5-HYDROXYTRYPTAMINE RECEPTORS INVOLVED IN VASODILATATION IN THE PITHED RAT

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5-Carboxyamidotryptamine (5-CT) is a ligand at 5-HT binding sites and produces vasodilatation in anaesthetised cats (Connor et al., 1986). We have examined the depressor actions of this and other 5-hydroxytryptamine (5-HT) receptor agonists in the pithed rat.

Diastolic blood pressure (DBP) and heart rate (HR) were recorded in pentobarbitone anaesthetised animals or animals pithed under ether anaesthesia. Spontaneously Hypertensive Rats (SHR) were employed since pithed SHR have a higher resting DBP than pithed Wistar. Mianserin (1 mg kg-1) was employed to eliminate 5-HT-2 receptor actions of agonists, and had the additional advantage of slightly increasing the resting DBP of pithed SHR.

In anaesthetised SHR, the putative 5-HT-1A receptor agonist 8-OHDPAT (Hjorth et al., 1982) had little effect on DBP at doses of up to 10 μg kg-1, whereas 5-CT markedly lowered DBP at doses of as low as 10-100 ng kg-1.

In pithed SHR, 5-CT markedly lowered DBP at doses of 100 ng kg-1, and mianserin (1 mg kg-1) did not affect this depressor response to 5-CT and was used to eliminate 5-HT-2 receptor mediated pressor responses to other agonists. 5-HT was 30 times less potent than 5-CT at producing depressor responses, and 8-OHDPAT was ineffective at doses of up to 3 mg kg-1. Depressor responses to 5-CT were unaffected by 8OHDPAT (3 mg kg-1), so that a partial agonist action of 8OHDPAT can be ruled out. Depressor responses to 5-CT were antagonised by methysergide (1 mg kg-1) and by yohimbine (5 mg kg-1), but not by propranolol, cyanopindolol, spiperone and spiroxatrine (all 1 mg kg-1).

In pithed wistar rats, 5-CT potently lowered DBP, although the maximum fall in DBP was less than in SHR presumably due to the lower resting DBP of wistar rats.

In conclusion, 5-CT is a potent agonist at 5-HT depressor receptors at which methysergide is an antagonist, suggesting that these receptors can be loosely classed as 5-HT-1. However, this 5-HT-1 depressor receptor does not easily fit into the 5-HT-1A,1B,1C classification (see Engel et al., 1986).

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RECEPTOR MEDIATED MODULATION OF THE EVOKED RELEASE OF [^{3}H]-BETAXOLOL FROM RAT ATRIA

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The cardioselective β-adrenoceptor antagonist ^{3}H-betaxolol (BTXL) can be accumulated and released by electrical stimulation (E.S.) from the rat atria simultaneously with ^{3}H-NA (Arbilla et al., 1986a ; Petruzzo et al., 1986). Sympathetic denervation abolishes the release of ^{3}H-BTXL induced by E.S., but does not affect its accumulation, suggesting that in the rat atria, ^{3}H-BTXL is not retained in noradrenergic nerve terminals (Arbilla et al., 1986b ; Petruzzo et al., 1986). As an intact noradrenergic innervation is necessary to trigger the release of ^{3}H-BTXL by E.S., we explored the influence of adrenoceptor and purinergic agonists on the release by E.S. of ^{3}H-BTXL and ^{3}H-propranolol (PROP).
Atrial slices were labelled with ^{3}H-BTXL, ^{3}H-PROP or ^{3}H-NA, and perfused with Krebs containing 2.6 mM Ca^{2+} and 1 µM atropine. Two periods of E.S. (S_1 and S_2 : 5 Hz, 2 msec, 24 mA, 2 min) were applied. Drugs were added 20 min before S_2. The ratios of the percent of total tissue radioactivity released by E.S. in control experiments are shown in Table 1. The release of ^{3}H-BTXL was significantly increased ($p < 0.005$) by exposure to NA 1 µM (S_2/S_1 = 1.80 ± 0.32, n = 6), adrenaline 1 µM (S_2/S_1 = 1.98 ± 0.25, n = 7), db AMPc 100 µM (S_2/S_1 = 1.56 ± 0.15, n = 8) and isoprenaline 0.1 µM (S_2/S_1 = 2.10 ± 0.20, n = 13). The facilitation by isoprenaline 0.1 µM of ^{3}H-BTXL release was significantly antagonized by PROP 1 µM (S_2/S_1 = 1.38 ± 0.27, n = 10, $p < 0.05$). Similar results were obtained for the release of ^{3}H-PROP by E.S.

Table 1 : Effect of purines on the release of ^{3}H-BTXL by E.S.

Drugs (S_2)	µM	S_2/S_1 ^{3}H-BTXL	^{3}H-PROP	^{3}H-NA
Control		0.80 ± 0.10 (15)	0.80 ± 0.05 (14)	1.02 ± 0.05 (10)
2-Cl adenosine	1	0.32 ± 0.05 (8)*	0.42 ± 0.06 (6)*	0.52 ± 0.01 (4)*
+ theophylline	100	1.07 ± 0.17 (6)**	0.76 ± 0.10 (6)**	N.T.
ATP	10	0.48 ± 0.12 (5)	0.75 ± 0.12 (5)	0.64 ± 0.06 (4)*
ATP	100	0.23 ± 0.07 (5)*	0.31 ± 0.07 (8)*	0.46 ± 0.03 (3)*

Values are mean ± S.E.M. from ( ) experiments per group. ATP : adenosine 5'-triphosphate. N.T. not tested. * $p < 0.001$ vs control ** $p < 0.02$ when compared vs 2-Cl adenosine 1 µM

Table 1 shows that 2Cl-adenosine 1 µM inhibited the release by E.S. of ^{3}H-BTXL, ^{3}H-PROP and ^{3}H-NA. A concentration of ATP 100 times higher than that of 2Cl-adenosine was necessary to obtain a similar inhibitory effect on ^{3}H-BTXL and ^{3}H-PROP release (Table 1). The inhibition by 2Cl-adenosine 1 µM of ^{3}H-BTXL and ^{3}H-PROP release was antagonized by theophylline 100 µM (Table 1).
The present data indicate that stimulation of β-adrenoceptors enhances, while stimulation of purinoceptors probably of P1 subtype inhibits the release by E.S. of ^{3}H-BTXL or ^{3}H-PROP retained in the rat atria. The involvement of noradrenergic transmission in these interactions remains to be clarified.

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REPERFUSION-INDUCED ARRHYTHMIAS AND OXYGEN-DERIVED FREE RADICALS: EVIDENCE FOR INVOLVEMENT OF MULTIPLE SOURCES.

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Previously, we have suggested (Manning, 1984) that oxygen-derived free radicals may be a causative factor in the genesis of reperfusion-induced arrhythmias. This was based on our finding that inhibition of the superoxide producing enzyme xanthine oxidase by allopurinol reduced the incidence of reperfusion-induced arrhythmias in the anaesthetised rat. In this investigation we have attempted to assess whether xanthine oxidase is the sole source of oxygen-derived free radicals responsible for the genesis of reperfusion arrhythmias, or whether additional sources are also involved.

Using an anaesthetised rat preparation with transient (7 min) coronary artery occlusion (CAO) as described previously (Crome et al, 1985), we have compared the ability of three regimes to reduce the incidence of reperfusion-induced arrhythmias and resultant mortality, (i) allopurinol pretreatment (20 mg/kg orally 24 h prior to use plus 20 mg/kg i.v. 10 min prior to CAO);(ii) superoxide dismutase (SOD) (35,000 units/kg) and catalase (400,000 units/kg), these enzymes being administered i.v. 3 min prior to CAO and again 2 min prior to reperfusion; (iii) a combination treatment of allopurinol together with SOD + CAT.

Table 1. Effects of allopurinol, SOD + CAT, or combined administration of allopurinol, SOD + CAT on reperfusion-induced arrhythmias in the anaesthetised rat

	Incidence VF (%)	Incidence VT (%)	Mortality (%)	VF($\log_{10}$ duration)	Arrhythmia Score
Control (15)	87	87	47	$1.73^{\pm}0.18$	$4.8^{\pm}0.8$
Allopurinol (15)	40*	80	0***	1.36 ± 0.12*	2.4 ± 0.4**
SOD + CAT (15)	26***	80	0***	1.43 ± 0.21	2.1 ± 0.3**
Allopurinol + SOD + CAT (15)	13^{+}	47*	0***	$0.44\pm0.04^{++}$	$1.2\pm0.26^{+}$

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ v. control; $^{+}$ $p<0.05$; $^{++}$ $p<0.01$ v. allopurinol or SOD + CAT alone; VF = ventricular fibrillation; VT = ventricular tachycardia; (n) = group size

While all regimes reduced the incidence of VF, and eliminated mortality, only combination therapy reduced VT significantly. Furthermore, additional protection by combination therapy was demonstrated by significant decreases in log duration VF and mean arrhythmia score compared with allopurinol or SOD + CAT alone. We conclude from these results that, although in the rat heart xanthine oxidase is an important source of oxygen-derived free radicals involved in the genesis of reperfusion-induced arrhythmias, there are additional sources of free radicals that may also be partly responsible for the onset of these rhythm disturbances.

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INTERACTIONS BETWEEN THE NEW CLASS 1 ANTIARRHYTHMIC AGENT, BW A256C, AND LIDOCAINE *IN VIVO* AND *IN VITRO*.

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A common property of class 1 antiarrhythmic agents is that their use can result in arrhythmogenesis (Goldstein *et al*, 1984). A recent clinical report has demonstrated that the class 1 antiarrhythmic agent, lidocaine, can suppress the proarrhythmic activity induced by another class 1 agent, flecainide (Maza, 1986). *In vitro* studies have shown that class 1 agents may compete at a common binding site on the sodium channel (e.g. Clarkson & Hondeghem, 1985); the resultant apparent displacement of one agent by another could explain the clinical observation. These findings have led us to study the interaction between the structurally novel class 1 antiarrhythmic agent, BW A256C (Allan *et al*, 1986) and lidocaine, both *in vivo* and *in vitro*.

BW A256C was infused intravenously (0.01 or 0.02 $mgkg^{-1}min^{-1}$) to 17 conscious dogs following a surgically induced myocardial infarction (Allan *et al*, 1986) until proarrhythmic activity was evident (mean ± sem total dose = 1.87 ± 0.11 $mgkg^{-1}$). This activity was manifest by atrioventricular dissociation, single or multiple ventricular complexes or ventricular flutter/fibrillation. On the appearance of proarrhythmic activity, rapid administration of lidocaine (2-4 $mgkg^{-1}$ i.v. bolus) restored normal sinus rhythm in 11 of 17 dogs. The effect was evident within 1 min of lidocaine administration but tended to be short lived (< 10 min) necessitating further administration of lidocaine (2 $mgkg^{-1}$ i.v.). The proarrhythmic activity of BW A256C observed in the remaining 6 dogs was not suppressed by lidocaine, possibly due to circulatory failure in these animals.

Interactions *in vitro* were studied in guinea-pig right ventricular tissue using conventional electrophysiological techniques (Donoghue *et al*, 1986). BW A256C (10^{-6} or 3 x 10^{-6}M) reduced maximum rate of depolarisation (V_{max}) without changing effective refractory period (ERP). Addition of lidocaine (10^{-5} - 10^{-4}M) did not cause any reversal of the effect of BW A256C on V_{max}; rather, additional decreases were observed, particularly at high stimulation frequencies. Addition of lidocaine also caused a dose-dependent increase in ERP, as seen with this drug alone. In the presence of both drugs, recovery from use-dependent reduction in V_{max} occurred in two phases: the first was rapid and attributable to recovery of lidocaine-blocked channels, the second was slow and not significantly different from that with BW A256C alone (Donoghue *et al*, 1986).

The results of our study suggest that the suppression of BW A256C-induced arrhythmias by lidocaine is not due to interaction of the two drugs at a common binding site in the sodium channel. An alternative explanation is that an increase in ERP by lidocaine may be responsible for suppressing the proarrhythmic activity of BW A256C. The potential clinical utility of our observations and how they may apply to the use of other class 1 antiarrhythmic agents awaits further study.

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POSITIVE INOTROPIC EFFECTS OF PALMITOYL CARNITINE ON EMBRYONIC CHICK HEART CELL AGGREGATES

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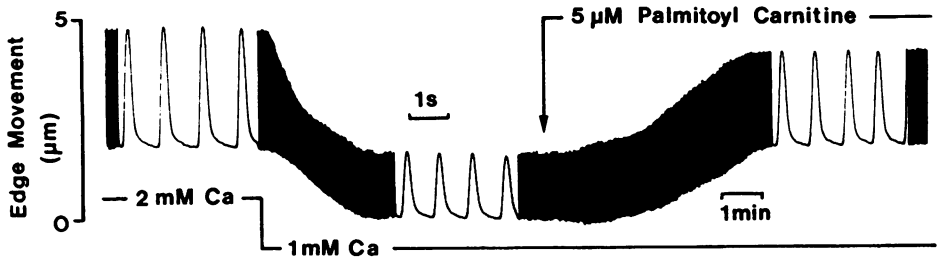
Palmitoyl carnitine is a lipid metabolite that accumulates in the heart following ischaemia and has been suggested to be responsible for some aspects of ischaemic cell damage (Neely & Feuvray, 1981). Palmitoyl carnitine has recently been shown to resemble Bay k 8644 as an activator of calcium channels in smooth muscle (Mir & Spedding, 1986). The present study investigates the possible facilitatory action of palmitoyl carnitine on calcium channels in embroynic chick heart.

We have previously shown that the calcium facilitator Bay k 8644 reverses nisoldipine (or verapamil) induced inhibition of beating of cultured embryonic chick heart cell aggregates (Duncan & Patmore, 1986). Palmitoyl carnitine (10-300 µM) reversed nisoldipine or verapamil induced inhibition of beating with pEC_{50} values of 3.8 and 4.1 respectively. Unlike Bay k 8644 (pEC_{50} = 7.1) reversal of antagonist action was not complete reaching a maximum of only 75% of control. At concentrations greater than 300 µM antagonism was observed. Contractions of these aggregates can be quantified by measuring edge movement as described previously (Patmore & Whiting, 1985). Since 2 mM calcium is near the peak of the extracellular calcium - contraction relationship, external calcium was reduced to 1 mM to allow demonstration of positive inotropy. Palmitoyl carnitine displayed positive inotropic activity at 5 µM (fig 1) increasing contractions to 157 +- 11% (s.e.mean, n=5) of control. The maximum attainable in this tissue is approx. 190% of 1 mM calcium contractions.Higher concentrations of palmitoyl carnitine (10-30 µM) were also positively chronotropic but ultimately inhibited automaticity.

Figure 1 Effects of palmitoyl carnitine on contractility



These effects are consistent with activation of calcium channels in heart muscle by palmitoyl carnitine. Positive inotropism was observed at lower concentrations than those previously reported to increase contractility and the rate of rise of calcium dependent action potentials in avian heart muscle (30-300 µM) (Inoue & Papanno, 1983).

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GX 1048 (GR 43659X), A NEW DIHYDROPYRIDINE CALCIUM ANTAGONIST WITH POTENT AND LONG-LASTING ANTIHYPERTENSIVE ACTION.

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GX 1048 (GR 43659X) (E)-4-(2-(3-(1,1-Dimethylethoxy)-3-oxo-1-propenyl)phenyl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid, diethyl ester) is a new vascular selective calcium channel blocker with potent and long-lasting antihypertensive activity, now under ongoing study in patients.

In vitro the compound antagonized Ca^{2+}-induced contraction of K^+ depolarized rabbit ear and basilar artery in a competitive manner (pA_2 8.4 and 9.2 respectively), and its effect was not reversed by washout over 10 h. On electrically driven guinea pig left atria, GX 1048 induced a negative inotropic effect at a higher concentration than the vascular ones.

In vivo on spontaneously hypertensive rats (SHR), the compound exerted a dose-related BP reduction both after oral and i.v. administration with a potency as ED_{25} (25% fall in BP) of 0.33 mg/kg and 0.009 mg/kg respectively. The duration of action was at least 12 h orally and 5 h i.v. Concomitant with the BP reduction, a short-lasting heart rate increase was detected.

In conscious renal hypertensive dogs (RHD), GX 1048 confirmed its powerful and long-lasting activity. In fact ED_{25} values were 0.5 mg/kg p.o. and 0.008 mg/kg i.v., and the antihypertensive effect lasted at least 9 h and 5 h after oral and i.v. administration.
The compound administered once a day for 5 consecutive days in SHR rats and RHD dogs at ED_{25} doses did not show any evidence of tolerance.

In anaesthetized dogs after intravenous administration (0.001-0.03 mg/kg), GX 1048 reduced BP, systemic and coronary vascular resistances and concomitantly increased stroke volume, cardiac output and coronary blood flow. These effects did not decline over 3 h after dosing. No changes in heart rate were detected.

As far as other pharmacological actions are concerned, GX 1048 produced a dose-related inhibition of gastrointestinal transit in rats at doses 10 times higher than the antihypertensive ones. No behavioural changes were detected after single oral administration in rats (1-30 mg/kg) and in dogs (2-50 mg/kg). Single (1-100 mg/kg) and repeated (1-10 mg/kg) oral administration in rats did not significantly effect pentobarbitone-induced hypnosis. In mice the compound proved to be devoid of any muscle relaxant effect, as well as of anticholinergic, central analgesic and anticonvulsant activity.

From a toxicological point of view, the compound was non-mutagenic, devoid of any toxic effect on the reproductive system and endowed with a good safety margin in chronic studies.

These results suggest GX 1048 is a new vascular selective calcium channel blocker, useful as an antihypertensive agent with long-lasting action and potential for once daily therapy.

THE DIFFERENTIAL POTENTIATION OF THE EFFECTS OF CALCIUM CHANNEL BLOCKERS BY CONDITIONS IN VITRO WHICH MIMIC ISCHAEMIA IN VIVO

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It has been shown (Lumley & Robertson, 1986) that a combination of acidosis, raised extracellular K^+ concentration and hypoxia produced a significant 4-fold potentiation of the negative inotropic effect of verapamil on the guinea-pig electrically stimulated papillary muscle in vitro. We have now further investigated this phenomenon and also tested whether these 'ischaemic' conditions potentiated the effects of other Ca^{++} channel blockers. Diltiazem and nifedipine were chosen as representatives of the structurally diverse series of compounds which are believed to exert their action predominantly through an action at the slow calcium channel (Spedding, 1985).

Right ventricular papillary muscles from male guinea-pigs were electrically stimulated (3 Hz; threshold voltage (~ 1V); 5 msec duration) in a non-depolarising modified Krebs solution, pH 7.4, at 32°C and bubbled with 95% O_2, 5% CO_2. The potency of the various Ca^{++} channel blockers was then determined either by measuring their ability to displace Ca^{++} concentration (0.6 - 9 mM) -response curves to the right or by measuring the direct negative inotropic effect of the drugs.

