IN VIVO EVIDENCE FOR AUTORECEPTOR CONTROL OF MESO-PREFRONTAL CORTICAL DOPAMINE FUNCTION

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There is evidence for autoreceptor-mediated modulation of dopamine (DA) neurotransmission in the nigrostriatal and mesolimbic systems, both at the level of the DA terminals and cell bodies (see Talmaciu et al., 1986). The situation in the meso-prefrontal cortex DA system, however, is less clear. It has been reported that these neurones lack both impulse-regulating and synthesis-modulating autoreceptors (Chiodo et al., 1984), but more recently release-modulating terminal autoreceptors have been demonstrated in the medial prefrontal cortex (Talmaciu et al., 1986). We report the use of intracerebral dialysis to monitor changes in DA metabolism in the meso-prefrontal cortex following injection of dopaminergic drugs into the ventral tegmental area (VTA) to further investigate the presence of somatodendritic autoreceptors on these neurones.

Male Sprague Dawley rats (270-340 g), anaesthetised with 2-3% halothane in O_2/N_2O (1:1) throughout the experiment, were implanted with a 23G stainless steel guide cannula 3 mm above the VTA and a dialysis probe (300 μm o.d. similar to Johnson & Justice, 1983) in the ispilateral meso-prefrontal cortex continually perfused with artificial CSF at a rate of 0.5 or 1 μl/min. Dialysis samples were collected over 10 or 30 min periods and assayed for DA, DOPAC and HVA using a microbore HPLC system with a 20 μl injection loop providing a 10-15 min run time and a limit of detection with a glassy carbon electrode of approx. 5 fmoles. After a minimum 2.5 h stabilisation period N-propylapomorphine (NPA, 1 μg), haloperidol (2.5 μg) or lactic acid vehicle (6 mg/ml) was injected in a volume of 0.5 μl into the VTA over a 2 min period via a 31G injection cannula.

Basal extracellular levels of DOPAC and HVA were estimated at 3.3×10^{-7}M and 8.4×10^{-7}M respectively. DA was below the limit of detection in most experiments and proved too unstable in the remainder to allow drug-induced changes to be reliably measured. However, NPA produced an immediate decrease in extracellular DOPAC falling to 67±2% ±SEM of pre-injection control levels 1 h post-injection (n=5). A similar, but slightly delayed, effect was observed with HVA which fell to 68%±8 at 2 h. An increase in DOPAC was observed following haloperidol injection but was delayed in onset by 1.5 h and was maximal at 2.5 h (+78%±7, n=5). A significant increase in HVA was not observed until 2.5 h post-injection (55%±11, n=5). Lactic acid vehicle, while having no effect on HVA, produced an increase in extracellular DOPAC (+22%±10 at 2.5 h, n=5).

The rapid onset decrease in DA metabolism following NPA injection suggests the presence of an autoregulatory mechanism within the VTA directly controlling the function of the meso-prefrontal cortex DA cells - possibly via somatodendritic autoreceptors. The delayed onset of the haloperidol-induced increase in DA metabolism however might reflect a less direct mechanism of action.

We thank the Wellcome Trust for financial support.

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EFFECTS OF DIHYDROPYRIDINES AND FORSKOLIN ON K^+-INDUCED OR ELECTRICALLY-EVOKED ENDOGENOUS DOPAMINE RELEASE FROM RAT STRIATAL SLICES

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Voltage-sensitive calcium channels are widely distributed in brain and are thought to play a critical role in neurotransmitter release, but most studies have found only small effects of dihydropyridine (DHP) calcium agonists or antagonists on monoamine release in vitro (Spedding & Middlemiss, 1985). This could relate to a predominance of DHP-insensitive calcium channels in neurones (Miller, 1985), but it has also been suggested that the activation of DHP-sensitive calcium channels is dependent on their phosphorylation by cyclic AMP-dependent protein kinase (Curtis & Caterall, 1985). Since cyclic AMP itself is also thought to be of importance in the regulation of brain catecholamine synthesis and release (Mulder & Schoffelmeer, 1985), we have investigated whether the depolarisation-induced release of endogenous dopamine (DA) from striatal slices is modulated by DHPs and the adenylate cyclase activator forskolin. In addition, since the depolarisations induced by elevated K or electrical stimulation have been suggested to activate calcium channels to different extents (Mulder & Schoffelmeer, 1985), we have compared the effects of DHPs and forskolin on the release induced by these two different stimuli.

Rat striatal slices were prepared and superfused as described previously (Herdon et al., 1985; Herdon & Nahorski, 1987) and endogenous DA in superfusate fractions quantified by HPLC with electrochemical detection. DA release was evoked by two 4 min periods (40 min apart) of either electrical field stimulation (3Hz, 2msec pulses) or elevated K^+ (25mM); spontaneous release was measured before each stimulation. Neither the DHP calcium antagonist (+)PN 200-110 (1μM) nor the agonist Bay K8644 (1μM) produced any significant changes in either spontaneous or electrically-evoked DA release, but Bay K8644 (1μM) increased K^+-induced DA release by 20-30% ($p<0.005$)whilst (+)PN 200-110 (0.3 or 1μM) stereospecifically reduced release by 20-30% ($p<0.005$) and blocked the effects of Bay K8644. Forskolin (10μM) alone increased electrically-evoked release by 30-60% ($p<0.05$) and K^+-induced release by 20-40% ($p<0.01$); it also increased spontaneous release by 20-40% ($p<0.05$). However, the combination of Bay K8644 and forskolin did not increase release to any greater extent than forskolin alone.

These studies indicate that both agonist and antagonist DHPs are able to influence endogenous DA release under some conditions, presumably via effects on calcium influx. However, the small magnitude of their effects suggests that DHP-sensitive calcium channels are not of major importance in the regulation of DA release, particularly in response to electrical stimulation. The difference in DHP-sensitivity between K^+- and electrically stimulated release could relate to the continuous and sustained depolarisation produced by the K^+stimulus, though other explanations are possible. Although forskolin produced a substantial increase in DA release suggesting that cyclic AMP could be an important intracellular regulator of dopaminergic neurotransmission, these studies do not provide any evidence for a cyclic AMP-mediated increase in the effectiveness of Bay K8644.

This work was supported by the M.R.C.

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BRL 43694: A POTENT AND NOVEL 5-HT_3 RECEPTOR ANTAGONIST

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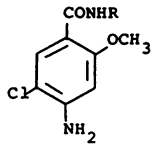
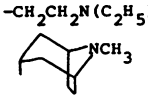
Metoclopramide (Mcp) is a gastric prokinetic agent and weak 5-HT_3 receptor antagonist (eg. antagonism of Bezold-Jarisch [B-J] reflex, ID_{50} 183µg/kg intravenously [i.v.], Donatsch et al, 1984). By restricting the conformational freedom of the diethylaminoethyl side chain of Mcp, BRL 24682 was identified as a potent antagonist of the B-J reflex (ID_{50} 0.8±0.2µg/kg i.v.; n=5; method of Dunbar et al, 1986) with gastric prokinetic activity (lowest active dose 0.1mg/kg subcutaneously (sc) in rats; method of McClelland et al, 1983).

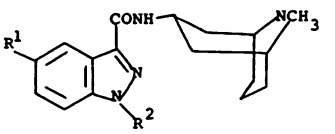
By changing the aromatic nucleus, the 3-indazole carboxamides (Table 1) were identified as compounds which retain the ability to block the B-J reflex but are poor stimulants of rat gastric motility.



Mcp; R = $-CH_2CH_2N(C_2H_5)_2$

BRL 24682; R =





(Compounds 1-5)

Table 1: Structure-activity relationships of 3-indazole carboxamides (1-5).

Compound	R^1	R^2	Antagonism of B-J reflex ID_{50} µg/kg i.v.
1	H	H	1.1±0.3 (n=3)
2	H	CH_3	0.7±0.2 (n=9)
3	H	(i)	>100 (n=3)
4	F	H	1.0±0.2 (n=3)
5	Cl	H	16±4.0 (n=3)

(i) 2-methyl isomer

The N-unsubstituted compound (1) is marginally less potent than the 1-methyl analogue (2) whereas the 2-methyl isomer (3) is much less potent. A large 5-substituent generally decreases potency.

Of this series BRL 43694, endo-N-(9-methyl-9-azabicyclo[3,3,1]non-3-yl)-1-methyl-indazole-3-carboxamide (2), was selected for further evaluation. At 0.5 or 1.0mg kg^{-1} sc, BRL 43694 did not consistently affect rat gastric motility. BRL 43694 antagonised 5-HT evoked contractions of the guinea pig isolated ileum (pA_2 8.1 ± 0.2; n=6) and 5-HT induced tachycardia of rabbit isolated heart (pA_2 10.7 ± 0.2; n=6) (methods of Sanger, 1987). In addition BRL 43694 has little or no affinity for 5-HT_1, 5-HT_2, dopamine (D_2), benzodiazepine, picrotoxin or α_1, α_2 and β-adrenoceptor binding sites in rat brain. Therefore, BRL 43694 appears to be a potent and selective 5-HT_3 receptor antagonist.

We acknowledge A.W. Dunbar, J. Bermudez, G. Pratt and G. Joiner for their assistance.

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EVIDENCE THAT SMOKING EXERTS REGIONALLY-SELECTIVE EFFECTS ON 5-HT SYSTEMS IN HUMAN BRAIN

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In rats, chronic treatment with nicotine causes reductions in the concentration and biosynthesis of 5-hydroxytryptamine (5-HT) in the hippocampus (Benwell and Balfour 1979,1982). In the present study the effects of smoking on the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) and on radioligand binding to 5-HT receptors in human brain tissue have been examined.

Tissue samples were obtained from 30 cadavers (7 male and 11 female non-smokers (mean age at death 74 ± 2 yr) and 6 male and 6 female smokers (mean age at death 71 ± 3 yr)) who had shown no evidence of neurological or psychiatric disease. Samples taken from the hippocampal formation including Ammon's horn and subiculum (HF), the lateral hippocampal gyrus (LHG) and the gyrus rectus (GR) were assayed for 5-HT and 5-HIAA using the method of Reinhard et al (1980). Radioligand binding to 5-HT_1, 5-HT_{1A} and 5-HT_2 binding sites was assayed with ^{3}H-5-HT (7nM), ^{3}H-8-hydroxy-(di-N-propylamino)-tetralin (^{3}H-8-OH-DPAT, 1nM) and ^{3}H-ketanserin (1nm) according to the method of Middlemiss and Fozard (1983). Statistical analysis was performed using a two-way ANOVA with smoking and sex as the independent factors and age and postmortem delay as covariates.

Smoking was associated with significant reductions in the levels of 5-HT (from 0.096 ± 0.009 to 0.066 ± 0.009 µg/g) and 5-HIAA (from 0.460 ± 0.041 to 0.332 ± 0.035 µg/g) in the HF (F smoking (1,23) = 5.5; $P<0.05$ and 4.7; $P<0.05$ respectively) and of 5-HIAA (from 0.392 ± 0.025 to 0.240 ± 0.020 µg/g) in the LHG (F smoking (1,23) = 12.9; $P<0.01$). There was also a significant effect of smoking on the concentration of 5-HT in the GR (F smoking (1,20) = 4.96; $P<0.05$) although, unlike its effects on 5-HT and 5-HIAA in the HF and LHG, there was an interaction with the sex of the subject (F smoking by sex (1,20) = 7.03; $P<0.05$). Subsequent analysis showed that smoking significantly increased 5-HT levels in the GR from 0.026 ± 0.006 to 0.045 ± 0.015 µg/g in males (F smoking (1,8) = 5.54; $P<0.05$) whereas it had no effect on the levels in females. ^{3}H-5-HT binding to LHG was increased significantly in smokers (F smoking (1,25) = 8.1; $P<0.01$) from 252 ± 24 to 385 ± 35 fmoles/mg protein. ^{3}H-8-OH-DPAT binding was increased signficantly in both HF (F smoking (1,25) = 7.1; $P<0.05$) and LHG (F smoking (1,25) = 10.3; $P<0.01$) from 124 ± 13 to 182 ± 16 fmoles/mg protein and from 66 ± 11 to 121 ± 11 fmoles/mg protein respectively. Smoking had no effects on radioligand binding to GR or on the binding of ^{3}H-ketanserin to any of the brain regions studied. The effects of smoking on radioligand binding were not affected significantly by the sex of the subject. The data suggest that smoking may exert regionally-selective effects on the brain 5-HT systems and that its effects on hippocampal 5-HT are similar to those observed in rats treated chronically with nicotine.

The study was supported by grants from the Scottish Home and Health Department and Tenovus Tayside.

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INVOLVEMENT OF '5-HT-1 LIKE' RECEPTORS IN PURPOSELESS CHEWING IN RATS

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Pilocarpine-induced purposeless chewing behaviour in rats is dependent on intact stores of brain 5HT, but not dopamine or noradrenaline. Mianserin, a 5HT-1 and 5HT-2 receptor antagonist is an effective antagonist in this central cholinergic behaviour but ketanserin, a selective 5HT-2 antagonist is not. These data suggest that it may be possible to alter purposeless chewing through central 5HT-1 receptors. We now report on the ability of some putative 5HT-1 agonists to induce purposeless chewing in rats.

Male Wistar rats (150-350 g) were treated with m-chlorophenylpiperazine (mCPP) or trifluoromethylphenylpiperazine (TFMPP: 2-16 mg/kg i.p.). Individual chews were recorded manually for 5 mins at the time of peak drug effect, by an observer unaware of the treatments. Following administration of the putative 5HT-1A agonists, 8-hydroxy-N,N-dipropyl-2-aminotetralin (8-OH-DPAT: 0.025-4.0 mg/kg s.c.) and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT: 0.25-8.0 mg/kg s.c.) animals were observed for chewing behaviour for up to 30 mins. In antagonist studies, ketanserin (5 mg/kg), ICS-205-930 (10 mg/kg), mianserin (2.5 mg/kg) methiothepin (1 mg/kg), prazosin (2 mg/kg), idazoxan (2 mg/kg), trifluoperazine (2.5 mg/kg), scopolamine (1 mg/kg) and methylscopolamine (1 mg/kg) were administered 30 mins before mCPP (6 mg/kg s.c.). Chewing was recorded over a 5 min period 5 mins following mCPP administration.

mCPP (1-8 mg/kg i.p.) and TFMPP (2-16 mg/kg i.p.) increased the incidence of purposeless chewing but dose response curves were bell shaped. In contrast, 8-OH-DPAT (0.025-4 mg/kg s.c.) and 5-MeODMT (0.25-8 mg/kg s.c.) did not induce chewing behaviour. The effects of mCPP (6 mg/kg s.c.) were antagonised by administration of the 5HT antagonists, mianserin, methiothepin and by the anticholinergic, scopolamine (Table 1). In contrast, ketanserin, prazosin, idazoxan and trifluoperazine, were without effect; methylscopolamine produced a small but non-significant inhibition.

Table 1 Antagonism of mCPP-induced purposeless chewing

Treatment	Dose (mg/kg)	% Inhibition of mCPP chewing
Ketanserin	5	0
ICS-205-930	10	0
Mianserin	2.5	73*
Methiothepin	1	78*
Prazosin	2	0
Idazoxan	2	0
Trifluoperazine	2.5	0
Scopolamine	1	93*
Methylscopolamine	1	20

* $p < .05$ Mann Whitney U test

These data suggest that the 5HT agonists such as mCPP and TFMPP induce purposeless chewing in rats. Antagonist studies with mCPP suggest that the response is mediated via '5HT-1 like' receptors, possibly the 5HT-1B subtype. Significant antagonism of the action of mCPP by scopolamine, but not methylscopolamine, implies a link between the central 5HT and cholinergic systems involved in chewing behaviour.

