baseline tension decreased to about 30 ± 3 mg (n= 5) and the Bayer dose-response curve was displaced to the right by approximately 10 fold at the EC50% level. Greater concentrations of nicardipine (10 nM and 30 nM) further shifted the regression lines to the right (35 fold and 450 fold respectively) and also reduced the maximum response (to 80% and 50% of the control maximum). 100 nM nicardipine reduced the maximum to approximately 10% of control.
In pithed rats cumulatively increasing i.v. bolus injections of Bayer (3 ug/kg-300 ug/kg) produced step-wise increases in mean arterial blood pressure. An i.v. infusion of nicardipine (10ug/kg/min) lowered the blood pressure from initially 64 ± 6 mmHg (n= 5) to 52 ± 7 mmHg (n= 5) after 5 minutes of infusion and reduced the maximum Bayer-evoked pressor response of 108 ± 10 mmHg (n= 5) by 40 ± 6%. An Infusion of 30 ug/kg/min of nicardipine lowered baseline blood pressure from 57 ± 5 mmHg (n= 6) to 40 ± 1 mmHg (n= 6) and also reduced the Bayer-evoked maximum pressor response to 18 ± 5% of the control.

These findings show that nicardipine inhibits cardiovascular stimulation evoked by Bayer k 8644 and are consistent with the hypothesis that the two agents interact with the same site to modulate calcium entry into heart and arterial cells.

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CALCIUM AGONISTS CAN DIFFERENTLY INFLUENCE CARDIAC CONTRACTILITY AND DIASTOLIC BLOOD PRESSURE

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The 1.4 Dihydropyridines Bay k 8644 and CGP 28392 have been reported to act as positiv inotropic and vasoconstricting agents (Schramm et al., 1983; Preuss et al.,1984; Ciba Geigy information). In our investigations, the effects of these new agents were studied on cardiac contractility (LV-dp/dtmax) and diastolic blood pressure (dBP) in two small animal models.

Male rats and guinea pigs were anaesthetized with hexobarbitone sodium (150 mg/kg, i.p.), subsequently pithed and artificially respirated. LV-dp/dtmax was measured by a Millar tip manometer (PR-249) introduced into the left ventricle via the carotid artery. Bay k 8644 and CGP 28392 were intravenously administered via the jugular vein. Blood pressure was measured by a pressure transducer in a carotid artery. The Ca-antagonist nisoldipine was intraarterially applied in a dose of 100 µg/kg.

Bay k 8644 and CGP 28392 increased LV-dp/dtmax and diastolic blood pressure in pithed rats. CGP 28392 was about 25 fold less potent than Bay k 8644. However, CGP 28392 (10 - 3000 µg/kg) induced a maximal increase in LV-dp/dtmax of 8382 $\pm$ 383 mmHg/s (n=6) which was comparable with the effects of Bay k 8644 (8026 $\pm$ 454 mm Hg/s, n=6) in the dose range of 0.1-100 µg/kg. The maximal increase in diastolic blood pressure induced by Bay k 8644 was not significantly higher than observed for CGP 28392, 79 $\pm$ 14 mm Hg (n=6) and 58 $\pm$ 8 mm Hg (n=6), respectively. A marked difference between Bay k 8644 and CGP 28392 was observed in the relationship % change dBP versus % change LV-dp/dtmax. CGP 28392 exerted a stronger effect on LV-dp/dtmax than on dBP whereas Bay k 8644 was about equipotent on heart and blood vessels. Pretreatment with nisoldipine hardly affected this relationship for CGP 28392. On the other hand, nisoldipine predominantly attenuated the blood vessel effects of Bay k 8644 and made the substance more selective for the heart.

Both agents, Bay k 8644 and CGP 28392 increased LV-dp/dtmax and diastolic blood pressure in pithed guinea pigs. CGP 28392 was about 30 fold less potent than Bay k 8644. Bay k 8644 (3-1000 µg/kg) induced a maximal increase in LV-dp/dtmax up to 3700 $\pm$ 270 mm Hg/s (n=6) which was significantly higher than the effects of CGP 28392 (30-3000 µg/kg) on LV-dp/dtmax (2170 $\pm$ 180 mm Hg/s, n=6). The maximal increase in dBP induced by Bay k 8644 was also higher than observed for CGP 28392, 50 $\pm$ 9 mm Mg (n=6) and 27 $\pm$ 2 mm Hg (n=6), respectively. The relationship % change of LV-dp/dtmax versus % change of dBP was the same for Bay k 8644 and CGP 28392. Both substances were more active on the heart than on the blood vessels. Nisoldipine did not influence this relationship for either agonist.

These results demonstrate that the selectivity of a Ca-agonist of the dihydropyridine type for the haeart and blood vessels seemed to be species dependant. Pretreatment with nisoldipine markedly increased the relative activity of Bay k 8644 on the heart in pithed rats but did not markedly influence the selectivity of CGP 28392.

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ARE DIHYDROPYRIDINE CALCIUM CHANNEL ANTAGONISTS AND FACILITORS VASCULAR SMOOTH MUSCLE SELECTIVE?

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The antihypertensive efficacy of dihydropyridine calcium antagonists can be explained by selective inhibition of calcium entry in vascular smooth muscle. We have previously demonstrated, using isolated tissue preparations, that both nifedipine and nicardipine selectively inhibit contractures of vascular smooth muscle (Clarke et al, 1983). Three compounds, Bay k 8644, CGP 28392 and YC 170, all dihydropyridine derivatives, are purported to facilitate calcium entry (Schramm et al, 1983; Langs & Triggle, 1985; Takenaka & Maeno, 1982). The present study compares the actions of dihydropyridine antagonists and facilitators on contractility of cardiac and vascular smooth muscle preparations. Measurements of contractility of guinea-pig right ventricular papillary muscles were performed as described by Clarke et al (1983). IC_{50} or EC_{200} values were calculated for antagonists and facilitators respectively and are shown in table 1. Guinea-pig superior mesenteric artery and porcine coronary artery rings (4 mm) were contracted to 50% of maximum tension and effects of each concentration of facilitator were monitored over 30 min periods. The facilitators increased the contractures up to, but not above, the maximum tension induced by 120 mM K^+. EC_{50} values are shown in table 1. Data are from an iterative fit to concentration-response curves (n = 3 - 6).

Table 1 Effects of calcium modulators on cardiac and vascular smooth muscle

	Cardiac Muscle		Vascular Smooth Muscle			
	Papillary muscle		Mesenteric artery		Coronary artery	
Compound						
Antagonists	pIC_{50}	pEC_{200}	pIC_{50}	pEC_{50}	pIC_{50}	pEC_{50}
Nicardipine	**6.79**		**8.20**[a]		**8.96**[a]	
Nifedipine	**7.10**		**7.89**[a]		**7.59**[a]	
Facilitators						
Bay k 8644		**6.79**		**8.59**		**8.26**
CGP 28392		**5.18**		**7.29**		**7.43**
YC 170		**<5.00**		**6.05**		**6.40**

[a]from Eglen et al (1983)

The data show that the three facilitators are more active on vascular smooth muscle than cardiac muscle preparations. This profile is supported by the finding that, in rabbit Langendorff hearts, Bay k 8644 produced marked coronary vasoconstriction prior to the onset of positive inotropic activity. Thus these compounds, in common with dihydropyridine calcium antagonists, appear to be vascular selective calcium modulators.

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CALCIUM CHANNEL ANTAGONIST-FACILITATOR COMPETITION IN CULTURED EMBRYONIC CHICK CARDIAC CELLS

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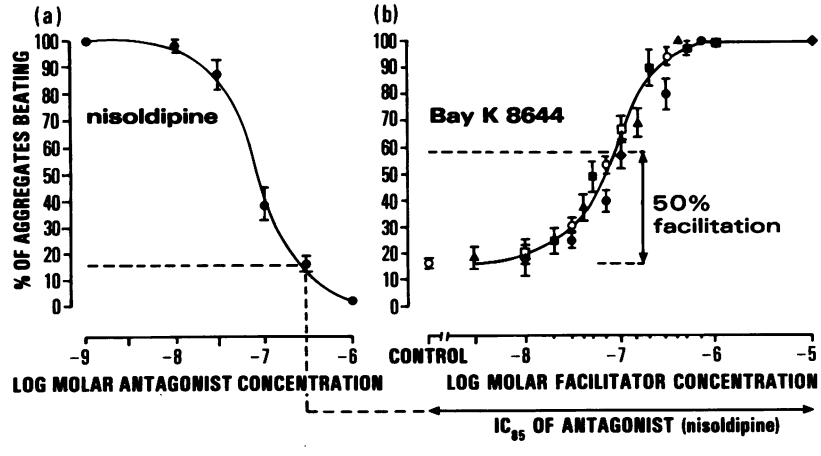
Binding studies using [^{3}H]-1,4-dihydropyridines have demonstrated a specific binding site in smooth muscle, cardiac muscle and neuronal tissue which has been suggested to be closely related to the calcium channel (Triggle & Janis, 1984). The affinity of these binding sites has been shown to be allosterically modulated, negatively by verapamil and positively by diltiazem whilst in functional studies allosteric modulation of the action of the dihydropyridine facilitator Bay k 8644 has not been observed (Spedding & Berg, 1984).

The present study investigates the functional competition between calcium channel antagonists and facilitators in cultured chick heart muscle. Primary embryonic chick heart cell aggregate cultures were prepared as described previously (Clarke et al, 1984). The effects of antagonists and facilitators on aggregate beating were monitored over 30 min periods. Data are expressed as % of control number of aggregates beating. In each experiment data for 4 separate cultures were pooled. Nisoldipine caused a concentration-dependent inhibition of aggregate contractility (fig 1a). Competition experiments were performed with concentrations of antagonists which produced 85% inhibition of beating. Complete reversal of nisoldipine-induced inhibition of beating was observed with Bay k 8644 (EC_{50} = 7.14 ± 0.04), fig 1b. Similar competition curves for Bay k 8644 were observed with verapamil and diltiazem as antagonists; pEC_{50} = 7.09 and 6.93 respectively, slopes for all three antagonists were similar (1.49 ± 0.06). When the facilitator CGP 28392 (Langs & Triggle, 1985) was used in place of Bay k 8644 lower but similar pEC_{50} values were obtained for nisoldipine, verapamil and diltiazem (pEC_{50} = 6.14, 6.08 and 5.99 respectively. However, lower slopes were observed (0.90 ± 0.03). These data demonstrate functional competition between calcium facilitators and antagonists in cardiac tissue.

Figure 1



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EFFECTS OF CALCIUM ANTAGONISTS ON NEURONAL CALCIUM AND POTASSIUM CURRENTS

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Dihydropyridine Ca antagonists are potent inhibitors of ion flow through Ca channels in cardiac and smooth muscle. Brain tissue is rich in high affinity binding sites for these drugs and may prove useful for isolating and purifying neuronal Ca channels. It must first be clearly determined, however, that the binding sites have a physiological correlate and represent a uniform class of Ca channels. We have examined the effects of the antagonists nifedipine and nisoldipine on Ca currents (I_{Ca}) recorded from several neuronal cell types, using the "whole-cell" variation of the patch-clamp technique (Hamill et al., 1981). These antagonists (<10 μM) had no effect on I_{Ca} recorded from neonatal rat superior cervical and dorsal root ganglion cells, or embryonic chick ciliary ganglion cells, dissociated and maintained in culture for 1-7 days. The drugs did, however, suppress the amplitude of I_{Ca} recorded from the neurosecretory bag cells of Aplysia californica; inhibition by nifedipine was half maximal at 1.7 ± 0.2 μM, a concentration 3-fold higher than that required for 50% suppression of Ca currents in cardiac muscle (Lee & Tsien, 1983; Gurney et al., 1985).

Similar mechanisms may underlie the blockade of I_{Ca} in bag cells and in cardiac muscle. Kinetic studies suggest that nifedipine and nisoldipine interact with the bag cell Ca channel in at least three states: closed, open, and inactivated. Thus the effects of dihydropyridine antagonists on I_{Ca} in bag cells, as in cardiac muscle (Gurney et al., 1985), can be described within the framework of the "modulated receptor" model of drug action (Hille, 1977), which proposes that the affinity of the drug for its receptor is modulated by the state of the channel. Nifedipine and nisoldipine, however, displayed poor selectivity for I_{Ca} in bag cells, also suppressing two outward K currents, identified as the "delayed rectifier" and the "A" current. Nifedipine concentrations as low as 3-5 μM were sufficient to cause 50% inhibition of either K current. Kinetic studies revealed several similarities between the effects of these drugs on the K currents and I_{Ca}, suggesting that the mechanisms of blockade may be similar. Interestingly, the dihydropyridine Ca agonist, BAY K 8644 (1-10 μM), was without effect on either current, but protected against the inhibitory effects of the antagonists on both Ca and K currents.

In bag cells, as in heart, the affinity of nifedipine was highest for Ca channels in the inactivated state, with a K_D <350nM. This value is much higher than dissociation constants measured in binding studies on brain tissue. Thus it seems unlikely that this study reveals the same binding sites.

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THE INHIBITORY EFFECTS OF LOCAL ANAESTHETICS IN SMOOTH MUSCLE ARE NOT AFFECTED BY THE Ca^{2+} CHANNEL ACTIVATOR, BAY K 8644

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There is some overlap between the ability of compounds to interfere with Na^+ and Ca^{2+} channel function, which has led to suggestions that there are structural similarities between the channels (Romey & Lazdunski, 1982). However, while toxins like batrachotoxin (Kongsamut et al., 1985) appear to interact directly at very low concentrations with both channels, it is not clear whether inhibitory effects of local anaesthetics are due to direct interactions with the ion channels (e.g. Strichartz, 1973 ; Hille 1984) or to less specific effects on membranal properties (Spedding & Berg, 1985). To further investigate this point I have tested whether the effects of local anaesthetics are influenced by the Ca^{2+} channel activator, Bay K 8644, which reverses the effects of selective Ca^{2+} channel antagonists (Spedding & Berg, 1984).