Verapamil (0.1, 1 and 3 μM) caused concentration-related rightward displacements of the Ca^{++} curve. This effect was significantly ($P < 0.05$) potentiated approximately 7 fold by the 'ischaemic' conditions (pH 6.9; K^+ 9.2 mM, bubbled with 55% O_2, 40% N_2, 5% CO_2). This potentiation was little affected by the presence of phentolamine (1 μM) and propranolol (300 nM). Diltiazem (0.6 - 20 μM) and nifedipine (0.03 - 1 μM) also produced rightward displacements of Ca^{++} concentration-effect curves. Under ischaemic conditions the effect of diltiazem was potentiated approximately 3-fold whilst that of nifedipine was unaltered. The effect of ischaemia upon the direct negative inotropic effect of the two drugs was also assessed. The geometric mean IC_{50} (95% confidence limits) (concentration to produce a 50% decrease in developed tension) for diltiazem and nifedipine in 'normal' conditions were 1.4 μM (0.5 - 3.7, n = 6) and 28 nM (22 - 35, n = 5) respectively. In the 'ischaemic' conditions these values were 0.57 μM (0.27 - 1.2, n = 7) and 24 nM (15 - 36, n = 4) respectively. Thus the negative inotropic effect of diltiazem was approximately 2 - 3 fold greater in 'ischaemic' conditions whereas that of nifedipine was unaltered. Whilst the 'ischaemia'-induced potentiation of the effect of diltiazem was not statistically significant, the small degree of potentiation was consistently observed using both protocols. Thus, the rank order of potentiation produced by the 'ischaemic' conditions was verapamil > diltiazem > nifedipine ~ 0. This rank order was the same when based upon either the direct negative inotropic effect of the compounds, or the ability of each compound to displace Ca^{++} concentration-effect curves.

In summary, conditions in vitro which mimic ischaemia in vivo do not potentiate the negative inotropic effect of verapamil, diltiazem and nifedipine equally. The reason for the variable potentiation is unclear but a differential potentiation of the negative inotropic effect of calcium channel blockers was also reported by Smith & Briscoe (1985) who measured the negative inotropic effects of these drugs on cat papillary muscles at pH 7.4 and 6.0.

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ALTERATION IN Na-HANDLING AND VOLUME STATUS BY CHRONIC NIFEDIPINE TREATMENT IN RATS WITH 1-KIDNEY, 1-CLIP RENAL HYPERTENSION

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It is generally acknowledged that calcium antagonists like nifedipine improve renal Na^+-excretion. Also, nifedipine was reported to affect red blood cell Na^+-transport in Dahl-S and DOCA-NaCl hypertension. We studied the influence of chronic treatment with nifedipine on plasma volume, red blood cell Na^+-concentration, Na^+,K^+-pump, Na^+,K^+-cotransport, passive permeability for Na^+, and plasma atrial natriuretic peptide (ANP) immunoreactivity in Wistar rats with 1-kidney, 1-clip renal hypertension. This form of hypertension is volume-expanded, and, unlike DOCA-NaCl hypertension, independent of exogenousmineralocorticoid. The right renal arteries of 9 - 11 week old female rats were constricted with silver clips of o.18 mm internal diameter. One week later, the contralateral kidney was excised. Control animals were sham operated. The rats were then fed on standard rat chow pellets with or without 300 ppm of nifedipine added. All measurements were done after 2 and 6 weeks post operation.

time	CONTROL				NIFEDIPINE			
post op.	BP mmHg	Na^+-pump h^{-1}	pl.vol. ml/kg bw.	ANP pg/ml	BP mmHg	Na^+-pump h^{-1}	pl.vol. ml/kg bw.	ANP pg/ml
sham	112 ± 3	0.360 ±0.013	29.8 ± 0.5	101.0 ± 15.9	91 ± 3	0.350 ±0.022	31.9 ± 0.8	82.8 ± 9.9
2 weeks RH	187** ± 5	0.314* ±0.012	35.8* ± 1.7	192.5* ± 40.7	139** ±11	0.384 n.s. ±0.014	31.7 n.s. ± 1.5	106.8 n.s. ±18.0
sham	112 ± 5	0.388 ±0.013	24.4 ± 0.1	88.4 ± 13.3	91 ±11	0.369 ±0.020	22.7 ± 0.9	56.8 ± 11.0
6 weeks RH	207** ± 8	0.399 n.s. ±0.016	25.7 n.s. ± 0.6	194.4* ± 35.7	161* ± 5	0.346 n.s. ±0.017	23.5 n.s. ± 0.5	102.4* ± 16.8

*P < 0.05; **P < 0.001; n.s. P > 0.05 vs. sham operated animals of corresponding group (6 -8 animals/group)

In untreated controls after 2 weeks plasma volume and plasma ANP were increased, and the rate constant of the Na^+-pump was diminished. After 6 weeks, only plasma ANP was still increased.

In animals treated with nifedipine, the development of hypertension was suppressed. There were no differences between sham operated animals and rats with renal hypertension in Na^+-pump activity, and plasma volume. Plasma ANP was low in all groups treated with nifedipine.

It is concluded that plasma volume expansion in 1-kidney, 1-clip renal hypertension is associated with red blood cell Na^+-pump depression and a sharp rise in plasma ANP immunoreactivity. Chronic antihypertensive treatment with nifedipine apparently normalizes the volume status and concomitant deviations in red blood cell Na^+-handling and release of ANP.

A STUDY IN THE RAT OF THE RENAL EFFECTS OF ATRIOPEPTIN III AND AN ATTEMPT TO LOCATE ITS SITE OF ACTION ALONG THE NEPHRON.

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The atrial natriuretic peptides isolated from the heart atria have now been synthesised and characterised (Needleman et al, 1985). Although these compounds have potent effects on the kidney, increasing sodium and water excretion, they also appear to be selective renal vasodilators. In this study an attempt was made to determine whether these two actions could be separated by infusing a number of different doses of the peptide, and to examine possible sites along the nephron at which the reabsorptive processes were inhibited.

Male Sprague-Dawley rats (375-420 g) were anaesthetized (60 mg/kg sodium pentobarbitone, i.p. plus 15 mg/kg/h, i.v.) and prepared for renal function measurements (Johns, 1985). Lithium clearance was measured and used to estimate proximal tubular reabsorption (Thomsen, 1984). Clearance periods of 15 min duration were used, two before and two following a period during which the peptide was infused. Atriopeptin III was given for a total of 25 min with a clearance period being taken over the last 15 min of infusion.

In a group of 9 renally denervated rats, atriopeptin III infused at 50, 100 and 200 ng/kg/min did not change blood pressure at 123 $\pm$ 6 mmHg, renal blood flow at 14.0 $\pm$ 1.5 ml/min/kg or glomerular filtration rate at 3.17 $\pm$ 0.31 ml/min/kg but significantly (all P's<0.05) increased urine flow from 64.0 $\pm$ 9.4 µl/min/kg by 36, 41 and 203%, respectively, absolute sodium excretion from 16.9 $\pm$ 2.8 µmol/min/kg by 42, 40 and 214%, respectively, fractional sodium excretion from 4.4 $\pm$ 0.8% by 35, 55 and 195%, respectively, and fractional lithium clearance from 23.4 $\pm$ 3.6% by 5, 34 and 44%, respectively. In 11 animals with intact renal nerves, atriopeptin III infused at 50, 100 and 200 ng/kg/min had no effect on blood pressure at 135 $\pm$ 5 mmHg, renal blood flow at 16.5 $\pm$ 1.3 ml/min/kg or glomerular filtration rate at 3.02 $\pm$ 0.19 ml/min/kg but significantly increased urine flow from 62.3 $\pm$ 9.5 µl/min/kg by 31, 54 and 188%, respectively, absolute sodium excretion from 15.4 $\pm$ 1.5 µmol/min/kg by 38, 115 and 222%, respectively, fractional sodium excretion from 3.7 $\pm$ 0.2% by 51, 89 and 221%, respectively and fractional lithium excretion from 22.1 $\pm$ 0.9% by 22, 39 and 56%, respectively.

These data show that under these experimental conditions, infusion of atriopeptin III had minimal effects on blood pressure, renal blood flow and glomerular filtration rate. However, the peptide caused dose related increases in sodium and water output. At 50 and 100 ng/kg/min atriopeptin III, the natriuretic actions could be attributed to an inhibition of proximal tubular reabsorption whereas at 200 ng/kg/min the reabsorption of sodium appeared to be inhibited at more distal tubular sites.

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ABSORPTION OF PHENYLBUTAZONE IN THE HORSE FOLLOWING ORAL ADMINISTRATION

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Phenylbutazone absorption following oral administration to horses is irregular, Cmax and Tmax showing considerable inter-animal variation (Moss,1972;Gerring et al.,1981;Rose et al.,1982;Sullivan & Snow,1982). The variable absorption pattern has not been explained but the herbivorous diet of the horse may be of importance.

In this study plasma concentration time curves for phenylbutazone were determined in six Welsh Mountain ponies housed in individual stalls and receiving a constant daily diet of concentrate (bran,200g; oats,300g; pony cubes, 150g) and hay in two portions (1.5 and 3.0kg) 5h apart. Phenylbutazone was administered orally as an aqueous suspension at a clinical dose rate (4.4mg/kg) to ponies under three feeding regimens : fasting before and access to hay after dosing (phase 1); access to hay before and after dosing (phase 2); fasting before and after dosing (phase 3). The drug was also administered intravenously for bioavailability determinations. Three weeks were allowed between each phase.

Table 1. Pharmacokinetic parameters for phenylbutazone (mean ± s.e. mean, n=6)

Parameter	Phase 1	Phase 2	Phase 3	Intravenous
Cmax	14.1±1.2	11.9±1.1	11.8±2.3	-
Tmax	5.3±1.5	13.2±1.2	5.9±1.8	-
AUC	134±20	124±11	141±20	187±24
Bioavailability	71±4	69±5	77±10	-

In contrast to previous reports of reduced bioavailability when phenylbutazone was administered to hay fed horses (Rose et al., 1982; Sullivan and Snow, 1982), the present study indicates that bioavailability is unchanged in horses with partial or full access to hay in comparison with those fasted before and after dosing. In vitro studies of phenylbutazone binding to hay in buffered solutions have shown that a high degree of binding occurs at several pH values. It therefore seems possible that binding to hay will occur in the gastrointestinal tract of horses. The very delayed mean Tmax value as well as the double peaks obtained in individual animals might be due to (a) passage into solution and rapid absorption of a proportion of the administered dose and (b) adsorption of the remainder onto the hay followed by release by fermentative digestion in the large intestine and a second absorptive phase from this site. These data may be significant therapeutically and toxicologically.

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STUDIES ON THE PHARMACOLOGY OF PHENYLBUTAZONE IN THE HORSE

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The enolic acid anti-inflammatory agent, phenylbutazone, has been used in equine medicine for more than 30 years and it is known that elimination half-life is dose dependent, ranging from 3.5 to 8.6h.

Using a carrageenin sponge implant system we have evaluated the use of phenylbutazone as an anti-inflammatory agent in the horse. In a cross-over study in six ponies three polyester sponge strips (50x25x5mm) soaked in sterile 2% carrageenin were implanted into a small subcutaneous pouch in the neck. Single sponges were removed at 6, 12 and 24h and the acute inflammatory exudate harvested. Samples were analysed for leucocyte numbers and, after centrifugation, for TXB_2, PGE_2 and 6-keto-$PGF_{1\alpha}$ by radioimmunoassay. Phenylbutazone at an i.v. dose of 4.4mg/kg produced a marked inhibition of eicosanoid synthesis up to 12h but exudate leucocyte numbers were not significantly affected. Phenylbutazone and its active metabolite oxyphenbutazone accumulated in and were cleared slowly from exudate as opposed to plasma. This might explain the relatively prolonged inhibition of eicosanoid synthesis in spite of the relatively short plasma half-life of 4.7h for phenylbutazone.

In a second study one group of 5 ponies received phenylbutazone orally as a paste formulation at a dose rate of 3.3mg/kg and a second group of 5 animals received equivalent doses of a placebo paste. Polyester sponges soaked in 2% carrageenin solution were inserted at the time of dosing and removed serially at 4,8,12 and 24h. Similar measurements to those reported on the first study were made and, additionally, the rise in skin temperature at the site of the acute inflammation was measured by infra-red thermometry.

Table 1. Actions and fate of phenylbutazone in a carrageenin-sponge model of inflammation

Time	Phenylbutazone concentration (µg/ml)		Rise in skin temperature (^{o}C)		Exudate leucocyte numbers (x 10^9/l)	
	Plasma	Exudate	Placebo	PBZ	Placebo	PBZ
4	9.09±3.17	3.17±1.20	2.7±0.5	0.4±0.4**	5±1	6± 2
8	7.45±2.22	8.12±2.55	n.d.	n.d.	15±5	17± 6
12	6.07±0.87	5.90±1.06	4.7±0.5	1.2±0.4**	12±2	10± 2
24	1.91±0.67	2.73±0.65	3.6±0.6	1.3±0.7*	34±6	43±13

Values are means s.e. mean (n=5) n.d. = not determined.*P<0.05:**P<0.01.

Phenylbutazone partially inhibited eicosanoid synthesis and attenuated the rise in skin temperature, but exudate numbers were unaffected. These findings indicate that recommended doses of phenylbutazone suppress eicosanoid synthesis and the heat generated by an acute inflammatory lesion, and the duration of action may be related to the accumulation of phenylbutazone and oxyphenbutazone in inflammatory exudate.

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INCREASED AIRWAY VASCULAR PERMEABILITY INDUCED BY PLATELET ACTIVATING FACTOR: EFFECT OF SPECIFIC ANTAGONISM AND PLATELET DEPLETION.

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Platelet-activating factor (PAF; acetyl glyceryl ether phosphorylcholine) is a potent mediator of inflammation, and may play an important role in the pathogenesis of asthma. It acuses acute bronchoconstriction and increases non-specific airways reactivity in several species including man (Chung et al, 1985; Mazzoni et al, 1985; Cuss et al, 1986).

Because oedema is a pathological feature found in the airways of patients dying of asthma (Dunnill, 1960), we examined the effect of PAF on the vascular permeability of the airways to macromolecules using Evans Blue (EB) dye. In anaesthetised Dunkin-Hartley guinea-pigs (300-500 Gm), EB dye (1% solution; 30 mg/kg) was injected via a jugular vein and allowed to circulate for 1 min. PAF, made up in 0.35% bovine serum albumen from a stock solution (1 mg/ml in ethanol) was given intravenously at the following doses: 1, 10, 50 and 100 ng/kg (volume of injection=0.3 to 0.8 ml). Five min later, the chest wall was opened and circulating EB dye was removed by the perfusion of citrate buffer (100 ml; pH 3.5; 120 mmHg) via the left ventricle. The lungs were removed en bloc; the trachea, main bronchi, proximal and distal intrapulmonary airways were prepared for assay of EB dye which was extracted in formamide (2 ml), and quantified by spectrophotometry (620 nm wavelength).

PAF caused dose dependent increases in EB extravasation in airway tissue (150%-400% of baseline at 50 ng/kg) and in oesophagus, bladder and nasal mucosa. To determine whether its effect was mediated through PAF receptors, we treated guinea-pigs with a specific PAF antagonist, the ginkgolide mixture BN-52063, for 2 min prior to PAF injection. BN-52063 at 2 mg/kg partially inhibited the effect of PAF (50 ng/kg) but at 5 mg/kg it caused complete inhibition. Because platelets accumulate rapidly in the lungs in response to PAF (Page et al, 1985), we determined whether PAF-induced microvascular leakage was mediated through the platelet. Guinea-pigs (n=5) were made thrombocytopenic after intraperitoneal injection of rabbit anti-platelet serum (mean platelet count 7,200/mm^3 compared to 585,500/mm^3 in controls); EB extravasation after PAF (50 ng/kg) was not significantly altered by platelet depletion in the intrapulmonary airways but was attenuated in the trachea.

We conclude that the increase in airway vascular permeability induced by PAF is mediated through PAF receptors, presumably on vascular endothelium, and is only partially dependent on platelets.

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INTERFERENCE OF A PAF-ACETHER ANTAGONIST WITH ADRENALINE-INDUCED ACTIVATION OF HUMAN PLATELETS.

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Adrenaline associated to PAF-acether triggers aggregation of human platelets at concentrations which are inactive when used alone (Fouque and Vargaftig, 1984). This synergism is not mediated by cyclooxygenase metabolites of arachidonate but is suppressed, as well as the direct effects of adrenaline, by alpha$_2$ adrenoceptor antagonists. The possibility that adrenaline and PAF-acether share pathways for platelet activation was now investigated. Human citrated platelet-rich plasma was aggregated with ADP (2.5 uM), arachidonate (0.4 mM), PAF-acether (0.1-1 uM) or adrenaline (0.5 - 5 uM), antagonists being added 1 min beforehand. To evaluate secretion, platelets were labelled with ^{14}C-5HT or secreted ATP was measured. The PAF-acether antagonist BN 52021 suppressed activation by PAF-acether (Braquet et al., 1985; Nunez et al., 1986), and was totally inactive against arachidonate, ADP and adrenaline when used up to 0.1 mM. In contrast, the PAF-acether antagonist and analogue Ro 19-3704 (3- 4 (R)-2-(methoxycarbonyl) oxy-3- (octadecylcarbamoyl) oxy = propoxy butyl thiazolium iodide; Burri et al., 1985) was not only effective against PAF-acether itself (IC_{50} of 0.4 + 0.05 uM) but also antagonized dose-dependently platelet aggregation and secretion by adrenaline, with an ICD_{50} of 5 + 2 uM. This antagonism differed from that due to aspirin-like drugs, since primary aggregation, which persists in spite of cyclooxygenase inhibition, was also suppressed. Furthermore, inhibition was restricted to PAF-acether and adrenaline, since Ro 19-3704 failed to interfere with ADP or arachidonate up to 0.1 mM. A chemical analogue of PAF-acether (1-O- octadecyl- 2-O- acetyl-glyceryl- 3-phosphoryl -morpholino- ethanol) which is not an antagonist (Coeffier et al.,in press), added to PRP up to 0.1 mM was inactive against adrenaline, whereas the reference PAF-acether antagonist compound CV-3988 (a phospholipid analogue of Ro 19-3704; Terashita et al., 1983) at 0.1 mM antagonized altogether the effects of PAF-acether and adrenaline. Finally, alpha$_2$ adrenoceptor antagonists which suppress the first and the second wave of aggregation by adrenaline are inactive against PAF-acether (Hydergine, dihydroergotamine) or only marginally efective (yohimbine) (Fouque and Vargaftig, 1985). Our results indicate that endogenous PAF-acether does not account for adrenaline-induced human platelet activation and that PAF-acether and adrenaline do not share a common receptor, since neither BN 52021 nor alpha$_2$ adrenoceptor antagonists antagonized adrenaline and PAF-acether, respectively. They also suggest that a component of the adrenaline receptor or pathway is affected by PAF-acether antagonists structurally related to the agonists.

