British Journal of Pharmacology

March 1991

Volume 102 Proceedings Supplement 1P–399P

1P DIFFERENTIAL EFFECT OF LITHIUM ON β-ADRENOCEPTOR DOWN-REGULATION IN LYMPHOBLAST CELL LINES FROM PATIENTS WITH BIPOLAR DEPRESSION AND HEALTHY CONTROL SUBJECTS

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Abnormalities of adrenergic function have been postulated as a biochemical basis for affective disorders (Werstiuk *et al*, 1990). Lymphoblast cell lines provide a useful model of human beta-adrenergic receptor (β-AR) function. A reduced β-AR number has been shown in lymphoblasts from patients with bipolar depression (BP) (Wright *et al* ,1984), but this was not seen by Berrettini *et al* (1987). We have previously shown that chronic coincubation with isoprenaline causes time and concentration dependent down-regulation of lymphoblast β-ARs (Kay & Elliott, 1988), with a parallel desensitization of the cAMP response (Kay & Elliott, 1990). In this study we have investigated lymphoblast β-AR number and down-regulation in BP patients and the effects of lithium on these parameters.

Cell lines from 9 subjects with bipolar depression (BP) and 9 sex and age matched controls were established. Confluent cells were coincubated with or without 1mM lithium chloride for 7 days and with or without 1nM isoprenaline for 24 hours prior to assay. Coincubations were stopped by centrifugation and the cells disrupted by homogenisation and sonication. The radioligand binding assay was carried out using [^{125}I]-iodocyanopindolol, a β–AR antagonist, as previously described (Kay & Elliott, 1988).

There was no difference in the basal β-AR binding capacity between BP cell lines and control cell lines (Table 1). However coincubation with isoprenaline caused a down-regulation of β-AR number by 40±5% in control cell lines, whereas in BP cell lines the down-regulation was only 10±12%. This difference was significant by Student's unpaired t-test ($P<0.05$). After 7 day lithium pretreatment there was no effect on basal Bmax, however the extent of down-regulation in response to isoprenaline in BP cell lines showed a significant increase to 40±7% when tested by Student's paired t-test ($P<0.005$). No equivalent change was seen in control cells.

Table 1: Basal binding capacity and %down-regulation in response to isoprenaline in control and BP cell lines with and without chronic coincubation with lithium chloride (results as mean ± s.e.mean).

		Bmax (fmol/mg protein)		% down-regulation		
	n	Control	BP	Control	BP	
No lithium	9	5.9 ± 0.7	4.8 ± 0.8	40 ± 5 %	10 ± 12 %*	* P<0.05 v control down-regulation
1mM lithium	9	4.3 ± 0.6	5.4 ± 0.9	30 ± 8 %	42 ± 7 %**	**P<0.005 v BP without lithium

These results show that lymphoblast cell lines from BP patients have a reduced sensitivity to isoprenaline down-regulation. Chronic coincubation with lithium chloride caused an increase in the extent of down-regulation in response to coincubation with isoprenaline in BP cell lines alone. These results provide a possible explanation of the therapeutic action of lithium in that it acts by normalising the regulation of β-ARs in bipolar depression.

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2P α_2-ADRENOCEPTOR BINDING SITES IN CORTICAL SAMPLES FROM DEPRESSED SUICIDE VICTIMS

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Studies of the growth hormone response to clonidine and of platelet α_2-adrenoceptors suggest that depression may be associated with altered α_2-adrenoceptor function (Katona et al., 1987). A recent study found the number of α_2-adrenoceptors to be higher in frontal cortex of depressed suicide victims (Meana & Garcia-Sevilla, 1987). To further investigate the role of α_2-adrenoceptors in depression, we have quantitated total α_2- and α_{2A}-adrenoceptor binding in post-mortem brain from antidepressant drug-free suicides (15M, 7F, mean age±SEM, 42±3 yrs; post-mortem delay, 35±4h) with a firm retrospective diagnosis of depression and matched controls (15M, 5F; 43±3 yrs; 40±3h). Saturation binding of [^{3}H]rauwolscine (6 conc., 0.125-4 nM) to well-washed membranes from four cortical regions was performed. Specific binding was defined with 100 μM phentolamine (total α_2-adrenoceptors) and 10 nM oxymetazoline (α_{2A}-subtype).

Kd values did not differ significantly between controls (1.04-1.57 nM) and suicides (1.06-1.60 nM). The number of total α_2-adrenoceptors was higher in suicides than controls (Table 1) and significantly so (by 24%, $p<0.05$, Wilcoxon rank sum test) in temporal cortex (Ba 21/22). Total α_2-adrenoceptor binding in the temporal cortex (Ba 21/22) of suicides who died by violent methods (n=12) was significantly higher than controls (by 39%, $p<0.05$), whereas non-violent suicides (n=10) did not differ significantly (higher by 9%, n.s.) from controls. The number of α_{2A}-adrenoceptor binding sites did not differ between suicides and controls (Table 1) or when suicides were divided according to violence of death.

Table 1. Bmax of α_2-adrenoceptor binding sites (fmol/mg protein, means ± SEM)

Brain regions	total α_2-adrenoceptors		α_{2A}-adrenoceptors	
	Controls	Suicides	Controls	Suicides
Frontal Cortex (Ba 11)	233 ± 13	260 ± 13	178 ± 11	199 ± 9
Occipital Cortex (Ba 17/18)	279 ± 10	314 ± 14	228 ± 10	242 ± 9
Temporal Cortex (Ba 21/22)	255 ± 13	316 ± 22 *	197 ± 12	214 ± 14
Temporal Cortex (Ba 38)	292 ± 12	281 ± 14	226 ± 9	201 ± 14

We thank the Sir Jules Thorn Charitable Trust for financial support.

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3P A PLASMA INHIBITOR OF PLATELET [^{3}H]IMIPRAMINE BINDING IS INCREASED IN DEPRESSIVE ILLNESS

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[^{3}H]Imipramine ([^{3}H]imip) has been widely used as a radioligand to label platelet 5-HT uptake sites. It has been proposed that plasma contains a factor(s) which modulates 5-HT transport and [^{3}H]imip binding. One candidate is α_1-acid glycoprotein (AGP) which inhibits [^{3}H]imip binding and enhances 5-HT uptake (Abraham et al., 1987). Platelet [^{3}H]imip binding has been widely studied as a marker of depression. Some studies have reported lower numbers of [^{3}H]imip binding sites in drug-free depressed patients compared to controls; other studies have found no differences (Mellerup & Plenge, 1988). It is possible that incomplete removal of an endogenous modulator from membrane preparations may contribute to these inconsistent findings. Indeed plasma AGP concentration has been reported recently to be higher in depressed patients than controls (Nemeroff et al., 1990). In this study we have compared the ability of plasma from depressed patients and controls to inhibit platelet [^{3}H]imip binding and have measured AGP concentration in the same samples.

Platelet-free plasma from 21 depressed patients (6 M, 15 F, age 34 ± 2 yrs, free of psychoactive drugs for at least 7 days) and 19 controls (5 M, 14 F, age 33 ± 2 yrs) inhibited specific [^{3}H]imip binding (defined by 100 μM desmethylimipramine) to well-washed platelet membranes in a volume dependent manner. Scatchard transformation of [^{3}H]imip (0.2-10 nM) binding in the presence of plasma revealed competitive inhibition (increased Kd, same Bmax). Plasma from depressed patients produced a greater inhibition of [^{3}H]imip binding compared to controls. The volume of plasma needed to inhibit specific binding by 50% (IV50) was 46 ± 2 μl for depressed subjects and 56 ± 3 μl for controls ($p<0.01$ Wilcoxon rank sum test). Plasma from depressed patients who had not previously received antidepressant drugs (n=13) was also more potent (IV50 44 ± 2 μl) at displacing [^{3}H]imip binding than plasma from matched controls (IV50 56 ± 2 μl, $p<0.01$). Thus differences are unlikely to be due to residual drug effects. Plasma AGP concentration (measured by radial immunodiffusion) did not differ between depressed patients (51 ± 3 mg/dl) and controls (56 ± 3 mg/dl). No correlation was found between AGP concentration and displacement of [^{3}H]imip.

This study confirms the ability of plasma to inhibit platelet [^{3}H]imip binding and suggests that the concentration of an endogenous factor is greater in depressed patients than controls, but that this factor is unlikely to be AGP. Contamination by an endogenous factor is unlikely to explain the discrepant findings in the number of [^{3}H]imip binding sites reported in studies of depressed patients since plasma increases the Kd of [^{3}H]imip binding without altering the number of sites.

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4P AUTORADIOGRAPHIC LOCALIZATION OF NON-ADRENOCEPTOR IDAZOXAN BINDING SITES (NAIBS) IN RAT BRAIN

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Idazoxan (IDX) is a potent and selective antagonist of α2-adrenoceptors (Stillings et al.,1985). Recently [3H]IDX has been reported to bind to sites that are insensitive to noradrenaline. These sites have been identified in tissue homogenates from several species and termed NAIBS (Michel & Insel, 1989). We now report their autoradiographical localisation in rat brain.

Anaesthetised (pentobarbitone 40mg/kg i.p.) male Wistar rats (250g) were perfused with phosphate buffered saline (intracardiac). Brains were removed, frozen, cut on a cryostat and 10μ sections thaw mounted onto subbed glass slides. For binding sections were prewashed in tris-HCl buffer (50 mM, pH 7.2, +1mM Mg^{++}), prior to incubation in fresh buffer containing 10 nM [3H]IDX (Amersham Int) with or without unlabelled drugs (20 min, 22°C). Sections were rinsed twice in fresh buffer (4°C), dried and apposed to [3H]-sensitive film for up to six weeks with appropriate [3H] standards to facilitate quantitative image analysis.

[3H]IDX autoradiography revealed a distribution of binding similar to that previously reported (Boyajian et al,1987). However when binding was performed in the presence of yohimbine (5μM), RX821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline) (3μM) or clonidine (3μM) there remained discrete areas of binding which corresponded to distinct brain nuclei, notably the area postrema and interpeduncular nucleus. This binding was not displaceble by 10μM noradrenaline or RX811059 (2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline) but was prevented by unlabelled IDX (1μM) or 3μM RX801023 (6-fluoro-2-[2-(1,4-benzodioxanyl)]-2-imidazoline) as shown in table 1 (fmol/mg, means ± s.e.m, n=3 rats). RX801023 is reported to have higher affinity for NAIBS than for α2-adrenoceptors (Mallard et al, this meeting).

[3H]idazoxan bound fmol/mg wet tissue	Area postrema	Interpeduncular nucleus	Arcuate nucleus	Ependyma	nucleus tractus solitarius	Frontal cortex	Ant Olfactory Nucleus
Total	222.0±12.9	178.5±5.7	116.7±2.3	149.1±23.4	136.1±6.8	131.4±3.5	252.6±7.6
+ Yohimbine 5μM	208±18.7	123.4±12.7	102.2±3.3	101.9±8.1	22.5±3.7	24.9±0.7	17.7±0.9
RX801023 3μM + Yohimbine 5μM	5.6±0.6	10.6±1.2	10.8±1.1	<10.0	8.1±1.3	18.8±0.9	11.0±2.8

Autoradiography of [3H]IDX in the presence of α2-adrenoceptor ligands allows the visualisation of NAIBS in the rat CNS. It is unclear whether these binding sites serve any physiological function, but their unique distribution in rat brain and ligand selectivity warrants further investigation.

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5P REGULATION OF ENDOGENOUS NORADRENALINE RELEASE IN RAT HIPPOCAMPUS AND FRONTAL CORTEX: *IN VIVO* MICRODIALYSIS

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The concentration of noradrenaline released into the synaptic cleft is regulated by a combination of autoreceptor activation and transmitter uptake. But the relative importance of each process in brain regions is not known. The present work uses in vivo microdialysis to examine the effects of inhibiting uptake (desmethylimipramine; DMI) and inhibiting α_2 receptors (idazoxan; IDX) on the release of endogenous NA from rat frontal cortex as compared to hippocampus.

Rats (250-350 g) were anaesthetized with chloral hydrate (400 mg/kg + 90 mg/kg ip as required) and dialysis probes were implanted stereotaxically into either brain area. Samples (15 min) were collected after stable basal release of NA was obtained. Three baseline samples were collected before IDX (1.0 - 20.0 mg/kg sc) or DMI (0.05 - 40 μM via the dialysis probe) were administered. Sampling continued for a further 75 min. NA concentrations were determined immediately after a sample was collected using HPLC-electrochemical detection.

Basal NA release was: frontal cortex 3.6 ± 0.1 pg/sample (n=23); hippocampus 3.8 ± 0.2 (n=23) pg/sample. Both IDX alone and DMI alone at all concentrations tested caused dose dependent increases in NA release into samples collected from both brain regions. However, the response to each drug differed markedly between the two brain regions. IDX caused a more rapid onset and a longer duration of increased NA release in frontal cortex versus hippocampus. NA content increased within 15 min of IDX administration in frontal cortex samples and the increase lasted for 45 to 75 min dependent on the dose. In hippocampus, a significant increase in NA release did not occur for 30 to 45 min after the drug and the increased release never lasted more than 30 min. In contrast, DMI caused greater increases in basal NA release in hippocampus versus frontal cortex. The hippocampus responded to concentrations of DMI as low as 0.1 μM, with the maximum increase in basal release occurring at 5 μM (22.9 ± 1.7 pg/sample). In frontal cortex, basal release increased only with concentrations of 1.0 μM DMI and above and the maximum response occurred at 20 μM (24.9 ± 1.8 pg/sample).

Both uptake and autoreceptor activation are important in the regulation of synaptic NA content, the influence of each differs between frontal cortex and hippocampus. In agreement with Dennis et al. (1987), our results suggest that the α_2 autoreceptor has a primary role in the frontal cortex. However, uptake process appears to predominate in hippocampus.

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6P CHARACTERISATION OF A HIGH-AFFINITY BINDING SITE FOR [^{3}H]UK 14304 IN RAT BRAIN MEMBRANES WHICH IS INSENSITIVE TO GTP

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Specific binding of the agonist ^{3}H-UK14304 in rat brain tissue is purported to identify a single high-affinity site characteristic of an α_2-adrenoceptor (Loftus et al, 1984). We have recently developed a novel method whereby specific binding of such agonist ligands is defined as that displaced by a saturating concentration of the stable GTP analogue guanylylimidodiphosphate (GppNHp) rather than a competing adrenergic ligand (Payvandi et al, 1990). In rat brain this led to the discrimination of ^{3}H-UK14304 binding into two high-affinity components, only one of which was sensitive to GTP. We have now further characterised binding at these two sites.

Membranes were prepared from 4 regions of the rat brain (cerebral cortex, thalamus/hypothalamus, pons/medulla, cerebellum) as described by Payvandi et al (1990). Specific binding of ^{3}H-UK14304 was defined using either GppNHp (100μM) or phentolamine (10μM) and resolved into GTP-sensitive, GTP-insensitive and aggregate specific binding (Payvandi et al., 1990).

Table 1:Binding capacity (fmol/mg protein; mean ± s.e.mean; n=4) in rat brain regions (*p<0.05, paired t-test v GTP-sens. component).

	^{3}H-UK14304			^{3}H-Rauwolscine
	GTP-sens.	GTP-insens.	Aggregate	
Cerebral cortex	117 ± 8	145 ± 7*	266 ± 15*	227 ± 15*
Pons/medulla	50 ± 6	89 ± 10*	137 ± 17*	101 ± 17*
Thalamus/hypothal.	87 ± 11	135 ± 21*	222 ± 32*	174 ± 17*
Cerebellum	23 ± 3	40 ± 6*	58 ± 5*	52 ± 5*

As shown in Table 1, binding capacity of the GTP-insensitive site was significantly greater than the GTP-sensitive site in all 4 brain areas and accounted for 55-70% aggregate agonist binding. GTP-sensitive binding constituted approximately 50% antagonist binding capacity in each brain area, as identified by ^{3}H-rauwolscine. Inhibition of ^{3}H-UK14304 binding by a range of adrenergic ligands was then tested in the presence or absence of 100μM GppNHp. None of the compounds tested showed significant selectivity for the GTP-insensitive site. However 100mM NaCl significantly reduced the affinity of ^{3}H-UK14304 at the GTP-insensitive site (Kd=6.70±0.75 nM; control Kd=1.99±0.50 nM, n=3, p<0.05) but not at the GTP-sensitive site (100mM NaCl Kd=1.36±0.23 nM; control Kd=1.30±0.34 nM). Treatment with DSP-4 (100mg/kg i.p.) reduced GTP-insenstive binding and increased GTP-sensitive binding.

We conclude that specific binding of ^{3}H-UK14304 to rat brain membranes as classically defined using excess adrenergic ligands can be differentiated into two high-affinity components on the basis of sensitivity to GTP and its analogues. We propose that the GTP-sensitive component represents binding to the α_2-adrenoceptor/G-protein complex whereas the nature of the GTP-insensitive component is unclear and is currently being investigated.

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Payvandi N., Elliott J.M. & Heal D.J. (1990) This meeting

7P GUANINE NUCLEOTIDES - A NOVEL METHOD FOR DEFINING SPECIFIC BINDING OF AGONIST RADIOLIGANDS TO α_2 ADRENOCEPTORS

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Specific binding of radioligands to the α_2-adrenoceptor is routinely defined as that displaced by excess concentration of unlabelled adrenergic ligand. Agonists differentiate the receptor population into high and low affinity sites (Hoffman & Lefkowitz, 1980). Addition of GTP converts all the receptors to the low-affinity form. In this study we have investigated the use of the stable GTP analogue guanylylimidodiphosphate (GppNHp) to discriminate the binding components of radiolabelled agonists at the α_2-adrenoceptor.

Membranes from fresh human platelets or rat cerebral cortex were prepared as described previously (Payvandi et al., 1990). Specific binding of the agonist radioligand ^{3}H-UK14304 defined using either phentolamine (10μM) or GppNHp (100μM) was resolved into 3 components: (i) aggregate binding, displaced by phentolamine and therefore corresponding with classical specific binding; (ii) GTP-sensitive component, displaced by GppNHp; (ii) GTP-insensitive component, displaced by phentolamine, but not by GppNHp.

Table 1: Components of specific binding of ^{3}H-UK14304 in human platelet and rat cerebral cortex membranes.

	Human platelet (n=6)		Rat cerebral cortex (n=10)	
	Kd	Bmax	Kd	Bmax
GTP-sensitive	1.08 ± 0.19	212 ± 13	1.27 ± 0.26	112 ± 10
GTP-insensitive	> 10	-	1.67 ± 0.54*	152 ± 8*
Aggregate	1.35 ± 0.13*	270 ± 23*	1.58 ± 0.37*	271 ± 16*

Values represent mean ± s. e. mean; Kd - nM ; Bmax - fmol/mg protein ; * $p<0.05$, paired t-test v GTP-sensitive component.

As shown in Table 1, the GTP-sensitive component of specific binding of ^{3}H-UK14304 to human platelet membranes demonstrates significantly lower capacity and higher affinity than aggregate (classical) specific binding. No high-affinity binding occurs at the GTP-insensitive site. In contrast, in rat brain ^{3}H-UK14304 binds with high affinity to both GTP-sensitive and GTP-insensitive components of aggregate specific binding. Binding to the GTP-sensitive site is of significantly lower capacity and higher affinity than either the GTP-insensitive or aggregate components. A similar pattern of specific binding components was observed using ^{3}H-p-aminoclonidine as the agonist radioligand.

We conclude that in human platelet membranes GppNHp can be used to define specific binding of agonist radioligands to the high-affinity state represented by the α_2-adrenoceptor/G-protein complex. In rat brain membranes, however, specific binding of agonist radioligands occurs with high affinity to both GTP-sensitive and GTP-insensitive sites, the nature of which will be further investigated.

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Payvandi N., Elliott J.M. & Heal D.J. (1990) Eur. J. Pharmacol. 183, 740-741

8P FERRITIN LEVELS IN THE BRAINS OF SUBJECTS WITH LEWY BODIES AT POST-MORTEM WHO DID NOT SHOW CLINICAL SYMPTOMS OF PARKINSON'S DISEASE IN LIFE

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Neuronal loss in Parkinson's disease (PD) is associated with a selective increase in total iron levels in substantia nigra, but a reduction in brain ferritin content, suggesting altered iron handling may occur in PD (Dexter et al, 1989, 1990). It is not known whether this represents a primary cause of dopamine cell death or whether iron accumulation is secondary to neuronal cell loss. For this reason we have determined brain ferritin levels in control subjects who at post-mortem were found to have Lewy bodies and cell loss in the substantia nigra but who had not shown clinical symptoms of PD during life and who may be considered to be in the early presymptomatic stages of PD. Post-mortem brain tissue was obtained from 6 normal control subjects with a mean age (68.3 ± 5.7 years), death to refrigeration time (2.9 ± 0.3hr), time between death and removal of the brain (14.3 ± 1.5hr) and from 6 Lewy body positive control subjects with a mean age (68.2 ± 5.8 years), death to refrigeration time (2.7 ± 0.4hr), time between death and removal of the brain (21.5 ± 2.2hr*).Brain ferritin levels were analysed using a radioimmunoassay technique as previously described (Dexter et al,1990) with the antibody being raised in rabbits using human spleen ferritin as the antigen. No differences in ferritin levels were observed in the cerebral cortex (Brodmann area 10), caudate nucleus, putamen, globus pallidus, substantia nigra and cerebellum when control and Lewy body +ve subjects were compared (see Table 1).

Table 1 Brain ferritin levels in normal controls and Lewy body +ve subjects

BRAIN REGION	FERRITIN LEVELS(ng/mg wet weight)		BRAIN REGION	FERRITIN LEVELS(ng/mg wet weight)	
	NORMAL	LEWY BODY +VE		NORMAL	LEWY BODY +VE
Cerebral cortex	47.9 ± 3.9	40.6 ± 5.0	Caudate nucleus	267.1 ± 35.2	227.9 ± 32.1
Putamen	388.4 ± 51.0	342.5 ± 49.4	Globus pallidus (M)	359.3 ± 64.6	292.9 ± 35.1
Substantia nigra (T)	365.3 ± 35.1	401.8 ± 57.2	Globus pallidus (L)	386.3 ± 20.4	334.6 ± 39.6
Cerebellum	41.4 ± 7.9	55.3 ± 6.1			

Values represent mean ± SEM. *P < 0.05 compared to normal controls (Student's t test). M = medial, L = lateral, T = total.

Coupled with our earlier finding of no changes in nigral iron levels in these patients (Dexter et al 1990), it would appear that iron metabolism is not affected during the early stages of the illness. This suggests that alterations in iron metabolism are not the cause of dopamine neurone degeneration but rather may contribute to the progress of the pathology.

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9P CHARACTERISTICS OF AUTORECEPTOR CONTROL OF DOPAMINE RELEASE IN OLFACTORY TUBERCLE IN THE ABSENCE OF UPTAKE INHIBITION

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Fast cyclic voltammetry detects dopamine (DA) overflow following single pulse electrical stimulation (0.1 ms, 20V) in the absence of a re-uptake inhibitor in rat brain slices (350 μm), superfused with oxygenated artificial cerebrospinal fluid at 32 °C (Palij *et al.*, 1990). The method is anatomically precise, and we have studied autoreceptor control of DA release at two sites: caudate putamen (CPu) and olfactory tubercle (OTu).

Peak overflow of DA by a single pulse was 0.10 μM in the CPu and 0.04 μM in the OTu. Overflow induced by trains of 20 pulses applied at different frequencies was expressed as a ratio relative to a single pulse. In the CPu there is a small range of response, and a maximum (about two-fold) increase in overflow is apparent with 20 pulses at 10 Hz and this does not change with increasing frequency. By contrast, a wide dynamic range is apparent in the OTu (Figure 1).

Addition of metoclopramide (0.3 μM) did not alter the train:single overflow ratio in the CPu. By contrast in the OTu, overflow was increased from 8.7 $\pm$ 0.5 to 18.0 $\pm$ 4.0 at 10 Hz, from 15.3 $\pm$ 1.0 to 25.8 $\pm$ 5.3 at 20 Hz and from 18.1 $\pm$ 1.9 to 23.0 $\pm$ 4.4 at 50 Hz.

The flatness of the frequency:overflow response in the CPu has been described previously using [^{3}H]-DA in the absence of re-uptake inhibition (Hoffmann & Cubeddu, 1982); this may indicate a narrow frequency response range in the CPu. The lack of effect of metoclopramide may indicate that DA autoreceptors do not modulate DA release in the CPu under these conditions. By contrast, a wide frequency range of DA release is observed in the OTu, and this may be regulated by DA autoreceptors, as metoclopramide increases overflow in response to trains of pulses but not to single pulses.

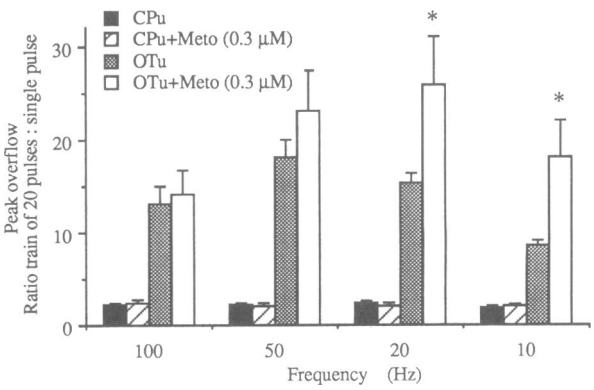


Figure 1 Effect of frequency on overflow evoked by 20 pulse trains in CPu & OTu in the absence and presence of metoclopramide (Meto, 0.3 μM). * $P < 0.01$ ($n \geq 5$).

Supported by the Wellcome Trust (SJT)

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10P THE PSYCHOPHARMACOLOGY OF THREE NEW, SELECTIVE D-1 DOPAMINE RECEPTOR ANTAGONISTS IN RELATION TO TWO DIRECTIONS OF D-1:D-2 INTERACTION

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The introduction of the first selective D-1 dopamine (DA) receptor antagonist SCH 23390 has generated the concept of D-1:D-2 receptor interactions in the regulation of DAergic behaviour (Waddington & O'Boyle, 1989). Recent studies indicate cooperative interactions in the regulation of typical behaviours but oppositional interactions in the genesis of atypical behaviours such as limb/body jerking (Murray & Waddington, 1989). As these concepts derive from studies using a single chemical class of D-1 antagonist, SCH 23390, it appears essential to substantiate and extend these notions using new, chemically distinct agents that have only recently become available.

Using previously described procedures (Murray & Waddington, 1989), rats were challenged with the selective D-2 agonist RU 24213 (15 mg/kg; Waddington & O'Boyle, 1989) after pretreatment with vehicle, SCH 23390 (0.01-1.0 mg/kg), the new putative selective D-1 antagonists SCH 39166 (0.1-10.0 mg/kg; Chipkin et al., 1989), NO 756 (0.01-1.0 mg/kg; Andersen et al., 1988) and A-69024 (1-25 mg/kg; Kerkman et al., 1989), or the selective D-2 antagonist YM-09151 (0.005-0.5 mg/kg; Waddington & O'Boyle, 1989) and behavioural responses assessed; selectivities of putative D-1 antagonists were clarified in binding studies using ^{3}H-SCH 23390 and ^{3}H-spiperone as ligands. Typical, non-stereotyped sniffing and locomotor responses to RU 24213 were readily antagonised ($P<0.05$) not only by YM-09151 but also by SCH 23390 and by each of the new, putative D-1 antagonists, in accordance with a permissive role for D-1 tone via cooperative D-1:D-2 interactions. Concurrently, atypical episodic jerking of the limbs or whole body in response to RU 24213 was released ($P<0.05$) by pretreatment with SCH 23390 and with each of the new, putative D-1 antagonists, in accordance with an inhibitory role for D-1 tone via oppositional D-1:D-2 interactions, but not with YM-09151. SCH 39166 and NO 756 shared with SCH 23390 a nM affinity and >1000-fold selectivity for the D-1 vs D-2 receptor, with A-69024 having some 20-fold less affinity and selectivity than its counterparts.

These studies indicate that SCH 39166, NO 756 and A-69024 share the psychopharmacological profile of SCH 23390. Critically, they demonstrate that these complex forms of behaviour are not idiosyncratic effects of SCH 23390 but appear to have their basis in two directions of D-1:D-2 interaction that can be revealed using all of the new, structurally distinct D-1 antagonists that are currently known.

This work was supported by the Wellcome Trust; we thank Abbott, Novo, Roussel, Schering and Yamanouchi.

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11P CENTRAL AND PERIPHERAL ACTIONS OF RECOMBINANT LIPOCORTIN-1 AND A FRAGMENT OF LIPOCORTIN-1 ON THE RESPONSES TO INTERLEUKIN-1B

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The anti-inflammatory actions of the glucocorticoids have been attributed to the induction of a group of phospholipase A_2-inhibiting proteins called lipocortins (LC, Di Rosa et al, 1984). The *in vivo* anti-inflammatory actions of LC-1 have been demonstrated using different preparations of LC-1. Inhibition of carrageenin-induced rat paw oedema was observed (Cirino et al, 1989) after peripheral administration of full length recombinant LC-1 molecule (1-345), while the central administration of a fragment of human LC-1 (N terminal, 1-188), caused an inhibition of interleukin-1ß (IL-1ß)-induced fever and thermogenesis in the rat (Carey et al, 1990). These observations indicate that LC-1 can act peripherally or within the brain and may inhibit cytokine action at either or both of these sites. The present study therefore investigated the central and peripheral actions of both recombinant LC-1 (rLC-1) and a fragment of LC-1 (fLC-1) on the pyrogenic and thermogenic responses to IL-1ß in the rat.

