NEUROMUSCULAR BLOCKADE BY VECURONIUM-VERAPAMIL AND REVERSAL BY EDROPHONIUM AT RAT NEUROMUSCULAR JUNCTION

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Vecuronium (Org.NC 45),a quaternary derivative of pancuronium,is known to produce a rapid onset of muscle block,of intermediate duration and a quick recovery (Durant,Marshall,Savage,Nelson,Sleigh & Carlyle,1979;Gencarelli & Miller,1982; Baird,Bowman &Kerr,1982;Engbaek,Ording,Ostergaard & Viby-Mogensen,1985).

In the present investigation,we have studied,in rat hemidiaphragm preparation,the effects and interactions of vecuronium with verapamil and reversal of combined neuromuscular blockade with edrophonium.The results were compared with those previously reported in other preparations (Bikhazi,Leung & Foldes,1982;Carpenter & Mulroy,1983).

Vecuronium (0.16-160 μM),reduced in a concentration-dependent manner,the indirectly-elicited twitch and tetanic contractions,evoked at 0.2-100 Hz with 5 V (maximal) and 0.2 msec square pulse duration. Verapamil (0.1-10 μM) had little effect on the twitch tension,but it significantly increased vecuronium-induced blockade of the twitch tension.The mean IC_{50} values (concentration to block 50% of maximum twitch tension) of vecuronium and vecuronium-verapamil blockade were 4.8±0.2 μM and 3.2±0.1 μM,respectively (means±S.E.,n=6,$P < 0.001$).The mean Freq.$_{30}$ values (i.e. frequency of stimulation which produced 30% of maximum tetanic contraction) of tetanic contractions in the control Krebs solution,and in the presence of vecuronium (8 μM) and vecuronium-verapamil (10 μM) were: 4.1±0.1, 10.5±0.2 and 15.2±0.1 Hz respectively. Edrophonium (1-50 nM),reversed the blockade of twitch tension by vecuronium and vecuronium-verapamil (Table 1) and decreased the mean Freq.$_{30}$ values to 2.8±0.1 and 8.1±0.2 Hz respectively (means±S.E.,n=6,$P < 0.001$).

Table 1. Effects and interactions of vecuronium-verapamil-edrophonium at the rat hemidiaphragm preparation.

	(1) Vecuronium(Vec) Mean±S.E.	(2) Vec+Verap. Mean±S.E.	% Change (1-2)/1	(3) Vec+Ver+Edro. Mean±S.E.	% Change (3-1)/1
IC_{50} value (μM)	4.8±0.2	3.2±0.1	33%	6.2±0.1	29%
Time to onset of action (sec)	25.5±5.0	15.0±2.0	41%	40.0±5.0	57%
Time to maximum block (min)	4.5±0.5	2.5±0.1	44%	7.0±0.5	56%
Time to 75% recovery (min)	8.5±0.5	12.2±0.4	43%	6.2±0.5	27%

The results showed that verapamil (10 μM) increased the vecuronium-induced blockade by about 33%,whereas edrophonium (50 nM) reversed this by about 29%. Verapamil decreased the time to onset of action of vecuronium,maximum block and prolonged time to 75% recovery of twitch tension.Edrophonium (50 nM) antagonized the blockade produced by vecuronium and by vecuronium-verapamil. The present results are in agreement with those reported previously and showed that edrophonium can easily reverse vecuronium and vecuronium-verapamil blockade without complication at the rat neuromuscular junction.

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LOCAL ANAESTHETICS INHIBIT INFLUX OF K^+, Na^+ AND Ca^{2+} INTO RAT ISOLATED ILEUM

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It is known that local anaesthetics can block nerve and muscle action potentials, by blocking the Na^+,and possibly K^+,channels (Aceves & Machne,1963;Ritchie,1975). Furthermore,local anaesthetics may also antagonize entry of Ca^{2+},cause release of Ca^{2+},or prevent re-uptake of Ca^{2+} into the sarcoplasmic reticulum (Kuperman, Altura & Chezar,1968;Bianchi & Bolton,1967).

In the present investigation,we have studied,in rat isolated ileum,the effects of 5 dufferent local anaesthetics,lignocaine,prilocaine,etidocaine,mepivacaine and bupivacaine on influx of K^+,Na^+ and Ca^{2+} to compare their potency and channel blocking activity at rest.
The preparation was incubated,for 10 min,in an organ bath containing 25 ml of Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in O_2 . Uptakes of K^+,Na^+ and Ca^{2+},into rat ileum,were measured using Kone Microlyte Analyser (Kone Corporation Instruments Division,SF-0231, ESP0032,Finland).The Kone Microlyte is an ion-selective electrode which first measures the ionic activity of electrolytes and then converts the activity into equivalent concentration (mM) using the formula: $I = \frac{1}{2} Z^2X.CX$, where I= ionic activity, Z=charge of ion X,and C=concentration of ion X.

The results showed that lignocaine (0.35-350 µM),prilocaine (0.39-391 µM),etidocaine (0.32-321 µM),mepivacaine (0.33-335 µM) and bupivacaine (0.29-292 µM) reduced the uptake of K^+,Na^+ and Ca^{2+} into the rat ileum (Table 1, means±S.E.,n=6,$P < 0.001$). In low concentrations (0.1-10 $\mu g.ml^{-1}$),the local anaesthetics did not significantly decrease the uptake of Ca^{2+},whereas they significantly ($P < 0.01$) reduced the K^+ and Na^+ influx.

Table 1. Effects of local anaesthetics on influx of K^+,Na^+ and Ca^{2+} into rat ileum.

Uptake	$ng.mg^{-1}\ min^{-1}$			$ng.mg^{-1}\ min^{-1}$			$ng.mg^{-1}\ min^{-1}$		
	K^+	K^+		Na^+	Na^+		Ca^{2+}	Ca^{2+}	
	Control	in LAs	(%)	Control	in LAs	(%)	Control	in LAs	(%)
Lignocaine	962±85	529±25	45%	31553±157	11260±103	64%	962±83	650±73	28%
Prilocaine	769±98	481±82	38%	31594±285	15960±180	49%	850±72	650±41	24%
Etidocaine	835±65	510±40	40%	33548±571	18891±218	44%	650±76	500±57	23%
Mepivacaine	795±35	417±96	48%	32571±428	14982±458	54%	700±80	500±51	29%
Bupivacaine	817±94	433±30	47%	71657±142	32571±428	55%	992±57	725±91	27%

The results showed that lignocaine,mepivacaine and bupivacaine were slightly more effective than prilocaine and etidocaine in reducing the influx of K^+,Na^+ and Ca^{2+}. Furthermore,the local anaesthetics were more effective in blocking the K^+ & Na^+ than the Ca^{2+}.These results are in agreement with the suggestion that,in high concentrations,local anaesthetics may have a calcium antagonist-like action.

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EFFECT OF NIFEDIPINE ON THE RESPONSES OF THE DIABETIC PREGNANT RAT UTERINE SMOOTH MUSCLE TO OXYTOCIN AND PROSTAGLANDINS

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Smooth muscle has been shown to be largely dependent on extracellular calcium which can enter into the cell via potential dependent or receptor operated channels (Bolton, 1979). In an earlier study we reported that verapamil, a calcium channel antagonist, is a potent inhibitor of oxytocin (OX) and prostaglandin (PG) induced uterine contraction in both control and diabetic day 21 pregnant rats and that verapamil may be useful in the treatment of premature labour (Desta & Senior, 1986). The present study investigates the effectiveness of another calcium channel antagonist, nifedipine in moderating the spasmogenic action of these uterine stimulants.

Dose-response curves for the effects of PG and OX were determined in the absence and presence of nifedipine (5×10^{-9}M and 2×10^{-8}M) on uterine strips from day 21 pregnant rats. Diabetes was induced by a single intravenous injection of streptozotocin 50 mg kg^{-1} on day 12 of pregnancy and was confirmed by blood glucose (BG) measurement on day 21 of gestation.

Diabetic (D) (BG 17±0.9 mmol L^{-1}) pregnant rats showed reduced ($P<0.001$) maternal body weights compared with euglycaemic (BG 4.2 ± 0.2 mmol L^{-1}) control (C) rats (D = 369 ± 10g, C = 433 ± 9g) and reduced ($P<0.05$) litter size (D = 14.5 ± 0.54, C = 16.5 ± 0.67). Responses of the diabetic uterine strips to OX were significantly depressed by this condition. Nifedipine (5×10^{-9}M and 2×10^{-8}M) produced a concentration dependent inhibition of PGE_2-induced responses in both the control (n=14) and diabetic (n=15) rat uteri. $PGF_{2\alpha}$- (n=15) and OX- (n=16) induced responses were significantly depressed only by the higher dose of nifedipine (2×10^{-8}). In uteri from diabetic rats, however, $PGF_{2\alpha}$-induced (n=15) contractions were highly depressed by both concentrations of nifedipine used. A further increase in the dose of nifedipine to 2×10^{-7}M and 2×10^{-6}M did not produce additional inhibition of OX and $PGF_{2\alpha}$ responses.

These results indicate that the contractile responses of rat uterine smooth muscle to OX and $PGF_{2\alpha}$ involve both extracellular and intracellular Ca. On the other hand the high sensitivity of PGE_2-induced responses to nifedipine suggests that an increase in Ca^{2+} influx from extracellular site may be the main mechanism of action of this compound.

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THE ROLE OF CALMODULIN IN THE CONTRACTILITY OF RAT TESTICULAR CAPSULE MYOFIBROBLASTS

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In a previous communication it was reported that nifedipine inhibited the mepyramine induced contractions of myofibroblasts present in the rat testicular capsule (Lal and Naylor, 1986). This effect contrasted with the lack of effect of another calcium antagonist, verapamil, which perhaps questioned attributing nifedipines' antagonistic effect solely to its effect on calcium ion movement. Since nifedipine has also been shown to antagonise calmodulin (Minocherhomjee et al, 1984) this investigation was to elucidate the role of calmodulin, using a number of calmodulin antagonists; trifluoperazine (Weiss et al, 1980; Spedding, 1983), compound 48/80 (Gietzen et al, 1983; Van Belle, 1984), fluphenazine (Roufogalis et al, 1983) and desipramine (Wakada et al, 1984) on the in vitro contractility of rat testicular capsule myofibroblasts.

Circular strips of the testicular capsule were prepared and arranged for superfusion as previously described (Lal and Naylor, 1986) and the following calmodulin antagonists were examined: trifluoperazine (10 - 100 μM), desipramine (10 - 100 μM), fluphenazine (10 - 50 μM) and compound 48/80 (20 -100 μg/ml). Appropriate dose response curves were constructed for each concentration of antagonist used (n = 6) and the degree of antagonism calculated.

Calmodulin antagonist	Base line	Effect
Trifluoperazine	No effect	Inhibitory $\geqslant$ 50 μM ($P<0.05$)
Compound 48/80	No effect	Inhibitory $\geqslant$ 40 μg/ml ($P<0.01$)
Fluphenazine	No effect	Inhibitory $\geqslant$ 50 μM ($P<0.05$)
Desipramine	Sustained increase	Inhibitory $\geqslant$ 50 μM ($P<0.05$)

Trifluoperazine and compound 48/80 both inhibited the magnitude of response induced by mepyramine in a dose dependent manner. Fluphenazine in addition to producing inhibitory effects on mepyramine responses also caused the duration of response to the prolonged (5 - 10 mins). The effects of fluphenazine were however difficult to reverse even after prolonged washing (>1 hr). Of the 'calmodulin antagonists' used only desipramine, (50 - 100 μM), caused an increase of 60 - 80 mg in the base line tension of the tissue in addition to antagonising the contractile effects of mepyramine. After washing, (> 1 hr), the base line tension returned to the original level and the magnitude of mepyramine returned to control responses.

These results indicate that if the calmodulin antagonists are specific then (a) myofibroblasts of the testicular capsule may contain calmodulin and (b) contractile processes activated by mepyramine are to some degree dependent on calmodulin. These findings suggest that testicular myofibroblasts have functional similarities to smooth muscle cells in respect to calmodulin.

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RELEASE OF OPIOID PEPTIDES FROM RAT STRIATAL SLICES AND CULTURED BOVINE CHROMAFFIN CELLS: SENSITIVITY TO DIHYDROPYRIDINES

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Pharmacological characterisation of neuronal Ca^{2+} channels involved in stimulus-secretion coupling indicates that dihydropyridine (DHP) Ca^{2+} channel blockers (antagonists) are generally ineffective in inhibiting Ca^{2+} influx and neurotransmitter release in response to depolarisation (e.g. Miller & Freedman, 1984; Creba & Karobath, 1986). DHP Ca^{2+} entry enhancers (agonists), however, may under certain experimental conditions potentiate depolarisation-induced Ca^{2+} influx and release of catecholamines and acetylcholine (e.g. Middlemiss, 1985; Woodward & Leslie, 1986). Here we investigate the role of DHP-sensitive Ca^{2+} channels in the stimulated release of peptides.

In this study we used two preparations, the bovine adrenal chromaffin cell (a widely used model of neuronal function) and rat striatal slices, and measured immunoreactivity to two proenkephalin products, (met)-enkephalyl-arg^6-phe^7 (YGGFMRF-IR) and (leu)-enkephalin (YGGFL-IR). Catecholamines were measured using high pressure liquid chromatography with electrochemical detection. Release was stimulated by either 30 mM or 50 mM K^+ (8 min. for striatal slices, 3 min. for chromaffin cells, static incubation conditions at 37°C). Where appropriate the preparations were preincubated with DHP (8 min. for striatal slices, 12 min. for chromaffin cells) which were also present during the stimulation period.

Table 1 Release in response to 30 mM K^+ (± SEM, n = 4)

TREATMENT (DHPs at 1 µM)	CHROMAFFIN CELLS Percent of cell content		STRIATAL SLICES Release in fmol	
	Noradrenaline	YGGFL-IR	YGGFL-IR	YGGFMRF-IR
Basal	2.87 ± 0.68	1.58 ± 0.24	12.2 ± 1.4	91.2 ± 11.3
30 mM K^+	13.32 ± 1.71	3.90 ± 0.10	69.8 ± 12.5	225 ± 19
" + BAY K 8644	22.38 ± 1.21	6.12 ± 0.32	85.7 ± 4.9	246 ± 13
" + (+)-202-791	21.45 ± 0.25	4.97 ± 0.27	75.5 ± 10.5	240 ± 7.5
" + (-)-202-791	3.22 ± 0.15	2.90 ± 0.32	56.0 ± 3.5	209 ± 10

The results show that both the YGGFL-IR and the noradrenaline released from chromaffin cells in response to stimulation with 30 mM K^+ is increased by the agonists BAY K 8644 and (+)-202-791, but is decreased by the Ca^{2+} channel antagonist (-)-202-791. Similar results were obtained with release of YGGFMRF-IR from chromaffin cells, and when the stimulus was 50 mM K^+. In parallel experiments on rat brain striatal slices, however, the presence of DHP resulted in no large changes in dopamine release in response to stimulation with 30 mM K^+ (not shown). Furthermore, we show in the data above that neither DHP agonists nor antagonists have any reproducible and significant effects on release of YGGFL-IR and YGGFMRF-IR from striatal slices. In these experiments 30 mM K^+ caused about half the release elicited by 50 mM K^+. Similar experiments using (+)-nicardipine and (+)-PN-200-110 showed a similar insensitivity of peptidergic neurones to DHP under our experimental conditions.