Taenia from the guinea-pig caecum were set up under isotonic conditions in Ca^{2+}-free K^+, 40 mM, Tyrode solution and cumulative concentration-response curves to Ca^{2+}, 30 - 10,000 µM, obtained at 40 min intervals (Spedding & Berg, 1984). Local anaesthetics were incubated with the preparations for 25 min, in the presence or absence of Bay K 8644, 1 µM, and caused parallel shifts to the right of the Ca^{2+} concentration-response curves, irrespective of the presence of Bay K 8644. Apparent pA_2 values were calculated from Schild plots and are listed in Table I.

Table 1. Potency of local anaesthetics as "calcium-antagonists" in the absence and presence of Bay K 8644, 1 µM.

	Control		Bay K 8644		
Drug	Apparent pA_2	Slope	Apparent pA_2	Slope	n
(±) propranolol	4.8 ± 0.1	1.86 ± 0.07	4.8 ± 0.1	1.54 ± 0.03	5
lignocaine	3.5 ± 0.2	1.68 ± 0.27	3.2 ± 0.1	1.72 ± 0.15	4
procaine	2.9 ± 0.2	1.74 ± 0.14	2.8 ± 0.1	1.57 ± 0.17	6

The effects of the neutral local anaesthetic benzocaine were very steeply concentration-dependent (>3 mM) and little affected by Bay K 8644, 1 µM.

Bay K 8644 does not appear to influence the inhibitory effects of local anaesthetics in smooth muscle. This is in marked contrast to the interaction with selective calcium-antagonists such as the dihydropyridines (Group I) or verapamil and diltiazem (Group II) where calcium-antagonistic activity is markedly reduced in the presence of Bay K 8644 (Spedding and Berg, 1984). These experiments show that the mode of action of local anaesthetics is clearly different from that of verapamil and may be due to more non-specific effects on membranes ; in this respect local anaesthetics resemble group III calcium-antagonists (Spedding, 1985).

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EFFECTS OF SOME CARDIOTONICS ON CALCIUM SENSITIVITY OF SKINNED MYOCARDIAL FIBRES

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Increasing myocardial contractility can be achieved by different mechanisms. A new mechanism of action to induce positive inotropic effects has recently been described for the cardiotonic agent sulmazole (Herzig et al., 1981; Solaro and Rüegg, 1982). Sulmazole induces an increase in the calcium sensitivity of cardiac muscle fibers and this effect is proposed to participate in the positive inotropic effects of the substance. Because such an effect represents a potentially useful new mechanism of action for cardiotonics, we investigated effects of some other positive inotropic agents of different structure on the calcium sensitivity of chemically skinned myocardial fibers.
The experiments in skinned cardiac muscle fibers were performed according to the method as described by Rüegg et al (1984). Subendothelial muscle fibers (about 4 mm in length and 0.1 mm in diameter) were prepared from porcine trabecula septomarginalis and extracted for 12 h in a solution containing 50 % glycerol and 50 % of a buffer (20 mmol/l imidazole, 10 mmol/l sodium azide 2 mmol/l dithioerythriol and 0.5 % Lubrol WX, pH = 7.0 at 4°C). Subsequently, the preparations were stored in the same solution but without Lubrol at -20°C for several days. After mounting, the fibers were relaxed by immersion in a solution containing 10 mmol/l ATP, 12.5 mmol/l $MgCl_2$, 5 mmol/l EGTA, 25 U/ml CPK, 20 mmol/l imidazole, 5 mmol/l NaN_3 and 10 mmol/l CP resulting in a free calcium concentration of pCa<8. Contraction was induced by immersion into a analogues solution in which EGTA was replaced by EGTA-calcium buffer. The effects were measured at pCa=5.78. Concentration of substances was 0.1 mmol/l. Data are presented as % tension development of maximal tension (obtained at pCa=4.84). Results are expressed as means ± SD.

substance	% tension	% tension in the presence of 0.1 mmol/l substance		
sulmazole (AR-L 115)	35.6 ± 4.6	38.8 ± 5.4	p<0.05	o
pimobendan (UD-CG 115)	63.3 ± 16.6	70.2 ± 16.5	p<0.05	
milrinone	41.1 ± 7.9	41.1 ± 7.9	ns	
amrinone	47.0 ± 4.5	47.0 ± 4.5	ns	
fenoximone(MDL 17043)	45.5 ± 6.1	47.3 ± 7.5	ns	
MDL 19205*	40.0 ± 4.0	38.9 ± 4.5	ns	
APP-201-533**	46.4 ± 2.1	48.9 ± 4.8	p<0.05	
CI-914***	35.2 ± .5.5	36.8 ± 8.4	ns	
OPC-8212****	53.0 ± 6.1	53.0 ± 6.1	ns	

o : paired t-test
* : 4-ethyl-1,3-dihydro-5-(4-pyridinyl-carbonyl)-2H-imidazol-2-one
** : 3-amino-6-methyl-5-phenyl-2(1H)-pyridinone
*** : 4,5-dihydro-6-(4-(1H-imidazol-1-yl)phenyl)3(2H)-pyridazinone
**** : 3,4-dihydro-6-(4-(3,4-dimethoxybenzoyl)-1-piperazinyl)-2-(1H)-quinolinone

The results demonstrate that sulmazole, pimobendan and APP-201-533 can induce an increase in calcium sensitivity of cardiac fibers which may represent a new mechanism of action for cardiotonic agents.

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PROSTAGLANDIN D_2 RELEASE IN LOCALISED HEAT URTICARIA

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Localised heat urticaria is one of the rarest forms of physical urticaria. Application of heat to an area of skin induces urticaria localised to that area. For 10 years, a 70 year old female developed immediate itching, erythema and oedema at the site of exposure of her skin to heat. The threshold temperature applied to her skin for 4 mins for induction of a localised weal was 43°C.

Previous studies of heat urticaria have demonstrated histamine release and mast cell degranulation in urticated skin (Greaves et al, 1974). Prostaglandin D_2 (PGD_2) is the major prostaglandin product of human mast cells (Lewis et al, 1982). It has a vasodilator action in skin and potentiates the permeability action of histamine (Flower et al, 1976). We therefore measured levels of histamine and PGD_2 released into the venous blood before and up to 32 mins after immersing the patient's forearm in water at 44.5°C for 5 mins. This resulted in a marked confluent weal of the forearm.

Histamine was measured by superfusion cascade bioassay and PGD_2 by radioimmunoassay. Histamine-like activity rose from a prechallenge level below the detectable limit of 2.5 ng/ml to a peak level of 12.5 ng/ml at 8 mins post immersion. PGD_2 levels showed a parallel rise from 18.5 pg/ml prechallenge to a peak level of 420.0 pg/ml 8 mins post challenge and was still elevated (87 pg/ml) at 32 mins. A similar heat challenge to a control normal subject showed no rise in histamine or PGD_2 concentrations, which remained below the limit of detection and between 10 and 25 pg/ml respectively.

Treatment by induction of tolerance by repeated exposure to hot water raised the threshold temperature for wealing to 47°C, with moderate clinical improvement. Addition of indomethacin, a prostaglandin synthetase inhibitor, 25 mg t.i.d. for two weeks, produced a slight additional clinical improvement. On repeat challenge only a few small weals appeared on her forearm. Histamine concentrations remained below 2.5 ng/ml and PGD_2 levels between 30 and 50 pg/ml.

The raised concentrations of PGD_2 in the venous blood from the markedly urticated forearm suggested that PGD_2 may have potentiated the effects of histamine. In view of the lack of rise of histamine and PGD_2 in the post treatment minimally wealed area, we could not exclude the involvement of other mediators.

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EFFECT OF LABETALOL ON INDICES OF MYOCARDIAL NECROSIS IN PATIENTS WITH SUSPECTED ACUTE INFARCTION

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Labetalol, a combined α and β blocker, has theoretical advantages over β blockade alone on left ventricular haemodynamics. Some recent work suggests that β blockers given early after myocardial infarction may limit infarction size. We conducted a randomized controlled trial to examine the effects of labetalol given during the first 6 hours after infarction. 166 patients, age range 39-74 were entered. Patients randomized to the treatment group were given an intravenous loading dose followed by an infusion for 6 hours. A 5 day period of oral therapy was commenced 1 hour before the end of the infusion. Dosages were adjusted to maintain systolic blood pressure (BP) between 100-120 mmHg. Heart rate and BP were measured at entryand hourly for 6 hours. Indices of infarct size were based upon cumulative CKMB enzyme release, R wave score, and scintigraphic left ventricular ejection fraction (LVEF) as a measure of ventricular function. Continuous ECG monitoring from Medilog recorders was available in 51 control and 49 treated patients. All patients were requested to return 6 weeks and 12 months after their initial event for further assessment. Symptoms of angina and dyspnoea were graded by NYHA classification and all patients underwent graded treadmill exercise testing and measurement of LVEF.

Applications of our dosage resulted in smaller dosages of labetalol than anticipated. Nevertheless this was sufficient to cause a significant reduction in BP and heart rate in the treated group. In the control group the mean initial BP was 139/87, the average pressure over the next 6 hours was 126/82. In the treated group the mean initial BP was 142/83, dropping to 112/76($p<0.001$). Heart rate fell from 77 to 71 in the control group and from 80 to 67 in the treatment grup ($p<0.001$). There was a significantly higher enzyme release in the treated group than in the control group ($p<0.05$). Time to peak enzyme activity was the same in both groups. Total enzyme release did not correlate with fall in BP. R wave score for treatment and control groups was not significantly different. No significant change in LVEF occurred in either group over the first 6 hrs. In those patients for which data was available, ectopic activity was significantly more common in the treated group. Hourly aberrant counts were 52 ± 132 in the treated group and 37 ± 115 in the control group. One patient in the control group and 5 in the treatment group died during hospital admission, of those who survived to leave hospital 13 died in the following year, 7 had received labetalol. There was no significant difference in effort tolerance on the treadmill, scintigraphic LVEF, or symptomatology between the two groups either at 6 weeks or 12 months.

The difficulty we experienced in drug administration according to our protocol suggests that the widespread use of labetalol during the acute phase of myocardial infarction is not practicable. Analysis of our results indicates that it is not beneficial for normotensive paients with AMI as a means of limiting myocardial necrosis, and that the induced lowering of BP may be harmful.

EFFECT OF ANTI-PLATELET COMPOUNDS ON EICOSANOID FORMATION *IN VIVO* FOLLOWING INTRAVENOUS THROMBIN CHALLENGE

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We have previously described a rat i.v. challenge model for evaluating inhibitors of eicosanoid metabolism *in vivo* (Haworth and Carey, 1985). In this study, we have investigated the effects of the anti-platelet compounds anagrelide, papaverine and ticlopidine on rat plasma TXB_2 and 6-oxo-$PGF_{1\alpha}$ levels following i.v. thrombin challenge. Their effects have been compared with the cyclo-oxygenase inhibitor aspirin (ASA) and the thromboxane synthase inhibitors dazoxiben (DAZ) and 2-isopropyl-3-(1-imidazolylmethyl) indole (UK 34787).

Anagrelide (10 $mgkg^{-1}$ p.o.), papaverine (20 $mgkg^{-1}$ i.v.) and ticlopidine (100 $mgkg^{-1}$ day^{-1} p.o. for 3 days) inhibited ADP and arachidonic acid (AA) induced platelet aggregation (PA) in rat heparinised platelet rich plasma *ex vivo* but did not significantly inhibit AA-induced TXB_2 formation. Thrombin challenge (40 NIH units kg^{-1} i.v) in control rats elevated plasma TXB_2 from 260 ± 15 pg ml^{-1} (basal, n = 41) to 1257 ± 75 pg ml^{-1} (n=54) whereas 6-oxo-$PGF_{1\alpha}$ levels were unchanged (basal, 305 ± 17 pg ml^{-1}; thrombin, 316 ± 17 pg ml^{-1}). None of the three anti-platelet agents had any significant effect on plasma TXB_2 or 6-oxo-$PGF_{1\alpha}$ after thrombin challenge (anagrelide, 25 ± 12% and 17 ± 8%; papaverine -19 ± 31% and -2 ± 17%; ticlopidine 23 ± 12% and 32 ± 7% inhibition respectively, n=6). In contrast, ASA (50 mg kg^{-1} p.o.) had no effect on ADP-induced PA but inhibited AA-induced PA and TXB_2 formation (97 ± 0%, n=3) and both plasma TXB_2 (88 ± 1%, n=6) and 6-oxo $PGE_{1\alpha}$ (54 ± 7%) following i.v. thrombin. DAZ (50 $mgkg^{-1}$ p.o.) had no effect on either ADP or AA induced PA but inhibited AA induced TXB_2 formation by 64 ± 4%. However, UK34787 (20 $mgkg^{-1}$ p.o.) inhibited AA-induced PA and TXB_2 formation (93 ± 4%). DAZ and UK34787 inhibited thrombin induced TXB_2 formation *in vivo* by 88 ± 2% and 81 ± 1% respectively but had no significant effect on plasma 6-oxo-$PGF_{1\alpha}$.