Recombinant IL-1ß, rLC-1 (1-345) or fLC-1 (N terminal, 1-188, Carey et al, 1990) were injected either peripherally (intravenously) or centrally (intracerebroventricularly) into conscious male Sprague-Dawley rats. Central or peripheral injection of rLC-1 or fLC-1 alone had no significant effect on either oxygen consumption (Vo_2) or colonic temperature (T_c). Injections of IL-1ß (20ng/kg central or 1µg/kg peripheral) produced significant increases in Vo_2 (20%) and T_c (1.5°C). Peripheral injections of rLC-1 or fLC-1 (62.5-500µg/kg) caused dose dependent inhibition of the increases in Vo_2 (up to 80%) and T_c (up to 90%) induced by peripheral IL-1ß. In contrast, peripheral injection of rLC-1 or fLC-1 did not modify the effects of centrally administered IL-1ß. Central administration of fLC-1 (1.6-4.8µg/kg) significantly inhibited central IL-1ß-induced fever and thermogenesis, whereas central rLC-1 (2.0-12.0µg/kg) did not affect these centrally-induced responses. Furthermore, central fLC-1 inhibited peripherally induced fever and thermogenesis, but these peripherally induced responses were not affected by centrally administered rLC-1.

The results demonstrate similar potent inhibitory actions of both rlipocortin-1 and lipocortin-1 fragment on the thermogenic and pyrogenic responses to IL-1ß. However, it appears that neither rLC-1 or fLC-1 is able to enter the brain from the periphery, and that when injected centrally, the ability to inhibit either centrally or peripherally-induced fever and thermogenesis depends on molecular size.

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12P PARADOXICAL THERMOREGULATORY OPERANT RESPONSES FOR HEAT IN RATS RECEIVING ISOPRENALINE

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The effects of maximal thermogenic doses ((75µg/kg; s.c) of (-)-isoprenaline (ISO) on operant-conditioned thermoregulatory responses in a cold (-8°C) environment were tested in rats trained to barpress for radiant heat (infrared lamps providing 180mW/cm^2 surface area). Changes in rectal temperature (Tr), thermal responses (R, secs heat/min) and barpress activity (BP, presses/min) were recorded over the 90min following injection of ISO or vehicle. Data on R and Tr were combined to provide an estimate of net thermal balance (NTB = body heat influx - body heat storage, kJ). Following injection of vehicle rats would barpress to raise Tr by 1.0-1.5°C over the first 30min, and then maintain this temperature over the next 60min. Typically, this would require 2-3 BP/min, to provide 10-12secs heat/min (R). Contrary to expectations, ISO rats required twice as much exogenous heat to maintain a smaller rise in Tr. In one experiment (n = 6), the results (mean±sem) for control and ISO, respectively, were: increase in Tr = 1.67±0.28 vs 0.80±0.19°C ($p<0.05$); R = 11.94±0.64 vs 22.97±0.59secs/min ($p<0.001$); NTB = 19.48±1.19 vs 39.80±1.85kJ ($p<0.001$); BP/min was reduced slightly, but press duration was increased by ISO from 4.24±0.42 to 11.04±0.81secs/press ($p<0.001$). The increased heat-seeking behaviour in cold-exposed rats suggests, paradoxically, that this thermogenic agonist induces hypothermia. The hypothermic effects of ISO could also be observed in rats in their home cages at normal room temperature (22°C). Following injection of vehicle, Tr rose by 0.5-0.8°C over the following 2.5h, whereas Tr decreased (maximum response = -0.76±0.27°C at 90min, $p<0.001$) after ISO. Unlike ISO, the $ß_3$-selective adrenergic agonists RO40-2148 (5mg/kg), BRL35135 (40µg/kg) and ICI/D7114 (1.4mg/kg) produced increases in Tr in rats at 22°C that were significantly greater than in rats receiving vehicle. Likewise, these thermogenic agents raised Tr and simultaneously reduced operant responses for exogenous heat in cold-exposed rats, and provide additional, behavioural evidence to support the existence of a $ß_3$-adrenoceptor. These results also suggest that the hypothermic effects of ISO are due to its actions on $ß_1$ and/or $ß_2$-adrenoceptors, a suggestion that has been tested in another series of experiments (Stock & Carlisle, 1990).

Stock, M.J. & Carlisle, H.J. (1990) This meeting

13P USE OF SELECTIVE β-ANTAGONISTS TO POTENTIATE THERMAL RESPONSES TO A NON-SELECTIVE β-AGONIST

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The hypothermic effects of (-)-isoprenaline (ISO) observed by Stock & Carlisle (1990) were presumed to be due to its $ß_1$ and/or $ß_2$-adrenergic activity negating its $ß_3$-mediated effects on thermogenesis. In the absence of a selective $ß_3$-antagonist, this suggestion was investigated further by taking advantage of the weak (pA_2 5.7-7.4; Stock & Sudera, 1989) inhibitory effects of conventional ß-adrenergic antagonists on brown fat (i.e. $ß_3$-mediated) thermogenesis, and attempting to use doses sufficient to block $ß_1$ and/or $ß_2$-mediated responses, but leave $ß_3$-mediated responses intact.

In rats injected with ISO (75µg/kg, s.c.), pretreatment with (±)-propranolol (100µg/kg) reversed the hypothermic effect of ISO on rectal temperature (increase in Tr at 90min = 0.59±0.15, c.f. -0.36±0.31°C for ISO alone, $p<0.05$). This dose of propranolol failed to affect Tr of control rats, or the thermogenic response to ISO, and compares with a dose of 20mg/kg normally required to block BAT thermogenesis in vivo. In anaesthetised (1.2g/kg urethane) rats, the initial (0-90min) rise in BAT temperature (Tbat) and Tr after ISO was significantly greater following pretreatment with propranolol, whereas the increase in heart rate was reduced. Low-dose pretreatment with a $ß_1$-selective antagonist ((±)-atenolol, 10µg/kg) resulted in profound reductions in Tbat (max = -1.27±0.11°C) and Tr (-1.64±0.17°C) following ISO, whereas pretreatment with a $ß_2$-selective antagonist (ICI118551, 100µg/kg) caused marked potentiation (max response: Tbat = 2.64±0.20, Tr = 1.27±0.23°C); over 2h, the mean Tbat response was 1.92±0.2, c.f. 1.0±0.14°C for ISO alone ($p<0.001$). ICI118551 had no effect on the heart rate response to ISO (116±4bpm), but atenolol reduced the response to 55±1bpm ($p<0.001$).

These experiments show the extent to which the effects of a non-selective agonist can be manipulated using appropriate doses of selective antagonists, and suggest that the $ß_2$ activity of ISO is responsible for its hypothermic effects, while its $ß_1$ activity protects against hypothermia.

Supported by a travel grant to MJS from Hoffman-LaRoche, Basel.

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14P THE INVOLVEMENT OF CORTICOTROPHIN RELEASING FACTOR (CRF) IN THE THERMOGENIC AND ANOREXIC ACTIONS OF SEROTONIN (5-HT)

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Serotonergic pathways exert dual actions in the regulation of energy balance, by both inhibiting energy intake and stimulating energy expenditure. Systemic and central injections of serotonergic compounds also stimulate the hypothalamic pituitary adrenal axis, through the hypothalamic release of corticotrophin releasing factor (CRF; Calogero et al, 1989). Anorexic and thermogenic actions of CRF have also been described (Morley & Levine, 1982; Le Feuvre et al, 1987). The present study therefore investigated the involvement of CRF in the thermogenic and anorexic actions of serotonin (5HT).

Serotonergic compounds and CRF antibody were injected directly into the third ventricle of conscious, male Sprague-Dawley rats via indwelling guide cannulae. Central injections of 5HT (0.5-50µg, icv) produced significant increases in resting oxygen consumption (VO_2) without obvious affects on behaviour. Maximum 12.5±2.3% increases in VO_2 were observed in response to 20µg 5HT 30-60 minutes post-injection. Similar (12-17%) increases in VO_2 were observed following injections of the 5HT precursors, tryptophan (14µg, icv), or 5-hydroxytryptophan (5-HTP, 20µg, icv), or the 5HT reuptake inhibitor, DL-fenfluramine (30µg, icv. or 10mg/kg, ip). Injections of vehicle (0.9% saline containing 0.1% ascorbic acid) or CRF antibody (3µl, icv) did not affect resting VO_2. Administration of CRF antibody (3µl, icv) ten minutes prior to serotonergic compounds, significantly reduced the increases in VO_2 observed in response to 20µg 5HTP (1.9±1.8%, $P<0.01$ vs 5HTP alone), 50µg 5HT (2.0±0.9%, $P<0.05$ vs 5HT alone) and fenfluramine (10mg/kg, ip, 1.2±2.4% $P<0.05$ vs fenfluramine alone). Voluntary food intake was measured for 6h (1000-1600h) in rats following 16h starvation. Injections (icv) of 20µg 5HT, 5HTP or 30µg fenfluramine reduced 6h food intake by 30% compared to saline-treated control rats. 45-54% reductions were observed following 40µg of 5HT or 5HTP, or 50µg fenfluramine (icv), and 67% reductions in response to peripheral injection of fenfluramine. Pretreatment with CRF antibody significantly attenuated the reductions in 6h food intake observed in response to 5HT, 40µg 5HTP, and fenfluramine. CRF antibody did not affect the anorexic actions of either 20µg 5HTP or peripheral injection of fenfluramine (10mg/kg). Central injections of non-immune rabbit serum (3µl) did not affect thermogenesis or food intake.

The central thermogenic and anorexic actions of serotonin and related compounds appear to be mediated, at least in part, by CRF.

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15P INTERACTIONS OF CORTICOTROPHIN AND GLUCOCORTICOIDS IN THE CONTROL OF THYMULIN RELEASE IN THE RAT

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Thymulin, a nonapeptide secreted by the thymus, is implicated in the regulation of T cell function. Reports that, in man, the plasma concentrations of thymulin, like those of cortisol, are increased both in the early morning and in conditions of stress (Safieh et al, 1990a) raise the possibility that the secretion of the peptide may be regulated by the stress hormones of the hypothalamo-pituitary-adrenal axis. We have therefore examined the influences of corticotrophin (ACTH) and dexamethasone on the secretion of thymulin by rat thymic tissue in vitro and in vivo.

Thymic tissue was removed post-mortem from male Sprague Dawley rats (150g) and pre-incubated under carefully controlled conditions for 2h before being challenged for 15 min with appropriate secretagogues. The thymulin content of the medium was determined by radioimmunoassay (Safieh et al, 1990b). ACTH (25-1000 pg/ml) produced significant ($P<0.01$) concentration dependent increases in thymulin release. Its effects were mimicked by K^+(56mM) and were Ca^{++} dependent. Although inclusion of dexamethasone (10^{-6}M) in the pre-incubation medium reduced ($P<0.05$) the basal release of thymulin, it potentiated significantly ($P<0.01$) the subsequent secretory responses to ACTH (500 & 1000 pg/ml). In vivo, weak thymulin immunoreactivity was apparent in the plasma of intact, non-stressed rats but it was below the level (300 fg/ml) which could be accurately quantified with our assay method. Adrenalectomy, which removed endogenous corticosterone (non detectable v 42±8 ng/ml in controls) and thus elevated the plasma ACTH concentration (936±121 pg/ml v 42±6 pg/ml in controls), raised the plasma thymulin concentration to 486±55 fg/ml. Treatment of adrenalectomized rats with dexamethasone (20mg/litre in the drinking water for 24h) prevented the hypersecretion of thymulin and reduced the plasma ACTH concentration to a level (7.8±0.2 pg/ml) below that found in intact controls.

The results indicate that ACTH stimulates the secretion of thymulin and that in appropriate conditions its effects are enhanced by glucocorticoids. They thus support a role for the hypothalamo-pituitary-adrenal axis in the regulation of the endocrine function of the thymus.

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16P EVIDENCE FOR ACTIVATION OF HISTAMINE-H_1 RECEPTORS BY SUMATRIPTAN

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Sumatriptan (GR43175) is a potent agonist at the 5-HT_1-like receptor mediating contraction of the dog saphenous vein (Humphrey et al, 1988) and in addition activates 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors (Schoeffter and Hoyer, 1989). We now report that under certain circumstances sumatriptan also activates histamine-H_1 receptors mediating smooth muscle contraction.

Experiments were performed using rings of rabbit aorta (RbA), rabbit femoral artery (RbFA) and the longitudinal muscle strip of the guinea-pig ileum (GPI). These were mounted in organ baths containing Krebs solution aerated with 95%O_2/5%CO_2 at 37°C (32°C GPI). In RbFA 5-HT contractions were mimicked by both sumatriptan and methysergide; the agonist potency order was (p[A_{50}] and maximum c.f. 5-HT): 5-HT (7.55±0.07;1.0) > methysergide (6.30±0.08; 0.9) > sumatriptan (5.30±0.17; 0.85). 5-HT effects were consistent with an action at a mixed population of 5-HT_1-like and 5-HT_2 receptors but responses to sumatriptan and methysergide could not be explained in these terms. However, whereas 5-HT effects were unaffected by mepyramine (0.3μM) this antagonist rendered concentration-effect curves to methysergide and sumatriptan overtly biphasic suggesting the involvement of histamine-H_1 receptors. This possibility was explored further in GPI and RbA. Affinity estimates for mepyramine (pK_B=8.58±0.05 (n=4); 8.39±0.02 (n=4); 9.17±0.05 (n=5)) and histamine itself (pK_A=4.94±0.10 (n=6); 4.07±0.21 (n=6); 4.40±0.21 (n=6)) in RbFA, RbA and GPI respectively suggest that all three tissues possess the same H_1 receptor type. In RbA sumatriptan and methysergide (<100μM) did not produce agonism but both antagonised histamine contractions (pA_2=4.63±0.06 (n=6) and 5.14±0.05 (n=4) respectively). In GPI sumatriptan antagonised histamine effects without producing agonism (pA_2=5.06±0.11, n=4), but methysergide now behaved as a partial agonist at the H_1 receptor (p[A_{50}]=5.82±0.09; max. c.f. histamine=0.24; pK_A = 5.45 ±0.14 (n=4)). This property of methysergide has been described previously (Gunning et al, 1988). Receptor alkylation studies using phenoxybenzamine showed that in both RbFA and GPI there exists for histamine a substantial receptor reserve which is not evident in RbA. Estimates of τ, an operational definition of efficacy, were 69, 18 and 71 (geometric mean of 6 estimates) in RbFA, RbA and GPI respectively. Whereas these tissue differences in efficacy offer an explanation for the failure of either sumatriptan or methysergide to display agonism in RbA, they do not explain the difference in agonist profile between GPI and RbFA. However in RbFA but not in GPI both compounds are agonists at 5-HT_1-like receptors mediating contraction. When these receptors were selectively and irreversibly alkylated using benextramine tetrahydrochloride (BHC, 10μM) this resulted in a profound reduction of the agonism induced by methysergide and sumatriptan but not histamine. These results suggest that sumatriptan, like methysergide, is a low affinity, low efficacy agonist at histamine-H_1 receptors, but when 5-HT_1-like receptors co-exist with H_1 receptors amplification of responses can result in the same manner that is observed for the interaction between 5-HT_1-like and thromboxane A_2 receptors (MacLennan et al, this meeting). These observations emphasise the need to consider the possibility of receptor 'cross-talk' when the receptor selectivity of pharmacological agents is being evaluated.

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17P FUNCTIONAL DISCRIMINATION OF α_{1A} AND α_{1B} ADRENOCEPTORS IN RAT VAS DEFERENS

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We have dissected the receptor types and sub-types involved in the response of the whole vas deferens of the rat to trains of electrical field stimuli (ToS, 10Hz, Pulse width 0.8ms for 10s). We have used prazosin, nifedipine, suramin a putative P_{2x} purinoceptor antagonist and Chloroethylclonidine (CEC, an α_{1B} adrenoceptor antagonist) alone and in combination. The whole time course of the mechanical response to each train was recorded using a computerised data collection and analysis system (Marshall & Spriggs,1985) which enabled mean responses from 6 to 8 tissues to be calculated and plotted.

Prazosin (10^{-8} - $3x10^{-7}$M) reduced both the first (<2s) and second (2-10s) phases of the response to ToS by approx. 50%. CEC ($3x10^{-6}$M) which blocks the noradrenergic phase of the response to a single electrical field stimulus (Mallard et al ,1990) reduced by around 50% the first phase of the response to ToS but failed to impair the second phase, indicating that the α_{1B}-adrenoceptor is not involved in this component of the response. Nifedipine ($3x10^{-6}$M) abolished the second phase of the response to ToS , implying that the adrenoceptor involved here is of the α_{1A} subtype. Suramin reduced the early phase of the response to ToS by 30% and the second phase by approximately 50%. CEC in the presence of suramin extinguished the remaining early phase of the response without further impairing the second phase. Both phases of the response to ToS were completely blocked by the combined presence of nifedipine and CEC.

We have shown that the two obvious physical components of the response to ToS can be further sub-divided on the basis of their pharmacology. Thus the 1st phase contains components attributable to P_{2X} and α_{1B} receptors whereas the later phase appears to be the result of P_{2X} & α_{1A} receptor activation. Thus although the noradrenergic component of the response to single stimulus appears to be due exclusively to α_{1B} adrenoceptor activation the second phase of the response to a train contains a significant contribution from α1A, but not α_{1B} adrenoceptors. One appealingly simple hypothesis to explain these observations is that α_{1B} adrenoceptors are confined to the synaptic region whereas α_{1A} adrenoceptors are excluded from this region. Trains of stimuli, unlike single stimuli, would allow NA to accumulate and spill out beyond the synaptic region to reach and activate the α_{1A} adrenoceptors.

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18P THE MUSCARINIC M1 AGONIST, McN-A-343, ACTIVATES RAT PROXIMAL AND DISTAL DUODENAL ELECTROGENIC SECRETION *IN VITRO* BY A POSTGANGLIONIC NEURAL PATHWAY

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There are few selective agonists for differentiating cholinergic receptor subtypes. McN-A-343 was proposed as an M_1 agonist, initially studied in the lower oesophageal sphincter (Goyal & Rattan, 1978). We have reported previously that McN-A-343 activates rat jejunal and ileal electrogenic chloride secretion *in vitro* indirectly via a neural pathway (Przyborski et al, 1990). In order to characterise the distribution of muscarinic receptors along the length of the small intestine, we have now examined the locus of action of this agonist in the rat proximal and distal duodenum.

Proximal duodenum (5cm segment immediately distal to the pyloric sphincter) and distal duodenum (5cm segment immediately proximal to the ligament of Trietz) were removed from rats anaesthetised with pentobarbitone (Sagatal, 60mg/kg b.wt. IP), mounted as whole thickness sheets in Ussing chambers and incubated in bicarbonate saline at 37°C, gassed with 95% O_2 5% CO_2. The short-circuit current (Isc, $\mu A/cm^2$) was obtained by standard procedures and the maximal increase in the Isc (Δ.Isc) used as the index of agonist-induced electrogenic secretion. Comparisons were made with bethanechol, a stable non-selective M_1/M_2 muscarinic agonist. Both agonists were added to the serosal side of the tissue after establishing baseline conditions for 15 minutes (n=6 for each experiment).

The 73-87% reduction in the electrogenic response to both agonists on incubation with a chloride-free buffer, indicated that the induced electrogenic secretion was carried mainly by chloride ions. Serosal atropine (0.1mM) inhibited completely the agonist (1mM) actions. Serosal hexamethonium (0.1mM), a nicotinic antagonist, had no effect on McN-A-343's action but completely blocked that of the nicotinic agonist DMPP (0.1mM). In both the proximal and distal duodenum, serosal tetrodotoxin ($1.25x10^{-6}$M) completely blocked the Isc increase induced by 1mM McN-A-343 and depressed by 33% that of bethanechol, but this was not statistically significant ($p>0.05$).

Thus rat duodenal enterocytes do not possess a functional cholinergic receptor with affinity for the muscarinic agonist McN-A-343. However, the enteric nervous innervation of the isolated *in vitro* duodenal preparation appears to have such receptors that, when activated, induce mainly electrogenic chloride secretion. Further, in agreement with previous work (Przyborski et al, 1990), the results suggest that these receptors are located on post-ganglionic neurons. Kilbinger and Nafziger (1985), working with guinea pig ileal myenteric plexus, proposed a similar location for an M_1 receptor which, when stimulated, facilitated a release of acetylcholine thus bringing about a response.

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SAP is supported by a SERC-CASE award.

19P PHARMACOLOGICAL AND BIOCHEMICAL ANALYSIS OF MUSCARINIC RECEPTORS IN GUINEA-PIG ILEUM *IN VITRO*

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Muscarinic receptor subtypes have been defined using both pharmacological (M_1, M_2 and M_3) and genomic cloning criteria (m_1, m_2, m_3, m_4 and m_5; Hulme et al., 1990). The guinea-pig ileum, classically utilised to study muscarinic receptor function (Ing, 1949) possesses a heterogeneous muscarinic receptor population (Michel and Whiting, 1990). This population was investigated using a) contractile studies to determine agonist potency and antagonist affinities (Clague et al., 1985); b) radioligand binding studies (Michel and Whiting, 1990) to assess heterogeneous populations, c) quantitative autoradiographical analysis (Sharif and Hughes, 1989) to visualize the distribution and d) second messenger studies (Ek and Nahorski, 1988) to characterize those receptors eliciting phosphatidylinositol (PI) hydrolysis. All values are mean, SEM <10%, n=4-8.

At receptors eliciting contractions, the following potencies (-log EC_{50}) were observed: (+)cis-dioxolane, 8.1, acetylcholine, 7.4, carbachol, 7.2, (±)methacholine, 7.2, arecoline, 7.1, pilocarpine, 6.3, bethanechol, 5.7 and McN-A-343, 5.2. At receptors mediating PI hydrolysis, the -log EC_{50} values were: (+)cis-dioxolane, 5.6, acetylcholine, 5.0, carbachol, 4.7, (±)methacholine, 4.8, arecoline, 5.2 and bethanechol, 3.7. Pilocarpine and McN-A-343 acted as antagonists (-log K_B=4.7 and 4.8, respectively).

The receptors eliciting contraction were antagonised in a competitive fashion (pA_2 values in parentheses) by atropine, (9.1), pirenzepine (6.8), p-F-HHSiD (7.9), HHSiD (hexahydro-siladifenidol, 8.5), 4-DAMP (4-diphenylacetoxy-N-methylpiperidine methiodide, 9.0), pentamethylene-bis-4-DAMP (7.8), methoctramine (6.0) and gallamine (<5.0). The -log K_B values for compounds antagonising PI responses were: atropine (8.9), pirenzepine (7.7), p-F-HHSiD (7.0), 4-DAMP (8.8), methoctramine (6.2) and gallamine (<5.0).

Binding studies using methoctramine displacement of [^{3}H]-N-methyl scopolamine ([^{3}H]-NMS) revealed the presence of both M_2 (-log K_i=7.7, 70%) and M_3(-log K_i=6.2, 30%) receptors. Quantitative autoradiography using [^{3}H]pirenzepine, [^{3}H]NMS and [^{3}H]4DAMP, respectively, revealed the following density of binding sites in longitudinal muscle: M_1, 1260, M_2, 8500 and M_3, 2300 amol.mm^{-2}.

These data indicate that the ileum is a highly complex tissue with respect to the muscarinic receptor population. Although M_2 receptors predominate, M_3 receptors appear to mediate both contraction and PI hydrolysis with high and low receptor reserves, respectively. The function of the majority M_2 population remains unknown.

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20P CHARACTERIZATION OF MUSCARINIC RECEPTORS MEDIATING RELEASE OF EPITHELIAL DERIVED RELAXANT FACTOR (EPDRF) *IN VITRO*

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Contractile responses in guinea-pig isolated tracheal strips to acetylcholine and (+) methacholine, but not carbachol, are enhanced upon epithelial denudation. Therefore, acetic but not carbamic acid choline esters may release EpDRF (see Small et al., 1990, for references). These responses, although inhibited by atropine (Guc et al., 1988), are mediated via a muscarinic receptor subtype that remains to be classified. We have characterized this receptor using the coaxial bioassay (guinea-pig trachea-donor; rat anococcygeus-recipient; Guc et al., 1988). Guinea-pig tracheal tubes (with epithelium intact) were placed over rat anococcygeus muscle in Krebs solution (pH 7.4, 30°C) containing indomethacin (1 μM) and physostigmine (0.1 μM). After 60 min equilibration, the latter tissue was precontracted with 30 μM phenylephrine. Once the contracture was sustained (approx. 10 min), muscarinic agonists were added on a cumulative basis. Relaxant responses were calculated as a percentage of the phenylephrine contracture. All values are mean, s.e mean <10%, n=4-8.

The rank order of agonist potency with -log EC_{50} values in parenthesis, was L660,863 (3-(3-amino-1,2,4-oxadiazol-5-yl)quinucline, 7.8), OXA-22, ((+)cis-2-methyl-5-trimethyl-1,3-oxathiolane, 7.5) (+)cis-dioxolane (7.2), acetylcholine (6.9), carbachol (6.9), (±)methacholine (6.6), ethoxyethyltrimethylammonium (6.4), arecoline (6.1) and bethanechol (5.5). No relaxant response was observed with trachea denuded of epithelium. The anococcygeus alone exhibited no significant fade in the contracture during the experimental period. Removal of the tracheal tube reversed the relaxation even though the muscarinic agonists were still present. The receptor subtype mediating release of EpDRF was characterized by determining affinities (-log K_B values in parenthesis) to the following antagonists: atropine (8.8), pirenzepine (6.5), methoctramine (5.4) 4-DAMP (4-diphenylacetoxy-N-methylpiperidine methiodide, (8.4) p-F-HHSiD (para-fluoro-hexahydro-siladifenidol; 7.3) and gallamine (4.9).

The ability of muscarinic agonists to release EpDRF, therefore, was a general property and not restricted to acetic acid choline esters. It is concluded from the antagonist -log K_B values that, in a similar fashion to the contractile response, (Eglen and Whiting, 1988), M_3 receptors also mediate the release of EpDRF.

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21P DOES HYPOXIA ACCOUNT FOR EPITHELIAL DERIVED INHIBITORY FACTOR (EPDIF) USING A CO-AXIAL BIOASSAY?

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We have demonstrated previously that vascular tissues relax when surrounded by rabbit epithelium-intact bronchus in response to methacholine using a co-axial bioassay (Spina & Page, 1989). Recently, it has been suggested that the inhibitory responses observed using co-axial assemblies may in part be due to hypoxia (Gunn & Piper, 1990). Hypoxia-induced vasodilation which may be due to the opening of ATP-sensitive potassium channels, is inhibited by glibenclamide (Daut et al., 1990). We have investigated whether the relaxant responses observed in co-axial assemblies can be inhibited with this sulfonylurea drug.
Rat endothelium-denuded aorta were suspended in an organ bath containing Krebs-Henseleit solution as described previously (Spina & Page, 1989). Following plateau of the contractile response to phenylephrine (0.05 μM; Phe), hypoxia was induced with 95% N_2 and 5% CO_2 and responses recorded over a 25 min period. Hypoxia was also induced following a 30 min incubation with glibenclamide (100 μM) or solvent (dimethyl sulfoxide; DMSO) and a third time, in the same tissues but in the absence of glibenclamide (100 μM), to investigate the irreversible nature of this drug. In other experiments, co-axial assemblies were prepared as described previously (Spina & Page, 1989). Relaxant responses to methacholine (100 μM) were performed in the absence or presence of glibenclamide (100 μM) or DMSO. Phe-contracted rat aorta relaxed under conditions of hypoxia as expressed in terms of % reversal of tone and area under the % relaxation versus time curve ($AUC_{0\text{-}25\ min}$; Table). The hypoxia-induced reversal of tone was significantly attenuated in the presence of glibenclamide (Table). When hypoxia was induced in tissues previously exposed to glibenclamide, the inhibitory effects were more pronounced (washout; Table). Hypoxia-induced relaxation was not inhibited in tissues treated with DMSO.

The affect of drug treatment on hypoxia induced relaxation (* $P < 0.01$ cf control)

Treatment	% reversal Phe tone	$AUC_{0\text{-}25\ min}$ (mm^2)	n
Control	81.4 ± 1.8	3382 ± 135	24
DMSO	75.3 ± 2.9	3185 ± 94	12
Glibenclamide	64.4 ± 3.3*	4208 ± 208*	12
Washout	35.4 ± 4.6*	4972 ± 217*	12

In co-axial assemblies, methacholine (100 μM) reversed phenylephrine (10 μM)-induced tone by 81 ± 9 % (n = 22) which was not significantly inhibited by DMSO (65 ± 11 %, n = 11, $P > 0.05$) or glibenclamide (70 ± 12 %, n = 11; $P > 0.05$). These studies demonstrate that under conditions where glibenclamide attenuated hypoxia, no inhibition of EpDIF responses was observed, suggesting that hypoxia does not account for EpDIF detected in co-axial assemblies using rabbit bronchus.

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22P DIFFERENCES BETWEEN THE THROMBOXANE RECEPTORS OF RAT AND GUINEA-PIG LUNG. 1. STUDIES WITH AGONISTS

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Studies with thromboxane (TX)A_2 antagonists suggest that the TXA_2 sensitive receptors of rat and guinea-pig lung are different (Cuthbert et al., this meeting). This report describes studies on rat lung strip with the prostanoid agonists we have previously used on guinea-pig lung strip (McKenniff et al., 1988) and also compares three TXA_2 mimetics; I-BOP (Morinelli et al., 1989), U44069 and CTA_2 (Lefer et al., 1980), on both tissues.

Both rat and guinea-pig lung strips were set up as previously described for the latter (McKenniff et al., 1988). Cumulative concentration-responses curves were constructed to all agonists using U46619 as the standard agonist. Maximal responses were determined relative to 50mM KCL, EC_{50} values were calculated by non-linear regression and expressed as pD_2s ± s.e. mean of 5 preparations.

On the rat lung strip all four TXA_2 mimetics were full agonists with maximal responses 75% of that produced by KCl with pD_2 values : I-BOP, 8.3 ± 0.1; CTA_2, 6.9 ± 0.1; U44069, 6.7 ± 0.1; U46619, 6.6 ± 0.1. PGD_2 and $PGF_{2\alpha}$ produced responses <10% of that produced by U46619 and 16,16 dimethyl PGE_2 only gave a response 20% of the U46619 maximum. On the guinea-pig lung strip only I-BOP and U46619 were full agonists (82% KCL) with pD_2 values 8.9 ± 0.1 and 6.4 ± 0.1 respectively. Both U44069 and CTA_2 produced maximal responses significantly less than that produced by U46619 - 65 ± 5 and 38 ± 6% respectively with respective pD_2s; of 6.4 ± 0.1 and 6.5 ± 0.3. The maximal responses produced by 16,16-dimethyl PGE_2 and PGD_2 were 65 ± 2% and 43 ± 8% respectively. (Concentration-response curves to the TXA_2 mimetics were not, in general, parallel).