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HEMODYNAMIC COMPARISON OF VARIOUS CALCIUM ENTRY BLOCKERS IN THE HEART-LUNG PREPARATION (HLP) OF THE GUINEA-PIG

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In vitro models have been used frequently to investigate and quantify the effects of calcium entry blockers (CEB). Using the heart-lung preparation of the guinea-pig we have attempted in the present study to compare several CEB in a more physiological model, approaching in vivo conditions. The following CEB were investigated: nifedipine (dihydropyridine), verapamil (phenylalkylamine), diltiazem (benzothiazepine), flunarizine, lidoflazine (both diphenylalkylamines) and bepridil.
Male guinea-pigs (375-450 g) were anesthetized with urethane (1.5 g/kg). The animals were subjected to artificial respiration with carbogen (95% O_2 + 5% CO_2) at positive pressure, via a tracheal cannula. After bilateral thoracotomy the vena cava inferior, aorta and vena cava superior were cannulated. The vena cava inferior was connected to a reservoir containing donor blood obtained from other animals and kept at 37°C. Left ventricular pressure and $\frac{dp}{dt\ max}$ were measured via a transducer connected with the lumen of the left ventricle. Heart rate was kept constant at 5 Hz by pacing. Similarly, aortic pressure (AoP) and cardiac output (CO) were measured at constant heart rate, preload and afterload. Blood gases were kept constant at values of approximately 7.3 (pH), 300 mm Hg (pO_2) and 40 mm Hg (pCO_2). In separate experiments the influence of the drugs on heart rate were studied in spontaneously beating preparations. Drugs to be studied were injected into the reservoir.
All calcium entry blockers studied caused dose-dependent decreases of LVP, $\frac{dp}{dt\ max}$ AoP and CO, which could be quantified by means of concentration-response curves. The following rank order of potency was found for the calcium entry blockers studied: nifedipine > verapamil > diltiazem > lidoflazine > flunarizine > bepridil. This sequence holds for all four hemodynamic parameters established. For the reduction in heart rate by the CEB the rank order found was: nifedipine > diltiazem > verapamil > lidoflazine > flunarizine > bepridil. The concentration-response curve (CRC) of bepridil differed in shape from that of the other drugs; the initial slope proved relatively flat in comparison with that of the other CEB.
In conclusion, hemodynamic effects of CEB can be demonstrated and quantified in the heart-lung preparation. The rank order of potency for the various drugs is similar for all parameters which were established. Nifedipine proved the most potent drug, flunarizine and bepridil the least active compounds. Data obtained are in accordance with the findings in in vitro models, described in the literature.

EFFECTS OF UD-CG-115 BS ON CONTRACTILE FORCE AND ^{45}Ca IN VASCULAR SMOOTH MUSCLE

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The benzimidazole-substituted pyridazine UD-CG-115 BS (Pimobendan) is a new orally effective cardiotonic drug which also exhibits vasodilator properties in animal preparations (Diederen et al., 1982; Honerjager et al., 1984). The purpose of this study was to examine the effects of UD-CG-115 BS on contractile responses induced by various stimuli and ^{45}Ca movements on isolated rabbit aortae. The influence of removing endothelial cells on the effect of UD-CG-115BS was also evaluated.

Experiments were undertaken on rings of rabbit aortae. Tissues were set up in organ baths containing Krebs solution (34°C) and gassed with 95% O_2- 5% CO_2. ^{45}Ca movements were determined in aortic rings placed in Hepes buffer (pH 7.3) gassed with 100% O_2 as described elsewhere (Meisheri et al., 1981).

UD-CG-115 BS, $10^{-6}M$ - $5x10^{-4}M$, caused a dose-dependent inhibition of noradrenaline (NA, $10^{-6}M$) or high-K^+ (80 mM)-induced contractions of rabbit aorta. NA-induced contractions were significantly more sensitive ($P < 0.05$) than high-K^+ to the inhibitory effect of the drug. The inhibitory effect of the drug was significantly decreased by removal of the endothelium. UD-CG-115 BS also reduced the contractile response induced by NA or caffeine (20 mM) in aortic strips incubated in Ca^{++} ion stored in the vascular smooth muscle fibres following repetitive applications of both agonists.

The effects of UD-CG-115 BS, $5x10^{-4}M$, on ^{45}Ca uptake were studied in resting preparations and in preparations stimuled by high-K^+ (80 mM) and NA ($10^{-6}M$). UD-CG-115 BS inhibited ^{45}Ca uptake both in resting (from 0.76 ± 0.06 to 0.52 ± 0.07 mmol $Ca^{++}.Kg^{-1}$. $P<0.05$) and NA-stimulated aortic rings (from 1.60 ± 0.19 to 1.09 ± 0.08 mmol Ca^{++}. Kg^{-1}. $P<0.05$). In contrast, it failed to cause inhibition of high-K^+-stimulated ^{45}Ca uptake (0.98 ± 0.18 as compared to 0.76 ± 0.08 mmol Ca^{++}. Kg^{-1}. $P>0.05$). Moreover, UD-CG-115 BS, $5x10^{-4}M$, caused a marked increase ($P<0.001$) in the rate of ^{45}Ca efflux caused by NA ($10^{-5}M$).

It is concluded that the action of UD-CG-115 BS on arterial contractility can be related to the blockade of Ca^{++} entry through leak and receptor-operated channels as well as to an intracellular action to decrease the availability of Ca^{++} required for activation.

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INFLUENCE OF MORPHINE AND U-50,488H ON THE CARDIOVASCULAR CHANGES ASSOCIATED WITH CENTRALLY ADMINISTERED BOMBESIN IN RATS

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Bombesin (BN) is a tetradecapeptide which is known to have different effects on the cardiovascular system depending on species, anaesthetic, and route of administration (Erspamer et al, 1972; Chahl and Walker, 1981; Fisher et al, 1985). We now wish to report the effect of centrally administered BN on heart rate (HR) and blood pressure (expressed as mean arterial pressure, MAP) in the anaesthetised rat.

Male Sprague Dawley albino rats (180-200 g; Zivic-Miller) were each implanted stereotaxically with a polyethylene cannula into the left lateral cerebral ventricle 7-10 days prior to experimentation . For measurement of HR and MAP, rats (n=4-8) were anaesthetised with urethane (1-1.5 g/kg, i.p.) and the femoral artery and vein cannulated. BN was given i.c.v. in a volume of 8 µl of water. In the interaction studies, animals were pretreated with test compound (2 µl i.c.v.) 5-6 min prior to BN. All agents were flushed in with 2 µl of water.

BN (1 pmol - 1 nmol; 1.6 ng - 1.6 µg) gave a dose-related effect which was biphasic for BP and monophasic for HR. The effect consisted of an immediate, transient hypotension (peak 1.5-2 min; duration 3-4 min) with little change in HR followed by a prolonged hypertension and tachycardia. The increase in BP was maximal by 7-10 min and lasted for approximately 20 min. Peak increases in HR occurred between 15 and 20 min and continued for up to 60 min post BN.

Tachyphylaxis develops rapidly to the cardiovascular actions of BN in rats. Indeed, we have observed that a very low dose of BN (1 fmol), inactive *per se*, prevents the cardiovascular effects of subsequent, higher doses of the peptide. Consequently, our studies were confined to single doses of BN in each animal. A dose of 0.10 nmol (0.16 µg) of BN produced near maximal cardiovascular changes (Table 1). This dose was therefore used in the interactional studies described below.

Table 1 Effects of bombesin on the rat cardiovascular system

Basal		**Bombesin** (0.10 nmol, i.c.v.)			
		Hypotension phase		Hypertension phase	
MAP (mm Hg)	**HR** (bpm)	**MAP** ↓	**Δ HR**	**MAP** ↑	**HR** ↑
68 ± 3.6	330 ± 13	12 ± 2.8*	-4.0 ± 3.0	16 ± 2.3*	53 ± 9.0*

Values are means and s.e.mean. *P<0.05 or better (paired t-test).

Previous work has shown that opioids can influence certain actions of BN *in vivo*. For example, *kappa* agonists such as 3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide (U-50,488H, U-50) antagonise the characteristic scratching induced by bombesin in rats (Hopkins et al, 1985). U-50 (0.10 nmol, i.c.v.) did not affect the hypotensive phase of the BN response (↓ MAP 12 ± 2.6) but markedly reduced both the hypertension (↑ MAP 4.0 ± 2.3) and tachycardia (↑ HR 29 ± 10). At 1 nmol, U-50 blocked both phases of the response (↓ MAP 3.0 ±3.1; ↑ MAP 8.2 ± 1.2, ↑ HR 17 ± 9.1). Neither dose of U-50 significantly altered basal BP or HR. In contrast, morphine sulphate (1 nmol, i.c.v.) did not consistently antagonise any component of BN action.

At 10 nmol, morphine prevented the initial hypotension but did not block the hypertension (↑ MAP 30 ± 1.7) or tachycardia (↑ HR 90 ± 8.1); indeed, at this dose, morphine potentiated BN-induced increases in BP and HR although, in the case of BP, this may have been an additive effect since morphine alone increased MAP (11 ± 1.7). The effects of BN on HR and BP were not antagonised by naloxone (1 nmol, i.c.v.) nor were they prevented by bilateral vagotomy.

Our results suggest that it may be possible to differentially influence the hypotensive and hypertensive/tachycardia phases of centrally administered BN by using morphine and U-50, respectively. [Grant DA 03681].

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COMPARISON OF THE RELAXATION OF RAT ARTERIES AND VEINS BY HUMAN α-CGRP, SODIUM NITROPRUSSIDE AND ACETYLCHOLINE

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Immunoreactive staining for calcitonin gene-related peptide (CGRP) was found in thin, beaded fibres associated with vascular smooth muscle (Rosenfeld et al,1983). Further studies revealed high levels of CGRP in certain rat blood vessels, the highest concentration being in the superior mesenteric artery (Mulderry et al, 1985). Relaxation of rat thoracic aortic rings was elicited by human and rat CGRP and the response was shown to be endothelium-dependent (Brain et al,1985). The present experiments studied the effects of human α-CGRP (hα-CGRP) on a range of arteries (superior mesenteric, renal, femoral, thoracic and abdominal aorta) and also the femoral and hepatic portal veins. The peptide was compared with sodium nitroprusside and acetylcholine.

With the arterial rings (in Krebs solution at 37°C, 0.5g resting tension), noradrenaline (10^{-7} or $3x10^{-7}$M) was used as a spasmogen. The induced tone was relaxed by hα-CGRP (10^{-9} - $3x10^{-6}$M), sodium nitroprusside (10^{-9}-10^{-6}M) and acetylcholine (10^{-9}-10^{-4}M). For the veins (also in Krebs solution at 37°C, 0.5g resting tension) hα-CGRP was added, followed by noradrenaline (10^{-7} or 10^{-6}M). In some mesenteric artery rings, gentle rubbing with wires was used to remove the endothelium and this was confirmed by lack of response to acetylcholine.

In all of the arteries studied, sodium nitroprusside and acetylcholine produced dose-dependent relaxation of the noradrenaline-induced tone. In most cases, they were more potent and had steeper dose-response curves than hα-CGRP e.g. in mesenteric artery rings, 10^{-6}M hα-CGRP evoked a relaxation of 52±7% (mean ± s.e. mean), whereas sodium nitroprusside and acetylcholine (both $3x10^{-8}$M) produced 93±4% and 89±9% relaxation respectively. In abdominal aortic rings, the same concentration (10^{-6}M) of hα-CGRP, sodium nitroprusside and acetylcholine elicited relaxations of 24±7%, 93±4% and 89±9% respectively. In rat thoracic aortic rings, sodium nitroprusside, acetylcholine and hα-CGRP all caused dose-dependent relaxations, but hα-CGRP had the lowest maximum. No detectable effect of hα-CGRP (10^{-8}-$3x10^{-6}$M) was seen in either the femoral vein or hepatic portal vein. When rings of mesenteric artery were denuded of endothelium, neither hα-CGRP (10^{-8}-10^{-6}M) nor acetylcholine (10^{-9}-10^{-6}M) altered the tone of the pre-constricted vessels, whereas sodium nitroprusside (10^{-9}-$3x10^{-8}$M) still elicited relaxation.

In conclusion, hα-CGRP showed some selectivity for relaxing arteries when compared with veins. In these experiments, using large arteries, sodium nitroprusside was always more potent than hα-CGRP, but this is not found in either perfused vascular beds (Marshall et al,1986, Holman et al,1986) or in vivo (Craig et al,1986). Human α-CGRP caused endothelium-dependent relaxation in rat mesenteric artery rings, as in thoracic aortic rings (Brain et al,1985), contrasting with the endothelial independent relaxation to CGRP in cat middle cerebral artery (Edvinsson et al, 1985).

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EFFECT OF VERAPAMIL ON THE CONTRACTIONS PRODUCED BY HIGH POTASSIUM AND NORADRENALINE IN HUMAN ISOLATED SAPHENOUS VEIN

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In the present investigation,we have studied,in human isolated saphenous vein,the effect of verapamil,which blocks voltage-dependent calcium channels,on the contractions produced by potassium chloride (KCl),which activates potential-operated calcium channels and is inhibited by calcium antagonists (Meisheri,Hwang & Van Breemen,1981),and by noradrenaline (NA),which activates receptor-operated calcium channels and/or can release calcium from intracellular stores (Karaki,Kubota & Urakawa,1979). The receptor-operated calcium channels are thought to be insensitive to calcium antagonists and are independent of membrane potential changes (Meisheri,et.al.,1979;Bolton,1979).

The saphenous vein was obtained from male patients.aged 38-69,undergoing coronary bypass operation,vein graft. Tissues were cut spirally into 2-3 mm wide and 20-30 mm long (Docherty & Hyland,1985),and the strips were set up in separate organ baths containing 20 ml of Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in O_2. Mechanical responses (contractions or relaxations) produced by drug action were recorded isometrically.