These results show that anagrelide, papaverine and ticlopidine, at doses which inhibited platelet aggregation, did not reduce platelet-derived thromboxane formation from endogenous precursors *in vivo*. If TXA_2 is involved in the development of ischaemic disorders, then thromboxane synthase inhibitors or receptor antagonists may be more effective than other anti-platelet agents by removing thromboxane-induced vasoconstriction without reducing the anti-aggregatory and vasodilator properties of PGI_2.

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INCREASED ACTIVATION OF CARDIAC β-ADRENOCEPTOR-LINKED ADENYLATE CYCLASE IN HYPERTENSION.

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Conflicting reports have appeared in recent years regarding the effects of raised blood pressure on peripheral β-adrenoceptor density and function. Thus decreases in cardiac β-receptor number have been reported in spontaneously hypertensive rats (Limas & Limas, 1978) and reduced β-receptor responsiveness (measured as the chronotropic response to isoprenaline) has been observed in hypertensive patients (Mc Allister et al., 1983). On the other hand, increased β-receptor density has been reported on intact mononuclear cells of patients with essential hypertension (Middeke et al., 1983). In the present study we have compared β-adrenoceptor number and catecholamine-linked adenylate cyclase (AC) activity in atrial appendage samples taken from patients undergoing coronary bypass graft surgery. Patients were subdivided into two groups, the first were normotensive while the second group had a prolonged history of hypertension. All patients had been treated for coronary insufficiency with either β-adrenergic blocking agents, calcium antagonists or vasodilators.

Strips of atrial appendage were collected immediately after atrial cannulation and were stored in liquid nitrogen. Cardiac membranes were prepared by a modification of the method of Brodde et al. (1983). Binding of ^{125}I-cyanopindolol (^{125}I-CYP) to membranes was performed at a protein concentration of 0.3 mg/ml in TME buffer (50 mM Tris-HCl, 2 mM Mg^{++}, 1 mM EDTA, pH 7.4) and involved incubation for 60 min at 37°C, follwed by filtration through glass-fibre filters (GF/C). Specific binding was displaceable by 1 μM (-)-propranolol and saturation curves were computer fitted using a nonlinear least squares curve fitting procedure (Metzler et al., 1974). AC activity was determined according to Salomon et al., 1974.

Estimated values for B_{max} and K_D for tissues from both groups of patients are shown in Table 1 (a). It can be seen that while K_D values were not different in the two groups, the tissue B_{max} values were significantly higher for the hypertensive group. The stimulation of AC activity (over basal) by isoprenaline (iso), iso + Gpp(NH)p and NaF is shown in Table 1 (b) and the values in all three cases are significantly higher for the hypertensive group. Since B_{max} values, are also higher for this group, the enhanced response to iso and iso + Gpp(NH)p would be expected, but the greater stimulation by NaF, at the level of the Ns protein would appear to reflect an overall increased level of activity in the β-adrenoceptor-linked AC system of atrial tissue taken from hypertensive patients.

Table 1. Comparison of β-adrenoceptor characteristics in human atrial tissue from normontensive (I) and hypertensive (II) patients (means ± s.e.m.).

(a)	B_{max}	K_D pM	n	(b)	AC activity over basal iso	iso+Gpp(NH)p	NaF	n
I	80±5.36	17.4±4.13	5		1.85±0.20	2.93±0.11	2.44±0.11	4
II	119±6.80	13.9±1.54	5		2.65±0.22	3.54±0.22	3.33±0.22	4

B_{max}: f.mol/mg protein; AC activity over basal: p.mol cAMP/mg/min.

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CHEMOKINETIC ACTIVITY OF 12(S) AND 12(R) HYDROXYEICOSATETRAENOIC ACIDS FOR HUMAN POLYMORPHONUCLEAR LEUCOCYTES

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12-hydroxy 5,8,10,14-eicosatetraenoic acid (12-HETE) has been identified in the lesional skin of patients with psoriasis (Camp et al., 1983), and may contribute to the inflammatory aspects of the disease since it is a chemoattractant for human polymorphonuclear leucocytes (PMNs), and causes erythema in human skin (Turner et al., 1975; Dowd et al., 1985). These properties have been determined by use of either platelet derived 12(S)-HETE or 12(RS)-HETE prepared by photooxidation of arachidonic acid (AA). Recently, the 12-HETE derived from lesional psoriatic scale has been shown to be stereochemically different from platelet 12(S)-HETE but chromatographically similar to 12(R)-HETE (Woollard, 1985). In the present study the (R) and (S) hydroxyl enantiomers of 12-HETE have been separated and their chemokinetic activity for human PMNs compared to that of 12(S)-HETE prepared from platelets.

12(RS)-HETE was prepared by photooxidation of AA. Resolution of the enantiomers was carried out by high performance liquid chromatography of the diastereomeric tert-butyldimethylsilyl ester dehydroabietyl urethane derivatives. Subsequent removal of the urethane and ester groups with trichlorosilane and tetrabutylammonium fluoride respectively yielded 12(R) and 12(S)-HETE. Platelet derived 12(S)-HETE was prepared from human platelets incubated with AA.

The chemokinetic activity of the 12(R) and 12(S) enantiomers for human peripheral blood leucocytes (70 - 80% PMNs) was examined in an agarose microdroplet chemokinesis assay over the dose range 5 x 10^{-8}M to 2 x 10^{-5}M (n = 4).

Both 12(R) and 12(S)-HETE caused dose related chemokinetic responses in human PMNs, the ED_{50} values being 6 x 10^{-7}M and 1.2 x 10^{-6}M respectively. The maximum distance moved by the cells in response to 12(R)-HETE was about 50 percent further than that seen after maximal stimulation with 12(S)-HETE. The dose response curve to platelet derived 12(S)-HETE was no different to that obtained using 12(S)-HETE prepared by photooxidation of AA.

In view of the structural similarity between 12-HETE and the potent chemokinetic agent 5(S),12(R)-dihydroxy-6,8,10,14-eicosatetraenoic acid (leukotriene B_4; LTB_4), the possibility that the 12(R) and 12(S) enantiomers were acting through the LTB_4 receptor was examined. Dose response curves to LTB_4 were compared in the presence and absence of ED_{50} concentrations of 12(S) and 12(R)-HETE and the responses found to be less than additive.

Thus 12(R)-HETE, which has been suggested to be the major stereoisomer of 12-HETE present in lesional psoriatic scale, causes PMN migration in vitro, and appears to be more active than the 12(S) enantiomer which is formed by platelets. 12-HETE may cause PMN chemokinesis by acting as a weak agonist at the LTB_4 receptor.

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EFFECTS OF ONO-3144 ON NORMAL BODY TEMPERATURE AND FEVER IN RABBITS AT DIFFERENT AMBIENT TEMPERATURES

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ONO-3144 (2-aminomethyl-4-tert-butyl-6-propionylphenol hydrochloride) is a non-steroidal anti-inflammatory agent, which does not inhibit cyclo-oxygenase (Aishita et al., 1983). Prostaglandin (PG) biosynthesis is affected, however, by ONO-3144 stimulating PG hydroperoxidase activity converting PGG2 to PGH2. Despite the facilitated formation of PGH2, the precursor of PGE2, a putative mediator of fever (Milton, 1982), ONO-3144 has been reported to produce anti-pyresis in febrile rats (Aishita et al., 1982). The present study investigates the effects of ONO-3144 on body temperature in febrile and afebrile rabbits at different ambient temperatures.

Thermistors were used to measure colonic and ear skin temperatures in restrained half lop rabbits (3-4 kg) at ambient temperatures of 15, 20 and 26°C. A sub-maximal fever was induced by injection i.v. of Salmonella abortus equi lipopoly-saccharide (LPS) 1 µg/kg. Afebrile rabbits received vehicle (0.9% saline 0.2 ml/kg i.v.). ONO-3144 or vehicle (water for injection 1 ml/kg) was administered i.p. 1 h after i.v. injection and body temperatures monitored for a further 4 h. Temperature responses were assessed as the area under the fever curve (change in temperature, °C x time, h) and tested for significance by a Student's t-test.

ONO-3144 10 mg/kg, but not 2 mg/kg, reduced the febrile response to LPS in rabbits at an ambient temperature of 20°C ($P<0.05$, $n=6$). The decrease in colonic temperature was associated with a rise in skin temperature of 7.4±1.7°C (mean± s.e.m.) 1 h after injection of ONO-3144 compared to a fall of 0.2±2.0°C after vehicle. ONO-3144 (10 mg/kg i.p.) produced a similar rise in skin temperature and a fall in colonic temperature ($P>0.05$) in afebrile rabbits held at 20°C. The effects of ONO-3144 (10 mg/kg i.p.) on skin and colonic temperatures were accentuated ($P<0.05$, $n=5$) in afebrile, but not in febrile rabbits at an ambient temperature of 15°C. Cold-exposed rabbits had a skin temperature of 23.2±1.0°C before treatment compared to 26.9±0.7°C when held in a room at 20°C. At an ambient temperature of 26°C, skin temperature before treatment was 35.9±0.3°C, and ONO-3144 (10 mg/kg i.p.) did not have significant effect on skin or colonic temperature in either febrile or afebrile rabbits.

ONO-3144 (10 mg/kg i.p.) killed two rabbits in this study, both at an ambient temperature of 15°C. Vasodilatation of visceral blood vessels was evident at examination post mortem.

These data indicate that the reduction of fever in rabbits by ONO-3144 is attributable to heat loss secondary to cutaneous vasodilatation, as indicated by an increase in skin temperature, and not to a specific antipyretic effect independent of environmental temperature.

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PROSTAGLANDIN AND CARCINOGENESIS IN RAT COLON: EFFECT OF DIET AND INDOMETHACIN

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Cyclooxygenase inhibitors have been reported to inhibit carcinogenesis (Carter et al, 1983). The gut contains cyclooxygenase and prostaglandins have been implicated in large bowel cancer (Bennett et al, 1977). This study examines whether a high fat diet, a known dietary risk factor for colonic cancer, affects colonic tissue PGE_2 levels and evaluates the influence of cyclo-oxygenase inhibition on colonic carcinogenesis.

Colonic carcinogenesis was induced in male Swiss albino rats by 12 weekly injections s.c. of azoxymethane (10 mg/kg). These animals were fed either normal rat diet (Oxoid) containing 4% crude fat or a high fat diet containing 25.2% crude fat (10.9% unsaturated). Groups received in their drinking water, either indomethacin (Sigma Ltd) for 4 or 24 weeks ($30mg.ml^{-1}$), 0.25% ethanol in water as a solvent control, or water alone. Prostaglandin content of colonic tissue was measured as ng PGE_2 per g wet weight of tissue by radio-immunoassay (Coker et al, 1982).

Table 1 The effect of diet and cyclooxygenase inhibition on mean tissue levels of PGE ($ng.g^{-1}$) and on tumour induction in rat large bowel. Brackets indicate standard deviations.

Diet		Normal	Normal	Normal	High Fat
Treatment		-	Alcohol	Indometh.	-
Number		6-12	4-5	6-11	4-10
	Wks.				
Caecum	4	66 (32)	110 (27)	48 (20)	59 (17)
	24	76 (12)	141 (27)	56 (21)	34 (11)
Dist.Colon	4	133 (94)	192 (55)	70 (52)	239 (289)
	24	163 (59)	256 (110)	153 (48)	128 (92)
Rectum	4	134 (96)	273 (67)	88 (48)	102 (36)
	24	167 (40)	260 (72)	159 (55)	184 (51)
Tumour Incidence	24	11/12	0/5	5/11	3/10

In untreated healthy animals (Table 1), the mean PGE_2 content was higher in the distal colon and rectum, where the incidence of tumours was greatest in azoxymethane treated animals. While the number of tumours was reduced in the high fat group, the mean PGE_2 content was unchanged. While ethanol alone had no significant effect on tissue PGE_2 levels, PGE_2 content was reduced at all levels of the large bowel in the animals given indomethacin ($P < 0.05$). Although tumour incidence in both ethanol and indomethacin groups was significantly less than in the untreated group ($P < 0.05$), it was not possible to demonstrate a greater effect of indomethacin over ethanol.

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ANTAGONISM OF THROMBOXANE AND PROSTAGLANDIN D_2 RESPONSES BY N-0164 IN GUINEA-PIG PLATELETS

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The experimental compound N-0164 is considered a selective antagonist of the anti-aggregatory actions of PGD_2 on human platelets (MacIntyre & Gordon, 1977; Whittle et al., 1978). Previous studies in guinea-pig platelet-rich-plasma (PRP) have shown that PGD_2 produces a bell-shaped dose-response relationship, with complete inhibition of platelet aggregation not being achieved (Hamid & Whittle, 1985). In the present study, the activity of N-0164 as an antagonist of these PGD_2 responses in guinea-pig PRP has therefore been studied. We have also investigated whether interaction of PGD_2 at the thromboxane receptor sites in guinea-pig platelets could explain the nature of the dose-response relationship using two selective thromboxane antagonists of divergent structures, 13-aza prostanoic acid (13-AZA; Venton et al., 1979) and BM 13.177 (Patscheke et al., 1984).

Blood was collected from the abdominal aorta of anaesthetised guinea-pigs into tubes containing tri-sodium citrate (final concentration 0.315%) and centrifuged at 3000 r.p.m. for 2 min to obtain PRP (Hamid and Whittle, 1985). Aggregation was induced by a submaximal concentration of ADP (2-4 μM) and dose-inhibition curves were constructed for PGD_2.