The potency of the TXA_2 mimetics and the low efficacy of PGD_2 and $PGF_{2\alpha}$ on rat lung strip indicate that the contractile response is mediated via TXA_2 receptors as it is on guinea-pig lung strip (McKenniff et al., 1988). The low efficacy of PGD_2 and $PGF_{2\alpha}$ suggests that rat lung contains a lower concentration of TXA_2 receptors than guinea-pig lung but the lower efficacy of both CTA_2 and U44069 on guinea-pig lung suggests the converse.

This data strengthens results obtained with TXA_2 antagonists indicating, that rat lung and guinea-pig lung contain distinct TXA_2 receptor subtypes.

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23P DIFFERENCES BETWEEN THE THROMBOXANE RECEPTORS OF RAT AND GUINEA-PIG LUNG. 2. STUDIES WITH THROMBOXANE ANTAGONISTS

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We have suggested that human bronchial muscle contains a novel thromboxane (TX)A_2 receptor subtype which is insensitive to some TXA_2 antagonists (McKenniff et al., 1988). More recently we have reported similar results on rat lung strip (Cuthbert et al., 1990). We have now compared a larger selection of, structurally diverse, TXA_2 antagonists against U46619-induced contractions of both rat and guinea-pig lung parenchymal strip.

Lung strip preparations were prepared as previously described (McKenniff et al., 1988). Cumulative concentration-response curves were constructed to U46619 after 60min equilibration with test antagonist or vehicle control. Antagonist affinities (pK_B) were generally obtained from Schild analysis.

Antagonist affinities (pK_B) observed on rat and guinea-pig lung strip.

Antagonist	Rat	Guinea-pig	Antagonist	Rat	Guinea-pig
BAY u3405	8.6 ± 0.1	7.7 ± 0.5	EP092	7.1 ± 0.2+	8.5 ± 0.4
L654664	7.5 ± 0.2	7.2 ± 0.3	AH23848	7.1 ± 0.1+	8.7 ± 0.3
L691840	7.0 ± 0.1	5.3 ± 0.4	BM13505	7.1 ± 0.2*	7.0 ± 0.5*
SQ29548	7.2 ± 0.4*	7.7 ± 0.1*	ICI185282	7.2 ± 0.3*	7.9 ± 0.2*

Values are the mean ± s.e. from 5 preparations. Some antagonists determined at 10^{-7}M (*) or 10^{-6}M (+) only.

Two of the tetrahydrocarbazoles (BAY u3405, and L691840) were more potent on the rat lung strip than on the guinea-pig lung strip. The tetrahydrocarbazole (L654664) and the sulphonamide (BM13505) displayed equal affinity on both preparations. In contrast, all four prostanoid-like antagonists were more potent on the guinea-pig lung strip. There was no correlation in pK_B values on the two preparations considering either rank order of potency ($r = 0.15$) or linear correlation ($r = 0.12$).

The potency of at least some of, the TXA_2 antagonists on each tissue supports the conclusion that U46619-induced contractions are TXA_2-receptor mediated on both rat and guinea-pig lung. The lack of correlation of antagonist potency between the two tissues indicates that their TXA_2 receptors differ. This is further evidence in support of the hypothesis that subtypes of TXA_2 receptors exist.

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24P AH13205 - A NOVEL SELECTIVE PROSTANOID EP_2 AGONIST

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Prostanoid EP-receptors have been classified into three subtypes, EP_1, EP_2 and EP_3, evidence for which has been obtained with both selective agonists and antagonists (Coleman et al, 1987). We now describe a novel prostanoid, AH13205 (trans-2-[4-(1-hydroxyhexyl)phenyl]-5-oxocyclo pentaneheptanoic acid) with selective agonist activity at EP_2-receptors. We have compared the agonist activity of AH13205 with that of PGE_2 and analogues on preparations containing EP_1, EP_2 and EP_3-receptors, and we have also tested it on preparations containing DP, FP, IP and TP-receptors.

Guinea-pig fundus (GPF-EP_1), cat trachea (CT-EP_2), guinea-pig vas deferens (GPVD-EP_3), dog iris (DI-FP), rat aorta (RA-TP) and human washed platelets (HWP-DP and IP) were prepared as described previously (Coleman, 1987; Coleman et al., 1987; Keery & Lumley, 1988).

The results obtained on the EP-receptor containing preparations are summarised in the Table. PGE_2 was a potent agonist on all three preparations, but the other agonists were all more selective. Although AH13205 was moderately potent on CT, it was virtually inactive on GPF and GPVD. In addition, in concentrations up to 10μM, AH13205 had no agonist activity on DI, RA or HWP, and therefore has no significant agonist activity at FP, TP, DP or IP-receptors.

Preparation (Response)	Receptor	PGE_2 (EC_{50}nM)	Equipotent Concentration (PGE_2 = 1) Sulprostone	Misoprostol	Enprostil	AH13205
GPF (↑)	EP_1	7.6 (4.8–11.9)	2.5 (1.5–4.0)	43 (20–92)	1.8 (1.1–2.9)	>1500
CT (↓)	EP_2	19.7 (12.2–31.2)	>10,000	3.7 (1.4–9.4)	[850–1580]	29 (11–74)
GPVD (↓ NT)	EP_3	1.5 (1.1–2.0)	0.16 (0.12–0.22)	1.0 (0.5–2.0)	0.08 (0.03–0.22)	2030 (500–8350)

↑ – contraction ↓ – relaxation ↓ NT – inhibition of neuronally-mediated contractions
Each value is the geometric mean of at least 4 determinations (95% C.L.), or [range] where n <4

AH13205 appears to be a moderately potent and highly selective, prostanoid EP_2-receptor agonist. Confirmation of these findings awaits evaluation on other EP_2-receptor containing preparations, and the development of potent, selective prostanoid EP_2-receptor blocking drugs.

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25P RELATION BETWEEN *IN VIVO* AND *IN VITRO* BRONCHOCONSTRICTION TO SUBSTANCE P IN AEROSOL-SENSITIZED GUINEA-PIGS

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Our purpose was to study in parrallel in vivo and in vitro bronchoconstriction to the sensory neuropeptide substance P (SP) in guinea-pigs sensitized to ovalbumin (OA) by aerosol. Following OA-aerosol challenge, these animals presented bronchial hyperresponsiveness to acetylcholine and 5-hydroxytryptamine and airways inflammation characterized by predominant peribronchial eosinophilic infiltrate (Boichot et al., 1990 and in press). We studied 3 groups of guinea-pigs : Group 1 was sensitized by OA-aerosol and challenged by OA-aerosol 48 hours before the experiment ; Group 2 was sensitized by OA-aerosol and challenged with saline-aerosol ; Group 3 served as control and inhaled saline-aerosol twice.

Guinea-pigs were anaesthetized by intraperitoneal injection of urethane (1.2 g/kg) and ventilated. Both cervical vagi nerves were transected at the level of the neck. Insufflation pressure was continuously monitored. The initial pressure was adjusted to 10 cm H_2O according to the method of Konsett-Rössler. Aerosols were generated with a DeVilbiss "Pulmosonic" nebulizer. Enhanced bronchopulmonary responses to aerosolized SP (1 mg/ml, 1 min) were observed in groups 1 (36 ± 11 %) and 2 (33 ± 15 %) comparatively to controls (20 ± 4 %). After pretreatment with aerosolized phosphoramidon (0.1 mM, 15 min) preventing SP degradation, the bronchopulmonary response to SP (0.1 mg/ml) was significantly enhanced in group 1 (58 ± 12 %) comparatively to groups 2 (28 ± 11 %) and 3 (28 ± 8 %). Bronchopulmonary responses to aerosolized acetylcholine and histamine were significantly increased in group 1 versus groups 2 and 3.

The in vitro response of guinea-pig trachea to substance P whose contractile effects are largely modulated by airway epithelium was studied isometrically in organ baths under a basal tension of 2 g. Cumulative concentration-response curves to SP were constructed in the presence and absence of airway epithelium. In the presence of epithelium, the response of tracheal smooth muscle to exogenous SP in vitro was similar in all 3 groups (EC50 > 10 μM). When epithelium was removed by gentle rubbing of the luminal surface with a cotton swab, a similar leftward shift was observed in all 3 groups (EC_{50} = 0.16 ± 0.08 μM). Pretreatment with phosphoramidon (10 μM) considerably enhanced contraction of the intact trachea to SP as reflected by a significant shift to the left of the concentration-response curves for all 3 groups. However, the plateau of contraction was not achieved. In epithelium-free trachea, a plateau of contraction was obtained at 1 μM SP and the leftward shift of SP-curves was still present. A significant increase in EC_{50}s was observed for all groups (39.8, 50.1 and 22.4 nM for groups 1, 2 and 3 respectively). Concentration-response curves to acetylcholine and histamine were the same for all 3 groups.

Our results show that in vivo bronchial hyperresponsiveness to SP in aerosolized OA-sensitized guinea-pigs is not associated with increased in vitro responsiveness of bronchial smooth muscle to exogenous SP, whether epithelium is present or not. In addition, our results with phosphoramidon suggest that loss of endopeptidase activity cannot account for the in vivo bronchial hyperresponsiveness to SP presently observed.

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26P THE RELATIONSHIP BETWEEN THE EFFECTS OF LEMAKALIM ON TENSION AND ITS EFFECTS ON MEMBRANE POTENTIAL AND K PERMEABILITY IN BOVINE TRACHEAL SMOOTH MUSCLE

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Low concentrations of K channel opening drugs relax smooth muscle whereas higher concentrations are required to produce membrane hyperpolarisation and to increase K efflux (e.g. Bray et al., 1990). In the present experiments we examined the relationship between the relaxant effects of lemakalim (LK) and its effects on membrane potential and K permeability (PK) in bovine trachea. Strips of bovine tracheal smooth muscle were mounted on a manifold for ^{42}K efflux studies (Bray et al., 1990) or in a tissue bath for the recording of either isometric tension or membrane potential. For the relaxant experiments the tissues were precontracted with 25mM KCl. For the calculation of PK see Quast & Baumlim (1988). In the presence of glibenclamide (GB), lemakalim (5μM) produced relaxation although changes in membrane potential and PK were smaller than those produced by lemakalim 1 or 5μM alone. In the presence of glibenclamide, lemakalim 10μM produced effects comparable to those produced by lemakalim 1μM alone. However, the time to maximum effect (tmax) was increased. For details see Table 1 (mean ± s.e.mean n=3-6). 25mM KCl depolarised the membrane to -34.9 ± 0.7mV (n=18 cells) and subsequent addition of lemakalim 5μM caused full relaxation and raised the membrane potential to -41.5 ± 0.8mV (n=9 cells).

Table 1

	Tension	Membrane potential		K Permeability	
	% KCl	mV	tmax (min)	U	tmax (min)
Basal	-	-60.2±2.3		8.4±0.6	-
LK 1μM	7.5± 3.5	-76.8±2.8	13.0±1.2	29.5±2.6	>20
LK 5μM	4.0± 3.0	-81.9±1.7	6.9±0.6	60.9±5.6	12-16
LK 10μM	4.5± 3.5	-78.3±1.0	8.3±1.9	51.3±7.4	12-16
LK 5μM & GB (1μM)	33.5±10.0	-70.0±2.5	16.8±4.5	19.7±1.7	>24
LK 10μM & GB (1μM)	4.8± 6.9	-80.4±1.2	15.0±3.2	24.7±1.8	16-20

Glibenclamide (1μM), inhibited responses evoked by lemakalim and also increased the tmax values. It appeared that glibenclamide was similarly effective in inhibiting the effects of lemakalim (5μM), on membrane potential, PK and tension. The results also show that complete relaxation may not necessarily coincide with maximum changes in membrane potential or PK. Indeed, a small change in membrane potential (i.e. from -35 to -41mV) is associated with complete relaxation.

J. Longmore is supported by a grant from SmithKline Beecham.

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27P FURTHER STUDIES OF THE EOSINOPHIL-DEPENDENT ENHANCEMENT OF THE PERMEABILITY OF THE BRONCHIAL MUCOSA TO ALBUMIN

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We have previously reported that eosinophils stimulated by ionophore A23187 or by opsonic adherence are capable of damaging the basolateral side of the bronchial mucosa to promote an increase in the basolateral-apical flux of albumin (Herbert et al., 1990a,b). To extend these observations we have performed experiments in which stimulated eosinophils were maintained in contact with the apical side of bovine tracheo-bronchial epithelium and the vector of albumin flux was measured in the apical-basolateral direction. With the exception of these modifications, experiments were performed as described previously, using guinea pig peritoneal eosinophils at 3.3 x 10^6 ml^{-1} and an eosinophil-mucosal contact time of 1h.

In the presence of unstimulated eosinophils the net apical-basolateral flux of albumin was 1.78 ± 0.20 fmol cm^{-2} min^{-1} in the bronchial mucosa. Stimulation of eosinophils with 5μM A23187 resulted in an augmentation of albumin flux to 5.95 ± 1.50 fmol cm^{-2} min^{-1} ($P<0.05$, $n=4$). However, in the tracheal mucosa, the net flux was unaltered by this procedure, with rates of 0.72 ± 0.15 and 0.65 ± 0.09 fmol cm^{-2} min^{-1} being recorded respectively ($n=4$). A similar effect was seen in mucosal sheets which had been opsonized by an antibody preparation raised against components of the airways mucosa. The measured flux after exposure of eosinophils to the opsonized bronchial mucosa was 3.27 ± 0.54 fmol cm^{-2} min^{-1} compared to 1.58 ± 0.21 fmol cm^{-2} min^{-1} with the sham-opsonized control ($P<0.05$, $n=5$). Again, the tracheal tissues were resistant to the effects of eosinophils with rates of 0.73 ± 0.07 and 0.67 ± 0.07 fmol cm^{-2} min^{-1} ($n=5$) in the opsonized and sham-opsonized tissues respectively.

Eosinophil-induced bronchial mucosal injury which results in an augmented permeability to albumin is thus independent of the side of the mucosa to which the cells are added. These results suggest that eosinophils in the airway lumen may retain the capacity to cause epithelial injury. Our findings will also be of potential value in elucidating the mechanism of this acute injury process.

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28P EXTRACELLULAR MAGNESIUM REDUCES THE SECRETORY RESPONSE OF RAT PERITONEAL MAST CELLS TO COMPOUND 48/80 IN A CALCIUM-FREE MEDIUM

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Magnesium is known to inhibit secretory processes that occur by exocytosis and is dependent on extracellular calcium (Rubin, 1970). This includes the anaphylactic histamine release (Foreman, 1972). The non-immunological histamine secretion induced by compound 48/80, however, can occur in a calcium-free medium. In the present investigation, the effect of physiological concentrations of magnesium on compound 48/80-induced histamine secretion from rat peritoneal mast cells in a calcium-free medium was investigated. Preincubation of the cells in a calcium-free buffered salt solution with magnesium resulted in a time- and dose-dependent decrease of the secretory response. In the presence of magnesium (1.2 mM) histamine secretion decreased from 63% after preincubation for 10 min to 18% after preincubation for 90 min ($p<0.001$). In the absence of magnesium histamine secretion was 72% after 10 min of preincubation and 65% after 90 min of preincubation ($p>0.05$). IC_{50} for the decrease in the secretory response caused by preincubation of the cells with magnesium for 90 min was 0.3 mM. Addition of calcium (0.1 mM) to the preincubation medium prevented the decrease in the secretory response due to magnesium (0.15-4.8 mM). And the addition of calcium (0.3-3.0 mM) to the cells simultaneously with compound 48/80 dose-dependently restored the secretory response. It is possible that magnesium decreases the secretory response by inducing a loss of cellular calcium that is involved in stimulus secretion coupling. This view is supported by results demonstrating an increased loss of cellular $^{45}Ca^{2+}$ from mast cells incubated with magnesium. The cellular $^{45}Ca^{2+}$ content of mast cells incubated for 30 min with magnesium, 1.0 mM, was equivalent to 3100 cpm/10^6 cells while the cellular $^{45}Ca^{2+}$ content of cells incubated without magnesium was equivalent to 4500 cpm/10^6 cells.

The mechanism by which cellular calcium is lost is not known. However, the loss of $^{45}Ca^{2+}$ from the mast cells in this investigation was temperature-dependent as also reported by Pearce & White (1984), who provided evidence for the existence of a sodium-calcium exchange mechanism in the plasma membrane of mast cells.

In conclusion, these results support the view that magnesium reduces compound 48/80-induced histamine secretion from rat peritoneal mast cells in a calcium-free medium by displacement of a cellular pool of calcium that is involved in the stimulus-secretion coupling.

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29P IDENTIFICATION AND PARTIAL CHARACTERISATION OF CYCLIC NUCLEOTIDE-DEPENDENT PHOSPHODIESTERASE ACTIVITIES IN GUINEA-PIG PERITONEAL EOSINOPHILS

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We have previously reported (Dent *et al.*, 1990) that serum-opsonised zymosan-induced respiratory burst in guinea-pig peritoneal eosinophils is potently inhibited by Type IV- but not Type III-selective phosphodiesterase (PDE) inhibitors (see Reeves *et al.*, 1987 for classification). We now report the results of studies designed to investigate the identity and nature of the cyclic nucleotide-dependent PDE isoform(s) present in these cells.

Guinea-pig peritoneal eosinophils were harvested and purified using well established methods. Cells were suspended in lysis buffer (10 mM TEA - pH 8; 1 mM EDTA) and frozen in liquid nitrogen. After thawing the eosinophil suspension was extruded (10-times) through a 23G needle and sonicated. Triton X-100 was then added to a concentration of 0.5% (v/v). The eosinophil slurry was either subjected to differential centrifugation or used immediately for PDE activity measurements.

Table I. Effect of Non-selective and Isoenzyme-Selective PDE Inhibitors on Cyclic AMP Hydrolysis by Eosinophil Lysates

PDE Inhibitor	'n'	Isoenzyme Selectivity	IC_{50} (μM)*	n_H
Denbufylline	9	IV	0.102 ± 0.016	0.454 ± 0.033
Rolipram	10	IV	0.16 ± 0.044	0.444 ± 0.035
Zardaverine	3	III/IV	1.63 ± 0.38	0.866 ± 0.035
AH 21-132	4	III/IV	2.83 ± 0.54	0.971 ± 0.055
ORG 30029	3	III/IV	9.28 ± 1.81	0.774 ± 0.121
IBMX	4	NS	11.2 ± 2.16	0.764 ± 0.053
Zaprinast	4	I	224.6 ± 37.3	0.956 ± 0.088
Theophylline	3	NS	476.5 ± 98.9	1.175 ± 0.167
Imazodan	3	III	35.5 ± 8.3%§	
SK & F 94120	4	III	32.7 ± 12.1%§	

* Cyclic AMP at 1 μM; NS: Non selective; § % inhibition at 1 mM

At a substrate concentration of 1 μM, eosinophil lysates preferentially hydrolysed cyclic AMP (1.81 ± 0.19 pmol. min^{-1}. 10^6 cells, n = 37) at a velocity 19-fold greater than when cyclic GMP (1 μM) was used as substrate (0.11 ± 0.001 pmol min^{-1}. 10^6 $cells^{-1}$, n = 6). This catalysis was not affected by cyclic GMP (10 μM) or by calmodulin. Cyclic AMP hydrolysis was, however, potently inhibited by Type IV-selective PDE inhibitors but with slope factors (n_H) significantly less that unity (Table 1). Type III/IV- and non-selective PDE inhibitors similarly inhibited cyclic AMP hydrolysis whereas Type III PDE inhibitors were inactive at concentrations at which they retained isoenzyme selectivity. Differential centrifugation of eosinophil suspensions revealed that the PDE was predominantly a membrane-associated enzyme that was exceedingly resistant to detergent solubilisation. Analysis of cyclic AMP hydrolysis by the method of Hofstee showed non-linear kinetics that could be divided into high and low affinity components. It is concluded that guinea-pig eosinophils express only a Type IV PDE isoenzyme that is responsible for regulating the intracellular cyclic AMP content. The shallow inhibition curves obtained for the Type IV-selective PDE inhibitors together with the non-linear kinetics of cyclic AMP hydrolysis suggests the presence of at least two Type IV PDE isoenzymes in guinea-pig eosinophils for which rolipram and denbufylline do not readily discriminate.

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30P EVIDENCE FOR TWO DISTINCT Ca/CALMODULIN STIMULATED CYCLIC NUCLEOTIDE PHOSPHODIESTERASES (PDES) IN RABBIT CARDIAC VENTRICLE

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The existence of several PDE isoenzyme families each consisting of different isoforms is now well recognised. In brain and testes multiple forms of Ca^{2+}/calmodulin dependent PDE (PDE I) have been identified (Beavo, 1988). Our aim was to further characterise the PDE isoenzymes in rabbit heart and in particular test for the presence of PDE V (cyclic GMP specific PDE), which often co-elutes with PDE I (Beavo, 1988), and multiple forms of PDE I. Previous studies have demonstrated the presence of four PDE isoenzymes in rabbit heart. PDE isoenzymes were separated using DEAE sepharose and assayed according to Shahid and Nicholson (1990). The first two activity peaks were stimulated by Ca^{2+}/calmodulin, the second being additionally stimulated by cyclic GMP. Calmodulin agarose chromatography showed the first peak to entirely consist of a calmodulin dependent isoenzyme (PDE Ia). The second peak, however, consisted of a calmodulin dependent PDE (PDE Ib), which was not stimulated by cyclic GMP, and an isoenzyme which had little affinity for calmodulin but was stimulated by cyclic GMP (PDE II). PDE Ib could be distinguished from PDE Ia as it had a higher affinity for cyclic GMP and was less effectively inhibited by zaprinast (table 1). Two further activity peaks were obtained from DEAE sepharose; the third peak (PDE IV) was potently inhibited by rolipram, but not cyclic GMP whilst the fourth peak (PDE III) was inhibited by cyclic GMP and milrinone but not by rolipram (table 1).

Table 1 Kinetic properties and inhibitor sensitivity of rabbit cardiac ventricle PDE isoenzymes

PDE Isoenzyme	Km (μM)		Ratio of Vmax	IC_{50} (μM)		
	cyclic AMP	cyclic GMP	cyclic AMP/cyclic GMP	zaprinast	milrinone	rolipram
PDE Ia	4.4[a]	21[a]	0.2	9.5	>250	>250
PDE Ib	11[a]	3.3[a]	0.4	73	>250	>250
PDE II	57[b]	35	1.8	209	>250	>250
PDE III	2.9	1.0	10	136	4.2	114
PDE IV	3.7	26	1.7	119	43	1.7

[a] In the presence of Ca^{2+} (20 μM)/calmodulin (0.3 μg/ml); [b] In the presence of cyclic GMP (1 μM);
[c] Inhibitor assays performed at 1 μM cyclic AMP except for PDE Ib where cyclic GMP was used.

The results show rabbit cardiac muscle to contain multiple PDE I isoforms. Furthermore, there is no evidence for the presence of the zaprinast-sensitive PDE V, which shows high activity in tissues such as platelets and smooth muscle.

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31P VANADIUM COMPOUNDS, BUT NOT HEPARIN, INHIBIT MECHANICAL RESPONSES TO IP_3 IN SAPONIN-TREATED GUINEA-PIG ILEUM LONGITUDINAL SMOOTH MUSCLE

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Mechanical responses to inositol 1,4,5-trisphosphate (IP_3) were examined in saponin-permeabilised strips of guinea-pig ileum longitudinal smooth muscle as previously described (Allen & Carpenter, 1989). IP_3 (1-100 μmol/l) and GTPgammaS (1-100 μmol/l), but not GTP (1-1000 μmol/l) elicited contractions in permeabilised strips.

Sodium decavanadate ($Na_6V_{10}O_{28}$) has previously been shown to inhibit IP_3-induced calcium release in permeabilised cells with an IC50 of 5 μmol/l (Fohr *et al.*, 1989). In our study decavanadate caused a concentration dependent inhibition of responses to both IP_3 (100 μmol/l) and GTPgammaS (100 μmol/l) with IC50 values of 3.6 μmol/l & 18.1 μmol/l respectively. Decavanadate (100 μmol/l) had no effect on the contractile response of permeabilised strips to calcium. Vanadium chloride (VCl_3), sodium metavanadate ($NaVO_3$) and vanadyl sulphate ($VOSO_4$) also caused concentration-dependent inhibition of the response to IP_3 with IC50 values of 88 μmol/l, 26 μmol/l & 65 μmol/l respectively. None of these compounds affected the response to calcium. These results are consistent with these vanadium-containing compounds being IP_3 antagonists at calcium release sites.

The putative IP_3 antagonist, heparin, and its De-N-sulphated derivative were also tested. On this preparation neither heparin (10-1000 μg/ml) nor De-N-sulphated heparin (10-1000 μg/ml) had significant effects on responses to IP_3 (100 μmol/l). Heparin has previously been shown to bind to IP_3 receptors in tracheal smooth muscle microsomes (IC50 7.6 μg/ml, Chilvers *et al.*, 1990) and to inhibit calcium-release from saponin-permeabilised rabbit main pulmonary artery (IC50 5 μg/ml, Kobayashi *et al.*, 1988). The difference in results may reflect a genuine difference in IP_3 receptors between smooth muscle types.

M.J.Allen is in receipt of a Fisons studentship.

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32P FORSKOLIN, BUT NOT 8 BROMO CYCLIC GMP, MODULATES THROMBIN-INDUCED ELEVATIONS OF CYTOSOLIC CALCIUM IN BOVINE AORTIC ENDOTHELIAL CELLS

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The cyclic nucleotides, cyclic AMP and cyclic GMP, both inhibit mobilisation of calcium in smooth muscle (Meisheri & Van Breemen, 1982; Cornwell & Lincoln, 1989). We wished to determine if the cyclic nucleotides similarly inhibit calcium mobilisation in vascular endothelium.

Primary cultures of bovine aortic endothelial cells (BAEC) were seeded onto sterile glass coverslips and loaded with fura-2/AM (2μM) for 45 min. Following a 20 min wash period, coverslips were suspended across the diagonal of a quartz cuvette containing HEPES (10mM)-buffered Krebs at 37°C. Excitation was alternated between 340 and 380nm every 3s and emission collected at 509nm. Intracellular calcium $[Ca^{2+}]_i$ was calculated according to Grykiewicz et al (1985).

In the presence of 1.8mM extracellular calcium, resting $[Ca^{2+}]_i$ was 75±6nM (n=20). Thrombin ($1Uml^{-1}$) induced a rapid, transient elevation of $[Ca^{2+}]_i$ to a peak of 347±34nM (n=11), which decayed to a sustained plateau of 197±19nM after 5 min. Treatment with forskolin (10μM, 5 min) had no effect on resting $[Ca^{2+}]_i$ or the magnitude of the thrombin-induced peak elevation of $[Ca^{2+}]_i$ (362±52nM; n=9). In contrast, addition of forskolin during the thrombin-induced sustained plateau resulted in a further elevation of $[Ca^{2+}]_i$; the maximum increase was 66±8nM (n=9) and this decayed to 41±6nM after 5 min. Isoprenaline ($10^{-5}M$) induced a similar biphasic elevation of $[Ca^{2+}]_i$ when added during the plateau phase; the maximum increase was 61±6nM (n=6) and this decayed to 25±6nM after 5 min.

In a second series of experiments, thrombin ($1Uml^{-1}$) elevated $[Ca^{2+}]_i$ to a peak of 228±16nM which decayed to a plateau of 181±6nM after 5 min (n=9). 8 bromo cyclic GMP (30μM, 5 min) had no effect on resting $[Ca^{2+}]_i$ or on the magnitude of the thrombin-induced initial transient elevation of $[Ca^{2+}]_i$ (238±28nM; n=6). 8 bromo cyclic GMP (30μM) also had no effect when added during the plateau phase of the thrombin-induced elevation of $[Ca^{2+}]_i$. Sodium nitroprusside (1μM), which acts via the formation of cyclic GMP, also had no effect when added on the plateau phase of the thrombin-induced increase in $[Ca^{2+}]_i$.

In conclusion, our study suggests that cyclic GMP has no effect on resting $[Ca^{2+}]_i$ or thrombin-induced mobilisation of calcium. Cyclic AMP is also without effect on resting $[Ca^{2+}]_i$ and on the initial thrombin-induced transient elevation of $[Ca^{2+}]_i$. The ability of forskolin and isoprenaline to augment the plateau phase of the thrombin-induced elevation of $[Ca^{2+}]_i$ suggests that cyclic AMP enhances calcium mobilisation in BAEC.

This work was supported by the British Heart Foundation.

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33P LECTIN-STIMULATED PROLIFERATION AND SECOND MESSENGER GENERATION IN HUMAN LYMPHOCYTES FOLLOWING ISOPRENALINE INFUSIONS

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It has previously been demonstrated that acute increases in sympathetic activity or infusions of catecholamines are accompanied by a redistribution of circulating lymphocyte subsets and a decreased proliferative response to mitogenic lectins in vitro (Crary et al. 1983, van Tits et al. 1990). Since the activation of resting lymphocytes involves generation of inositol phosphates (IP) and influx of extracellular Ca^{++} (Hadden 1988), we have now investigated whether the lymphocyte subset redistribution and the depressed proliferative response is accompanied by an altered signal transduction.

Isoprenaline was infused into healthy volunteers at increasing doses of 3.5, 7, 17.5, 35 and 70 ng/kg/min (5 min each) and blood samples for the isolation of lymphocytes were obtained before and after the infusion. Phenotypes of circulating lymphocytes were determined by indirect immunofluorescence using monoclonal antibodies (van Tits et al. 1990), intracellular free Ca^{++} was measured with the fluorescent dye Fura-2, formation of IP was determined in cells pre-labeled with [^{3}H]-myoinositol, and proliferation was assessed by the incorporation of [^{3}H]-thymidine.