Verapamil (0.1-10 µM) relaxed,whereas KCl (18-180 mM) and NA (0.2-200 µM) contracted the human isolated saphenous vein in a concentration-dependent manner.A mean maximum relaxation of 1.2±0.2 g and contraction of 1.5±0.1 g , 3.4±0.3 g tension was produced by verapamil (10 µM),KCl (180 mM) and NA (200 µM),respectively. The mean EC_{50} values (concentration to produce 50% maximum effect) of verapamil, KCl and NA-induced responses were: 1.0±0.1 µM, 32±2.3 mM and 9±0.5 µM,respectively (means±s.e.,n=6).

Verapamil (1 µM) reduced the contractions produced by KCl and NA and shifted their concentration-effect curves to the right. The mean EC_{50} values of KCl and NA-induced contractions were increased in the presence of verapamil (1 µM) to 147± 10.4 mM and 19±1.5 µM,respectively. Thus verapamil produced greater reductions in the KCl than NA-induced contractions in human saphenous vein (a reduction of 5 versus 2 -fold was obtained respectively). Verapamil (1 µM) reduced the maximum KCl and NA-induced contractions by 60±3.1% and 21±2.5%,respectively (means±s.e., n=6, $P < 0.001$, $P < 0.05$).

It is possible that by blocking calcium influx through voltage-dependent calcium channels (Fleckenstein,1977),verapamil relaxed the human saphenous vein,and this confirmed the well-known vasodilator effect of verapamil. Furthermore,verapamil produced a differential block of KCl and NA-induced contractions,greater reductions were obtained in the KCl-induced contractions, and this shows that verapamil was more effective in blocking voltage-dependent than receptor-operated calcium channels in the human isolated saphenous vein.

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DIFFERENT INVOLVEMENT OF CALCIUM IN PGE_2 GENERATION AND TRANSEPITHELIAL P.D. IN COLON OF RAT VERSUS RABBIT

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Calcium may be an intracellular messenger in intestinal secretory processes, including those of colon, but the functional relationships of Ca^{2+} to potent secretagogue eicosanoids such as prostaglandin E_2 have not yet been fully established. We have investigated Ca^{2+} involvement in the changes of transepithelial p.d. and generation of PGE_2 in colon of rat and rabbit caused by calcium ionophore A23187 and lysyl-bradykinin (LBK) used as agonist secretagogues.

Colonic sheets stripped of underlying muscle were mounted in Ussing-type chambers for measurement of p.d. and release of immunoassayable PGE_2 (Hoult & Phillips, 1986). PGE_2 is released mainly into the serosal bathing medium; these values are shown below, expressed as ng min^{-1} cm^{-2} after 15 min exposure to secretagogue. The (lower) values for PGE_2 in mucosal medium are omitted.

LBK at 10^{-6}M induced p.d. changes (due to net chloride secretion, see Cuthbert & Margolius, 1982) and PGE_2 release in both rat and rabbit colon. The p.d. response but not PGE_2 release was prevented in rat colon by removal of extracellular Ca^{2+} (1mM EGTA, 30 min pretreatment), whereas in rabbit colon both the p.d. and PGE_2 responses were substantially reduced (Table). A23187 10^{-6}M caused a rise in transepithelial p.d. in both tissues, but it was much more marked in rabbit (88% of the LBK response) than in rat (39% of LBK). A23187 also caused substantial PGE_2 release into rabbit serosal bathing medium (x 3.8 increase), but not in that of rat.

	PGE2 release			change in p.d., mV	
	control	LBK	LBK,Ca-free	LBK	LBK,Ca-free
rat	0.46 ± 0.10	2.46 ± 0.48	2.68 ± 0.86	+4.1 ± 1.5	0.0 ± 0.1
rabbit	0.42 ± 0.10	3.00 ± 0.66	1.03 ± 0.16	+8.9 ± 2.2	0.3 ± 0.5
	control	A23187		A23187	
rat	0.62 ± 0.12	0.76 ± 0.15		+1.6 ± 0.6	
rabbit	0.47 ± 0.10	1.80 ± 0.30		+7.8 ± 2.0	

The results show species variation in the Ca^{2+} dependence of colonic mucosal PGE_2 synthesis. The difference in the magnitude of the p.d. response to A23187 in the two tissues implies that in rabbit colon prostaglandins may be involved, but not in rat. Thus care must be taken in interpreting the effects of A23187 in different intestinal transporting systems so as to include the possible involvement of endogenous cyclo-oxygenase products.

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A POTENTIAL SOURCE OF ERROR IN THE QUANTITATION OF PLATELET-ACTIVATING FACTOR (PAF) IN BIOLOGICAL FLUIDS

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Both PAF and lyso-PAF have been found to be released from a variety of biological sources. The release of PAF can be quantitated using in vitro aggregation of rabbit platelets; however, lyso-PAF exhibits little biological activity and must be chemically acetylated to PAF before bioassay.

Bovine serum albumin, (BSA Fraction V) 0.2% is frequently used as a trapping agent for PAF to prevent binding to biological tissue and glassware. Extraction efficiency and acetylation of PAF and lyso-PAF from Tyrode had been assessed using ^{14}C-radiolabelled hexadecyl-PAF and lyso-PAF, dissolved in 0.2% BSA in Tyrode. Recovery of PAF after extraction was 80%, as assessed by both radiolabel and bioassay. However after acetylation of lyso-PAF, radiolabel estimates of recovery were still 80% whilst bioassay estimates were more than 100%. The potential cause of the discrepancy between radiolabel and bioassay estimates was thought to be the presence of acetylated BSA. Samples of RIA Grade Fraction V BSA, fatty-acid-free BSA, and ovalbumin were extracted from Tyrode and acetylated. The biological activity of these samples was compared.

1 ml samples of Tyrode alone or Tyrode plus 0.2% protein were extracted using a modification of the method of Clancy & Hugli, (1983), with isopropyl alcohol, (0.5 mls), 5N formic acid, (30 µl), ethyl acetate, (1.5 mls). The dry samples were acetylated using 100 µl pyridine and 100 µl acetic acid anhydride, incubated for 1 hour at room temperature. The samples were blown to dryness and dissolved in calcium-free Tyrode containing 0.2% BSA. The material was assayed for aggregatory activity on a washed suspension of rabbit platelets in vitro. Platelets were pre-incubated with indomethacin 1 µM, creatine phosphate, 31.25 µg/ml, and creatine phosphokinase, 15.25 µg/ml. When used the selective PAF receptor antagonist MSD L652,731 (2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrofuran) was incubated with the platelet suspension for 5 mins at 37°C.

The results show that only acetylated RIA Grade Fraction V BSA elicited a PAF-like aggregatory response from rabbit platelets which was inhibited by pre-incubation of platelets with L652,731, 10 µM, a selective PAF receptor antagonist, (n=8). RIA Grade Fraction V BSA may therefore contain a lyso-PAF-like material that would interfere with the quantitation by bioassay of lyso-PAF released from biological sources after stimulation. It seems that either fatty acid-free BSA or ovalbumin would be suitable trapping agents for lyso-PAF. Alternatively, any protein used to trap lyso-PAF in biological fluids should be precipitated before acetylation.

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IS PAF INDUCED BRONCHOCONSTRICTION INITIATED BY LIPOXYGENASE PRODUCTS?

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Platelet Activating Factor (PAF) administered intravenously to guinea-pigs produces bronchoconstriction and long lasting hypotension. It has been suggested (1) that the bronchoconstriction is largely due to the secondary actions of lipoxygenase products. The present study was performed to further investigate this hypothesis.

The bronchoconstriction to intravenously administered PAF, was examined in anaesthetised (pentobarbitone sodium 38 mg/kg i.p.) ventilated guinea pigs by the method of Konzett and Rossler(2).

PAF (in 0.2% BSA) was administered at a dose of 100 ng/kg i.v. causing marked bronchoconstriction and cardiovascular effects.

In this study the ability of various compounds to reduce a PAF induced bronchoconstriction was investigated. The MSD PAF antagonist L652,731 (2,5-bis(3,4,5-trimethoxyphenyl)tetra-hydrofuran) (5 mg/kg, 0.5 min predose) caused a 66% ($p<0.001$) inhibition of the PAF induced response, a dose that caused no inhibition of a LTD_4 induced bronchospasm. The leukotriene end-organ receptor antagonist FPL55712 (1 mg/kg, 0.5 min predose) caused a 53% ($p<0.05$) reduction in the PAF induced bronchoconstriction. However the lipoxygenase inhibitor AA861 (Takeda) (5 mg/kg, 2 min predose) did not have a significant effect. The cyclooxygenase inhibitor indomethacin (3 mg/kg i.v. 10 minutes prior to PAF administration) caused a 70% ($p<0.001$) reduction of the PAF induced bronchoconstriction and the selective thromboxane A_2 receptor antagonist EP092 (rac 5-endo(6'-carboxyhex-2'Z enyl)-6-oxo(1"-[N-(phenylthiocarbamoyl) hydrazone] ethyl)-bicyclo[2.2.1]heptane) (1 mg/kg, 5 min predose) caused a 70-80% reduction ($p<0.001$) of the PAF induced bronchoconstriction.

FPL55712(10 μM)was also shown to cause a 75% inhibition of the *in vitro* aggregation of rabbit platelets to a sub maximal concentration of the thromboxane receptor agonist U46619 (1μM)(9,11-dideoxy,9α-11α-methanoepoxy-$PGF_{2\alpha}$); a similar concentration did not affect the aggregation induced by PAF (3×10^{-10}M).

The inhibition of the bronchoconstriction by FPL55712 supports the previous suggestion for a lipoxygenase product involvement in PAF induced bronchospasm. However the lipoxygenase inhibitor AA861, at a dose shown to cause good inhibition of an antigen induced bronchospasm in sensitised guinea pigs caused no inhibition of the PAF induced bronchoconstriction. In addition the present results obtained with indomethacin strongly suggest a significant involvement of a cyclooxygenase product in an intravenous PAF induced bronchoconstriction in the Konzett Rossler model. Further studies with EP092 and the ability of FPL55712 to inhibit U46619 mediated platelet aggregation suggest that this cyclooxygenase product is most likely thromboxane A_2.

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ANAPHYLACTIC RELEASE OF PROSTAGLANDINS AND THROMBOXANE B_2 FROM GUINEA-PIG LUNG

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Anaphylaxis in mammalian lung results in activation of arachidonate metabolism with production of prostaglandins D_2, E_2, $F_{2\alpha}$, 6-oxo-$F_{1\alpha}$ and thromboxane B_2 (Ogunbiye & Eyre, 1985). It has frequently been suggested,however, that the prostanoids are secondary or indirect mediators of immediate sensitivity reactions particularly since they are also released from the lung by other mediators (Piper & Samhoun, 1982). The present investigation explores this hypothesis and examines their functional role in immediate bronchospasm in guinea-pig lung.

Male guinea-pigs (300-500 g) were sensitised with ovalbumin (50 mg i.p and 50 mg s.c. boosted 3 days later with 50 mg i.p.) and used 21-28 days later. The animals were killed by cervical dislocation the pulmonary artery cannulated and the lung placed in a heated jacket. The lungs were perfused at 10 ml/min with oxygenated (95% O_2; 5% CO_2) Krebs solution at 37°C and left to stabilise for 30 min prior to challenge with bolus injections of ovalbumin (1 µg-10 mg). In some experiments the pulmonary artery cannula was removed and the lung perfused through the trachea. The lung effluent was collected and analysed for histamine (fluorimetry), prostanoids (GC-NICI/MS) and leukotriene D_4 (post extraction RIA).

Following antigen challenge histamine release reached a peak within 1 min and was virtually complete by 10 min. The kinetics of prostaglandin release were almost indistinguishable from that of histamine except that maximum rates were seen 2 min post challenge and declined to resting within 15 min. TXB_2 and LTD_4 release was relatively delayed with peak release 2-4 min post challenge and was more sustained as was the appearance of the major prostanoid metabolites. At peak rates of release mediator levels were histamine (ng/min) 7400 ± 240; LTD_4 20.7 ± 7.6; PGD_2 259 ± 60; TXB_2 474 ± 48; 6-oxo-$PGF_{1\alpha}$ 220 ± 40; $PGF_{2\alpha}$ 48 ± 10; PGE_2 52 ± 14. Mediator release was related to antigen dose and there were significant linear correlations ($P < 0.05$) between histamine and PGD_2 ($r = 0.97$) histamine and LTD_4 ($r = 0.91$) LTD_4 and PGD_2 ($r = 0.91$) and LTD_4 and TXB_2 ($r = 0.89$). At low provocation doses (10 µg/ml) histamine and eicosaniod release were greater when the challenge was via the airway, at near maximal challenge (1 mg ovalbumin) there was no difference between mediator release by either route. Eicosaniod concentrations relative to histamine were greater at the lower of the two doses. Indomethacin (2.79 µM) inhibited TXB_2 production in response to a 10 µg ovalbumin challenge by >98% and abolished release of the other prostanoids measured; there was no attenuation of the increase in airways perfusion pressure following challenge and LTD_4 release was enhanced (0.34 ± 0.04 ng/ml compared to 1.79 ± 0.8 ng/ml).

We are unable to descriminate between the possibility that prostaglandin release is secondary to mast cell activation or is a primary event; the correlation between LTD_4 and TXB_2 concentrations, however, suggests that thromboxane release is secondary to leukotriene production. The data suggests that prostanoids may have a greater importance at lower degrees of mast cell activation although they do not appear to be involved in the immediate bronchospasm.

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DIFFERENCES IN ANTAGONIST BUT NOT AGONIST POTENCIES ON THE THROMBOXANE-SENSITIVE SYSTEMS OF HUMAN, RAT AND RABBIT PLATELETS

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There is considerable interest in the concept that different receptors may mediate the biological actions typical of TXA_2. Recently Narumiya et al (1986) reported that rabbit platelets (as PRP) were insensitive to the aggregatory action of the thromboxane analogue STA_2 when compared with human platelets. They also showed that the thromboxane receptor antagonist ONO 11120 (Katsura et al, 1983) was 100 times less effective against STA_2 in rabbit as opposed to human PRP.

Being very much aware that plasma protein binding may give a false picture of relative activities, we have investigated the sensitivity of washed platelet suspensions from man, rat and rabbit to both thromboxane agonists and antagonists. Platelet suspensions were prepared using PGI_2 as described previously (Armstrong et al, 1985). The results are shown in Table 1.

The ranking of agonist activity is similar in the three platelet systems. Of note is the high aggregatory activity of STA_2 on washed rabbit platelets; the EC_{50} value of 8 nM is about 1000 times lower than that reported for rabbit PRP.