HYPERACTIVITY FOLLOWING WITHDRAWAL OF MESOLIMBIC DOPAMINE INFUSION AND NEUROLEPTIC TREATMENT IS REVERSED BY GR38032F

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GR38032F, a selective antagonist of $5HT_3$ receptors (Brittain et al., 1987) has been shown to inhibit the immediate behavioural consequences of a mesolimbic dopamine excess. Thus, GR38032F inhibits the peaks of hyperactivity responding which result when dopamine is infused into the mesolimbic nucleus accumbens or amygdala of the rat, or nucleus accumbens of the marmoset (Costall et al, 1987a, b). Further, such inhibition is achieved without changing normal dopamine function. These observations have lead to the hypothesis that GR38032F may provide the first of a new class of antipsychotic agents exerting antischizophrenic action with an absence of side effects normally associated with neuroleptics.

In the treatment of schizophrenia patients have frequently received numerous treatment regimes, and the question may be raised as to the effectiveness of GR38032F in patients which have previously been exposed to classical neuroleptic therapy. In order to mimic this situation in the rodent, male Sprague-Dawley rats were subjected to standard stereotaxic surgery for the implantation of chronically indwelling guide cannulae for subsequent infusion of dopamine (25µg/24h, 0.48µl/h) into the nucleus accumbens for 13 days. Concurrent with the dopamine infusion rats received haloperidol, 0.15mg/kg twice daily by the intraperitoneal route (7.00am and 7.00pm). This treatment not only suppressed the usual peaks of hyperactivity responding to dopamine, but suppressed locomotor activity to below normal values. When the combined dopamine infusion and haloperidol treatments were abruptly withdrawn on the 13th day, after a delay of 1-2 days, a marked rebound hyperactivity developed (347 ± 43 counts/60min compared with control values of 86 ± 8.9 counts/60min, $p<0.001$) which persisted for at least 70 days. GR38032F (0.001-0.1mg/kg) given during the phase of marked rebound hyperactivity was shown to reduce this to control values (79 ± 8-86 ± 9 counts/60min, $P<0.001$). Further, the inhibitory effects of a single dose challenge with GR38032F could be seen to persist for at least 9 days, regardless of whether intervention was early or late in the withdrawal period (day 3, 15 or 60 of withdrawal).

It is therefore concluded that GR38032F not only has an ability to reverse a raised mesolimbic dopamine function in rodent and primate, but is also able to control the behavioural consequences of withdrawal from a period of mesolimbic dopamine excess combined with classical neuroleptic suppression. Thus, the potential antischizophrenic action of GR38032F should not be compromised by prior neuroleptic treatment, indeed, the duration of antagonism effected by a single dose of GR38032F after a period of mesolimbic dopamine excess/neuroleptic treatment is remarkably prolonged which augurs well for the potential of GR38032F as an antischizophrenic agent.

Dr. A.M. Domeney is a Senior Glaxo Fellow.

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SHAM FEEDING IN THE RAT FOLLOWING THE ADMINISTRATION OF(+)-FENFLURAMINE AND THE PUTATIVE 5-HT_{1A} AGONIST, 8-OH-DPAT

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Sham feeding studies in the rat, in which animals have an open gastric fistula, provide an important opportunity to analyse factors that contribute to the arousal, maintenance and satiation of feeding responses (Smith and Gibbs, 1979). Sham feeding rats show a pronounced satiety deficit, and Weingarten and Watson (1982) demonstrated that sham feeding is a valuable procedure for evaluating the effect of diet palatability on the level of food ingestion.

Fenfluramine produces loss of body weight in obese patients, and reduces meal size in human volunteer subjects; considerable evidence links its anorectic action to serotonergic mechanisms (for a review, see Rowland and Carlton, 1986). Recently, it has been demonstrated that the more potent isomer, (+)-fenfluramine (1.25 and 2.5 $mg.kg^{-1}$) reduced sucrose intake in freely fed rats (Borsini et al., 1985). Hence, the sham feeding preparation can be used to test whether or not oropharyngeal factors are sufficient to mediate, at least in part, the anorectic effect of (+)-fenfluramine.

Adult male hooded rats (General strain, bred in our laboratory) were operated under general anaesthesia and equipped with a gastric fistula, which when open, would allow a liquid diet to flow directly out of the stomach, to be collected in a tray placed beneath the test chamber. Methods are described in detail, elsewhere (Kirkham and Cooper, 1987). After recovery from surgery, the animals were trained to sham feed a 5% sucrose solution, following 4h food deprivation. A control group of nonfistulated rats was also tested. (+)-Fenfluramine hydrochloride was administered in doses of 1.0, 1.75, 3.0 and 10.0 $mg.kg^{-1}$ i.p. 30 min before the 2 hour sucrose consumption test to each rat, the order of testing being balanced across rats.

In the first 60 min of the test, following vehicle injection, animals with an open gastric fistula (N = 9) consumed 107.8 $\pm$ 5.2 ml (mean $\pm$ S.E.M.) of 5% sucrose, compared to 29.6 $\pm$ 1.9 ml consumed by the nonfistulated group (N = 10). By the end of the 2 hour test, intake for the two groups had risen to 173.1 $\pm$ 11.1 and 41.4 $\pm$ 3.7, respectively. (+) Fenfluramine reduced sucrose consumption in both groups. Decreases occurred at 1.75 $mg.kg^{-1}$, and at 3.0 $mg.kg^{-1}$ when sham feeding was reduced by about 50%, although there was substantially less decrease in the nonfistulated animals (19.6% reduction in the first 60 min) at this dose. At 10.0 $mg.kg^{-1}$, consumption was markedly depressed and animals displayed a flat-body posture.

Experiments were also carried out in other animals with the 5-HT_{1A} agonist, 8-OH-DPAT, which has been shown to increase consumption of dry food pellets in freely-feeding rats (Dourish et al., 1985). Both groups of animals were tested following administration of 8-OH-DPAT hydrobromide at doses 3 - 300 $\mu g.kg^{-1}$ s.c. There was evidence for some increase in sham feeding at 30 and 100 $\mu g.kg^{-1}$ 8-OH-DPAT, but no effect at 300 $\mu g.kg^{-1}$.

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INVOLVEMENT OF THE HALOPERIDOL-SENSITIVE SIGMA RECOGNITION SITE IN THE BEHAVIOURS INDUCED IN THE RAT BY (+)-SKF 10,047

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The complex behavioural syndrome of lateral head weaving, hyperlocomotion and ataxia, induced in the rat by the prototypical sigma receptor ligand, (+)-SKF 10,047 ((+)-N-allylnormetazocine), is thought to reflect activation of the sigma receptor (Shannon, 1983). However, recent evidence indicates the existence of distinct 'high' and 'low' affinity sigma recognition sites (Largent at al, 1986). We have investigated the involvement of the high affinity, haloperidol-sensitive sigma recognition site in the behaviours induced by (+)-SKF 10,047.

The total number of lateral head movements (head weaves) induced by the subcutaneous administration of (+)-SKF 10,047 in male Sprague-Dawley rats (250-300g) was counted during five observation periods of 45 sec duration, commencing 10 min after injection and repeated at 4 min intervals thereafter. Ambulation was quantified simultaneously by counting the number of quadrants of the observation cage (43x29x16 cm high) entered. Ataxia was scored using a ranked intensity scale (0 = absent; 1 = equivocal; 2 = present; 3 = severe) by (a) assessing motor co-ordination during ambulation and (b) assessing the delay in righting when placed in the supine position at the end of the final observation period. Putative antagonists were administered subcutaneously at doses devoid of overt behavioural effects, 30 min before (+)-SKF 10,047.

Head weaving, ambulation and ataxia all increased dose-dependently following injection of (+)-SKF 10,047 (1 - 60 mg/kg). (Side-to-side rolling of the hind quarters was also seen, though too infrequently to allow meaningful analysis. No other behaviour was consistently observed in this dose range.) Head weaving and ambulation, but not ataxia, induced by a submaximal dose of (+)-SKF 10,047 (10 mg/kg) were antagonised dose-dependently by the dopamine receptor antagonists, spiperone (0.025 - 0.1 mg/kg) and (+)-butaclamol (0.1 - 0.4 mg/kg) and by the mixed dopamine/sigma receptor ligand, haloperidol (0.1 - 0.4 mg/kg), consistent with an involvement of both dopamine and sigma receptors in the expression of the behaviours. However, (-)-butaclamol (0.4 - 4 mg/kg), which, unlike the (+)-isomer, has high affinity for the haloperidol-sensitive sigma recognition site and low affinity for dopamine receptors (Tam and Cook, 1984) was without effect on all behaviours induced by (+)-SKF 10,047. Similarly, head weaving, hyperlocomotion and ataxia were not antagonised by (+)-3-(3-hydroxyphenyl)-N-n-propylpiperidine ((+)-3-PPP, 1-4 mg/kg), which also has nanomolar affinity for the haloperidol-sensitive sigma recognition site (Largent et al, 1986) and antagonises the discriminative stimulus properties of (+)-SKF 10,047 (Balster, 1986).

The results indicate that head weaving and hyperlocomotion induced by (+)-SKF 10,047 are dependent on dopamine systems for their expression, and are inconsistent with involvement of the high affinity, haloperidol-sensitive sigma recognition site. Ataxia induced by (+)-SKF 10,047 seems not to involve the activation of either dopamine or high affinity sigma receptors.

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$GABA_B$ BINDING SITES IN THE CORTEX AND HIPPOCAMPUS OF DEPRESSED SUICIDE VICTIMS

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$GABA_B$ receptors may be involved in the mechanism of action of antidepressant treatments since chronic administration of a number of antidepressant drugs and repeated electroconvulsive shocks has been reported to markedly increase $GABA_B$ binding sites in the rat frontal cortex (Lloyd et al., 1985).

In this study we have measured $GABA_B$ binding sites in the frontal (Brodman area 11) and temporal (Brodman areas 21 & 22) cortex and hippocampus of suicide victims and age- and sex-matched control subjects. The suicide victims studied included only those in which a firm diagnosis of depression could be made; subjects in whom the diagnosis was unclear or there was a history of schizophrenia, epilepsy, alcoholism or drug abuse were specifically excluded. Control subjects died suddenly of causes not involving the CNS.

Membranes were prepared, stored and $GABA_B$ binding performed essentially as Bowery et al., 1983. For cortical regions, saturation binding (8 GABA concentrations, 1-300nM) was performed; for the hippocampus binding was determined at 1 and 51nM GABA. Specific binding was defined with 10^{-4}M (±) baclofen. Assays were performed on coded samples.

Bmax values in human post-mortem cortical samples were a little lower than the rat cortex, K_D values were very similar (Cross & Horton, 1986). There was no significant correlation between post-mortem delay and $GABA_B$ binding, indicating the stability of these binding sites up to 65h after death. K_D and Bmax values did not differ significantly between depressed suicide victims and controls (Table 1).

Table 1: $GABA_B$ binding sites in the cortex of controls and depressed suicides.

		Age	Sex	K_D	Bmax
Frontal cortex	Controls (n=8)	43±4	6M,2F	32±3	0.98±0.09
	Suicides (n=8)	43±4	6M,2F	32±4	1.01±0.11
Temporal cortex	Controls (n=9)	42±4	7M,2F	25±4	0.70±0.09
	Suicides (n=9)	43±4	7M,2F	33±3	0.78±0.09

Age = yrs, K_D = nM, Bmax = pmole/mg protein. Results are means ± S.E.M.

Similarly in the hippocampus, $GABA_B$ binding did not differ significantly between the two groups (1nM GABA: controls 15±1, suicides 17±2; 51nM GABA: controls 290±37, suicides 309±33 fmoles/mg protein, n=10).

Thus, in these largely drug-free depressed suicides (one subject was receiving mianserin, another diazepam), we found no evidence that the $GABA_B$ binding differed from well-matched control subjects in 3 brain regions.

We thank the Wellcome Trust for financial support. J.A.C. is an S.E.R.C. scholar.

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[^{3}H]-MUSCIMOL BINDING TO POST-MORTEM BRAIN TISSUE IN SCHIZOPHRENIA

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An increase of striatal dopamine D_2 receptors in schizophrenia is well established. However, it is unclear whether this increase is a result of the disease process or of drug treatment, since chronic neuroleptic administration to animals induces an up-regulation of D_2 receptor number (Clow et al. 1980). Other receptors are also affected by these antipsychotic drugs. Gale (1980) has found (3H)-GABA binding to the substantia nigra to be increased after chronic neuroleptic treatment of animals. In order to obtain a better understanding of the possible contribution of drug treatment to neurochemical abnormalities in schizophrenia, we have investigated the binding of (3H)-muscimol to post-mortem brain tissue from schizophrenic patients and age-matched control subjects.

Membranes from brain tissue supplied by the Cambridge Brain Bank Laboratory were prepared from nigral and medial pallidal regions using the method of Quast and Brenner (1983) modified by a Triton X-100 incubation to remove endogenous GABA. Saturation analysis was performed at 37°C with (3H)-muscimol in the range of 2-64 nM using 10^{-3}M GABA to define non-specific binding.

Table 1 (3H)-Muscimol binding to post-mortem brain tissue in schizophrenia

	Substantia Nigra		Medial Pallidum	
	Bmax	K_D	Bmax	K_D
Controls	456 ± 184	15.9 ± 5.8	513 ± 175	32.9 ± 20.2
Schizophrenics	455 ± 218	15.6 ± 6.9	529 ± 246	31.4 ± 15.9

Values are means ± s.d. of 10-12 samples. Bmax = fmol/mg protein. K_D = nM.

Results shown in Table 1 indicate no differences in saturable muscimol binding (presumably to GABA receptors) between controls and schizophrenics in the two structures studied, each of which receive strong GABAergic innervation from the striatum. Nor did we find any asymmetries of receptor density, no significant differences being apparent in comparing tissues from left and right nigras from a series of eight of each of these control and schizophrenic brains.

Thus it would appear that the antipsychotic drug treatment inevitably received by the schizophrenic patients studied here had no effect on (3H)-muscimol binding, despite the increase in dopamine D_2 receptors which occurs in these brains.

We thank the Schizophrenia Research Fund, Wellcome Trust and MRC for financial support.

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MICROINJECTION OF HISTAMINE INTO THE NUCLEUS ACCUMBENS ALTERS BEHAVIOURAL ACTIVITY IN RATS

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The high levels of histamine (HA) in the hypothalamus, nucleus accumbens and hippocampus (Bennett et al., 1983), and the presence of histaminergic fibres innervating these areas (Prell & Green, 1986), suggest a neuroregulatory role for HA. Various studies have implicated HA in arousal mechanisms; for example, microinjection of HA into rat cerebral ventricles affects locomotor activity and behaviours such as grooming, sniffing and rearing (Kalivas, 1982). More particularly, iontophoretic application of histamine agonists into rabbit nucleus accumbens alters neuronal activity in this region (Chronister et al., 1982), thus the present study has investigated the effects of intra-accumbens microinjections of HA on behavioural activity in rats.

Male, Wistar rats (~280 g) were anaesthetised with pentobarbitone (60 mg/kg i.p.) and bilaterally implanted with guide cannulae for microinjections into the accumbens. Following 7 days recovery, rats were injected with 0.9% saline (2x1 µl) or histamine dihydrochloride (1-200 µg, 2x1 µl). Activity was then monitored in 15 min periods for 2 h using an Actimat doppler shift radar activity meter (Sharp et al., 1984). In addition, animals were also observed and rated for the presence or absence of several behavioural parameters. To investigate whether histaminergic receptors mediate these behaviours, mepyramine or SKF 93479 (1 µg or 10 µg, 2x0.5 µl) were bilaterally microinjected 10 mins before HA (20 µg, 2x0.5 µl) or saline.