Infusion of isoprenaline decreased the number of T_{helper}- (T_h) cells and increased that of $T_{suppressor/cytolytic}$- ($T_{s/c}$) and natural killer cells. Thus $T_h/T_{s/c}$-cell ratio decreased significantly from 1.6 ± 0.1 to 1.0 ± 0.1 (n = 5). Incorporation of [^{3}H]-thymidine upon stimulation with the lectins phytohemagglutinin (PHA) and concanavalin A (Con A) was depressed by 30 to 50%. Infusion of isoprenaline did not affect basal levels of intracellular Ca^{++} (60 ± 3 vs. 64 ± 2, n = 5) and of IP. Lectin-stimulated formation of IP, however, was 250 to 500% greater in lymphocytes obtained after isoprenaline-infusion. On the other hand, PHA-stimulated increases in intracellular Ca^{++} were only slightly but significantly increased and Con A-stimulated increases in intracellular Ca^{++} were not altered at all.

We conclude that the isoprenaline infusion-induced redistribution of circulating lymphocyte subsets is accompanied by opposite changes of lectin-stimulated proliferation and second messenger responses.

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34P ADP-RIBOSYLTRANSFERASE IN NG108-15 CELLS AND ITS ROLE IN THE REGULATION OF Gsα ACTIVITY AND ABUNDANCE

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Prostacyclin and adenosine stimulate adenylate cyclase in NG108-15 hybrid cells. Prolonged exposure of these cells to iloprost (a stable prostacyclin analogue) led to a decrease in both iloprost and adenosine-stimulated adenylate cyclase activity. This example of heterologous desensitization is accompanied by a decrease in the capacity of cholera toxin to ADP-ribosylate Gsα, which may be due to iloprost-stimulated endogenous ADP-ribosylation of Gsα (Molina y Vedia et al. 1989). We have now examined endogenous ADP-ribosyltransferase activity, and its possible role in these cells.

NG108-15 cells (approx. 1.8 x 10^7 cells) were suspended in 300 µl of 50% (w/v) PEG 1500 containing 200 µCi [^{32}P]-NAD for 10 min. The cells were washed throughly with PBS, and seeded into six 35 mm plates. The cells were treated with either 10 µM iloprost, 50 mM nicotinamide or a combination of both for 18 h. Cell proteins were separated by polyacrylamide gel electrophoresis. Labelled bands were identified autoradiographically. Adenylate cyclase activity was determined in cell homogenates (Kelly et al. 1990). The level of Gsα in the membranes was determined by immunoblotting with an anti-Gsα antibody (Milligan and Unson, 1989). The capacity of cholera toxin to ADP-ribosylate membrane proteins was also measured (Kelly et al. 1990).

Endogenous ADP-ribosyltransferase activity resulted in five major bands on autoradiography with molecular weights of 52K, 44K, 35K, 30K and 25K. Enzyme activity was not increased by iloprost, but was inhibited by nicotinamide. Nicotinamide pretreatment had no effect on responsiveness of adenylate cyclase to iloprost or heterologous desensitization of responses to NaF or Gpp(NH)p. However, nicotinamide increased basal adenylate cyclase activity 2-3 fold.

Prolonged exposure of cells to iloprost reduced the capacity of cholera toxin to ADP-ribosylate Gsα, whereas nicotinamide enhanced cholera substrate. This effect of nicotinamide might be due to the inhibition of endogenous ADP-ribosyltransferase. Immunoblots of membranes also indicated removal of Gsα from the membrane by iloprost. In contrast, nicotinamide appeared to increase slightly the amount of Gsα, which is consistent with the increase in basal adenylate cyclase activity (measured in the presence of GTP) reported above.

This endogenous ADP-ribosylation is not implicated directly in heterologous desensitization in NG108-15 cells. However, the results indicate that this enzyme may have a role in regulating the abundance of Gsα.

This work was supported by a grant from the Wellcome Trust.

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35P THE TUMOUR-PROMOTER OKADAIC ACID INDUCES THE PHOSPHORYLATION AND INACTIVATION OF G_i-2

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The receptor-dependent stimulation and inhibition of adenylate cyclase are mediated by two distinct G-proteins termed G_s and G_i respectively. A family of "G_i-like" proteins exists which is comprised of three pertussis toxin-sensitive G-proteins termed G_i-1, G_i-2 and G_i-3, and the pertussis toxin-insensitive G_z. In addition to mediating inhibition of adenylate cyclase, members of this family are involved in the regulation of a variety of signalling systems including phosphoinositidase C, phospholipase A_2 and K^+ channels (Friessmuth et al, 1989).

G-proteins can serve as potential key points for the initiation of "cross-talk" between different signalling pathways reulting in the desensitization and/or potentiation of specific signalling systems. Such "cross-talk" represents an important mechanism for the control of cellular responsiveness and may be mediated by hormone-induced G-protein phosphorylation. Indeed, we have shown that treatment of intact hepatocytes with a range of ligands capable of activating protein kinase C and/or cyclic AMP-dependent protein kinase induced the selective phosphorylation and inactivation of α-G_i-2, but did not affect either α-G_i-3 or α-G_s.

Recently, a novel tool has become available for the study of protein phosphorylation in intact cells. This is okadaic acid, a tumour promoter which acts to inhibit protein phosphatases 1 and 2A, thereby increasing the phosphorylation of key regulatory proteins (Cohen et al, 1990). In the present study we have examined the effects of okadaic acid treatment on the phosphorylation and functioning of α-G_i-2 in isolated rat hepatocytes. Hepatocytes were prepared from male Sprague-Dawley rats and G_i phosphorylation and functioning were examined as described (Bushfield et al, 1990). Okadaic acid (10^{-10} to 10^{-5} M) caused a rapid time- and concentration-dependent phosphorylation of α-G_i-2. This effect was significant with 10^{-9} M okadaic acid and the EC_{50} was 10^{-8} M. The elevation of G_i phosphorylation reached a peak after 5 to 10 minutes of incubation with 10^{-7} M okadaic acid. Under these conditions, okadaic acid treatment also caused a complete blockade of G_i functioning as monitored by guanine-nucleotide-dependent inhibition of adenylate cyclase in hepatocyte plasma membranes. These results demonstrate that inhibition of protein phosphatase activity under basal conditions, i.e. in the absence of hormonal challenge, suffices to increase the phosphorylation of α-G_i-2. This, we propose, exposes a dynamic phosphorylation/dephosphorylation cycle which controls the activity of this key guanine nucleotide regulatory protein.

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36P MODULATIONS OF CYCLIC AMP FORMATION IN HUMAN CEREBRO-CORTICAL SLICES

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Second messenger formation in the brain can be modified indirectly by a number of so-called neuromodulators. In many cases these modulations are species selective (Hill and Kendall,1989) and studies using experimental animals can be misleading with regard to effects in human brain. We have therefore examined the properties of some potential modulators of cyclic AMP formation in human brain slices.

Samples of tissue (generally 200-300mg wet weight)from a variety of cortical areas were taken during trans-cortical neurosurgical procedures. The mean age of the donors was 54yrs (ranging from 4 to 74) and 55% were male. The tissue samples were rapidly transfered to ice-cold Krebs-Henseleit buffer and transported to the laboratory (<5mins.) for preparation. 350 m cross-chopped slices were pre-labelled with ^{3}H-adenine and ^{3}H-cyclic AMP formation was monitored in 10µl aliqots of packed slices. Agonist stimulations were for 10mins. after which the incubations were stopped with 1M HCl (Robinson and Kendall,1989).

Responses to maximally effective concentrations of the following agents were recorded (expressed as fold basal stimulation) adenosine ($3x10^{-4}$M), 48±3 (n=3); isoprenaline ($3x10^{-6}$M), 28±4 (n=14); forskolin (10^{-5}M), 3±0.3 (n=3). 5HT ($3x10^{-4}$M), histamine ($1x10^{-3}$M), glutamate ($3x10^{-3}$M) and quisqualate ($1x10^{-4}$M) all failed to produce significant stimulations. The maximum response to noradrenaline ($3x10^{-4}$M; α+ βadrenoceptor stimulation) exceeded that to isoprenaline (selective β-stimulation) by 1.5±0.08 fold (n=9). This response to noradrenaline was reduced to the level of the maximum isoprenaline stimulation by prazosin (IC_{50} =3±$1x10^{-8}$M, n=6) and yohimbine (IC_{50} =1.5±$0.6x10^{-7}$M,n=5) these relative affinities being close to those found using rat brain slices (Robinson and Kendall,1989). Despite having no effect alone histamine (10^{-3}M) ptentiated the responses to isoprenaline ($1x10^{-6}$M) by 1.8±0.08 fold (n=3) and to forskolin ($1x10^{-5}$M) by 1.9 fold (n=2) and these enhancements were abolished in the presence of mepyramine ($1x10^{-6}$M). Neither glutamate nor quisqualate altered the response to adenosine.

It appears therefore that some, but not all, of the modulations of cyclic AMP formation that have been observed in experimental animal brains exist in human cerebral cortex and these findings emphasize the potential dangers in extrapolating data from one species to another.

Funded by the Trent RHA.

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37P A CRUDE BOVINE BRAIN EXTRACT CONTAINING CLONIDINE DISPLACING SUBSTANCE INHIBITS CYCLIC AMP FORMATION IN BRAIN SLICES

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We reported recently (Young et al.1990) that a crude extract of bovine brain displaced ^{3}H-clonidine binding from brain membranes and stimulated inositol phospholipid hydrolysis whilst reducing the uptake of myo-inositol. Here we describe the effects of similar extracts on cyclic AMP formation in brain slices.

Extracts containing "clonidine displacing substance" were prepared as described by Atlas and Burstein (1984) from 250g fresh bovine brain (minus cerebellum) and dissolved in 5ml distilled water. ^{3}H-clonidine binding to bovine brain membranes was as described by Rouot and Snyder (1979) and ^{3}H-cyclic AMP formation in rat, guinea-pig and human cerebral cortex slices was measured by a ^{3}H-adenine pre-labelling assay (Robinson and Kendall,1989).

The extract inhibited the binding of 0.5nM ^{3}H-clonidine with an IC_{50} of 6.3 µl/ml (n=4). The cyclic AMP response to 2-chloroadenosine (2-CA, $3x10^{-5}$M) in rat cortical slices was abolished by the higher concentrations of the extract used and the IC_{50} was 2.7±0.2 µl/ml (n=3). At a concentration of 30 µl/ml the extract also abolished the responses to forskolin (10^{-5}M) and isoprenaline (10^{-6}M) and the inhibition was unsurmountable at a concentration of 10^{-4}M isoprenaline. The inhibition was partly reversible, the response to 2-CA returning to 37% (n=2) of control after a 15 min incubation with 16 µl/ml of the extract followed by a 60 min washout and was unaffected by omission of Ca^{2+} from the medium or by the inclusion of the phosphodiesterase inhibitor rolipram (10^{-5}M). The inhibition of the 2-CA response due to the presence of 16 µl/ml of the extract was unaffected by either idazoxan or atropine (both 10^{-6}M). The extract was equally effective in a single experiment using human cerebral cortex slices stimulated by 2-CA or isoprenaline, but even at a concentration of 60 µl/ml the extract failed to inhibit the response to 2-CA in guinea-pig cerebral cortex slices (n=2).

The effects of the extract cannot be accounted for by cell toxicity or, given the lack of effect of idazoxan, by interaction with alpha$_2$ adrenoceptors or "imidazoline prefering sites" and it seems likely that a direct inhibition of adenylate cyclase is involved although the species selectivity is puzzling. Identification of the active agent(s) in these extracts is now a priority.

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38P CHARACTERIZATION OF GUINEA-PIG EOSINOPHIL CYCLIC AMP PHOSPHODIESTERASE (PDE)

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Cyclic AMP (cAMP) exerts a negative influence on eosinophil activation. Recently, it has been reported that inhibitors of Ro-20-1724-inhibited (Type IV) cAMP PDE, but not cGMP-inhibited (Type III) cAMP PDE, reduce eosinophil superoxide generation (Dent et al, 1990). We have undertaken studies to characterize the PDE subtype(s) in guinea-pig peritoneal eosinophils and evaluate the effects of selective and non-selective inhibitors on cAMP accumulation.

Cells were harvested from the peritoneal cavity of horse-serum treated (0.5ml i.p., biweekly) guinea-pigs and centrifuged on discontinuous metrizamide gradients. Eosinophils (100-200 X 10^6) were homogenized in isotonic buffer containing a cocktail of protease inhibitors. The homogenate was centrifuged at 105,000 x g for 60 min and the pellet resuspended in homogenization buffer. PDE activity in the homogenate, cytosol and particulate fraction was determined according to Thompson et al (1979). In intact cell studies, cAMP was extracted with 5% trichloroacetic acid and quantified by RIA (NEN).

Eosinophils contained a predominantly membrane-bound cAMP PDE (93 ± 2.4% of total activity, n = 4, cAMP substrate - 2µM). This activity was not influenced by cGMP(1µM) or $CaCl_2$(2µM) + calmodulin (10U/ml). The enzyme(s) exhibited complex kinetics (km's = 0.6µM and 21µM). Treatment of membranes with Triton X-100 (1%) failed to solubilize any cAMP PDE. The Type IV PDE inhibitors, rolipram (IC_{50} = 0.12 ± 0.04µM, mean ± s.e.mean, n = 4) and denbufylline (IC_{50} = 0.1 ± 0.01µM, n = 3) were potent inhibitors of particulate cAMP PDE. Non-selective inhibitors, trequinsin (IC_{50} = 0.11 ± 0.02µM, n = 3) and AH-21-132 (IC_{50} = 2.6 ± 1.2µM, n = 3) also inhibited activity but the Type III PDE inhibitor, SK&F 94120, had no effect. PDE inhibitors, alone, had little effect on cAMP levels in intact cells; however, in the presence of isoprenaline (10µM), rolipram and denbufylline potently increased cAMP accumulation (EC_{50}'s = 0.45 ± 0.16µM and 0.28 ± 0.08µM, n = 3, respectively) with a maximal increase >500%. In contrast to their actions on PDE activity, trequinsin (EC_{50} = 4.3 ± 2.0µM, n = 3) and AH-21-132 (EC_{50} > 10µM, n = 3) were poor stimulators of cAMP accumulation.

We conclude that guinea-pig eosinophils contain a tightly-bound, particulate Type IV cAMP PDE. Inhibition of this activity by certain compounds correlates poorly with their ability to elevate cAMP, suggesting the presence of a barrier in intact cells impeding their access to the enzyme.

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39P EFFECTS OF PHOSPHODIESTERASE INHIBITORS ON GRANULOCYTE SUPEROXIDE GENERATION

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We have found (Souness et al 1990) that although certain selective and non-selective phosphodiesterase (PDE) inhibitors potently inhibit the guinea-pig eosinophil type IV cyclic adenosine monophosphate (cAMP) PDE with IC_{50}'s in the sub-micromolar range, this activity does not correlate with their ability to enhance isoprenaline induced cAMP accumulation in whole cell preparations. In order to determine if the effects of PDE inhibitors on cAMP accumulation are associated with inhibition of cell function we have investigated the effects of PDE inhibitors on granulocyte superoxide generation (SOG).

Guinea-pig eosinophils harvested by peritoneal lavage of horse serum treated animals (0.5 ml i.p. biweekly) and human granulocytes prepared from dextran sedimented whole blood, were purified by centrifugation on discontinuous metrizamide gradients. Cells (10^6 $50\mu l^{-1}$ $well^{-1}$) were washed, resuspended in HBSS containing Ca^{++} and Mg^{++} and added to 96-well plates (GIBCO) to a final volume of 250µl of HBSS containing 0.5 mg ml^{-1} p-iodonitrotetrazolium violet (INTV) in the presence or absence of drugs. Cells were pre-incubated with the PDE inhibitors or vehicle (0.1% DMSO) for 15min at 37°C prior to challenge for 30min with opsonized zymozan (OPZ) or buffer. SOG was measured as the superoxide dismutase (100µg ml^{-1}) inhibitable reduction of INTV at 492nm following solubilization of the cell pellet in DMSO-5% HCl.

In guinea-pig eosinophils (n=4-6) rolipram and denbufylline were potent inhibitors of basal (incubation induced) SOG (mean IC_{50}'s 0.02 and 0.03µM respectively), trequinsin was a weak inhibitor (IC_{50} 8µM) and AH21-132 (0.01 - 10µM) was without effect. In human granulocytes (n=4) rolipram was a potent inhibitor of background and OPZ (0.01mg ml^{-1}) stimulated SOG (IC_{50} 0.05µM), both denbufylline and trequinsin (<50% inhibition at 10µM) were relatively weak inhibitors. The maximum inhibition of superoxide dismutase inhibitable SOG by rolipram (10µM) in cells stimulated with 0.01 mg ml^{-1} OPZ was 83 ± 13.8% but this was reduced to 13 ± 7.8% in cells stimulated with 1mg ml^{-1} OPZ (n=9).

In conclusion, inhibition of basal guinea-pig eosinophil SOG is exhibited by type IV selective and non-selective cAMP-PDE inhibitors with an order of potency that correlates with their efficacy in enhancing isoprenaline-induced increases in cAMP accumulation (Souness et al 1990). Unlike their effects on cAMP accumulation inhibition of SOG by rolipram and denbufylline did not require activation of adenylate cyclase by an exogenous stimulus and was seen at concentrations that alone had no detectable effect on intracellular cAMP levels. Thus it is likely that only small changes in cAMP levels are necessary to regulate SOG. In human granulocytes the inhibitory effects of PDE inhibitors are similar to those observed in guinea-pig eosinophils but are attenuated by a potent stimulus to superoxide generation.

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40P RENAL ACTIONS OF ANGIOTENSIN II ARE MODIFIED BY AN INHIBITOR OF CYTOCHROME P450-DEPENDENT ARACHIDONIC ACID METABOLISM

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Several non-cyclooxygenase arachidonic acid (AA) metabolites, synthesized via lipoxygenase (LO) and cytochrome P450 (cyt P450) enzymatic pathways, have taken on special significance as potential regulators of vasomotion and ion transport. For example, the 20-hydroxy AA metabolite inhibits rubidium uptake by rabbit thick ascending limb of Henle cells (Escalante, 1990) and produces natriuresis, independent of changes in GFR, in the anesthetized rat (Takahashi et al., 1990). The 5,6 epoxide of cyt P450-AA metabolism (5,6-EET), reduces GFR, whereas during cyclooxygenase (CO) inhibition, GFR is increased (Takahashi et al., 1990). In rat isolated proximal tubules, release of 5,6-EET, which is stimulated by angiotensin II (AII), inhibits sodium reabsorption and may participate in the natriuretic response to high-dose AII (Romero et al., 1990). We have previously found that infusion of high levels of AII increased the excretion of salt and water. Concomitant inhibition of CO and LO pathways of AA metabolism during AII infusion enhanced fractional water and sodium excretion rate as well as increasing GFR. We therefore studied the effect of 7-ethoxyresorufin (7-ER), a potent inhibitor of cyt P450-AA metabolism, on renal responses to AII infusion using the rat-isolated kidney perfused with oncotic agents. In all experiments indomethacin (I) was used to block cyclooxygenase; renal perfusion pressure was kept constant at 90 mmHg during 4 consecutive 15 min clearance periods. Four groups were studied (n=5-8); 1) I (10µM); 2) I+7ER (2µM); 3) I+AII infusion (10 pmol/min) and 4) I+7ER+AII.

AII increased urine volume from 44.2±21.5 and 44.9±13.1 µl/min (groups 1 and 2) to 134.1±25.9 µl/min (group 3), an effect reduced by 7ER (62.6±16.3 ul/min, group 4). Similarly, 7ER reduced the AII-induced increases sodium and chloride excretion rates being 9.6±2.4 and 10.0±2.2 versus 5.0±1.0 and 4.9±1.3 µEq/min in the I+AII and I+7ER+AII groups, respectively. The increase in potassium excretion during AII infusion was completely prevented by 7ER. I+AII increased renal vascular resistance (RVR) to a maximum of 7.0±2 mmHg/ml/min and increased filtration fraction to 8.8±3.7 without affecting GFR. 7ER further increased RVR to 9.6±3.4 associated with a reduction in filtration fraction and GFR. As fractional electrolyte excretion rates were not different between I+AII and I+7ER+AII treated kidneys, the reduction in the natriuretic response to AII by an inhibitor of cyt P450-AA metabolism could be entirely accounted for by changes in GFR. These results are compatible with a role for cyt P450-AA metabolism in the renal actions of angiotensin II in the rat isolated kidney.

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41P ACTION OF STAUROSPORINE ON Ca SIGNAL AND CONTRACTION EVOKED BY PHENYLEPHRINE IN RAT AORTA

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Adrenoceptors stimulation evokes the contraction of rat aorta by mobilisation of both intracellular and extracellular calcium pools (Godfraind *et al.*, 1986). A question still controversial is the nature of receptor(s) involved in this process. The present experiments were designed in order to see how phenylephrine could affect calcium signal and contraction in rat aorta.
Rat aorta rings from male rats (290-300 g) were lightly rubbed to remove the endothelium. They were preincubated during 6 h in physiological solution containing fura-2 AM (5µM) and nitroarginine (0.1 mM) The rings were inverted and mounted between two hooks under a tension of 2 g in 7 ml organ bath filled with Krebs solution at 37°C gassed with 95% O_2: 5 % CO_2. This bath was part of a fluorimeter (CAF100,JASCO, Tokyo) which allowed an estimate of the calcium signal. Tissues were contracted with two successive 72.5 mM K-depolarisations followed by phenylephrine 30 nM. Contraction and fluorescence were recorded simultaneously and were expressed in per cent of the second KCl response.
Phenylephrine 30 nM evoked a sustained Ca signal and a simultaneous tonic contraction on which were superimposed rhythmic activities occurring at a frequency of 10 min^{-1}. The peak contractions and Ca signals were respectively equal to 96.2±9.2 %(n=7) and to 64.8±5.4% (n=7). The rhythmic activity was sustained as long as phenylephrine was present in the bath and was abolished by nimodipine 1 µM. We observed that the contraction resistant to nimodipine was equal to 19.9±3.7 % (n=6) and that the calcium signal recorded in the presence of nimodipine was equal to 11.1±3.7 %(n=6). Preparations (n=5) were treated during 10 min by staurosporine 100 nM after the second K-evoked contraction and were therafter stimulated by phenylephrine. In staurosporine treated muscles, the contractile response but not the Ca signal evoked by phenylephrine was delayed. The initial Ca signal recorded before development of contraction was equal to 5.4±0.9 % .The delayed peak contractions and Ca signals were respectively equal to 53.4.3±11.8 % and to 28.3±6.4 % ($P< 0.01$ from controls). After nimodipine, tonic contraction and sustained Ca signal were respectively equal to 7.7±2.7 % ($P<0.01$ from controls) and 10.4±1.8 % (n.s.from controls). (It is likely that staurosporine was partly denaturated by the illumination required to measure the Ca signal).
In summary, staurosporine left unaltered the nimodipine resistant Ca signal but reduced the nimodipine-resistant Ca contraction and the nimodipine-sensitive Ca signal and contraction. This confirms previous reports showing that the activation of protein kinase C contributes to agonists evoked contraction (see Karaki, 1989). Furthermore, this indicates that the activation of protein kinase C could be involved in the opening of voltage-dependent Ca channels operated by alpha1 adrenoceptors (Morel and Godfraind, 1990).

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42P UNDERSTANDING HOW INTERLEUKIN 1 ACTIVATES CELLS: INDUCTION OF THE TRANSCRIPTION FACTOR KFKB BY IL1 IN THE MURINE THYMOMA LINE EL4 NOB-1

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Despite intense investigation into the effects of interleukin 1 (IL1) upon immune and inflammatory responses, the basic mechanism by which it activates cells remains a mystery (O'Neill *et al.*, 1990). The ultimate response of a cell to IL1 is a change in the expression of specific genes. Expression of a gene is regulated by the binding of transcription factors to DNA motifs in its 5' untranslated region. NFkB is a transcription factor involved in the expression of some IL1 responsive genes. Here we report an investigation into the activation of NFkB in an IL1-responsive cell-murine thymoma EL4 NOB-1 cells.

Nuclear extracts were prepared from EL4 cells using the method of Osborn *et al.*, (1989). NFkB activity was determined by a standard electrophoretic mobility shift assay using DNA probes containing the NFkB motif (5'-GGGACTTTCC-3') as described previously (Edbrooke *et al.*, 1989). IL1α caused an early induction of NFkB, detectable from 5 minutes. The effect was transient, peaking at 15 minutes and no longer detectable by 4h. Studies with mutant and synthetic oligonucleotide probes showed that the response was specific for NFkB. The activation was detectable from 0.1ng/ml IL1α and was maximal at 100ng/ml. Inhibition of the induction by an antagonist which blocks binding to the 80kD IL1 receptor indicated that the signal was transduced via this receptor.

Latent NFkB in cells can be activated by protein kinase C. Protein kinase C appears not to be involved in the action of IL1 because (a) phorbol myristate acetate (PMA) did not mimic the effect (b) staurosporine was not inhibitory and (c) IL1 could still induce NFkB after depletion of protein kinase C. Protein kinase A is also not involved. IL1 fails to elevate cAMP levels or activate adenylyl cyclase in the cells and stimulation of adenylyl cyclase by forskolin does not activate NFkB. These results show that in EL4 cells, activation of the transcription factor NFkB by IL1 is an early receptor-linked event possibly regulated by an as yet undefined protein kinase. This kinase may be similar to that suggested in fibroblasts (Bird & Saklatvala, 1989) and could be an important early signal in the steps leading to enhanced gene expression induced by IL1.

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We have shown the transfer rate of anticonvulsants from the maternal (M) to fetal (F) circulation depends on umbilical blood flow, drug lipid solubility and M & F protein binding (Knott & Reynolds, 1986; 1989). We now examine the F to M transfer of anticonvulsants in drug naive anaesthetised NZW does at 26 days gestation. One placenta from each doe was perfused *in situ* with Krebs bicarbonate buffer gassed with 5% CO_2 in O_2 at 37°C, containing either phenytoin (DPH) 10µg/ml, n=3, carbamazepine (CAZ) 7µg/ml, n=3 or valproate (VAL) 75µg/ml, n=3 (ua) and either Dextran (3%) [●], or albumin (10 [■] or 20 [▲] g/L). Umbilical perfusates (uv) were collected at flow rates between 0.5 and 4.0ml/min, and drugs were measured by GLC and EMIT. Does were given isotonic solutions of DPH (12mg/h; n-15), VAL (150mg/h; n=7) or CAZ (12mg/h; n=7) intravenously after loading. At M steady state, fetuses were removed and blood sampled for plasma total and unbound drug measurements. These data are used to derive the observed and predicted equilibrium F:M drug concentration ratios (Table 1). Drug transfer measured as clearance by maternal blood of umbilical perfusate: (ua-uv x Perfusion flow/ua), increased with flow rate (Fig.1), and the addition of albumin to the perfusate reduced drug transfer at all rates.

Table 1 Total F:M Ratios (mean ± SEM)		
Predicted	**Observed**	**n**
DPH 0.602	0.766 ± 0.07	15
CAZ 0.714	0.772 ± 0.10	7
VAL 0.638	0.526 ± 0.09	5

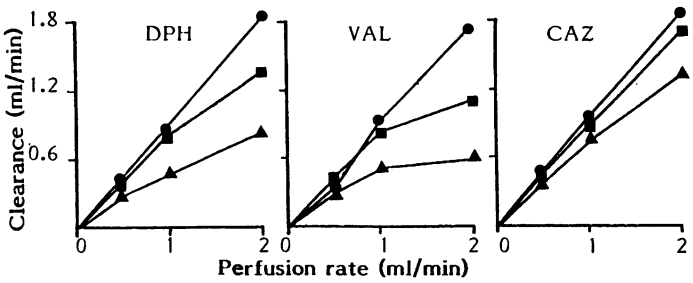


Fig.1

At 20g/L albumin, (measured fetal plasma albumin; 23.7 ± 1.2g/L), the F to M clearance matched our previously reported placental clearance values (1986) for CBZ (b=0.931; r=0.982) and DPH (b=1.282; r=0.999) suggesting there is no preferential direction for transfer. For VAL, placental clearance exceed F to M clearance (b=0.407; r=0.972), in accord with clinical findings of high F:M ratios (Knott 1985).

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44P DISTRIBUTION OF CHOLECYSTOKININ RECEPTORS IN POST-MORTEM SAMPLES OF MEDIAL TEMPORAL LOBE FROM CONTROL SUBJECTS AND SCHIZOPHRENIC PATIENTS

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The medial temporal lobe is a focus of pathological abnormality in schizophrenia and abnormalities in CCK and other peptides have been found in brain homogenates from schizophrenics' temporal lobe (for reviews see Kerwin, 1989, Roberts, 1990). Relatively little is known about the intact distribution of CCK and oxytocin receptors in human medial temporal lobe, nor of the microanatomical localisation of the CCK receptor abnormality in schizophrenia.
Using quantitative receptor autoradiography and image analysis we have studied the distribution of CCK and oxytocin receptors in bilateral medial temporal lobe material from nine controls and nine age and sex matched schizophrenics.
Fresh frozen (20um) cryostat sections were used. Incubations were performed under appropriate conditions of pH and temperature with 400pM 125 I-CCK8 sulphated or 4nM ^{3}H oxytocin. Non specific binding was determined by 10^{-7} M CCK8 sulphated and 10^{-5} CCK8 desulphated or 4uM oxytocin.

Displacement studies reveal CCK receptor binding was of the B type. The receptors are highly localised to the subicular region and layer 2 of the entorhinal cortex. Low levels were found in the body of the hippocampus. Highest levels of oxytocin sites were found in CA_3/CA_4. Non parametric evaluation of data revealed bilateral losses of CCK receptors in schizophrenic entorhinal cortex bilaterally (e.g. 4780 ± 1676, 2754 ± 1156 control right/left respectively 1191 ± 422 ± 1717 ± 639 schizophrenics right/left respectively $p < 0.05$). MANOVA failed to reveal any side x diagnosis interaction. No differences between control and schizophrenics were found for oxytocin.