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EFFECTS OF LEUKOTRIENES ON EOSINOPHIL EMIGRATION INTO CONJUNCTIVAL TISSUE

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The conjunctiva has the ability to synthesize SRS-A (Kulkarni & Srinivasan, 1983; Williams et al., 1983), and conjunctival microvascular permeability can be altered by exogenously applied leukotrienes (LTs) (Woodward & Ledgard, 1985). This study examined the effects of topically administered leukotrienes (LTB_4, LTC_4, LTD_4 and LTE_4) on leukocyte infiltration into the guinea-pig conjunctiva. The effects of histamine (HA), bradykinin (BK), and the L-alanylglycyl-L-seryl-L-glutamic acid and L-valylglycyl-L-seryl-L-glutamic acid forms of eosinophil chemotactic factor of anaphylaxis (ECF-A) were also evaluated for comparative purposes.

20µl of test solution were topically applied to the left eye and 20µl of vehicle solution to the right eye of Hartley strain albino guinea-pigs. LTs were evaluated over the dose range 10ng-1000ng; histamine, bradykinin and ECF-As over the dose range 10µg-1000µg. Animals were killed at 6 hours post-treatment time by intracardiac injection of T-61 Euthanasia solution. Eosinophil counts were obtained from 6µm paraffin sections, taken from formalin-fixed eyes enucleated immediately after sacrifice, at 500x magnification.

LTD_4 and LTE_4 demonstrated dose-dependent eosinophil emigration over the tested dose range, but LTB_4 elicited marked eosinophil infiltrates only at the highest dose. LTC_4 was essentially inactive. Scattered neutrophils were observed at the highest doses of LTB_4 and LTE_4. Relative potencies, with 95% confidence limits (based on 50% maximal response to LTB_4): LTB_4, 562.9pMol (210.4-2382.4); LTD_4, 33.6pMol (2.2-96.3); LTE_4, 32,8pMol (4.5-80.3); HA, 33,802 pMol (15,992-57,217). Eosinophil emigration was directional for the conjunctival epithelium. The cells appeared intact and no evidence of tissue damage was observed in any LT treatment group. HA-induced eosinophilia showed extensive cell degranulation and fragmentation with attendant epithelial damage. Contralateral control eyes showed no eosinophilia and no tissue damage. In contrast to the LTs and HA, BK, both forms of ECF-A, and BK/ECF-A combinations failed to evoke any significant leukocyte response. LT-induced eosinophil infiltration was virtually abolished by the SRS-A antagonist, FPL 55712 (200µg topical), but was not affected by pretreatment with the cyclo-oxygenase inhibitor, indomethacin (16mg/kg).

Reports of chemotactic stimulation of eosinophils by ECF-A are based largely on *in vitro* studies of isolated cells. The *in vivo* studies reported here indicate that ECF-A, alone or in combination with BK, does not elicit eosinophil emigration into the conjunctiva. Moreover, the lack of eosinophil emigration with BK treatment demonstrates that simply increasing microvascular permeability is insufficient to cause appreciable cellular infiltration. In addition to involvement in other aspects of the inflammatory process, the peptidoleukotrienes may also evoke eosinophil infiltration, which is a salient characteristic of type I allergy in the eye.

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HUMAN BRONCHIAL SECRETION: EFFECT OF SUBSTANCE P, MUSCARINIC AND ADRENERGIC STIMULATION IN VITRO

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Airway mucus secretion is regulated by autonomic nerves and abnormalities in autonomic control may contribute to abnormalities in mucus secretion in disease. We have measured the secretion of mucus from human bronchi in vitro using fucose as a specific marker of mucus secretion and hexose and protein as non-specific markers of secretion (Lopez-Vidriero et al,1977;Jeffery et al,1984). The sensory neuropeptide substance P (SP) is a potent secretagogue in canine trachea (Coles et al,1984) and we have now compared its effects on secretion in man with those of the muscarinic agonist methacholine (MeCh), the alpha-agonist phenylephrine (Phen) and the beta-agonists terbutaline (Terb:beta 2-selective) and dobutamine (Dob:beta 1-selective)

Main bronchi from cigarette smokers, ex-smokers and non-smokers, which had been resected for carcinoma were dissected and oxygenated in ice-cold Krebs-Henseleit solution overnight. The following day, segments of the opened airways were mounted flat between the two halves of Ussing chambers and bathed in oxygenated Krebs at 37°C. Every 30 min the Krebs (containing secretions) was collected from the lumenal side of the chamber. After equilibration for 2.5h SP, MeCh, Phen, Terb or Dob (100μM final conc.) was added to both halves of the chambers for a 30min period before collection. One h later a second drug was tested. By electron microscopy the tissue, taken from the chambers at the end of the experiment, appeared well preserved with the cells free of vacuolation or disrupted mitochondria. After equilibration, the mean basal rate of secretion (μg/cm^2 tissue per $^1/_2$h) for each marker was fucose (ie.mucus) 8 (n=22), hexose 278 (n=22) and protein 307 (n=17). SP and MeCh, but not the adrenergic drugs, significantly ($p<0.05$*, sign test) increased the rates of secretion of fucose and hexose above basal. SP also significantly ($p<0.05$*) protein secretion. Atropine (100μM) blocked the secretory responses to MeCh.

Mean % change in rate of secretion (SEM)

	SP (n=5)	MeCh (n=11)	Phen (n=7)	Dob (n=5)	Terb (n=5)
Fucose	+199* (67)	+94* (34)	-34 (15)	+7 (34)	+248 (176)
Hexose	+17* (9)	+68* (38)	-7 (21)	+3 (10)	+459 (544)
Protein	+71* (68)	+64 (44)	+29 (32)	+5 (24)	+42 (58)

We conclude that human bronchi show differential responses to autonomic agonists in terms of secretion. The different components of the stimulated secretions may represent a balance between different secretory rates (Quinton,1979) and the secretory structure stimulated. SP appeared to be most potent which suggests that it may be involved in secretion in human airways, possibly via an axon reflex mechanism (Barnes,1986).

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IMMUNISATION INDUCES BRONCHIAL HYPER-REACTIVITY AND INCREASED MEDIATOR RELEASE FROM GUINEA-PIG LUNGS

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Bronchial hyper-reactivity of asthma may be due to increased generation of mediators. Since PAF-acether (1-O-alkyl-2-acetyl -sn-glyceryl-3-phosphorylcholine) induces bronchoconstriction (Vargaftig et al., 1980) and hyper-responsiveness to unrelated bronchoconstrictor agents in guinea-pigs (review by Page et. al., 1984), we investigated whether actively sensitized guinea-pigs responded more to PAF-acether, to leukotriene (LT) D4 and to arachidonic acid (AA) as compared to control Al(OH)3-treated animals. Hartley guinea-pigs were sensitized i.p. with 10 µg of ovalbumin in 1 mg of Al(OH)3 (modified by Detsouli et al., 1985 from Andersson & Bergstrand, 1981). Seven to 10 days later the animals were anaesthetized with pentobarbitone, thoracotomized and the lungs were perfused through the pulmonary artery with 50 ml of a Krebs solution containing 2.5 g/l of bovine serum albumin (Krebs-BSA). The lungs were transfered into a chamber, ventilated and perfused with oxygenated Krebs-BSA. After a 10 min period of equilibration, PAF-acether or LTD4 were administered at 10 min intervals at 3 concentrations: 1, 3 and 10 ng or 10, 30 and 100 ng or with AA at 10 µg. At the end of each experiment 10 µg of ovalbumin were injected. One-minute fractions of the effluent were collected during the first 3 minutes and at the tenth minute after the injections for the determination of histamine (fluorometrically) and of thromboxane (TX) B2 and 6-oxo-PGF1$_\alpha$, by an RIA. PAF-acether or LTD4 down to 1-10 ng induced a dose-dependent bronchoconstriction in lungs from Al(OH)3-treated animals (controls), but 10 ng evoked a maximum irreversible bronchoconstriction on those from sensitized guinea-pigs. The basal secretion of histamine, of TxB2 and of 6-oxo-PGF1$_\alpha$ by control lungs was of 0.30 ± 0.1, 0.7 ± 0.07 and 0.35 ± 0.05 ng/ml, respectively, and of 2.3 ± 0.7, 1.6 ± 0.3 and 0.6 ± 0.1 by the sensitized lungs, respectively (n = 4-5). Stimulation of sensitized lungs with PAF-acether or LTD4 resulted in the formation of significantly higher amounts of TxB2 and 6-oxo-PGF1$_\alpha$ as compared to control animals. Increased formation was particularly marked for histamine, since in a series of experiments when the background of histamine release was of 0.5 ng/ml, PAF-acether at 1 ng released 0.75 ± 0.43 ng/ml from control lungs, and 12.0 ± 4.3 ng/ml from sensitized ones, and LTD4 1.5 ± 0.6 and 15.0 ± 5 ng/ml, respectively. Dose-dependency was maintained when 1-100 ng of both mediators were used. 10 µg of AA were transformed into 0.8 ± 0.2 and 19.0 ± 6 ng/ml of TxB2 when injected into control or sensitized lungs, respectively. Lungs from the sensitized guinea-pigs challenged with ovalbumin released 160.4 ± 17.0 ng/ml histamine, 184.8 ± 18.0 ng/ml TXB2 and 8.4 ± 0.10 ng/ml 6-oxo-PGF1$_\alpha$. Our results show that lungs from actively sensitized guinea-pigs are hyper-responsive to PAF-acether, to LTD4 and to AA, with respect to the cyclooxygenase -dependent metabolites, to histamine and to bronchoconstriction. Studies with inhibitors (anti-histamine, LT antagonists, and enzyme inhibitors) should indicate if one or more pathways account for these effects. At this stage, our results demonstrate unequivocally that anti-allergic drugs should be tested in allergic animals, since immunization modifies qualitatively the pattern of responses to putative mediators.

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SOME CHARACTERISTICS OF THE AMINE OXIDASE ACTIVITIES FOUND IN TISSUES OF THE RACEHORSE

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The ingenuity of those engaged in the illicit administration of agents affecting the performance of racehorses provides a stimulus to the development of various methods of detection. For example, it may be possible, in readily obtainable blood samples, to measure changes in the activities of enzymes or in concentrations of endogenous substances, to provide clues to the identity of the administered agent. In view of the importance of biogenic amines in racing performance, it was decided to examine the amine oxidase activities of blood and other selected tissues of the horse to see if they might be of value in this respect. An amine oxidase, provisionally called benzylamine oxidase (BZAO; Bergeret et al, 1957) has been found in horse plasma, but little appears to be known about monoamine oxidase (MAO) in blood or other tissues of the horse.

Blood samples in either citrate or lithium-heparin were obtained by jugular venepuncture from racehorses while tissue samples came from horses destroyed following racing accidents. Blood samples were divided into platelets, erythrocytes, buffy coat and plasma while tissues were homogenised in 1mM potassium phosphate buffer, pH 7.8. MAO and BZAO activities were measured by the use of radioactively labelled benzylamine, tyramine and 5-hydroxytryptamine (5-HT) together with unlabelled potential substrates and inhibitors.

Very little amine oxidase activity towards tyramine and 5-HT could be found in any blood fraction. However deamination of benzylamine was readily detected in the plasma by an enzyme with a Km of 106 ± 4.6 μM with an apparent Vmax value of 821 ± 78 $nmol.h^{-1}.ml^{-1}$ plasma (uncorrected for haematocrit; n = 11). This activity was unaffected by preincubation with 1mM clorgyline or deprenyl (selective irreversible inhibitors of MAO-A and -B respectively) but was completely inhibited by 2×10^{-4}M semicarbazide, an agent active against non-flavin dependent amine oxidases.

When other tissue samples were examined, high activities of MAO were found in the liver with Km and Vmax values for 5-HT and benzylamine respectively of 455 ± 28 μM and 117 ± 15 $nmol.h^{-1}.mg^{-1}$protein (n = 7) and 104 μM and 721 $nmol.h^{-1}.mg^{-1}$protein (n = 2). Use of clorgyline indicated that while benzylamine and tyramine appeared to be substrates for MAO-B alone, 5-HT was deaminated mainly by MAO-A. When similar experiments were undertaken with lung and kidney, substantial deviation from linearity of the kinetic plots was seen at low concentrations of 5-HT but not with other substrates. Since both these tissue homogenates were contaminated by blood, various amounts of blood were added to liver homogenates and the incubations with substrates repeated. With 5-HT as substrate, the kinetic plots were again found to be non-linear. This deviation was produced both with haemoglobin in solution and with intact red cells added to homogenates in isotonic media. Conversion of haemoglobin to methaemoglobin removed this efffect.

The ease with which plasma BZAO can be measured and some observations showing its activity increasing following exercise, suggests that this enzyme might be used to reveal manipulations of amine metabolism. In addition, if the interaction between MAO-A and the red blood cell takes place in vivo, it may be important in local responses to 5-HT in organs such as the lung and kidney.

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ALTERATIONS IN H_1-AGONIST AFFINITY AND EFFICACY INDUCED BY 1,4-DITHIOTHREITOL IN GUINEA-PIG ILEUM.

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We have previously reported that the disulphide bond reducing agent 1,4-dithiothreitol (DTT) selectively potentiates H_1-receptor mediated contractile activity in the longitudinal smooth muscle of guinea-pig ileum (Donaldson & Hill, 1986a). In these studies, however, it was not clear whether the potentiating effects of DTT were due to an increase in agonist efficacy and/or affinity. We have now undertaken a combination of contractile and radioligand binding studies to investigate these possibilities.

Agonist-induced contractions were recorded isotonically in Kreb's-Henseleit solution at 37°C gassed with O_2/CO_2 (95:5). DTT (1 mM) was added to the reservoir solution and allowed to equilibrate with the tissue for 30 min prior to the determination of subsequent dose-response curves. The irreversible H_1-receptor antagonist phenoxybenzamine (POB; 5 μM) was incubated with the tissue for 30 min. Muscle strips were then washed with Kreb's containing 1 mM sodium thiosulphate every 15 min for a period of 1 h, before the re-determination of agonist dose-response curves. The effect of DTT (1 mM) on the agonist inhibition of ^{3}H-mepyramine binding to a membrane fraction of guinea-pig ileum was determined essentially as described previously (Donaldson & Hill, 1986b).

Studies using POB to eliminate the spare receptor reserve suggested that DTT altered both agonist efficacy and affinity. The affinity constant for histamine, calculated from these experiments using the method of Furchgott & Bursztyn (1967), increased significantly from $1.8\pm0.2 \times 10^5 M^{-1}$ (n=7) in the absence of DTT to $4.9\pm1.1 \times 10^5 M^{-1}$ (n=6; P<0.002 Mann-Whitney U-test) in the presence of DTT. Agonist efficacy similarly appeared to increase in the presence of DTT since the maximal response to histamine following POB treatment increased significantly in the presence of DTT from 59.9±5.9% to 85.7±4.4% (n=7) of the response to histamine prior to POB treatment (P<0.01, Wilcoxon signed rank). This increase in maximum was accompanied by a leftward shift of the dose-response curve (EC_{50}[-DTT]/EC_{50}[+DTT]=4.1±0.7, P<0.02 Wilcoxon signed rank test). Further evidence for an increase in agonist efficacy was provided by studies using two partial H_1-agonists, SKF 71473 (Durant et al., 1973) and N-N-diethyl-2-pyridylethylamine (DE-2P, Kenakin & Cook, 1980). In the presence of DTT, the maximum response to SKF 71473 increased from 58.3±7.6% to 98.2±1.2% (SKF 71473, n=6, P<0.05 Wilcoxon signed rank test) of the response to histamine in the absence of DTT, and the dose-response curves, shifted to the left (3.7±0.9 fold, P<0.05 Wilcoxon signed rank test). Similar results were obtained with DE-2P. ^{3}H-Mepyramine binding studies confirmed that DTT increased agonist affinity. DTT produced a significant parallel shift to the left of the displacement curves for histamine (IC_{50}[-DTT]/IC_{50}[+DTT]=3.4±0.7, n=4, P<0.005 analysis of variance according to Delean et al., 1978).

The results of this study therefore suggest that DTT potentiates H_1-receptor mediated contractile activity in guinea-pig ileum by increasing both agonist efficacy and affinity.

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ADENOSINE AUGMENTS HISTAMINE-INDUCED INOSITOL PHOSPHOLIPID HYDROLYSIS IN GUINEA-PIG CEREBRAL CORTICAL SLICES

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Adenosine is an inhibitory modulator of both central and peripheral neurotransmission (Snyder, 1985). Adenosine can both inhibit or stimulate adenylate cyclase activity via A_1 and A_2 receptors respectively (Snyder, 1985). In guinea-pig cerebral cortical slices the A_2-receptor mediated stimulation of cyclic AMP accumulation can be augmented by histamine H_1-receptor stimulation (Hill et al., 1981). We have now examined the converse relationship i.e. the effects of adenosine on H_1-receptor function, by determining the effect of adenosine on histamine stimulated inositol phospholipid hydrolysis.

Guinea-pig (Hartley strain) cerebral cortical slices were prepared and preincubated for 60 min with [^{3}H]myo-inositol in the presence of 5 mM LiCl. Adenosine and histamine were added simultaneously and the incubation terminated after a further 45 min with 10% (w/v) perchloric acid. The accumulation of [^{3}H]inositol phosphates was then measured using ion exchange chromatography (Donaldson & Hill, 1985).

Adenosine had no significant effect alone, but it enhanced the accumulation of total [^{3}H]inositol phosphates elicited by histamine (0.1 mM) in a concentration dependent manner (EC_{50} of adenosine = 30 μM). There was a 2.3$\pm$0.2 fold (n=14) increase in [^{3}H]inositol phosphate accumulation in the presence of 0.1 mM histamine, and a 4.0$\pm$0.3 fold (n=14) rise when 0.1 mM adenosine was also present. The increased response to histamine was due to an elevation of the maximal effect and no change in the EC_{50} value was observed. The stable adenosine analogue, 2-chloroadenosine (0.1 mM) also potentiated the response to histamine (0.1 mM) by 2.3$\pm$0.1 fold (n=3). However, adenosine (0.1 mM) had no effect on the accumulation of [^{3}H]inositol phosphates elicited by carbachol (100 μM), noradrenaline (300 μM), 5-hydroxytryptamine (300 μM) or elevated K^+ ion concentration (31 mM).

The augmentation of the histamine response by adenosine (0.1 mM) was completely inhibited by the adenosine receptor antagonists theophylline (1 mM) and isobutylmethylxanthine (1 mM) while the response to histamine (0.1 mM) alone was unchanged. The A_1-selective agonist 1-N^6-phenylisopropyl adenosine (l-PIA) enhanced the response to histamine with an EC_{50} of 0.6 μM. This concentration, however, is greatly in excess of that required to occupy A_1 receptors selectively (Schwabe & Trost, 1980).