Administration of HA induced dose dependent biphasic effects on spontaneous activity. In the first 15 mins, HA produced a significant hypoactivity compared to saline controls (-73% at 20 µg, n=8, $P<0.01$; -82% at 50 µg, n=9, $P<0.01$, Wilcoxon Signed Rank Test). No hypoactivity was observed following bilateral injection of 1 µg HA. The hypoactivity was immediately followed by a marked, inverse dose related, hyperactivity phase, peaking between 45 and 60 mins and lasting the duration of the experiment (+373% at 20 µg, n=8, $P<0.01$; +141% at 50 µg, n=9, $P<0.01$, compared to saline controls). In contrast, microinjection of the inactive metabolite N-tele-methylhistamine (20 µg, 2x1 µl) did not induce these behavioural effects. Changes in activity scores were accompanied by changes in individual behavioural parameters; the hypoactivity phase was associated with reductions in locomotion, rearing, sniffing and head-forepaw movements, and the hyperactivity phase with intermittent increases in these parameters. Pretreatment with the H_1 antagonist mepyramine (10 µg) significantly attenuated both the initial hypoactivity response and the entire hyperactivity phase, but had no effect itself on behaviour ($P<0.05$, n=9). Administration of a lower dose of mepyramine (1 µg) did not alter hypoactivity but significantly reduced hyperactivity during the 30-45 min period ($P<0.05$, n=9). The H_2 antagonist SKF 93479, at similar doses, did not affect histamine-induced hypo- or hyperactivities.

These results suggest that HA in the accumbens induces arousal behaviour following an initial hypoactivity response and that these behaviours may be mediated by H_1-receptors.

L.J. Bristow is an SERC CASE student with SKF.

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SELECTIVE MAO INHIBITORS - EFFECTS ON 5-HYDROXYTRYPTAMINE RELEASE IN THE FREELY MOVING RAT

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Administration of l-tryptophan to rats pretreated with unselective monoamine oxidase (MAO) inhibitors evokes a characteristic behavioural syndrome (Grahame-Smith, 1971). This behaviour is associated with increased 5HT tissue levels. However it is not known whether there is a correlation between increased extracellular 5HT and the occurrence of the syndrome. Furthermore, selective inhibitors of MAO A fail to produce the behavioural syndrome (Green & Youdim, 1975) despite 5HT being predominantly metabolised by MAO A (Johnston, 1968). Therefore, it was the purpose of this study to determine if there is a correlation between the behavioural syndome and extracellular 5HT, as measured by intracranial dialysis in the freely moving rat (Brazell et al., 1985) and to examine the effect of selective MAO A and MAO B inhibition on extracellular 5HT levels.

Dialysis probes consisting of cellulose tubing supported by two stainless steel cannulae were perfused with artificial CSF (pH 7.4) at a rate of 1 µl/min and implanted into the ventromedial hypothalami of rats under halothane anaesthesia. The animals were given 3 h to recover from the anaesthesia after which time dialysis samples were collected and 5HT and 5HIAA were measured using HPLC with electrochemical detection. The animals were given either saline, the MAO A inhibitor clorgyline (5 mg/kg i.p.) (Johnston, 1968), the MAO B inhibitor deprenyl (10 mg/kg i.p.) (Yang & Neff, 1973) or both clorgyline and deprenyl. Two hours later they were given l-tryptophan (50 mg/kg i.p.). Dialysis samples were collected every 30 mins. Separate groups of 5 rats were treated in the same way as above and the behavioural parameters scored on a scale of 0-3 for 1 h following l-trytophan (0 = absent and 3 = continuous). MAO A and MAO B activity were determined using [^{14}C] 5HT and [^{14}C] phenylethylamine respectively.

Rats treated with either clorgyline or deprenyl plus l-tryptophan failed to exhibit the behavioural syndrome. Deprenyl plus l-tryptophan significantly inhibited MAO B but caused no significant changes in MAO A activity or extracellular 5HT levels however clorgyline plus l-tryptophan significantly increased (+150%) extracellular 5HT and decreased MAO A activity. Co-administration of clorgyline and deprenyl plus l-tryptophan induced the behavioural syndrome, inhibited MAO A and B and also caused a large increase in extracellular 5HT (+580%).

These results suggest that although selective inhibition of MAO A increases extracellular 5HT inhibition of both MAO A and MAO B are required to cause substantial rises in extracellular 5HT levels. Furthermore it appears that the behavioural syndrome is associated with large increases in extracellular 5HT. However previous work has shown that the syndrome is not necessarily dependent upon such increases (Marsden et al., 1987).

AJS is supported by an SERC CASE Studentship. CAM is a Wellcome Senior Lecturer.

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EFFECT OF CHRONIC LOFEPRAMINE AND DESMETHYLIMIPRAMINE ADMINISTRATION ON TRYPTOPHAN METABOLISM AND DISPOSITION IN THE RAT

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We have shown (Badawy, 1986) that acute administration of the tricyclic antidepressant lofepramine or its major metabolite desmethylimipramine (DMI) increases rat cerebral 5-hydroxytryptamine (5-HT) concentration and that of its major metabolite 5-hydroxyindol-3-ylacetic acid (5-HIAA) by enhancing tryptophan availability to the brain secondarily to inhibition of activity of the major tryptophan-degrading enzyme, hepatic tryptophan pyrrolase, and that lofepramine exerts these effects directly, rather than through metabolism to DMI. It was considered of interest to find out if similar changes also occur after chronic administration of either drug.

Locally bred male Wistar rats (224-314g at the start of experiments) were maintained on cube diet 41B (Oxoid) and water under standard conditions. The hydrochlorides of lofepramine and DMI (both gifts from E. Merck) were administered intraperitoneally once daily at two dose levels (3 and 6mg/kg body wt.) for up to 4 weeks. Control rats received an equal volume(s) of the vehicle (dimethylformamide:saline; 1:3, v/v) by the same route. All chemical, enzymic and other determinations were performed by standard procedures (for references, see Badawy & Evans, 1982).

After one week of single daily injections of lofepramine or DMI at the above two dose levels, liver tryptophan pyrrolase activity was significantly inhibited. Thus the total enzyme activity was decreased by 35% and 55% by the 3 and 6mg/kg doses respectively of either drug. The corresponding decreases in apoenzyme activity were 64% and 91% respectively. As a result of this inhibition, tryptophan accumulated in liver and blood. The increase in free (ultrafiltrable) serum tryptophan concentration caused by the 3 and 6mg/kg doses of lofepramine was 19% and 39% respectively, whereas that by the corresponding doses of DMI was 18% and 92% respectively. Total (acid-soluble) tryptophan concentration, by contrast, was elevated equally effectively by both doses of the two drugs (by 28% by the 3, and 43-47% by the 6, mg/kg doses). Accordingly, only the 6mg/kg dose of DMI significantly decreased tryptophan binding to serum proteins and this was associated with a significant increase in serum non-esterified fatty acid concentration.

The concentrations of brain tryptophan, 5-HT and 5-HIAA were all significantly increased, by 18-32% and 42-56% by the 3 and 6mg/kg doses of lofepramine respectively and by 26-48% and 61-122% respectively by the corresponding doses of DMI.

There were no significant changes in concentrations of serum albumin and glucose, whereas that of corticosterone was decreased by 52-71% by lofepramine and by 28-30% by DMI.

Similar results to those described above were obtained in rats treated with lofepramine or DMI for longer periods.

These results demonstrate that chronic administration of lofepramine or DMI enhances rat cerebral 5-HT and 5-HIAA concentrations by increasing tryptophan availability to the brain, mainly secondarily to inhibition of liver tryptophan pyrrolase activity. Because this inhibition is a direct effect, it is suggested that the chronic effects of lofepramine described here are also direct and not secondary to metabolism to DMI.

We thank E. Merck for their support.

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EVIDENCE AGAINST BENZODIAZEPINE SITE MEDIATION OF 'DEFEAT' ANALGESIA IN MALE MICE

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Compounds classed as inverse agonists at benzodiazepine recognition sites (e.g. CGS8216, FG7142, DMCM) produce dose-dependent analgesia on the mouse tail-flick test(Randall & Rodgers, 1986). These effects are reversed by the competitive antagonist Ro15-1788 and by full agonists(chlordiazepoxide, diazepam), agents which per se are devoid of activity on the tail-flick assay. Furthermore, Ro15-1788 (10-40mg/kg) and diazepam(0.5-2mg/kg) were found to block non-opioid 'defeat' analgesia in male intruder mice, leading to the suggestion that the latter reaction may be mediated by an endogenous anxiogenic ligand for benzodiazepine sites. However, in contradiction of this tentative hypothesis was the finding(Rodgers & Randall, 1987) that 'defeat' analgesia is unaffected by chlordiazepoxide(5-30mg/kg) and midazolam(1.25-5mg/kg). In an attempt to clarify this inconsistent profile, the present study assessed the effects of ZK93423(a β-carboline full agonist), ZK91296(a β-carboline partial agonist) and CGS9896(a pyrazoloquinoline partial agonist) on basal nociception and the analgesic response to defeat experience.

8-10 week old male DBA/2 mice(Bantin & Kingman, Hull) were housed in groups of ten, and maintained in a temperature-controlled room(24±1°C) in which a reversed 12hr light cycle was operative. All testing was conducted under dim red light during the dark phase of the cycle. Nociceptive latencies were assessed by tail-flick assay, using a cut-off of 10 seconds. Pre-injection baseline latencies provided within-groups control data. For defeat experience, intruder mice were placed into the home cage of an aggressive resident conspecific and removed immediately upon display of the species-characteristic upright submissive posture. ZK93423(0.5-10mg/kg), ZK91296(2.5-20mg/kg) and CGS9896(2.5-20mg/kg) were ultrasonically dispersed in distilled water to which Tween 80 had been added. All drugs were administered intraperitoneally(i.p., 10ml/kg) thirty minutes before testing. Statistical analysis(ANOVA) failed to reveal significant effects of any compound on basal tail-flick latencies:- ZK93423($F(4,35)=0.03$,NS), ZK91296($F(4,65)=0.77$,NS), CGS9896($F(4,35)=0.55$,NS). Similarly, none of the compounds tested was found to alter the analgesic reaction of intruder mice to defeat experience:- ZK93423($F(4,35)=0.36$,NS), ZK91296($F(4,35)=0.55$,NS), CGS9896($F(4,35)=0.58$,NS).

The lack of effect of ZK93423, ZK91296 and CGS9896 on basal nociception generally corroborates our previous finding that compounds classed as agonists at benzodiazepine sites are devoid of activity on the mouse tail-flick assay. The ineffectiveness of the two partial agonists in blocking 'defeat' analgesia further suggests that the ability of Ro15-1788 to prevent this reaction(Rodgers & Randall, 1987) is not due to partial agonist activity of this compound at the doses used. However, the failure of ZK93423(a high-affinity agonist) to block 'defeat' analgesia markedly contrasts with the total inhibition of this reaction by diazepam. In conclusion, the overall profile of results to date strongly suggests that the efficacy of Ro15-1788 and diazepam in blocking 'defeat' analgesia may depend upon interaction with mechanisms other than benzodiazepine recognition sites.

This work was supported by M.R.C. The authors wish to thank Dr Dai Stephens (Schering AG, Berlin) and Dr Charles Brownley (Ciba-Geigy Corp., USA) for the kind gifts of compounds used in this study.

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ANTAGONISM OF PIRENZEPINE-INDUCED TURNING IN MICE: A NEW MODEL FOR THE ASSESSMENT OF A CHOLINOMIMETIC ACTIVITY

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The behavioural assessment of selective muscarinic agonistic activities is presently limited by the lack of specific tools (agonists and antagonists) and by the poor brain disposition of most reference drugs. We already reported that the intrastriatal injection of muscarinic agonists in mice leads to ipsilateral turning (Biziere et al., 1986). In the present study, we describe the effect of an intrastriatal injection of pirenzepine (PZ), a selective antagonist of M_1 muscarinic receptors, which does not penetrate into the brain (Hammer et al., 1980).

Female Swiss CD_1 mice (Charles River France) weighing 25 to 30 g were used. For intrastriatal injections, PZ hydrochloride was solubilized in a pH6 phosphate buffer and injected directly into the right striatum (volume 1 µl) using the free-hand technique described by Worms et al. (1986). Rotations were then counted during 3 periods of 2 min (2-4, 8-10 and 13-15 min.) after injection. Complete ispilateral rotations were noted as +, contralateral rotations were noted as -. For interaction experiments, the drugs or vehicle were injected intraperitoneally (i.p.) 15 or 30 min. before the intrastriatal injection of PZ (1 µg). Turning behaviour was then assessed as described above.

Buffer-injected mice exhibited a weak ipsilateral turning. Intrastriatal PZ induced a dose-dependent contralateral turning (mean ± SEM cumulated number of turns/6 min.. 0.001 µg : - 1.2 ± 0.3 ; 0.01 µg : - 5.4 ± 0.7 ; 0.1 µg : - 8.8 ± 0.8 ; 1 µg : - 12.2 ± 1.1). The contralateral turning induced by 1 µg PZ was dose-dependently antagonized by the muscarinic agonists arecoline (mean ± SEM % of controls . 0.3 mg/kg i.p. : 66 ± 7** ; 1 mg/kg : 46 ± 7** ; 3 mg/kg : 14 ± 3 **), pilocarpine (0.3 mg/kg : 67 ± 11* ; 1 mg/kg : 43 ± 9** ; 3 mg/kg : 7 ± 3**) and oxotremorine (0.003 mg/kg : 53 ± 5** ; 0.01 mg/kg : 24 ± 7** ; 0.03 mg/kg : 1 ± 1**). The cholinesterase inhibitor physostigmine also decreased PZ-induced rotations (0.01 mg/kg i.p. : 76 ± 8 %* ; 0.03 mg/kg : 46 ± 3** ; 0.1 mg/kg : 22 ± 5**). Haloperidol, weakly but significantly, antagonized PZ-induced turning (0.03 mg/kg i.p. : 86 ± 4%* ; 0.1 mg/kg : 81 ± 5** ; 0.3 mg/kg : 56 ± 4** with strong motor impairment), whereas (±)-sulpiride (3 to 100 mg/kg i.p.) did not modify the effect of PZ. Finally, (+)-amphetamine (0.1 to 3 mg/kg : 112 to 84 % of control), citalopram (1 to 30 mg/kg i.p. : 102 to 95 % of controls) and muscimol (0.03 to 0.3 mg/kg i.p. : 101 to 107 % of controls) failed to affect the PZ-induced turning behaviour.

These data indicate that, when injected directly into the mouse striatum, the selective M_1 muscarinic antagonist PZ induces contralateral rotations which are selectively antagonized by cholinomimetic drugs. This suggests an involvement of muscarinic receptors of the M_1 type in rotational behaviour. In addition, this effect of PZ provides a new model for studying the central activities of cholinergic agonists.

* $p < 0.05$; ** $p < 0.01$ vs. PZ controls (Student's t-test).

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EFFECTS OF BAY K 8644 ON α-ADRENOCEPTOR-MEDIATED VASOCONSTRICTION IN RAT AUTOPERFUSED HINDLIMB AND MESENTERY

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The calcium entry promoter, Bay K 8644, has been reported to potentiate selectively systemic pressor responses to i.v. bolus doses of α_2-adrenoceptor agonists in the pithed rat (Wilffert et al., 1984). It is not yet clear whether this increase results from the appearance of an α_2-adrenoceptor mediated response in vascular beds previously unresponsive to α_2-adrenoceptor agonists, or whether the response is only increased in regions showing an α_2-adrenoceptor-mediated response under control conditions. We have investigated the effects of Bay K 8644 on vasoconstrictor responses to the selective α_2-adrenoceptor agonist, B-HT 933, and to the selective α_1-adrenoceptor agonist, methoxamine, in the autoperfused hindlimb and mesenteric vascular beds in the pithed rat. The mesenteric vasculature is unresponsive to α_2-adrenoceptor agonists in the rat (Nichols and Hiley, 1985).

Male Sprague-Dawley rats (300-400g) were anaesthetized with methohexitone, artificially ventilated and pithed. Diastolic blood pressure (DBP) was recorded from the left carotid artery. Blood was pumped at a rate of 2 ml/min from the right carotid artery into the right femoral or the superior mesenteric artery for recording of hindlimb or mesenteric perfusion pressure (PP), respectively. Drug injection was *via* the left jugular vein. Atropine and propranolol (each 1 mg/kg, i.v.) were administered and PP allowed to stabilize for 30 min before i.a. infusion of the α-adrenoceptor agonists was commenced. Bay K 8644 was given i.v. 10 min prior to the start of agonist infusion.