Table 1 Activities of prostanoids on washed platelet suspensions

	human	rat	rabbit
Agonists	Equipotent molar ratios for aggregation ($n \geq 4$)		
EP 171	0.041	0.034	0.045
STA_2	0.31	0.17	0.13
9,11-azo PGH_2	0.47	0.57	0.24
9,11-endoxy-10a-homo PGH_2	0.93	1.00	0.47
11,9-epoxymethano PGH_2	1.00 (60 nM)*	1.00 (150 nM)	1.00 (60 nM)
PGH_2	1.86	1.57	1.33
EP 109	2.5	1.35	2.09
Antagonists	pA_{10} values for block of aggregation ($n = \geq 4$)		
EP 092	6.52 (6.48-6.55)	6.48 (6.43-6.55)	5.63 (5.62-5.64)
ONO 11120	6.53 (6.45-6.61)	6.42 (6.39-6.85)	4.99 (4.97-5.00)
BM 13177	5.25 (5.19-5.31)	5.14 (5.12-5.15)	4.91 (4.87-4.95)

* EC_{50} values. Limits for the pA_{10} values are given. See Wilson & Jones (1985) for structures of the EP analogues.

The three antagonists, EP 092 (Armstrong et al,1985), ONO 11120 and BM 13177 (Patscheke & Stegmeier, 1984), produced parallel shifts of the log concentration-response curves to 11,9-epoxymethano PGH_2 on the three platelet systems. The pA_{10} values are similar for the human and rat platelets. However, EP 092 and in particular ONO 11120 are less effective antagonists on rabbit platelets. We have seen similar trends using PGH_2 and STA_2 as agonists. However, with STA_2 we have evidence for an inhibitory action opposing its pro-aggregatory action at the higher doses required to overcome receptor blockade.

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A ROUTINE METHOD FOR STUDYING PULMONARY PRESSOR RESPONSES TO THROMBOXANE MIMETICS IN THE ANAESTHETIZED RAT

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The bronchoconstrictor and pulmonary vasoconstrictor actions of TXA_2 and its stable mimetics are well known. The former action can be measured in the anaesthetized guinea-pig by the Konzett-Rossler method. However we felt that there was a need for a simple method of assessing the pulmonary vascular response in a small laboratory animal, for example the rat.

Male PVG rats (210-280 g) were anaesthetized with i.p. pentobarbitone sodium (70 mg kg^{-1}). A femoral artery was cannulated for BP measurements and a femoral vein for injection of drugs. The trachea was cannulated and artificial ventilation applied (10 ml kg^{-1} x 72/min, 40% oxygen). The heart was exposed by a sternal split and the pericardium opened. A 'hooked' needle (23 gauge) attached to silastic tubing was inserted into the pulmonary artery for continuous pressure recording. In addition to this basic procedure cardiac output was measured in some rats with an electromagnetic flow probe (Statham Gould, 1.5 or 2 mm) placed around the ascending aorta.

11,9-Epoxymethano PGH_2 (U-46619) was used as the standard agonist. On i.v. injection it produced dose-dependent and transient rises in pulmonary artery pressure (PAP). In 12 rats the dose required for a rise of 10 mm Hg ranged between 0.37 and 0.95 µg kg^{-1}. During larger PAP rises both cardiac output and BP fell. Using the basic procedure, the rats remained in good condition for at least 5h. Dose-response curves to other prostanoids were constructed and potency ratios calculated (Table 1).

Table 1 Comparison of pulmonary pressor activities in the rat

Prostanoid	Equipotent molar ratio (± s.e.m.)	n
EP171	0.14 ± 0.010	4
9,11-azo PGH_2	0.32 ± 0.007	4
STA_2	0.92 ± 0.067	4
11,9-epoxymethano PGH_2	1.00	
PGH_2*	2.81 ± 0.26	4
PTA_2	170 ± 25	4

* Prepared from 9β,11β-dibromo-9,11-dideoxy $PGF_{2\alpha}$ (Porter et al 1979).

The most active compound EP 171 (Jones et al, 1985) differed from the other analogues in having a prolonged duration of action. At the highest dose level tested (400 ng kg^{-1}) both cardiac output and BP fell steadily and the animal's condition deteriorated irretrievably. The mechanisms underlying the toxic action of EP 171 are being investigated.

The PAP rises produced by all the analogues were blocked by the thromboxane receptor antagonist EP 092 (Armstrong et al, 1985) at 2 mg kg^{-1} i.v., whereas the peripheral vasoconstrictor action of Angiotensin II was unaffected.

We thank the Ono Company, Japan for the gift of STA_2.

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ICI 185282; A SELECTIVE, POTENT THROMBOXANE A_2 RECEPTOR ANTAGONIST ON SMOOTH MUSCLE

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We have previously demonstrated that ICI 159995 is a selective thromboxane A_2 (TxA_2) receptor antagonist which acts at vascular, pulmonary and platelet receptors (Jessup et al, 1985). We now describe the in vitro smooth muscle antagonist profile of ICI 185282 (5(Z)-7-([2,4,5-cis]-4-O-hydroxyphenyl-2-trifluoromethyl-1,3-dioxan-5-yl)heptenoic acid) a more potent analogue of ICI 159995.

Isolated smooth muscle strips were prepared and antagonist activity determined as previously described (Jessup et al, 1986).

ICI 185282 caused concentration-dependent parallel shifts to the right of U-46619 response curves of both rat and rabbit thoracic aortae. Analysis of these results (Arunlakshana and Schild, 1959) gave mean pA_2 values ($\pm$ s.e, n = 12) of 8.48 $\pm$ 0.05 and 7.90 $\pm$ 0.18 respectively, with Schild plot slopes (1.15 and 1.06) not significantly different from unity ($p>0.05$). The compound (0.1μM) also antagonised responses of rat and rabbit aortae to $PGF_{2\alpha}$, PGE_2 and PGD_2 giving concentration ratios ($\pm$s.e. n=4) of 22 $\pm$ 4.3 and 13 $\pm$ 1.5, 16 $\pm$ 2.5 and 32 $\pm$ 4 and 20 $\pm$ 1.9 and 9.5 $\pm$ 1.0 respectively. The TXA_2 receptor selectivity of ICI 185282 in rat and rabbit aortae was confirmed by the observations that at a final concentration of 20μM the compound yielded mean concentration ratios ($\pm$ s.e, n=4) of 2.2 $\pm$ 0.2 and 3.3 $\pm$ 0.75 for noradrenaline and 1.35 $\pm$ 0.1 and 1.58 $\pm$ 0.2 for 5-hydroxytryptamine. ICI 185282 (0.1μM) caused inhibition of U-46619 responses of canine, porcine and bovine coronary artery strips giving mean concentration ratios ($\pm$s.e, n=4) of 3.9 $\pm$ 0.3, 5.5 $\pm$ 0.9 and 7.9 $\pm$ 2.8 respectively. On rabbit mesenteric artery strips ICI 185282 (0.1μM) antagonised PGE_2 and PGD_2-induced contractions with concentration ratios ($\pm$s.e,n=4) of 8.5 $\pm$ 1.9 (PGE_2) and 12.5 $\pm$ 0.9 (PGD_2). In contrast ICI 185282 (10μM) did not modify PGE_2-induced relaxation of this tissue. The antagonist (0.1μM) inhibited contractile responses of guinea pig tracheal chain preparations to U-46619 and PGD_2 giving mean concentration ratios ($\pm$s.e, n=4) of 8.3 $\pm$ 1.9 and 14.1 $\pm$ 1.1. Similarly, antagonist activity was observed on guinea pig lung parenchyma preparations, where mean concentration ratios ($\pm$s.e, n=4) of 18.1 $\pm$ 2.3 (U-46619), 10.5 $\pm$ 1.9 ($PGF_{2\alpha}$) and 4.2 $\pm$ 0.6 (PGD_2) were achieved with ICI 185282 (0.1μM). However histamine-induced contractions of both guinea pig trachea and lung parenchyma were unaffected by ICI 185282 (0.1 - 10μM). The antagonist had no significant activity at other prostanoid receptors. Thus ICI 185282 (10μM) had no effect on responses of guinea pig ileum to PGE_2, $PGF_{2\alpha}$, PDG_2, histamine and acetycholine. Furthermore it did not modify responses of dog iris to $PGF_{2\alpha}$, neither did it inhibit contractions of rat colon to $PGF_{2\alpha}$, PGE_2 and acetylcholine.

These results indicate that ICI 185282 is a potent, selective, competitive TXA_2 receptor antagonist which acts at vascular and pulmonary receptors.

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ICI 185282 A POTENT THROMBOXANE RECEPTOR ANTAGONIST ON PLATELETS

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We have previously shown that ICI 185282 is a potent, selective, competitive thromboxane A_2 (TXA_2) receptor antagonist on smooth muscle (Byland et al, 1986). We now describe the antagonist activity of this compound on platelet preparations from a variety of species.

The methodology used for in vitro platelet aggregation has been previously described (Jessup et al, 1986). For ex vivo studies animals were dosed orally with either ICI 185282 (5–50mg/kg) or vechicle alone. Rats were anaesthetised (anaesthetic ether) and bled from the abdominal aorta, and rabbits bled sequentially from an ear artery. Human platelet microsomal enzyme activity was measured using the method of Howarth et al, 1982.

In vitro, U–46619 caused concentration–dependent aggregation of heparinised rat, and citratedguinea pig, rabbit and human platelets. Incubation of platelet preparations with ICI 185282 (0.1 – 100μM) caused concentration – dependent parallel shiftsto the rightof U–46619 aggregation curves. Analysis of this data(Arunlakshana & Schild, 1959) yielded mean $_pA_2$ values (±s.e.) of 6.70±0.07 (rat, n=8), 7.11±0.04 (guinea–pig, n=7), 5.8±0.07(rabbit, n =4) and 7.02±0.03 (human, n=4) with Schild plot slopes (1.22, 1.03, 0.96 and 1.09 respectively) not significantly different from unity (P>0.05). ICI 185282 (100μM) did not modify the primary phase (mean concentration ratios, ±s.e. 1.09±0.03 and 1.06±0.05, n=4) but did inhibit the secondary phase,of both ADP and adrenaline induced human plateletaggregation. ICI 185282 (0.1,1 and 10μM) caused concentration–dependent inhibition of collagen–induced human platelet aggregation, yielding mean concentration ratios(±s.e., n=4) of 4.8 ±1.2, 10.7±3.4 and 19.8±8.5 respectively. However the antagonist (10μM) did not effect PGI_2, PGD_2 or PGE_1 inhibition of ADP–induced human platelet aggregation neither did it significantly modify human platelet microsomal thromboxane synthetase, cyclooxygenase or 12–lipoxygenase. When dosed orally to conscious rats ICI185282 (5,10 and 20mg/kg) antagonised U–46619 induced platelet aggregation ex vivo giving mean(±s.e.,n≥4) peak concentration ratios of 7.5±2.1, 14.7±4.7 and >98.0 respectively. Significant (P<0.005, House 1986) activity persisted for 5 hours (10 and 20mg/kg,p.o.) When administered to conscious rabbits ICI 185282 (5,20 and 50mg/kg,p.o.) caused dose–related antagonism of U–46619 induced platelet aggregation ex vivo giving mean (±s.e.,n=4) peak concentration ratios of 5.8±1.5, 15.2±4.3 and 115.4±47.5 respectively. Significant (P<0.05, House 1986) inhibition of platelet aggregation was observedfor 8 hours (5mg/kg,p.o.), 12 hours (20mg/kg,p.o.) and 24 hours (50mg/kg,p.o.).

We conclude that ICI 185282 is a potent, selective, competitive TxA_2 blocking drug whose activity is expressed at platelet receptors. In addition, ICI 185282 is orally active in both rats and rabbits and has a prolongedduration ofactivity in both species.

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MODIFICATION OF UTERINE RESPONSES IN A NORMOTENSIVE AND A HYPOTENSIVE STRAIN OF RAT BY A THROMBOXANE ANTAGONIST

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There is a known involvement of prostanoids and other humoral mediators in the early oestrogen-induced uterine hyperaemic and uterotrophic responses in the rat (Phaily & Senior, 1978). In this study AH23848 ([1α(Z),2β,5α]-(±)-7-[5-[[1,1'-biphenyl]-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptanoic acid), a thromboxane (TXA_2) receptor antagonist, was used to assess any additional contribution of TXA_2 to these oestrogen-induced uterine effects. Age matched CD derived (blood pressure (BP) 120±9 mmHg) and spontaneously hypertensive rats (SHR, Okamoto strain, BP 205± 10 mmHg) were used.

Blood flow (BF) was measured in anaesthetised (pentobarbitone sodium 60 mg kg^{-1} i.p.) animals, bilaterally ovariectomised 21 days prior to experimentation, using the labelled microsphere technique (SC^{46}, 15 μm). BF was expressed, using tissue wet weight, as ml min^{-1} $100g^{-1}$. AH23848 (2.5 mg kg^{-1} i.v.) in a sodium bicarbonate (1% w/v)/sodium chloride (0.9% w/v) vehicle, was administered 10 minutes before oestradiol - 17β (E_2) (0.5 μg kg^{-1} i.v.) in propylene glycol (10% v/v)/sodium chloride (0.9% w/v).

3 hours after E_2 treatment, AH23848 had reduced the E_2-induced rise in uterine dry weight (mg) in both CD and SHR (CD: E_2 alone 27±1; E_2 + AH23848 19±1***; SHR: E_2 alone 28±1; E_2 + AH23848 19±1*** $P<0.001$). Similarly, AH23848 depressed the E_2-induced increase in uterine wet weight (mg), but only in the SHR (E_2 alone 111±9; E_2 + AH23848 92±3** $P<0.01$). The 3 hour E_2-induced uterine BF was also significantly lowered by AH23848 again only in the SHR (E_2 alone 227±50; E_2 + AH23848 160±11*** $P<0.001$). No change in BF or weight was noted in any other organ examined.

AH23848 has been shown to act as a TXA_2 receptor antagonist 'in vitro' and 'in vivo' (Brittain et al, 1985). Results obtained suggest that TXA_2 augments the E_2-induced early uterotrophic response, since AH23848 effectively reduced dry weight in both the CD and SHR strains. Oestrogen may mobilise TXA_2 which in turn contributes to the initiation of biosynthetic events including protein synthesis. Additionally it would appear that TXA_2 plays a role in the water imbibition response induced by E_2 since in the SHR AH23848 significantly reduced uterine wet weight 3 hours after E_2-treatment.

The lack of effect of AH23848 on the uterine hyperaemic response in the CD strain suggests that TXA_2 is not involved in the E_2 induced uterine blood flow. In the SHR pretreatment with AH23848 reduced the hyperaemic response, this effect could be due to the weak partial agonist property reported for this TXA_2 antagonist (Brittain et al, 1985). The results may reflect an increased sensitivity to TXA_2 in the SHR strain.