It is not yet clear whether the interaction between adenosine and histamine occurs at the receptor or post receptor level. However, whatever the exact mechanism the results of this study indicate a novel property of adenosine which might be representative of a more general form of interaction between modulators and neurotransmitter substances.

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THE DESIGN OF BRAIN-PENETRATING H_2 RECEPTOR HISTAMINE ANTAGONISTS.

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The study of H_2 histaminergic mechanisms in brain would be greatly facilitated by the availability of suitably potent and selective antagonists which readily cross the blood-brain barrier. Most H_2 receptor antagonists prepared to date, however, are typically polar, hydrophilic compounds lacking the ability to enter the brain in more than very small amounts. In order to overcome this problem, studies were carried out to identify physicochemical properties of existing antagonists which could be modified to increase brain penetration in analogues.

Biological membranes are generally considered to be lipoidal, and permeable to uncharged chemical species, and the relative ability of these species to diffuse through membranes is thought to depend on their lipid solubilities and size. In seeking a model solvent system which closely resembles the partitioning characteristics of the blood-brain barrier, comparisons were initially made of the partition coefficients of 3 H_2 antagonists for which measurements could be made in a variety of solvent systems, with clonidine, mepyramine and imipramine which readily cross the blood-brain barrier. In contrast with earlier observations (Glave and Hansch, 1972; Rapoport et al, 1979), logarithms of the partition coefficients (logP), measured in the octanol/water system and corrected for ionisation, showed no correlation with the logarithms of the equilibrium brain/blood concentration ratios for the isotopically-labelled compounds, estimated in the anaesthetised male rat. The difference between the octanol/water and cyclohexane/water logP values (ΔlogP), which is regarded as an approximate measure of hydrogen-bonding ability (Seiler, 1974), however, showed a good correlation. This result is in accord with Overton's rules relating to the effect of polar groups on the ability of a compound to pass through membranes (Wright and Diamond, 1969).

Structural modification of different structural types of H_2 antagonist namely cimetidine, tiotidine and ranitidine have been made with the aim of reducing overall hydrogen-bonding ability. In each case large increases in brain penetration were obtained, but the best combinations of brain penetration and H_2 antagonist activity were obtained in a series of compounds possessing the 3-(3-(1-piperidinylmethyl)phenoxy)propyl side chain, in particular, SK&F 95282, the 2-aminobenzothiazole derivative, which has a brain/blood concentration ratio of 1.4 and a pA_2 value in the guinea pig atrium against histamine of 7.46 (Calcutt et al, 1986).

Comparison of the brain penetration and ΔlogP values of 20 compounds for which data is available shows a highly significant correlation, and it is suggested that this relationship might have more general utility in the design of other types of drugs where brain penetration is either to be maximised or minimised.

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SK&F 95282 IS A POTENT SELECTIVE BRAIN-PENETRATING HISTAMINE H_2-RECEPTOR ANTAGONIST.

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Histamine has been identified in the brain of many species, including man. The amine does not readily penetrate the brain from the cerebral circulation, and therefore its occurrence, and the identification of specific enzymes for its synthesis (histidine decarboxylase) and catabolism (histamine N-methyltransferase) in brain of various mammalian species supports the contention that locally synthesised histamine mediates specific functions in the tissue. There is also clear evidence for histamine receptors in brain; H_1-receptors (^{3}H-mepyramine binding; histamine-stimulated glycogenolysis; phosphatidylinositol turnover; cAMP accumulation), H_2-receptors (histamine-stimulated adenylate cyclase and cAMP accumulation; ^{3}H-tiotidine binding; electrophysiological studies), and H_3-autoreceptors (mediating feedback inhibition of neurotransmitter histamine release) have been identified.

What functions might histamine control in brain? Studies to this end would be facilitated by the availability of suitable receptor agonists and antagonists. While the advent of selective H_2-receptor antagonists has helped to clarify the role of histamine in gastric acid secretion, the currently available agents do not readily penetrate the brain, limiting their utility in investigations on the functions of histamine acting at central H_2-receptors. Here we describe the identification of 2-[3-[3-(piperidinomethyl)phenoxy]propylamino]benzthiazole (SK&F 95282) as a potent selective brain-penetrating histamine H_2-receptor antagonist.

SK&F 95282 antagonised histamine-stimulated tachycardia at the guinea-pig isolated right atrium, yielding pA_2 7.46 (cf. cimetidine pA_2 6.1), and the inhibition by histamine of KCl-induced contraction of the isolated rat uterus (pA_2 7.26). The compound also inhibited histamine-stimulated adenylate cyclase in cardiac ventricle homogenate (pK_i 7.3). The potency of SK&F 95282 at central H_2-receptors was demonstrated by: inhibition of ^{3}H-tiotidine binding to H_2-receptors in guinea-pig cortex and striatum (pK_i 7.2), inhibition of histamine-stimulated adenylate cyclase in guinea-pig hippocampal homogenates (pK_i 7.4) and of dimaprit-stimulated cAMP accumulation in guinea-pig hippocampal slices (pA_2 7.63). In addition, the compound showed good selectivity relative to various other central and peripheral receptors where it was at least 30-1000 less potent.

Evidence that SK&F 95282 crosses the blood-brain barrier was obtained from infusion of ^{14}C-SK&F 95282 into rats. After a period of infusion (approx. 2 hours) to establish a constant blood concentration, the concentrations of radio-activity in the brain and blood were measured, and a brain/blood ratio of 1.4 was calculated (cf. cimetidine 0.036).

Thus, SK&F 95282 has been characterised as a potent, selective brain penetrating H_2-receptor antagonist. The compound should help to clarify the role of histamine acting at H_2-receptors in brain.

TACHYKININ RECEPTORS IN RAT CEREBRAL CORTEX: [^{125}I]BOLTON-HUNTER-LABELLED-ELEDOISIN AND -NEUROKININ A LABEL A COMMON SITE.

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Radioligand binding studies have identified two types of binding sites for tachykinin peptides in the mammalian central nervous system. [^{125}I] Bolton -Hunter-labelled (BH)-substance P (SP) binds to sites having the pharmacological specificity of SP-P receptors, whereas [^{125}I]BH-eledoisin labels sites which resemble SP-E receptors (Cascieri et al, 1984). A third type of binding site specificity has been reported by Buck et al (1984) in mammalian peripheral tissues which has a high affinity for neurokinin A, can be labelled using [^{125}I]BH-neurokinin A and is designated an SP-K type. The present study was undertaken to evaluate the existence in the CNS of the SP-K type of tachykinin receptor specificity. To this end, we have examined the binding characteristics of [^{125}I]BH-neurokinin A using synaptic plasma membranes (SPMs) from rat cerebral cortex and compared these to the binding sites labelled by [^{125}I]BH-eledoisin in the same membranes.

Rat cerebral cortex SPMs were prepared and binding assays performed as described by Cascieri et al (1984). As shown in Table 1, there is a close agreement between the potencies of a number of tachykinin peptides as inhibitors of [^{125}I]BH-neurokinin A (0.5nM) and [^{125}I]BH-eledoisin (0.2nM) binding to rat cortex SPMs (correlation coefficient = 0.98). For both radioligands, neurokinin B was the most potent displacer and neurokinin A was >70 times less potent. The SP-P specific compound, substance P methyl ester, was inactive against both radioligands. A further indication of the similarity between the binding of [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin was inhibition by the GTP analogue guanylyl-5'-(ß-γ-imido)diphosphate (Gpp(NH)p; Cascieri et al, 1984) which gave IC_{50} values of 202 and 201nM, respectively.

Tachykinin	[^{125}I]BH-neurokinin A	[^{125}I]BH-eledoisin
	IC_{50}(nM)*	IC_{50}(nM)*
Neurokinin B	0.7(0.5,1.1)	0.7(0.4,1.0)
Kassinin	16.0(15.1,17.1)	21.7(18.6,25.2)
Eledoisin	23.2(20.2,26.6)	37.4(31.6,44.4)
Substance P	31.2(23.0,42.4)	71.8(59.0,87.3)
Neurokinin A	47.3(38.1,58.7)	64.1(52.8,77.8)
Physalaemin	90.8(78.0,105.7)	96.4(64.3,144.5)
$DiMeC_7$	203.2(116.4,354.8)	247.2(210.4,290.4)
Substance P methyl ester	>1800	>1800

* values are mean (-SEM, + SEM) of 3-4 experiments.

These data demonstrate that [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin bind to a common site in rat cerebral cortex SPMs. The potency of neurokinin B relative to neurokinin A indicates that the site labelled is not the SP-K site as defined by Buck et al (1984), but closely resembles the SP-N site described in guinea pig ileum myenteric plexus neurons by Laufer et al (1985).

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THE DISTRIBUTION OF [^{3}H]-MK801 BINDING SITES IN RAT BRAIN DETERMINED BY IN VITRO RECEPTOR AUTORADIOGRAPHY

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It has recently been reported that the novel anticonvulsant MK801 ((±)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine maleate) is a selective excitatory amino acid antagonist at N-methyl D-aspartate (NMDA) receptors (Wong et al 1986). However, MK801 appears to act allosterically rather than at the recognition site for NMDA. In an attempt to define this possible association with NMDA sites we have examined the distribution of ^{3}H-MK801 receptor binding sites in the central nervous system using a quantitative receptor autoradiographic procedure.

Sprague-Dawley rats were decapitated and 10µm cryostat sections of brain prepared. These were stored at -20°C for up to 2 weeks. Thawed sections were incubated in 50mM tris-HCl containing 190mM sucrose for 60 min prior to the application of ^{3}H MK801 (22.5 Ci/mmol) (30 nM) for 20 min at room temperature. Sections were then rinsed twice for 20 sec in fresh solution at room temperature. Dried sections were apposed to LKB Ultrofilm at 4°C for 3-4 weeks. Non-specific binding was determined by the addition of 100µM unlabelled MK801 to the incubation solution. Specific binding formed 50-60% of the total ligand bound to parasagittal sections.

Specific binding of ^{3}HMK801 was prevented by ketamine and also by the sigma receptor ligands dextrorphan and SKF 10047 (all at 100µM). NMDA and haloperidol failed to reduce the binding.

The highest concentrations of ^{3}HMK801 binding sites were detected in the hippocampus, particularly the CA1 region, the molecular layer of the dentate gyrus and superficial layers of the cerebral cortex. Moderate levels were observed in the caudate putamen and thalamic medial and lateral geniculate nuclei. Very low levels were present in the cerebellum, substantia nigra and corpus callosum. This pattern concurs with that for NMDA site labelling with ^{3}H-glutamate (Monaghan & Cotman 1985) or D-^{3}H-aminophosphonovaleric acid (Monaghan et al 1984). Linear comparison of the concentrations of NMDA and MK801 binding sites in 26 regions of rat brain indicated a positive correlation ($r = 0.78$).

The similar patterns of distribution of ^{3}H-MK801 & NMDA sites in rat brain would support the contention of Wong et al (1986) that MK801 acts at sites associated with NMDA receptors.

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ANTAGONISM OF QUISQUALATE RESPONSES BY BARBITURATES

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The interaction of barbiturates with amino acid transmitter systems extends beyond the well-documented enhancement of γ-aminobutyric acid (GABA). In particular, there is evidence for inhibition of excitatory amino acid responses (Barker & Ransom, 1978). This may be due to an effect at quisqualate or kainate receptors (Sadawa & Yamamoto, 1985; Harrison, 1985). We report here a quantitative study of the effect of barbiturates on quisqualate-induced responses in slices of rat cerebral cortex.

Segments from coronal sections of cortex + corpus callosum were arranged in two-compartment baths as described before (Harrison & Simmonds, 1985), such that population responses from pyramidal cells could be recorded. The compartment containing the cortex was perfused with Krebs medium from which Mg^{2+} had been omitted. Additions of quisqualate for 2 min periods evoked dose-dependent depolarizations and the amplitude of these responses was measured. Control dose-response lines were constructed for which the concentrations of quisqualate were usually 5 and 10 μM, i.e. in the low-middle part of the full dose-response curve. The dose-response line was redetermined in the presence of various concentrations of each barbiturate after at least 45 min perfusion with the barbiturate. The dose-response lines were parallel for up to 10-fold shifts to the right, allowing antagonism to be expressed as quisqualate dose ratios. From these, Schild plots were constructed. Each was approximately linear and the gradients and pA_2 values are given below.

	Concentration range	Slope	pA_2	n
Quinalbarbitone	10- 100 μM	1.23 ± 0.08	4.79	17
Pentobarbitone	30- 300 μM	1.00 ± 0.15	4.39	16
Phenobarbitone	30-1000 μM	0.84 ± 0.12	3.96	20
Butobarbitone	100-1000 μM	1.07 ± 0.08	3.71	12

The slopes of the Schild plots were close to 1 which would be compatible with a competitive action of the barbiturates. However, reductions in the slope of the dose-response curve to quisqualate were produced by higher concentrations of barbiturate. This suggests that the antagonism may, in fact, be non-competitive with the consequence that the pA_2 values cannot be interpreted as dissociation constants for the barbiturates. Nevertheless, the pA_2 values still provide a useful indication of the relative potencies of barbiturates in antagonizing quisqualate.

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HYPERPOLARISING RESPONSES TO N-METHYL-D-ASPARTATE(NMDA) RECEPTOR AGONISTS.

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Recently, we have described (Kemp et al, 1986) the selective, agonist-dependent antagonism of NMDA-receptor mediated depolarising responses by MK-801, a novel anticonvulsant (Clineschmidt et al., 1982). During the course of these experiments it became apparent that MK-801 attenuated the depolarising component of the NMDA response to a much greater extent than the "after hyperpolarisation". In this communication we describe the results of subsequent experiments in which relatively pure hyperpolarising responses to NMDA were obtained from cortical slice preparations continuously perfused with MK-801. Experiments were carried out using the rat cortical slice preparation of Harrison & Simmonds (1985), modified as previously described (Kemp et al., 1986).

Application of increasing concentrations of NMDA (5 - 20μM) or quisqualic acid (3 - 30μM) to cortical slices produced characteristic biphasic responses. Both the initial depolarisation and subsequent hyperpolarisation, to either agonist, were concentration-dependent with the depolarising phase predominating. When the same tissues were perfused continously with MK-801 (10μM) depolarisations to NMDA were progressively attenuated whilst responses to quisqualate acid remained unaffected. Further increases in the concentration of NMDA (40μM-6.4mM) produced responses in which the hyperpolarising phase predominated, often in the absence of any obvious preceding depolarisation. NMDA-induced hyperpolarisations were slow in onset (time to peak 1-3 min) and duration (up to 15min) and have been observed up to 1.5 mV in amplitude. Trans-2,3-piperidine dicarboxylic acid (40-320μM), quinolinic acid (1-8mM) and N-methyl-L-aspartic acid (2mM), other NMDA-receptor agonists, evoked similar hyperpolarising responses in the presence of MK-801 (10μM).

It is unlikely that the described hyperpolarising responses were due to an effect on either $GABA_A$- or $_B$-receptors or to an alteration in Cl^- conductance, since both isoguvacine (30μM) and baclofen (3μM) had little effect. Similarly, L-2-amino-4-phosphonobutyrate (10μM), a potent depressant at certain excitatory synapses (Koerner & Cotman, 1981), was without effect.

The mechanism underlying the hyperpolarising response to NMDA-receptor agonists is at present obscure. After hyperpolarisations following large depolarisations to glutamate have been attributed to activation of the electrogenic Na^+-K^+ pump, resulting from increases in Na^+ permeability (Padjen and Smith, 1983, Thompson & Prince, 1985). Although such a mechanism cannot be excluded, it would be necessary for NMDA-receptor agonists to directly stimulate the Na^+-K^+ pump in order to explain our observations. It remains to be determined whether the hyperpolarising response represents an action of acidic amino acids at a novel receptor subtype.

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COMPARISON OF PHENCYCLIDINE, THIENYLCYCLOHEXYLPIPERIDINE AND MK801 AS NMDA ANTAGONISTS ON RAT SPINAL AND CORTICAL NEURONES

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Phencyclidine (PCP) and thienylcyclohexylpiperidine (TCP) are amongst the most potent ligands for brain PCP/sigma receptors (Zukin & Zukin, 1981) and for reducing the excitatory action of N-methylaspartate (NMA) on spinal neurones (Berry & Lodge, 1985). The recent description of the NMA antagonist properties of the anticonvulsant dibenzocycloalkenimine, MK801, (Kemp et al. 1986) prompted us to compare the actions of MK801, PCP and TCP on spinal neurones in vivo and cortical neurones in vitro.

Using the technique of microelectrophoresis on 14 spinal neurones recorded from pentobarbitone-anaesthetised rats, MK801 (2-5nA) and PCP (5-20nA), ejected from 5mM solutions in 200mM NaCl, showed similar selectivity in blocking excitation by NMDA compared with that by quisqualate or kainate. With MK801, the action of NMDA was reduced by 67±17% (mean ± S.D) whereas the mean reduction of both quisqualate and kainate was 8%. With PCP the equivalent figures were 68±20% for NMDA and less than 3% for quisqualate and kainate. On individual cells the equieffective currents for MK801 were 2-5 (mean 3.5) times lower than for PCP. We have previously shown that TCP was 1.5-2 times more effective than PCP as an NMA antagonist on spinal neurones (Berry & Lodge, 1985). On a further 8 cells, MK801 and PCP only weakly differentiated between the excitant actions of L-aspartate, L-glutamate and L-homocysteate, supporting previous suggestions that these endogenous agonists act on more than one receptor subtype. Following electrophoretic ejection, recovery from the submaximal doses of MK801 requires about twice as long as from PCP. Intravenously MK801 is more potent and longer lasting than PCP; doses of MK801 between 0.1 and 0.3 mg/kg produced a substantial and selective block of NMA action which took several minutes to reach a maximum and at least 4 hours to achieve even 40% recovery.

Using rat cortical wedges perfused with a magnesium-free artificial CSF, each of the three substances selectively reduced the depolarising action of NMDA and not that of quisqualate or kainate. Dose-response curves for NMDA were shifted to the right in a non-parallel fashion and with reduced maxima. Apparent pA_2 values were equivalent to concentrations of 0.13μM, 0.79μM and 1.5μM for MK801, TCP and PCP respectively. Unlike D-2-amino-5-phosphonovalerate for which an equieffective dose would be about 5μM, the times to reach a maximum response and for recovery with all three compounds, and especially with MK801, were very slow. Epileptiform potentials recorded from these slices were also blocked by these drugs at doses equivalent to those for NMA antagonism.

The high potency of MK801 as both an NMA antagonist and an anticonvulsant in vivo (Clineschmidt et al. 1982) and in vitro agrees with our previous correlation between these two effects in a series of PCP/sigma ligands (Church et al. 1986). It will be interesting to see whether MK801 also has other behavioural effects common to such drugs.

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IS CALCIUM REQUIRED FOR THE INDUCTION OF LONG-TERM SYNAPTIC POTENTIATION IN THE HIPPOCAMPUS?