Resting hindlimb PP was 73±2 mmHg (n=28). Resting mesenteric PP was 48±5 mmHg (n=17). In the hindlimb, B-HT 933 produced dose-dependent vasoconstriction: the ED_{50} was 2.6±0.8 μg/kg/min and the maximum response was 61±9 mmHg above resting PP (n=6). In contrast, in the mesenteric bed, B-HT 933 was inactive. Hindlimb vasoconstrictor responses to B-HT 933 were increased in a dose-dependent manner by Bay K 8644. When measured at the control ED_{50} response level, leftward shifts of the dose-response curve to B-HT 933 amounted to 0.26 and 0.81 log units with 10 and 100 μg/kg Bay K 8644, respectively. The maximum response to B-HT 933 was also significantly increased with these doses of Bay K 8644 to 86±6 (n=6) and 116±7 mmHg (n=4), respectively. In contrast, even after 100 μg/kg Bay K 8644, the mesenteric bed remained unresponsive to B-HT 933. For methoxamine in the hindlimb, the ED_{50} was 2.0±0.3 μg/kg/min and the maximum response was 209±10 mmHg (n=5), while in the mesenteric bed the ED_{50} was 3.8±0.1 μg/kg/min and the maximum response was 138±17 mmHg (n=4). Hindlimb vasoconstrictor responses to methoxamine were only increased by the higher dose of Bay K 8644 (leftward shift 0.25 log unit), and the maximum response was unaffected. In the mesenteric bed, Bay K 8644 did not significantly shift the methoxamine dose-response curve. Increases in DBP were smaller for both agonists than their corresponding effects on PP. Bay K 8644 increased DBP responses to B-HT 933 but not to methoxamine.

The results of the present study indicate that α_2-adrenoceptors are present in the hindlimb, but not in the mesenteric vasculature of the rat. The calcium entry promoter, Bay K 8644, selectively increased α_2-adrenoceptor-mediated vasoconstrictor responses, but did not reveal an α_2-adrenoceptor-mediated response in vascular smooth muscle normally unresponsive to α_2-adrenoceptor agonists.

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NIFEDIPINE, AT HIGH DOSES, REDUCES THE ARRHYTHMIAS INDUCED BY MYOCARDIAL ISCHAEMIA

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In a previous series of experiments, we investigated the antiarrhythmic activity of calcium antagonists in our conscious rat model of myocardial ischaemia and infarction (Curtis et al., 1984, 1985, Curtis & Walker 1986). Although the racemate and the optical enantiomers of verapamil had pronounced antiarrhythmic activity, the vascular selective dihydropyridine, felodipine, was much less effective, suggesting that depression of ventricular slow calcium currents may be responsible for the observed antiarrhythmic actions. In order to test this hypothesis, we have administered a range of doses of nifedipine to conscious rats, and have compared cardiovascular effects with actions on arrhythmias induced by occlusion of the left coronary artery.

Rats were surgically prepared, according to previously described techniques (Curtis et al., 1984), 7 days prior to administration of nifedipine i.v. and subsequent occlusion of the left coronary artery. Blood pressure, heart rate and the ECG were recorded for 30 min before, and for 4 h after occlusion. Arrhythmia scores (AS) were calculated. After 24 h, hearts were removed for estimation of zone at risk (OZ) and infarct size (IZ) (both expressed as % of ventricle weight). The results are summarised in Table 1.

Table 1.

Dose	Haemodynamic changes				OZ	IZ	Occlusion-induced arrhythmias					
$mgkg^{-1}$	Mean BP		Heart rate				0-30 min			0-240 min		
	-1min	+2h	-1min	+2h			AS	VT	VF	AS	VT	VF
0	108±2	88±4	410±20	380±30	39±3	32±2	3.2±0.3	9/9	6/9	3.9±0.3	9/9	8/9
0.5	89±3*	91±4	500±20*	410±20	37±3	29±2	2.3±0.4	7/9	4/9	4.1±0.3	9/9	8/9
2.0	79±3*	88±6	480±20*	390±20	38±2	26±3	2.9±0.4	9/9	4/9	3.7±0.3	8/9	7/9
10.0	80±3*	74±4*	450±20	440±30	36±2	28±4	1.1±0.5*	4/9*	0/9*	1.8±0.5*	4/9*	3/9*

Values are mean±s.e.mean or incidence out of n; n=9; *p=less than 0.05

Nifedipine had no effect on PR or QRS at 1 min before occlusion. Only the highest dose of nifedipine delayed ischaemia-induced changes in R-wave and ST amplitude (not shown). Higher doses (30 mg/kg) could not be investigated, owing to death from asystole (sinus arrest) before occlusion.

These findings were taken as further evidence that blockade of ischaemic ventricular calcium currents is antiarrhythmic; nifedipine only exhibited significant antiarrhythmic actions at doses in excess of those producing vasodilatation, and without affecting conduction in the non-ischaemic myocardium.

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ICI 169,369 IS BOTH A COMPETITIVE ANTAGONIST AND AN ALLOSTERIC MODULATOR OF ARTERIAL 5-HT_2 RECEPTORS

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The effects of 5-hydroxytryptamine (5-HT) can be blocked in a surmountable manner (eg ketanserin) or in an insurmountable but reversible manner (eg methysergide). Paradoxically ketanserin can restore methysergide-depressed contractions of arterial and tracheal smooth muscle induced with 5-HT (Kaumann et al., 1985; Lemoine & Kaumann, 1986). We have proposed that the 5-HT_2 receptor interconverts between a highly active state R and a low active state R' (Kaumann & Frenken, 1985). The interconversion R⇌R' is modulated by an allosteric site. We investigated the mode of action of ICI 169,369 (2-(2-dimethylaminoethylthio)-3-phenylquinoline), a new 5-HT_2 receptor antagonist (Blackburn et al., 1986). What is the nature of the antagonism by ICI 169,369? Does ICI 169,369 exert allosteric effects? To answer these questions we used strips of calf coronary artery and rat tail artery with the endothelium removed (Krebs solution, 6 µM cocaine, 0.2 mM ascorbate, 32.5°C). Concentration-effect curves (cec) for the contractile effects of 5-HT were determined. ICI 169,369 (incubated for 3 h) shifted the cec in a parallel and surmountable manner. The slope of Schild-plots was 0.99 (1-1000 nM) on 32 strips of 6 calves and 1.11 (3.3000 nM) on 16 strips of 4 rats. pK_B values were ($\bar{x}\pm$SD) 9.1 ± 0.2 for coronary artery and 8.8 ± 0.2 for tail artery.

The methysergide-induced depression of the maximum response to 5-HT was prevented by ICI 169,369, as estimated from cec (Table 1). ICI 169,369 and methysergide were incubated simultaneously for 3 h before the determination of a cec for 5-HT. High concentrations of methysergide overcame the protective effect of ICI 169,369 by again depressing 5-HT-induced responses.

Table 1

Percentage of maximum response to 5-HT ($\bar{x}$ ± SEM, n ≥ 4)

Coronary artery		Tail artery	
Methysergide	Methysergide plus 100 nM ICI 169,369	Methysergide	Methysergide plus 300 nM ICI 169,369
0.01 nM 100 ± 4		0.1 nM 94 ± 1	
0.1 nM 64 ± 4		1 nM 76 ± 3	
2 nM 31 ± 2	106 ± 7	10 nM 62 ± 1	104 ± 2
20 nM 30 ± 3	106 ± 9	100 nM 55 ± 3	91 ± 2
200 nM 29 ± 3	50 ± 4	300 nM 56 ± 2	67 ± 2

ICI 169,369 100 nM also restored to 92 ± 5% (n=6) the maximum response to 5-HT in the presence of 20 nM methysergide after it had been depressed to 30 ± 3% in coronary artery. Similarly, ICI 169,369 300 nM restored to 91 ±2% (n=4) the maximum response to 5-HT in the presence of 100 nM methysergide after it had been depressed to 63 ± 3% in tail artery.

Conclusions: 1) ICI 169,369 competes with 5-HT for the 5-HT_2 receptor. 2) ICI 169,369 competes with methysergide for the allosteric site. 3) Through its interaction with the allosteric site, ICI 169,369 favours the highly active state R of the 5-HT_2 receptor. 4) The interaction of ICI 169,369 with the 5-HT_2 receptor system resembles that of ketanserin.

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DOPAMINE RELEASES RENIN FROM ISOLATED RAT JUXTAGLOMERULAR EPITHELIOID CELLS BY STIMULATION OF DA_1 RECEPTORS BUT NOT β-ADRENOCEPTORS

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There are conflicting results regarding the effects of dopamine on renin release. For instance, in rat kidney slices and in vitro perfused kidneys dopamine liberates renin through stimulation of β-adrenoceptors. However, in anesthetized dogs a dopaminergic mechanism was suggested to be responsible for this effect of dopamine (see : Lokhandwala and Barrett, 1984). The aim of this communication is to demonstrate that dopamine, like fenoldopam (Cavero et al., 1987), increases renin secretion from a primary culture of rat epithelioid juxtaglomerular cells via stimulation of DA-1 dopamine receptors.

Isolation of juxtaglomerular cells was carried out as described by Kurtz et al. (1985,1986). Briefly, rat kidneys from young Sprague-Dawley rats (80-120 g) were perfused in situ with citrate buffer and then removed. The renal cortex was minced and subsequently incubated with a collagenase-trypsin solution. The suspension was sieved over a 22 µm screen and the recovered cells were washed with culture medium and centrifuged in a Percoll-density gradient. The density band III (1.06 g/ml) was incubated with culture medium and shown to contain 80-90% juxtaglomerular cells. All experiments described herein were performed on the second day of culture. Renin activity was determined by its ability to generate angiotensin I (AI) from the plasma of bilaterally nephrectomized rats by means of a radioimmunoassay. The renin secretion rate (ng AI/h/mg protein/30 min) was calculated from the linear increase measured in the cell-conditioned buffer solution after a 30 min incubation with no drug (control : CO), dopamine (DA : 1 µM), isoproterenol (ISO : 3 µM), SCH 23390 (SCH : 10 nM), propranolol (PR : 0.1 µM), SCH + DA, PR + DA and PR + ISO. Results are given as means ± s.e. mean of at least 4 independent determinations.

DA (1 µM) increased markedly the renin release from juxtaglomerular cells (CO : 18.2 ± 3.1, DA : 44.7 ± 7.5 ng AI/h/mg protein/30 min). This effect was inhibited by SCH 23390 (DA + SCH : 19.7 ± 2.7) which itself did not modify renin secretion. In another series of experiments DA (1 µM) and ISO (3 µM) were found to produce a similar significant ($P < 0.05$) increase in renin release (CO : 8.4 ± 0.8 ; DA : 27.0 ± 3.2 ; ISO : 31.5 ± 5.5 ng AI/h/mg protein/30 min). Propranolol, which itself failed to affect base-line renin secretion blocked the effects of ISO (PR + ISO : 11.6 ± 0.6) but not those of dopamine (PR + DA : 23.9 ± 2.2).

In conclusion, dopamine like fenoldopam, a relatively selective DA-1 receptor agonist (Cavero et al., 1987), has the ability to increase renin secretion rate from cultured juxtaglomerular epithelioid cells through stimulation of DA-1 dopamine receptors , but not β-adrenoceptors. Thus, these cells are endowed with the DA-1 subtype dopamine receptor which may be responsible at least partly for the increase in plasma renin activity produced by fenoldopam in man (Harvey et al., 1985).

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THE EFFECTS OF DOPEXAMINE AND DOPAMINE IN MYOCARDIAL ISCHAEMIA

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Dopexamine is a recently developed agonist at peripheral dopamine receptors and β_2-adrenoceptors (Brown et al, 1985a) which reduces blood pressure and slightly increases heart rate and contractility in dogs (Brown et al, 1985b) and which may also improve cardiac function in patients with chronic heart failure (Dawson et al, 1985). The aim of the present study was to determine whether dopexamine (and dopamine) induce ventricular ectopic activity in the ischaemic myocardium and also whether they can modify the severity of arrhythmias induced by acute coronary artery occlusion. The studies were performed in both anaesthetised rats and greyhounds.

Male rats, anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.) were prepared for coronary artery ligation using the technique described by Clark et al (1980). The greyhounds, anaesthetised with chloralose, were thoracotomised and prepared for occlusion of the left anterior descending coronary artery (LAD). For the first study rats received dopexamine (0.1-1.0 µg kg min or dopamine (0.1-0.5 µg kg min) from 15 minutes prior to occlusion until the end of the experiment to determine the effects on early (0-30 min) ischaemic arrhythmias (Table 1). For the second study both greyhounds and rats received infusions of dopexamine, dopamine and noradrenaline (in doses which produced significant haemodynamic responses) for 10 minute periods after the early ischaemic arrhythmias had subsided but at a time when the myocardium is especially sensitive to the arrhythmogenic effects of catecholamines (Table

Table 1. The effects of dopexamine and dopamine (0.25 µg Kg^{-1} min^{-1}) on early ischaemic arrhythmias in rats.

	n	VEB	VT(%)	VF(%)	Mortality (%)
Control	10	1252 ± 346	100	60	40
Dopamine	10	586 ± 114	100	78	50
Dopexamine	10	380 ± 182*	60	25*	10*

Values are mean ± s.e.m. *P<0.05. (Mann Whitney; Fischer's Exact)

Table 2. Sensitivity of the ischaemic myocardium; appearance of arrhythmias during infusion of dopexamine, dopamine or noradrenaline 0.5-1.5h after commencment of ischaemia.

	% Incidence.		Number VEB's	
	Rats	Dogs	Rats	Dogs
Control	30	0	2 ± 1	0
Dopexamine	10	60	2 ± 1	15 ± 10
Dopamine	40	90*	2 ± 1	30 ± 5
Noradrenaline	90*	100*	33 ± 6	110 ± 10*

*P<0.05 compared to controls. (Mann Whitney; Fischer's Exact)

These results suggest that dopexamine, in doses which have positive inotropic and chronotropic activity, is less likely than either dopamine or noradrenaline to induce severe ventricular arrhythmias. Further, dopexamine possesses antiarrhythmic activity against early ischaemic arrhythmias in anaesthetised rats.

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CARDIOVASCULAR EFFECTS OF ICV OR INTRACISTERNAL (IC) VASOPRESSIN (AVP) IN CONSCIOUS LONG EVANS AND BRATTLEBORO RATS

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Icv administration of AVP to anaesthetised Brattleboro rats deficient in hypothalamic AVP, produces an increase in blood pressure (BP) which is not different from that of control rats (Pittman et al., 1982). However, the cardiovascular effects of centrally administered AVP in rats appear to be influenced by the presence or absence of anaesthesia (Zerbe & Feuerstein, 1985). In the present study, we have examined the effects of centrally administered AVP on BP and heart rate (HR) in conscious Long Evans and Brattleboro rats.

Male Long Evans (360±10 g, n=12, mean±s.e.m.) and Brattleboro (349±13 g, n=17) rats were prepared, under sodium methohexitone anaesthesia (60 mg kg^{-1} i.p.) with 23 gauge stainless steel guide cannulae for injection into the right lateral cerebral ventricle or into the cisterna magna. Seven days later, the animals were re-anaesthetised with sodium methohexitone and a catheter placed in the abdominal aorta via the caudal artery. Continuous recordings of systolic and diastolic BP and HR were begun following overnight recovery (17-18 hr).

Icv and ic administration of AVP (0.03-100 ng in 5 µl saline) increased BP of both Long Evans and Brattleboro rats in a dose dependent manner. In Long Evans rats, 3 ng AVP icv increased BP by 9±1/8±2 mmHg (Δ systolic BP/Δ diastolic BP, n=6), whereas in Brattleboro rats BP increased by 24±6/16±4 mmHg (n=7) following this dose of AVP ($P<0.05$ for systolic). HR did not change significantly in Long Evans rats but in Brattleboro rats HR rose by 56±11 beats min^{-1} at the time of the peak pressor response. It was not possible to construct a full dose response curve to icv AVP in Brattleboro rats due to the behavioural effects observed at higher doses. The pressor response to the highest icv dose of AVP tested in Brattleboro rats (30 ng) was 46±13/21±6 mmHg (n=6). The dose response curve to icv AVP in Long Evans rats was shallow, with a maximum pressor response of 13±2/13±1 mmHg (n=6) following 100 ng AVP. There was a tendency for HR to increase at higher doses of AVP. Increases in HR following icv AVP in Brattleboro rats were not dose dependent.