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EFFECT OF INDOMETHACIN ON HUMAN MYOMETRIAL RESPONSES TO PROSTAGLANDINS $F_{2\alpha}$ E_2 AND E ANALOGUES

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Prostaglandins (PG) $F_{2\alpha}$ and E_2 have been shown to stimulate human myometrium in the non-pregnant and pregnant subject in vivo. However, in vitro, whilst $PGF_{2\alpha}$ remains stimulant the response to PGE_2 is an initial stimulant effect followed by a dose-related inhibitory effect on myometrial strips. The myometrium is taken from the anterior wall of the corpus uteri in non-pregnant subjects at hysterectomy and the lower segment from pregnant subjects at Caesarean section. In the present study two analogues of PGE, enprostil and misoprostol, have been compared to the effects of PGE_2 and $PGF_{2\alpha}$ on myometrium taken from both non-pregnant and pregnant subjects. The tissue obtained from pregnant subjects was from patients at full term who were not in labour. Tissue from non-pregnant subjects was taken from pre-menopausal patients undergoing hysterectomy and from histological examination of the endometrium was classified as being either in the follicular or luteal phase of the menstrual cycle. The myometrial strips were set up as previously described (Massele and Senior, 1981) and were superfused with Krebs' solution at 2ml min^{-1}. In some experiments indomethacin (2.79×10^{-6}M) was added to the superfusate, this dose of indomethacin had no effect on the spontaneous activity of the tissue.

$PGF_{2\alpha}$ produced a dose-related increase in myometrial tension in myometrium taken from pregnant subjects and from non-pregnant subjects in the luteal phase of the cycle; tissue obtained during the follicular phase of the cycle responded to $PGF_{2\alpha}$ with an increase in tension but showed little dose related effect. The inclusion of indomethacin in the superfusate significantly potentiated ($P<0.05$-$P<0.001$) the response to $PGF_{2\alpha}$ on all tissues studied but the effect was most marked on tissues from the pregnant donors. The PGE_2 analogue, enprostil, was also spasmogenic and showed a marked dose-response relationship on tissues from pregnant donors which was not potentiated in the presence of indomethacin. The response to enprostil on tissue from non-pregnant donors was significantly potentiated by the presence of indomethacin ($P<0.05$-$P<0.005$).

PGE_2 produced a dose-related inhibition of spontaneous activity on tissue taken during either follicular or luteal phases of the menstrual cycle and on tissue from pregnant donors. This response was preceded by a dose-independent spasmogenic effect. The PGE_1 analogue, misoprostol, produced a qualitatively similar response on all tissues to that observed for PGE_2. The inhibitory responses to PGE_2 and misoprostol were unaffected by the presence of indomethacin in the superfusate.

The PGE_2 analogue, enprostil, appears to be acting through a different receptor population on the human myometrium in vitro to the PGE_1 analogue, misoprostol. From the experiments conducted so far the similarity in the responses between PGE_2 and misoprostol suggest the possibility of these PGs acting through one receptor. $PGF_{2\alpha}$ and enprostil may be acting through a further receptor and this suggestion is strengthened by the finding that both $PGF_{2\alpha}$ and enprostil responses may be potentiated by the presence of indomethacin in the superfusate. This potentiation may indicate that $PGF_{2\alpha}$ and enprostil are releasing a spasmolytic substance from the PG synthesis pathway or that a further mechanism is involved in the indomethacin effect. This is being currently investigated by the use of other PG synthesis inhibitors.

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THE EFFECT OF ISOENZYME SELECTIVE PHOSPHODIESTERASE INHIBITOR ON ACID SECRETION IN THE RAT ISOLATED STOMACH AND FUNDUS STRIP

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Non selective phosphodiesterase (PDE) inhibitors e.g. isobutylmethylxanthine (IBMX) stimulate acid secretion in a variety of preparations including the rat isolated stomach (McCarthy et al., 1981). In this study PDE isoenzyme selective inhibitors were used to further investigate this effect in the rat isolated stomach (Bunce & Parsons, 1976) and on gastric smooth muscle using the rat fundus strip (Vane 1957). Inhibitor potencies towards PDE's from cat cardiac ventricle (England et al., 1986) are shown in Table 1.

Table 1 IC_{50}'s (x 10^{-6}M) for PDE's

	Ca^{2+}/CALM stim. PDE	Low K_m PDE	cAS PDE
IBMX	11.5	4.2	13.0
M+B 22948	10	~400	~300
Rolipram	N.I.	96	1.0
Milrinone	~100	2.2	~30
SKF 94120	N.I.	11	~500
RO-20-1724	N.I.	~100	5.0

N.I. = No inhibition at 10^{-4}M
Ca^{2+}/CALM stim = Ca^{2+}/calmodulin stimulated
cAS = cAMP selective
Low K_m PDE = Low K_m cAMP selective PDE

Table 2 shows results obtained in the rat isolated stomach and rat fundus strip (EC_{50} for stimulation of acid output; IC_{50} for inhibition of contraction to KCl (50mM) and carbachol (10^{-6}M)).

Table 2 Activity of PDE inhibitors in vitro in the rat

	Rat stomach(n=5-7)	Rat fundus (n = 4-6)	
	EC_{50} (M)	IC_{50} vs KCl(M)	IC_{50}vs Carb (M)
IBMX	1.9×10^{-5}	1.2×10^{-5}	2.6×10^{-5}
M+B 22948	Inactive to 10^{-4}	5.0×10^{-4}	3.3×10^{-4}
Rolipram	8.4×10^{-8}	3.1×10^{-4}	-
Milrinone	9.5×10^{-5}	-	7.7×10^{-4}
SK&F 94120	Inactive to 10^{-3}	~10% IBMX Max at 10^{-3}	~20% IBMX Max at 10^{-3}
RO-20-1724	1.5×10^{-7}	1.6×10^{-4}	-

Rolipram and RO-20-1724 (cAS PDE inhibitors) were potent stimulants of acid secretion, whereas inhibitors of Ca^{2+}/CALM PDE and low K_m PDE were inactive. In the rat fundus strip cumulative dose response curves were constructed after a plateau to carbachol (10^{-6}M) or KCl (50mM) was established. Inhibitors of the three isoenzymes were less active than IBMX, and at concentrations required to elicit a response would be acting non-selectively. Therefore no conclusion could be made regarding the isoenzyme involved in the relaxant response to IBMX in the rat fundus strip.

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THE EFFECT OF U46619 ON RAT GASTRIC BLOOD FLOW USING LASER DOPPLER VELOCIMETRY

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Gastric mucosal ischaemia has been implicated in the pathogenesis of experimental ulcer and more recently thromboxane (TXA_2) has been implicated as a mediator of mucosal damage (Whittle et al., 1985). In this study the gastric vasoconstrictor effects of the TXA_2 mimetic U46619 in the rat (Whittle et al., 1985) have been further investigated. A range of antagonists was used to investigate the selectivity of the response and whether U46619 is acting directly or indirectly on the gastric vasculature (e.g. by the reflex consequences of vasoconstriction elsewhere).

Male rats (300g) were starved for 18 hours but were allowed free access to water. Rats were anaesthetised with pentobarbitone (60mg/kg i.p. + 6mg/kg/h i.v.) and the trachea cannulated. Both jugular veins were cannulated for drug administration and the carotid artery for measurement of blood pressure and heart rate. The abdomen was opened and the stomach exteriorised and placed in a chamber. Body temperature was maintained at 37°C. Blood flow was continuously measured by Laser Doppler Velocimetry which provides a linear measurement of gastric blood flow to the mucosa (Kiel et al., 1985). The laser probe was positioned and held above the mucosa using a micromanipulator and was protected from light. Two sequential dose response curves (SDR) to U46619 i.v. were constructed in each animal, with 5 minutes preincubation with antagonist or 0.9% saline i.v. prior to the second curve. Equivalent volumes of 0.9% saline were also given prior to each SDR to assess effects of volume on the measured parameters. Results were expressed as % change compared to predose values. Values are mean ± S.E.M.

U46619 caused a dose-dependent reduction in blood flow. At doses 0.04, 0.11, and 0.33 µg i.v. these changes were not accompanied by changes in blood pressure or heart rate. Blood flow was reduced by 10±3, 17±5 and 35±4%(1st SDR) and 16±6, 30±7 and 41±6%(2nd SDR) (n=6) respectively. (At higher doses U46619 produced a reduction in blood pressure. Responses were short-lived with a maximum recovery time of 3 minutes.) The ganglion blocker pempidine (2mg/kg i.v.), prazosin (0.1mg/kg i.v.) and propranolol (1mg/kg i.v.) did not inhibit U46619 induced reductions in blood flow either alone or in combination. In the absence and presence of the mixture of antagonist blockers % reductions in blood flow were 14±3, 34±7, 59±5 (1st SDR) and 28±4, 51±7 and 75±6 (2nd SDR) (n=6) respectively. The TXA_2 antagonist SK&F 88046 (20mg/kg i.v.) inhibited the effect of U46619 on blood flow (12±3, 29±4, 46±7 (1st SDR) and 10±5, 16±5, 13±6 % (2nd SDR) (n=6). These data are consistent with a direct effect on the gastric vasculature.

These data indicate that at low doses (0.04 - 0.33 mg i.v.) U46619 may act directly on the gastric vasculature by stimulating TXA_2 receptors.

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DIFFERENCES IN VASOLIDATOR ACTIVITY OF SOME ISOENZYME SELECTIVE PHOSPHODIESTERASE INHIBITORS

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In vascular smooth muscle, changes in the intracellular concentrations of cyclic nucleotides mediate the effects of many drugs and hormones (Vegesna & Diamond, 1986, Itoh et al., 1985). Using selective adenylate and guanylate cyclase stimulants, forskolin (F) and sodium nitroprusside (SN) respectively and selective phosphodiesterase (PDE) isoenzyme inhibitors, we have examined the role of these nucleotides in producing vasodilatation. Changes in perfusion pressure of isolated rabbit ear arteries preconstricted by perfusion at constant flow with 80 mM KCl Kreb's solution were used to assess changes in vasomotor tone (for F/SN studies n=8 and for PDE inhibitor studies n=8).

Bolus injections of either F or SN produced dose dependent vasodilatations with similar threshold doses, $3x10^{-10}$ moles and dilatations at the maximum concentration of $3x10^{-8}$ moles, limited by solubility, of 106.3±11.6 and 129.4±23.4 mmHg respectively. At all doses, F-induced dilatations were of longer duration than those induced with SN. At $3x10^{-8}$moles, times to 50% recovery were 14.4±2.8 and 6.4±2.5 minutes respectively. For F and SN the respective doses required to produce a fall in perfusion pressure of 50 mmHg were $2.4±0.1x10^{-9}$ and $2.3±0.2x10^{-9}$ moles. Four PDE inhibitors were tested for their direct vasodilator activity by bolus injection. SK&F 94120, a low Km PDE inhibitor and Rolipram selective for the cyclic AMP selective PDE, produced very small dilatations of 8.7±2.4 and 7.5±2.5 mmHg at the highest dose used $1x10^{-7}$ moles, limited by solubility. M&B 22948 selective for the Ca^{2+}/calmodulin PDE produced a larger dilatation, 37.5±5.6 mmHg at the same maximal dose with time course to 50% recovery, 9.7±4.7 minutes. This was more sustained than the much larger dilatation produced by isobutylmethylxanthine, a non-selective PDE inhibitor which at the same maximum dose produced a dilatation 103.1±4.4 mmHg with a 50% recovery time, 2.0±1.1 minutes and threshold dose of $3x10^{-9}$ moles.

These studies with the cyclic nucleotide stimulants confirm that in the perfused rabbit ear artery both cyclic AMP and cyclic GMP are associated with vasodilatation. Studies with PDE inhibitors indicated that non-selective inhibition of the isoenzymes and inhibition of the Ca^{2+}/calmodulin isoenzyme result in dilatation whereas inhibition of the other isoenzymes did not. Experiments perfusing the arteries with different concentrations of the inhibitors are being undertaken to ensure that the lack of response to inhibition of some PDE isoenzymes is not due to equilibration/access problems of these compounds.

These results compliment those from the myocardium, platelets and acid secretion (Leigh et al., 1986) by demonstrating differential functional consequences of selective PDE inhibitors.

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LEUKOTRIENE B_4 AND ACUTE INFLAMMATION IN THE RABBIT

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It has been speculated that 5-lipoxygenase inhibitors may be novel anti-inflammatory drugs by reducing the levels of leukotriene B_4 (LTB_4), a potent chemotactic agent for leukocytes. Previous work from this laboratory has cast doubt on the rat as a suitable species in which to demonstrate the anti-inflammatory efficacy of such agents (Foster et al, 1986). We have, therefore, investigated the rabbit as an alternative species in which to try and establish an LTB_4 'driven' model of inflammation.

Sterile polyester sponges soaked in 0.5% λ carrageenin were implanted subcutaneously, under anaesthesia, into the backs of New Zealand White rabbits. At various times after implantation the rabbits were killed with a lethal i.v. dose of sodium pentobarbitone and the sponges were removed. The inflammatory exudate was recovered from the sponges and the leukocyte and LTB_4 content were determined by cell counting and radioimmunoassay respectively (Carey and Forder, 1986). The immunoreactive LTB_4 was shown to be authentic by HPLC fractionation of a methylformate extract of the inflammatory exudate.

Following a 2h lag period, leukocytes (>95% PMNL) rapidly infiltrated the sponges attaining a maximal density ($20.0 \pm 4.0 \times 10^6$ cells/ml) by approximately 10h and remained elevated for at least 48h. The infiltration of leukocytes was preceded by a rapid accumulation of LTB_4 into the sponge exudate with peak levels (6.05 ± 2.25ng/ml; N = 4 experiments) at 4h, returning almost to background by 8h. We were interested to see if the levels of LTB_4 measured in the inflammatory exudate induced leukocyte infiltration into sponges. No statistical significant difference was detected in the leukocyte content of sponges that had been soaked either in saline or LTB_4 (range 1-1000ng/ml) following analysis 4h after their subcutaneous implantation. Quantitative recovery of LTB_4, was achieved from the LTB_4-soaked sponges. Histological examination of sections taken through LTB_4 and saline-soaked sponges and the surrounding tissues also revealed no difference in their leukocyte content. In contrast, 1ng of LTB_4 injected intradermally caused a time dependent infiltration of leukocytes into the skin.