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Recent studies conducted in our laboratory showed that long-term potentiation (LTP) of excitatory postsynaptic potentials (EPSPs) in CA_1 neurones in the hippocampus could be induced if an activation of the input is paired with a depolarization of the CA_1 neurones (Sastry, Goh and Auyeung, 1986). It has been reported in the literature that Ca^{++} is required to induce LTP (Dunwiddie and Lynch, 1979). In the present study we, therefore, examined whether LTP can be induced if a depolarization of the postsynaptic neurone is paired with an activation of the presynaptic terminals in the absence of Ca^{++} in the medium.

Rat hippocampal slices were superfused with a medium containing 120 mM NaCl, 3.1 mM KCl, 26 mM $NaHCO_3$, 4 mM $CaCl_2$, 4 mM $MgCl_2$, 10 mM dextrose, 200 µM EDTA and 10 µM picrotoxin (to block the GABA-ergic inhibition). Population EPSPs or intracellular EPSPs were recorded from the CA_1 neurones in response to the stimulation of stratum radiatum at 0.2 Hz. After obtaining stable controls, the slices were exposed to media containing 1 mM $MnCl_2$ and 7 mM $MgCl_2$ (no $CaCl_2$), or 1 mM $CoCl_2$ and 7 mM $MgCl_2$ (no $CaCl_2$), or 4 mM $CaCl_2$ and 4 mM $MgCl_2$ with the concentration of KCl at 3.1-80 mM, for 3-5 min and the population EPSP followed for 30 min post-treatment. While the population EPSP was increased in size 15 min after the exposure to high K^+ in Ca^{++}-free media, it was actually depressed at the same time after the exposure to Ca^{++}-containing medium with high concentration of K^+ (see Table 1). In 6 CA_1 neurones in which the intracellular EPSPs were recorded, depolarizing commands (150 msec, 10 nA, 10 commands at 0.2 Hz) that were paired with an activation of the input fibres, were given during the exposure of the slices to Mn^{++}-Mg^{++} containing medium with 3.1 mM K^+. The EPSP was potentiated to 121-167% (139.4 ± 5.1 SEM, 15 min after paired depolarizing commands) of control.

Table 1

Population EPSP as a % of control 15 min after exposure to media with $[K^+]$ in mM

		3.1	10	20	40	80
Mn^{++}-Mg^{++} medium	Range	100-119	106-128	109-129	120-155	122-197.5
	Mean±SEM	110.5±2.96	116.5±4.79	120.4±3.28	136.3±4.6	153.7±9.93
	n	8	4	6	7	7
Co^{++}-Mg^{++} medium	Range	87-102	--	--	--	112-192
	Mean±SEM	92.5±3.43	--	--	--	142.2±13.5
	n	4	--	--	--	5
Ca^{++}-Mg^{++} medium	Range	--	103-111	94-115	86-109	61-114
	Mean±SEM	100±0	106.3±1.7	104.4±3.39	101±3.27	90.1±7.63
	n	8	4	5	6	7

These results suggest that, as long as the activation of presynaptic terminals is paired with sufficient depolarization of the postsynaptic neurone, extracellular Ca^{++} and transmitter release are not required for the induction of LTP.

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INHIBITION OF CALCIUM CURRENTS BY THE ADENOSINE ANALOGUE 2-CHLOROADENOSINE INVOLVES GTP BINDING PROTEINS.

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Voltage-activated inward calcium currents (I_{Ca}) were recorded from cultured rat dorsal root ganglion neurones using the whole cell recording technique. These currents are markedly reduced by the adenosine analogue 2-chloroadenosine (0.05μM). This appears to involve a direct inhibition of Ca^{2+} channels rather than an indirect effect due to an increase in K^+ conductance. Outward K^+ currents $I_{K(Ca)}$ and $I_{K(Vt)}$ were reduced by 2-CA (0.05μM to 0.5μM). Furthermore addition of a further K^+ channel blocker 4-aminopyridine (4-AP) applied externally did not reduce the action of 2-CA.

Inclusion of the GTP and GDP analogues guanosine 5'-0-3-thiotriphosphate (GTP-γ-S) and guanosine 5'-0-2-thiodiphosphate (GDP-ß-S) in the patch solution has previously been found to alter the contributrion of T,N and L type Ca^{2+} channels to the whole cell current and change the sensitivity to the $GABA_B$ receptor agonist (-)-baclofen (Scott and Dolphin, 1986). In the presence of GTP-γ-S, 2-CA (0.05μM) reduced I_{Ca} by 69±8% (mean±S.E. n=5). In comparison, smaller reductions, 33±6% (n=5) and 49±4% (n=5) respectively, were seen in the presence of GDP-ß-S and under control conditions. These data suggest that 2-CA induced inhibition of I_{Ca} involves a guanine nucleotide binding protein.

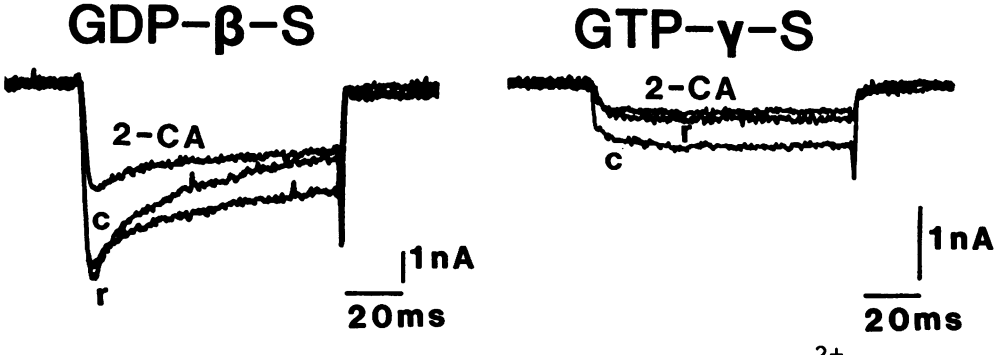

Figure The maximum inward currents, (carried by Ba^{2+}) were recorded with intracellular GDP-ß-S or GTP-γ-S at a concentration of 500μM. C = control; 2-CA = in the presence of 0.05μM 2-CA; and r = after 5 minutes recovery. Note little recovery occurs after 5 minutes in the presence of GTP-γ-S.

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ASCORBIC ACID-DEPENDENT BINDING OF [^{125}I]-IODOCYANOPINDOLOL TO NON-β-ADRENOCEPTOR SITES IN GUINEA-PIG TRACHEA.

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We have previously shown that the specific binding of the potent, highly selective beta-adrenoceptor radioligand [^{125}I]-iodocyanopindolol (I-CYP, 5-320 pM) (Engel et al., 1981) in 16 μm transverse frozen sections of guinea-pig trachea was saturable and involved a single class of non-interacting binding sites of high affinity (K_D = 75 ± 16 pM) (Goldie et al., 1986a). The beta-adrenoceptor antagonist propranolol (10 μM) reduced total I-CYP binding to background levels. However, ascorbic acid (10 nM - 10 μM) caused a concentration-related increase in total I-CYP (50 pM) binding of from 1.4 ± 1.3 fold to 11.1 ± 1.3 fold. Similar increases were produced in the presence of other reducing agents including dithiothreitol and L-cysteine. No such enhancement of binding was observed in pig bronchus (Goldie et al., 1986b). Autoradiography (10 μm frozen sections) demonstrated that this large increase in I-CYP binding was primarily associated with the sub-epithelial mucosal cell layer. Ascorbic acid-induced I-CYP binding was unaffected by simultaneous exposure to propranolol, ICI-118551 ($beta_2$-selective) or atenolol ($beta_1$-selective) at concentrations up to 100 μM. Conversely, this enhanced binding was reduced in a concentration-dependent manner to background levels, in the presence of (+) or (-)-isoprenaline, (-)-adrenaline, (-)-noradrenaline, (±)-fenoterol, 5-hydroxy-tryptamine, dopamine or phentolamine (0.1-10 μM). IC_{50} values were similar for each of these drugs (0.7 - 2.5 μM). Histamine was inactive, while clonidine (10 μM) and yohimbine (10 μM) caused reductions in binding of only 22 ± 6% and 43 ± 3% respectively. Thus, these alternate binding sites for I-CYP were not readily identifiable by pharmacological means. Ascorbic acid-enhanced binding was not reduced by repeated washout at 15 min intervals over a 2 hour period. Furthermore, (-)-adrenaline (10 μM) failed to reduce such binding when it was added to the incubation mixture at equilibrium. Dialysis of a digest of guinea-pig tracheal sections exposed to I-CYP (50pM) in the presence of ascorbic acid (10 μM) indicated that the majority of the radioactivity in the sections was strongly bound to protein.

Results indicate that in guinea-pig trachea, ascorbic acid and some other reducing agents may reveal a large population of alternate binding sites for I-CYP, making this radioligand less selective for beta-adrenoceptors than previously thought.

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β-AGONIST-INDUCED DESENSITIZATION OF GUINEA-PIG TRACHEAL β-ADRENOCEPTORS IN VITRO

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In severe asthma, the bronchial response to beta-agonists may be reduced as a direct consequence of the disease and this trend may be reversed by treatment with glucocorticoids (Ellul-Micallef & Fenech, 1975; Paterson et al., 1982). Attenuated bronchodilator responsiveness may also result from prolonged therapy with beta-agonists (Tashkin et al., 1982). We have assessed an in vitro airway model in which $beta_2$-adrenoceptors were down-regulated experimentally, in an attempt to mimic the dysfunction in asthma.

Pretreatment of guinea-pig tracheal ring preparations with isoprenaline (Iso) caused a time and concentration-related decrease in both the relaxant potency (pD_2) and maximal relaxant effect (Emax) of Iso, (-)-noradrenaline and (±)-fenoterol. Desensitization was stereo-selectively induced by the (-)-isomer of Iso, with the (+)-isomer virtually inactive . Relaxant responsiveness to beta-agonists recovered spontaneously and completely within 1 h of the withdrawal of Iso. Exposure to (-)-Iso (5 μM, 3 h) caused a 7 fold reduction in Iso pD_2 and a reduction in Iso Emax (% maximal carbachol-induced tone) from 62.6 ± 5.1% (control) to 51.9 ± 5.0% (desensitized) ($P < 0.02$, $n = 23$; Student's paired t-test). Exposure to 25 μM Iso for 1 h reduced Iso Emax from 75.1 ± 3.6% to 17.6 ± 2.8% ($P < 0.001$, $n = 20$). However, this pronounced loss of relaxant responsiveness prohibited accurate estimation of the change in Iso pD_2. In contrast, pD_2 and Emax values for the non-beta-agonist relaxants theophylline and nitroprusside were not significantly reduced ($P > 0.1$). Furthermore, pretreatment with theophylline failed to modify responsiveness to Iso. The beta-adrenoceptor antagonists propranolol (0.2 μM, non-selective) and ICI-118551 (0.2 μM, $beta_2$-selective) protected tracheal preparations from Iso-induced desensitization, while atenolol (10 μM, $beta_1$-selective) was ineffective. The use of light microscopic autoradiography demonstrated that exposure to 25 μM (-)-Iso for 1 h caused reductions in the densities of autoradiographic grains derived from the beta-adrenoceptor radioligand [^{125}I]-iodocyanopindolol, in airway smooth muscle and epithelial structures of 70.5 ± 8.8% and 60.4 ± 5.0% respectively. Taken together, these data indicate that beta-agonist-induced desensitization was homologous, involving selective dysfunction of tracheal $beta_2$-adrenoceptors. Neither the cyclooxygenase inhibitor indomethacin, nor the phospholipase A_2 inhibitor mepacrine, had any significant effect on desensitization. Conversely, pretreatment with cortisol (25 μM) significantly reduced desensitization and enhanced the rate of spontaneous recovery.

The present study indicates that guinea-pig tracheal preparations with experimentally-induced beta-adrenoceptor desensitization may provide a useful model of the beta-adrenoceptor dysfunction seen in asthma.

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LEUKOTRIENE C_4 ELICITS INOSITOL PHOSPHATE FORMATION AND INHIBITS ADENYLATE CYCLASE IN GUINEA-PIG LUNG.

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Leukotrienes are potent bronchoconstrictor agents of guinea-pig (GP) lung and trachea. It has been suggested that bronchoconstriction to leukotrienes may be mediated by intracellular calcium mobilization (Raeburn and Roger, 1984) or adenylate cyclase inhibition (Anderson et al., 1982). We investigated whether leukotriene C_4(LTC_4) elicited the formation of inositol phosphates (IP), which may be responsible for mobilization of calcium from intracellular stores (Hashimoto et al., 1985) and adenylate cyclase inhibition in guinea-pig parenchyma and trachea.

GP peripheral lung or trachea were removed and chopped immediately after slaughter, and incubated in the presence of 10^{-5}M indomethacin and 7.5 mM lithium chloride (LiCl), with 0.5 M [^{3}H]inositol for 90 min at 37°C, in order to label membrane phospholipids. To trigger phospholipid breakdown and IP formation, LTC_4 (10^{-9}M - 10^{-5}M) was added for 5 min to the tissue in the presence or absence of serine borate 45 mM. The reaction was stopped by addition of chloroform / methanol / trichloracetic acid (v/v, 100/200/2), and water was added to separate the phases. The aqueous phase was applied to a Dowex-formate chromatography column and inositol mono-, bi-, and trisphosphate were collected in ammonium formate buffers (0.1 to 0.75 M) and 0.1 M formic acid. To identify inositol tetrakisphosphate, tissue was incubated with 5 mM [^{3}H]inositol and collection was made in 1 M ammonium formate and 0.1 M formic acid. There was a basal formation of all inositol phosphates, and basal level of IP formation was 5 to 6 times higher in parenchyma than trachea. In the presence of LiCl LTC_4 elicited a concentration-dependent increase in inositol monophosphate in trachea (EC_{50}: 7.5×10^{-8} M; maximal increase above basal: 200 ± 25%; mean ± SEM; n=6) and in parenchyma (EC_{50}: 3×10^{-8}M; maximal increase above basal 81 ± 11%). In parenchyma, the formation of inositol bi-, tris-, and tetrakisphosphate was also enhanced (65, 185, and 239% above basal respectively). Serine borate did not modify the LTC_4-induced IP formation. Cyclic adenosine monophosphate (cAMP) was measured by radio-immunoassay on an homogenate of whole trachea, in the presence or absence of isoprenaline 10^{-6}M, and the effect of a three-minute incubation with LTC_4(10^{-9} to 10^{-7}M) was assessed. Basal level of cAMP was 28 ± 9 pM/ mg protein (mean ± SEM; n=3) and LTC_4 10^{-7}M inhibited cAMP formation by 37 ± 10%; in the presence of isoprenaline, cAMP level was 56 ± 18 pM. and LTC_4 elicited a decrease of cAMP of 42% in the presence of indomethacin 10^{-5}M only.

We conclude that LTC_4-induced contraction in guinea-pig lung may be mediated through inositol phosphate formation and adenylate cyclase inhibition. Comparison with the effect of other LT will allow the calculation of their relative potencies in inhibiting cAMP and stimulating inositol phosphate formation.

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A CONVENIENT PROCEDURE FOR THE ASSAY OF [^{3}H]-LABELLED INOSITOL PHOSPHATES.

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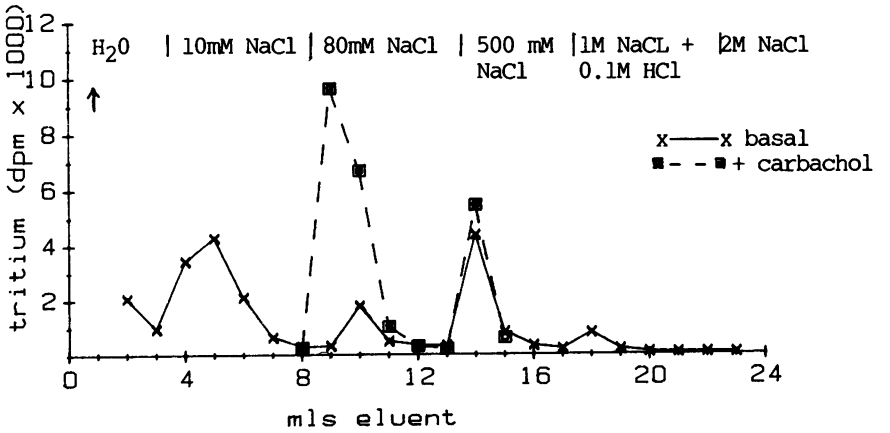
The receptor stimulated hydrolysis of inositol phospholipids to generate diacyl glycerol and inositol phosphates is an important signal transducing system (see Berridge ,1984). Pharmacological characterisation of this response is hampered, however, by the lengthy chromatographic separation of labelled inositol phosphates. We have therefore developed a rapid, simple assay for the separation of inositol phosphates using bonded silica ion-exchange chromatography.

Rat cortical slices were incubated with ^{3}H-myo-inositol and stimulated with 5mM carbachol in the presence of 10mM Li^+ as described by Brown et al (1984) and Batty and Nahorski (1985). The inositol phosphates were separated on SAX-Cl^- (quaternary amine anion exchanger) bonded silica columns (Jones Chromatography) in a 10-place manifold under reduced pressure. Products of the separation were identified using ^{3}H-labelled standards (Amersham) with ^{3}H-glycerophosphoinositol (GPI) prepared according to Downes and Michell (1981). The columns were activated (1 ml methanol,1ml H_2O), sample applied, and eluted as follows: inositol, H_2O wash; GPI, 10mM NaCl; inositol phosphate (IP_1), 80mM NaCl; inositol bisphosphate (IP_2), 500 mM NaCl; inositol trisphosphate (IP_3), 1M NaCl + 0.1 M HCl (see Fig 1). Recovery was greater than 80%, and the columns were regenerated with 2M NaCl without affecting the separation or recovery.

Carbachol stimulation for 45 min elevated IP_1 levels by 3-4 fold (Fig 1). With short incubation times (5 min) in the absence of Li^+, carbachol elevated IP_3 levels from 740 to 1023 dpm (mean of two experiments). The inositol phosphates were also separated satisfactorally using single 5 ml washes of the chloride solutions. Total inositol phosphates were eluted in only 3 steps: 5ml 10 mM NaCl to remove inositol and GPI, followed by 2 x 0.5 ml 2M NaCl to elute the phosphates.

Figure 1 Stimulation of inositol phospholipid hydrolysis by carbachol (5mM) in rat cortical slices in the presence of 10mM LiCl.



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ACTIVATORS OF PROTEIN KINASE C ACT AT A POST-RECEPTOR SITE TO AMPLIFY β-ADRENOCEPTOR STIMULATION OF PINEAL CYCLIC AMP.

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Activation of α_1-adrenoceptors amplifies β-adrenergic stimulation of pinealocyte cyclic AMP at least 10-fold by a mechanism involving activation of protein kinase C(PKC; Sugden et al., 1985). This response is physiologically important as noradrenaline(NA), a mixed α_1- and β-adrenergic agonist is the neurotransmitter in this tissue and cyclic AMP regulates the activity of N-acetyltransferase, a key enzyme in the synthesis of the pineal hormone, melatonin.