These results confirm the unusual localisation of CCK receptors in human brain (Kohler & Chan Palay 1988) and partly confirm losses of CCK receptors in schizophrenia and localise this to the entorhinal cortex.

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45P CENTRAL ADMINISTRATION OF A CCK-B RECEPTOR AGONIST INDUCES ANXIETY

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Recently we demonstrated that the selective CCK-B receptor antagonist PD 134308 (CI-988), produces anxiolytic-like actions in several experimental models of anxiety (Hughes et al., 1990; Field et al., This meeting). We now examine the effect of a selective CCK-B receptor agonist pentagastrin in the mouse Light/Dark (L/D) box and the rat elevated X-maze.

Male To mice (25-30g; Bantin and Kingman, U.K.) and Hooded Lister rats (250-300g; Olac, U.K.) were used. The effect of pentagastrin in the two models was examined during the 5 min observation period. Intracerebroventricular (icv) administration of pentagastrin (in 5 ul of 50mM $NaHCo_3$), 15 min before test, dose-dependently decreased the time spent on the illuminated side of the L/D box and the time spent on the open arms of the X-maze. In both species, pentagastrin up to 8ug per animal did not affect locomotor activity.

Table 1. The anxiogenic-like actions of pentagastrin.

	Pentagastrin (ug/animal) Veh.	0.08	0.8	8.0
L/D box	108±21	84±9	56±5*	34±9*
X-maze	57±8	38±7	29±6*	27±7*

Data shown as the mean time (s) spent on either the illuminated side of the L/D box or the open arms of the X-maze ± SEM of at least 10 animals per group. *P<0.05 compared with the vehicle controls (Dunnett's t-test after significant ANOVA).

Consistent with an involvement of the CCK-B receptor, CI-988 (0.3-1.0 mg/kg) dose-dependently (administered p.o. 40 min before test) antagonised the effect of a submaximal dose of pentagastrin (0.8 ug/mouse; mean inhibition following 1 mg/kg CI-988 = 98% and 113% in the L/D box and the X-maze respectively).

In conclusion, central administration of the selective CCK-B receptor agonist pentagastrin leads to an anxiogenic-like action in two species, whilst the selective antagonist of this receptor CI-988, produces potent anxiolysis. Thus, these results strongly indicate an involvement of the CCK-B receptor in anxiety.

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46P THE EFFECTS OF CCK-B/GASTRIN ANTAGONISTS ON STIMULATED GASTRIC ACID SECRETION IN THE ANAESTHETISED RAT

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The importance of gastrin, in relation to that of the other secretagogues acetylcholine and histamine,in the control of gastric acid secretion, has remained unresolved because of the lack of antagonists of high affinity and selectivity for the cholecystokinin-B (CCK-B)/gastrin receptor.

We have now investigated the effects of CI-988 and PD 136450, novel highly selective CCK-B/gastrin antagonists (Hughes *et al.*,1990; Horwell *et al.*,1991), on acid secretion in the anaesthetised rat evoked by infusions of pentagastrin, histamine or bethanecol. For comparison we also examined the effects of the CCK-A antagonist devazepide, and the relatively selective CCK-B antagonist, L-365,260.

Using the modification by Parsons (1969) of the preparation for the *in situ* perfusion of the stomach (Ghosh & Schild 1958), the subcutaneous administration of CI-988 or PD 136450 produced a dose-dependent inhibition of pentagastrin-induced acid secretion. The potency of CI-988 was comparable to that of the H_2-antagonist ranitidine, with PD 136450 4-5 times more potent; after intraperitoneal administration L-365,260 was comparatively weak, while devazapide was inactive (Table 1).

Table 1 Potency (ED_{50} $\mu mol.kg^{-1}$, 95% confidence limits) of agents to inhibit gastric acid secretion

	Pentagastrin	Histamine	Bethanecol
CI-988	0.25 (0.06-1.05)	>25	>25
PD 136450	0.05 (0.01-0.26)	>5	>5
L-365,260	1.61 (0.61-4.21)	>160	>160
Devazepide	>300	>300	>300
Ranitidine	0.19 (0.11-0.36)	0.20 (0.08-0.49)	0.25 (0.03-2.30)
Atropine	>0.2	>0.2	0.002 (0.0003-0.012)
Omeprazole	0.47 (0.17-1.33)	0.23 (0.03-1.96)	0.19 (0.09-0.40)

Against histamine or bethanecol-induced acid secretion CI-988 and PD 136450 were virtually inactive; ranitidine or omeprazole inhibited acid secretion by either secretagogue, with values for ED_{50} comparable to those seen against pentagastrin. Atropine powerfully blocked acid secretion by bethanechol, but was virtually inactive against pentagstrin or histamine (Table 1).

In summary, these studies illustrate that CI-988 and PD 136450 are powerful and selective CCK-B/gastrin antagonists. The inability of either compound to inhibit acid secretion by histamine or bethanechol supports the theory that for the anaesthetised, vagotomised rat at least, histamine is the final common chemostimulant in convergant pathways for the induction of acid secretion in the stomach.

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47P THE EFFECT OF INTRAVENOUS CHOLECYSTOKININ ON VASOPRESSIN AND CORTISOL RELEASE IN THE CONSCIOUS PIG

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Vasopressin release is associated with nausea and emesis in species exhibiting an emetic reflex (Rowe et al, 1979). The inhibition of a variety of behavioural responses may be produced in pigs by the administration of cholecystokinin octapeptide (CCK) (Baldwin et al, 1983), and to ascertain whether this is due to nausea, the effect of CCK on vasopressin release has been studied.

Prepubertal Large White pigs (n=7), each with a jugular venous catheter previously implanted under halothane anaesthesia, were injected with either 0.15M NaCl or CCK in doses of 0.3, 0.7 or 1.3 µg/kg body weight. Blood samples were taken 10 min before and 2, 5, 10, 20 and 30 min after the injection for determination of plasma lysine vasopressin (LVP) and cortisol by radioimmunoassay. The 0.7 and 1.3 µg/kg doses produced a rapid and significant increase in plasma LVP concentration from 0.54 $\pm$ 0.08 pg/ml to a peak at 2 min of 1.8 $\pm$ 0.32 and 4.0 $\pm$ 0.92 pg/ml respectively (mean $\pm$ s.e.m., $P<0.05$ and $P<0.01$, 2 tailed paired t-test). There was a later increase in cortisol concentration increasing from 26.2 $\pm$ 5.1 ng/ml to 55.0 $\pm$ 2.4 and 74.7 $\pm$ 7.7 ng/ml at 20 min ($P<0.05$ and $P<0.02$). These results are similar to those described in man (Miaskiewics et al, 1989). It is also of interest to note that the peak in plasma LVP corresponded with the period of behavioural inhibition previously observed in pigs making operant responses for food after receiving the same doses of CCK (Baldwin et al, 1983). Furthermore, in preliminary studies, the attenuation of plasma levels of vasopressin in pigs given metoclopramide (0.3 mg/kg, i.v.) 10 min before administration of CCK (1.3 µg/kg, i.v.) suggests that dopaminergic pathways may be involved in the LVP response.

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48P 5-HYDROXYTRYPTAMINE AND CHOLECYSTOKININ INTERACT IN THE BRAIN VIA 5-HT_3 RECEPTORS

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Serotonin (5-HT) and cholecystokinin (CCK) have been implicated in the control of anxiety (Chopin & Briley, 1987; Ravard & Dourish, 1990). This similar behavior prompted us to investigate on a possible interaction between the two compounds in the brain. The question was approached by studying the action of 5-HT on the release of cholecystokinin-like immunoreactivity (CCK-LI) from rat cerebral tissue.

Synaptosomes were prepared from the cerebral cortex of adult male Wistar rats (200 - 250 g) and superfused in parallel chambers as previously described (Raiteri et al., 1974) with a physiological medium having the following composition (mM): NaCl 125; KCl 3; $MgSO_4$ 1.2; $CaCl_2$ 1.2; NaH_2PO_4 1.0; $NaHCO_3$ 22; glucose 10 (aeration with 95% O_2 and 5% CO_2 at 37° C); pH 7.2 to 7.4. Depolarization was produced by 15 mM K^+ and the CCK-LI released was determined by radioimmunoassay.

5-HT (0.1 - 10 nM) increased CCK-LI release in a concentration-dependent manner (EC_{50} = 0.4 nM). The 5-HT_1/5-HT_2 receptor antagonist methiothepin (300 µM) did not affect the release of CCK-LI elicited by 10 nM 5-HT. In contrast, the releasing action of 10 nM 5-HT was counteracted in a concentration-dependent manner by the selective 5-HT_3 receptor antagonist ICS 205-930 (0.1 - 100 nM; IC_{50} = 3.56 nM) and abolished by 100 nM of MDL 72222. The 5-HT_3 receptor agonist 1-phenylbiguanide, tested at 1 or 10 nM, mimicked 5-HT as a CCK-LI releaser.

It is concluded that 5-HT can evoke release of CCK-LI from rat cerebrocortex nerve terminals by acting on receptors of the 5-HT_3 type. The results seem to provide a rationale to the clinical development of both 5-HT_3 receptor antagonists (Lecrubier et al., 1990) and CCK receptor antagonists (Ravard & Dourish, 1990) as novel anxiolytic drugs.

Supported by Italian C.N.R. (Target Project on Biotechnology and Bioinstrumentation).

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49P LEUKOTRIENES C_4 AND D_4 ARE POTENT VASOCONSTRICTORS OF THE HUMAN SAPHENOUS VEIN *IN VITRO*

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The Cysteinyl leukotrienes (LTs), C_4 and D_4 are potent vasoconstrictors in anaesthetized experimental animals, (Michelassi, et al, 1983), but there is limited information about their action on human blood vessels. The human saphenous vein (hSV) is the most commonly used autologous graft in reconstructive vascular surgery. We have therefore investigated the actions of LTs C_4 and D_4 on human saphenous vein in vitro.

Experiments were conducted on 141 ring segments of hSV from 33 patients undergoing coronary artery bypass grafts (CABG). Endothelium intact vascular rings (3-5mm) were suspended in 5ml organ baths containing modified Tyrodes solution at 37°C, and gassed with 95%02/5%CO2. The LTD_4 receptor antagonist ICI-198615 ($3x10^{-8}$M) was dissolved in dimethyl sulfoxide (DMSO) ($3x10^{-8}$M). Indomethacin (10^{-5}M) was dissolved in sodium carbonate ($NaCO_3$) (100mM).

Leukotrienes C_4 and D_4 (10^{-9} to 10^{-7}M) produced potent dose-dependent contractile responses in the hSV. The potency (EC_{50}± s.e. mean) and the maximum response (E_{max}± s.e. mean) to LTC_4 were EC_{50}: 1.46±0.22x10^{-8}M, E_{max}: 25.75±4.01mN; and for LTD_4 EC_{50}: 1.46±0.18x10^{-8}M, E_{max}: 27.71±3.11mN. The maximum response of LTC_4 and LTD_4 when compared as a percentage of the maximum response elicited by 90mM potassium chloride was 80.62±14.2% and 76.4±10.0% respectively.

When hSV was pretreated (30 min) with the LTD_4 receptor antagonist ICI-198615 ($3x10^{-8}$M) the dose response curve to LTD_4 was significantly shifted to the right ($p<0.005$). However, ICI-198615 also antagonised LTC_4 ($3x10^{-8}$ to $3x10^{-7}$M) ($p<0.05$) but had no significant effect on low doses of this leukotriene. Contractions of hSV to LTC_4 and LTD_4 were not affected by DMSO. Incubation of hSV with indomethacin (10^{-5}M) for 30 minutes did not alter responses to LTC_4 or LTD_4 (10^{-7}M) or affect the dose response curve to both LTs (10^{-9} to 10^{-7}M). $NaCO_3$ did not alter LT-induced responses.

We conclude that LTs C_4 and D_4 are potent constrictors of hSV in vitro. This effect was inhibited by a specific LTD_4 antagonist but not affected by indomethacin, indicating that cyclo-oxygenase products do not contribute to these responses. This data may be of relevance to coronary artery bypass surgery.

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50P CHARACTERISATION OF NEUROKININ RECEPTORS MEDIATING RELAXATION OF CANINE ISOLATED MIDDLE CEREBRAL ARTERY

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The aim of this study was to characterise the neurokinin (NK) receptor subtype mediating relaxation of canine isolated middle cerebral artery using selective agonists and antagonists. Beagle dogs were killed with pentobarbitone sodium (100mg/kg i.v.); the middle cerebral arteries were removed and stored overnight in modified Krebs-Henseleit solution (Apperley et al., 1976). Arteries were cut into ring segments (2-3cm in length) and suspended in 10ml organ baths containing modified Krebs-Henseleit solution gassed with 5% CO_2 in O_2 at a final resting tension of 1g. In order to assess vasodilator effects vessels were pre-contracted with a submaximal concentration of PGF2α (1-10μM) to produced an active tone of approximately 1g. Cumulative concentration-effect curves to agonists were constructed. When antagonists were used a 30 minute contact time was allowed. Responses were expressed as % papaverine (200μM)-induced relaxation. Substance P (SP) caused concentration-related relaxations with an EC_{30} value of 0.23nM (95% confidence limits 0.1-0.6nM, n=13). The selective NK-1 receptor agonists GR73632 (Hagan et al, 1989) and SP methylester (SPOMe) also caused concentration-related relaxations with similar maximum effects to SP. The selective NK-2 receptor agonist GR64349 (Hagan et al, 1989) and the selective NK-3 receptor agonist senktide were only weakly active at causing relaxation. Relative potencies and maximum responses are shown below:-

Agonist	EPCR	% PAPAVERINE MAXIMUM
SP	1	64.9±5
GR73632	20.7(6.7-63.9)	51.9±4
SPOMe	5.9(0.8-41.5)	62.7±16
GR64349	>425(211-857)	23.8±7
Senktide	>245(65-922)	16.3±6

Values are geometric means (95% confidence limits) or mean ± s.e.m. from 3-7 experiments. Equi-potent concentration ratios (EPCR) were calculated at the level of 30% papaverine-induced relaxation.

The relaxant effects of SP were antagonised by the selective NK-1 receptor antagonist GR82334 (Hagan et al, this meeting; pA_2 7.4±0.2, slope not significantly different from unity, n=10). However, the selective NK-2 receptor antagonist, Ac-Leu-Asp-Gln-Trp-Phe-Gly-NH_2 (10μM; Maggi et al 1990) did not affect SP-induced relaxations (concentration ratio 0.7 (0.08-6.1), n=3). These results suggest that in the canine middle cerebral artery SP-induced relaxations are mediated through NK-1 receptors.

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51P THE EFFECT OF MONOCLONAL ANTIBODIES TO CGRP ON CGRP-INDUCED VASODILATATION IN PIG CORONARY ARTERY RINGS

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Calcitonin-gene-related peptide (CGRP) is a 37 amino acid peptide and a vasodilator in pig coronary artery rings (PCA). These effects are receptor mediated, however, the regions of the peptide which are important for its activity are unclear. A potential means of investigation would be to treat CGRP with monoclonal antibodies (MAbs), which are specific for discrete regions of the peptide, and to determine how these affect its pharmacological profile. Hence in the present study we used a range of MAbs to modify the vasodilatory effect of CGRP in PCAs. The MAbs used (and the amino acid sequences recognised) were CN1 (1-13); CRA3 (1-13); CRA5 (1-13); CRA2 (27-37); CB3 (32-37); CRA8 (19-28), (Andrew et al, 1990).

PCAs of diameter < 1mm were mounted between two hooks, under a resting tension of 2g, in a organ bath containing Krebs solution gassed with 95% O_2 and 5%CO_2 and maintained at 37 ± 1°C. Changes in tension were measured using a Grass FT03 force-displacement transducer and recorded on a Gould BS273 flat-bed recorder. Tissues were pre-constricted using a sub-maximal dose of acetylcholine (3 x 10^{-7}M). A control concentration-response curve (CRC) was obtained by cumulatively administering CGRP (2 x 10^{-10} - 2.56 x 10^{-8}M) to the bath using a dose cycle of 6 minutes. Subsequent CRCs to CGRP were obtained in the presence of each MAb. MAbs in a 10 x fold excess (10^{-6}M) to CGRP were incubated with the peptide for 30 minutes prior to administration. All data were expressed as a % reversal in tone induced by ACh and was analysed using one-way ANOVA.

CGRP caused a concentration-dependent relaxation of PCAs which reached a maximum (98.2 = ± 4.8%, n=25) at 1.28 x 10^{-8}M and gave an EC_{50} of 3.8 ± 0.8 x 10^{-9}M. In the presence of CN1, CRA2 and CRA3, CGRP gave EC_{50}S (CN1, 4.1 ± 0.9 x 10^{-9}M, n=6; CRA2, 3 ± 0.6 x 10^{-9}M, n=7; CRA3, 1.8 ± 0.5 x 10^{-9}M, n=4) and maximum reversals in tone which were not significantly different to CGRP alone. However, in the presence of CRA5, significant inhibition of the CGRP response was observed; the concentration of CGRP to give a 25% relaxation being 3.1 ± 0.7 x 10^{-9}M (n=25) in the absence and 9.9 ± 2 x 10^{-9}M (n=7) in the presence of CRA5, $p < 0.05$. In contrast, potentiation of the CGRP response was observed in the presence of CB3 and CRA8, the EC_{50}S (CB3, 7.5 ± 1.6 x 10^{-10}M, n=5; CRA8, 1.1 ± 0.2 x 10^{-9}M, n=8) being significantly less than ($p < 0.05$) CGRP alone.

These data show that MAbs can be utilised to modify the vasodilatory effect of CGRP, causing either inhibition (CRA5, N-terminal) or potentiation (CB3, C-terminal, CRA8, middle region) of the response and could imply that epitopes in these regions may be involved in biological activity.

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52P THE PHASIC AND TONIC RESPONSES TO PHENYLEPHRINE MAY REFLECT A DIVERGENCE IN THE EXCITATION-CONTRACTION COUPLING OF α_1-ADRENOCEPTORS IN RAT PORTAL VEIN

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A few types of blood vessels when mounted in an organ bath display spontaneous myogenic activity like for instance the portal vein and coronary arteries of several animal species. In particular, the isolated rat portal vein has been used frequently as a model for resistance vessels and also been studied intensively because of its spontaneous myogenic activity (Ljung, 1970).

Portal veins of male Wistar rats (350-400 g) were mounted longitudinally under a passive force of 1 g in thermostatically controlled organ baths (37°C). Tissues were bathed in a Tyrode solution (NaCl 124, KCl 4.0, $MgCl_2$ 1.1, $NaHCO_3$ 24.9, Na_2HPO_4 0.42, Glucose 5.5, Ca^{2+} 0.9 mM) and gassed with 95 % O_2 and 5 % CO_2. Isometric contractile activity was measured via force displacement transducers.

Phenylephrine administered in a cumulative manner caused a concentration dependent response in rat portal vein, which consisted of an amplification of spontaneous phasic myogenic activity at low concentrations (0.1 µM-1 µM), while at higher concentrations (3µM-30 µM) a sustained tonic contracture developed. Both the phasic and tonic types of responses to phenylephrine were competitively blocked by the α-adrenoceptor antagonists prazosin (9.04±0.07; 8.88±0.11), phentolamine (8.14±0.08; 8.15±0.24), corynanthine (7.12±0.16; 7.39±0.18), yohimbine (6.41±0.01; 6.37±0.01), idazoxan (6.74±0.32; 6.39±0.01), rauwolscine (6.15±0.06; 6.09±0.28) and 5-methyl-urapidil (8.32±0.14; 8.30±0.02). pA_2 values ± s.e.m., obtained from Schild plots are given between brackets for the phasic and tonic types of contraction, respectively. Slopes of the Schild plots did not differ from unity. For yohimbine a pK_b value was obtained. The pA_2 values for the competitive antagonists studied and the pK_b value for yohimbine were not significantly different for the phasic and tonic types of contraction in response to phenylephrine. In other words, the two types of contraction elicited by phenylephrine were probably mediated by the same subtype of α_1-adrenoceptor as revealed by Schild analysis with competitive α-adrenoceptor antagonists which have been employed by other investigators to discriminate between α_1-adrenoceptor subtypes in vascular and other tissues (Hanft & Gross, 1989; Minneman, 1988). The irreversibly acting α-adrenoceptor antagonists phenoxybenzamine and chlorethylclonidine could indeed discriminate between the phasic and tonic types of contraction in response to α_1-adrenoceptor stimulation in the rat portal vein. For phenylephrine two different affinity constants (pK_a's, 5.53±0.13; 4.51±0.29) and receptor reserves (q's, 0.14±0.02; 0.33±0.04) for the phasic and tonic types of contraction, respectively, could be calculated, using the method of receptor alkylation with phenoxybenzamine (Furchgott & Bursztyn, 1967).

These data suggest that there may be a divergence in the excitation-contraction coupling of α_1-adrenoceptors in rat portal vein, which is located behind the receptor and also reflected by the two types of contraction (phasic vs tonic). The two receptor-operated pathways stimulated by phenylephrine probably differ in their efficiency of coupling.

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53P ABNORMAL VASCULAR RESPONSIVENESS FOLLOWING ONE WEEK OF RAPID VENTRICULAR PACING IN DOGS

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Rapid ventricular pacing in the dog produces congestive heart failure (CHF; Armstrong et al., 1986). In addition, vascular smooth muscle reactivity to α-agonists is altered at severe CHF (Forster et al., 1988). Specifically severe CHF resulted in increased responsiveness of the saphenous vein and the dorsal pedal artery to selective α_1-agonists and either no change or decreased responsiveness to α_2-agonists (Forster and Armstrong, 1990). The temporal evolution of this finding is unknown and therefore the responsiveness of the saphenous vein and the dorsal pedal artery to α-agonists was evaluated 1 wk following the onset of pacing at which time animals present with mild cardiomegally and moderate elevations in plasma noradrenaline.

Five male mongrel dogs were included in the study, saphenous vein and dorsal pedal artery segments were dissected and removed at pacemaker implantation and contralateral vascular segments were removed 1 wk following the onset of rapid ventricular pacing (250 bpm delivered asynchronously). All vessels were treated in an identical fashion and were endothelial-intact. Vascular rings (6 x 3mm rings/vascular segment) were mounted in organ baths. Following equilibration, endothelial function was assessed with acetylcholine. Following a further 1h washout period, concentration effect curves were constructed on pairs of rings to noradrenaline (NA), phenylephrine (PE) and BHT 920 (B2) in a cumulative manner. The results are shown in the table below where maximum tension developed (max; mean $\pm$ sem) in $g.mm^{-2}$ and EC_{50} [uM] (geometric mean with 95% confidence limits in parentheses; *$p<0.05$ before vs 1 wk).

Maximum tension and EC_{50} for α-agonists before and 1 wk following pacing

	SAPHENOUS VEIN						DORSAL PEDAL ARTERY					
	before NA	1 wk	before PE	1 wk	before B2	1 wk	before NA	1 wk	before PE	1 wk	before B2	1 wk
max	5.0±0.8	10.6±2.4*	5.9±1.0	9.0±1.8*	2.7±0.7	3.3±0.6	7.0±0.4	13.3±2.8*	5.7±1.0	10.1±0.7*	1.2±0.4	2.7±1.3
EC	0.7(0.2-1.9)	0.2(0.1-0.6)	4.5(3.1-6.9)	3.9(2.0-7.0)	0.6(0.2-1.9)	0.5(0.3-0.9)	7.4(1.9-16.0)	0.7(0.1-1.7)*	4.3(1.9-7.6)	2.2(0.7-6.5)	36(8-80)	41(7-260)

These results from peripheral blood vessels before and 1 wk following ventricular pacing show 1) at 1 wk, maximal tension developed to NA and PE is elevated in both the vein and the artery but EC_{50} is decreased only to NA in the artery. 2) at 1 wk, tension developed to B2 remains unchanged in both the vein and the artery but the EC_{50} is significantly higher in artery compared to the vein. 3) Finally no change in the responsiveness to acetylcholine occurred at the 2 time points studied. The alteration in max to NA and PE we demonstrate at 1 wk is identical to our previous findings in severe CHF (4.4 wk) and suggests that α_1-adrenergic receptor abnormalities occur early in CHF development and most likely initially reflect a change in receptor number rather than affinity. The vein/artery differences in α_2-activation support different α-receptor populations within vascular beds.

Supported by the Heart and Stroke Foundation of Ontario.

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54P MODIFICATION OF BOVINE AND RAT SODIUM/POTASSIUM ATPase BY OXIDISED AND REDUCED GLUTATHIONE AND OTHER SULPHYDRYL COMPOUNDS

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Glutathione is one of the main endogenous protective agents used under oxidative stress. It has been shown to protect against reperfusion induced arrhythmias, attenuate potassium loss during reperfusion and delay the onset of an ischaemically induced contracture. We now report on the action of reduced and oxidised glutathione on isolated bovine Na/K-ATPase and on a histochemical analysis of Na/K-ATPase on tissue slices from rat heart previously subjected to a 30 minute ischaemic insult.

The addition of 1mM reduced glutathione (GSH) to the isolated Na/K-ATPase assay system resulted in a stimulation of activity by +35.7±0.61%. In contrast oxidised glutathione (GSSG) produced a concentration dependent reduction in activity such that at 1mM a 56.4±0.37% inhibition was seen (n=6).

In view of the fact that glutathione is a tripeptide composed of glutamate, glycine and cysteine, we investigated the individual effect of each of these amino acids (at 1mM) on isolated Na/K-ATPase. Neither glutamate nor glycine had any significant effect whereas cysteine caused a significant increase in activity (+15.0±0.61%). 1mM Cystine (oxidised cysteine) had no significant effect on activity. The sulphydryl compounds dithiothreitol, penicillamine and captopril (all at 1mM) all resulted in a stimulation of isolated Na/K-ATPase of 25±0.63, 21.4±0.62 and 20±0.62% respectively. We have also shown that GSH (0.25-1.0mM) can overcome, in a concentration dependent manner, the inhibition of isolated Na/K-ATPase produced by 1mM GSSG (n=4).

In the histochemical analysis of Na/K-ATPase activity in rat heart tissue slices control activity readings (mean integrated extinction x 100) were 20.89±0.07. Following 30 min. global ischaemia and 5 min. subsequent reperfusion the enzyme activity was significantly reduced to 15.36±2.23 (MIE x 100). In hearts perfused with 1mM GSH prior to and during the ischaemic insult the loss of Na/K-ATPase activity was attenuated. In control hearts the activity was 17.25±2.11 whereas in the presence of 1mM GSH the activity was increased to 26.44±2.2 which represents a significant increase in activity with respect to the non-GSH treated hearts as well as hearts not subjected to an ischaemic insult. We have, therefore, shown that bovine Na/K-ATPase activity can be altered by physiological concentrations of GSH/GSSG such that a fall in this ratio results in an inhibition of the enzyme. We propose that changes in the GSH/GSSG ratio, that occur during oxidative stress, could be a regulator of Na/K-ATPase.

P.S Haddock is supported by NIH grant No.372780

55P INVESTIGATION OF THE EFFECT OF CYCLOHEXIMIDE ON ^{111}IN-NEUTROPHIL ACCUMULATION IN ISCHAEMIC/REPERFUSED RABBIT MYOCARDIUM

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Myocardial tissue which has undergone ischaemia shows characteristic features of an inflammatory response, one component of which is neutrophil infiltration. This has been the subject of considerable interest following reports that depletion of circulating neutrophils results in a reduction in myocardial infarct size (Lucchesi & Mullane, 1986). We have previously shown that the CD18 adhesion glycoprotein complex expressed on the surface of leukocytes is involved in neutrophil accumulation into ischaemic/reperfused myocardium (Williams et al 1990). Further we have shown that neutrophil accumulation in response to the intradermal injection of the cytokine interleukin 1 (IL-1), can be blocked by local inhibition of protein biosynthesis (Rampart and Williams, 1988). In the experiments described below we tested intravenous cycloheximide (28mg/kg) against neutrophil accumulation in the heart. At this dose cycloheximide abolished neutrophil accumulation induced by intradermal IL-1.
Male New Zealand White rabbits (2.7-3.4kg) were anaesthetised with sodium pentobarbitone (45-60mg/kg i.v.). The trachea was cannulated and the animals were ventilated with room air. Blood pressure was recorded from the carotid artery and the ECG was measured using lead I electrodes. Neutrophils isolated from donor rabbit blood were labelled with ^{111}In and administered i.v. A ligature was positioned around the left main coronary artery. Animals received cycloheximide (28mg/kg i.v.) or saline 30mins prior to coronary artery occlusion (CAO). A period of 45 mins CAO followed by 3 hours reperfusion was used. Animals were killed with an overdose of barbiturate. Heart tissue was divided into normal zone (NZ) and area at risk (AR) by dye exclusion and levels of ^{111}In measured by gamma counting.
In control animals there was an increase in the number of ^{111}In-neutrophils of 126±20% in the AR compared with the NZ (19075±868 vs. 8594±851 ^{111}In-neutrophils/g respectively, $P<0.01$, $n=5$). A similar increase of 165±17% was seen in the cycloheximide treated animals (AR = 46834 ± 2148 and NZ 17865 ± 689 ^{111}In-neutrophils/g, $p<0.01$, $n=6$). Therefore cycloheximide did not suppress neutrophil accumulation in ischaemic/reperfused myocardium in this model. This would suggest that the neutrophil infiltration is not dependent on a protein biosynthetic mechanism and may not involve endogenous IL-1 under the conditions used. Interestingly there was a marked difference in ^{111}In-neutrophil numbers in the NZ for the saline and cycloheximide groups. This was reflected in the % of ^{111}In-neutrophils administered still circulating at the end of the experiment (9.2±2% in controls and 40.0±8.2% in cycloheximide group). Thus, it appears that cycloheximide does not affect the percentage of ^{111}In-neutrophils accumulating in ischaemic/reperfused myocardium but cycloheximide does affect neutrophil circulation possibly by inhibiting basal attachment of the marginating pool.

This work was supported by Roche Products Ltd, British Heart Foundation and National Asthma Campaign.