N-0164 at a low concentration (75 μM) converted the PGD_2 bell-shaped curve to a sigmoid dose-inhibition curve, exposing it as a full inhibitor of aggregation. Similar effects were observed with the selective thromboxane antagonists. Both 13-AZA (16-64.4 μM) and BM 13.177 (5.9-29.8 μM) converted PGD_2 to a full inhibitor of aggregation in a dose-related manner. Higher doses of these thromboxane antagonists did not further shift the PGD_2 curves. In contrast, increased concentrations of N-0164 (380 and 760 μM) caused a dose-related rightward shift of PGD_2 dose-inhibitory curves, with the ID_{50} value (0.29 ± 0.02 μM, n=9) being increased to 0.9 ± 0.18 and 2 ± 0.6 μM respectively ($P<0.001$). Although N-0164 (760 μM) did not significantly shift the dose-inhibition curve for PGI_2, it did cause a rightward shift of the curve for BW245C, which is considered to act on PGD_2 receptors (Hamid & Whittle, 1985); the ID_{50} value for BW245C was increased from 46 ± 4 nM to 277 ± 55 nM ($P < 0.01$).

To investigate further whether N-0164 was acting as a thromboxane receptor antagonist, its effects on aggregation induced by U-46619, a thromboxane mimetic (Coleman et al., 1981) was evaluated. N-0164 (38 - 760 μM) shifted the U-46619 dose-aggregation curve to the right in a concentration-related manner, with a pA_2 value of 4.8 and a slope of 1.05.

These results, using selective thromboxane antagonists, indicate that at high concentrations PGD_2 can interact at guinea-pig platelet thromboxane receptors. Previous studies on platelets have shown that N-0164 acts as an antagonist of PGD_2, as confirmed in the present study. However, our findings also demonstrate that N-0164 can also act as a thromboxane receptor antagonist in guinea-pig platelets.

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STIMULANT EFFECTS OF CIGARETTE SMOKING ON EEG

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Tobacco smoking has been shown to decrease EEG theta and alpha power and increase dominant alpha frequency in smokers deprived of cigarettes for at least 10 hours (Herning et al., 1983). However there is little systematic evidence concerning the effects of cigarette smoking on EEG under short periods of tobacco deprivation, and a question arises as to whether such results can be generalised to more naturalistic periods of smoking deprivation (Golding and Mangan, 1982; Ashton and Stepney, 1982; Mangan and Golding, 1984). The present study examines the effects of cigarette smoking on EEG, visual evoked potentials (VEP) and photic driving (PD) in habitual cigarette smokers allowed to smoke ad lib up to one hour prior to experimentation.

Healthy male and female volunteers (mean age 23.6±3.9 years) habitual smokers were allocated to (n = 15) real smoking (1.3 mg nicotine cigarette) or (n = 15) sham smoking (0 mg nicotine cigarette) groups; sex and average cigarette smoking were balanced between groups. Mean number of cigarettes smoked prior to experimentation since waking (3.3±3.1 cigs) was equivalent across groups. Bipolar electrode positions were midway between C_z-O_z to linked mastoids, with earth on forehead mid-hairline. EEG, with eyes open and closed, VEP, PD, heart rate (HR) and exhaled carbon monoxide (CO) were recorded before and after real or sham smoking.

Real as opposed to sham smoking significantly increased relative power in all the beta bands, decreased alpha and theta activity and increased dominant alpha frequency. The effects in the alpha and theta bands depended on whether eyes were open or closed. Mean effects of smoking for delta bands, VEP and PD failed statistical significance at the 0.05 level. HR and CO were significantly elevated by real smoking. Correlational analysis indicated that variables such as the number of prior cigarettes smoked, basal CO level, personality and basal EEG activity level significantly predicted variation in the effect of smoking on EEG.

Although individual differences in response occurred, the overall effect of cigarette smoking on tonic (but not phasic) EEG under conditions of minimal tobacco deprivation was predominantly stimulant. Thus, the stimulant effect of smoking cannot be entirely due to a simple reversal of a tobacco withdrawal syndrome.

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ICI 159995; INHIBITION OF BRONCHOCONSTRICTION IN THE GUINEA-PIG

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Antigen-induced contractions of human bronchus in vitro are mediated, at least in part, by cyclo-oxygenase product release (Dunlop and Smith, 1975). However the role played by arachidonic acid metabolites in the bronchoconstriction, mucus hypersecretion and oedema formation characteristic of human allergic asthma remains controversial. We have investigated the effects of ICI 159995 (5(z)-7- [2,2-dimethyl-4-phenyl-1,3-dioxan-cis-5-yl]heptenoic acid), a specific competitive thromboxane(TXA_2) receptor antagonist (Jessupet al, 1985) on pulmonary function in the guinea-pig during experimental provocation tests.

Dunkin-Hartley guinea-pigs (♂, 250-350g) were anaesthetised with pentobarbitone, artificially ventilated, and airflow was measured by the method of Konzett and Rossler (1940). The TXA_2 mimetic U-46619 caused reproducible, dose-related bronchoconstriction when administered cumulatively (0.2-4 µg/kg i.v.) at 30 minute intervals. ICI 159995 (5 mg/kg i.v.) inhibited this response, yielding dose-ratios of 25.3±2.9, 12.3±4.1, 6.9±3.0 and 2.9±1.0 (mean ± s.e.m., n=4) at 2,30,60 and 120 minutes respectively. ICI 159995 (5 mg/kg i.v.) also modified leukotriene D_4-induced bronchospasm with a maximum dose-ratio (57.4±13.5, n=5) recorded 2 minutes after antagonist administration. Similarly the bronchospastic activities of both $PGF_{2\alpha}$ and PGD_2 were inhibited by ICI 159995 (5 mg/kg i.v.) whereas responses to histamine were unaffected.

We also measured airway resistance (Raw) and dynamic compliance (Cdyn) in lightly anaesthetised (alphaxalone, alphadolone acetate) spontaneously breathing guinea- pigs. While not itself causing bronchospasm in this model, ICI 159995 (5 mg/kg i.v.) did modify changes in Raw and Cdyn produced by U-46619, yielding a dose-ratio of 13.4±2.2 (n=5) 15 minutes after antagonist administration. This dose of ICI 159995 also reversed ongoing bronchoconstriction to U-46619, respiratory parameters returning to baseline in 1 minute, compared with > 10 minutes in control animals. Leukotriene D_4, $PGF_{2\alpha}$ and arachidonic acid-induced changes in Raw and Cdyn were also inhibited by ICI 159995, whereas bronchoconstriction produced by acetylcholine and histamine and PGE_2 bronchodilatory responses were unaffected.

These data suggest that if arachidonic acid metabolites play a role in bronchial asthma in man, then thromboxane antagonists with the pharmacological profile of ICI 159995 may have therapeutic value.

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LYMPHOCYTE β-ADRENOCEPTORS AND MULTIPLE PROSTAGLANDIN RECEPTORS - EFFECTS OF AGEING AND SMOKING

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The predominance of the β_2-subtype within the lymphocyte β-adrenoceptor population and its capacity to undergo changes in density and responsiveness have been established. Lymphocyte prostaglandin receptors have received less attention, however, and the full range of lymphocyte prostaglandin receptors is not yet determined.

Results of an investigation of the effects of ageing and smoking on lymphocyte β-adrenoceptor and prostaglandin receptors will be presentedin addition to results of a preliminary investigation of lymphocyte adenylatecyclase linked prostaglandin receptors.

Circulating lymphocytes were isolated by ficoll density centrifugation following removal of platelet rich plasma. Isolated lymphocytes were then washed twice in phosphate buffered saline with centrifugation for 10 minutes at 150g and 4°C. Dose response curves of agonist induced cyclic AMP accumulation were obtained following five minute incubations of intact cells in the presence of isobutylmethylxanthine (5×10^{-4} M). β-adrenoceptor density was measured in cell membranes using (-)-[^{125}I]-iodocyanopindolol.

β-adrenoceptor density increased with age over the range 18-65 years (p 0.001, n=37), while maximum β-adrenoceptor response to isoprenaline decreased with age (p 0.05, n=37). Maximum responses to epoprostenol (PGI_2) were not related to age. β-adrenoceptor density was similar in male smokers (n=12) and age matched male nonsmokers (n=12). Maximum responses to isoprenaline and PGI_2 were reduced in the smoking group, but without statistical significance. In a comparison of the prostaglandins PGE_2, PGD_2 and PGI_2 responses of similar magnitude were obtained from all three, but with low Hill coefficients; 0.36, 0.36 and 0.45 respectively (n=4). EC_{50} values ranged between 10^{-8}-10^{-6} M, with the following order of potency: $PGE_2 > PGD_2 > PGI_2$. β-adrenoceptor responses to (±) isoprenaline exhibited a Hill coefficient of 0.96 and an EC_{50} of 3×10^{-8} M (n=8).

The findings in ageing are consistent with previous reports (Krall *et al*, 1981; Doyle *et al*, 1984) and support the concept of reduced coupling of lymphocyte β-adrenoceptors with advancing age in man (Feldman *et al*, 1984). Responses to all three protaglandins indicates the presence of EP, DP and IP receptors, according to classification by Coleman *et al*, (1984). Interaction at more than one binding site is indicated by the low Hill coefficient obtained with the three prostaglandins and could be attributed to loss of selectivity at higher concentrations.

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STEREOCHEMICAL RELATIONSHIP OF ORGANIC NITRATES FOR CORONARY PGI_2 STIMULATION, VESSEL TONE AND PLATELET FUNCTION

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Earlier investigations have demonstrated a stimulation of coronary vascular prostacyclin (PGI_2) formation by a number of nitrates including glyceryltrinitrate (Schrör et al. 1981) and isosorbidedinitrate (Darius et al. 1984). The present study was designed (I) to investigate the PGI_2 stimulating action of the new organic nitrate teopranitol (2-[γ-theophylline-7-ylpropylamino]-2-desoxy-L-isoidide-5-nitrate-bifumarate) and a number of structurally related isohexides, (II) to determine the relationship between PGI_2 stimulation and vessel relaxation, (III) to investigate the significance of a PGI_2-associated mechanism of organic nitrates for inhibition of platelet secretion in human volunteers ex vivo.

Bovine coronary arteries (BCA) were prepared and either helically cut into strips or punched into slices. Strips were used for detecting changes in active vessel tone, measured isotonically. Slices were used for bioassaying PGI_2 by measuring the antiaggregatory effect of vessel incubates on ADP-induced aggregation of human platelets in platelet rich plasma (Darius et al. 1984). Inhibition of collagen (1.2µg/ml) induced ATP-secretion was measured ex vivo in whole blood taken from 6 healthy volunteers before and 1 h after ingestion of 20 mg teopranitol or placebo (double blind, crossover design).

All of the nitrates studied produced a dose-dependent relaxation of the BCA. The isoidide teopranitol was the most effective compound, followed by the isosorbide derivatives with the free nitro-group in [exo]-position (KC-116) and [endo]-position (KC-144), whereas the isomannide analogue (KC-146) was the least effective compound. With teopranitol, there was a clear biphasic dose-response relationship, exhibiting a first peak at 2µmoles/l and maximum at 0.1 mmoles/l. The decrease in vessel tone at 2 µmoles/l teopranitol amounted to 70% of maximum response, 50% with KC-144 but only 35% with KC-146. Removal of the caffeine residue with (KC-087) or without (KC-070)additional removal of the ethyl chain resulted in a disappearance of the biphasic reaction and a shift of the threshold concentration from 10 nmoles/l to 100 nmoles/l with both compounds ($P < 0.05$).

Teopranitol increased PGI_2 2-3 fold above control within 20 min of incubation at 2 µmoles/l ($P < 0.05$, $n = 4-7$). At the same concentration, there was also a significant stimulation by KC-116 (n=6) while KC-144, KC-146, KC-087 and KC-070 were ineffective. Administration of 20 mg teopranitol to human volunteers resulted in a 50% inhibition of ATP secretion ($P < 0.05$) while placebo was ineffective. There was no direct antiplatelet action by teopranitol in vitro up to 20 µmoles/l.

The data suggest an important role for the steric position of the free nitro-group of the isohexides investigated for PGI_2-stimulation. It also concluded that the methylxanthine component enhances both vessel relaxation and PGI_2 production by teopranitol. The significance of nitrate stimulated PGI_2 in vivo might mainly be an inhibition of platelet secretion.

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THE EFFECT OF PLATELET-DERIVED SUBSTANCES ON BRONCHOCONSTRICTION IN GUINEA PIGS

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There is much evidence to suggest the involvement of platelets in allergic bronchoconstriction since it has been shown that substances released from activated platelets, including thromboxane A_2 (TxA_2), prostaglandin endoperoxides, PAF-acether and histamine, are potent bronchoconstrictors.

In guinea-pigs (300-800g) anaesthetised with a mixture of urethane (290 mg/kg) and allobarbitone (70 mg/kg), lung inflation pressure (LIP) was recorded using a modified Konzett-Rossler system. The carotid artery was cannulated to allow continuous blood sampling for simultaneously monitoring intravascular platelet aggregation. (1)

Collagen (80 µg/kg i.v.) produced reproducible increases in LIP and platelet aggregation. The effects of indomethacin, FPL 55712 a leukotriene antagonist, dazoxiben a thromboxane synthetase inhibitor and ketotifen a drug used clinically in prophylactic asthma treatment, were then studied on collagen-induced bronchoconstriction and platelet aggregation. After obtaining two control responses to collagen these drugs were administered 5 mins before a third dose of collagen, except for FPL 55712 which was given 1 min prior to collagen since it has a short plasma half-life.

Compound	Dose mg/kg	% Inhibition of increase in LIP	% Inhibition of aggregation
Indomethacin	0.5	80.26 ± 10.88	50.45 ± 8.78
FPL 55712	1	N.S.	18.23 ± 6.44
	10	83.58 ± 4.97	40.10 ± 26.5
Dazoxiben	1	65.88 ± 4.15	30.7 ± 7.30
Ketotifen	0.5	61.42 ± 11.71	N.S.