Dispersed pinealocytes were prepared by trypsinization of rat pineal glands and then incubated (37°C, 95% air/5% CO_2) in suspension culture for 24 hours before treatment. Cyclic AMP was determined by radioimmunoassay.

As previously reported, the potent PKC activator, 4β-phorbol 12-myristate 13-acetate (PMA, 10^{-7} M) increased (~6-fold) the cyclic AMP response to isoprenaline(ISO). However, examination of the ISO dose-response curve revealed that PMA did not alter the EC_{50} of the response (1.7×10^{-8} M), indicating that PKC activation does not alter β-adrenoceptor sensitivity. Cholera toxin(CT) bypasses β-adrenoceptor activation and stimulates cyclic AMP production by stabilizing the active form of the stimulatory guanine nucleotide regulatory protein (Ns) which regulates adenylate cyclase activity. CT treatment(1 ug/ml, 60 to 90 min) alone caused a small (~2-fold) increase in cyclic AMP; activation of PKC with α_1-adrenergic agonists, phorbol esters or diacylglycerol, had no effect alone. However, cyclic AMP in CT-treated cells was increased(>10-fold) by selective α_1-adrenergic agonists; the effect of phenylephrine(PE) was antagonized by selective α_1-adrenergic antagonists. Similarly, cyclic AMP in CT-treated cells was markedly and rapidly increased by phorbol esters (PMA> 4β-phorbol 12,13-dibutyrate >> 4α-phorbol 12,13-didecanoate) and synthetic diacylglycerols (1,2-dioctanoylglycerol> 1-oleoyl 2-acetylglycerol>> diolein). The cyclic AMP response to forskolin (10^{-5} to 10^{-3} M), which acts directly on Ns and/or adenylate cyclase, was also increased by PE (3×10^{-6}M) or PMA (10^{-7}M). The results of these studies point to a site of action of PKC activators beyond β-adrenoceptor stimulation. To examine whether cyclic AMP efflux was altered by PKC activators, cyclic AMP in the culture medium was examined. Neither PMA (10^{-7}M) nor PE (10^{-6} M) inhibited cyclic AMP efflux. To examine whether cyclic nucleotide phosphodiesterase(PDE) was involved, the effect of PKC activators on the β-adrenergic-cyclic AMP response was examined in the presence of a combination of PDE inhibitors (isobutylmethylxanthine [10^{-3} M] and Ro 20-1724 [10^{-4} M]). PMA or PE amplified the β-adrenergic stimulation of cyclic AMP even in the presence of PDE inhibitors, indicating that PDE inhibition is probably not the basis of potentiation.

Together these data indicate that activators of PKC amplify the β-adrenergic cyclic AMP response in pinealocytes by enhancing cyclic AMP production, probably through an action on Ns or adenylate cyclase.

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DIFFERENCES BETWEEN ADRENERGIC AND PURINERGIC INHIBITION IN THE MOUSE VAS DEFERENS IN VITRO

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It has been suggested that noradrenaline and ATP act as co-transmitters in the mouse vas deferens (Stjärne & Åstrand, 1984), and there are presynaptic inhibitory α_2 adrenergic (Illes & Starke, 1983) and P_1 purinergic (Burnstock & Sneddon, 1984) receptors present on the sympathetic nerve terminals which may be involved in a negative feedback regulatory system. We have compared the inhibitory actions of the α_2 agonist clonidine with the P_1 agonists 2-chloroadenosine (2ClA) and 1-phenylisopropyladenosine (PIA) on both the mechanical (twitch) response and junction potential (e.j.p) of the field stimulated mouse vas deferens. All experiments were carried out in Krebs solution containing 1.1mM Mg^{2+} and 2.5mM Ca^{2+} at 37°C. Electrical stimulation was produced by a pair of ring electrodes around the prostatic end of the vas. Isometric twitch responses were elicited by supramaximal stimulation (5 pulses 0.1ms, 5Hz every 60s). Trains of junction potentials (5 at 2Hz every 20s) were recorded intracellularly, using a stimulating voltage which gave an initial e.j.p. amplitude of approximately 10mV. Dose response curves were analysed using an iterative curve fitting program (Allfit), with a minimum of 4 observations at each concentration.

Clonidine (0.3 to 30nM) produced a concentration dependent inhibition of the mechanical response with an EC_{50} (± s.e.m.) of 1.9 ± 0.2 nM, and at a concentration of 10nM reduced the response to <10% of control. Clonidine also inhibited the e.j.p., with an EC_{50} of 3.9 ± 0.6nM, and reduced the response to <10% of control at a concentration of 30nM.

2ClA (0.3 to 30μM) produced a concentration dependent inhibition at the mechanical response with an EC_{50} of 1.4 ± 0.2μM and a maximum of >85% inhibition. 2ClA also produced a decrease in the e.j.p. amplitude, however the effect varied greatly from cell to cell, with 25% inhibition being observed at concentrations from 0.1 to 10μM, and a maximal effect of 20 to 75% inhibition. The component of the e.j.p. which was resistant to 2ClA was abolished by clonidine (30nM). Similar results to those obtained with 2ClA were obtained using another P_1 agonist PIA. However, in the presence of PIA, e.j.ps were not only reduced in amplitude, but were also changed in shape, with the appearance of a component with a shorter latency, indicating that PIA may have both inhibitory and facilitatory effects.

Although purinergic and adrenergic agonists have similar effects on the mechanical response of the vas deferens, our results show differences between the effects of these agonists on the e.j.p. Furthermore, the variability in the effects of the purinergic agonists suggests a heterogeneous population of sympathetic neurones which are distinguishable by their sensitivity to P_1 agonists.

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FORSKOLIN POTENTIATES SECRETORY RESPONSES TO SUBSTANCE P AND ISOPRENALINE IN RAT PAROTID GLANDS.

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Rat parotid tissue responds to secretagogues with the production of electrolytes and the enzyme amylase. This response is thought to be brought about by either an increase in cAMP or in inositol-1,4,5-phosphate leading to Ca^{2+} mobilisation. We have previously shown (Arkle et al 1985) that substance P and isoprenaline act synergistically on amylase secretion. The present paper reports the ability of forskolin to enhance secretory responses to both isoprenaline and substance P.

Parotid glands were dissected free from fat, ducts and blood vessels and cross chopped on a McIlwain tissue chopper (60° at 150mm). Following preincubation in Krebs' bicarbonate buffer, the slices were incubated with test compounds for 30 min at 30°C. The reaction was terminated by rapid centrifugation and amylase activity measured in the supernatant (Bernfeld 1955) and protein in the pellet. Phosphatidyl inositide metabolism was assessed by measuring the formation of [^{3}H]-inositol-1-phosphate in slice preparations prelabelled with [^{3}H]-inositol. The metabolism of [^{3}H]-inositol-1-phosphate was inhibited by the addition of LiCl (10mM) to the incubation medium, and the radioactive product was isolated by ion exchange chromatography. cAMP was measured after 30min incubation at 30°C using a bovine adrenal binding protein.

Isoprenaline, substance P and forskolin all stimulated amylase release. Forskolin and isoprenaline caused an increase some 8-10 fold over basal levels (EC_{50} for forskolin 230 nM and isoprenaline 45nM) whilst substance P gave a doubling of the response (EC_{50} = 10nM). The addition of a low concentration (1nM) of forskolin which had no effect by itself significantly enhanced the production of amylase by substance P. Thus the EC_{50} for substance P in the presence of forskolin was 0.17 nM compared with 9.3 nM in its absence. Forskolin did not alter the maximum response. At higher concentrations, (40 nM) forskolin also potentiated the response to isoprenaline (EC_{50} = 99nM in the presence of forskolin compared with 181 nM in its absence). Both forskolin and isoprenaline stimulated parotid cAMP production at concentrations where enhancement of the secretory response was observed (40 nM forskolin); the combination of the two drugs produced a greater than additive effect on cAMP production. This was not observed in the case of substance P: 1nM forskolin did not increase cAMP formation in either the presence or absence of substance P. Substance P stimulated parotid P.I. metabolism (control 2703 $\pm$ 306 dpm per mg protein; substance P at 100 nM 10136 $\pm$ 120 dpm per mg protein;n=6) with an EC_{50} of 9.3 nM. The addition of low concentrations of either isoprenaline or forskolin had no effect on substance P stimulated P.I. metabolism.

The results demonstrate that a number of synergistic interactions between secretagogues can occur in rat parotid. The interaction between substance P and isoprenaline does not involve changes in the amounts of second messengers (cyclic AMP or inositol-1,4,5-phosphate)formed, while the potentiating effects of forskolin can only partially be explained in this way.

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DO μ- AND κ-SELECTIVE OPIOIDS INHIBIT OXYTOCIN RELEASE IN THE SAME WAY?

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Opioids suppress suckling-evoked oxytocin release. Both mu and kappa-selective agonists have this property (Wright et. al., 1982), but it is not known whether the underlying mechanisms are similar. Recently we have found that inhibition of reflex milk-ejection by the kappa agonist U50488H can be antagonized by propranolol (Clarke & Wright, 1986) and hence may be adrenergically mediated. We have now examined the effect of the mu-selective opiate fentanyl to see whether a similar mechanism is involved.

Experiments were carried out on lactating Wistar rats seperated from all but one of their pups overnight and anaesthetized with urethane (1.0g/kg, i.p.). All surgical procedures which included saphenous vein, intrathecal and mammary gland cannulations were carried out under halothane (0.5-1.5% in O_2/NO_2). Three hours after induction of anaesthesia ten hungry pups were placed on the nipples. Reflex release of oxytocin, which usually commences within 30 min and occurs at regular intervals (approximately 5 min) for several hours, was detected by increased intramammary pressure and/or pup behaviour. Intraperitoneal or intrathecal injections were made after 5 such milk-ejection responses and significant inhibition was defined as an increase in the interval to this subsequent response (postinjection interval) greater than the 99% confidence limit of the mean basal interval.

Fentanyl significantly suppressed reflex milk-ejection in 4/5 (80%) and 5/10 (50%) rats after intraperitoneal (100μg) and intrathecal (0.5μg) injection respectively. A dose of propranolol (1mg/kg, i.p.) which has previously been shown to abolish the effect of the kappa-opiate U50488H was ineffective against fentanyl. When the postinjection intervals were compared the effect of fentanyl given alone (mean = 33.3 ± 9.1 min) and to propranolol-treated animals (mean = 39.6 ± 8.2 min, n=5) were not significantly different (P>0.05); saline control mean = 8.2 ± 1.2 min, (n=8). Similarly following intrathecal opiate administration the effect of fentanyl alone (mean postinjection interval = 14.0 ± 2.0 min) was similar to that in propranolol-treated animals (12.7 ± 1.4 min, n=14); saline control mean = 7.7 ± 3.0 min (n=6). Further studies with U50488H showed that in contrast to the effects of low doses a considerably larger intrathecal dose (100μg) could, like fentanyl, also cause a propranolol-insensitive inhibition. U50488H (100μg) significantly inhibited milk-ejection in 4/6 (66.7%) rats when given alone and the mean postinjection interval (29.0 ± 7.6 min) was not significantly different from that after administration to propranolol-treated rats (27.7 ± 7.3 min, n=5). The opiate antagonist naloxone (1mg/kg, i.p.) however, reduced the effect of U50488H to a level at which it was not significantly different from that of saline controls.

The results of experiments reported here and earlier (Clarke & Wright, 1986) have demonstrated a fundamental difference in the mechanisms underlying the inhibition of milk-ejection by U50488H (kappa selective) at low, but not high, doses and fentanyl (mu selective).

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COMPARISON OF THE EFFECTS OF GABA RECEPTOR AGONISTS ON THE SOMATIC MUSCLE RECEPTORS OF ASCARIS SUUM.

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Gamma-aminobutyric acid (GABA), >0.1 μM, produces hyperpolarisation of the somatic muscle cell bellies of Ascaris (del Castillo et al., 1964; Brading and Caldwell, 1971) which is mediated by GABA receptor/Cl^- channels (Martin, 1980). The pharmacology of this GABA receptor has not been studied previously and in this study the effects of five GABA receptor agonists, muscimol, isoguvacine, THIP, Piperidine-4-Sulphonic Acid (P4S) and 3-Aminopropane Sulphonic Acid (3APS) were tested.

A 2cm section (just anterior to the genital ring) of Ascaris body wall was excised, cut along one of the lateral lines and pinned out on the Sylgard base of a tissue bath (5ml volume). Muscle cell bellies were impaled with two microelectrodes filled with 4M K-acetate of resistances 10 - 30MΩ. Current pulse parameters for the determination of input conductance were typically 20 nA, 500 ms, 0.2 Hz. Conventional electrophysiological recording techniques were used to amplify and display the results. The bath was continually perfused (7 ml/min) with Artificial Perienteric Fluid (APF) of composition: NaCl 67mM; Na acetate 67mM; $MgCl_2$ 15mM; KCl 3mM; $CaCl_2$ 3mM; Tris 5mM; pH 7.6 at 21 ± 1°C. Drugs were diluted to the required concentration in 20ml volumes of APF and superfused for 2.5 min, normal APF perfusion recommencing after this period.

GABA (>0.1 μM) produced a reversible hyperpolarisation and increase in input conductance. The threshold concentration for a response varied greatly and some cells were not sensitive to GABA concentrations below 10 μM. 300μ M GABA produced the maximum hyperpolarisation (19mV) which matched the 20mV change expected from a membrane behaving as a Cl^- electrode. The EC_{50} for GABA was 3.46 ± 0.71 x 10^{-5}M ($\bar{X}$ ± 1 x S.E., n = 5). Three other agonists tested produced similar hyperpolarisations and increases in conductance. Relative potencies (determined from parallel portions of the dose-conductance curves) were 0.25 ± 0.13 (n = 5) for muscimol, 0.19 ± 0.07 (n = 3) for isoguvacine and 0.006 ± 0.0006 (n = 3) for THIP. P4S and 3APS were inactive at 1mM.

The GABA receptor in Ascaris muscle clearly does not recognise some $GABA_A$ receptor agonists. Also the rank order of potencies of agonists to which it is sensitive is different to that of other $GABA_A$ receptor systems (see e.g. Nistri and Constanti, 1979; Wheal and Kerkut, 1976; Desarmenien et al., 1984).

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INVOLVEMENT OF CYCLIC NUCLEOTIDES IN PREJUNCTIONAL ADRENOCEPTOR MODULATION OF NORADRENALINE RELEASE

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Activation of β-adrenoceptors stimulates adenylate cyclase and activation of α-adrenoceptors inhibits adenylate cyclase (Schultz & Jakobs, 1981). The aim of this study was to investigate the possible role of cyclic AMP in the modulation of noradrenaline release from mouse atria and to determine if changes in adenylate cyclase activity and the formation of cyclic AMP are an integral part of prejunctional β-adrenoceptor facilitation and prejunctional α-adrenoceptor inhibition of noradrenaline release.

Mouse atria were preincubated with (^{3}H)-noradrenaline and then subjected to field stimulation (5 Hz, 60 s). The β-adrenoceptor agonist isoprenaline (0.003 and 0.01 μmol/l) increased the stimulation-induced (S-I) efflux of radioactivity to 113.5% (s.e.m. = 5.1, n = 4) and 144.2% (s.e.m. = 15.7, n = 8), respectively, of control. Similar to isoprenaline, the cell permeable analogue of cyclic AMP, 8-bromo cyclic AMP, produced a concentration dependent increase in S-I efflux, from 126.5% (s.e.m. = 7.4, n = 4) for 30 μmol/l to 179.1% (s.e.m. = 25.2, n = 6) for 270 μmol/l. In the presence of 8-bromo cyclic AMP (90 μmol/l), isoprenaline (0.01 μmol/l) failed to produce a facilitation of the S-I efflux. This lack of effect of isoprenaline may be due to the high concentration of 8-bromo cyclic AMP and suggests that these two drugs may act through the same end-mechanisms. Inhibition of the breakdown of cyclic AMP by a phosphodiesterase inhibitor, ICI 63,197 (30 μmol/l, 2-amino-6-methyl-5-oxo-4-n-propyl-4,5-dihydro-5-triazolo (1,5-a) pyrimidine) enhanced the S-I efflux to 122.5% (s.e.m. = 3.9, n = 4) of control. In the presence of ICI 63,197 (30 μmol/l), isoprenaline (0.003 μmol/l) produced a much larger facilitation of the S-I efflux than in the absence of ICI 63,197 (171.9% s.e.m. = 17.3, n = 4). This may be because in the presence of ICI 63,197 any cyclic AMP generated by isoprenaline would not be readily broken down.

The α_2-adrenoceptor agonist clonidine (0.03 μmol/l) inhibited the S-I efflux of radioactivity to 64.8% (s.e.m. = 3.8, n = 7). In the presence of 8-bromo cyclic AMP (90 μmol/l) the inhibitory effect of clonidine was unaltered. Similarly in the presence of ICI 63,197 the inhibitory effect of clonidine was also unaltered.

These results suggest that β-adrenoceptor facilitation of noradrenaline release may be due to stimulation of cyclic AMP production. The lack of interaction between clonidine and 8-bromo cyclic AMP and ICI 63,197 suggests that α-adrenoceptor inhibition of noradrenaline release is not due to inhibition of adenylate cyclase.

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DIFFERENTIAL EFFECTS OF PHORBOL 12-MYRISTATE 13-ACETATE ON PGD_2- AND PGI_2-INDUCED CYCLIC AMP FORMATION IN PLATELETS.

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Platelet reactivity is controlled by the opposing actions of at least two distinct second messenger systems. Inhibitory agonists stimulate adenylate cyclase and elevate intracellular cyclic AMP content [cAMP] (Feinstein et al, 1981). Stimulatory agonists act to increase the hydrolysis of inositol phospholipids resulting in increased cellular levels of 1,2-diacylglycerol (DG) and elevation of cytosolic free calcium ion concentration (Caf) (Rink et al, 1984). DG stimulates protein kinase C (PKC), an effect which is mimicked by phorbol 12-myristate 13-acetate (PMA). Activated PKC functions as a bidirectional regulator of platelet reactivity to (a) synergise with elevated Caf to mediate platelet activation (Rink et al, 1984) and (b) feed back to inhibit or reverse agonist-induced inositol phospholipid hydrolysis and elevation of Caf (MacIntyre et al, 1985). We have previously reported that activation of PKC by PMA directly inhibits prostaglandin (PG) D_2-induced cAMP formation by platelets (Bushfield et al, 1986).