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56P EFFECTS OF NIFEDIPINE IN ISOLATED HEARTS FROM DIABETIC AND CONTROL RATS DURING GLOBAL ISCHAEMIA AND REPERFUSION

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We have investigated the effect of nifedipine (N) on the time of onset of ischaemic contracture (IC) and the recovery of the left ventricular pressure (LVP) and coronary flow (CF) in hearts from diabetic rats (DR) and control rats (CR) during global ischaemia and reperfusion.

Male Wistar rats (275-300 g) were divided into two groups. The diabetic group received via i.v. injection 50 mg/kg of streptozotocin, dissolved in 0.1 M citrate buffer. The control group was injected with buffer alone. The diabetic state was assessed by measuring the blood glucose values twice, resp. at t=7 and at t=45 days. Blood glucose values proved significantly elevated (23.1 vs 6.0 mM, $p < 0.05$). At t=45 days the hearts from both groups were perfused according to Langendorff and stimulated at 5 Hz. The left ventricular pressure (LVP) was measured by means of an intraventricular balloon. Hearts were subjected to a period of 45 min of global ischaemia followed by a reperfusion period of 60 min.

The time of onset of IC in hearts from DR was significantly postponed compared with those from CR (20.3 ± 1.0 vs 15.5 ± 0.6, $p < 0.05$). After pretreatment with N (3.10^{-8} M) the period of time until onset of IC was relatively more prolonged in hearts from DR than in hearts from CR, 20.3 ± 1.0 to 28.1 ± 1.2 min and 15.5 ± 0.6 to 18.3 ± 0.9 min, respectively. The prolongation of the period of time until onset of IC was not accompanied by a better functional recovery of the LVP or CF. However, LVP in hearts from DR was significantly reduced compared with those from CR (23.4 ± 8.2% vs 81.6 ± 13.5% of the preischaemic value, $p < 0.05$). Recovery of the CF was not significantly different for both groups. Pretreatment with N (3.10^{-8} M) did not significantly change functional recovery, as reflected by LVP and CF, respectively.

The present data demonstrate, that nifedipine can prolong the period of time until onset of ischaemic contracture in both types of hearts. However, both in the presence and absence of nifedipine there was no relationship between the time of onset of ischaemic contracture and functional recovery.

57P EFFECT OF DIPYRIDAMOLE AND NIFEDIPINE ON CELLULAR AND INTERSTITIAL ADENINE NUCLEOTIDE CATABOLITES IN ISCHAEMIC GUINEA-PIG HEARTS

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Interstitial fluid (IF) can directly be obtained from ischemic hearts (Scheufler et al., 1988). Increased levels of inosine and other metabolites were found in IF of ischemic guinea-pig hearts (Hugtenburg et al.1990). Nucleoside transport inhibitors like dipyridamole (Dip) but not the Ca entry blocker nifedipine (Nif) reduced inosine concentrations in IF. The aim of the present study was to measure concentrations of adenine nucleotide catabolites in IF and tissue in the presence and absence of these drugs.

Isovolumically perfused guinea pig Langendorff hearts were made ischemic for 45 minutes at 37°C. IF was sampled from the tip of the heart between 30 and 45 min of ischemia and analysed for inosine monophosphate (IMP), inosine (INO) and adenosine (ADO) by HPLC and UV detection. Drugs were given 20 min before ischemia (Nif: 2E-8, Dip: 1E-6 mol/l). Significant differences to ischemic controls were calculated by ANOVA ($p<0.05$;*)

mean±S.D.	ADO	(µmol/kg)	or (µmol/l)	INO	(µmol/kg)	or (µmol/l	IMP	(µmol/kg)	or (µmol/l)
* p<0.05	tissue	IF	tissue/IF	tissue	IF	tissue/IF	tissue	IF	tissue/IF
ischemia	172±54	<0.5	>344	5710±1340	370±80	15	2550±380	9.9±0.5	259
isch+Nif	155±55	<0.5	>310	5980±1036	298±76	20	1950±920	8.5±2.0	231
isch+Dip	4880±1660*	<0.5	>9760	2840±640*	60±32*	47	1750±470	<0.25*	>7000

A compilation of the levels of INO, ADO and IMP in tissue (µmol/kg DW) as well as IF (µmol/l) is given in the table. The tissue concentrations primarily reflect cellular levels of nucleotide metabolites in myocytes. To give an idea about gradients across the sarcolemma the ratio between tissue concentrations and IF are given in the table. Gradients are increased for INO in the group treated with Dip. Concomittantly, the levels of INO in the IF of Dip treated hearts were lower than in ischemic hearts. ADO levels were too low to be quantitatively detected in the IF of either treated or untreated hearts. However, ADO in tissue was markedly increased in the Dip group. Therefore, gradients across the sarcolemma are expected to be larger in the latter group. Nif did not markedly alter gradients.- It is concluded that Dip inhibits the release of nucleosides from ischemic guinea-pig myocytes. Another feature of the results is the appearance of IMP concentrations in IF of ischemic guinea pig hearts. Dip lowered these concentrations to nondetectable levels and increased the gradients. Therefore, Dip could also inhibit the passage of IMP through the sarcolemma in an as yet unknown manner.

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58P FURTHER EVIDENCE THAT THE CARDIOPROTECTIVE ACTION OF DEFIBROTIDE IN ACUTE MYOCARDIAL ISCHAEMIA IS PROSTACYCLIN-MEDIATED

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Defibrotide (DEF), a DNA fraction, protects reperfused ischaemic myocardium and stimulates endogenous PGI_2 formation (Thiemermann et al., 1985; Hohlfeld et al., 1990). This study investigates the effects of indomethacin (IND) on DEF induced alterations in infarct size and ischemia-induced neutrophil accumulation.

Anaesthetized minipigs (25-35 kg bw) were subjected to 60 min of coronary artery occlusion (LAD) and 180 min of reperfusion. DEF or vehicle (VEH) were infused i.v. (32 mg/kg·h), starting with a loading dose (32 mg/kg) 20 min before ischaemia. IND (3 mg/kg) was given i.v. 60 min before ischaemia to VEH- and DEF- treated pigs. Transcardiac gradients (coronary venous-arterial plasma concentrations) of PGI_2 (6-oxo-$PGF_{1\alpha}$) and thromboxane (TX) B_2 were measured by RIA before DEF or IND infusion (Control) and during ischaemia and reperfusion. After 3 h of reperfusion, PMN infiltration in the ischaemic border zone (light microscopy) and infarct size (percent of risk area; Evans blue/tetrazolium stain) were determined.

The transcardiac 6-oxo-$PGF_{1\alpha}$ gradients were increased by DEF (table) ($p<0.05$ at 180 min of reperfusion). IND reduced the gradients of PGI_2 during ischaemia and the first hour of reperfusion. TX gradients were not changed by DEF and IND decreased TX plasma levels below the detection limit (not shown). The accumulation of PMN in reperfused ischaemic myocardium was reduced by DEF (VEH: 37 ± 10, DEF: 10 ± 6 cells/mm^2; $p<0.05$). This effect was not seen after IND pretreatment. DEF markedly reduced infarct size. IND prevented this effect of DEF and increased infarct size above the values seen in VEH treated animals.

		6-oxo-$PGF_{1\alpha}$ gradients [pg/ml] (plasma concentrations)				Infarct
		Control	Ischaemia	Reperfusion		size [% of
	n		(60 min)	(60 min)	(180 min)	risk area]
VEH	7	107 ± 26	100 ± 18	289 ± 54	347 ± 77	44 ± 6
DEF	7	112 ± 38	321 ± 177	854 ± 507	628 ± 89*	23 ± 4*
IND	4	99 ± 34	<15*	57 ± 54	536 ± 133	75 ± 4*
DEF+IND	4	63 ± 47	<15*	52 ± 21*	489 ± 71	70 ± 2*

(Mean ± s.e.mean; *:$p<0.05$ vs VEH)

The protective action of DEF on reperfused ischaemic myocardium, i.e. the substantial reduction of infarct size and inflammatory reaction, is associated with an enhancement of cardiocoronary PGI_2 formation. The prevention of these effects by IND adds further evidence to the assumption that the cardioprotective action of DEF is mediated by an increase of local PGI_2 generation.

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59P INCREASED mRNA LEVEL OF THE α-SUBUNIT OF INHIBITORY G-PROTEINS IN END-STAGE MYOCARDIAL FAILURE

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In failing human hearts the positive inotropic and cAMP elevating effects of both β-adrenoceptor agonists and phosphodiesterase inhibitors are diminished (Schmitz et al., 1987). Beside β-adrenoceptor downregulation this might be attributed to an increase in the inhibitory signal transducing G-protein, G_i (Feldman et al., 1988, Neumann et al., 1988). The aim of the present study was to get more insight into the cellular level of regulation of αG-subunits in human heart failure. Therefore, we measured mRNA levels of α-subunits of G_i (αG_i) and of the stimulatory G-protein (αG_s) in left ventricular myocardium of patients undergoing heart transplantation because of endstage myocardial failure due to idiopathic dilated cardiomyopathy (IDC, n=8) or ischemic cardiomyopathy (ICM, n=6) in comparison to nonfailing hearts (NF, n=8).

Northern blotting of left ventricular total cellular RNA with ^{32}P-nick-translated rat cDNA gave specific signals for αG_s- and αG_{i2}-mRNA. In contrast, αG_{i1}- and αG_{i3}-mRNA were not detectable. Slot blot quantification revealed an increase in αG_{i2}-mRNA of 75±26% ($p<0.05$) in IDC and 90±26% ($p<0.05$) in ICM, as compared to NF (fig. 1A). αG_s-mRNA levels were similar in the three groups (fig. 1B).

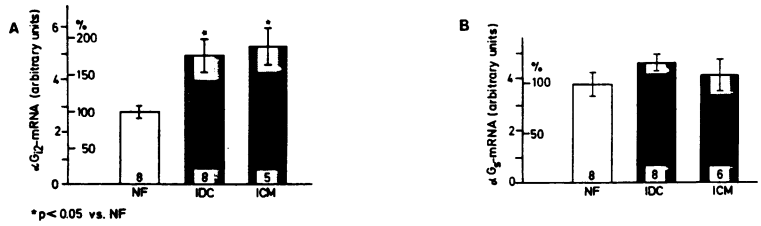


Figure 1: αG_{i2}-mRNA (A) and αG_s-mRNA steady state levels in human heart

It is suggested that an upregulation of mRNA of the α-subunit of G_i is involved in the pathophysiological process leading to reduced responsiveness to cAMP-increasing agents in endstage heart failure. (Supported by the DFG)

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60P AUTORADIOGRAPHIC MAPPING OF NON-NMDA GLUTAMATE RECEPTOR SUBTYPES IN THE HUMAN HIPPOCAMPUS: ALTERATIONS IN ALZHEIMER'S DISEASE

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Non-N-methyl-D-aspartate (NMDA) glutamate receptor subtypes can be divided into those associated with receptor-operated ion channels (ionotropic), namely, 2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate, and those linked to phosphoinositide hydrolysis (metabotropic). An autoradiographic method for mapping this metabotropic glutamate binding site has recently been published (Cha et al. 1990). We have examined the distribution and levels of this glutamate binding site in conjunction with the AMPA and kainate sites, using autoradiography, in the hippocampal region of normal elderly subjects and patients with neuropathologically-confirmed Alzheimer's Disease (AD).

Serial 20μm cryostat sections of the hippocampal region from six control subjects (age = 75 ± 3 years; postmortem delay = 10 ± 2 hours; mean ± SEM) and six patients with AD (age = 82 ± 3; postmortem delay = 11 ± 2 hours; mean ± SEM) were used for autoradiography. Sections were incubated with ^{3}H-glutamate (100nM + 100μM AMPA + 100μM NMDA + 100mM KSCN + 2.5mM $CaCl_2$) to label glutamate metabotropic sites; with ^{3}H-AMPA (150nM + 100mM KSCN) to label AMPA sites and with ^{3}H-kainate (50nM) to label kainate sites (Chalmers et al. 1990). Autoradiograms were analysed by image analysis and binding values calculated by reference to ^{3}H-microscales. Subicular neurones were counted in adjacent cresyl violet-stained sections.

	METABOTROPIC		AMPA		KAINATE	
REGION	CON	AD	CON	AD	CON	AD
SUB	31.7 ± 3.7	12.6 ± 3.6*	208.3 ± 36.1	101.9 ± 11.2*	26.5 ± 2.4	18.6 ± 2.7*
CA1	21.8 ± 1.9	11.9 ± 2.5*	195.0 ± 40	98.1 ± 10.9*	25.7 ± 1.5	20.8 ± 2.3
PHG	16.9 ± 4.1	14.6 ± 2.6	76 ± 10.5	47.8 ± 7.0*	51.5 ± 1.2	43.0 ± 2.2*
CA3	12.2 ± 2.4	11.2 ± 1.4	103.5 ± 14.1	87.0 ± 11.0	44.1 ± 2.5	39.6 ± 3.2
DG	18.4 ± 2.2	15.6 ± 2.4	162.3 ± 29.6	126.7 ± 18.4	27.1 ± 2.6	28.4 ± 1.8

Values are mean ± SEM pmol/g tissue, n=6 in control and AD groups. *P<0.05, unpaired t-test.
Abbreviations: SUB = subiculum; PHG = parahippocampal gyrus; DG = dentate gyrus.

The density of subicular neurones was reduced in the AD patients (81 ± 16 cells/mm^2, mean ± SEM) compared to controls (149 ± 9 cells/mm^2). The ratio of binding sites to cell densities in the AD group was reduced for glutamate metabotropic sites, slightly increased for kainate sites and unchanged for AMPA sites. These three glutamate binding site subtypes may be located on neuronal elements which are differentially affected by the pathological process of AD.

This investigation was supported by the Wellcome Trust.

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61P THE NEUROPROTECTIVE ACTION OF 2,3-DIHYDROXY-6-NITRO-7-SULFAMOYL-BENZO(F)QUINOXALINE (NBQX) IN A RAT FOCAL ISCHAEMIA MODEL

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Glutamate is the main excitatory neurotransmitter in the brain, which acts on N-methyl-D-aspartate (NMDA) and non-NMDA subtypes of excitatory amino acid receptors. Excessive release of glutamate which follows a period of cerebral ischaemia is thought to mediate the resulting neurodegeneration. NMDA receptor antagonists have been shown to be potent neuroprotective agents in focal ischaemia models (Park et al., 1988a) but not universally protective in global ischaemia models (Choi, 1988), suggesting that other receptor subtypes may be involved. Recently it has been shown that NBQX (Sheardown et al., 1990) a selective antagonist of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors was neuroprotective in the gerbil model of transient forebrain ischaemia. Therefore we have investigated the neuroprotective action of NBQX in a rat focal ischaemia model.

Male Sprague-Dawley rats (340-380g) were anaesthetised with a mixture of 2% halothane, 30% oxygen and 70% nitrous oxide and subjected to permanent occlusion of the middle cerebral artery (MCA) as described by Park et al., (1988b). The animals were allowed to survive for 24h following which time they were perfused using 2,3,5-triphenyltetrazolium chloride (TTC) and then fixed using 10% formalin in saline (Park et al., 1988b). Following perfusion with TTC normal areas are stained red, whereas ischaemic areas remain white. The brains were cut into 9 coronal slices each being 1.5mm in thickness and photographed.

The areas of damage were mapped onto scale diagrams and measured using an image analyzer. Areas of ischaemic damage were used to determine the total volume of ischaemic damage in each brain. These same slices were also processed for histological analysis. NBQX was administered at doses of 3, 10 and 30mg/kg i.v. at the time of occlusion and 1h post MCA occlusion (N=13-14/group). The control group (N=13) of animals received 0.1ml/kg of saline.

Permanent MCA occlusion with 24h survival resulted in ischaemic damage in the dorsolateral cortex and in the neostriatum. The mean (±s.e.mean) volume of ischaemic damage in the hemisphere and cortex of the control animals was 166.6±5mm^3 and 123.1±4mm^3 respectively. NBQX at a dose of 2x30mg/kg gave significant ($P \leq 0.05$) protection in the hemisphere (126.8±5mm^3) and cortex (89.5±4mm^3). NBQX at 2x3mg/kg and 2x10mg/kg did not produce significant protection with volumes of ischaemic damage of 156±7mm^3 and 161±6mm^3 respectively in the hemisphere and 117.9±6mm^3 and 121.3±4mm^3 in the cortex. There was no protection against caudate damage by any of the doses of NBQX tested.

Therefore NBQX is neuroprotective in a rat model of focal ischaemia. We can conclude that excitotoxic damage following a focal insult involves NMDA and non-NMDA subtypes of excitatory amino acid receptors.

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62P REGIONAL INHIBITION OF [^{3}H]MK801 BINDING IN RAT BRAIN BY CPP AND 7-CL-KYNURENATE: A RECEPTOR AUTORADIOGRAPHY STUDY

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The N-methyl D-aspartate (NMDA) receptor complex for excitatory amino acids on mammalian neurones has a multi-component organization of different interrelated functional domains (see Monaghan et al., 1989). These include the transmitter recognition site, at which NMDA and glutamate act as agonists and 3-(2-carboxypiperazin-4-yl)propyl-1-phosphinic acid (CPP) and 2-amino-5-phosphonopentanoic acid (AP5) as antagonists, as well as allosteric sites which appear to modulate transmitter function. Among these are the dizocilpine (MK801) and glycine binding sites (Wong et al., 1986; Johnson and Ascher, 1987). Activation of the latter site positively modulates the action of glutamate whereas MK801 decreases it. Furthermore glycine can facilitate the ^{3}H-MK801 binding to synaptic membranes (Wong et al., 1987). The effect of glycine can be antagonised in a competitive manner by 7-chlorokynurenate (7-Cl kyn), (Kemp et al., 1988). In the present study we have considered whether the glutamate and glycine dependency of ^{3}H-MK801 binding is comparable throughout different regions of the rat brain. For this purpose we have used in vitro receptor autoradiography examining the patterns of inhibitions of binding produced by 7-Cl kyn and CPP.

Rat brain sections (10μm) were incubated with ^{3}H-MK801 (40nM, 53 Ci/mmol) in the presence of increasing concentrations of CPP (0.1-100μM) or 7-Cl kyn (1-1000μM) and prepared for receptor autoradiography as described previously (Bowery et al., 1988). Autoradiograms were obtained by apposing dried sections to tritium sensitive film. Background (non-specific) binding was obtained with excess (100μM) unlabelled MK801. Autoradiograms were analyzed by regional computerized densitometry (Quantimet 970). The cerebellum was not considered in the present study because of the low binding of ^{3}H-MK801 in this region (Bowery et al., 1988). ^{3}H-MK801 binding was inhibited by increasing concentrations of 7-Cl kyn (IC_{50}: 29μM) and CPP (IC_{50}: 22μM). High concentrations of both drugs (100μM CPP or 1000μM 7-Cl kyn) completely inhibited specific ^{3}H-MK801 binding in all brain regions. No regional differences in the IC_{50} values for either 7-Cl kyn or CPP were evident and glutamate (100μM) and glycine (100μM) could completely reverse the inhibition of ^{3}H-MK801 binding induced by CPP (10μM) and 7-Cl kyn (100μM) respectively.

In conclusion, the presence of glycine (and glutamate) appears to be uniformly required for the binding of ^{3}H-MK801 to occur throughout the rat brain suggesting that glycine sites are always present to the same extent in the NMDA receptor complex although we cannot include or exclude the complex in the cerebellum at present.

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63P MK801 INHIBITS ISCHAEMIA-INDUCED RELEASE OF GLUTAMATE, ASPARTATE AND GABA FROM THE RAT RETINA *IN VITRO*

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Retinal ischaemia in man may be caused by central vein occlusion, or more rarely, by central artery occlusion. These occurences are serious ophthalmological problems for which there is little or no treatment at present. Less dramatic, but of considerable clinical importance, diabetic retinopathy is believed to involve a relative ischaemia of the retina. The eye provides an advantageous situation for the trial of drugs that may be effective in preventing ischaemic damage because it is possible to apply drugs to the retina locally by intravitreal injection.

In the present experiments we have examined the effects of "ischaemia" on the release of amino acids from the rat retina in vitro. Isolated retinas were incubated for 30 min at 32°C in Krebs bicarbonate medium gassed with $95\%O_2/5\%CO_2$ (controls) or in glucose-free medium gassed with $95\%N_2/5\%CO_2$ (ischaemia). Amino acids were then measured by HPLC.

When control retinas were incubated for a period of 30 min the release of glutamate, aspartate and GABA was 1489±211; 193±26.5 and 2066±147 pmol/retina/30 min (mean ± s.e. mean, n=5). The release of glutamate, aspartate and GABA from retinas incubated under ischaemic conditions for 30 min was increased 2.45-fold, 2.9-fold and 3.2-fold respectively (n=9 $P<0.001$).

The NMDA antagonist, MK801 (0.1uM) totally prevented the ischaemia induced release of glutamate and aspartate and reduced the increase in GABA release by approximately 75%. The ischaemia evoked release of GABA remained significant ($P<0.01$). The kainate/AMPA antagonist, DNQX, had no effect at a concentration of 2uM but at 100uM it prevented the ischaemia induced release of all three amino acids. Mannitol (1 or 5mM) appeared to reduce the ischaemia induced release of amino acids by about 50% but this effect was significant only for aspartate ($P<0.02$).

It has generally been assumed that EAA antagonists protect against ischaemic damage by preventing the action of released excitotoxins such as glutamate. However, in the retina it seems that MK801 is a potent inhibitor of EAA and GABA release under ischaemic conditions.

64P ACTIVITIES OF 4-METHYL DERIVATIVES OF HA-966 AT THE GLYCINE SITE OF THE N-METHYL-D-ASPARTATE RECEPTOR FROM RAT BRAIN

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HA-966 (3-amino-1-hydroxypyrrol-2-one) antagonises N-methyl-D-aspartate (NMDA) receptor responses through competition at the glycine site and may be a low efficacy partial agonist (Foster and Kemp, 1989). We have investigated the affinity and efficacy of 4-methyl derivatives of HA-966 in two radioligand binding assays. Washed P2 membranes from rat cortex were used to measure the specific binding of [^{3}H]glycine (Foster and Kemp, 1989) and [^{3}H]MK-801 (Foster and Wong, 1987).

Compound	[^{3}H]Glycine IC50 (μM)	[^{3}H]MK-801 (+ 100μM NMDA) EC50 (μM)	%Im	IC50 and EC50 values are geometric means (-SEM,+SEM) and %Im is maximum inhibition as the arithmetic mean ±SEM. N ≥ 3. * Results from Singh et al (1990).
(+)HA-966	12.5*	6.99 (4.07,12.0)	21.8±5.7	
(-)HA-966	339*			
(+)cis-4-methyl-HA-966	1.37 (0.86,2.19)	1.54 (1.28,1.86)	48.0±3.44	
(±)cis-4-methyl-HA-966	2.91 (1.77,4.78)			
(-)cis-4-methyl-HA-966	85.7 (67.1,109)			
(±)trans-4-methyl-HA-966	>100			

Cis-substitution of a methyl group in the 4-position of HA-966 increased affinity in the [^{3}H]glycine binding assay, whereas affinity was reduced with trans-substitution. As previously reported for HA-966 (Singh et al, 1990), glycine site affinity resided principally in the 3R (+) enantiomer of cis-4-methyl-HA-966. Glycine increased specific [^{3}H]MK-801 binding with an EC50 (geometric mean (-SEM,+SEM), N = 3) of 56 (52,62) nM. The glycine site antagonist 7-chlorokynurenic acid inhibited [^{3}H]MK-801 binding with an IC50 of 3.07 (2.66,3.54) μM and a maximum inhibition of 91.6±1.74% (N = 3). Both (+)HA-966 and its cis-4-methyl derivative inhibited [^{3}H]MK-801 binding, and these effects were reversed by addition of glycine (0.1 - 10 μM). Consistent with its action as a low efficacy partial agonist (Singh et al, 1990), (+)HA-966 inhibited maximally only 22% of [^{3}H]MK-801 binding, however, (+)cis-4-methyl-HA-966 inhibited to a greater maximal extent, but less than the maximum caused by 7-chlorokynurenic acid. These results indicate that cis-substitution of a methyl group in the 4-position of HA-966 leads to increased affinity for the glycine site on the NMDA receptor but reduced partial agonist activity.

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65P FUNCTIONAL ASSESSMENT OF THE ACTIONS OF 4-METHYL DERIVATIVES OF HA-966 AT THE GLYCINE SITE OF THE N-METHYL-D-ASPARTATE RECEPTOR

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The N-methyl-D-aspartate (NMDA) receptor antagonist effects of HA-966 (3-amino-1-hydroxypyrrolid-2-one) are mediated by an action at the glycine-site, where it acts as a low efficacy, partial agonist (Foster & Kemp, 1989). Here we report on the affinity and efficacy of a more potent analogue of HA-966, R-(+)- *cis* 4-methyl-HA-966, L-687,414 (Leeson et al, 1990), as determined using functional electrophysiological studies.

Excitatory amino acid-induced depolarizations of rat cortical tissue and whole-cell voltage-clamp recordings from rat cultured cortical neurones were performed as previously described (Foster & Kemp, 1989).

On rat cortical slices, racemic *cis* 4-methyl-HA-966 antagonised NMDA responses, in a D-serine reversible manner, with an apparent Kb of 24 μM. As with HA-966 itself, the glycine site activity resided mainly in the R-(+)-enantiomer, L-687,414, (and see Foster at al, this meeting) which had an apparent Kb = 15 μM. In contrast, the *trans* 4-methyl analogue was much weaker with an apparent Kb > 1mM. On voltage-clamped rat cultured cortical neurones, racemic *cis* 4-methyl-HA-966 (10 μM) and L-687,414 (3 μM) produced parallel shifts to the right of the glycine concentration-response curve leading to estimated pKb values of 5.9 ± 0.09 (n = 5) and 6.2 ± 0.12 (n = 5), respectively. Increasing concentrations of L-687,414 led to a progressive inhibition of NMDA (30μM)/glycine (300 nM) responses with a pIC_{50} = 5.3 and a calculated pKi = 5.83. However, like HA-966, L-687,414 failed to produce a complete block of these responses and reached a plateau at 90% inhibition. By comparison the inhibition produced by (+)HA-966 reached a plateau at 79%.

In conclusion, these results suggest that L-687,414 is a higher affinity analogue of HA-966 but possesses a slightly lower level of intrinsic efficacy.

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66P THE ANTICONVULSANT PROPERTIES IN THE MOUSE OF THE GLYCINE/NMDA RECEPTOR ANTAGONIST, L-687,414

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R(+)-HA-966 antagonises the action of NMDA by a selective blockade of the glycine modulatory site on the NMDA receptor complex and is weakly anticonvulsant against seizures induced by N-methyl-DL-aspartate (NMDLA, Singh et al, 1990). We now show that L-687,414, the cis-β-methyl-analogue of (+)-HA-966 and a selective glycine receptor antagonist *in vitro* (Kemp et al, this meeting), is a potent anticonvulsant in the mouse.

Tonic seizures were induced by NMDLA (500 mg/kg, s.c.), pentylenetetrazol (PTZ, 120 mg/kg, s.c.), and electroshock (0.2 mA, 0.2 s) in male Swiss Webster mice (20-25 g), and by sound (120 dB, 1.4 kHz bell for 30 s) in male audiogenic seizure prone DBA/2 mice (8-10 g). For determination of anticonvulsant potency, four or five doses of compound were administered to groups of 8 or more mice. Protection was defined as the absence of tonic seizures during the 30 min following injection of NMDLA or PTZ, immediately following the application of electroshock or during the 30 s of sound exposure. The ED_{50} dose of L-687,414 protecting 50% of the animals tested was calculated by probit analysis. In addition, the effect of L-687,414 on motor coordination was assessed using a rotarod. Mice were first trained to remain for 120 s on the rod revolving at 15 rpm. Following drug administration, latency to fall from the rod was determined.

L-687,414 dose-dependently antagonised seizures induced by NMDLA, PTZ and electroshock (ED_{50} = 19.7, 13.0 and 26.1 mg/kg, i.v. 15 min before test, respectively), but was most potent against audiogenic seizures (ED_{50} = 5.1 mg/kg, i.p., 15 min before test). Given orally, 30 min before NMDLA, the ED_{50} increased to 44 mg/kg. The minimum effective dose of L-687,414 to induce a significant decrease in rotarod performance 15 min post-injection was 44 mg/kg, i.v. and 27.3 mg/kg, i.p. in Swiss Webster and DBA/2 mice respectively. When the glycine receptor agonist, D-serine (100 μg/mouse) was administered i.c.v. (Singh et al, 1990) immediately after the i.v. injection of 60 mg/kg L-687,414 and 15 min before NMDLA, the latency to convulse decreased from 30 + 0 min (mean ± s.e. mean, n = 8 per group) to 9.9 ± 1.7 min ($p<0.05$, Dunnett's t test). The seizure latency of animals given only saline or D-serine was 7.6 ± 0.5 min and 6.4 ± 0.5 min respectively. D-Serine similarly decreased the rotarod performance deficit induced by L-687,414 (mean latency to fall from the rotarod ± s.e. mean, n = 8 per group: controls, 109 ± 11 s; D-serine, 120 ± 0 s; L-687,414, 14 ± 12 s; L-687,414 ± D-serine, 107 ± 13 s, $p< 0.05$ vs. L-687,414 alone).

Thus, L-687,414 is a potent, orally active anticonvulsant. Motor incoordination, though present, occurs only at doses two to five fold greater than those blocking seizures. Both effects appear to result from antagonism of the glycine/NMDA receptor.

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67P R(+)-HA-966, A GLYCINE/NMDA RECEPTOR ANTAGONIST, BLOCKS INCREASED MESOLIMBIC DOPAMINE METABOLISM INDUCED BY PSYCHOSTIMULANT DRUGS

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The R(+) -enantiomer of HA-966 (3-amino-1-hydroxypyrrolid-2-one) attenuates glutamate neurotransmission by an antagonist action at the glycine modulatory site on the N-methyl-D-aspartate (NMDA) receptor complex (Singh *et al*, 1990). In view of the facilitatory role that glutamate can play on dopaminergic (DA) systems (e.g. Jhamandas and Marien, 1987), we have examined the effects of (+)-HA-966 on amphetamine- and phencyclidine (PCP)-stimulated DA synthesis and metabolism in rodent brain.