With 3 ng AVP ic, BP increased by 25±4/23±4 mmHg (n=6) in Long Evans rats and by 60±13/46±12 mmHg (n=6) in Brattleboro rats; this was the highest ic dose of AVP tested in Brattleboro rats. HR fell in a dose dependent manner following ic AVP in Long Evans rats. Changes in HR were variable in Brattleboro rats with some decrease at lower doses (300 pg: -24±10 beats min^{-1}, n=10), but an increase at the highest dose (3 ng: 34±15 beats min^{-1}, n=6). Doses as low as 1 ng ic and 3 ng icv caused 'barrel-rolling' behaviour in some Brattleboro rats. This response was not observed in Long Evans rats which had received 10-fold higher doses of AVP.

These results suggest that Brattleboro rats are more sensitive to the central pressor actions of AVP than are Long Evans rats. Furthermore, AVP may exert differential effects on the cardiovascular system of conscious rats depending on the site of injection into the cerebrospinal fluid.

Supported by grant 85/86 from the British Heart Foundation.

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THREE DAY INTRAVENOUS INFUSION OF HUMAN α-CALCITONIN GENE-RELATED PEPTIDE (HUMAN α-CGRP) CAUSES SUSTAINED HYPOTENSION IN RATS

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Human α-calcitonin gene-related peptide (human α-CGRP) lowered blood pressure after bolus i.v. administration to conscious normotensive or spontaneously hypertensive rats (Craig et al, 1986). No tachyphylaxis to the hypotensive effect was observed when human α-CGRP was infused i.v. for up to 4h (Al-Kazwini et al, 1987). The present study examines the cardiovascular effect of chronic administration of human α-CGRP (up to 72h).

Female normotensive Sprague-Dawley rats (175-200g) or Wistar Kyoto derived spontaneously hypertensive rats (165-210g) were anaesthetised using halothane and the carotid artery and external jugular vein were cannulated, the former being exteriorised at the back of the neck. An Alzet mini-osmotic pump ($1\mu l h^{-1}$) was implanted and connected by a cannula to the jugular vein. The contents of the cannula were arranged so that the pump would initially deliver saline followed by human α-CGRP for 24h (or 72h in some experiments) which in turn was followed by saline. This procedure allowed the determination of recovery from the peptide without the mini-pump having to be removed. Control rats received an infusion of saline from the mini-pump throughout the experiment. Mean arterial pressure (MAP) and heart rate (HR) were recorded from the carotid artery.

Human α-CGRP 3×10^{-10} mol kg^{-1} min^{-1} reduced the pre-drug MAP from 108±5 mm Hg (mean ± s.e. mean) to 82±5 mm Hg after 2h and 87±2 mm Hg after 24h. Within 6h following the subsequent infusion of saline the blood pressure was not significantly different from the pre-CGRP level (t-test; $P > 0.05$). In the same animals the heart rate was increased by human α-CGRP from 338±10 b. min^{-1} to 489±17 b. min^{-1} at 2h. However, after 18h of CGRP infusion the heart rate was not significantly different from the initial value ($P>0.05$).

In spontaneously hypertensive rats, human α-CGRP lowered MAP from 176±5 mm Hg to 100±5 mm Hg and 114±6 mm Hg after 2 and 24h infusion respectively. However, the significant increase in heart rate (pre-CGRP 435±10 b. min^{-1} to 473±10 b. min^{-1} at 2h) was not maintained and was not significantly different ($P > 0.05$) from the pre-CGRP level after 24h of peptide infusion (439±19 b. min^{-1}). Control rats were not significantly different in either MAP or HR before and after a 24h saline infusion.

Another group of spontaneously hypertensive rats were infused with human α-CGRP, 3×10^{-10} mol kg^{-1} min^{-1} for 72h followed by saline for a further 24h. The peptide evoked a fall in MAP from the pre-drug value of 160±7 mm Hg to 102±3 mm Hg at 2h, 113±6mm Hg at 24h, and 108±3 mm Hg at 72h. Subsequent infusion of saline showed no recovery of MAP over the following 6h although recovery was complete within 20h of the end of CGRP infusion.

In conclusion, infusion of human α-CGRP for 24h or 72h produced sustained falls in blood pressure which gradually recovered (with no sign of a rebound) when the peptide was substituted by saline. In contrast the associated increase in heart rate evoked by human α-CGRP in these conscious rats was less well maintained.

We thank the Medical Research Council and Celltech Limited for support.

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THE CARDIOVASCULAR SIGNIFICANCE OF CENTRALLY ADMINISTERED NEUROPEPTIDE Y

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Neuropeptide Y (NPY) is a 36 amino acid peptide which has a widespread distribution in the peripheral and central nervous systems. It is putatively involved in cardiovascular control and has been located immunohistochemically in many important cardiovascular centres in the brain. Intracisternal (i.c.) administration of NPY (1.25 nmols in 5ul sterile saline) in anaesthetised rats gave rise to a fall in blood pressure from a preinjection value of 101±3.6 (mean ± s.e. mean n=8) to 78.6±3 mmHg 1 hour post-injection. This result confirms an earlier report (Fuxe et al, 1983) but in addition was accompanied by a fall in heart rate from 423±12 to 340± 12 beats/min. The hypotension and bradycardia produced by i.c. NPY were significantly different ($p<0.05$) from control responses with i.c. saline which was without cardiovascular effect.

A more specific study has been carried out on one important cardiovascular centre - the caudal ventrolateral medulla where NPY-immunoreactive nerve terminals have been located around the A1 noradrenergic cell group which controls sympathetic outflow and the nucleus ambiguus which controls cardiac parasympathetic outflow. Discrete stereotaxic microinjections of NPY (25 pmols in 0.2 ul sterile saline) into this area in anaesthetised rats resulted in a significant fall in BP from 97.8±4.8 mmHg prior to microinjection to 89.4±3.6 mmHg 1.5 hours post-injection (n=12). This was concomitant with a significant fall in heart rate from 363±13 to 287±18 beats/min. Microinjection of 50 pmoles NPY gave rise to more profound changes; BP fell from 116.5±3.6 to 93.2±5.9 mmHg and heart rate from 397±14 to 265±31 beats/min 1.5 hours post-injection (n=7). Three hours following NPY administration cardiovascular parameters had returned or were close to pre-injection values. Methylatropine (1mg/kg i.a.) a peripherally acting muscarinic antagonist was administered prior to NPY microinjection in order to investigate a possible involvement of cardiac vagal fibres from the nucleus ambiguus. In the presence of methylatropine, the bradycardic response to 50pmols NPY was markedly attenuated and the maximum fall in BP reduced and shifted from 1.5 hours to 30 mins. It is therefore concluded that NPY microinjected into the caudal ventrolateral medulla exerts potent effects on blood pressure and particularly heart rate in the anaesthetised rat. The bradycardic response would appear to be due to activation of cardiac vagal fibres and although part of the hypotensive response is a result of this, the remaining fall in BP in the presence of methylatropine provides evidence for a second cardiovascular component which may involve the A1 cell group.

The support of the British Heart Foundation is gratefully acknowledged.

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PROLONGATION OF REFACTORY PERIODS IN ISCHAEMIC VENTRICULAR MYOCARDIUM OF THE ANAESTHETISED RAT BY AMIODARONE

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Amiodarone has been demonstrated to prolong the refractory period of cardiac tissues in vitro (Rizzon & Di Biase, 1975) and in vivo (Smeets et al, 1983). This study examines the effects of the drug on refractory periods in ischaemic ventricular myocardium of rats anaesthetised with urethane (1.5g/Kg body weight).

Refractory periods were measured as previously described by Northover (1986). Rats were pre-treated intravenously with saline, polysorbate 80 (20mg/Kg) or amiodarone (10mg/Kg, administered as diluted solutions of Cordarone X Injection which contains amiodarone 50mg/ml and polysorbate 80 100mg/ml) five minutes before permanent left coronary ligation (CL). Amiodarone significantly prolonged effective refractory periods (ERP) by 5.7% ($P<0.001$) and functional refractory periods (FRP) by 10.5% ($P<0.001$) in the non-ischaemic tissues compared with the values obtained from saline pre-treated rats, while polysorbate alone shortened ERP by 4.1% ($P<0.001$) but prolonged FRP by 5.6% ($P<0.001$).

CL produced an initial increase in both ERP and FRP followed immediately by a gradual shortening to significantly below pre-occlusion values from 4 to 30min (ERP) or 8 to 20 min (FRP) in the ischaemic ventricular myocardium of saline treated rats. ERP and FRP declined to minima at around 10 min after the initiation of CL which was coincidental with the highest observed level of arrhythmia in these animals. Amiodarone significantly prolonged both ERP and FRP at all times in the ischaemic areas compared with values at corresponding times after CL in saline treated rats. After 10 min of CL ERP was increased by 28.3% ($P<0.001$) and FRP by 17.2% ($P<0.001$) in amiodarone treated rats compared with the values in saline treated rats. Polysorbate 80 significantly shortened ERP by 11.3% ($P<0.05$) but produced no significant change in FRP.

The protective effects of amiodarone against cardiac arrhythmias will be discussed in the light of these electrophysiological effects of the drug.

We thank Sanofi U.K. Ltd for materials and information.

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SOME EFFECTS OF THE ENANTIOMERS OF THE POTASSIUM CHANNEL OPENERS, BRL34915 AND PINACIDIL, ON RAT BLOOD VESSELS

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BRL34915(± 6-cyano-3,4-dihydro-2,2-dimethyl-trans-4-(2-oxo-1-pyrrolidyl)-2H-benzo[b]pyran-3-ol and pinacidil (± N-cyano-N-4-pyridyl-N-1,2,2-trimethylpropyl-guanidine monohydrate) are recently developed antihypertensive agents which share a common mechanism of action. Both produce smooth muscle relaxation by opening K^+-channels in the membrane of vascular and other smooth muscle cells, an action which raises the membrane potential towards the potassium equilibrium potential (Hamilton et al., 1986; Southerton et al., 1987). In the present study, the effects of the two enantiomeric forms of BRL34915 and of pinacidil, alone and in combination have been examined on rat aorta and portal vein. Using this approach it was hoped to learn more about the mechanism and site(s) of action of these potassium channel opening drugs.

Whole portal veins and endothelium-free segments of rat aorta were removed from male Sprague Dawley rats (300-500g) and mounted for isometric tension recording in MOPS-buffered physiological salt solution at 37°C. Some portal veins were mounted under similar conditions in a glass capillary containing Pt ring electrodes for the extracellular measurement of electrical changes.

In portal vein, both (-) and (+) BRL34915 produced complete inhibition of spontaneous electrical and mechanical activity with IC50 values of 16 nM [(-)] and 3.1 μM [(+)]. Similar results were obtained with pinacidil (IC50 values of 81 nM [(-)] and 2.1 μM[(+)]. Pre-incubation for 6 min with either 100 nM (+) BRL34915 or 100 nM (+) pinacidil (the maximum concentration of the (+) enantiomers which failed to produced detectable inhibition) had no effect on inhibitory responses to either (-) BRL34915 or (-) pinacidil when these were re-examined in the continuing presence of the (+) enantiomers. In segments of rat aorta both (-) and (+) BRL34915 and (-) and (+) pinacidil were each capable of producing total relaxation of the contraction produced by 20 mM KCl (IC50 values 23.7 nM, 7.4 μM, 112 nM and 2.3 μM, respectively). In aortic segments contracted with 20mM KCl, exposure for 6 min to either 800 nM (+) BRL34915 or 400 nM (+) pinacidil had no effect on inhibitory responses elicited by either (-) BRL 34915 or (-) pinacidil in the continuing presence of the (+) enantiomers. These results confirm and extend the observations of Arrigoni-Martelli et al. (1980) and of Buckingham et al. (1986) on the isomers of pinacidil and BRL34915, respectively.

It is concluded that the differences in the potencies of the (+) and (-) enantiomeric forms of both BRL34915 and pinacidil result from differences in the affinities of the enantiomers for their site(s) of action, rather than from differences in their intrinsic efficacies if these were relatively low. The question of whether the two K-channel opening drugs share a common site of action remains unanswered.

Supported by the SmithKline Foundation, and by an SERC Case Award to D.T.N.. K.M.B. holds a Ciba-Geigy Studentship. The help of Leo Laboratories is gratefully acknowledged.

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EFFECTS OF ACIDOSIS AND Na^+/H^+ EXCHANGE ON ANOXIC NORADRENALINE RELEASE FROM THE HEART

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Investigation of noradrenaline (NA) release within the heart during myocardial ischaemia is complicated by concomitant change in a number of physiological processes and the severe degree of flow reduction required to induce nerve-impulse independent NA efflux. These problems can be partly overcome by studying anoxia at unchanged flow rate[1]. We have now studied the effects of acidosis, an early and marked feature of ischaemia, on anoxic NA release.

Experiments were performed on rat hearts perfused at constant flow (approx 5ml/g/min) by the Langendorff method with sampling of venous effluent for NA (radio - enzymatic) determination. All group sizes were >6 and values (pmol/g/min) are mean ± sem. Following a stabilization perfusion (pO_2>500mmHg, pH 7.4, glucose 5.5mM) hearts were subjected to substrate free, anoxic (PO_2< 1mmHg) perfusion without change in flow. Perfusate Na^+ concentration was 144.5mM unless otherwise stated.

NA overflow during normoxic perfusion was <2.5pmol/g/min. As reported previously anoxic substrate free perfusion leads to enhanced NA overflow after 15min (9.8 ± 3.5) rising to 46.6 ± 3.3 after 30 min. With a calcium free perfusate, or following a period of normoxic substrate free pre-perfusion (substrate-depleted), early anoxic NA overflow is increased. Reduction of pH (by altering HCO_3^- content) under these conditions suppresses this early NA overflow. Thus after 10 min anoxic perfusion, following substrate depletion, NA overflow was 22.5 ± 3.8 (pH 7.4) and 7.2 ± 2.3 (pH 6.5); after 15 min anoxic calcium free perfusion overflow was 18.3 ± 3.7 (pH 7.4) and 1.4 ± 0.6 (pH 6.5). At later times acidosis increased NA overflow - after 30 min anoxia (pH 7.4) overflows were 39.4 ± 4.3 (substrated depleted) and 59.6 ± 5.6 (calcium free) in contrast to 92.4 ± 16.9 and 97.5 ± 8.5 respectively during acidosis. Substitution of perfusate NaCl by LiCl leads to marked early overflow (145.9 ± 19.0 at 5 min, 82.6 ± 4.5 at 10 min) which is suppressed at pH 6.5 (14.5 ± 2.1 and 69.2 ± 12.0 respectively). At 15 min overflows were 70.0 ± 5.1 (pH 7.4) and 98.8 ± 6.9 (pH 6.5).

Amiloride (1mM) suppressed anoxic NA release (pH 7.4) both with 144.5mM and 25mM Na^+ (substituted with Li^+). Overflows (144.5mM Na^+) were 28.5 ± 4.0 (20 min) and 42.4 ± 3.9 (40 min) in the absence of amiloride and 11.8 ± 0.8 and 31.3 ± 4.3 respectively in the presence of amiloride.

These experiments demonstrate that during myocardial ischaemia concomitant acidosis would not be expected to lead to earlier non-exocytotic (carrier-mediated) NA efflux although later peak efflux may be increased. The mechanism for the biphasic effect of acidosis is not apparent. Suppression of anoxic release by amiloride may indicate a contribution to this release from Na^+ (Li^+) entry via the Na^+/H^+ antiporter.

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EVIDENCE FOR 5-HT_3-RECEPTOR MEDIATED RELEASE OF AN INHIBITORY TRANSMITTER IN GUINEA-PIG ISOLATED ILEUM

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Peripheral 'M' or $5HT_3$ receptors appear to mediate neuronal depolarisation (Bradley et al., 1986) which in the guinea-pig ileum leads to release of acetylcholine and substance P which initiate smooth muscle contraction (Buchheit et al., 1985). However, depolarisation of inhibitory neurones by activation of this receptor would cause smooth muscle relaxation. We have investigated this possibility in the guinea-pig ileum.