In the present study we have examined the effects of PMA on cAMP formation induced by PGI_2, PGE_1 and 6ketoPGE$_1$. Experiments were performed using plasma-free suspensions of human platelets in a modified Hepes-buffered Tyrodes solution and platelet [cAMP] was measured by radioimmunoassay (Bushfield et al, 1985). Incubation ($\leq$4 min, 37°C) with PMA ($\leq$300nM) did not alter the level of cAMP in control (8.3$\pm$0.4pmol/10^8 cells; mean $\pm$ S.E.; n=7) or in IBMX (1mm)-treated platelets (20$\pm$3.5 pmol/10^8 cells; n=3). PGD_2 ($\leq$3μM), PGI_2 ($\leq$300nM), PGE_1 ($\leq$10μM) and 6ketoPGE$_1$ ($\leq$1μM) induced concentration-dependent elevation of platelet [cAMP]. PMA (1-300nM, 2 min preincubation, 37°C) inhibited PGD_2 (300nM)-induced cAMP formation (I_{50}~7.5 nM) but had no significant effect on PGE_1 (0.1-10μM), 6ketoPGE$_1$ (1μM) or PGI_2 (300 nM)-induced cAMP formation. This inhibitory effect of PMA was not dependent upon ADP release, thromboxane A_2 formation or active phosphodiesterase and was not mimicked by the non-tumour promoting 4α-phorbol didecanoate.

These findings indicate that activation of PKC by PMA inhibits PGD_2 but not PGI_2, PGE_1 or 6ketoPGE$_1$-induced cAMP formation. Increased levels of cAMP in response to these agonists occur as a consequence of interaction of PGD_2 with the DP receptor or of PGI_2, PGE_1 or 6ketoPGE$_1$ with the IP receptor (Kennedy et al, 1983). The effects reported here could occur as a consequence of specific PKC-mediated inhibitory phosphorylation of the DP receptor. Indeed, PKC-induced receptor phosphorylation occurs in a number of different cell types (e.g. Sibley et al, 1984). The specificity of this effect suggests that activation of PKC in platelets may provide a regulatory mechanism to abrogate the effects of the endogenous adenylate cyclase stimulant, PGD_2, without compromising the actions of exogenous prostanoid stimulants of adenylate cyclase (e.g. PGI_2, 6-ketoPGE$_1$). Whether this phenomenon is of physiological or patho-physiological importance in the regulation of platelet reactivity in vivo remains to be ascertained.

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THE EFFECTS OF PROLONGED AMINISTRATION OF ASPIRIN ON RABBIT PLATELETS

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Patients with rheumatoid arthritis receive aspirin at high dose levels over prolonged periods of time. The present experiments determine the effect of prolonged administration of aspirin on rabbit cyclooxygenase activity, measured by ex vivo arachidonic acid (AA) induced platelet aggregation.

5 ml citrated blood was centrifuged (180 g, 10 min, 37°C) to prepare platelet rich plasma (PRP). The residue was again centrifuged (200 g, 10 min, 37°C) to prepare platelet poor plasma which was stored at -70°C for determination of plasma salicylate levels. Aggregation of PRP at 37°C was initiated by the addition of 200 g ml^{-1} AA in a Payton dual channel aggregometer over a period of 4 min, after which 10 $\mu g\ ml^{-1}$ indomethacin was added to stop further AA metabolism. The PRP was then centrifuged (1000 g, 3 mins) and the supernatent stored at -70°C for thromboxane B_2 (TXB_2) assay (RIA, New England nuclear).

Soluable aspirin was dissolved in 80 ml tap water and administered to the rabbits as drinking water, consumed over several hours and then replaced by tap water ad libitum . After initial control experiments, one group of 3 rabbits received a dose of 40 mg Kg^{-1} day^{-1} (low dose) and a second group of 3 received 90 mg Kg^{-1} day^{-1} (high dose) aspirin. One rabbit received tap water only. They were bled weekly over a period of three months to determine platelet cyclooxygenase activity and plasma levels of salicylate and TXB_2. The results showed that the rabbits treated with low dose aspirin achieved a plasma level of about 30 $\mu g\ ml^{-1}$ salicylate and a significant ($p < 0.05$) reduction (40%) in platelet aggregation and TXB_2 concentration (75%), maintained throughout the 80 day treatment period. Rabbits treated with high dose aspirin showed an even greater reduction in platelet aggregation (80%) and TXB_2 levels (97%). However, in this group after day 38 of treatment, the platelet aggregation response returned towards the control value seen at day zero (inhibition reduced from 80% to 40%). Levels of TXB_2 followed a similar pattern (97% to 65%) even though throughout the period the plasma level of salicylate remained constant. The data also showed that platelets from rabbits receiving high dose aspirin were more sensitive to TXB_2 (10 ± 4 ng ml^{-1} TXB_2 = 20 ± 6% aggregation: 45 ± 20 ng ml^{-1} TXB_2 = 58 ± 8% aggregation) compared to control rabbits (17 ± 8 ng ml^{-1} TXB_2 =0% aggregation: 190 ± 28 mg ml^{-1} TXB_2 = 64 ± 7% aggregation).

These results might indicate that rabbits receiving high dose aspirin over a prolonged period develop a tolerance to the drug, as displayed by AA induced platelet aggregation, which may involve cyclooxygenase and the sensitivity of the platelets to TXA_2.

ACTIVATION OF D_1 DOPAMINE RECEPTORS INHIBITS THE EVOKED RELEASE OF [^{3}H]-SEROTONIN IN THE RAT SUBSTANTIA NIGRA.

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In the rat substantia nigra, D_1 dopamine (DA) receptors appear to be present in the nerve terminals of neuronal afferents (Dawson et al., 1985 ; Napier et al., 1986), while D_2 DA receptors seem to be localized predominantly in the dopaminergic neuron (Gale et al., 1977). Since serotonergic nerve terminals are known to interact with intranigral dopaminergic dendrites (Dray et al., 1976), we have explored whether DA receptors could be involved in the modulation of the evoked release of serotonin (5HT). The whole substantia nigra was labelled with ^{3}H-5HT. After 60 min of perfusion at 1 ml/min with Krebs medium, two periods of electrical stimulation (S_1 and S_2) at 3 Hz, 2 msec, 24 mA, 2 min were applied with an interval of 44 min. Drugs were added 20 min before S_2, or when indicated (S_1 + S_2) 20 min before S_1. Total radioactivity released during S_1 represented 1.44 ± 0.07 % (n = 26) of tissue stores, and the ratio between the two periods of stimulation (S_2 and S_1) was close to unity (Table 1).

Table 1 : Electrically-evoked release of ^{3}H-5HT in the rat substantia nigra

Drug (S_2)	µM	S_2/S_1			
		-	Sulpiride 0.1 µM (S_1+S_2)	SCH23390 0.1 µM (S_1+S_2)	Idazoxan 1 µM (S_1+S_2)
Control	-	1.01 ± 0.04	1.13 ± 0.16	1.20 ± 0.04	0.91 ± 0.06
Dopamine	0.3	0.52 ± 0.03*	0.46 ± 0.07*	1.02 ± 0.10	0.58 ± 0.02*
SKF 82526	1	0.34 ± 0.02*	N.T.	0.99 ± 0.10	N.T.

Values are mean ± S.E.M. from 3-22 experiments per group. N.T. : not tested. * $p < 0.001$ vs the corresponding control.

The electrically-evoked release of ^{3}H-5HT was significantly inhibited by DA 0.3 µM (Table 1) and in the presence of nomifensine 3 µM (S_2/S_1 = 0.52 ± 0.16, n = 4, $p < 0.05$), when added before S_2. The inhibitory effect of DA 0.3 µM was antagonized by the selective D_1 receptor antagonist (Napier et al., 1986) SCH 23390 0.1 µM, but not by sulpiride 0.1 µM or idazoxan 1 µM (Table 1). The release of ^{3}H-5HT was not modified by the D_2 receptor agonist (Tsuruta et al., 1981) LY 141865 1 µM (S_2/S_1 = 0.81 ± 0.08, n = 3), but it was inhibited in a concentration-dependent manner by the D_1 receptor agonist (Hahn et al., 1982) SKF 82526 (0.03 - 1 µM). The inhibition by SKF 82526 1 µM was antagonized by SCH 23390 0.1 µM (Table 1). SCH 23390 0.1 µM did not modify on its own the release of ^{3}H-5HT, but it facilitated ^{3}H-5HT release in the presence of nomifensine 3 µM (S_2/S_1 = 1.54 ± 0.19, n = 4, $p < 0.05$; control : 1.06 ± 0.08, n = 3).

In conclusion, DA receptors of D_1 but not of D_2 subtype are involved in the modulation of the electrically-evoked release of ^{3}H-5HT from the rat substantia nigra. These receptors may be physiologically activated by endogenously released DA. The present results may provide the first evidence for an inhibitory modulatory role of D_1 DA receptors on neurotransmitter release.

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PHARMACOLOGICAL MANIPULATION OF PILOCARPINE-INDUCED PURPOSELESS CHEWING IN RATS.

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The pharmacological manipulation of neuroleptic-induced purposeless chewing movements in rats closely resemble that of acute dystonia (Rupniak et al. 1986). The administration of cholinergic agonists, such as pilocarpine, also induces purposeless chewing (Salamone et al. 1986; Jenner et al. 1986) and we now report the effects of drug manipulation.

Purposeless chewing was stimulated in male Wistar rats (150-380g) by the administration of pilocarpine (4 mg/kg ip) and measured as previously described (Jenner et al. 1986). In some experiments benzhexol (0.75-2.5 mg/kg), secoverine (0.5-10 mg/kg sc), trifluoperazine (2.5 mg/kg sc), thioridazine (30 mg/kg sc), clozapine (1-20 mg/kg sc), idazoxan (2 mg/kg sc), prazosin (2 mg/kg sc), ketanserin (2 mg/kg sc) and tetrabenazine (10 mg/kg sc) were administered 30 min prior to pilocarpine. Pimozide (1 mg/kg sc) and reserpine (5 mg/kg ip) were administered 3 and 15h respectively before pilocarpine. Chewing was assessed over a 5 min period 30 min following pilocarpine administration.

Pilocarpine (4 mg/kg ip) markedly increased the incidence of purposeless chewing in rats (control 2 ± 1; pilocarpine 140 ± 26). Pilocarpine-induced chewing was dose-dependently inhibited by benzhexol (0.75-2.5 mg/kg) and secoverine (0.5-10 mg/kg). Pretreatment with trifluoperazine (2.5 mg/kg) or thioridazine (30 mg/kg) or pimozide (1 mg/kg) did not alter the intensity of pilocarpine-induced chewing. However, administration of clozapine (20 mg/kg) produced marked inhibition. The adrenergic antagonists idazoxan (2.0 mg/kg) and prazosin (2.0 mg/kg) and 5HT antagonist ketanserin (2.0 mg/kg) were ineffective. In contrast, both reserpine (5.0 mg/kg) and tetrabenazine (10 mg/kg) caused a reduction in pilocarpine-induced chewing.

Table 1 Drug manipulation of pilocarpine (4 mg/kg ip)-induced chewing in rats

	Number of chewing movements/5 min		
Drug (mg/kg sc)	Vehicle	Treatment	% Inhibition
Benzhexol (2.5)	164 ± 35	6 ± 3*	96
Secoverine (10)	242 ± 11	24 ± 5*	90
Trifluoperazine (2.5)	132 ± 31	105 ± 26	20
Thioridazine (30)	145 ± 33	151 ± 14	0
Pimozide (1)	157 ± 20	191 ± 20	0
Clozapine (20)	141 ± 22	25 ± 10*	82
Tetrabenazine (10)	261 ± 20	134 ± 25*	49
Reserpine (5)	234 ± 21	119 ± 23*	49

(N = 6) * $P < .05$ Mann Whitney U test + given I.P.

Pilocarpine-induced chewing is antagonised by anticholinergic drugs but only by neuroleptic drugs with high inherent anticholinergic activity. While monoamine antagonists in general did not inhibit the effects of pilocarpine, the actions of reserpine and tetrabenazine suggest the necessity for intact catecholamine stores for the mediation of this behaviour.

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A SINGLE PRETREATMENT WITH ACTH PREVENTS THE DEVELOPMENT OF TOLERANCE TO MORPHINE ANALGESIA IN MICE

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In recent years, several groups have suggested that Adrenocorticotropin (ACTH) may act as an endogenous opioid antagonist (for review see Hendrie, 1985). Further, as opioids/opiates stimulate the release of ACTH, one model of tolerance would be that, following chronic morphine administration, stimulated ACTH release antagonises the actions of morphine, thereby preventing the display of analgesia. On this view, it may be predicted that the development of tolerance to the analgesic effects of morphine should be prevented if released ACTH was rendered inactive. It has been suggested that one method of deactivating endogenous peptides is to stimulate antibody formation by pretreatment with a structurally similar peptide derived from the tissue of a different species (McLaughlin and Baile, 1985). The present study was conducted in order to investigate the influence of a single administration of porcine ACTH on the development of tolerance to the analgesic effects of morphine in mice.

Thirty-six male DBA/2 mice (Bantin & Kingman, Hull), weighing between 20 and 30g, were injected in randomised counter-balanced order with 10 i.u. porcine ACTH (Sigma, UK) or 0.9% saline, and left undisturbed for 4 days. Following this period, animals were administered 5mg/kg morphine sulphate or saline; all injections were performed intraperitoneally in a volume of 10ml/kg. Four experimental groups were used (n=8) saline/saline;ACTH/saline;saline/morphine and ACTH/morphine. Administration of saline and morphine continued on a daily basis for a further 12 days with tail flick latencies being recorded 1 hr post-injection on days 4,8,12 and 16 following ACTH or saline pretreatment. Data (mean ± SEM) are presented below.

Number of days		TREATMENT			
(a)	(b)	saline/saline	saline/morphine	ACTH/saline	ACTH/morphine
4	1	3.95(± 0.36)	5.8(± 0.45)***	4.75(± 0.49)	7.1(± 0.63)***
8	4	3.4(± 0.17)	4.3(± 0.3)	3.1(± 0.46)	8.4(± 0.5)***
12	8	3.7(± 0.32)	3.6(± 0.5)	3.8(± 0.5)	7.1(± 0.9)***
16	12	3.5(± 0.2)	3.8(± 0.4)	3.4(± 0.2)	8.1(± 0.9)***

(a) = days following ACTH or saline pretreatment
(b) = morphine or saline treatment ***p<0.01

Analysis of variance and orthogonal contrasts revealed significant analgesia in saline/morphine ($F(1,28)=10.13, p<0.01$) and ACTH/morphine ($F(1,28)=13.6, p<0.01$) groups following initial opiate administration. Analgesia was apparent only in ACTH/morphine treated animals on post-ACTH treatment day 8 ($F(1,28)=106.89, p<0.01$), day 12 ($F(1,28=18.56, p<0.01$) and day 16 ($F(1,28)=38.56, p<0.01$).

These data clearly demonstrate (1) an initial analgesic reaction to morphine in saline pretreated animals to which tolerance had developed by the fourth day and (2) the complete prevention of the development of tolerance by ACTH pretreatment, with the analgesic response to morphine following 12 consecutive days of chronic treatment being at least as great as that observed following the first administration. These data may be interpreted as being consistent with the view that pretreatment with porcine ACTH stimulates the formation of an 'anti-ACTH' autoimmune response, although in the absence of further experimentation other possibilities cannot be discounted.

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DOES CHRONIC BENZODIAZEPINE ADMINISTRATION CAUSE A "WITHDRAWAL SHIFT" ACROSS THE WHOLE BENZODIAZEPINE LIGAND SPECTRUM?

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Clinical use of benzodiazepines is complicated by the development of tolerance to their effects and subsequent withdrawal signs. It is clear that changes in benzodiazepine receptor number or affinity do not explain these phenomena (Braestrup et al, 1979). We have shown previously that in flurazepam tolerant animals the effects of the partial agonist FG7142 were enhanced (Little et al, 1984). The present study investigates the effects of a short course of the benzodiazepine agonist, flurazepam, on the actions of the antagonists Ro 15-1788 and ZK 93426 and the inverse agonists CGS 8216 and β-CCM.

Male CD1 mice (Charles Rivers), weight 30 - 35g, were given flurazepam. 40 mg kg^{-1} once daily for seven days; controls received saline injections. 24h or 48h later the above drugs (given i.p., 15 min pretreatment for the infusions) were tested on seizure threshold using i.v. infusion of bicuculline (Nutt et al, 1980) or by observation. Baseline seizure thresholds to bicuculline 24h (and 48h) after the last dose of flurazepam were unchanged. Clear tolerance was found to the anticonvulsant effects of flurazepam at the 24h interval. Ro 15-1788 and ZK 93426 had no effect on seizure thresholds in control mice, but became proconvulsant 24h after flurazepam treatment i.e. they behaved like partial inverse agonists. At 48h only ZK93426 caused a significant change, in flurazepam-treated mice.

Seizure thresholds	bicuculline mg kg^{-1}, mean ± s.e.m., (n), 24h interval			
Acute treatment: Tween	Ro 15-1788 (10 mg kg^{-1})	Tween	ZK 93426 (1 mg kg^{-1})	
Chronic saline	0.43 ± 0.03 (10)	0.40 ± 0.02 (10)	0.48 ± 0.03 (9)	0.45 ± 0.01 (8)
Chronic FZ	0.48 ± 0.04 (9)	0.38 ± 0.02* (9)	0.52 ± 0.03 (7)	0.39 ± 0.03**(7)

Convulsion incidence	Acute treatment: CGS 8216 (10 mg kg^{-1})		β-CCM (3 mg kg^{-1})	
Chronic saline	24h: 0/10	48h: 0/8	24h: 0/8	48h: 3/8
Chronic FZ	24h: 5/10+	48h: 7/8+	24h: 7/8+	48h: 8/8+

* $p < 0.05$, cf. chronic FZ/acute tween, ** $p < 0.01$ cf. chronic saline/acute ZK 93426, $p < 0.02$ cf. other three groups.(Mann-Whitney 'U' test), + $p < 0.05$ cf chronic saline (Fisher's exact test)

CGS 8216 is a weak partial inverse agonist, which causes no seizures in control animals. β-CCM is a higher efficacy inverse agonist; it normally causes convulsions. However, 24h and 48h after flurazepam treatment CGS 8216 caused full seizures and the effects of β-CCM were increased.

A similar pattern was found after diazepam treatment. The increased effect of the inverse agonists is unlikely to have been due to precipitated withdrawal as firstly, no measurable quantities (by gas chromatography, nitrogen detection, ethanol/ether extraction) of flurazepam, desalkylflurazepam or ethylhydroxyflurazepam remained in the brain 24h after the last flurazepam dose and, secondly, residual benzodiazepine agonist would have been expected to decrease rather than to increase the effects of the inverse agonists. These results show a shift across the whole spectrum of benzodiazepine ligands towards the inverse agonist direction. Such a shift may explain both tolerance and withdrawal.

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ANTINOCICEPTIVE EFFECTS OF BENZODIAZEPINE INVERSE AGONISTS: POSSIBLE RELEVANCE FOR 'DEFEAT' ANALGESIA IN MALE MICE?