Paired student's 't' test used to assess significance ($P<0.05$). (n=5-8)

Collagen acts directly on the platelet membrane to stimulate the platelet activation and release reaction but has no direct effect upon airway tissue. In the guinea-pig up to 60% of the aggregating response is due to the action of endogenous TxA_2 (2) which is also a potent bronchoconstrictor, as indicated by the inhibiting effects of indomethacin and dazoxiben on both LIP and platelet aggregation. It has been suggested that NSAIDs enhance bronchoconstriction by a mechanism involving increased arachidonate metabolism being diverted via the lipoxygenase enzyme to leukctrienes. These results show indomethacin to be acting via platelet arachidonate metabolism to inhibit TxA_2 production and consequently the bronchoconstrictor response.

Ketotifen acts predominantly by inhibiting mediator release, particularly the leukotrienes, during anaphylaxis, it also has strong antihistaminic properties. These results are consistent with an action inhibiting bronchoconstriction while having little effect on platelet aggregation. The selectivity of FPL 55712 as a leukotriene antagonist in the guinea-pig has already been questioned (3) and the antiaggregatory effects of both low and high doses again brings this into question.

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FLUFENAMIC ACID INHIBITS PROSTAGLANDIN $F_{2\alpha}$, E_2 AND D_2 BIOSYNTHESIS IN SENSITISED GUINEA-PIG LUNG

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The non-steroidal anti-inflammatory drug (NSAID) flufenamic acid has recently been shown to inhibit arachidonic acid metabolism associated with lung microsomes (McNamee et al., 1985), RBL-1 cell homogenates (Sturton et al., 1984) and soyabeans (Davis et al., 1984). The ability of flufenamic acid to inhibit both the cyclo-oxygenase and lipoxygenase pathways may be important in the control of antigen-induced bronchoconstriction. The present study investigates the effect of this NSAID on the cyclo-oxygenase pathway associated with sensitised lung tissue.

Lung microsomes were prepared by the method of Remmer et al. (1967) from Duncan-Hartley guinea-pigs (500-800g) which had become sensitised following the administration of 25mg S.C. and 50mg I.P. ovalbumin in 0.9%(w/v) NaCl. Cyclo-oxygenase activity was determined by measuring the formation of prostaglandins (PG) $F_{2\alpha}$, E_2 and D_2 from ^{3}H-arachidonic acid according to the methods of Blackwell et al. (1975) and McNamee et al. (1985).

In the absence of flufenamic acid, microsomal fractions from sensitised guinea-pig lungs converted ^{3}H-arachidonic acid to 0.57 ± 0.09pmol $PGF_{2\alpha}$, min^{-1} mg $protein^{-1}$, 0.37 ± 0.05pmol PGE_2 min^{-1} mg $protein^{-1}$ and 0.1 ± 0.03pmol PGD_2 min^{-1} mg $protein^{-1}$ (all n=5), in the ratio of 5.7 : 3.7 : 1 respectively. The optimum substrate concentration was 150nM ^{3}H-arachidonic acid, a concentration three times lower than the corresponding optimum used in a previous study using non-sensitised lungs.

Table 1. Inhibition of $PGF_{2\alpha}$, PGE_2 and PGD_2 Formation From Microsomal Fractions of Sensitised Guinea-Pig Lung by Flufenamic Acid.

Source of Microsomal Fraction	Prostaglandins.		
	$PGF_{2\alpha}$ IC_{50} (μM)	PGE_2 IC_{50} (μM)	PGD_2 IC_{50} (μM)
Untreated Lung	1.4 ± 0.2	2.8 ± 0.5	1.3 ± 0.1
Sensitised Lung	0.6 ± 0.04	0.7 ± 0.02	0.9 ± 0.10

Results are the means of five different experiments ± s.e.mean.

In the presence of 1-10μM flufenamic acid there was a concentration-dependent inhibition of $PGF_{2\alpha}$, PGE_2 and PGD_2 formation. In terms of IC_{50} values, (the concentration required to reduce the maximum response by 50%), flufenamic acid was found to be equipotent in preventing the formation of $PGF_{2\alpha}$, PGE_2 and PGD_2 (Table 1). Microsomal preparations from non-sensitised animals were found to be less sensitive to flufenamic acid and showed a differential effect, with the formation of PGE_2 being less sensitive to the anti-inflammatory agent (Table 1).

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CHRONIC LITHIUM TREATMENT INHIBITS 5-HYDROXYTRYPTAMINE AND CARBACHOL STIMULATED PHOSPHOINOSITIDE METABOLISM IN RAT BRAIN SLICES

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The pharmacological mechanism by which lithium salts act to produce their therapeutic effect in affective disorders is unknown. Grahame-Smith and Green (1974) showed that Li^+ increased certain aspects of the functional activity of 5-hydroxytryptamine (5-HT) in rat brain, probably on the basis of increased 5-HT release. Recently 5-HT has been shown to increase inositol phospholipid breakdown in brain, probably mediated through 5-HT_2 receptors (Kendall and Nahorski, 1985; Minchin, 1985). Sherman et al (1981) showed that Li^+ inhibited myo-inositol-1-phosphatase resulting in an increase in brain inositol-1-phosphate and a decrease in myo-inositol. Berridge et al.(1982) postulated that Li^+ might exert its therapeutic effect by interfering with inositol lipid metabolism in brain. The complexities of the interactions between 5-HT function, lithium and phosphoinositide metabolism prompted us to investigate, in rat brain slices, the effect of Li^+ administration on inositol lipid breakdown, produced by 5-HT and, as a comparison, the muscarinic cholinergic agonist carbachol.

Rats were given LiCl or NaCl (3 mEq/kg s.c.) twice daily for either 3 or 18 days, and 16h after the last dose cerebral cortex slices were prepared, preincubated with ^{3}H-inositol and stimulated with 5-HT or carbachol. ^{3}H-inositol phosphates (^{3}H-IP) were separated and assayed as described previously (Godfrey et al.,1985). Other rats were given one dose of 10mEq/kg LiCl or NaCl 4h prior to removal of the brain and estimation of phospholipid responses. Plasma Li^+ concentrations were determined by atomic absorption spectrometry.

Both carbachol and 5-HT increased the formation of ^{3}H-IP in a dose-dependent fashion with maximal increases of 300% and 100% respectively. Treatment with LiCl for 3 days decreased the maximal response to carbachol by 35% and to 5-HT by 50% (plasma Li^+ 2.4 ± 0.5 mM 16h after the last dose). Treatment with LiCl for 18 days reduced the maximal responses to carbachol and 5-HT by 30% and 40% respectively. A single dose of 10mEq/kg LiCl caused no significant change in inositol phospholipid breakdown in response to either agonist. Chronic lithium treatment did not affect the labelling, in the brain slices, of the lipids with ^{3}H-inositol.

These experiments show that chronic lithium treatment in rats reduces the inositol phospholipid breakdown not only in response to 5-HT but also in response to carbachol. It remains to be seen whether these effects are receptor mediated or whether they are the result of a more generalised effect of Li^+ on phosphoinositide metabolism.

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RESPONSES OF RAT LYMPHOID TISSUE TO A PERFLUOROCARBON BLOOD SUBSTITUTE

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Previous work has shown that emulsified perflurocarbons (PFC) can be used as oxygen-carrying blood substitutes and some of their physiological effects have been studied in several species, including man (Lowe, 1984a,b; Lowe & Bollands, 1985). The most widely tested emulsions are the proprietary preparations, Fluosol-DA 20% (F-DA) and Fluosol-43 (FC-43; Green Cross, Japan). However, F-DA can impair normal reticuloendothelial system (RES) clearance function in rats (Castro et al., 1984) while both F-DA and FC-43 can have adverse effects on mammalian phagocytic and other cells in vitro (Bucala et al., 1983; Lowe et al., 1984; Wake et al., 1985). These observations therefore raise the question of whether treatment with PFC can alter animals' ability to respond to immunological 'challenges' and this has been investigated in the present experiments.

Adult female Wistar rats (body weight: 122-166g; n = 32) were used. Animals were initially injected intraperitoneally (i.p.) or intravenously (i.v.) via a tail vein with either 5ml.kg ^{1} (low dose) or 10 ml.kg ^{1} (high dose) F-DA; control animals received comparable doses of physiological saline (0.9% w/v NaCl). After 24h, all animals were then injected i.p. with a single bolus of 5×10^{8} washed sheep erythrocytes suspended in 1.0 ml Hank's balanced saline solution. Animals were subsequently sacrificed for post-mortem after 7 days and the weights of liver, spleen, thymus and gut mesenteric lymph nodes (MLN) recorded. Measurements were also made of haematocrit (Hct) and 'fluorocrit' (Fct). The specific antibody titre to sheep red cells was estimated in plasma samples using a conventional serial-dilution haemagglutination test.

Spleen weight was increased significantly ($P<0.01$) following injection with both doses of F-DA whereas liver and thymus weights were unchanged in each case. MLN weights were significantly ($P<0.02$) greater in animals receiving i.v. F-DA at both low and high doses. A consistent fall in Hct occurred in both F-DA-treated ($P<0.05$) and control animals injected with the high dose of saline ($P<0.05$) during the course of the experiments. No detectable Fct was measured in animals receiving F-DA. The mean plasma antibody titre against sheep red cells was significantly ($P<0.05$) increased in animals pre-treated with high dose i.p. F-DA but was similar to that in control animals in all other cases.

These results are in conflict with previous preliminary findings that pre-treatment of rats with comparable doses of either F-DA or FC-43 markedly reduced in vivo antibody production to sheep erythrocytes (Shah et al., 1984). However, this difference may reflect species variations in the physiological responses to emulsified PFC although this has not been studied systematically. The present experiments nevertheless demonstrate that administration of F-DA in doses sufficient to produce increases in spleen and MLN weights is able to significantly increase antibody production against sheep red cells in rats.

A.D.B. is an M.R.C. Scholar.

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KINETICS OF METHAEMOGLOBIN PRODUCTION BY PAPP OR DMAP

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After administration of methaemoglobin-producing substances, methaemoglobin levels are determined by the rate of the reaction haemoglobin to methaemoglobin as catalysed by the xenobiotic, by the rate of spontaneous oxidation of haemoglobin and by the rate of the back reaction catalysed by the enzyme methaemoglobin reductase. Although there is evidence both in vitro (Bright and Marrs, 1982) and in vivo (Klimmek et al, 1983) that dog and human haemoglobin differs in some respects in sensitivity to methaemoglobin producers, the greater part of interspecies differences in response to these compounds appears to be caused by different levels of NADH-linked methaemoglobin reductase in the red cell (Calabrese, 1983). The similarity of NADH-linked methaemoglobin reductase activity in the dog and human and the dissimilarity of rodents has caused the dog to be used as a model for human drug-induced methaemoglobinaemia.

Methaemoglobin estimations were carried out on beagle bitches using an IL 282 CO-oximeter modified to read out absorbances; the data thus obtained were correlated with a computer program designed to output methaemoglobin levels. After intravenous injection of 4-aminopropiophenone (p-aminopropiophenone, PAPP) or dimethylaminophenol (DMAP) further methaemoglobin estimations were carried at intervals of not more than 10 min. Differential equations describing the kinetic models were solved for each animal using a Gould-SEL 32/27 micro-computer and an ISIS simulation package. Assuming $\overline{PAPP}$ is the total concentration of PAPP and its active metabolites, k_e their effective rate of elimination, k_1 the catalysed rate of oxidation of haemoglobin, k_{1S} the spontaneous rate of oxidation, k_2 the rate of reduction of methaemoglobin and $\overline{PAPP}^1$ and met^1 are rates of change of these species:-

$$\overline{PAPP}^1 = -k_e \times \overline{PAPP}$$

and $$Met^1 = k_1 \times \overline{PAPP} \times Hb + k_{1S} \times Hb - k_2 \times met$$

moreover at time zero $k_{1S} \times Hb\ \emptyset = k_2 \times Met\ \emptyset$, where Hb Ø and Met Ø are haemoglobin and methaemoglobin levels at this time.

Using intravenous PAPP at doses of 0.2-0.5 mg kg^{-1} profiles were analysed and rate coefficients calculated giving the least sum of squares of differences between observed and calculated points. k_2 was almost constant at 0.012 min^{-1} while k_1 was 0.018-0.028 min^{-1} mg^{-1} kg and k_e 0.018-0.033 min^{-1} in the different animals (range of results). DMAP was administered at a constant dose of 1.2 mg kg^{-1}: in spite of the much earlier peak methaemoglobin level observed (10 min as against 60 min for PAPP), the same model adequately described methaemoglobin profiles. An almost identical value for k_2 was obtained whilst k_e ranged from 0.15 to 0.17 min^{-1}. k_1 was constant at 0.040 min^{-1} mg^{-1} kg. Despite the difference between the mechanism of action of PAPP and DMAP (the former needs metabolic activation) the kinetic model gave a good description of methaemoglobin profiles in both cases; indeed, with PAPP, even when the dose was varied. The main difference between PAPP and DMAP lay in the very much higher k_e of the latter.

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CHRONIC ALCOHOL TREATMENT PREVENTS THE DIABETIC OBESITY SYNDROME OF MALE CBA MICE

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The CBA/CA mouse colony of the London Hospital Medical College has been reported to have developed a spontaneous diabetic obese syndrome in a proportion of males (Campbell & Das, 1982). An inbred colony of mice derived from them has been established at Bristol and, as previously reported (Connelly & Taberner 1985), has been found to exhibit hyperglycaemia, hyperinsulinaemia, hyperphagia and obesity in all males from 16-20 weeks of age. We have previously shown (Connelly et al.,1983) that C57BL mice, which are mildly hyperglycaemic and prefer ethanol solutions to water, respond to chronic alcohol treatment by a fall in plasma glucose level (PGL). This investigation has therefore studied the effects of chronic ethanol on the development of the diabetic obesity syndrome of the non-alcohol preferring male CBA mice.