Dunkin-Hartley guinea-pigs (200-300g) were humanely killed and whole ileal segments dissected out 30cm from the pyloric sphincter and mounted in organ baths containing a modified gassed Krebs-Henseleit solution. Changes in tension were measured isometrically.

In preparations precontracted with histamine ($6x10^{-7}M$), the selective $5HT_3$ receptor agonist, 2-Me-5HT ($3x10^{-6}M$) caused a biphasic response which usually consisted of an initial contraction that faded within about a minute to reveal a relaxation. Both phases of the response were tachyphylactic unless a 30min interval was left between doses. Pretreatment with tetrodotoxin ($1x10^{-7}M$) abolished both components of the response. The selective $5HT_3$ antagonist, GR38032F ($1x10^{-7}M$; Brittain et al., 1987) also abolished the relaxation induced by 2-Me-5HT and reduced the contraction phase. Increasing the concentration of GR38032F ($1x10^{-6}M$) virtually abolished both phases of the response as did metoclopramide ($1x10^{-5}M$). Atropine ($1x10^{-7}M$) abolished the relaxation and reduced the contraction by 60%.

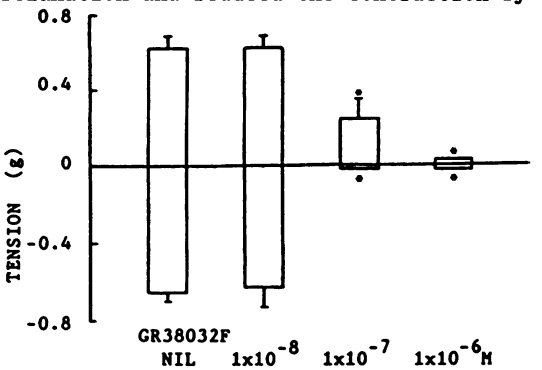

Fig. 1 The effect of GR38032F (30min pretreatment) on the biphasic response to 2-Me-5HT ($3x10^{-6}M$). Both phases of the response, namely contraction and relaxation, are shown. n=5-21. *$p<0.05$ (unpaired t-test; limits are s.e. mean).

We conclude that the quantification of the true potency of $5HT_3$ antagonists in this preparation is confounded by the complex motor response to 5HT mediated via neurones releasing both excitatory and inhibitory transmitters. The claim (Richardson & Engel, 1986) that the $5HT_3$ receptors in guinea-pig ileum are different to those in other preparations is unreasonable, on present evidence, since the putative $5HT_3$ receptor population(s) have yet to be individually characterised.

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CHARACTERISATION OF RECEPTORS FOR TACHYKININS IN GUINEA-PIG ISOLATED TRACHEA

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Tachykinins produce contraction in the isolated trachea of the guinea-pig, or bronchoconstriction in the whole animal, but the receptor mediating this effect has not been characterised in terms of the known receptor types: NK1 (SP-P), NK2 (SP-E), NK3 (SP-N). We have attempted such a classification by comparing the potencies of a number of tachykinins, paying particular attention to the determination of the relative potencies of [Glp^{6}L-Pro^9]SP-(6-11) and the D-Pro^9-analogue (Fletcher et al., 1987).

Spiral strips of trachea were suspended in organ baths containing Krebs solution at 37°C and responses were obtained by cumulative addition of agonists. Responses to most agonists were increased in the presence of indomethacin (1μM), suggesting the concomitant release of a prostanoid, possibly from epithelium, that produces relaxation; EC_{50} values were altered in some cases, but the rank order of potency was not much affected (Table 1).

Table 1 Potencies of tachykinins to contract the guinea-pig, isolated trachea

Tachykinin	EC_{50} (nM) control	n	EC_{50} (nM) indomethacin	n
Substance P	572	10	930	9
Neurokinin A	42	4	26	11
Neurokinin B	161	4	145	10
[Glp^6, L-Pro^9]SP-(6-11)	7	7	3	6
[Glp^6, D-Pro^9]SP-(6-11)	37	5	10	4
Suc-[Asp^6, Me-Phe^8]SP-(6-11)	10800	2	6020	4
GlpPhePhe(R)Gly[ANC-2]LeuMet.NH_2	11	6	2	6

With the mammalian tachykinins, the order of potency, NKA > NKB >> SP, is that for the NK2 receptor, e.g. in the rat vas deferens (McKnight and Maguire, 1978), but our findings with the other peptides, and in particular the exceptionally high potency of [Glp^6, L-Pro^9]SP-(6-11), make the conclusion that NK2 receptors are involved in mediating the contraction untenable (Fletcher et al., 1987). The low potency of Suc-[Asp^6, Me-Phe^8]SP-(6-11) (senktide), the selective agonist at NK3 receptors (Wormser et al., 1986), and the high potency of the lactam-containing peptide GlpPhePhe(R)Gly[ANC-2]LeuMet.NH_2 (Cascieri et al., 1986), make it difficult for us to reconcile our data with an important contribution of either NK3 or NK1 receptors. Although a definitive classification of receptors for tachykinins must await the development of selective antagonists, we feel that it would be not inappropriate for us to propose, on heuristic grounds, the existence of an NK4 receptor for tachykinins that is present in the trachea and mediates constriction of airway smooth muscle.

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EFFECTS OF NEUROPEPTIDES ON TRACHEAL VASCULAR RESISTANCE IN ANAESTHETIZED DOGS

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Neuropeptides may be responsible for the noncholinergic component of parasympathetic vasodilatation in the airway (Martling et al, 1985; Laitinen et al, 1986) and contribute to airway wall inflammation in asthma (Barnes, 1986). Recently, substance P and vasoactive intestinal peptide (VIP) were shown to be much more powerful than classical transmitters as dilators of tracheal vessels (Laitinen et al, 1987). We have studied neurokinin A (NKA), calcitonin gene-related peptide (CGRP), peptide histidine isoleucine (PHI) and neuropeptide tyrosine (NPY) on the same preparation.

Greyhounds (21-31 kg) of either sex were anaesthetized with pentobarbitone (30 mg. kg^{-1}), heparinized, and their lower tracheas were cannulated. We measured tracheal vascular resistance (R_{tv}) by perfusing the cranial tracheal arteries on both sides, as described previously (Laitinen et al, 1986), and tracheal muscle tone was assessed from pressure changes in a balloon in the cervical trachea. Neuropeptides were given as close arterial injections in 0.2 ml saline to the perfusion circuits on both sides of the trachea (n = 15-18).

All neuropeptides produced dose-related changes in R_{tv}. NKA (0.02-20 pmol) was the most powerful vasodilator, reducing ipsilateral R_{tv} by 1.7 ± 1.2 (mean ± S.E.M.) to 28.8 ± 3.6%. CGRP was about 10 times and PHI about 100 times less potent than NKA as a tracheal vasodilator. The corresponding reductions in R_{tv} were between 3.2 ± 2.7 and 37.2 ± 6.9% by CGRP (2-200 pmol) and between 1.9 ± 1.1 and 32.9 ± 7.1% by PHI (0.02-2 nmol). According to ED_{25} values (dose causing 25% decrease in R_{tv}) in our study and that of Laitinen et al (1987), the order of potency of vasodilator neuropeptides is NKA > VIP > CGRP ≥ substance P > PHI. NPY (0.02-2 nmol) constricted tracheal vessels, increasing R_{tv} by 3.9 ± 1.2 to 27.0 ± 7.3%. The largest doses of CGRP and NPY caused long-lasting vascular effects with more than half of the responses still present after 10 min. NKA and PHI responses usually subsided within 1-2 min. None of the neuropeptides influenced the systemic arterial blood pressure. Contralateral R_{tv} responses were usually 15-40% of the corresponding ipsilateral values. PHI (1 and 2 nmol) relaxed the tracheal smooth muscle, but the other neuropeptides were inactive.

In conclusion, the neuropeptides tested were strongly vasoactive. The longer-acting neuropeptides CGRP and NPY may be more important than the shorter-acting NKA and PHI in the regulation of airway blood flow.

*Supported by the Academy of Finland, the Kivi and Montin Foundations (Finland), and the Finnish Antituberculosis Association.

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INDENTIFICATION OF INTESTINAL ANGIOTENSIN II RECEPTORS WITH A RADIOLABELLED ANTAGONIST, ^{125}I-[SAR^{1}.THR^{8}]-AII

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Angiotensin II (AII) exerts a dual action upon gastrointestinal epithelia. At concentrations of 1pM-1nM electroneutral NaCl absorption is stimulated in rat intestine while NaCl secretion occurs at 1nM-1μM AII, apparently via both electroneutral (Levens et al., 1981) and electrogenic mechanisms (Cox et al. 1987). ^{125}I-AII binding sites have been identified in preparations of rat jejunum and descending colon epithelia (Cox et al., 1986) but these studies were severely restricted by ligand hydrolysis. In order to obtain more accurate estimations of binding affinity and capacity, a more stable ligand was sought. The monoiodinated antagonist [Sar^{1}.Thr^{8}]-AII was chosen since it exhibits resistance to enzymmatic hydrolysis in vitro and is a potent antagonist of AII responses in many target tissues including Cl secretion in rat jejunum (Cox et al., 1987).

Epithelial membranes were prepared according to Manning et al. (1982). Final pellets were resuspended in hypotonic solution containing 10mM triethanolamine HCl and 0.1mM phenylmethylsulphonyl fluoride (PMSF), pH 7.6. Membranes were incubated with ^{125}I-[Sar^{1}.Thr^{8}]-AII (s.a. 2000 Ci/mmol, N.E.N., Boston, USA) at 22°C in 20mM Tris-HCl buffer, pH 7.4, containing 120mM NaCl, 5mM Na_2 EDTA, 0.1mM PMSF, 0.2% BSA. Incubations were terminated by filtration over GF/B filters (pretreated with 0.1% polyethylenimine) under vacuum. Nonspecific binding was assessed by addition of 1μM unlabelled [Sar^{1}.Thr^{8}]-AII. Both free and membrane bound ligand were assessed by thin layer chromatography (t.l.c.) using a butanol: acetic acid: water (4:1:5) solvent system. Protein determinations were performed according to Bradford (1976).

Specific ^{125}I-[Sar^{1}.Thr^{8}]-AII binding was linear with increasing protein and reached equilibrium by 5 min at 22°C. Upon development of cellulose t.l.c. plates ^{125}I-[Sar^{1}.Thr^{8}]-AII ran with an Rf of 0.3±0.03 (n=3). After 5 min incubations at 22°C 59.2±19.2% of free and 92.6±2.2% of membrane bound peptide remained intact (n=3). Analysis of saturation data yielded K_D of 4.39±1.4nM (n=3) and B_{max} 414.2±124.7 fmol.mg^{-1} in preparations of jejunum epithelia while descending colon preparations exhibited a K_D of 2.55±0.6nM (n=3) and B_{max} 354.6± 118.6 fmol.mg^{-1}. The order and potency of a range of analogues at displacing antagonist binding was very similar to that achieved with ^{125}I-AII binding in jejunum epithelial membranes. All displacement curves were monophasic and (with the exception of AI, AII, AIII and renin substrate (RS)) exhibited Hill Coefficients close to 1.0. IC_{50} values in nM are given in parenthesis [Sar^{1}.Thr^{8}]-AII (4.5) AII (4.8) [Sar^{1}.Leu^{8}]-AII (5.0) [Sar^{1}.Ile^{8}]-AII (8.5) [Sar^{1}.Ala^{8}]-AII (18.5) [Ile^{8}]-AIII (66.0) AIII (175.0) AI (425.0) RS (1000.0).

^{125}I-[Sar^{1}.Thr^{8}]-AII has identified specific AII binding sites in epithelial preparations from rat intestine where AII elicits well characterised responses. The specificity of ligand binding corresponds closely with the order of antagonist analogue inhibition of AII induced Cl secretion in jejunum and therefore probably represents the receptor protein involved in this response.

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PROSTANOID CONTRACTILE RECEPTORS ON GUINEA-PIG AND HUMAN AIRWAYS

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The following compounds are invaluable tools for the classification of prostanoid receptors:- the Thromboxane A_2 (TXA_2) mimetic U46619, the TXA_2 antagonists AH23848 (Brittain et al 1985) and EP092 (Jones et al 1982), a selective agonist and an antagonist of PGE contractile receptors 16,16dimethylPGE$_2$ (DME_2) (Jones et al 1982) and AH6809 respectively (Coleman et al 1985) and fluprostenol a specific $PGF_{2\alpha}$ mimetic. These compounds were used to compare the prostanoid contractile receptors present in the human lung strip (HLS), guinea-pig lung strip (GPLS) and guinea-pig tracheal ring (GPTR). Tissues were set up in 10ml baths containing Tyrodes solution and flurbiprofen 8×10^{-6}M at 37°C, and gassed with carbogen. Tension generation was measured isometrically.

The rank order of agonist potency was U46619 > DME_2 > $PGF_{2\alpha}$ >> fluprostenol = 0 in the HLS and GPLS, and DME_2 > U46619 >> $PGF_{2\alpha}$ >> fluprostenol = 0 in the GPTR. Fluprostenol did not contract any of the tissues indicating the absence of a selective $PGF_{2\alpha}$ contractile receptor.

TABLE 1 Prostanoid antagonist potency

ANTAGONISTS	HLS	GPLS			GPTR		
	U46619	U46619	DME_2	$PGF_{2\alpha}$	U46619	DME_2	$PGF_{2\alpha}$
EP092	8.9	8.7	8.7*	+	8.7	< 6	< 6
AH23848	8.7*	8.7*	8.5	+	8.5*	< 6	< 6
AH6809	< 6	< 6	< 6	< 6	< 6	7.2	6.9

* pK_b values, + Marked block at 10^{-6}M.

U46619-induced contractions were blocked by the TXA_2 antagonists in all three tissues (TABLE 1). EC_{50} and pA_2 values were similar indicating that the TXA_2 receptors in these tissues are essentially the same. DME_2 was most potent in the GPTR where its effects were blocked by AH6809 demonstrating the presence of PGE contractile receptors. In the GPLS the contractile effects of DME_2 were blocked by the TXA_2 antagonists, but not by AH6809 indicating that in the GPLS DME_2 acts via a TXA_2 receptor. The contractile effects of $PGF_{2\alpha}$ were blocked by the TXA_2 antagonists in the GPLS and by AH6809 in the GPTR, demonstrating that $PGF_{2\alpha}$ can induce contractile effects through TXA_2 or PGE receptors.

The data suggests that prostanoid contractile effects are mainly mediated through TXA_2 receptors in the HLS and GPLS and through TXA_2 and PGE sensitive receptors in the GPTR. The TXA_2 receptors in all three tissues appeared to be very similar. The study also demonstrates that prostanoid agonists (excluding U46619) seem to act on more than one prostanoid contractile receptor or receptor subtypes whereas the antagonists are more selective.

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INTERLEUKIN 1, TUMOUR NECROSIS FACTOR, LEUKOTRIENE B_4 AND HUMAN POLYMORPHONUCLEAR LEUKOCYTE CHEMOKINESIS

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Interleukin-1 (IL-1) and tumour necrosis factor (TNF) are polypeptides which are secreted by stimulated macrophages and exhibit similar pro-inflammatory properties (Dayer et al., 1986; Pohlman et al., 1986). In this study we have compared IL-1, TNF and leukotriene (LT) B_4 as stimulators of human polymorphonuclear leukocyte (PMN) chemokinesis in the agarose microdroplet assay (Smith & Walker, 1980).

Human PMNs were prepared as described by Boyum (1968). Aliquots of $4x10^5$ PMNs suspended in a 2 μl droplet of agarose 0.2% (w/v) in medium RPMI 1640 containing 5% heat-inactivated foetal calf serum were placed in the centre of flat-bottomed microtitre wells (Sterilin 29AR). The agarose droplet was then overlaid with 90 μl medium RPMI 1640, followed by the addition of 10 μl medium containing test agents or appropriate vehicles. The microtitre plates were then incubated at 37°C (5% CO_2, 100% humidity) for 18 h. All results are expressed as migration index (MI) which is the migration distance in the presence of test agent divided by the distance in the presence of vehicle.