The failure of LTB_4 to cause leukocyte infiltration into subcutaneously implanted sponges at concentrations measured in inflammatory exudates is puzzling since such concentrations were chemotactic when injected intradermally. One possible interpretation of these observations is that LTB_4 injected into the dermis but not LTB_4 in the subcutaneously implanted sponges gains access to the microcirculation thus increasing adherence of PMNL to the local microvascular endothelium (a demonstrated property of LTB_4 in the rabbit) which is a prerequisite for chemotaxis. Our data also suggests that if LTB_4 contributes to leukocyte recruitment in this model then it must be generated by cells external to the sponge. The failure of LTB_4 within the sponge to induce chemotaxis however does not rule out sponge implantation in the rabbit as a potential model for evaluating the anti-inflammatory efficacy of 5-lipoxygenase inhibitors.

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COMPARISON OF ZOLPIDEM WITH DIAZEPINE HYPNOTICS ON RESPIRATORY FUNCTION IN THE RAT

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Although generally considered to have a large therapeutic ratio, diazepines (benzodiazepines and others) have a respiratory depressant effect which is especially undesirable when used as hypnotics in patients with chronic airway obstruction and in premedication or anaesthesia induction (Danneberg, 1986). Zolpidem(Z) is a new hypnotic unrelated structurally to diazepines (Depoortere et al,1986;Nicholson and Pascoe,1986). We investigated the effects of Z in comparison to various diazepines on respiratory parameters in adult (400-450g) male Sprague-Dawley rats (CD COBS, Charles River France).
The rats were anaesthetized (urethane, 1.2g/kg i.p.) and placed in a hermetic body plethysmograph. Normal respiration with the external air occurred via a endotrachial cannula. Respiratory parameters were measured with a Hewlett Packard 7754B. Respiratory frequency was measured as the number of respiratory cycles per min (controls 110-120). Respiratory volume was calculated by electronic integration of the flow rate(ml/respiratory cycle; controls 2.0-2.2).Pulmonary resistance is the ratio between the variations in transpulmonary pressure (Hewlett Packard 270 transducer) at a given flow rate(controls 0.065-0.083). Compliance is the ratio of the variation in respiratory volume to the variation in transpulmonary pressure(ml/cm H_2O controls 0.90-1.10). Measurements were taken at 1-5-15-30-60 and 90 min post injection.

Maximum effect (% Relative change as compared to solvent injection)

Respiratory Parameter	Compound;Dose (mg/kg iv)					
	Z	Flu	Mid		Triaz	
	10	1.0	10	30	0.1	1
Frequency	+7	-27**	-16**	-42**	-15	-20*
Volume	-13*	-7	-8*	-16*;-14*	+15*	+7
Pulm. Resist.	-13	+35**	+20	+31*	+10	+13
Pulm. Compl.	+16;-17	-28*	-30*	-12	-11	-7

*p<0.05; **p<0.01 relative to solvent; 6-7 rats per compound studied. Z=Zolpidem; Flu=Flunitrazepam; Mid=Midazolam; Triaz= Triazolam. Pulm. Resist.= Pulmonary Resistance; Pulm. Compl.=Pulmonary Compliance.

Z had very little effect on respiratory parameters, a small decrease in volume being the only significant action.Only Z did not significantly decrease the respiratory frequency. For Flu, Mid, and the high dose of Triaz this effect was immediate and lasted the duration of the experiment. Respiratory volume was slightly, but significantly decreased by Z and low dose Mid. High dose Mid exhibited an initial increase with a later decrease.Pulmonary resistance was increased significantly by Flu and Mid whereas compliance was significantly decreased by Flu and low dose Mid.
Thus at pharmacologically equivalent doses, Z has fewer effects on respiratory parameters in the rat than do diazepine hypnotics such as Mid, Flu, or Triaz. Whether this difference is related to the specificity of Z for the BZD_1, receptor (Arbilla et al,1985) is unknown.

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SELECTIVE MESOLIMBIC NEUROCHEMICAL RESPONSIVENESS TO NEUROTENSIN MICROINJECTION INTO RAT MESENCEPHALIC DOPAMINE CELL BODIES

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Neurotensin (NT), on central administration, displays many pharmacological properties similar to those reported for typical neuroleptics (Nemeroff, 1980), including induction of hypothermia, muscle relaxation, diminution of locomotor activity, blockade of amphetamine-induced locomotion and rearing and the potentiation of barbiturate sedation. Moreover, NT alters the dopaminergic activity of both nigrostriatal and mesolimbic pathways as its injection into A9 and A10 cell body regions reportedly increases dopamine turnover in the corresponding terminal areas upon post mortem determinations of amine and metabolites (Nemeroff and Cain, 1985). Presented here are results from studies performed in vivo which demonstrate that low doses of NT injected into A9 and A10 perikarya increase dopamine (DA) neurotransmission at mesolimbic but not nigrostriatal terminal areas.

Male Sprague-Dawley rats (270-360 g) were anaesthetised using halothane (2-3% in 1:1 O_2/N_2O) and stereotaxically implanted with two electrically-pretreated (peak 2) carbon fibre microelectrodes (Sharp et al., 1984), one into the left nucleus accumbens and the other into the right striatum; reference and auxiliary electrodes were positioned beneath the dura. Extracellular DOPAC levels were monitored via differential pulse voltammograms which were recorded from each electrode every 5min. After a minimum stabilisation period of 1h, stereotaxically guided microinjections of NT (0.1µg) or saline were introduced into the left ventral tegmental area (VTA) and right substantia nigra zona compacta (SNc), via a 31 gauge steel cannula. Approximately 1h later further injections of NT (1 µg), or saline again, were performed, followed in all cases approximately 1.5h later by 2.5 ug injections of haloperidol. All injection volumes were 0.5 µl . Finally lesions were produced at each electrode tip and brains were removed for histological verification of injection and recording sites.

Injections of saline or NT (0.1 and 1 µg) into the ipsilateral SNc produced no changes in voltammetric DOPAC signals in the striatum; while injection of haloperidol following saline or NT produced an immediate and significant ($p<0.05$) increase in DOPAC levels of 100% above pre-injection levels in each group. In the accumbens, DOPAC levels were increased by 20% ($p<0.05$ cf. saline) and 60% ($p<0.05$) following injection of NT at 0.1 and 1.0 µg respectively. In control and NT injected groups, subsequent injection of haloperidol again produced an increase in DOPAC levels equivalent to 100% of pre-injection level.

It is concluded that, despite the demonstration of NT receptors and immunoreactivity in both SNc and VTA (Quirion et al., 1982; Cooper et al., 1981), a functional neurochemical responsiveness to exogenous NT exists only within the mesolimbic system in vivo.

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APOMORPHINE-INDUCED CLIMBING BEHAVIOUR IN MICE: A D_1 AND D_2 DOPAMINE RECEPTOR INTERACTION

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The inhibition of apomorphine induced climbing behaviour in mice has been widely used as a test to detect potential antipsychotic agents. The observation that atypical agents such as the selective D2 antagonist sulpiride are active in the test (Costall et al, 1978) suggests that D2 dopamine receptor mechanisms are primarily implicated. However, recently it has been found that the selective D1 dopamine receptor antagonist SCH23390 ((R)-(+)-8-chloro 2,3,4,5,-tetrahydro-3-methyl-5-phenyl-1H-3 benzazepin -7-ol)will antagonise the climbing response (Iorio et al, 1983). It has also been shown that climbing behaviour is not observed following the administration of all dopamine agonists (Gianutsos and Palmeri, 1983). In view of these facts we have investigated the possibility that the behaviour is induced by an interaction at both types of dopamine receptor.

Groups of eight female CF1 mice (20-25g) were used in the study. Each animal was placed in a cylindrical wire mesh cage for one hour prior to drug treatment. They then received either SKF38393 (2,3,4,5-tetrahydro -7,8-dihydroxy-1-phenyl 1H 3-benzazepine)(D1 agonist), LY171555 (trans-(-)-4aR-4,4a,5,6,7,8,8a,9 octahydro-6-propyl-2H-pyrazolo (3,4-g)quinoline diHCl)(D2 agonist), apomorphine (D1/D2 agonist), combinations of LY171555 and SKF38393, or vehicle. Climbing behaviour was assessed at 5 min intervals for 2 h starting 10 min after drug administration by counting the number of paws each animal had on the mesh of the cage.

Apomorphine produced a marked increase in climbing behaviour (climbing index = 3.7±0.2, control climbing index = 0±0). While LY171555 (1.25 and 2.5 mg/kg s.c.) and SKF38393 (20 mg/kg s.c.), given alone, failed to produce any behavioural changes. However, when LY171555 and SKF38393 were given in combination intense stereotyped climbing was observed which was indistinguisable from that induced by apomorphine (climbing index = 3.7±0.2 and 4.0±0.0 for SKF38393 20 mg/kg s.c. + LY171555 1.25 and 2.5 mg/kg s.c. respectively). This observation supports the proposal of Gianutsos and Palmeri (1983) that D1 receptor stimulation is necessary for climbing behaviour, but D2 receptor stimulation must also be present. These results also add support to the hypothesis that both D1 and D2 receptors have a functional role in the mediation of motor behaviours.

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ANTAGONISM OF THE PHARMACOLOGICAL EFFECTS OF ZOLPIDEM BY A NEW AMINO-ß-CARBOLINE

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Recently, 3-(methoxycarbonyl) amino-ß-carboline (ß-CMC) has been reported to act as a selective antagonist of the sedative effects of diazepam (Dodd et al, 1985). In order to investigate whether ß-CMC could selectively antagonise the sedative actions of other benzodiazepine (BZ) receptor agonists, we studied the potential antagonism by ß-CMC of different pharmacological effects of zolpidem, an imidazopyridine hypnotic (Nicholson and Pascoe, 1985) which binds preferentially to the type I BZ receptor (Arbilla et al, 1985; Depoortere et al, 1986).

In mice, anticonvulsant effects were evaluated by observing the presence of tonic convulsions induced by pentetrazole (125 mg/kg, s.c.) or supramaximal electroshock (60 mA, 50 Hz, 0.4 s) as well as by measuring the delay of the first convulsion after isoniazid (800 mg/kg, s.c.). For assessing the central depressant effects we measured the decrease in spontaneous locomotor activity.

In rats, the anticonflict effect of zolpidem was studied on punished drinking, and we also investigated the stimulus properties of this compound in rats trained to discriminate zolpidem (2 mg/kg, i.p.) from saline as previously described (Sanger and Zivkovic, 1986).

ß-CMC neither exerted an anticonvulsant effect nor antagonised the anticonvulsant action of zolpidem against pentetrazole or electroshock-induced maximal convulsions (up to 60 mg/kg, s.c.).

Doses of ß-CMC up to 120 mg/kg, s.c. did not block isoniazid-induced convulsions. However, ß-CMC (40 mg/kg, s.c.) antagonised the zolpidem-induced increase in the delay of appearance of the first convulsion.

Locomotor activity was not changed by doses of ß-CMC up to 120 mg/kg, s.c., but administered at 40 mg/kg, s.c. it strongly antagonised the depressant action of zolpidem.

ß-CMC (30-60 mg/kg, s.c.) also fully antagonised the anticonflict effect of zolpidem in rats, and produced a dose-related (1-100 mg/kg, i.p.) antagonism of the discriminative stimulus induced by zolpidem.

These results demonstrate that ß-CMC antagonises some but not all of the pharmacological effects of zolpidem. In general, these findings are consistent with the suggestion that ß-CMC preferentially antagonises effects of BZ receptor ligands related to sedation. The antagonism of the antipunishment effect of zolpidem by ß-CMC may indicate that this effect of zolpidem is linked to its sedative properties.

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MODULATION OF INTRASYNAPTOSOMAL FREE CALCIUM CONCENTRATION BY α_2-ADRENOCEPTORS AND SEROTONIN RECEPTORS

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The evoked release of noradrenaline from synaptosomal preparations of rat brain is dependent on the availability of intrasynaptosomal free calcium (De Langen & Mulder, 1980). However the mechanisms associated with the inhibition of noradrenaline release by the activation of presynaptic α_2-adrenoceptors and serotonin receptors are not understood.

In this study we have monitored intrasynaptosomal free calcium levels in cortical synaptosomes during incubation with selective α_2-adrenergic and serotonergic agonists/antagonists, by a modification of the method of Ashley et al, 1984, using the fluorescent probe, quin-2.

In these experiments, crude synaptosomes (P2, Gray & Whittaker, 1962) prepared from cortices of male Wistar rats (200g), were further purified by centrifugation on a percoll discontinuous density gradient (Dunkley et al, 1986). These synaptosomes were then washed and resuspended, at a tissue concentration of 0.7g. eq/ml in Krebs buffer containing 1.0mM $CaCl_2$ to which 50µM quin 2 acetoxymethylester was added and incubated at 37°C for 40 mins. At this point intrasynaptosomal calcium was measured in the presence of modulating compounds.

The control calcium level (no drug addition) was 107.8 ± 5.3nM (mean ± SEM, n = 28). Addition of clonidine (100µM) reduced this level by 37.1% (n = 5, $p < 0.001$, paired t test). Inclusion of idazoxan (200µM) antagonised the effect and produced a 25.7% increase in intrasynaptosomal calcium, compared with controls (n = 4, $p < 0.05$, paired t test). Serotonin (100µM) and serotonin (100µM) and cyproheptadine (100µM) failed to produce significant changes in intra synaptosomal free calcium (n = 5, $p > 0.05$, paired t test). In experiments where antagonists were used, these were added first.

These preliminary results show that clonidine apparently reduces the free intrasynaptosomal calcium concentration; this may cause an inhibition of neurotransmitter release mechanisms. This effect is reversed by the antagonist leading to an "overshoot" phenomenon, suggesting that high concentrations of antagonist may also displace endogenously bound noradrenaline.

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IS THE DOPAMINE UPTAKE SYSTEM REQUIRED FOR MPTP TO INDUCE NEUROTOXICITY IN THE PRIMATE BRAIN?

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1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) given systemically or intracerebrally to primates induces a Parkinson-like syndrome (Barnes et al, 1985). The selective neurotoxicity of MPTP to the dopamine (DA) neurones is thought to depend, firstly, on conversion of MPTP to MPP^+ by monoamine oxidase B, and subsequently, in the rat at least, accumulation of MPP^+ in the DA neurones due to the high affinity of MPP^+ for the DA uptake process, (Javitch et el, 1985). In the present study we investigate the actions of the DA uptake inhibitor benztropine on the neurotoxic effects of MPTP in the primate.

Common marmosets (male, 350-400g) were given, i.p., (a) MPTP, 4mg/kg on day 1; 2mg/kg on days 2, 3 and 4, (b) MPTP + benztropine (10mg/kg/day) 2 days prior, during and for 2 days after the MPTP treatment (n = 5 for each group). For the intracerebral infusion of MPTP marmosets were subject to standard stereotaxic surgery for implantation of stainless steel guide cannulae (Barnes et al, 1985) allowing for a unilateral infusion of MPTP, via osmotic minipumps, into either the substantia nigra, SN, (10µg/24h for 13 days) or the central area of the caudate nucleus, CN (40µg/24h for 13 days).