Adult mice (8 weeks old) were maintained on 20%(w/v) ethanol as their sole fluid source for 12 weeks. Controls had free access to water. The PGLs were monitored by tail bleeding under ether anaesthesia, and the extracted plasma assayed using a Beckman Glucose Analyser 2. Serum insulin was assayed by the RIA method of Herbert et al.(1965). Liver plasma membranes were prepared using Percol self-forming gradients and incubated at 4° for 24h with A^{14}-I^{125} insulin for measurement of displacement by cold insulin.

Table 1. Values are means ± SEM of ≥8 mice per group.

Age (wks)	Treatment	Body wt (g)	PGL (mM)	Serum insulin (μU/ml)
8	-	26.4 ± 0.8	10.66 ± 0.30	16.6 ± 2.9
25	-	38.7 ± 1.2*	13.38 ± 0.77	195.0 ± 40*
25	Ethanol	27.8 ± 0.9	12.93 ± 0.55	22.0 ± 3.6

Controls > ethanol group, $P < 0.01$ * (uncorrelated 2-tailed t-test).

The results shown in the table indicate that the chronic ethanol treatment prevented the obesity and hyperinsulinaemia that occurred in aging control mice. During ethanol treatment the food intake was reduced by about 20% from control. The high affinity binding of insulin to liver plasma membranes (Bmax) was significantly reduced in untreated obese mice compared to controls; insulin affinity (1/Kd) was not significantly altered. We conclude that the effect of the ethanol on obesity could be dietary, but that the effects on insulin output are more likely to be a metabolic effect exerted directly or indirectly on the pancreas.

DMC is an MRC scholar.

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FURTHER STUDIES WITH BUPROPION-INDUCED GLUCOSE UPTAKE INTO THE RAT ISOLATED HEMIDIAPHRAGM

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Previous work shows that drugs which promote glucose uptake into skeletal muscle can be catagorised: a) fenfluramine type, insulin dependent antagonised by methysergide (Kirby and Turner, 1977); b) ciclazindol type, insulin independent antagonised by membrane stabilising agents (Kirby and Williams, 1978). Bupropion also increases glucose uptake (Kirby, Pleece and Redfern, 1983) and it is of interest to determine to which category its actions fall.

Hemidiaphragms were prepared from male CFY rats (University of Bath strain) (120-140g) previously fasted overnight (method of Kirby and Turner, 1975). All results are within rat comparisons expressed as mean ±s.e.m. and change in glucose uptake as mg glucose taken up/g wet weight of tissue/90min. and carried out in the presence of 100μU.ml^{-1} insulin, as in its absence no significant effect was found using the 500ng maximal concentration of bupropion; change in glucose uptake being - 1.46±2.79, n=6. This maximal concentration of 500ng.ml^{-1} was used in all antagonist studies, while a concentration of 250ng.ml^{-1} of each antagonist was used. At this concentration no antagonist itself altered glucose uptake significantly.

Table 1 - Effect of antagonists on bupropion induced glucose uptake

Antagonist	Change in glucose uptake	n	Significance
Atenolol	+0.27±0.02	6	n.s.
Atropine	-0.27±0.99	6	n.s.
Cimetidine	-0.05±3.67	6	n.s.
Haloperidol	-0.77±0.46	5	n.s.
Indomethacin	+0.01±1.29	6	n.s.
Lignocaine	-0.01±0.36	6	n.s.
Mepyramine	+0.57±2.80	6	n.s.
Methysergide	+1.41±3.52	6	n.s.
Propranolol	-1.41±1.43	6	n.s.
Suxamethonium	+0.84±0.60	6	n.s.
Tubocurarine	+0.77±1.01	6	n.s.
Verapamil	+0.37±3.48	6	n.s.

Thus no antagonist inhibited bupropion. Finally a direct comparison of fenfluramine and bupropion was made using 250ng.ml^{-1} of each. No difference in activity was found (difference in uptake -0.14±3.95 n=6, n.s.). Thus bupropions effect on glucose uptake is similar to fenfluramine, the two compounds being equipotent and insulin dependent. But it is not antagonised by methysergide nor indeed any of the antagonists used in this study.

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ANATOMICAL DEPOSITION AND EARLY DISTRIBUTION OF AN INHALED RADIOAEROSOL IN CONSCIOUS AND ANAESTHETISED RATS

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Small rodents, particularly rats, are often the subject of pharmacological and toxicological investigations of the effects of inhaled aerosols. In the present study we have used a simple and generally applicable isotopic method to compare the relative anatomical deposition and early distribution of aerosolised particles following head-only exposure of either conscious or anaesthetised rats.

Conscious or anaesthetised (Inactin 75mg^{-1} i.p.) male Wistar strain rats (235-265g) were positioned by cervical entrapment in individual vented chambers. Each animal was exposed for 10 min to a radioaerosol of distilled water containing a tracer amount (0.3 mCi ml^{-1}) of ^{99m} Technicium pertechnate. The aerosol was generated by a Turret jet-nebuliser (Medic-Acid, Chichester) driven by compressed air (14 p.s.i., flow rate 4L min^{-1}) delivering approximately 0.2 ml min^{-1}. The aerosol particle size distribution (measured with a TSI aerodynamic particle sizer) ranged from 0.7 - 15μm (mode 0.9μm, weight mean diameter of 9μm). At the end of the exposure period each animal was sacrificed by passing CO_2 into the exposure chamber. The carcase was removed and head, thorax and stomach regions scanned for radioemission of γ-particles (energy peak 139-140 KeV) using a Minishield hand held detector (Oakfield Instruments Oxford) connected to a MS 310E counter/ratemeter (J.P Engineering Reading Ltd). Further readings were also taken from dissected tissues. (Table 1). Whole body scans were taken of representative animals using a Nuclear Enterprises NE 8960 Scinticam γ-camera.

Table 1. Regional γ-radioemission (c sec^{-1}) following aerosol exposure

Field/Tissue	Conscious	% total	Anaesthetised	% total
Head	1116 (± 206)	36.8	724 (± 53)	73.6
Thorax	380 (± 73)	12.8	135 (± 26)	13.8
Trachea	32 (± 15)	1.1	15 (± 9)	1.6
Lungs	134 (± 44)	4.5	60 (± 9)	6.2
Oesophagus	135 (± 18)	4.5	5 (± 2)	0.6
Stomach	1169 (± 621)	38.6	26 (± 19)	3.0
Duodenum	29 (± 20)	1.0	5 (± 1)	0.5
Heart	28 (± 11)	1.0	6 (± 2)	0.7

All values represent the mean (± s.e. mean) result from 5 animals in each group.

In conscious animals relatively little deposition and subsequent retention of radioaerosol mass occurred the lower respiratory tract (LRT) compared with either the head region or the gastrointestinal tract (GIT). Although the overall count rate was less in anaesthetised animals (probably reflecting reduced ventilation) the proportion of counts in the LRT was very similar. However, there was a very marked reduction in radioemission from the GIT both in actual and percentage terms, coupled with an increase in the proportion of radioaerosol retained in the head region. The most obvious explanation for this is a depression of the swallowing reflex. This factor must be taken into consideration in the design and interpretation of aerosol exposure experiments.

STUDIES ON THE PROTECTIVE ACTIONS OF SUCRALFATE ON THE RAT GASTRIC MUCOSA

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Sucralfate (an aluminium salt of sucrose octasulphate) has been shown to enhance peptic ulcer healing in man (Miyake et al, 1980) and reduce the severity of gastric mucosal damage induced by absolute ethanol in the rat (Harrington et al, 1981). The effects of sucralfate on gastric acid secretion and ethanol-induced gastric mucosal damage (macroscopic subjective scoring) have now been investigated in the rat.

Doses of 1514mg/kg p.o. sucralfate, (Antepsin tablets ground and suspended in 0.5% methyl cellulose in water) did not significantly protect against ethanol-induced gastric mucosal damage when administered orally 0.5h or 1h prior to 1ml absolute ethanol/rat p.o. (16%, 22%, $p>0.05$ respectively). In view of this lack of activity of sucralfate, 3h pre-dosing was carried out.

Doses of 76mg and 152mg/kg p.o. sucralfate, did not significantly effect gastric secretion or ethanol-induced gastric mucosal damage. However, doses of 304mg-2.27g/kg p.o. sucralfate, given 3h prior to the ethanol challenge reduced gastric mucosal damage (89-96%, $p<0.001$). This protective effect was accompanied by a marked stimulation of the volume and hydrogen ion concentration of the gastric juice. The secretory effect was also confirmed in a Shay (2h pylorus-ligated) test; sucralfate 1514mg/kg p.o. dosed 1h prior to pyloric ligation resulted in a stimulation of gastric secretory volume (194%, $p<0.05$) and hydrogen ion concentration (73%, $p<0.05$).

The secretory effects of 1514mg/kg sucralfate p.o. (pre-dosed by 3h) were reduced, with atropine (0.1mg/kg s.c. 3h and 1.5h prior to ethanol) by 53% ($p<0.05$) and 75% ($p<0.001$) respectively for volume and hydrogen ion concentration. The gastric mucosal protective effects were not significantly reduced by atropine pretreatment. Thus, stimulation of gastric acid secretion by sucralfate may be mediated via a cholinergic pathway. Since atropine did not influence the mucosal protective effect of sucralfate, dilution of the ethanol by increased gastric secretion would not seem to be responsible for the gastric mucosal protection.

The results show that, in the rat, sucralfate may possess a relatively slow onset of action with respect to its mucosal protective activity. They also show that this activity may be accompanied by stimulation of both the volume and hydrogen ion concentration of gastric secretion. However, these two effects did not appear to be inter-dependent, since atropine reduced the acid secretory effect without reducing the mucosal protective effect.

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LACK OF EVIDENCE FOR Na^+-COUPLED ACTIVE TRANSPORT OF MELPHALAN ACROSS THE RAT INTESTINAL EPITHELIUM

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Melphalan (L-phenylalanine mustard) is an alkylating agent used in the treatment of multiple myeloma and other neoplastic diseases. Uptake into a variety of cells, including human lymphocytes and L1210 murine leukaemia cells, is mediated by an active process involving two separate amino acid transport systems, one of which is sodium dependent (Goldenberg, G.J. *et al*, 1979). The present study was designed to investigate the factors governing the transport of melphalan across the rat isolated ileum, since studies in patients suggest that both the rate and extent of absorption vary widely following oral administration of the drug (Bosanquet & Gilby, 1984).

The method chosen was a modification of the Ussing technique (Ussing & Zerahn, 1951), in which isolated terminal ileum was mounted as a flat sheet between two halves of a perfusion chamber containing modified Ringer's solution (Okada *et al*, 1975). The potential difference (PD) and short-circuit current (I_{sc}) across the gut wall were monitored continuously. A marked increase in both of these parameters occurs during Na^+-coupled active transport. The technique can be readily adapted to the study of transport mechanisms in human intestinal tissue.

Typical increases in potential difference resulting from the active transport of (a) D-glucose and (c) L-phenylalanine are shown in Figure 1. The addition of melphalan to the mucosal side of the chamber is indicated at (b).

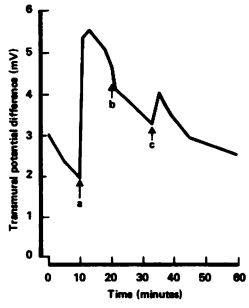

Figure 1 Changes in PD across rat ileum following addition of substrate to mucosal side of chamber.

Increases in PD (mean ± S.D., n=6) following the sequential addition of D-glucose (11mM) and L-phenylalanine (10mM) were 1.9 ± 0.8mV and 0.8 ± 0.2mV respectively. The corresponding increases in I_{sc} were 27 ± 11μA and 12 ± 3μA. The addition of melphalan (4.5mM) to the mucosal side of the chamber produced no measurable change in PD or I_{sc}.

There is therefore no evidence in these experiments for a Na^+-coupled active transport system for melphalan in the rat ileum. Na^+-independent active transport is being studied using high specific activity (^3H)-melphalan.

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THE EFFECT OF THE ANTIHISTAMINES DIPHENHYDRAMINE AND CYPROHEPTADINE ON RAT ANOCOCCYGEUS MUSCLE

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The antihistamines cyproheptadine and diphenhydramine were studied for their effects on motor responses of the rat anococcygeus muscle (Gillespie ,1972) to field stimulation, noradrenaline (NA), tyramine (Tyr), adenosine (Aden) and ATP. The type of response of anococcygeus muscle to field stimulation in presence of diphenhydramine or cyproheptadine was dependent on the concentration of antihistaminic used. Thus, diphenhydramine (3-10 μM) increased both amplitude and duration of responses to field stimulation and to NA but reduced that to Tyr. Cyproheptadine (0.6-6 μM) reduced responses to field stimulation, NA and Tyr. Higher concentrations of diphenhydramine (30-60 μM) and cyproheptadine (7-15 μM) resulted in a biphasic response to field stimulation, an initial inhibition followed by a contractile response.

Further increase in concentration of cyproheptadine (16-21 μM) and diphenhydramine (60-85μM) raised muscle tone and in this case field stimulation resulted only in inhibitory response which was not mimcked by ATP or Aden and was not affected by atropine or phentolamine indicating non-adrenergic non-cholinergic inhibitory responses (NANC).