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We have recently reported an acute non-opioid analgesic reaction in male intruder mice exposed to either social defeat or the scent of an isolated male conspecific (Rodgers & Randall, 1986). As both stimulus situations are aversive to intruders, we hypothesized that anxiety may be an important factor in the activation of non-opioid intrinsic analgesia mechanisms. In view of a highly inconsistent literature, we examined the effects of a variety of benzodiazepine (BZP) ligands on basal nociception before proceeding to an analysis of effects upon 'defeat' analgesia.

6-8 week old DBA/2 male mice (Bantin & Kingman, Hull) were housed in groups of ten, and maintained in a temperature-controlled room ($24\pm1^{o}C$) in which a 12hr reversed LD cycle was operative. All testing was conducted under dim red light during the dark phase of the cycle. Nociceptive latencies (TFL) were assessed by traditional (radiant heat) tail-flick assay, using a cut-off 10 seconds. Pre-injection baseline latencies were recorded to provide within-groups control measures. Drugs used were as follows- chlordiazepoxide hydrochloride (CDP), midazolam bimaleate (MDZ), diazepam (DZ), Ro15-1788, CGS8216, FG7142 and DMCM. CDP and MDZ were dissolved in saline (0.9%); DZ, Ro15-1788, CGS8216 and FG7142 were ultrasonically dispersed in distilled water to which Tween 80 had been added; DMCM was dissolved in acidified (0.01N HCl) water. Corresponding vehicle solutions were used throughout and all injections were performed intraperitoneally (i.p.) in a volume of 10ml/kg. Statistical analysis was conducted by analysis of variance.

Results indicated that CDP(5-30mg/kg), MDZ (0.625-5mg/kg), DZ(0.5-4mg/kg) and Ro15-1788(5-80mg/kg) were ineffective in altering basal tail-flick latencies. However, CGS8216, FG7142 and DMCM all induced significant dose-dependent analgesia (minimum effective doses = 10mg/kg, $\leq$5mg/kg and 1mg/kg, respectively). Drug interaction studies indicated that Ro15-1788 (20mg/kg) completely reversed the analgesic effects of CGS8216 (20mg/kg), FG7142 (10mg/kg) and DMCM (1mg/kg). Furthermore, CDP(20mg/kg) also readily and completely reversed the analgesic effects of FG7142 and DMCM whilst CGS8216 analgesia was substantially attenuated by diazepam (5mg/kg).

Present data are consistent with the proposal that compounds classed as inverse agonists (contragonists, agonists with negative efficacy) induce analgesia through interaction with BZP recognition sites. In recent social interaction studies, we have found that the analgesic reaction induced in intruder mice by acute defeat experience is dose-dependently blocked by Ro15-1788 (10-40mg/kg) and by diazepam (0.5-2mg/kg). Since social defeat is an anxiety-provoking experience, and as BZP inverse agonists are reported to exert anxiogenic effects (Pellow & File, 1984), present findings would not be inconsistent with a defeat-induced release of an endogenous anxiogenic ligand for BZP sites. Further studies are currently underway to test this preliminary hypothesis.

This work was supported by the Medical Research Council. The authors wish to thank Roche Products Ltd (UK), Hoffmann-La-Roche (Basel), Ciba-Geigy Corporation (USA) and Schering AG (Berlin) for the kind gifts of chlordiazepoxide and midazolam; Ro15-1788; CGS8216; DMCM,respectively. FG7142 was purchased from Research Biochemicals Inc. (USA).

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DO AGENTS ENHANCING GABA RECEPTOR-COUPLED CHLORIDE IONOPHORE FUNCTION HAVE ANXIOLYTIC PROPERTIES?

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Biochemical evidence strongly supports the existence of a supramolecular receptor complex consisting of recognition sites for GABA, benzodiazepines and picrotoxin. Also associated with this complex is a chloride ion channel which is gated by the other components of this system (Olsen, 1981; Neilsen et al., 1985).

Recently a number of agents have been found to interact with the picrotoxin recognition site as labelled by [^{35}S] TBPS (Wong et al., 1984). Six agents which have such activity were tested in the stress induced ultrasonic vocalisation model using 11 day old CFHB rat pups, which has been shown to detect the anxiolytic effects of benzodiazepines (Gardner, 1985). Additionally, a subjective measure of muscle relaxation was used to assess this side effect of drugs. All experiments were performed with the observer blind to the treatments.

Log dose-response curves were constructed and an ED_{50} value for inhibition of the stress response was calculated for each agent tested. Diazepam was tested as a reference anxiolytic.

DRUG	ED_{50} (mg/kg i.p.)
Diazepam	0.06 (0.03-0.11)
Tracazolate	1.1 (0.8-1.52)
Tofisopam	8.0 (5.52-11.6)
Methaqualone	1.5 (0.9-3.6)
Etomidate	0.9 (0.54-1.51)
LY 81067	0.61 (0.29-1.28)
Pentobarbitone	0.52 (0.31-0.88)

All compounds tested appeared to possess anxiolytic activity similar to that exhibited by diazepam but at higher doses.

Diazepam, etomidate, LY81067 and pentobarbitone also displayed dose-related induction of muscle relaxation at doses 10-20 times greater than those inhibiting ultrasounds. However, tracazolate, tofisopam and methaqualone did not induce marked muscle relaxation.

We conclude that the compounds tested may induce anxiolytic activity like that of diazepam, but mediated through enhancement of chloride ionophore function via the picrotoxin recognition site.

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EVIDENCE FOR INCREASED $GABA_B$ RECEPTOR FUNCTION IN MOUSE FRONTAL CORTEX FOLLOWING ANTIDEPRESSANT ADMINISTRATION

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Baclofen, an agonist selective for the $GABA_B$ receptor (Hill and Bowery 1981) has been shown to inhibit the release of 5-hydroxytryptamine (5-HT) from slices of frontal cortex of the rat (Bowery et al 1980) and mouse (Gray et al 1986). It has been shown recently that repeated administration of several antidepressant drugs and electroconvulsive shock (ECS) increases $GABA_B$ receptor number in rat frontal cortex (Lloyd et al 1985). We have examined the effect of single and repeated administration of amitriptyline, mianserin, zimeldine, and ECS on the (±)-baclofen-mediated inhibition of potassium evoked release of endogenous 5-HT from slices of mouse frontal cortex.

Groups of mice were treated for 24 hours or 14 days with the following drugs (administered intraperitoneally): amitriptyline (10 $mg.kg^{-1}$ twice daily), zimeldine (10 $mg.kg^{-1}$ once daily), mianserin (10 $mg.kg^{-1}$ once daily). Control animals received 0.9 % NaCl. Further groups of mice received either a single ECS under halothane anaesthesia or 5 ECS spread over 10 days; control animals received halothane only. Twenty four hours after the last treatment animals were killed and slices of frontal cortex were prepared and incubated as described previously, in the presence of pargyline (50 µM) and fluoxetine (5 µM) (Gray et al 1985). 5-HT was measured by high pressure liquid chromatography with electrochemical detection.

Repeated administration of ECS, amitriptyline, mianserin and zimeldine all led to a significant increase in the ability of (±)-baclofen to inhibit the potassium evoked release of 5-HT (Table). One day of administration of either ECS or any of the antidepressant drugs did not alter the response.

	Percentage inhibition of K^+ evoked release of 5-HT by (±)-baclofen (10 µM)	
Treatment	Control	Treated
ECS x 5/10	52 ± 6 (4)	81 ± 7* (4)
Amitriptyline	47 ± 5 (4)	74 ± 6** (4)
Zimeldine	35 ± 4 (4)	63 ± 6* (4)
Mianserin	32 ± 4 (4)	71 ± 6* (4)

Different from control *p < 0.05; **p < 0.01

These data suggest that $GABA_B$ receptor function has been increased by repeated administration of a variety of antidepressant treatments. This increased sensitivity to (±)-baclofen is consistent with the increase in receptor number (B_{max}) in the frontal cortex reported by Lloyd et al (1985). The mechanism for this change is unknown. However the occurence of this increased sensitivity after repeated administration of antidepressants of widely different pharmacological profiles suggests the need to consider further the role of GABA in their mode of action.

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800P

β-ADRENOCEPTOR UPREGULATION AFTER FG7142 IS ABOLISHED BY CHRONIC TREATMENT WITH THE ANTIDEPRESSANT DESMETHYLIMIPRAMINE

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We have shown previously that a single injection of the benzodiazepine partial inverse agonist,FG7142,causes a significant increase in β-adrenoceptor number in mouse cerebral cortex. This increase is found seven days, but not 24h,after the injection of FG7142 (Little et al,1986).FG7142 has also been shown to promote "learned helplessness" following inescapable shock treatment, a behavioural syndrome used as a model for depression.This effect, and the receptor changes, outlasted the acute actions of FG7142,such as lowering of seizure threshold and hypothermia (Little et al,1984;Taylor et al,1985).β-receptor desensitisation can be induced by antidepressant treatments,which are also effective in preventing the development of "learned helplessness".We now report that chronic treatment with the antidepressant,desmethylimipramine (DMI),prevented the rise in β-adrenoceptor binding.

Male CDI mice, 30-35g were used.DMI (10mg kg^{-1},dissolved in saline) or saline (Treatment A) were given once daily,i.p. for 14 days.24h after the last dose,either FG7142 (40mg kg^{-1},suspended in Tween 80) or Tween (treatment B) was injected,i.p. The animals were killed after a further 7 days and the cerebral cortices removed. [^{3}H]-dihydroalprenolol (0.2-2.0nM) was used as a radioligand for β-adrenoceptor binding.Nonspecific binding was assessed by incubation in the presence of (±) propanolol (10μM).Binding data was analysed using the computer programme "Ligand" (Munson & Rodbard,1980).

Treatment A	Treatment B	n	Bmax	% paired controls	kd	% paired controls
saline	tween	20	106±7		1.0±0.3	
DMI	tween	17	108±8	102	1.0±0.2	117
saline	FG7142	20	160±24*	165	1.8±0.4	175
DMI	FG7142	18	104±8+	110	1.2±0.2	156

Bmax pMoles g^{-1} ;KD nM.
*= P< 0.05 (saline/tween cf.saline/FG7142)
+= P< 0.05 (DMI/FG7142 cf. saline/FG7142) (Wilcoxon matched pairs signed ranks)

The results, which were similar whether or not DMI treatment was continued after the FG7142 injections, show that the β-adrenoceptor upregulation after FG7142 is abolished by treatment with DMI.DMI had no effect on adrenoceptor binding when given alone, showing that the prevention of FG7142-induced upregulation was not explained by opposing drug actions. There are numerous reports of β-adrenoceptor downregulation in rat brain after chronic treatment with DMI (e.g.Stanford et al, 1983), but we have repeated this experiment and failed to find any evidence of down-regulation in these CDI mice.This may be explained by a strain or species difference in the effects of DMI.The evidence supports the view that the receptor changes produced by FG7142 may underlie the effect of FG7142 in "learned helplessness"

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HUMAN PLATELET α_2-ADRENOCEPTOR BINDING AND FUNCTION DURING THE MENSTRUAL CYCLE.

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Administration of steroid sex hormones has been shown to alter the number of α_2 adrenoceptor binding sites in rabbit brain and platelets (Barnett et al, 1983; Bloomfield et al, 1985). Endogenous oestrogen may also influence platelet α_2 adrenoceptors since altered binding capacity during pregnancy and after parturition in women corresponds to the changes in plasma oestrogen (Metz et al, 1983). We now report platelet high affinity agonist α_2 adrenoceptor binding and adrenaline (Ad) - induced aggregation during the normal menstrual cycle.

Blood samples (60ml) were obtained at weekly intervals for 4 weeks from 14 healthy female volunteers (age 18-33 years) who had regular menstrual cycles and were not taking any medication or oral contraceptives. Ad-induced aggregation (10^{-8}-10^{-5}M) is expressed as the concentration of Ad producing 50% of the maximal initial rate of aggregation. High affinity agonist binding was determined with ^{3}H UK-14,304 (^{3}H-UK, 0.14-3.3nM) on freshly prepared membranes (Gibson et al, 1986) and plasma oestrogen and progesterone by radioimmunoassay.

The binding parameters and Ad-induced aggregation did not differ significantly (ANOVA) between cycle stages (Table 1).

Table 1. α_2 adrenoceptor binding, Ad-induced aggregation and plasma hormones during different stages of the menstrual cycle (in days).

Parameter/Cycle Stage	2-5	6-9	10-15	16-21	22-29
Oestrogen (pmoles/l)	130±28	178±48	455±101	434±59	470±73
Progesterone (nmoles/l)	1.0±0.4	0.9±0.4	0.6±0.3	20.4±7.2	24.7±5.0
Bmax ^{3}H UK	160±19	133±20	143±14	148±19	125±17
K_D ^{3}H UK (nM)	0.9±0.1	0.7±0.1	0.9±0.1	0.7±0.1	1.1±0.2
EC_{50} Ad (x10^{-7}M)	4.4±0.7	4.1±0.4	3.8±0.5	4.2±0.6	4.0±0.5

Bmax = fmoles/mg protein Values are means ± s.e.m. for 6-14 estimations

Analysis of the whole menstrual cycle using moving averages revealed several trends. Bmax decreased from 20 days to a minimum at 26 days and then increased up to day 6. K_D increased from day 23 to a maximum at day 2 and then decreased to day 7. These results differ somewhat from those of Barnett et al (1984) who reported a minimum number of α_2 adrenoceptors, labelled with ^{3}H yohimbine at day 7 and a maximum labelled with ^{3}H dihydroergocryptine at 28 days, a time when we find a minimum in high affinity agonist binding. Using time series analysis, changes in Bmax correlated strongest with oestrogen concentrations 20 days earlier and those of K_D with oestrogen (14 days earlier) and progesterone (5 days earlier).

While normal circulating concentrations of sex steroids may influence platelet high-affinity agonist α_2 adrenoceptor binding the magnitude of these effects is small.

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LOWER HIPPOCAMPAL, BUT UNCHANGED CORTICAL, 5HT$_2$ BINDING SITES IN DEPRESSED SUICIDE VICTIMS

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Altered serotinergic (5HT) function has long been implicated in depression and suicide. Post-mortem studies of 5HT receptors in suicide victims have yielded conflicting results. Stanley & Mann (1983) reported increased 5HT$_2$ binding in the frontal cortex of suicide victims, whereas Owen et al (1986) found no differences. These discrepancies may relate to heterogeneity in the presence and nature of psychiatric illness associated with suicide and to the effects of antidepressant or other drugs.

In this study 5HT$_2$ binding sites were measured in 5 brain regions (frontal, temporal and occipital cortex, hippocampus and amygdala) from suicide victims (8M, 2F, mean age $\pm$ s.e.m. 44.3$\pm$3.7 years) and a control group (8M, 2F, mean age $\pm$ s.e.m. 44.4$\pm$3.2 years) dying suddenly from non-psychiatric non-neurological causes. The suicide group included only subjects in which a firm diagnosis of depression could be made; subjects with a history of schizophrenia, epilepsy, alcohol or drug abuse and those in whom the diagnosis was unclear were excluded. ^{3}H Ketanserin binding (8 concentrations, 0.07-5.4nM) was performed on well-washed membranes using coded samples. Specific binding was defined with 5×10^{-6}M methysergide. Equilibrium dissociation constant (K_D) and the number of binding sites (Bmax) were determined by non-linear regression analysis.

Table 1 Ketanserin Binding Sites in Frontal Cortex and Hippocampus of Suicides and Controls

	FRONTAL CORTEX (n=9)		HIPPOCAMPUS (n=10)	
	K_D	Bmax	K_D	Bmax
Controls	0.76$\pm$0.19	311$\pm$12	1.01$\pm$0.12	128$\pm$9
Depressed Suicides	0.84$\pm$0.18	342$\pm$33	1.29$\pm$0.18	97$\pm$10*

Values are means $\pm$ s.e.m. K_D = nM Bmax = fmoles/mg protein *P<0.05 (t test).

The number of 5HT$_2$ binding sites was significantly lower (24%) but the K_D unaltered, in the hippocampus of the depressed suicide victims compared to the controls (Table 1). Bmax did not differ significantly between suicides and controls in the other brain regions studied. The reduced number of 5HT$_2$ binding sites in the hippocampus of suicide victims was of a similar magnitude (26%), but failed to reach statistical significance, after exclusion of two suicide victims with therapeutic levels of psychoactive drugs present in their blood at post-mortem. Although the numbers are small, lowered hippocampal 5HT$_2$ binding appeared to be more prominent in those suicide victims who died by violent means (hanging and jumping).

In this study we found no significantly increased cortical 5HT$_2$ binding in a small group of largely drug-free depressed suicide victims. The lowered hippocampal 5HT$_2$ binding and its relationship to suicide and/or depression is worthy of further study with a larger group of subjects.

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ELECTROPHYSIOLOGICAL STUDIES ON THE SITE OF ACTION OF MORPHINE IN THE SPINAL CORD OF THE ANAESTHETISED RAT

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Opiates are known to reduce the release of substance P from the terminals of primary afferent fibres (Jessell & Iversen, 1977; Yaksh et al, 1980) and much of the peptide is associated with primary, non-myelinated, afferent fibres in the spinal cord. Systemically administered or microiontophoretically applied opiates reduce the activation by noxious heat of convergent dorsal horn neurones. This has given rise to the hypothesis that a major of action of opiates in the production of analgesia is to reduce the release of transmitter from primary afferent fibres.

Pini & Ryall (1986) have recently shown that noxious radiant heat can inhibit, as well as excite, convergent dorsal horn neurones in rats and cats, and that the inhibition is mediated by a spinal GABAergic reflex: excitation or inhibition is obtained depending upon the location of the stimulus in the receptive field. We now report that morphine has no effect on the inhibition, whereas excitation is effectively diminished in spinal rats anaesthetised with thiobutabarbital, 100-120 mg kg^{-1} i.p., supplemented with i.v. administration as required.

Of 187 neurones studied in 29 rats, 176 were either excited or inhibited by noxious radiant heat. Of these, 27 cells (15%) were inhibited. Morphine, administed iontophoretically to four cells inhibited by noxious heat had no effect on the inhibitions. Morphine sulphate was administered intravenously to 16 cells whilst recording their responses to noxious heat. On nine cells which were excited by the stimulus the response was attenuated by 0.5 to 4 mg kg^{-1}. On seven cells which were inhibited, 0.5 to 6 mg kg^{-1} failed to have any effect. On two further cells in which the response to noxious heat was biphasic the excitation but not the inhibition was reduced by morphine (2-4 mg kg^{-1}).

It is probable that the same primary afferent fibres mediate both the excitation and the inhibition. The failure of morphine to attenutate the inhibition, whilst effectively diminishing the excitation indicates that the most important action of morphine is at a site located beyond the first synapse and not the reduction in transmitter release at the terminals of primary afferent fibres. Alternatively, there may be two subsets of primary afferent C-fibres, one of which is devoid of opiate receptors and which mediates the morphine-resistant inhibition.

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