Male BKTO mice (25-30 g) were pretreated with either vehicle (0.9% NaCl, i.p.) or (+)-HA-966 (30 mg/kg, i.p.). Twenty min later they were injected with either d-amphetamine SO_4 (2.5 mg/kg, s.c.) or vehicle. After a further 20 min, all mice received the DOPA decarboxylase inhibitor, NSD 1015 (100 mg/kg, i.p.) and were killed 30 min later. Striatum and nucleus accumbens (N.Ac.) were removed and analysed for DOPA content by HPLC. Male Sprague-Dawley rats (250 - 300 g) were pretreated with either vehicle (i.p.) or (+)-HA-966 (30 mg/kg, i.p.), followed 20 min later by PCP (10 mg/kg, i.p.) or vehicle (i.p.) and killed 60 min later. N.Ac. and medial prefrontal cortex (PFC) were analysed for DA, DOPAC and HVA content by HPLC.

Table 1.

	Amphetamine (DOPA, ng/g)		**PCP** ([DOPAC + HVA]/[DA])	
	N.Ac.	Striatum	N.Ac.	Prefrontal cortex
Vehicle/vehicle	1299 ± 54*	1297 ± 62*	0.25 ± 0.01*	0.81 ± 0.08*
Vehicle/stimulant	1829 ± 146	2117 ± 98	0.40 ± 0.02	2.10 ± 0.20
(+)-HA-966/stimulant	1432 ± 74*	2007 ± 133	0.27 ± 0.01*	0.88 ± 0.16*

Values are means ± s.e. mean of 6 - 12 animals/group. * $p<0.01$ cf. vehicle/stimulant group (ANOVA).

Results are shown in Table 1. In mice, d-amphetamine significantly increased DOPA in striatum and N.Ac. (+)-HA-966 alone did not alter DOPA in either brain region, but decreased the amphetamine-enhanced accumulation of DOPA in the N.Ac. without affecting that in striatum. In rats, (+)-HA-966 prevented the increase in DA turnover ([DOPAC + HVA]/[DA]) induced by PCP in N.AC. and PFC without altering turnover in non-stimulated animals. Striatal DA turnover was not affected by PCP.

Thus, NMDA receptors appear to be importantly involved in the feedback mechanisms which mediate changes in mesolimbic DA metabolism in response to psychostimulant drugs.

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68P R(+)-HA-966, A GLYCINE/NMDA RECEPTOR ANTAGONIST, ATTENUATES PCP AND AMPHETAMINE-INDUCED HYPERACTIVITY IN RODENTS

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Hutson et al (1990) demonstrated that the glycine/NMDA receptor antagonist, (+)-HA-966 attenuates the increase in mesolimbic DA metabolism induced by psychostimulant drugs. We now show that (+)-HA-966 also diminishes the hyperactivity response of rodents to the stimulants, amphetamine and phencyclidine (PCP).

Male BKTO mice (20-30g) were individually habituated to photocell activity cages (230 x 210 x 280 mm high) for 2 h prior to injection with (+)-HA-966 or saline followed 30 min later by saline or PCP (10 mg/kg, s.c.). Photocell beam breaks were then recorded at 10 min intervals for 60 min. Other mice were also assessed by direct observation in perspex observation cages (230 x 210 x 280 mm high) and the number of lateral head weaves and quadrants of the cage entered scored 10 - 20 min after giving PCP (3 mg/kg, s.c.). Male Sprague-Dawley rats (250 g) were bilaterally cannulated under pentobarbital anaesthesia for direct injection of amphetamine into the nucleus accumbens (AP, + 1.7 mm; L, ± 1.5 mm; V, -5 mm from the dura, Paxinos & Watson, 1982). Following a 2 day recovery period, the animals were habituated to photocell activity cages (215 x 315 x 300 mm high) for 3 h prior to injection of (+)-HA-966 (30 mg/kg,i.p.) or saline followed 30 min later by an infusion into the nucleus accumbens of either amphetamine (5 µg in 0.5 µl per side) or saline (0.5 µl per side). The number of photocell beam breaks were then recorded at 10 min intervals for 2 hours. Each rat received all treatments in a latin square design with a minimum of 2 days between successive tests. Injection sites were verified histologically on completion of the experiment.

PCP significantly increased the number of photocell beam breaks from 4 ± 0.8 counts/h (mean ± s.e. mean, n = 8 - 9 per group) in saline-treated controls to 284 ± 62 counts/h ($p<0.05$, Dunnett's t test). Doses of 10 and 30 mg/kg (+)-HA-966 dose-dependently reduced the PCP-induced locomotor response to 188 ± 55 counts/h and 105 ± 25 counts/h ($p<0.05$) respectively, without altering activity in saline-treated controls. The head weaving response to PCP was also antagonised by (+)-HA-966 (saline + PCP = 165 ± 37; (+)-HA-966 + PCP = 68 ± 25 head weaves/10 min, $p<0.05$). Cage crossing was similarly reduced (saline + PCP = 182 ± 46; (+)-HA-966 + PCP = 51 ± 12 quadrants/10 min, $p<0.05$). In the rat, infusion of amphetamine into the nucleus accumbens increased total photocell counts from 13 + 4 to 250 + 58 ($p<0.05$), an effect that was almost completely blocked by 30 mg/kg (i.p.) (+)-HA-966 (44 ± 15, $p<0.05$). (+)-HA-966 itself did not alter rat activity.

Thus, together with the ability of (+)-HA-966 to attenuate the stimulation of DA metabolism induced by amphetamine and PCP (Hutson et al, 1990) these results support a role for NMDA receptors in modulating the response of mesolimbic DA systems to psychostimulant drugs.

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69P MODIFICATION OF ARGININE RESIDUES REVEALS [^{3}H]STRYCHNINE BINDING SITES SENSITIVE TO USE-DEPENDENT CATION CHANNEL BLOCKERS IN MOUSE SPINAL CORD MEMBRANES

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Modification of mouse spinal cord membranes with the arginine specific reagent 2,3-butanedione (BD) reveals binding sites for strychnine additional to those already known to exist at the synaptic glycine receptor (O'Connor *et al.*, 1990). In an attempt to identify these additional strychnine binding sites, we have compared the potencies of a variety of compounds to inhibit specific [^{3}H]-strychnine binding to untreated and BD-treated membranes.

Well washed mouse spinal cord membranes were treated with BD at 80mM in phosphate buffered saline (PBS; 100mM NaCl, 50mM Na_2HPO_4, pH 7.4 at 20°C) for 40 min and the reaction terminated by addition of PBS (160 vols) containing bovine serum albumin, 0.1% w/v. The membranes were then washed three times with PBS before incubation for a minimum of 2h at 4°C with [^{3}H]-strychnine (6nM) and unlabelled compounds, as required. Specificity of binding was determined by addition of unlabelled strychnine (0.1mM). Incubations were terminated by filtration under vacuum through glass fibre filters pre-soaked in polyethylenimine (0.1% v/v).

Of a variety of compounds thought to act at neurotransmitter receptors in the central nervous system and screened in the present experiments at 0.1mM, only strychnine and closely related alkaloids inhibited [^{3}H]-strychnine binding to BD-treated membranes. However, the structure-activity relations for this inhibition were different to those observed for inhibition of [^{3}H]-strychnine binding to the glycine receptor in untreated membranes (Phelan *et al.*, 1989). In contrast to their binding at the glycine receptor, affinity of strychnine derivatives for the BD-induced site was decreased by substitution with an amino group at position 2 or by the opening of the lactam ring III (*e.g.* cacotheline, diaboline, isostrychninic acid, n-oxystrychninic acid and Weiland-Gumlich aldehyde).

In view of the cation channel blocking activity of strychnine (Shapiro, 1977), other such channel blockers were tested for their ability to inhibit BD-induced [^{3}H]-strychnine binding. Quinine, procainamide and several other use-dependent cation channel blockers were found to inhibit this binding, with IC_{50} values in the μM range. Modification of arginine residues may, therefore, induce conformational changes in channel proteins that reveal strychnine binding. Although their function remains unclear, the preponderance of these BD-induced binding sites in brainstem and spinal cord (O'Connor *et al.*, 1990) could account for the central side effects of anti-arrythmic drugs.

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70P AUTORADIOGRAPHICAL DETERMINATION OF STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR MODULATION OF [^{3}H]MK801 BINDING IN RAT CNS

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The distribution of binding sites in rat brain for radiolabelled dizocilpine (MK-801), the non-competitive N-methyl D-aspartate (NMDA) antagonist (Wong *et al.*,1986) correlates with that of NMDA receptors (Bowery *et al.*,1988) and strychnine -insensitive glycine sites (Bristow *et al.*,1986). In addition [^{3}H]-MK-801 binding to rat brain synaptic membranes is dependent on NMDA receptor activation (Wong *et al.*,1987) which in turn is glycine dependent (Kleckner and Dingledine,1988). The aim of the present study was to use [^{3}H]-MK-801 receptor autoradiography to determine whether this interdependence is uniform throughout the brain.

Autoradiographical investigation was performed as described previously by Bowery *et al.*,(1988). Ten micron cryostat sections of rat brain were preincubated in 50mM Tris-HCl buffer (pH 7.4 24°C) for periods of between 2 and 24 hours in an attempt to decrease the endogenous levels of glutamic acid and glycine. The sections were then dried and incubated for 20 min in fresh buffer containing 30nM [^{3}H]-MK-801 (53 Ci/mmol) with or without glycine(10μM) and/or glutamate(1μM). Sections were rinsed (2 X 30s) in fresh buffer (4°C) and dried rapidly in a stream of cool air. Autoradiograms were generated by apposing the sections to tritium-sensitive film for 3-4 weeks. Non-specific binding (which represented 10-15% of total) was defined by the addition of 100μM MK-801 to the incubation solution. Image analysis of the autoradiograms from all total binding sections revealed a heterogeneous distribution which was consistent with previous reports. However the density of binding was related inversely to the period of preincubation. The highest density observed in all sections was in the CA1 radiatum of the hippocampus. After 2 hours preincubation, binding in this region was significantly enhanced by glycine (126% of control, n=4 $p<.05$) and glutamate (132% n=4 $p<.05$) in a dose dependent manner. Combination of glutamate and glycine potentiated the binding still further to a level (187% n=4 $p<.05$) which was significantly greater than for either amino acid alone. Extending the preincubation time enhanced the potentiation with maximal effects observed after 12 hours (glycine alone 140%, glutamate alone 205%, combination 285%). This enhancement was evident in all the regions studied and occurred to the same extent.

In conclusion these data provide an autoradiographical demonstration of glycine and glutamate modulation of [^{3}H]-MK-801 binding in rat brain. The additive effects of these amino acids is consistent with their actions at distinct sites on the NMDA receptor complex, and the similar degree of potentiation observed throughout the brain suggests that, at least in the regions studied, the relationship between these sites is uniform. Financial support by Wellcome Trust is gratefully acknowledged.

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71P FLUNARIZINE AND R 56865 SUPPRESS VERATRIDINE-INDUCED INCREASE IN O_2 CONSUMPTION AND ^{45}Ca UPTAKE IN RAT CORTICAL SYNAPTOSOMES

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R 56865 (N-[1-[4-(4-fluorophenoxy)butyl]-piperidinyl]-N-methyl-2-benzothiazolamine) and flunarizine diminish ischemia-induced dysfunction. Since ischemia triggers cellular Na and Ca uptake, the effect of R 56865 and flunarizine on Na and Ca uptake elicited by veratridine in rat cortical synaptosomes was investigated. The veratridine-induced increase in Na uptake was measured via synaptosomal O_2 consumption due to an enhanced Na/K ATPase activity and the Ca uptake was determined with the ^{45}Ca uptake method.
Rat cortical synaptosomes were suspended in Tyrode solution (app. 1 mg of synaptosomal protein per ml) and equilibrated with 95% O_2/5% CO_2 (pH 7.4). The suspensions were preincubated for 20 min at 36°C in the presence or absence of the compound studied. The synaptosomes were incubated for 3 min with or without veratridine (10^{-5} M) in ^{45}Ca (1 µCi/ml) containing Tyrode solution. After addition of ice cold Tyrode solution and rapid filtration through a membrane filter, the remaining ^{45}Ca was determined (dpm/mg protein). The veratridine-induced O_2 consumption measured by a continuous polarographical recording of the O_2 tension at 30°C was corrected for the basic O_2 consumption observed in the presence of oligomycin (4 mg/ml). The effects on mitochondrial function were investigated by detection of O_2 consumption upon treatment with FCCP (5×10^{-6} M). The effects on the Na/K ATPase were investigated in separate experiments. Data are given as mean ± SEM. Statistical significance ($p < 0.05$) was calculated with the Wilcoxon, Mann, Whitney-U-test.
Tetrodotoxin (10^{-5} M) suppressed veratridine-induced O_2 consumption by 87 ± 2% (n = 4) and the ^{45}Ca uptake by 98 ± 3% (n = 12), respectively. Nitrendipine (10^{-5} M) and omega-conotoxin (5×10^{-7} M) exhibited no effect on veratridine-induced O_2 consumption and ^{45}Ca uptake. Flunarizine (10^{-6} - 10^{-5} M) decreased the veratridine-induced O_2 consumption by 38 ± 4% and ^{45}Ca uptake by 96 ± 1% (n = 5 - 10).
R 56865 (10^{-6} - 10^{-5} M) supressed the veratridine-induced O_2 consumption by 62 ± 5% and ^{45}Ca uptake by 90 ± 2% (n = 6 - 9). None of the compounds tested altered Na/K ATPase activity and mitochondrial function.
Tetrodotoxin effectively inhibited both the veratridine-induced O_2 consumption (Na influx) and ^{45}Ca uptake, thus, confirming that Na and Ca influxes induced by veratridine are closely associated. Neither nitrendipine nor omega-conotoxin modified Na influx and ^{45}Ca uptake produced by veratridine, suggesting that L- and N-type Ca channels are not involved. However, flunarizine and R 56865 clearly suppressed both veratrine-induced Na influx and ^{45}Ca uptake. Therefore, we propose that flunarizine and R 56865 might exert their protective effects against ischemia-induced dysfunctions via reducing both cellular Na and Ca load.

72P THE EFFECT OF CI-977 ON POTASSIUM-STIMULATED GLUTAMATE RELEASE FROM RAT CORTICAL SLICES

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It has been shown previously that compounds which possess agonist activity at the κ-opioid receptor are both anticonvulsant and neuroprotective in animal models of ischaemia (Tang et al, 1985). The novel arylacetamide, CI-977 is a potent and selective agonist for the κ-opioid receptor (Hunter et al, 1990) and has been shown to be an effective anticonvulsant against NMDLA-evoked seizures in mice (Singh et al, 1990). The mechanism of action of CI-977 remains unclear but it would appear to involve a specific interaction between the κ-opioid receptor and the NMDA receptor complex. The aim of this study, therefore, was to determine if a component of this interaction might involve kappa agonist pre-synaptic modulation of excitatory amino-acid release.

350µm coronal slices from brains of male Wistar rats(180-220g) were cut using a vibratome and the area corresponding to parietal cortex was dissected out. The tissue was then placed in a perfusion chamber and allowed to equilibrate for 60 minutes before being exposed to the first pulse of 50mM potassium (S1). CI-977 was perfused for two minutes prior to the second K^+ stimulation(S2). Amino-acid release was measured by HPLC with fluorimetric detection.

Table1:Effect of CI-977 on the S2/S1 ratio for glutamate, aspartate, glycine, taurine and GABA (ratio+SEM)

		[CI-977]			
	CONTROL	10nM	30nM	50nM	100nM
ASP	0.93+0.21	1.28+0.28	0.60+0.07	0.48+0.10	0.22+0.20
GLU	0.85+0.09	0.91+0.13	0.72+0.06	0.22+0.05	0.30+0.18
GLY	1.10+0.28	0.83+0.23	1.39+0.31	1.78+0.25	0.75+0.20
TAU	0.57+0.03	0.58+0.07	0.38+0.08	0.32+0.04	0.92+0.37
GABA	0.96+0.47	0.46+0.08	0.76+0.15	1.21+0.18	-

CI-977 dose-dependently reduced the K^+-stimulated release of aspartate, glutamate and taurine from slices of parietal cortex with an IC_{50} of 46 (±15) nM, 51 (±8) nM and 48 (±5) nM respectively. There was no significant effect observed on the release of glycine or GABA. PD129289(1µM), the corresponding (+)-enantiomer to CI-977, had no effect on K^+-stimulated amino-acid release;MK801 (3µM) was also ineffective. The effect observed with a submaximal concentration of CI-977 (50nM) was completely attenuated by naloxone(1µM) and the κ-selective antagonist norbinaltorphimine(10nM). Collectively, these observations are consistant with CI-977 producing its effect via the κ-opioid receptor and may form the molecular basis for the anti-convulsant and neuroprotective properties of κ-agonists.

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73P BEHAVIOURAL ACTIVITY OF NEUROKININ AGONISTS FOLLOWING INTRACEREBROVENTRICULAR INJECTION IN MICE

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Intracerebroventricular (ICV) injection of substance P (SP) and other neurokinins (NKs) in rodents are known to increase locomotor activity (LMA). However, the NK receptor(s) involved in this response and the mechanism through which the LMA is induced are unclear (Elliott et al 1990). Recently, peptidase-resistant and receptor-selective agonists and antagonists have been developed which can be used to investigate which NK receptors are involved in SP-induced locomotion. In order to determine the behavioural profiles of these synthetic compounds, we have investigated their activity following ICV injection in two test situations; (1) activity in an open field arena and (2) activity in photocell cages.

Male albino mice (Glaxo), weighing 12-16g, were used in all studies. Drug and vehicle injections were carried out using a previously described method (Brittain & Handley, 1967) in a 3μl volume. Locomotor activity of mice was recorded in cages (26 x 41 x 16 cm) equipped with 14 photocells over a 60 min test session. Mice were habituated to the apparatus for a minimum of 60 min prior to drug injection. Behavioural activity of mice in the novel open field (60 x 60 x 30 cm) was recorded on video tape and analysed later by observers blind to the drug treatments. Test sessions were performed under bright lighting for 45 min. The agonists used were : NK-1, Ava[L-Pro^9,N-Me-Leu^{10}]SP(7-11) (GR73632; Hagan et al, 1989); NK-2, Ava[D-Pro^9]SP(7-11) (GR51667) and [Lys^3,Gly^8-R-γ-lactam-Leu^9]NKA(3-10) (GR64349; Hagan et al, 1989); NK-3, senktide. An NK-1 antagonist was also used in this study : [D-Pro^9[Spiro-γ-lactam]Leu^{10},Trp^{11}]Physalaemin(1-11) (GR82334; Hagan et al 1990).

The NK-1 agonist, GR73632 (0.001-3nmol) increased LMA in a dose-dependent manner. This increase was attenuated by simultaneous ICV injection of the NK-1 antagonist, GR82334 (0.01- 10nmol). In addition to the elevated LMA, GR73632 (1-10 nmol) also elicited scratching behaviour. Agonists at NK-2 and NK-3 receptors were found to increase LMA only at high doses and these responses were antagonised by GR82334 (10nmol). No obvious behavioural responses were observed following ICV injection of the NK-2 agonists. However, ICV injection of the NK-3 agonist, senktide, induced a number of behaviours, including flattened body posture, tail-rattle, straub tail and splayed hind limbs.

The present data suggest that, following ICV injection, the NK's increase LMA via activation of NK-1 receptors. The location of these receptors remains to be determined.

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74P MODULATION BY CAPSAICIN-SENSITIVE SENSORY NEURONES OF GASTRIC SECRETORY RESPONSES INDUCED BY PEPTONE

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The acid secretory response induced by a meal is directly related to its protein content. The present report analyzes if the stimulation of gastric acid secretion by peptone, a pancreatic digest of casein containing a mixture of amino acids and peptides, (a) could be the consequence of a nervous reflex involving capsaicin-sensitive afferent fibres; (b) the anatomical pathways of such reflex and (c) the role played by gastrin.

Capsaicin (50 mg kg^{-1}) or vehicle was administered s.c. in 2 day old rats and the animals used 3-4 months later. After fasting for 24h rats were anaesthetized with urethane (1.5 g kg^{-1}, i.p.), and two polyethylene cannulae inserted into the gastric lumen. The stomach was continuously perfused with saline (0.9 ml min^{-1}) and acid output was determined by titration of the perfusate to pH 7 with 0.01 N NaOH. Once the acid output had remained constant for 60 min (basal), acid secretion was stimulated by intraluminal perfusion (60 min) with an isotonic solution of peptone (0.5%, 1% and 2.5%). In some experiments the vagus was cut bilaterally, the coeliac ganglion removed or both. Gastrin concentration in arterial blood was measured by radioimmunoassay in basal conditions and during the infusion of peptone.

Basal acid secretion was not statistically different in control rats (4.2 ± 0.7 μEq 100^{-1} 60 min^{-1}, n=25) or capsaicin pretreated (3.1 ± 0.9 μEq 100^{-1} 60 min^{-1}, n=11). Intragastric peptone, at the concentrations of 0.5%, 1% and 2.5% induced a concentration dependent stimulus of acid production, respectively 10.1 ± 1 μEq 100^{-1} 60 min^{-1} (n=11), 19.4 ± 1.6 μEq 100^{-1} 60 min^{-1} (n=13) and 28.2 ± 2.5 μEq 100^{-1} 60 min^{-1} (n=17). In capsaicin treated rats acid responses were significantly ($p<0.05$) lower. Acid output stimulated by peptone 0.5% decreased by 55.5% ± 11.45% (n=7), peptone 1% by 48.6% ± 7.3% (n=7) and peptone 2.4% by 68.7% ± 7.7% (n=7). Acid responses to peptone 1% were reduced by vagotomy, coeliac ganglionectomy, or the combination of both to levels not different from those present in capsaicin-treated animals. These surgical procedures did not modify the acid responses to peptone in capsaicin-treated rats. The concentration of plama gastrin was not altered by intragastric perfusion with peptone 1%.

These findings suggest that peptone stimulates acid secretion in the rat (a) through a nervous reflex mediated by capsaicin-sensitive neurones, which involves (b) both the vagus and the coeliac ganglion and (c) is independent of increases in plasma levels of gastrin.

75P CIGARETTE SMOKE-INDUCED GOBLET CELL SECRETION IN GUINEA-PIG TRACHEA *IN VIVO*: UNDILUTED VAPOUR PHASE ACTIVATION OF CAPSAICIN-SENSITIVE NERVES

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Goblet cells in the surface epithelium of guinea-pig airways can be shown to have discharged their intracellular mucin by using a morphometric measure, the mucus score (MS), which is inversely related to degree of discharge: the greater the MS, the greater the content of mucin and, therefore, the lower the degree of discharge, and *vice versa*. Using this parameter, we have shown previously that goblet cells discharge in response to activation of cholinergic and capsaicin-sensitive nerves, as well as to injection of sensory neuropeptides, predominantely substance P (Tokuyama *et al.*, 1990; Kuo *et al.*, 1990a). We have also found that the particulate phase of cigarette smoke (CS), diluted 1:10 in air, induced goblet cell secretion via a hexamethonium-sensitive but capsaicin-insensitive mechanism (Kuo *et al.*, 1990b). In contrast, Lundberg *et al.* (1983) found that the vapour phase of CS induced airway plasma leakage in rat airways via activation of capsaicin-sensitive nerves. In the present study we sought to determine the role of these nerves in inducing goblet cell secretion in guinea pig trachea *in vivo* in response to undiluted CS.

One tidal volume of undiluted middle tar CS was blown into the trachea of urethane-anaesthetized, artificially ventilated guinea pigs every 5 or 6 ventilated breaths for a total of 20 tidal volumes of CS. Fifteen min later, the trachea and lungs were removed for histology. The intracellular mucin of the goblet cells was stained using Alcian blue (pH 2.6) and periodic acid-Schiff. The amount of intracellular mucin was graded and a mucus score (MS), which represents the total amount of intracellular mucin, was derived for 40 high power fields in the lower trachea.

Undiluted CS caused a significant decrease in MS from 627 (SE 59, n=6) in sham air controls to 351 (SEM 58, n=5), indicating that CS caused goblet cell secretion. The effect of CS was not inhibited by autonomic receptor antagonists (combined atropine 1mg/kg, propranolol 1 mg/kg and phentolamine 0.1 mg/kg, i.v.). Capsaicin pretreatment inhibited the response to CS (578, SE 70, n=5) compared with controls (287, SE 67, n=6). Neither histamine antagonists (cimetidine 10mg/kg + mepyramine 10 mg/kg, i.p.) nor indomethacin (10mg/kg, i.p.) modified the effect of CS. Removing the particulate phase of CS using a Cambridge filter failed to attenuate the effect of CS (341, SE 50, n=6). The response to the vapour phase (after removing the particulate phase) was significantly inhibited by capsaicin pretreatment (565, SE 36, n=5) but not the ganglionic blocking agent, hexamethonium (381, SE 70, n=5).

We conclude that 20 breaths of undiluted CS induced goblet cell secretion via vapour phase activation of capsaicin-sensitive nerves.

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76P THE RELEASE OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) BY CAPSAICIN IN THE RABBIT SKIN *IN VIVO*

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The neuropeptide CGRP, localised in sensory C-fibres, is a potent vasodilator in rabbit skin (Brain et al 1985). We have recently shown that intradermal (i.d.) injection of capsaicin, which selectively activates C-fibre nerves, stimulates increased blood flow in rabbit skin (Buckley et al 1990). In the present study using a multiple site ^{133}xenon clearance technique, we investigated whether an antibody to CGRP inhibited capsaicin-induced vasodilator activity (Louis et al 1988). A goat anti-human CGRP IgG antibody was raised, purified and papain digested to yield Fab fragments. The anti-CGRP Fab was pre-injected (100μl/site i.d.) for 40 min into rabbit dorsal skin at a concentration equivalent to that in the original goat serum. Control sites received saline (100μl/site i.d.). Anti-CGRP Fab neutralised the increased blood flow induced by i.d. injection of CGRP (CGRP 3 x 10^{-12}mol/site 87.9 $\pm$ 18.3%, CGRP + anti-CGRP Fab 18.4 $\pm$ 10.2%, anti-CGRP Fab 3.6 $\pm$ 5.8% increase in blood flow n=6 replicate sites). However, responses to the vasodilator prostaglandin, PGE_2 (10^{-10}mol/site) and the neuropeptide vasoactive intestinal peptide (VIP; 10^{-11}mol/site) were unaffected (PGE_2 89.8 $\pm$ 9.6%; PGE_2 + anti-CGRP Fab 78.3 $\pm$ 9.5%; VIP 106.5 $\pm$ 9.6% ; VIP + anti-CGRP Fab 125.0 $\pm$ 12.4% increase in blood flow n=6 replicate sites). All results are expressed as % increase in blood flow (mean $\pm$ SEM) compared with that at saline-injected sites.
The effect of anti-CGRP on the vasodilator response of capsaicin is shown in table 1.

Table 1

Agent	Increase in blood flow(%) control	+Fab	n (rabbits)
Capsaicin ($3x10^{-8}$mol/site)	45.2 ± 6.0	36.8 ± 6.2	4
Capsaicin (10^{-7}mol/site)	63.3 ± 15.5	43.5 ± 9.2	4
Capsaicin ($3x10^{-7}$mol/site)	96.9 ± 15.3	63.6 ± 12.2 **	7
saline control	0	9.1 ± 5.9	6

** significant inhibition of capsaicin response ($p<0.05$, two way ANOVA)

These results suggest that capsaicin activates sensory C-fibres in the rabbit skin leading to the release of CGRP. However, anti-CGRP Fab only partially inhibited responses induced by capsaicin. It is possible that the nerves containing CGRP are in close spatial arrangement with vascular receptors in the microvasculature, which prevents complete neutralisation of endogenous CGRP by the anti-CGRP Fab. In addition, the existence of other neuropeptide vasodilators in sensory C-fibres cannot be discounted.

We would like to thank the Wellcome Trust for financial support. TLB is an MRC scholar.
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77P DEVELOPMENT OF A COMPETITIVE ANTAGONIST FOR THE SENSORY NEURONE EXCITANT, CAPSAICIN

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Capsaicin (N-methyl-N-vanillyl-6-nonenamide) is a specific excitant of a subset of primary afferent, sensory neurones that includes the polymodal nociceptors. Capsaicin acts on a putative membrane receptor to open a membrane ion channel that is permeable to both monovalent and divalent cations (see Bevan & Szolcsanyi, 1990). The dye, ruthenium red has recently been used as a capsaicin antagonist, but this agent is not specific and can affect other membrane receptors and ion channels. Here we report the discovery of a compound, capsazepine (2-[2-(4-chlorphenyl)ethylamino-thiocarbonyl]-7,8-dihydroxy-2,3,4,5- tetrahydro-1H-2-benzazepine), that acts as a potent and specific antagonist of the actions of capsaicin on sensory neurones.

Electrophysiological experiments were made on dissociated dorsal root ganglion (DRG) neurones from neonatal rats and whole cell currents recorded under voltage clamp. Capsazepine (0.5-1μM) reversibly reduced or abolished the response to 0.1-0.5μM capsaicin, but had no obvious effect on the response to GABA.

The antagonism between capsazepine and capsaicin was studied quantitatively in ion flux studies on both neonatal rat cultured DRG neurones and adult rat vagus nerves. Capsazepine antagonized the capsaicin induced uptake and accumulation of $^{45}Ca^{2+}$ in DRG neurones, with an IC_{50} of 0.5μM. Capsaicin dose-response curves for both $^{86}Rb^{+}$ efflux from DRG neurones and ^{14}[C]-guanidinium efflux from vagus nerves provided evidence that the antagonism was competitive. Log concentration-effect curves were shifted in a parallel manner with increasing concentrations of capsazepine. Schild plots of these data were linear with a slope very close to 1, and gave K_d estimates of 100nM (DRG neurones) and 690nM (vagus nerve).