The elevation of tone by diphenhydramine and cyproheptadine was not affected by phentolamine or reserpine treatment and could not be demonstrated in Ca^{2+} - free Krebs solution.

Diphenhydramine, but not cyproheptadine, potentiated the contractile response of anococcygeus muscle to ATP. Adenosine did not contract the muscle with resting tone but contracted that whose tone was elevated by diphenhydramine.

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INTERACTION OF A POLYACRYLATE, CARBOMER, WITH GASTRIC MUCUS AND PEPSIN

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An acidic polyacrylate, carbomer 934P, of molecular weight c.$3x10^6$, with carbenoxolone (50 and 5 $mg.kg^{-1}$ respectively, neither active alone), will protect the gastric mucosa against ethanol induced necrosis (Dettmar et al, 1985). The gastroduodenal mucus:bicarbonate barrier is the first line of defence against mucosal ulceration (Allen and Garner, 1980, Allen et al, 1984) and this study describes the interaction of carbomer with the mucus barrier.

The protective properties of the mucus are determined by its ability to form a water insoluble gel layer adherent to the mucosal surface. This gel forming ability of mucus is reflected in the viscosity of its glycoprotein components in dilute solution. Addition of carbomer caused a synergistic increase in the viscosity of gastric mucus glycoprotein, i.e. the measured viscosity (η^{MC}) of a mixture of gastric mucus (η^{M}) and carbomer (η^{C}) was substantially greater than that calculated from the summation of the individual viscosities alone ($\eta^{MC} >> \eta^{M} + \eta^{C}$). For example carbomer (10 $mg.ml^{-1}$) with purified gastric mucus glycoprotein (pig, 2-10 $mg.ml^{-1}$) in isotonic buffer at pH 2.2, had a viscosity over sixty-fold greater than the sum of mucus and carbomer viscosities alone.

An important factor in the erosion of the adherent mucus gel <u>in vivo</u> is luminal pepsin which fragments the polymeric gel forming structure of the mucus glycoprotein into lower molecular weight glycoprotein components. These degraded glycoproteins are soluble in the gastric juice, they cannot form a gel (Allen et al, 1984, Pearson et al, 1985) and they have a lower viscosity than the polymeric gel forming glycoprotein (specific viscosity about one fifth at 5 $mg.ml^{-1}$). Carbomer (10 $mg.ml^{-1}$) enhanced the viscosity of the degraded glycoprotein (4 mg. ml^{-1}) only about two-fold in contrast to its sixty-fold increase in viscosity with the polymeric glycoprotein.

Carbomer was found to inhibit pepsin hydrolysis of serum albumin substrate. For example, at a carbomer concentration of 4 $mg.ml^{-1}$, pepsin (1 $\mu g.ml^{-1}$) hydrolysis was inhibited by about 50% (pH 2.2). The mechanism of inhibition appears to be through reversible binding of pepsin by carbomer. With increasing pepsin concentration (1 to 5 $mg.ml^{-1}$) and fixed carbomer concentration (4 $mg.ml^{-1}$) there was no change in the percentage of bound pepsin. At a fixed pepsin concentration (2 $mg.ml^{-1}$) the amount bound rose with increasing carbomer concentration; 73% and 31% pepsin was bound at a carbomer concentration of 4 or 0.4 $mg.ml^{-1}$, respectively. The serum albumin substrate also bound to carbomer. The percentage of serum albumin bound to carbomer rose with increasing carbomer concentration and fell with increasing albumin concentration. Carbomer is insoluble in aqueous solution, existing in the form of a fine dispersion: the inhibition of enzymatic activity is associated with the removal of both pepsin and substrate from the solution.

The above results show that polyacrylates, such as carbomer, could reinforce the gastroduodenal mucus:bicarbonate barrier against autodigestion by physically reinforcing the adherent mucus gel layer and by reducing peptic degradation of that layer.

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HISTAMINE DIRECTLY STIMULATES RELEASE OF RENIN FROM SUPERFUSED RAT RENAL CORTICAL CELLS IN VITRO

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Histamine (H) stimulates the release of renin from the isolated perfused rat kidney via H2 receptors (Schwertschlag & Hackenthal, 1982). In rat glomeruli, H is synthesised (Abboud et al, 1982), and H2-receptors are present (Chansel et al, 1982). The aim of this study, therefore, was to investigate if H acts directly on the juxtaglomerular (JG) cells to stimulate the release of renin.

Rat (Wistar, ♀, b.w. 180-220g) renal cortical cells, prepared by the method of Williams et al (1983), were loaded onto Biogel in PTFE columns (1 kidney equivalent per column). After 2 hours equilibration, at 37°C (perfusion medium Krebs bicarbonate buffer + 0.2% BSA), 5 minute samples of the perfusate were collected for estimation of renin activity (angiotensin I was measured by radioimmunoassay after incubation with excess renin substrate from nephrectomised sheep). H1- and H2-receptor agonists were infused for 10 minutes on a 40 minute dose-cycle. The effects of H1 and H2 antagonists were studied either alone, or in conjunction with H, in which case the antagonists were infused 10 minutes prior to H infusion. The results were expressed as stimulation ratios: calculated as the mean of the 3 peak samples compared with the 3 basal samples before and after the peak.

Table 1: The effects of H agonists and antagonists on basal release of renin

Drug concentration	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M
Histamine	111.5 ± 5.8	161.9 ± 8.6	175.0 ± 9.7	145.2 ± 10.9
Dimaprit	97.4 ± 1.6	128.9 ± 11.1	158.1 ± 15.3	185.6 ± 14.1
PEA	-	106.2 ± 4.7	104.0 ± 3.9	113.1 ± 2.2
DPH	-	97.5 ± 4.3	107.8 ± 1.4	105.4 ± 4.1
SKF 93479	-	101.5 ± 1.8	91.0 ± 2.6	97.7 ± 3.8

(Results are expressed as stimulation ratios, mean ± SEM, n = 3 experiments)

H and the H2-receptor agonist, dimaprit, showed a concentration-dependent increase in release of renin, whereas the H1-receptor agonist, 2-pyridylethylamine (PEA), had no significant effect on the basal release of renin. The H1 and H2-receptor antagonists, diphenhydramine (DPH) and SKF 93479 (Blakemore et al, 1981) respectively, also had no significant effect on basal release of renin. DPH (10^{-6}M) did not significantly alter the release of renin in response to H(10^{-7}M) (control H stimulation ration = 164.2 ± 7.0:H + DPH stimulation ratio = 159.9 ± 10.2, n = 3 experiments). In the presence of SKF 93479 (10^{-6}M), the release of renin in response to H(10^{-7}M) was not significantly different to basal release (stimulation ratio = 94.2 ± 3.8, n = 3 experiments).

In summary, stimulation of release or renin was observed with H and dimaprit but not with PEA. SKF 93479 antagonised the response to H but DPH was without effect. These results suggest that, in the rat, H stimulates the release of renin by acting directly on the H2 receptors on the JG cells. We postulate that H, synthesised in the kidney glomeruli, could play a physiological role in the regulation of release of renin.

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MODULATION OF FORSKOLIN-INDUCED GASTRIC ACID SECRETION IN VITRO; POTENTIATION OF SECRETAGOGUE RESPONSES BY SUB-THRESHOLD DOSES

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Forskolin potently stimulates gastric acid secretion in the rat in vivo and in vitro (Main & Wilson 1985). Previous studies have shown that sub-threshold concentrations of forskolin can potentiate responses to many stimuli in a number of tissues, implying the responses are cAMP-linked (Seamon & Daly 1983). A recent report on the effect of forskolin on responses to isoprenaline has challenged this assumption (Waldeck & Widmark 1985). We have investigated the interaction of a number of compounds with forskolin to further examine the role of cAMP in stimulated acid secretion.

Experiments were carried out on the rat isolated gastric mucosal preparation of Main & Pearce (1978), which provides a test and control tissue from each individual. Omeprazole 1 μM, ProstaglandinE_2 (PGE_2) 2 μM, isobutylmethylxanthine (IMX) 10 μM, Indomethacin (Indo) 1 μM and cimetidine 4 μM, were each given 30 minutes before cumulative doses of 0.5 then 2 μM forskolin. For potentiation experiments a sub-threshold dose of forskolin (100 nM) was given to the test preparation 30 minutes before secretagogue. Either 10 then 100 μM histamine, given cumulatively, or 1 μM methacholine (MeCh), with effect of endogenous histamine inhibited by 8 μM cimetidine, was used. Each response was followed for one hour. Results are expressed as mean ±s.e.m. Statistical analysis was by paired Student's t-test.

Acid secretory responses to forskolin were significantly inhibited by both omeprazole and PGE_2, but cimetidine had no effect. Mean % age inhibition by omeprazole of responses to 0.5 and 2 μM forskolin was 96.8 ± 1.9 and 56.6 ± 15.5 % respectively. PGE_2 inhibited the same responses by 69.8 ± 7.8 and 67.8 ± 16.0 % (n=4). In the presence of 10 μM IMX, responses to 0.5 and 2 μM forskolin were increased from control values of 1.68 ± 0.7 and 5.59 ± 1.36 μequiv/cm^2/hr to 6.46 ± 1.87 ($p<0.05$) and 10.87 ± 2.16 μequiv/cm^2/hr ($p<0.01$) respectively (n=5). Similarily, Indo significantly increased responses to both doses from control values of 3.72 ± 0.62 and 5.84 ± 0.90 μequiv/cm^2/hr to 4.09 ± 0.64 ($p<0.05$) and 7.19 ± 1.03 μequiv/cm^2/hr ($p<0.05$) respectively (n=11).

The mean response to 10 μM histamine was not significantly altered by the presence of 100nM forskolin. Responses to 100 μM histamine were increased from a mean control value of 0.64 ± 0.15 to 2.29 ± 0.70 μequiv/cm^2/hr ($p<0.05$, n=6). Mean response to MeCh was potentiated from a control of 4.81 ± 0.39 to 7.18 ± 0.90 μequiv/cm^2/hr ($p<0.05$, n=9).

We conclude that forskolin has a similar profile of activity to dibutyrylcAMP; H_2-receptor antagonists do not inhibit the response, and it is potentiated by both Indo and IMX. A sub-threshold conc. of forskolin potentiates responses to both histamine, which is mediated by cAMP, and MeCh. According to the assumptions of Seamon & Daly this implies that cholinergic stimulation is at least partially cAMP-linked.

G.W. is an M.R.C. scholar

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STEREOSELECTIVE INTERACTION OF MIANSERIN WITH 5-HT BINDING SITES IN THE RAT BRAIN

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The tetracyclic antidepressant mianserin possess anti-5-hydroxytryptamine (5-HT) properties and inhibits the binding of ^{3}H-ketanserin to the 5-HT_2 site and that of ^{3}H-5-HT to the 5-HT_1 site. The presence of three 5-HT_1 subsites has been proposed (Pazos et al, 1985), with mianserin selectively blocking the 5-HT_{1C} site. We have studied whether the enantiomers of mianserin interact stereoselectively with the 5-HT_2 and 5-HT_{1C} and 5HT_{1B} sites.

Binding of ^{3}H-5-HT (2nM) was studied according to Nelson et al (1983) using rat striatal membranes, ^{3}H-ketanserin (1nM) binding to frontal cortical membranes was performed according to Leysen et al (1981). Specific binding was defined as that displaced by 10 µM methysergide. Data were analysed using the non-linear curve-fitting programs ALLFIT and LIGAND.

Both stereoisomers of mainserin inhibited ^{3}H-ketanserin binding in a competitive manner with slope factors close to unity. The (+) stereoisomer however, was 30 times more potent than the (-) isomer at the 5-HT_2 site (Table 1).

The mianserin stereoisomers both inhibited striatal ^{3}H-5-HT binding with slope factors significantly less than unity (0.54±.02 and 0.53±.02 for the (+) and (-) isomer respectively). These results were best described by a two-site model with both isomers showing a high affinity for the 1C site; the (+) isomer being 3-4 fold more potent than the (-) isomer. The ^{3}H-5-HT displaced by mianserin with low potency corresponded to the 5-HT_{1B} site (Alexander et al, this meeting) and this was also more sensitive to the (+) than the (-) isomer.

Table 1. Interaction of mianserin enantiomers with 5-HT binding sites

	5-HT_2	5HT_{1C}		5HT_{1B}	
	IC_{50}(nM)	IC_{50}	Bmax	IC_{50}	Bmax
(+) mianserin	2.6±0.3	13.9±4.8	32.6±3.5	2930±740	62.9±1.9
(-) mianserin	70.0±19*	95.2±2.7*	38.3±5.7	13900±1650*	64.0±5.5

5-HT_2 binding was studied using ^{3}H-ketanserin; 5-HT_1 binding was studied using ^{3}H-5-HT. Mianserin displaced ^{3}H-5-HT from two apparent sites, the high affinity site being 5-HT_{1C} and the low-affinity site being 5-HT_{1B} IC_{50} values in nM, Bmax in % specific binding, *$p < 0.05$ compared to (+) isomer, Student's t-test results are mean±SEM from 4-5 separate experiments.

These results show that mianserin interacts with the 5-HT_2, the 5-HT_{1B} and the 5-HT_{1C} binding sites in a stereoselective manner. At all three binding sites the (+) isomer was more potent than the (-) isomer, although this difference was less marked at the 5-HT_{1B} and 5-HT_{1C} sites than at the 5-HT_2 site. These results support the suggestion that most of the biological activity of mianserin resides in the (+) isomer (Nickolson & Wieringa, 1981).