In those animals receiving i.p. MPTP regimes, locomotor activity was assessed using infrared photocell cages prior to, during and after the MPTP administration. In those animals receiving a unilateral MPTP intracerebral infusion asymmetry was assessed using a 0-7 point scoring system (severity of changes in head posture, front and hind limb usage, trunk twisting and circling movements). Animals were visually observed via remote video recordings.

Following peripheral treatments with MPTP alone or MPTP + benztropine marmosets showed significant ($P<0.001$) reductions in locomotor activity, from 80-100 counts/60 mins to 0-5 counts/60 mins during MPTP treatment and for 77 days post-MPTP treatment. Animals receiving MPTP infusion into the SN developed marked contralateral asymmetry (score 4) within 12h of commencing infusion. The asymmetry increased to score 7 in all animals by day 6 of infusion and was maintained at this score for the remainder of the 13 day infusion. 21 days after discontinuing infusion animals became ipsilaterally asymmetric (score 5-6) and this persisted for at least 12 months. Animals co-treated with benztropine (10mg/kg/day i.p.) also exhibited marked contraversive asymmetry (score 7) during MPTP infusion into the SN, this persisting for 21 days after terminating the MPTP infusion with subsequent development of an ipsilateral asymmetry (score 4-6), again apparent for at least 12 months. Infusion of MPTP (40µg/24h for 13 days) into the CN caused an ipsilateral asymmetry after 6 days of infusion (score 2); this was maintained (up to score 7) for the duration and for 56 days after the infusion. Co-treatment with benztropine (10mg/kg/day i.p.) failed to modify the development or intensity of this ipsilateral asymmetry.

The long-lasting motor changes caused by MPTP administered peripherally or centrally, associated with a loss in striatal DA (Barnes et al, unpublished data), reflects a neurotoxic action on the nigrostriatal DA system. The failure of benztropine to inhibit such changes questions the importance of the DA uptake system for the neurotoxic action of MPTP in primates.

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LATERALITY OF BRAIN NEUROCHEMISTRY IN THE RAT: RELATIONSHIP BETWEEN DOPAMINE AND 5-HYDROXYTRYPTAMINE

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It has been shown that in trained circling rats, hemispheric differences in dopamine (DA) and 5-hydroxytryptamine (5HT) metabolism in limbic and nigrostriatal areas of the brain can be measured during circling behaviour (Yamamoto & Freed, 1984). In untrained animals, an asymmetry of brain biochemistry is not so well recognised and therefore in these studies we have measured levels of DA and 5HT in brain areas taken from rats which were selected according to postural-motor asymmetry in a turn preference test.

Male Sprague-Dawley rats were categorised in terms of left or right hemispheric dominance by measuring spontaneous turn preference in an open field (see Bradbury et al, 1985 for experimental details). After assessing functional asymmetry on 5 consecutive days the animals were killed by cervical dislocation and structures from the left and right hemispheres dissected for separate analysis of DA and 5HT using HPLC with electrochemical detection. Areas taken included the striatum (CP), amygdala [separated into individual nuclei; central (ACE), medial (AME), basolateral (ABL) by punch dissection], nucleus accumbens (ACB), and frontal cortex (FC).

Table 1: Hemispheric differences in levels of DA and 5HT in the right (R) and left (L) forebrain areas taken from rats showing right (RD) or left (LD) hemispheric dominance.

		ACE		ABL		AME	
		RD	LD	RD	LD	RD	LD
DA	R	0.9±0.1	0.4±0.04	1.8±0.2	2.0±0.2	ND	ND
	L	0.3±0.03***	0.8±0.1*	1.4±0.1*	1.5±0.2*	ND	ND
5HT	R	0.9±0.07	0.9±0.07	1.5±0.1	2.0±0.08	0.5±0.05	1.6±0.2
	L	0.9±0.06	0.6±0.03***	2.1±0.1**	2.6±0.2*	1.0±0.05***	1.1±0.08**
		CP		ACB		FC	
		RD	LD	RD	LD	RD	LD
DA	R	9.5±0.5	9.1±0.5	0.6±0.08	1.8±0.2	54±4	38±3
	L	7.0±0.3***	10.7±0.2**	0.6±0.07	1.6±0.1	51±5	43±5
5HT	R	0.3±0.02	0.3±0.01	0.09±0.01	0.3±0.02	452±25	320±9
	L	0.5±0.02***	0.2±0.02***	0.1±0.01	0.4±0.02**	481±22	333±10

Values expressed as ng/mg wet weight of tissue except for FC, pg/mg. n = 7-11. A significant difference between L and R hemispheres is indicated as *P<0.05, **P<0.01, ***P<0.001 (Mann-Whitney U test). ND, not detectable.

The major biochemical findings shown in Table 1 were significantly increased levels of DA in the right hemisphere in the CP, ACE and ABL in RD animals and in the left hemisphere in the CP and ACE of LD animals. Differences in the levels of 5HT between the hemispheres were also shown and, where the 5HT levels in a structure were lower, the dopamine levels were higher, e.g. the ACE (LD), ABL (LD and RD) and CP (RD and LD).

It is concluded that a functional asymmetry measured as a turning preference may be correlated with raised dopamine and reduced 5HT levels in the CP, ACE and ABL in the dominant hemisphere.

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EFFECTS OF GR38032F ON HYPERACTIVITY INDUCED BY UNILATERAL INFUSIONS OF DOPAMINE INTO THE CENTRAL AMYGDALA OF THE RAT

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GR38032F is a selective $5HT_3$ antagonist (Brittain et al, this meeting) and has been shown to antagonise a raised dopamine function in the limbic nucleus accumbens of rat brain (Costall et al, this meeting). Locomotor hyperactivity can also be induced by the unilateral infusion of dopamine into the amygdala of rat brain (Bradbury et al, 1985) and in the present study we assess whether the amygdala is a further site of action of GR38032F to modify limbic dopamine function.

The studies used male Sprague-Dawley rats (Bradford strain) subject to standard stereotaxic surgery for the implantation of chronically indwelling guide cannulae for subsequent infusion/injection into the central area of the amygdala complex. Rats were preselected for hemispheric dominance using a turn preference test: rats released into an open field and immediately turning right were considered to have left hemispheric dominance and vice versa. Dopamine (25ug/24h for 10-13 days) was infused unilaterally into the amygdala of animals (n=6 minimum for each group) having right or left hemispheric dominance and marked hyperactivity only resulted when dopamine was infused into the left amygdala of rats having right hemispheric dominance [measurement in individual cages equipped with photocells, control (vehicle infused) values 72±9 counts/60 min, dopamine infused animals 206±19 counts/60 min (maximum hyperactivity responses)]. Rats demonstrating such hyperactivity were given (on days 3 or 8 of dopamine infusion) an acute injection into the left or right amygdala of fluphenazine (25-500pg), sulpiride (5-250pg) or GR38032F (0.1-100ng).For these injections the infusion units were replaced by injection units via which drug or vehicle was delivered over a 60s period whilst animals were manually restrained. On injection into the left amygdala, all three compounds antagonised the dopamine induced hyperactivity, with the highest doses of fluphenazine, sulpiride and GR38032F reducing the hyperactivity by 73%, 82% and 62% respectively ($p<0.001$). Injection of the three agents into the right amygdala also significantly reduced hyperactivity by 62-84% ($p<0.001$).

The hyperactivity induced by the infusion of dopamine into the left amygdala was also antagonised by twice daily (7.0 am and 7.0 pm) peripheral administrations of sulpiride (5.0mg/kg i.p.), fluphenazine (0.1mg/kg i.p.) and GR38032F (0.1mg/kg i.p.), which reduced activity to control levels (53-96 counts/60 min). Non-sedative doses of diazepam (1.0mg/kg i.p.) also reduced the dopamine-induced hyperactivity to the same degree.

It is concluded that dopamine can initiate a hyperactivity from the amygdala of the rat with a laterality of action to the left amygdala which is sensitive to antagonism by neuroleptic agents and GR38032F from both hemispheres. Diazepam was also shown to antagonise the dopamine response. Thus the ability of GR38032F (similarly to neuroleptics) to antagonise limbic dopamine function can be shown in the amygdala, with the indications that such actions may be relevant in either or both, schizophrenia or anxiety.

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INTERACTION OF BENZAZIPINE ENANTIOMERS RELATED TO SCH 23390 WITH RAT BRAIN SERETONIN RECEPTORS

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Several derivatives of phenyl-1H-3-benzazepine have been shown to interact selectively with dopamine D1 receptors. Thus SK & F 38393 acts as a selective D1 agonist and SCH 23390 is a selective D1 antagonist (Iorio et al 1983). The usefulness of SCH 23390 in the study of D1 receptor function may be compromised by its relatively high affinity for serotonin S2 receptors in ligand binding assays (Cross et al 1983, Hyttel et al 1983) and in vitro preparations (Hicks et al 1984). In this study we have examined the stereospecificity of benzazepine interactions with serotonin S1 and S2 ligand binding sites.

Ligand binding assays were performed on membrane prepartions of the forebrain of male S.D. rats, as described by Cross et al (1985). Serotonin S1 receptors were studied using 4 nM ^{3}H-serotonin, and S2 receptors with 1 nM ^{3}H-ketanserin. The non-specific binding of both ligands was defined with 10 μM methysergide.

SCH 23390 inhibited ^{3}H-ketanserin binding, but was only weakly active at ^{3}H-serotonin binding (Table 1). The related bromo-analogue SK & F 83566 was less potent at S2 receptors and showed less than 10 fold stereoselectivity.

Table 1 Inhibition of serotonin receptors by benzazepines

Compound	IC_{50}(μM)	
	^{3}H-serotonin	^{3}H-ketanserin
SCH 23390	1·4 ± 0·4	0·34 ± 0·05
R(+) SK & F 83566	10 ± 2	0·86 ± 0·05
S(-) SK & F 83566	33 ± 6	7·6 ± 1·4
R(+) SK & F 38393	17 ± 5	25 ± 9
S(-) SK & F 38393	38 ± 15	18 ± 4

The present results suggest that the R-enantiomer of SK & F 82566 may be considerably less potent at serotonin S2 receptors than the closely related compound SCH 23390. Despite this difference, SK & F 83566 retains potent activity at the D1 site (O'Boyle & Waddington 1984). Moreover the stereoselectivity of SK & F 83566 at S2 receptors (10 fold) is considerably less than the 300 fold effect at D1 receptors. The effects may be useful in the determination of the relative contribution of D1 and S2 antagonism in the effects of phenylbenzazepines.

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[^{125}I]-BOLTON-HUNTER LABELLED ELEDOISIN BINDING SITES IN RAT DORSAL HORN AND CEREBRAL CORTEX ARE SIMILAR

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The presence of two categories of ^{125}I-Bolton-Hunter conjugated eledoisin (^{125}I-BHE) binding sites has been suggested in rat brain and peripheral smooth muscle. These are differentiated by the relative potencies of tachykinins to compete for the ^{125}I-BHE specific binding sites in various tissue membrane preparations. One site, (NK-2), exhibited by the rat duodenum, has the following rank order of affinity : neurokinin B (NKB) ≃ eledoisin (ELE) ≃ neurokinin A (NKA) >> physalaemin (PHYS) ≃ substance P (SP) (Buck et al., 1984) while the other, (NK-3), exhibited by the rat cortex, has a rank order of affinity of : NKB > ELE >> PHYS ≃ NKA > SP (Buck et al., 1984). In the rat spinal cord the nature of the ^{125}I-BHE binding sites is unknown although they appear to be differentially located to those of ^{125}I-Bolton-Hunter SP (Ninkovic et al., 1984). We have therefore compared the characteristics of ^{125}I-BHE specific binding in the rat spinal cord with the rat cerebral cortex.

The preparation of rat cerebral cortex membranes and the incubation assay conditions were similar to those described by Buck et al (1984). Eledoisin, NKB, NKA and SP all produced a concentration-dependent inhibition of ^{125}I-BHE specific binding to rat cortex membranes. The rank order of affinity with IC_{50} values was : NKB (6.8±1.1nM) > ELE (29.6±3.4nM) >> NKA (172±13nM) > SP (228±25nM). This profile confirms previous observations in this tissue (Buck et al., 1984). In contrast, in preliminary experiments we observed no significant ^{125}I-BHE specific binding to a rat spinal cord membrane preparation. This is probably due to the discrete localization of ^{125}I-BHE binding sites in the rat dorsal horn resulting in a high degree of tissue dilution in the membrane assay. We therefore decided to investigate ^{125}I-BHE binding to rat spinal cord by autoradiography.

The preparation of rat spinal cord sections (10μm) and the incubation assay conditions were similar to those described by Ninkovic et al (1984). To characterize the ^{125}I-BHE binding site in the rat spinal cord we used information obtained from the analysis of the competition curves in the cerebral cortex membrane assay. Concentrations of the competing tachykinin agonists were NKB, 30nM, NKA, 100nM, and SP 100nM. These were chosen such that if the spinal cord binding site was similar to that in cerebral cortex only NKB would inhibit specific ^{125}I-BHE binding whereas if the spinal cord was similar to rat duodenum, both NKA and NKB would inhibit specific binding. SP was not expected to displace from either type of binding site. NKB was the only peptide of the group which produced a maximal displacement at the concentrations tested. Thus, although a more quantitative method of analysis is ultimately required it would appear that, on the basis of this preliminary evidence, the ^{125}I-BHE binding sites in rat spinal cord resemble those in the rat cerebral cortex in contrast to those in the rat duodenum.

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THE EFFECTS OF 8-HYDROXY-2-(DI-N-PROPYLAMINO) TETRALIN (8-OH-DPAT) ON SPATIAL LEARNING IN THE MORRIS WATER MAZE TASK

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Although there is a marked loss of presynaptic cholinergic markers in the cortex of patients suffering from Alzheimer's disease, reductions in other aminergic systems have also been reported (Cross et al, 1984). The binding of the selective $5HT_{1A}$ ligand 8OH-DPAT is significantly reduced in patients with Alzheimer's disease (Middlemiss et al, 1986) although the relationship between this loss and the cognitive deficits remains to be determined. As the effects of serotonergic compounds on different types of learning are still poorly understood, we investigated the effects of 8OH-DPAT on a test of spatial learning, the Morris Water Maze.