IL-1 (Genzyme Ultrapure, 0.1 to 10 LAF units/ml, specific activity $8x10^9$ units/mg) did not produce a significant ($p > 0.05$) alteration in MI, while LTB_4 (0.1 and 1.0 nM) produced MIs of 1.38 ± 0.15 ($p \leq 0.01$) and 2.97 ± 0.37 ($p < 0.005$) respectively (n = 2 - 9 donors). In a second series of experiments, LTB_4 at 1.0 and 10 nM produced MIs of 3.86 ± 0.39 ($p < 0.05$) and 7.77 ± 2.32 respectively; however, human recombinant (hr) IL-1α (Hoffman La Roche; 48-4800 pg/ml; specific activity $2.1x10^7$ LAF units/mg), hrIL-1β (Ciba-Geigy; 48-4800 pg/ml; $2-4x10^7$ LAF units/mg), and hrTNFα (Boehringer Institute; 0.5-50 ng/ml; $6x10^7$ cytolytic units/mg) failed to produce a significant ($p > 0.05$) increase in MI compared to control (n = 2 - 5 donors). The bioactivity of the TNF and IL-1 preparations was confirmed by their ability to stimulate PGE_2 production by synovial cells in culture and cause PMN accumulation in rabbit skin 4 h after intradermal injection (assessed by histological techniques).

Furthermore, hrIL-1α or hrIL-1β or hrTNFα (480 pg/ml) added to the PMNs with LTB_4 (0.1-10 nM) did not significantly modify the dose-related LTB_4-induced migration index. These results indicate that IL-1 and TNF at biologically active concentrations do not directly induce human PMN chemokinesis *in vitro*, in contrast to LTB_4 (Ford-Hutchinson et al., 1980). Thus secondary cells are probably responsible for the ability of IL-1 to induce PMN infiltration *in vivo* (Pettipher et al., 1986).

We are most grateful to Drs A Stern and PT Lomedico (Hoffman La Roche, USA), Dr GR Adolf (Boehringer Institute, FRG) and Dr K Muller (Ciba-Geigy, Switzerland) for the gifts of hr IL-1α, hrTNFα and hrIL-1β respectively. We thank Ciba-Geigy (USA) for financial assistance. MW is an MRC student.

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THE EFFECT OF N-ACETYL LEUKOTRIENE E_4 ON GUINEA-PIG AIRWAY SMOOTH MUSCLE *IN VITRO*

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Leukotriene (LT) E_4 is a metabolite of LTC_4 and can be further converted to N-acetyl LTE_4 (N-acLTE_4). Recently this N-acetylated metabolite has been isolated from subcellular fractions of liver and microsomal preparations of spleen, kidney, skin and lung in the rat (Bernström & Hammarström, 1986). Since the cysteinyl-containing LTs are very potent bronchoconstrictors, we found it of interest to investigate whether N-acLTE_4 retained biological activity in guinea-pig respiratory smooth muscle *in vitro*.

Strips of guinea-pig lung parenchyma (GPP) were prepared from the major lobes. Guinea-pig isolated tracheae (GPT) were opened by a longitudinal cut on the ventral side and cut in a zig-zag fashion. Experimental tissues were superfused in series with oxygenated Tyrode's solution at 37°C and LTs were administered as bolus injections into the superfusing buffer. In addition, responses due to N-acLTE_4 on GPP were also studied in the presence of FPL 55712 and indomethacin, the cysteinyl-containing LT antagonist and cyclo-oxygenase inhibitor respectively, which were administered as continuous infusions over the tissues. In all cases, n ranges between 5 and 15.

N-acLTE_4 (0.1 - 10 nmol) was approximately two orders of magnitude less active than LTD_4 (3 - 100 pmol) in causing dose-related contractions of GPT. Contractions induced by N-acLTE_4 were of similar duration to those elicited by LTD_4 and considerably longer-lasting than responses due to histamine. In the presence of indomethacin (2.8 uM), contractions caused by N-acLTE_4 were enhanced, as previously described for other cysteinyl-containing LTs (see Samhoun & Piper, 1986).

N-acLTE_4 was more active on GPP than on GPT and contracted the GPP in a dose-dependent manner when administered at 0.01 to 3 nmol. It was approximately equiactive with LTE_4 (0.01 - 1 nmol) and was 10 times less active than LTD_4 (1 - 300 pmol). Further, contractions induced by both N-acLTE_4 and LTE_4 were almost identical and more sustained than those produced by either LTD_4 or histamine. FPL 55712 (1.9 uM) antagonised the action of N-acLTE_4 on the GPP as already reported for the other cysteinyl-containing LTs. Indomethacin (2.8 uM) inhibited contractions caused by N-acLTE_4; contractions caused by 100pmol and 300pmol N-acLTE_4 were inhibited by $77\pm6\%$ and $58\pm5\%$ respectively, indicating that cyclo-oxygenase products are involved in its action on GPP.

It is of interest that metabolism of LTC_4 to N-acLTE_4 does not represent a major inactivation step as the latter still retains considerable biological activity in the preparations described above. In the event of the formation of N-acLTE_4 in the airways or its presence in the circulation, this LT metabolite may have a pathological role in respiratory diseases by virtue of its activity and long-lasting actions in isolated airways.

We thank the Frances and Augustus Newman Foundation for financial support and Dr J Rokach, Merck Frosst, for the gift of leukotrienes.

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EFFECT OF LEUCOPAENIA ON THE DEVELOPMENT OF ANTIGEN-INDUCED ARTHRITIS

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Leucocyte infiltration and cartilage degradation are characteristic features of rheumatoid joints, but it is not known whether there is a causal relationship between these two parameters. We have investigated the effect of depleting the circulating polymorphonuclear leucocytes (PMNs) and monocytes on the development of antigen-induced arthritis in the rabbit (Dumonde and Glynn, 1962).

After sensitisation of New Zealand White rabbits (2.5-3.0 kg) to ovalbumin in Freund's complete adjuvant, some animals received an intravenous injection of 1.0 - 1.75 mg/kg nitrogen mustard (Mustine; Boots; Nottingham). Three days after injection of Mustine, the animals were challenged by injection of 5 mg ovalbumin into one knee joint. Circulating leucocyte levels were monitored before and after Mustine-treatment and during the period of arthritis. Joint diameters were measured by calipers at regular intervals after challenge. Animals were sacrificed at 1 or 4 days after challenge and leucocyte infiltration into the joint fluid assessed. The loss of proteoglycan from the cartilage of challenged joints was determined as previously described (Pettipher et al., 1986).

A single injection of Mustine reduced the circulating levels of PMNs from $5.3 \pm 1.6 \times 10^6$ cells/ml (mean ± SEM, n=6) to $<10^5$ cells/ml (n=13) by 3 days after injection. The circulating monocytes were reduced from $6.1 \pm 1.1 \times 10^5$ cells/ml (n=10) to $1.1 \pm 0.2 \times 10^5$ (n=13). The circulating PMN and monocytes remained depressed for a further 2 days. In contrast, the numbers of circulating platelets were unaffected at these times and the numbers of circulating lymphocytes were only partially reduced from $5.1 \pm 0.6 \times 10^6$ cells/ml (n=6) before injection to $2.2 \pm 0.3 \times 10^6$ cells/ml (n=6) at day 4 after injection (the lowest level measured). A further injection of Mustine at the time of challenge maintained the suppression of the circulating PMNs and monocytes while the lymphocytes were relatively spared ($1.2 \pm 0.6 \times 10^6$ cells/ml, n=3, at day 4 in the arthritis).

Intra-articular challenge with ovalbumin resulted in high PMN infiltration ($29.3 \pm 5.8 \times 10^6$ cells/ml, n=7) into the joints of control animals and this was accompanied by joint swelling of 5.9 ± 0.3mm (n=11). In contrast, animals pretreated with Mustine had few PMN in the challenged joints ($0.8 \pm 0.3 \times 10^6$ cells/ml, n=10) and significantly ($p < 0.01$) diminished joint swelling (0.7 ± 0.3mm, n=13) at day 1 after antigen challenge. Four days after antigen challenge of Mustine-treated animals the numbers of leucocytes infiltrating the joints returned to control values but this was not accompanied by joint swelling which remained inhibited up to 17 days after challenge. Despite the markedly diminished PMN infiltration there was no inhibition of the cartilage proteoglycan degradation at day 1. At day 4 after antigen challenge the cartilage proteoglycan degradation was significantly ($p < 0.05$) enhanced compared to control animals at this time (24.4 ± 5.4%, n=6, loss of proteoglycan in control animals compared to 38.8 ± 1.5%, n=5, loss in animals treated with 2 doses of Mustine).

These results are consistent with the hypothesis that leucocytes contribute to acute joint swelling but suggest that the infiltrating PMNs and monocytes are unlikely to be mediating the cartilage degradation.

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INDUCTION OF RAT GASTRIC DAMAGE BY CLOSE-ARTERIAL INFUSION OF PAF AND THE THROMBOXANE MIMETIC, U-46619

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PAF-acether induces extensive gastric mucosal damage following intravenous infusion in the rat (Rosam et al, 1986). In order to investigate the local actions of low doses of PAF on gastric mucosal integrity, we have now developed a technique for the close-arterial infusion of such pro-ulcerogenic mediators directly into the rat left gastric artery. Using this technique, we have also investigated the local actions of the thromboxane mimetic, U-46619, which is a potent vasoconstrictor in the rat gastric microcirculation (Whittle et al, 1985).

Male rats (250g) were anaesthetised with pentobarbitone and the stomach exposed by a mid-line incision. The left gastric artery was freed from connective tissue under a steriomicroscope and cannulated with a modified short 23g plastic cannula. Patency of the cannula was assured by observing the back flow of blood derived from the mucosal and submucosal vascular network.

Infusion of the vehicle, 0.25% bovine serum albumen - 0.9% saline (12.5 µl/min) into the left gastric artery for 10 min, with the stomach being removed 20 min later, did not cause any macroscopic damage or histological disruption, determined in 4µM wax-embedded sections stained with haemotoxylin and eosin. Close-arterial infusion of PAF (5-25ng/kg/min for 10 min) induced significant ($P<0.05$) dose-related damage, macroscopically apparent as diffuse vasocongestion, with areas of haemorrhage at the higher doses, located predominantly in the upper corpus region. With doses of PAF (10 and 25ng/kg/min) this damage involved 7±1% (n=10) and 21±4% (n=8) of the total mucosal area respectively, as determined by computerized planimetry. Histologically, there was dose-related epithelial cell disruption, deep vasocongestion and necrosis. Intravenous infusion of PAF (25 ng/kg/min) caused a fall in systemic arterial blood pressure (BP; 34±5 mmHg, n=7) which was comparable to that following local intra-arterial infusion of this dose, yet induced only minimal mucosal damage.

Instillation of 20% ethanol (1ml) into the gastric lumen caused no macroscopically apparent mucosal damage. However concurrent close-arterial infusion of PAF (0.25 and 1 ng/kg/min for 10 min) in doses having no significant effect in BP, induced significant ($P<0.05$), haemorrhagic damage in the corpus mucosa, involving 14±5% (n=4) and 22±5% (n=5) of the mucosa respectively. Intra-arterial infusion of U-46619 (25-50 ng/kg/min) under these conditions likewise induced areas of localised haemorrhagic mucosal damage but did not affect BP. With U-46619 (50ng/kg/min for 10 min) the damage involved 16±6 % (n=4) of the mucosa. In addition, distinct focal lesions in the antral region were observed.

The current study indicates that low doses of either PAF or the thromboxane mimetic can induce or potentiate gastric mucosal damage following close-arterial administration in the rat. These observations support the concept that the local release of such mediators may be involved in the pathogenesis of gastric ulceration.

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LEUKOTRIENES POTENTIATE PARASYMPATHETIC NEUROTRANSMISSION IN GUINEA-PIG TRACHEA BY PRE- AND POST-JUNCTIONAL MECHANISMS

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Apart from their direct bronchospastic properties, leukotrienes are known to sensitize bronchial smooth muscle to several inflammatory mediators. However, the effect of the leukotrienes on transmission in the pulmonary parasympathetic nerves, which exert a dominant controlling action on airway smooth muscle, has not been studied. The present experiments were designed to test the effect of the peptidoleukotrienes, LTC4 and LTD4 on contractions of tracheal smooth muscle elicited by stimulation of the parasympathetic nerves. The effects on contractions elicited by exogenous ACh and by histamine were studied for comparison.

Guinea-pigs (200-300g) were anaesthetised with urethane (1.5g/kg) and the trachea removed with the right vagus attached (Blackman & McCaig, 1983). The trachea was suspended in Krebs solution oxygenated with 95% O / 5% CO_2 at 37°C. Contractions of the trachealis muscle were elicited either by vagal nerve (preganglionic) or transmural stimulation in the presence of hexamethonium (post-ganglionic), (30Hz, 0.2 ms, 150 pulses). Responses were measured as increases in intraluminal pressure with a Statham (p23A) transducer. Indomethacin (20 μM) was present throughout.

Under these conditions, LTC4 and LTD4 caused contraction of the trachealis muscle; the leukotrienes were approximately 10 to 50 fold more potent than ACh and 100 fold more potent than histamine. LTC4 and LTD4, in a concentration of 5 nM which caused only a small increase in tone, produced 40-50% potentiation of the contractile responses to both preganglionic and transmural stimulation. The contractile response to exogenous ACh and methacholine were also potentiated but histamine-induced contractions were unaltered. When the leukotriene concentration was increased to 50 nM, the response to exogenous ACh declined to control values but the response to nerve stimulation was still potentiated. These effects were abolished by the leukotriene antagonist, L 649, 923 (2 μM).

The results indicate that the peptidoleukotrienes, in doses which do not elicit significant contraction of tracheal smooth muscle, potentiate parasympathetic neurotransmission in the trachea. This is due (i) to a pre-junctional action, possible via an increase in transmitter output, and (ii) to a post-junctional action. The latter effect appears to involve mechanisms secondary to muscarinic receptor activation as histamine-induced contraction was unaltered.

We wish to thank D.J. Rokach of Merck Frosst, Canada for gifts of leukotrienes and L 649, 923.

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BRADYKININ STIMULATES ELECTROGENIC BICARBONATE SECRETION BY GUINEA-PIG GALLBLADDER

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The actions of bradykinin (BK) on mammalian gallbladder include contraction of smooth muscle (Kubata et al., 1985) and stimulation of sensory neurones (Ordway & Longhurst, 1983). Since electrolyte-dependent fluid secretion occurs across gallbladder epithelium and BK is known to affect such processes elsewhere, we studied the effects of BK on ion transport in this tissue. Hemi gallbladders from female guinea pigs were mounted in Ussing chambers (window area 0.6 cm^2) bathed on either side with identical Krebs-Henseleit solutions. The electrical potential difference was maintained at zero by continuous passage of short circuit current (SCC). Resting SCC was -0.4±0.9 μA (n=36) and the transepithelial conductance was 24.5±1.7 mS (n=36). Bradykinin (BK) added to the bathing solution stimulated, within a minute, a sustained inward current characteristic of anion secretion, cation absorption, or both. BK was more effective when added mucosally (ED_{50} = 0.2 μM) than serosally (ED_{50}=10 μM). The SCC response to 1 μM BK (mucosal) was 40.9±2.6 μA (n=30) with no change in conductance. Cumulative dose response curves to BK (0.01-10 μM) were unaffected by 1 mM amiloride on the mucosal side or 1 mM piretanide on the serosal side but were attenuated by acetazolamide added to both bathing solutions. 1 mM of this carbonic anhydrase inhibitor reduced the maximal response to BK to 56% of the corresponding control responses (n=5) and the dose response curves were different ($P<0.01$) when compared by 2-way analysis of variance. Collectively, these results establish that BK stimulation of gallbladder SCC is due, at least in part, to electrogenic bicarbonate secretion. The effects of BK were not altered by tetrodotoxin (1 μM)) suggesting neurones are not involved in mediating this phenomenon.