Stereoselectivity is an important criterion in identifying whether or not the binding of a radioligand is to a receptor. The greater potency of (+) mianserin than the (-) isomer at the 5-HT_2, 5-HT_{1B} and 5-HT_{1C} sites, therefore, provides further support for a functional role for these sites.

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EFFECT OF SEROTONIN UPTAKE INHIBITORS ON THE IMMOBILITY OF MICE IN THE TAIL SUSPENSION TEST

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Screening methods for potential antidepressant drugs are largely restricted to pharmacological tests which are based upon the mechanism involved in the action of these drugs. A "behavioural despair" test has been described as a simple procedure sensitive to antidepressants, but this test generally fails to detect activity of serotonin uptake inhibitors (Porsolt et al, 1977). Recently Steru et al (1985) described a new experimental procedure conceptually similar to "behavioural despair" in which different antidepressants decrease the frequency and the duration of the phase of immobility of a mouse suspended by its tail. In the present study, we used this test to investigate the effect of several antidepressants which selectively inhibit serotonin uptake.

Mice (male NMRI, weight 22 ± 2 g, Charles River, France) were individually suspended by the tail from a thread attached to a force displacement transducer in a sound attenuating box. Alternations of agitated and immobile periods were recorded by a pen recorder. Drugs were injected i.p., 30 min before the test. Results are expressed as the time which the mice spent immobile during a 6 min test period.

Table 1. Effect of serotonin uptake inhibitors on the immobility time in the tail suspension test in mice.

Drugs	MED	Immobility Time (sec)			
	mg/kg, ip	Control		Treated	
Paroxetine	4	79.0 ± 9.6	(20)	23.8 ± 6.6**	(20)
Fluoxetine	16	113.2 ± 12.9	(10)	51.6 ± 13.4**	(10)
Zimelidine	16	138.8 ± 14.4	(20)	78.4 ± 11.9**	(20)
Fluvoxamine	20	98.6 ± 14.1	(18)	51.7 ± 9.9*	(18)
Indalpine	10	149.0 ± 11.0	(19)	78.5 ± 11.4**	(19)

*p < 0.05 ; **p < 0.01 (Dunnett test) MED : Minimal effective dose
Number of mice tested in parentheses

All the compounds listed in Table 1 produced a dose dependant decrease in the immobility time. The minimal doses which induced a statistically significant decrease in the immobility time failed to influence spontaneous locomotor activity. These effects of serotonin uptake inhibitors appear to depend on endogenous serotonin as depletion of brain serotonin by p-chlorophenylalanine (PCPA, 300 mg/kg, i.p., 72, 48 and 24 h before the test) antagonized the effect of fluoxetine FLU, (immobility time in sec ; saline = 121.1± 12.4 ; PCPA = 92.8 ± 12.8 ; FLU 16 mg/kg = 67.7 ± 13.2**; FLU 32 mg/kg = 52.3 ± 12.2**; FLU 16 mg/kg + PCPA = 95.9 ± 13.4; FLU 32 mg/kg + PCPA = 90.0 ± 18.2). Pretreatment with PCPA however failed to antagonize the effect of maprotiline (16 mg/kg, i.p.), an antidepressant which selectively inhibits noradrenaline uptake.

These results demonstrate that the tail suspension test in the mouse is a behavioural procedure sensitive to serotonin uptake inhibitors and that the activity of these compounds is related to serotoninergic mechanisms.

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THE INTERACTION OF 5-HYDROXYTRYPTAMINE AND METHYSERGIDE WITH METHIOTHEPIN AT "5-HT_1 -LIKE" RECEPTORS IN DOG SAPHENOUS VEIN

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The 5-HT receptor which mediates the contractile action of 5-hydroxytryptamine (5-HT) in dog saphenous vein is not of the 5-HT_2 type, and has been recently described as "5-HT_1-like" (Apperley & Humphrey, 1977; Apperley et al., 1980; Feniuk et al., 1985). In this preparation we now describe the antagonistic effects of methiothepin (metitepine) which has a high affinity for the 5-HT_1 ligand binding site (Leysen et al., 1981).

Dog saphenous veins were set up in glass organ baths as previously described so that four preparations were prepared from a single vessel (Apperley et al., 1980). Preliminary experiments showed that methiothepin could not be fully washed from the baths between experiments and hence the same bath was always used for a particular concentration of methiothepin (or control). Agonist concentration-effect curves in the presence of three different concentrations of methiothepin were compared with that in the absence of antagonist on the fourth preparation.

The concentration-effect curves for 5-HT in the presence of methiothepin were displaced to the right in a parallel, concentration-dependent manner compared to those in control preparations (see Table 1). However, the antagonism was not apparently of a competitive nature since the slope of the Schild plot was markedly greater than unity (pA_2 value estimate [slope] was 8.1 [1.5]). The contractile action of methysergide was antagonised to a similar degree to that of 5-HT whilst contractile responses to potassium chloride were unaffected (Table 1).

Table 1: Antagonistic effects of methiothepin against contractile actions of 5-HT, methysergide and potassium chloride.

Agonist	Agonist concentration-ratio* at methiothepin concentration of:		
	5×10^{-8}M	1×10^{-7}M	5×10^{-7}M
5-Hydroxytryptamine	13.2 (6.8 - 25.4)	62.2 (37.5 - 103)	442 (256 - 760)
Methysergide	16.6 (99 - 27.9)	71.7 (34.8 - 148)	464 (298 - 724)
Potassium chloride	1.1 (0.8 - 1.6)	0.9 (0.7 - 1.2)	0.9 (0.7 - 1.2)

Each value is the geometric mean (95% confidence limits) of 4-8 observations.
*Calculated at level of 50% response to potassium chloride (3×10^{-2}M), except methysergide for which 10% level was used.

The use of methiothepin is of limited value in such experiments because it markedly sticks to glass and does not behave as a competitive antagonist. Nevertheless the data is consistent with our previous postulate that 5-HT and methysergide are agonists at the same "5-HT_1-like" receptor in the dog saphenous vein (Apperley et al., 1980; Feniuk et al., 1985).

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$5HT_1$ AND $5HT_2$ RECEPTORS IN THE ISOLATED PIG CORONARY ARTERY PREPARATION

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It has been reported that 5-hydroxytryptamine (5HT) can cause contraction of vascular smooth muscle preparations in some species and that this is probably due to stimulation of $5HT_2$ receptors (Müller-Schweinitzer & Engel, 1983; Humphrey 1984). It has also been reported that 5HT can produce a vasodilation and that this is due to stimulation of $5HT_1$ receptors (Cocks & Angus, 1983; Saxena & Verdouw, 1985). The effects of 5HT in the pig coronary artery were examined in order to determine whether or not this preparation possessed $5HT_2$ and $5HT_1$ receptors.

Left anterior descending coronary arteries were dissected from pig hearts obtained from the local abattoir. The arteries were cut into four spiral strips (3 x 15 mm) and suspended in 30 ml organ baths containing Krebs-Henseleit solution at 37°C and gassed continuously with 5% CO_2 in oxygen. The strips were stretched to an initial tension of 500 mg and then allowed to equilibrate for 2 h in the bathing medium which was changed every 15 min. Tension was recorded isometrically. Pargyline (1×10^{-6} mol.litre^{-1}) was added to the baths 20 min before the agonists to inhibit MAO.

$5HT_2$ receptor study: Two cumulative concentration-response curves (CRC) for 5HT were established. Increasing concentrations of antagonist were added to three of the strips and allowed to equilibrate for 45 min before a third 5HT CRC was established, the fourth strip acted as a control. The EC_{50} was estimated for each curve and the pA_2 was determined by the method of Arunlakshana & Schild (1959). The EC_{50} for 5HT was 3×10^{-7} mol.litre^{-1} and the pA_2 values were ketanserin 8.64, slope 0.9; methysergide 8.31, slope 1.0; and cyproheptadine 8.9, slope 1.1.

$5HT_1$ receptor study: The strips were prepared as above except that the Krebs now contained 3×10^{-6} mol.litre^{-1} phentolamine and ketanserin to inhibit the α- and $5HT_2$ receptors respectively. $PGF_2\alpha$ (1×10^{-6} mol.litre^{-1}) was added to the baths together with the pargyline 20 min prior to addition of the agonists. The $PGF_2\alpha$ contracted the arteries and once a steady level of contraction was achieved, CRCs to 5HT or 5-carboxamide tryptamine (5CT) were constructed. When the agonists had produced the maximum response, papaverine (2×10^{-4} mol.litre^{-1}) was added to relax maximally the preparation. The relaxation produced by these agonists was expressed as a % of the total relaxable tone. The EC_{50} values for 5HT and 5CT are respectively 4×10^{-7} and 4×10^{-7} mol. litre^{-1}. The % relaxation was greater with 5CT than with 5HT.

The antagonism of the 5HT contraction responses by the above antagonists demonstrates the presence of $5HT_2$ receptors in this preparation. Further, the relaxation in the $5HT_2$-blocked and $PGF_2\alpha$-contracted preparation by 5CT would indicate the presence of $5HT_1$ receptors. We therefore suggest that this preparation possesses both $5HT_2$ and $5HT_1$ receptors.

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CARDIOVASCULAR RESPONSES TO FIVE DAY ADMINISTRATION OF ATENOLOL IN COMBINATION WITH NIFEDIPINE IN CONSCIOUS DOGS

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The combination of a calcium antagonist and a beta-adrenoceptor antagonist appears to provide useful therapy both in hypertension (Lederballe Pederson, et al 1980) and in angina (Lynch et al 1980).

We have compared the effects of oral administration of a combination of nifedipine ('Adalat' Retard, 3 $mg.kg^{-1}$) and atenolol (7.5 $mg.kg^{-1}$) with placebo on blood pressure and heart rate in conscious hypertensive dogs. These dogs had been made hypertensive using the Goldblatt technique (unilateral nephrectomy and clipping of the contralateral renal artery). Since responses to acute drug administration often differ from chronic effects and duration of action is important in evaluating antihypertensive agents, we have used a radiotelemetry system to measure cardiovascular responses continuously for five days. Drug or placebo were administered orally in a gelatin capsule to separate groups of four dogs at 12 hourly intervals. A total of nine doses were administered to each animal and measurements were made continuously and for an additional 30 hours after the last dose. Systolic, and diastolic blood pressure (DBP) and heart rate (HR) were captured and stored using HECTOR (an in house data capture system) which calculated values as means ± the standard error of the mean for all data collected in twenty minute blocks. All data were expressed as per cent change from pre-dose values obtained on day one.

The control values for DBP and HR were 93±7 mmHg and 87±2 $bts.min^{-1}$ respectively in the placebo group and 96±13 mmHg and 88±15$bts.min^{-1}$ in the drug treated group. Administration of placebo had no significant effect on heart rate throughout the five day dosing period. There was a small decrease in DBP of 13±4% on days 4 and 5 which did not correlate with the times of dosing. The administration of the atenolol, nifedipine combination reduced systolic and diastolic blood pressure by 23±4% and increased heart rate by 50±19% one hour after dosing on day one. The decrease in DBP was maintained for approximately five hours. After each dose was administered systolic and diastolic blood pressure decreased and as the study progressed there was an increase in duration and magnitude of the blood pressure effect which lasted for 12 hrs on day 4 and decreased by 44±4%. In addition the magnitude of the reflex tachycardia was reduced with time so that on day 4 the heart rate, although increased from the immediate pre-dose value did not increase above the pre-study control value. There was no rebound increase in blood pressure after the last dose. This dose of the combination produced maximal effects on blood pressure on day 4.

The results are consistent with a vasodilator action of nifedipine and attenuation by atenolol of the beta-adrenoceptor mediated component of the tachycardia since the increase in heart rate was less marked than was observed with nifedipine alone. The use of continuous measurement of blood pressure and heart rate has been useful in determining the magnitude and duration of the responses to this combination of atenolol and nifedipine.

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DECREASED DYNORPHIN-LIKE IMMUNOREACTIVITY IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a hereditary condition transmitted by an autosomal dominant gene which has been localised on chromosome 4. The main symptons of the disease are cognitive deficits, psychiatric abnormalities and Parkinsonian-like dystonia. The neuropathology is confined mainly to the caudate nucleus and putamen where cell loss can be up to 80% (Bruyn, 1968). Previous studies have shown neurochemical changes within the neostriatum. The contents of substance P and methionine-enkephalin (ME) are markedly reduced in HD caudate nucleus, putamen and substantia nigra (Emson et al., 1980) while the contents of somatostatin and neuropeptide Y are increased in HD caudate and putamen (Dawbarn et al., 1985). This study was designed to investigate the content of ME-Arg-Gly-Leu, dynorphin A, dynorphin B and α-neoendorphin in HD brains and in unaffected normal brain tissue.

Tissue samples from control and HD brains were stored at -70°C. For assay of opioid peptide content, samples were weighed and extracted by boiling in 0.1M HCl for 10 minutes. The homogenates were centrifuged at 4°C and the supernatants removed and lyophilized. Radioimmunoassays were performed with specific antisera directed against ME-Arg-Gly-Leu, dynorphin A, dynorphin B and α-neoendorphin. For immunocytochemical studies sections of formalin fixed control and HD brain were processed for visualisation of dynorphin A like immunoreactivity using the peroxidase antiperoxidase techniques.

ME-Arg-Gly-leu immunoreactivity was significantly depleted from the caudate nucleus, putamen substantia nigra (NS) and globus pallidus (GP) but not from the hypothalamus or cortex in HD. Similarly the prodynorphin produces (dynorphin A, dynorphin B and α-neoendorphin) were decreased in content in the caudate nucleus, putamen, SN and GP but not from cortex or hypothalamus in HD.

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