In this test rats learn to locate a hidden platform (island) in a square tank (122 x 122cm) of opaque water, using visual cues placed around the tank. The platform could be placed in one of four positions. On each trial the rat was given a maximim of 100 seconds to find the island, and the following measured: speed, time spent in the island quadrant, annulus crossings. The latter are the number of times the rat crossed the annulus around the exact island position. Male Lister hooded rats weighing approximately 300g, previously trained on the water maze to find one island position, were treated with either saline, 60, 100 or 200µg/kg i.p. of 8OH-DPAT. Twenty minutes later they then received 6 consecutive trials to find a new island position. After these 6 trials, they were given a trial of 100 seconds with the island removed from the tank.

If the rat has learnt where the island is in trials 1-6, it will tend to swim around the position where the island used to be in the 7th "no island" trial. As a result, the time spent in the island quadrant and the number of times the rat crosses this annulus will be increased. As shown in Table 1 below, both these measures were decreased dose-dependently:-

TABLE 1. The effects of 8OH-DPAT in the extinction trial (mean ± S.E.M.)

Treatment	(n)	Time in Island Quadrant %	Annulus crossings %
Saline	15	42.6±3.2	53.9±5.1
60µg 8OH-DPAT	7	38.1±4.3 p>0.05	50.0±5.6 p>0.05
100µg 8OH-DPAT	9	32.0±2.8 p<0.001	44.5±5.3 p>0.05
200µg 8OH-DPAT	9	33.2±1.8 p<0.001	34.7±8.2 p<0.05

(P values calculated by Analysis of Variance)

The changes shown above may be associated with the dose-dependent increase in swimming speed seen in all the trials: 23.3±2.4(15)cm.sec^{-1}(mean±S.D. of (n) determinations) saline; 24.8±2.5(7) 60µg 8OH-DPAT; 27.6±2.9(9) 100µg 8OH-DPAT; 28.2±2.7(9) 200µg 8OH-DPAT. In conclusion, these results show that although 8OH-DPAT appears to impair learning on the Morris Water Maze, this may be indirect.

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TOLERANCE TO THE POTENTIATING EFFECTS OF SPINAL 5-HT_2 RECEPTOR ACTIVATION ON A FLEXOR REFLEX IN SPINAL RATS

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Injection of paroxetine to rats pretreated with an MAO inhibitor evoked many wet-dog shakes (WDS) but the rats were tolerant of a second injection given 3 h later (Koshikawa et al., 1985). WDS are generated by brainstem/spinal mechanisms and are selectively antagonized by $5HT_2$ receptor antagonists (Green, 1984). It was suspected that this tolerance was due to down-regulation of spinal $5HT_2$ receptors because of a temporal relation between decline in cortical $5HT_2$ receptor numbers, which persisted for at least 24 h, and the decline in WDS. Unfortunately this could not be tested directly because of the low density of spinal 5HT receptors. Rapid tolerance also develops to the behavioural effects of repeated injections of 5-methoxydimethyltryptamine (5-MeODMT) in non-pretreated rats and this is associated with a reduction in central (^3H)-5HT binding sites (Trulson & Keltch, 1985). The present study describes the effects of these treatments on a flexor reflex in spinal rats.

The spinal cord of male Wistar rats (250 - 350 g) was transected at C_1 under ether anaesthesia and the brain destroyed. Anaesthesia was discontinued and artificial respiration commenced. Reflex contractions of the right anterior tibialis muscle evoked by supramaximal electrical stimulation of the proximal end of the divided ipsilateral posterior tibial nerve (one 200 us pulse/ 10 s) were recorded with an isometric transducer. In other spinal rats, the lumbar roots were exposed and a recording and a stimulating electrode placed on the central stumps of two adjacent ipsilateral roots (usually L_5 and L_6). Ventral root reflexes were evoked every 2 s by supramaximal electrical stimulation of the adjacent root and recorded on an oscilloscope. Movement artefacts were minimised by gallamine (1 mg/kg i.v.).

5-MeODMT (0.1 - 0.2 mg/kg i.v.) markedly potentiated the flexor reflex within 1 min as seen by increased contractions of the anterior tibialis and by increased monosynaptic potentials and polysynaptic discharges; the effect gradually lessened over the ensuing 20 min. Similar, but much longer lasting effects were obtained after i.p. injection of paroxetine (12 mg/kg) in rats pretreated with phenelzine (18 h, 50 mg/kg, and 1.5 h, 12 mg/kg, before paroxetine). The induced potentiations could be prevented by the selective $5HT_2$ receptor antagonist, pirenperone (0.5 mg/kg i.v.) but not by (-)-propranolol (20 mg/kg i.v.), used here as a $5HT_1$ antagonist (Green, 1984). In contrast, 5MeODMT (0.1 mg/kg) was almost without effect when given 24 h after paroxetine (in rats pretreated with an MAO inhibitor) and when given 30 min after the last of 4 desensitizing injections of 5MeODMT (2 mg/kg) given at 30 min intervals; sensitivity to 5-MeODMT returned within 2 h of the last desensitizing injection of 5MeODMT.

Thus tolerance to the effects of $5HT_2$ receptor activation on a spinal reflex pathway occurs and disappears at similar times to tolerance to the behavioural effects seen in intact rats after the same treatments. Whether tolerance within the spinal cord is due to 5-HT receptor down-regulation remains to be proven.

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THE CHARACTERISATION AND LOCALISATION OF [^{3}H]- FLUNITRAZEPAM BINDING SITES IN THE PRIMATE BRAIN

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The distribution of benzodiazepine receptors in monkey brain has been examined for comparison with the distribution of low affinity GABA receptors as revealed by [3H]-muscimol binding. Cortical samples, from three macaques which had been killed by barbiturate overdose, were homogenised in 50mM Tris HCl, pH7.4 at 4°C, washed twice in buffer and stored at -70°C until required. The pellets were then thawed, washed four times in buffer and the membrane suspension incubated for 40 min at 4°C in 50mM Tris HCl, pH7.4 at 4°C containing a range of concentrations (0.25-8.0nM) of [3H]-flunitrazepam and 1μM flunitrazepam as the displacing ligand. Incubations were terminated by filtration through Whatman GF/C filters using a cell harvester, and the filters analysed using scintillation counting techniques. All assays were performed in triplicate, and the results were analysed using the computerised EBDA/LIGAND programme (McPherson, 1985). For autoradiography, 20μm brain sections were prepared from the frozen brains of three animals (Stuart et al., 1986). The sections were washed in buffer for 2 hours and then incubated for 40 min in 50mM Tris HCl, pH7.4 at 4°C containing 1nM [3H]-flunitrazepam in the presence or absence of 1μM flunitrazepam as the displacer. Incubations were terminated by a 2 min wash in ice-cold buffer followed by a dip into ice-cold water. The sections were freeze-dried and exposed to tritium-sensitive film for 6 weeks. Optical densities of the brain sections were compared using a microcomputer-based image analysis system (Slater, 1985).

[3H]-Flunitrazepam appears to bind to a single class of binding site in monkey neocortex with a Kd of 2.55nM (± 0.03 s.e.mean) and Bmax of 2.86fmol/mg (± 0.595 s.e.mean). Autoradiographically, high densities of binding sites were observed in the cerebral cortex, amygdaloid complex, dentate gyrus, mammillary bodies and the superficial layers of the superior colliculus, with moderate levels being observed in the basal ganglia (including the caudate nucleus, putamen and globus pallidus) and in the substantia nigra, cerebellum and thalamus.

There is a striking parallelism between the benzodiazepine and low affinity GABA receptors in the monkey brain (Crossman et al., 1986). However, preliminary investigations in monkeys with experimentally induced parkinsonism indicate that changes in low affinity GABA receptor status are not necessarily reflected by changes in benzodiazepine binding.

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THE DISTRIBUTION OF [^{3}H]-MUSCIMOL RECEPTOR SUBTYPES IN THE PRIMATE CENTRAL NERVOUS SYSTEM

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The distribution of GABA receptor subtypes in monkey brain has been examined using autoradiography. Previously a relative impoverishment of high affinity GABA receptors has been noted in certain areas (Stuart et al., 1984), viz. the globus pallidus and substantia nigra, which receive substantial GABAergic innervation from the striatum; latterly, the presence of a low affinity GABA receptor has been established within these and other structures.

Cortical samples, from three macaques which had been killed by barbiturate overdose, were homogenised and washed in 50mM · Tris HCl, pH7.4 at 4°C, washed twice in 50mM Tris citrate, pH7.0 at 4°C and frozen at -70°C until required. The pellets were then thawed, resuspended in buffer and washed twice. The membrane suspension was then incubated for 40 min at 4°C in 50mM Tris citrate, pH7.0 containing a range of [3H]-muscimol concentrations (1-300nM) with 1mM GABA used to define the non-specific binding. Incubations were terminated by filtration over Whatman GF/C filters using a cell harvester, and the amount of bound ligand was measured using standard scintillation techniques. All assays were performed in triplicate, and the results analysed using the EBDA/LIGAND software program (McPherson, 1985). For the autoradiographic study 20μm brain sections were prepared from the frozen brains of three animals (Stuart et al., 1986). The sections were washed in buffer for 2 hours to remove endogenous GABA, and then incubated for 40 min at 4°C in 50mM Tris citrate, pH7.0 containing either 5 or 1000nM [3H]-muscimol. 10mM GABA was used as the displacing ligand. Incubations were terminated by 3x3 sec washes in ice-cold buffer, followed by a dip into ice-cold water. The sections were freeze-dried and exposed to tritium-sensitive film for 8-16 weeks.

[3H]-muscimol binds to two sites in the primate neocortex; a high affinity component with a Kd of approx 3.85nM (± 2.06 s.e.mean) and Bmax of 3.81fmol/mg (± 0.33 s.e.mean), and a low affinity component with a Kd in excess of 100 nM and Bmax in excess of 30fmol/mg. Low affinity sites were observed in many areas of the brain including the globus pallidus, substantia nigra and other nuclei. The importance of the high affinity GABA receptor in basal ganglia function is currently being examined; preliminary studies of tissue from monkeys with experimentally induced parkinsonism show major changes in low affinity GABA receptor status within the basal ganglia structures, and in the nuclei to which they project.

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AUTORADIOGRAPHIC DEMONSTRATION OF INCREASED [^{3}H] - SPIPERONE BINDING IN THE MPTP-TREATED MONKEY

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Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to primates is known to induce a profound parkinsonian state characterised by hypokinesia, rigidity and tremor (Burns et al, 1983; Langston et al, 1984), however,the status of dopamine receptors is by no means clear. We now report the effects of MPTP treatment on the regional distribution of [^{3}H]-spiperone binding in brain using in vitro semi-quantitative autoradiography.

Four female monkeys (Macaca fascicularis) received daily injections of MPTP over 7-8 days. Total doses ranged from 0.3-0.7mg/kg i.v. Animals were killed 15-20 days later by a barbiturate overdose and brains were immediately frozen in isopentane at -50°C. Tissue was blocked stereotactically and 20µm sections were thaw-mounted on gelatin-coated glass slides. Three untreated animals served as controls. Sections were washed twice in phosphate buffer, pH7.4, for 30min at 20°C. The sections were incubated at 20°C for 60min in 50mM Tris-HCl, pH7.4, containing 120mM NaCl to which had been added 0.3nM [^{3}H]-spiperone (76Ci/mmol) and 0.1µM ketanserin. Specific binding was defined as that displaced by 1µM haloperidol. Following incubation sections were washed in ice-cold buffer for 2x1 min, air dried and exposed to tritium-sensitive film for 34 days at -20°C. Autoradiographs were analysed using a computer-based image analysis system.

Brains from monkeys treated with MPTP showed a loss of dopaminergic neurons from substantia nigra pars compacta and ventral tegmental area, together with a >90% depletion of striatal and accumbens dopamine levels (Mitchell et al, 1985). In the control monkey brains, high levels of specific binding were found in the caudate and putamen using [^{3}H]-spiperone as the ligand. There was a heterogeneous distribution of binding, the highest levels being found in the dorsolateral part of the anterior putamen and the ventrolateral part of the posterior putamen. The caudate displayed a more even distribution. Only moderate binding was found in the nucleus accumbens. Autoradiographs from monkeys treated with MPTP demonstrated a marked enhancement (40-180%) in the specific binding of [^{3}H]-spiperone in the caudate and putamen compared to control animals. No change was found in the nucleus accumbens.

Thus, the neurotoxic action of MPTP in the macaque monkey causes severe dopamine depletion in the caudate and putamen resulting in increased [^{3}H]-spiperone binding, suggesting the development of dopamine supersensitivity.

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$GABA_B$ RECEPTOR MEDIATED INHIBITION OF 5-HT STIMULATED PHOSPHATIDYLINOSITOL TURNOVER IN MOUSE CEREBRAL CORTEX

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Baclofen, the $GABA_B$ agonist (Bowery et al 1981) has been shown to enhance the stimulation of adenylate cyclase in cerebral cortex slices by noradrenaline (Karbon et al 1984), isoprenaline, histamine and adenosine (Enna et al 1985) and vasoactive intestinal polypeptide (Watling and Bristow 1986). We have investigated whether baclofen and GABA have similar modulatory effects upon agonist stimulated inositol phospholipid (PI) turnover in the cerebral cortex.

Adult male C56/B1/601a mice (Olac, Bicester) weighing 25-30g were used throughout. Cortical slices from 4 mice were pooled and PI turnover was determined by the method of Brown et al (1984). The slices were incubated with ^{3}H-inositol (0.5 Ci/ml) for 30 min and for a further 45 min in the presence of LiCl 10mM and agonist (5-HT 10 μM, noradrenaline 100 μM or Carbachol 100 μM). Baclofen, GABA and/or bicuculline methiodide were added 10 min prior to addition of the agonist.

(-)-Baclofen produced a dose dependent inhibition of the stimulation of PI metabolism by 5-HT. The ED_{50} of the inhibition by baclofen was 100 nM, and at a maximally effective concentration of 1 μM baclofen, the response was inhibited by 45 ± 5%. The inhibitory effect of baclofen was stereospecific, the (+)-enantiomer being devoid of effect at a concentration of 1 μM. GABA also inhibited the 5-HT mediated PI response.The inhibitory effect of GABA was not antagonised by (-)-bicuculline methiodide (10 μM). The $GABA_A$ agonist, 4,5,6,7 tetrahydrioisoxazol (5,4-c) pyridin-3-ol (THIP), at a concentration of 100 μM, did not inhibit the 5-HT mediated stimulation of PI turnover.

Neither GABA (100 μM) nor baclofen (100 μM) had an inhibitory effect on the PI stimulation evoked by noradrenaline or carbachol.

These data suggest that stimulation of PI metabolism by 5-HT may be inhibited by $GABA_B$ receptor activation. The results provide further evidence that GABA might modulate function of other neurotransmitters through alteration of their second messenger responses.

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