Kinins also stimulate electrogenic chloride secretion in rat colon (Cuthbert & Margolius, 1982) partially mediated by eicosanoid production (Cuthbert et al., 1984). The SCC response to BK in gallbladder was abolished by the cyclooxygenase inhibitor piroxicam (1 μM). This action of piroxicam was completely reversible. PGE_2 stimulated SCC equally well regardless of the side to which PGE_2 was added (ED_{50}=0.01 μM). Mucosal side application of PGE_2 (0.1 μM) produced SCC values of 74.8±7.4 μA (n=5), a greater stimulation than that achieved with BK even at ten times higher concentrations. Thus BK appears to exert its effect on gallbladder bicarbonate secretion by stimulation of eicosanoid synthesis.

The gallbladder response to BK differs from that of intestinal tissue (Cuthbert et al., 1984) making this an interesting site for the study of effects of kinins on epithelial ion transport.

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DEFICIENCY DOWNSTREAM OF CAMP GENERATION PREVENTS CHLORIDE SECRETION IN COLONY 3 EPITHELIAL CELLS

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Colonic epithelia exhibit electrogenic chloride secretion in response to a variety of secretagogues which either increase intracellular cAMP, intracellular Ca or both. We describe here a human cell line with a very reduced potential for responding to cAMP generating stimuli, a characteristic typical of epithelia in cystic fibrosis.

Two stable cell lines, derived from a single human colonic adenocarcinoma, designated HCA-7 and Colony 3 were cultured on collagen coated Millipore filters and used to investigate transepithelial ion transport, measured by recording short circuit current (SCC). All the SCC responses were inhibitable by piretanide and therefore are presumed to represent anion secretion, presumably of chloride.

Using near-maximally effective concentrations of three secretagogues the responses in HCA-7 monolayers were compared with those prepared with Colony 3 cells. The responses in Colony 3 cells were 0%, 8.5% and 29.5% respectively of those with HCA-7 using lysylbradykinin (LBK, 100nM), carbachol (10μM) and vasoactive intestinal polypeptide (VIP, 10nM). All agents were added to the basolateral side of the tissue.

As these results may have been dependent upon different receptor reserves we bypassed receptors by using forskolin (10μM) and A23187 (10μM) respectively to increase intracellular cAMP and Ca_i. Both types of monolayer gave identical responses to A23187 yet the responses to forskolin in Colony 3 were only 13.6% of those measured with HCA-7. Inhibition of PDE with IBMX did not improve the SCC responses to forskolin in colony 3 cells.

In HCA-7 monolayers it appears there is synergism between stimuli which generate calcium and cAMP signals. For example carbachol and LBK, which generate calcium signals, are potentiated by forskolin and VIP. Similar potentiation could not be demonstrated with Colony 3 monolayers. For example forskolin did not potentiate the responses to carbachol, even though the former increased tissue cAMP.

We conclude there is a defect downstream of cAMP generation, which is not due to excess phosphodiesterase, that prevents Colony 3 cells from secreting chloride. Apical chloride channels and the basolateral NaKCl triporter are presumably present as these cells secrete chloride in response to A23187 and this effect is inhibited by piretanide.

Supported by grants from CRC, NIH (HL-17705) and Cystic Fibrosis Research Trust.

THROMBIN STIMULATES THE INFLUX OF DIVALENT CATIONS INTO HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS LOADED WITH FURA-2

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Thrombin causes a sustained increase in the concentration of cytoplasmic free calcium, $[Ca^{2+}]_i$, in human umbilical vein endothelial cells in the presence but not in the absence of extracellular calcium (Hallam & Pearson, 1986a). One possible explanation is that thrombin promotes a maintained influx of Ca^{2+} across the plasma membrane. I have tested this hypothesis by examining the effects of removing the thrombin and changing external Ca^{2+}, Mn^{2+} and Ni^{2+} concentrations on the fluorescence of intracellular fura-2.

Human umbilical vein endothelial cells grown to confluence on glass coverslips were loaded with fura-2 and placed in a cuvette containing 145mM NaCl, 5mM KCl, 1mM $MgSO_4$, 10mM glucose, 10mM Hepes at pH 7.4 with 1mM $CaCl_2$ or 1mM EGTA in a dual wavelength excitation fluorimeter at 37°C. Fluorescence and $[Ca^{2+}]_i$ were measured as previously described (Hallam & Pearson, 1986b). In 1mM external Ca^{2+} thrombin, 0.5U/ml, caused a rapid increase in $[Ca^{2+}]_i$ to 2-3uM. $[Ca^{2+}]_i$ then fell to about 1uM and this level was maintained for at least 10 minutes. Addition of 2mM EGTA or 1U/ml hirudin, a thrombin ligand, during this maintained steady-state phase curtailed the response and $[Ca^{2+}]_i$ decreased back to the resting pre-stimulated level in about 30s. With 1mM EGTA, thrombin caused a rapid transient rise in $[Ca^{2+}]_i$ that returned to the basal pre-stimulated level within 90s despite the continued presence of agonist. Addition of 2mM $CaCl_2$ now caused a rise in $[Ca^{2+}]_i$ and a maintained elevated level of c. 1uM was established. Subsequent addition of excess hirudin decreased $[Ca^{2+}]_i$ to the resting level again. These results show that the maintained elevated $[Ca^{2+}]_i$ needs both agonist and external Ca^{2+}.

Mn^{2+} and Ni^{2+} both bind avidly to fura-2 and quench its fluorescence. It has been shown in human blood platelets loaded with quin2 that thrombin can cause the stimulated quench of cytoplasmic dye fluorescence in the presence of extracellular Mn^{2+} but not Ni^{2+}, demonstrating that stimulated influx of Mn^{2+} but not Ni^{2+} can occur (Hallam & Rink, 1985). Similar results were obtained with fura-2 -loaded endothelial cells. In the presence of 0.5mM Mn^{2+} thrombin caused a rapid quench of the intracellular fura-2 fluorescence. No stimulated quench could be produced using 2mM Ni^{2+}. In Ca^{2+}-containing medium in the presence of 2mM Ni^{2+}, thrombin caused a transient elevation in $[Ca^{2+}]_i$ that returned to basal levels a response similar to that observed in the absence of extracellular Ca^{2+}. 2mM Ni^{2+} also inhibited the stimulated quench of fura-2 fluorescence normally seen with 0.5mM Mn^{2+}, suggesting that Ni^{2+} competes at the translocation site with Ca^{2+} or Mn^{2+} and prevents their influx. These results provide evidence that thrombin causes the influx of Ca^{2+} (and Mn^{2+}) and are consistent with the proposal that a receptor-operated Ca^{2+} channel is present in the endothelial cell plasma membrane.

I thank Dr J.L. Gordon and colleagues at the MRC C.R.C., Harrow for cells.

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EFFECT OF FORSKOLIN, DIDEOXYFORSKOLIN AND PGE_1 ON STIMULUS SECRETION COUPLING IN CHROMAFFIN CELLS

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Looking at noradrenaline release from cultured chromaffin cells we have previously reported that forskolin (10^{-5} M) treatment reduced nicotine-stimulated release but increased high K^+ or calcium ionophore-stimulated release (Adams et al. 1986). These results suggested that the effect of forskolin on nicotine stimulus secretion coupling was at the nicotinic receptor, or proximal to it. Here we report the effect of forskolin, dideoxyforskolin (DDF) and prostaglandin E_1 (PGE_1) on cyclic AMP accumulation and stimulus secretion coupling. This is part of a study investigating the hypothesis that stimulation of cyclic AMP dependent protein kinase reduces the efficacy of nicotinic stimulus secretion coupling. DDF is a forskolin analogue reported to be ineffective in stimulating adenylate cyclase (Seamon et al. 1984)

Bovine adrenal medulla cells were maintained in culture in 24 well multiwell plates for 4-7 days prior to use. A preincubation of 12 min. preceded the 3 min. stimulation period, with forskolin, DDF or PGE_1 present throughout where appropriate. Cyclic AMP in cell extracts was measured with a protein binding assay, while noradrenaline released into the supernatants and in cell extracts was measured by high pressure liquid chromatography with electrochemical detection.

Both forskolin and DDF at 10^{-5} M reduced nicotine stimulated release, while only forskolin stimulated cyclic AMP levels, shown in the table.

Table 1 Release of noradrenaline and content of cyclic AMP

	Nicotine (3×10^{-5} M)	Nicotine + Forskolin (10^{-5} M)	Nicotine + DDF (10^{-5} M)
Noradrenaline release (% of cell content)	20.40 ± 0.37	6.69 ± 0.37	3.50 ± 0.34
Cyclic AMP (pmol/well)	2.67 ± 0.14	30.48 ± 0.61	2.37 ± 0.08

Mean ± SEM, n = 4

Forskolin gave an EC_{50} for noradrenaline release attenuation of about 6×10^{-5} M, and for cyclic AMP content of about 10^{-6}M; for DDF the EC_{50} for inhibition of noradrenaline release was about 2.5×10^{-6} M, with no effect on cyclic AMP accumulation. DDF also inhibited 50 mM K^+ stimulated release to about 30% of control, the opposite effect to that seen with forskolin. PGE_1 inhibited nicotine stimulated release by about 50% and increased cyclic AMP accumulation (EC_{50} about 3.5×10^{-6} and 2×10^{-6} respectively). Potentiation of PGE_1 stimulation of adenylate cyclase by 0.2×10^{-6} M forskolin enhances the accumulation of cyclic AMP in response to PGE_1, but does not lead to a substantial change in inhibition of noradrenaline release in response to nicotine.

The results show that DDF has an inhibitory influence on release which is different from that of forskolin in that it applies to high K^+ as well as nicotine stimulation. The dose-response curves to forskolin and PGE_1 show that the relationship between inhibition of nicotinic stimulus secretion coupling and cyclic AMP accumulation is not a simple one.

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THE EFFECT OF BUMETANIDE, OUABAIN AND METABOLIC INHIBITORS ON MAGNESIUM TRANSPORT IN THE GUINEA-PIG JEJUNUM

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The mechanisms of magnesium transport in the intestine have been described as diffusion (Behar, 1974), carrier mediated transport (Ross, 1962) or as a combined carrier and diffusion system (O'Donnell and Smith, 1973). In the experiments described here the intestinal absorption of magnesium in the guinea pig jejunum was investigated.

Isolated sheets of jejunal mucosae were prepared using the method of Lauterbach (1977). In these studies the absorptive epithelium is separated from the kinetically rate limiting connective tissue layers found in the jejunal wall. The absorptive epithelium is then mounted between halves of a micro flux chamber where it forms a separating membrane. Bathing media are then introduced to either side as required. The tissue is oxygenated throughout the experimental period (45 minutes). At the end of the experiment the bathing solutions were removed and assayed using AAS or scintillation counting as required. The viability of the mucosal sheets used was determined using (i) glucose transport (S/M ratio 1.4 $\pm$ 0.1 n = 18), (ii) lactate dehydrogenase release (0.7 $\pm$ 0.1% serosal, 0.9 $\pm$ 0.1% mucosal at 45 minutes, n = 10), (iii) polyethylene glycol 900 permeability (porosity> 0.5% being rejected as leaky), (iv) histological examination (data not shown). All data indicate the mucosal sheets used in these studies were viable.

Kinetic analysis of magnesium transport in the lumen to blood direction (over the range of 0 - 20 mM Mg) indicated that absorption saturated at luminal magnesium concentrations above 2 mM. There was no correlation between magnesium transport and (^{3}H) polyethylene glycol 900 permeability. Cyanide (1 mM) and 2.4 dinitrophenol (1 mM) significantly reduced magnesium transport and tissue magnesium concentration (p 0.05). This suggests metabolic energy is required for magnesium transport and to maintain tissue magnesium levels. Despite the use of metabolic inhibitors magnesium transport is not halted, although it is possible this residual magnesium transport is tissue efflux caused by the metabolic inhibitors used. The effect of bumetanide (1 mM) on magnesium transport was investigated. Luminal addition of bumetanide significantly reduced (p 0.05) magnesium transport to a level comparable with cyanide and 2.4 D.N.P. However, serosal addition of bumetanide had no effect on magnesium transport. The serosal or luminal addition of bumetanide had no effect on tissue magnesium concentration. This data suggests a bumetanide sensitive magnesium transporter is present in the luminal membrane. Bumetanide sensitive magnesium transport has been reported previously in isolated cells (Gunter and Vorman, 1985). The effect of ouabain (1 mM) on magnesium transport was studied. Serosal addition of ouabain significantly reduced magnesium transport (p70.05) but did not reduce tissue magnesium concentrations. This suggests that the favourable electrochemical gradient present in the intestine is involved in magnesium absorption. This effect has been observed in sheep rumen (Martens, 1985).

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EFFECTS OF NEOMYCIN SULPHATE ON SECRETION IN RAT PANCREATIC ACINI

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The observation that it is possible to evoke a full secretory response in pancreatic acinar cells (e.g. with phorbol 12-myristate 13-acetate (TPA) in the absence of phosphatidylinositol 4,5-bisphosphate ($PtdInsP_2$) hydrolysis and with no change in intracellular [Ca^{2+}] (Merritt & Rubin, 1985) raises the question of which, if any, of the known responses to physiological agonists are obligatory for secretion. We therefore investigated the effect of amylase secretion stimulated by cholecystokinin octapeptide (CCK_8), bethanechol or bombesin of neomycin sulphate (NS), an inhibitor of $PtdInsP_2$ hydrolysis.

Acini isolated from rat pancreas by collagenase digestion (Peikin et al., 1978) were suspended at a protein concentration of approx. 10mg.ml^{-1} in HEPES-buffered medium (composition (mM) NaCl (103), KCl (4.8), $MgCl_2$ (1.2), $CaCl_2$ (2.0), NaH_2PO_4 (1.2), $NaHCO_3$ (0.25), glucose (14), HEPES (25), bovine serum albumin (2mg.ml^{-1})). After stimulation with agonists for 15 min. in the presence or absence of NS, amylase released from the acini was measured as a percentage of total cellular amylase.

NS inhibited amylase secretion stimulated by optimal concentrations of CCK_8 (10^{-10}M), bethanechol (10^{-4}M) or bombesin (10^{-8}M) in a dose-dependent manner but the sensitivity of the responses to inhibition differed. CCK-induced secretion was inhibited by NS in the concentration range 10^{-11}-10^{-6}M, with an IC_{50} of 0.3nM (n=6). Secretory responses to bethanechol and bombesin were also inhibited by NS at concentrations 10^{-9}M, but the dose-inhibition curves were broader, secretion was only reduced to 10% of maximum by NS 10^{-3}M and the approximate IC_{50} for bethanechol stimulation was 12nM and for bombesin stimulation 300nM. Since secretory responses to A23187 (10^{-6}M) and TPA (10^{-7}M) were unaffected by NS at concentrations up to 10^{-3}M, it is concluded that the cellular signalling process and not the secretory mechanism *per se* is affected by NS. This conclusion is supported by the observation that the inhibition of secretion by NS correlated with a decrease in stimulated inositol trisphosphate ($InsP_3$) production measured in ^{3}H-inositol-loaded acini in a manner consistent with the major effect of NS being to bind preferentially to $PtdInsP_2$ and thus prevent phospholipase C action. NS (10^{-3}M) had no effect on the transient rise in intracellular Ca^{2+} measured in quin 2- loaded acini stimulated by CCK_8, bethanechol or bombesin. This suggests that the initial Ca^{2+} transient may arise directly from ligand-receptor interaction rather than from $InsP_3$-induced release of Ca^{2+} from intracellular stores.

In conclusion we have shown that NS is a potent inhibitor of amylase secretion and $PtdInsP_2$ hydrolysis stimulated by Ca^{2+}-mobilizing agonists in pancreatic acinar cells. The greater sensitivity of CCK_8 stimulation, by comparison with bombesin or bethanechol, to NS inhibition may reflect differences in the coupling of the respective receptors to phosphoinositide turnover.

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