Capsazepine is the first competitive capsaicin antagonist to be reported and should be a useful pharmacological tool in studies on the mechanisms of capsaicin action and the functions of capsaicin sensitive neurones. The competitive antagonism also provides good additional evidence that capsaicin acts on a specific membrane receptor.

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78P ANTAGONISM OF CAPSAICIN-INDUCED ACTIVATION OF C-FIBRES BY A SELECTIVE CAPSAICIN ANTAGONIST, CAPSAZEPINE

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Several studies have suggested that the selective action of capsaicin on primary sensory neurones is produced by an interaction with a specific receptor (eg see Bevan & Szolcsanyi, 1990). This suggestion has now been confirmed using a competitive capsaicin antagonist, capsazepine (2-[2-(4-chlorophenyl) ethylamino - thiocarbonyl] - 7,8-dihydroxy - 2,3,4,5 - tetrahydro - 1H-2- benzapine) (Bevan et al, this meeting). In the following studies we have further characterized the action of capsazepine, showing selective antagonism of the capsaicin-induced activation of sensory C-fibres in a number of preparations.

Capsaicin-induced activation of primary sensory neurones was evoked in the following preparations: 1) the isolated guinea-pig ileum in which capsaicin produces contractions mediated by the release of sensory neuropeptides; 2) the spinal cord-tail preparation from the neonatal rat, maintained in vitro, in which activation of peripheral fibres in the tail evokes a depolarizing response in a lumbar spinal ventral root (Dray et al 1989); 3) the isolated, hemisected mouse (12-20 day old) spinal cord in which capsaicin activates C-fibre afferent terminals to depolarize single dorsal horn neurones; 4) an isolated preparation of the rat saphenous nerve and hind paw skin in which activation of single C-fibre units were recorded following capsaicin administration on to the receptive field of the skin (Reeh 1986); 5) capsaicin-induced reflex blood pressure fall, recorded in an anaesthetised rat, following close injection into a femoral artery (Donnerer & Lembeck 1983); 6) capsaicin-evoked pain in man, scored subjectively, following intradermal injection of capsaicin in the forearm.

In each of these preparations capsazepine administered alone was inactive but it consistently produced a concentration dependent (ileum IC_{50}= 10-50nM; cord-tail IC_{50}= 0.2μM; mouse cord 1-5μM; skin-nerve 1.0μM; blood pressure 10 nmol,i.a.; pain response = 10-100 pmol, intradermally), reversible and selective reduction of capsaicin-evoked activity.

These data show that capsazepine acts as a selective capsaicin antagonist and strongly support the concept that capsaicin activates C-fibres, including nociceptors, by interacting with a specific membrane receptor.

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79P THE SELECTIVE CAPSAICIN ANTAGONIST CAPSAZEPINE INHIBITS CAPSAICIN-INDUCED ANTINOCICEPTION: ELECTROPHYSIOLOGICAL STUDIES IN RODENTS

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Systemically administered capsaicin at low to moderate doses produces a reversible antinociceptive effect that can be demonstrated in a number of conventional behavioural tests (Hayes et al 1984; Campbell et al 1989). This effect of capsaicin appears to be exerted mainly by an action on capsaicin-sensitive primary afferent nerve terminals in the spinal cord (Dickenson et al 1990). The probability that the effects of capsaicin on sensory neurones are mediated by a specific receptor has been strongly supported by studies using the competitive and selective antagonist capsazepine,(2-[2-(4-cholorophenyl) ethylamino- thiocarbonyl] - 7,8-dihydroxy - 2,3,4,5 - tetrahydro -1H-2-benzapine) (Bevan et al; Dray et al; this meeting). In the present study we have used this compound to show that capsaicin-induced antinociception is also mediated through a capsaicin receptor mechanism.

The discharges of wide dynamic range dorsal horn neurones, in response to electrical stimulation of their peripheral cutaneous receptive field was recorded in halothane (1-5%) anaesthetized rats (Dickenson & Sullivan, 1990). Systemic capsaicin (20 µmol/kg s.c.) reversibly reduced the discharge in response to stimulation of C-fibres, but not that produced by Ab-fibres. This inhibitory effect of systemic capsaicin was significantly reduced by capsazepine administered s.c. (100µmol/kg) and by capsazepine administered directly to the spinal cord (5nmol in 50µl). Capsazepine did not affect neuronal responses to peripheral stimuli when administered alone by the s.c. route (400µmol/kg, s.c.) or when applied directly to the spinal cord (5nmol in 50µl). Local injection of capsaicin (200pmol in 10µl) into the peripheral receptive field first activated C-fibres and then reduced the electrical excitability of C-fibres (Dickenson et al 1990). Capsazepine (1nmol in 10µl) reduced both these effects of capsaicin when it was co-injected with capsaicin.

These results suggest that capsaicin-induced antinociception is mediated by an effect on a specific capsaicin receptor. In addition the data further support the hypothesis that antinociception, following systemic capsaicin, is likely due to an effect via capsaicin receptors on primary afferent fibres in the spinal cord.

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80P NITRIC OXIDE MODULATES CHOLINERGIC NEUROTRANSMISSION IN GUINEA-PIG TRACHEAL SMOOTH MUSCLE

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Airway smooth muscle possesses an inhibitory non-adrenergic, non-cholinergic (i-NANC) innervation and evidence suggests that the neurotransmitter of airway i-NANC effects may be partly due to nitric oxide (NO) (Tucker *et al.*, 1990). Since i-NANC mechanisms may coexist with cholinergic innervation, we have used L-N^G-nitro-arginine-methyl-ester (L-NAME), a potent, specific, competitive inhibitor of NO synthase (Hobbs & Gibson, 1990), to examine whether endogenous NO modulates cholinergic neurotransmission in the airways.

Guinea-pig tracheal strips were suspended in organ baths containing Krebs solution (37°C, pH 7.4) between platinum field electrodes for electrical field stimulation (EFS, 40V, 0.5ms, 0.5, 4 and 32Hz for 15s every 4min). When constant responses to EFS were obtained, L-NAME (10^{-7}-10^{-4}M) was added. When the enhancement was maximal, L-arginine (L-Arg, 10^{-3}M) or D-arginine (D-Arg, 10^{-3}M) was added. The effect of L-NAME (10^{-4}M) on concentration-response relationships to acetylcholine (ACh, 10^{-8}-10^{-2}M) was also studied.

L-NAME (10^{-7}-10^{-4}) had no effect on resting tone in guinea-pig trachea, but significantly enhanced the cholinergic contractile responses to EFS (40V, 0.5ms, 4Hz) in a concentration-dependent manner. L-NAME (10^{-4}M) increased cholinergic responses by 33.3 $\pm$ 5.7% (n = 10, $p < 0.001$). This enhancement was partially reversed by L-Arg (10^{-3}M, 59.7 $\pm$ 14.9% reversal, n = 5) but not D-Arg. The enhancement by L-NAME was greater at higher stimulation frequencies (46.1 $\pm$ 6.5% at 32Hz, n = 5, $p < 0.01$). L-NAME had no effect on the contractile responses to exogenous ACh with EC_{50} values of 2.6 $\pm$ 0.12 x 10^{-6}M in the absence and 2.8 $\pm$ 0.06 x 10^{-6}M in the presence of 10^{-4}M L-NAME.

L-NAME significantly enhanced the cholinergic contractile component to EFS in the guinea-pig trachea in a concentration-dependent manner and this effect was partially reversed by L-Arg but not D-Arg. L-NAME failed to affect the concentration-response relationships to exogenous ACh, suggesting a prejunctional site of action. The enhancement of the EFS-induced contractile component was frequency dependent with a greater effect at higher frequencies, which is consistent with i-NANC responses. NO may modulate cholinergic nerve induced contraction either by a functional antagonism of ACh at the level of airway smooth muscle or via a prejunctional effect to inhibit the release of ACh from cholinergic nerve terminals.

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81P THE NATURE OF α-CHYMOTRYPSIN-RESISTANT NANC RELAXATIONS IN GUINEA-PIG TRACHEA

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α-Chymotrypsin-resistant non-adrenergic, non-cholinergic (NANC) relaxations of guinea-pig trachea are reduced by L-N^G-nitro-arginine (L-NOARG), an inhibitor of L-arginine:NO synthase (Tucker *et al.*, 1990), suggesting that such NANC responses may be mediated by an endogenous nitrate, as has been proposed in the anococcygeus muscle (Hobbs & Gibson, 1990). Here we have investigated this possibility further, by determining the effect on trachea of 3 procedures previously found to modify NANC/NO relaxation in the anococcygeus: arginine analogues (Hobbs & Gibson, 1990), hydroquinone and anoxia (Gibson & Mirzazadeh, 1989).

Guinea-pig tracheal strips, consisting of two adjacent cartilage rings were suspended in organ baths containing Krebs-bicarbonate buffer at 37°C. Atropine (1μM), propranolol (1μM) and indomethacin (2μM) were present in the bathing medium. α-chymotrypsin (2units/ml) was also added to attenuate the actions of vasoactive intestinal peptide. To observe relaxations to drugs and NANC stimulation tone was raised with U46619 (50nm).

Electrical field stimulation (10Hz; 30s trains) of pre-contracted tissues elicited reproducible, submaximal relaxations due to excitation of NANC nerves. L-N^G-monomethyl-arginine (L-NMMA; 5-100μM), L-N^G-nitro-arginine (L-NOARG; 1-100μM) and L-N^G-nitro-arginine-methyl-ester (L-NAME; 5-100μM) produced concentration-related inhibitions of NANC relaxations with an order of potency L-NOARG > L-NAME > L-NMMA. In the presence of 200μM L-arginine, the concentration-response curve for L-NOARG was shifted to the right by a factor of 8; 200μM D-arginine was without effect. L-NOARG (100μM), which reduced NANC responses by 90%, had no effect on relaxations induced by NO (150μM) or sodium azide (5μM). NANC responses obtained in the absence of α-chymotrypsin were also blocked by L-NOARG, although to a lesser extent (maximum inhibition 90% in the presence and 40% in the absence of α-chymotrypsin). Hydroquinone (100μM) reduced relaxations to NO by 75%, but had no effect on those to NANC stimulation or sodium azide. Conversely, anoxia (95%N_2: 5%CO_2 for 10 minutes) completely abolished responses to sodium azide, greatly reduced those to NANC stimulation, but potentiated those to NO.

These observations confirm that α-chymotrypsin-resistant NANC relaxations in guinea-pig trachea involve an endogenous nitrate; the NANC system is similar to that recently proposed in the anococcygeus, although the inhibitory effect of L-NOARG is less easily reversed by L-arginine in the trachea. The ability of hydroquinone and anoxia to differentiate between NANC stimulation and NO may suggest that free NO is not the actual substance released by NANC nerves (Gibson & Mirzazadeh, 1989; Gillespie & Sheng, 1989).

A.J. Hobbs is an SERC student. We thank the MRC for support.

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82P EVIDENCE FOR A ROLE OF EDRF IN PENILE ERECTION

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Penile erection is effected by parasymapthetic nerve stimulation, but the neurotransmitters involved have still to be elucidated. Current evidence for a role of EDRF in penile erection has related to a relaxant effect on isolated corpus cavernosum muscle (Azadzoi et al., 1990). We have used a pithed rat preparation to study the role of EDRF in the vasodilator response to parasympathetic stimulation in vivo.

Sprague-Dawley rats (220-280g) were pithed under halothane, and respired with oxygen-enriched air. A spinal electrode was localised at the area of the lumbo-sacral junction. Pressure was recorded from a carotid artery (BP), and from a needle inserted into one of the corpora cavernosa (CP). Application of square-wave stimuli (50V, ims, 2, 5, 10 and 20 Hz for 1 min) to the spinal electrode produced reproducible increases in CP. Corporal response to stimulation was calculated as the ratio CP/BP.

Intravenous injection of L-N^G-nitroarginine (NOARG; 25mg kg^{-1}) produced elevation in BP, and inhibition of the corporal response to spinal stimulation. The inhibition of the corporal response was greatest at lower frequencies of stimulation, and was maintained for more than 2h. At 30 min after NOARG, CP/BP ratio was reduced to 3±3% control at 5Hz, and 47±5% control at 20Hz (SEM, n=9). Intravenous injection of l-arginine (400 mg kg^{-1}) had no sustained effect on blood pressure or on corporal response to stimulation, but partially antagonised both the inhibitory effect of NOARG on the corporal response to spinal stimulation, and the pressor response to NOARG injection. Corporal response to stimulation at 5 and 20Hz respectively was 35±14% and 94±11% control after the combination of NOARG and L-arginine (n=8; $P<0.05$ and <0.01 respectively for difference from NOARG alone). In some experiments, L-arginine was capable of reversing an existing inhibition of corporal response to stimulation by NOARG.

These data provide preliminary evidence for a role of EDRF in mediation of the vasodilator response to pelvic nerve stimulation in the rat.

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83P EFFECTS OF N^G-SUBSTITUTED ANALOGUES OF L-ARGININE ON NANC RELAXATION OF THE ANOCOCCYGEUS, RETRACTOR PENIS AND PENILE ARTERY

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We have previously reported that L-N^G-monomethyl arginine (L-NMMA) inhibits, in a stereoselective and L-arginine-reversible manner, NANC relaxation of the rat anococcygeus but not the bovine retractor penis (BRP; Gillespie and Xiaorong, 1989; Gillespie et al, 1989). These findings suggested that the L-arginine-nitric oxide system could account for NANC relaxation in the anococcygeus but not in the BRP. More recently, another analogue, L-N^G-nitro arginine (L-NOARG), has been shown to be a more potent and effective inhibitor of NANC transmission in the rat anococcygeus (Hobbs and Gibson, 1990). We have now re-examined the role of the L-arginine-nitric oxide system in NANC relaxation of the BRP using L-NOARG. We have also examined the effects of L-NOARG on the bovine penile artery to see whether the resistance to L-NMMA is species determined. The penile artery like the BRP possesses a powerful NANC innervation.

Rat anococcygeus and BRP muscle strips were bathed in oxygenated Krebs solution at 37°C for isometric tension recording. Pressure recordings were made from isolated segments of bovine penile artery perfused at constant flow (4 ml/min). Guanethidine (30 μM) was used to inhibit adrenergic motor responses, and in the penile artery 5-hydroxytryptamine (1 μM) was used to induce constriction. NANC relaxation was induced by electrical field stimulation at supramaximal voltage.

L-NMMA (10-100 μM) induced a concentration-dependent inhibition of NANC relaxation of the rat anococcygeus at all frequencies tested (0.1 - 20 Hz); at 10 Hz, the maximum inhibition was 55 ± 6%, n = 6. In contrast, L-NMMA (10 - 100 μM) had no effect on NANC relaxation of the BRP or penile artery. L-NOARG, however, abolished NANC relaxation of the rat anococcygeus, BRP and bovine penile artery at all frequencies tested: the IC_{50} on each tissue was 2 μM. The inhibition by L-NMMA in the rat anococcygeus was easily reversed by a 3-fold molar excess of L-arginine. In the BRP, inhibition by L-NOARG in concentrations up to 10 μM was reversed by a 30-fold molar excess of L-arginine, but that induced by higher concentrations was difficult to reverse.

The inhibition by L-NOARG in the anococcygeus, BRP and penile artery is consistent with the L-arginine-nitric oxide system mediating NANC responses in these tissues. The effectiveness of L-NMMA in the rat anococcygeus but not in the BRP or bovine penile artery suggests a difference in the nitric oxide synthase in the two species.

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84P NITRIC OXIDE RELEASED UPON STIMULATION OF NON-ADRENERGIC NON-CHOLINERGIC NERVES IN THE RAT GASTRIC FUNDUS

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The possible role of nitric oxide (NO) as inhibitory non-adrenergic non-cholinergic (NANC) neurotransmitter was studied in the rat gastric fundus. In a first series of experiments, longitudinal muscle strips of the gastric fundus of reserpinised rats (24 h before killing) were prepared and mounted in organ baths. During a 5-hydroxytryptamine (0.1 μM)-induced contraction and in the presence of atropine (1 μM), NO (0.1 - 30 μM) induced tetrodotoxin-resistant relaxations (pD_2 = 6.1 ± 0.1, n = 6), morphologically similar to those induced by electrical stimulation (ES, 0.5 - 8 Hz, 1 ms). N^G-monomethyl-L-arginine (L-NMMA, 0.3 mM) and N^G-nitro-L-arginine (L-NNA, 0.1 mM) induced an increase of the basal tension, 0.59 ± 0.15 g and 0.88 ± 0.16 g (n = 7) respectively, which was partly reversed by L-arginine (5 mM) but not by D-arginine (5 mM). L-NMMA and L-NNA inhibited the relaxations to ES, but not those induced by ATP (0.1 mM), vasoactive intestinal polypeptide (VIP, 0.1 - 10 nM), noradrenaline (0.01 - 1 μM) or NO (3 μM). This effect was prevented by L-arginine, but not by D-arginine.

In a second series of experiments, the gastric fundus served as donor tissue in a superfusion bioassay with deendothelialised rings of rabbit aorta contracted by noradrenaline (0.1 μM) as detector tissue. The fundus released a labile factor with vasodilator activity upon ES (1 - 8 Hz, 1 ms). The relaxation of the aortic rings in response to ES (4 Hz, 1 ms) of the gastric fundus was reduced from 21 ± 6 % to 5 ± 2 % (n = 5) and from 22 ± 4 % to 11 ± 4 % (n = 6) of the initial contraction by tetrodotoxin (1 μM) or L-NNA (0.1 mM) respectively, whereas it was increased by L-arginine. The relaxations of the bioassay tissue were enhanced from 7 ± 3 % to 26 ± 6 % (n = 7) by superoxide dismutase (20 units.ml^{-1}) and eliminated by hemoglobin (0.1 μM).

In conclusion, our results suggest NO or a NO releasing substance as inhibitory NANC neurotransmitter in the rat gastric fundus, as we previously described in the canine ileocolonic junction (Boeckxstaens et al., 1990; Bult et al., 1990).

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85P INHIBITION OF FIELD STIMULATED NON-CHOLINERGIC CONTRACTIONS OF THE GUINEA-PIG ILEUM BY HISTAMINE H_3 RECEPTOR ACTIVATION

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Activation of histamine H_3 receptors inhibits release of histamine and a variety of other neurotransmitters (see Schwartz et al., 1990) by a presynaptic mechanism. Ambache and Aboo Zar in 1970 noted that histamine inhibited non-cholinergic neurogenic contractions of the longitudinal muscle of the guinea-pig isolated ileum (GPILM). Menkveld and Timmerman (1990) provided evidence that this may be an H_3 mediated response. We now describe the effects of H3 receptor agonists and antagonists on this preparation.

Ileum from male Dunkin Hartley guinea-pigs (300-350g) was removed and myenteric plexus containing longitudinal muscle strips prepared as described by Rang (1964). Muscle strips were mounted in organ baths and bathed in aerated (95% O_2/ 5% CO_2) Krebs solution at 37°C, containing atropine sulphate (0.1μM), mepyramine maleate (1μM) and ranitidine hydrochloride (10μM). At one min intervals the tissues were stimulated by an electric field comprising a train of 20 pulses of 100 Hz frequency, 30V intensity and 0.5ms duration.

The above stimulation parameters elicited an initial rapid contraction followed by a sustained (>10s) secondary contraction. Histamine H_3 receptor agonists added cumulatively inhibited, in a dose dependent manner, the second contraction with minimal effect on the initial contraction (maximum inhibition 65-75%). Agonist pD_2 values were as follows: N-α-methylhistamine 7.6, (R)α-methylhistamine 7.5, Nα-Nα-dimethylhistamine 6.4 and histamine 5.8. Histamine H_3 receptor antagonists caused concentration-related rightward shifts in the (R)α-methylhistamine concentration-response curve, although the putative H_3 receptor antagonist betahistine was inactive (up to 30μM). Derived pA_2 values were as follows: thioperamide 8.2, burimamide 6.9, phenylbutanoylhistamine 5.6 and betahistine $\leq$4.0 (pK_B) (s.e. means <0.2; n>8; Schild slopes not significantly different from unity).

In conclusion, we have investigated the effects of H_3 receptor agonists and antagonists on non-cholinergic contractions of the GPILM. H_3 receptor agonists markedly inhibited these contractions in a dose-dependent manner. This effect was reversed by H_3 receptor antagonists although betahistine, which is reported to be a weak H_3 antagonist (Ki 6.9μM; Arrang et al., 1985) was inactive at the concentrations tested.

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86P GANGLION BLOCKING ACTIVITY OF DEQUALINIUM IN FROG AND RAT SYMPATHETIC GANGLIA *IN VITRO*

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Dequalinium (1,1'-decamethylenebis[4-aminoquinaldinium]) arose from a project to develop neuromuscular blocking agents, but it is better known for its antibacterial and antifungal actions (Collier *et al.*, 1959). More recent studies have shown that dequalinium is in fact a potent blocker of the response to nicotinic receptor activation in frog skeletal muscle (Castle, 1987). In this study, I have examined the ganglion blocking activity of this compound in frog paravertebral sympathetic ganglia, and compared this with its effect on the superior cervical ganglion of the rat.

Compound action potentials elicited by preganglionic stimulation, and d.c. potential changes in response to carbachol were recorded from frog and rat sympathetic ganglia, using the "grease-gap" technique, essentially as described by Brown & Dunn (1983). In addition, some experiments were carried out using conventional intracellular recording techniques.

In frog ganglia, dequalinium (0.3 to 10μM) produced a reversible, concentration dependent reduction in the amplitude of the postganglionic compound action potential, with an IC_{50} of 2.0 ± 0.2μM (mean ± s.e.mean, n=3). At similar concentrations, it caused a non-competitive antagonism of the ganglion depolarization evoked by carbachol (in the presence of 1μM atropine). At a concentration of 1μM, dequalinium reduced the response to 100μM carbachol by 74 ± 6% (mean ± s.e.mean n=7). Intracellular recordings from frog ganglia revealed that 1μM dequalinium produced a 55% reduction in the amplitude of evoked excitatory post synaptic potential (n=2), but did not affect the generation of action potentials by current injection. In rat ganglia, concentrations of dequalinium up to 100μM produced only 25 ± 5% (n=3) reduction in the amplitude of the postganglionic compound action potential, and a 17 ± 3% (n=3) reduction in the response to 100μM carbachol.

In conclusion, dequalinium is a moderately potent blocker of ganglionic transmission in frog sympathetic ganglia, due to a non-competitive action at the nicotinic ACh receptor/channel complex. However it is approximately 100 times less potent in rat ganglia, which is consistent with the view that the nicotinic ACh receptor/channel complex in frog ganglia represents a distinct class of neuronal nicotinic receptor (Lipscombe & Rang, 1988).

Supported by the Wellcome Trust.

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87P BENZODIAZEPINES CAN INHIBIT MOTOR ACTIVITY BY BLOCKING IMPULSE PROPAGATION

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Muscle relaxation produced by benzodiazepines *in vivo* is generally believed to be of central origin. *In vitro* twitch and tetanic contraction can be blocked by benzodiazepines both after indirect and after direct stimulation of curarized preparations (Eigenhuis *et al.*, 1990). Depression by diazepam of vagal nerve activity and synaptic potentials measured from dorsal and ventral roots in the isolated spinal cords have also been reported (Wesselman *et al.*, 1990). In the present study we have studied the effect of benzodiazepines on the compound action potentials and on the propagation of action potentials in the phrenic nerve of the rat. These results are compared with the effects of the local anaesthetic lidocaine.

Phrenic nerves were stimulated (0.1 Hz) with bipolar platinum electrodes using rectangular waves (0.5 ms) at supramaximal voltages. The nerve was pulled through a hole into a nerve chamber which was filled with Krebs solution. The hole connecting the two chambers was sealed with vaseline grease, such that the chamber with the muscle was completely separated from the chamber with the nerve and leakage was prevented. The twitch tension was registered as a measure of the effect of the drugs applied to the phrenic nerve. In another series of experiments the effect of midazolam and diazepam on the compound action potential of the phrenic nerve has been studied.

The solvents propyleneglycol and benzylalcohol have no effect on the impulse propagation and the compound potential. The four benzodiazepines and lidocaine applied to the phrenic nerve cause a reversible reduction of the twitch. Flurazepam (IC_{50} 0.1 mM), diazepam (IC_{50} 0.17 mM) and midazolam (IC_{50} 0.19 mM), cause complete inhibition of the contraction at about 0.3 mM. Chlordiazepoxide (IC_{50} 1 mM) has the weakest effect, in the same range as found for lidocaine (IC_{50} 1 mM). The benzodiazepines diazepam (0.08 mM) and midazolam (0.16 mM) reduce the amplitude of the evoked compound action potential, within an exposure period of 10 minutes, to 38.6% and 72% of the control values respectively (n=3).

In conclusion, benzodiazepines can block propagation of action potentials in concentrations being equal to ten times less compared with the local anaesthetic lidocaine.

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88P THE EFFECTS OF ALDOSE REDUCTASE INHIBITION ON NERVE CONDUCTION ABNORMALITIES OF DIABETIC RATS

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Diabetic neuropathy is characterised by reduced nerve conduction velocity (NCV) and an increased resistance to ischaemic blockade of action potential propagation. Both phenomena may be observed in rats with experimental diabetes mellitus. In diabetic rats the reduced NCV is prevented by aldose reductase inhibitors (ARI) (Tomlinson *et al.*, 1982), which attenuate flux through the sorbitol pathway. The present study was designed to examine the effect of a new ARI on NCV and to determine whether its protective effect extends to prevention of the exaggerated resistance to ischaemic (or hypoxic) conduction block. Twenty male Wistar rats made diabetic with streptozotocin (50 mg/kg i.p.) formed 2 groups - untreated and ARI-treated [1.0 mg/kg/day by gavage of imirestat (HOE 843) - spiro-(2,7-difluorofluorene-9,4'-imidazolidine)-2'-5'dione]; 10 non-diabetic rats formed a control group. Treatments were maintained for 4 weeks. Motor NCV was then measured under halothane anaesthesia in the sciatic/tibialis system; 48-72 h later rats were killed by a blow to the head and both sciatic nerves removed. One sciatic nerve was assayed for sorbitol pathway metabolites by gas chromatography. The other was desheathed and prepared for recording compound action potentials *in vitro* (maintained in Krebs-Henseleit bicarbonate buffered saline containing 5.0 mM glucose and 0.5 mM myo-inositol). Preparations were gassed initially with 95% O_2/5% CO_2, followed by a 40 min period during which the O_2 content was reduced to 8% (balance N_2), to study the effect of this period of hypoxia on compound nerve action potential amplitude. Motor NCV (m/sec ± SEM) in diabetic rats (42.5 ± 1.4) was slowed compared to controls (51.0 ± 2.3; $p<0.05$ by one-way ANOVA). This slowing was absent in the ARI-treated group (51.1 ± 2.6). The decline in action potential amplitude (% of initial value ± SEM) during the 40 min period of hypoxia was greater in controls (60.3 ± 3.9) than in diabetics (70.8 ± 1.9; $p<0.05$). In the ARI-treated group the decline was similar to that seen in the untreated diabetic rats (72.6 ± 2.7). This implicates different mechanisms in the development of these two diabetes-associated abnormalities of nerve function.

This study was supported by Hoechst Aktiengesellschaft, Pharmaceutical Division.

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89P EVIDENCE FOR A NORADRENERGIC INNERVATION OF "ATYPICAL" β-ADRENOCEPTORS IN GUINEA-PIG ILEUM

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Indirect evidence suggests that $beta_1$-adrenoceptors are innervated (Grassby & Broadley, 1986) but it is not yet determined whether ileal "atypical" beta-adrenoceptors (putative $beta_3$-adrenoceptors; Bond & Clarke, 1988; Blue et al., 1990) also receive a postganglionic sympathetic innervation. In order to answer this question, experiments were undertaken using periarterial sympathetic nerve stimulation (PSNS) to isolated proximal segments of guinea-pig ileum set up *in vitro* (Kreb's solution at 37°C).

Tension development in the ileal segments was induced either by transmural electrical field stimulation (0.2 Hz; 1.5 ms pulse duration; supramaximal voltage) to evoke the cholinergic "twitch" response, or by the use of a submaximal concentration of histamine (3μM) to produce a steady-state contracture. PSNS was evoked at frequencies ranging from 1 to 20 Hz in 40s trains (1.5 ms pulse duration; supramaximal voltage), and relaxation of ileal tension was measured.

PSNS evoked a frequency-dependent inhibition of the "twitch" response which was blocked by guanethidine (10μM) and restored by dexamphetamine (10-20 μM), indicating typical noradrenergic transmission.

In preparations contracted with histamine, and pretreated with benextramine (10μM for 30 min followed by washout), PSNS-evoked responses were reduced in amplitude by beta-adrenoceptor antagonists (e.g. bromoacetylalprenololmenthane at 1μM, or a combination of ICI 118, 551 & CGP 20712A at 0.3 and 0.1μM respectively), but were not abolished. Remaining responses were blocked by the "atypical" beta-adrenoceptor antagonists alprenolol (3μM) and nadolol (300μM), as well as the agonist isoprenaline (10μM; to saturate the "atypical" beta-adrenoceptors). However, relaxations to papaverine (30μM) were unaffected. Parallel control studies indicated that the frequency-response curve to PSNS did not vary significantly with time.

The role of potential co-transmitters in responses to PSNS (10Hz) was also examined. ATP (100μM) exerted a rapid relaxant effect followed by complete desensitization. Responses to PSNS were not affected. Both neuropeptide Y and somatostatin failed to evoke responses, and did not appear to modify responses to PSNS . The results suggest that co-transmitters do not play any detectable role in relaxations produced by PSNS and demonstrate, for the first time, that "atypical" beta-adrenoceptors in the guinea-pig ileum receive a noradrenergic innervation.

This work was supported, in part, by NIH grant GM 39621.

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90P 13,14-DIHYDRO-PGE_1, A POTENT INHIBITOR OF HUMAN PLATELET AND NEUTROPHIL ACTIVATION

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PGE_1 is an arterial vasodilator and inhibits platelet and polymorphonuclear granulocyte (PMN) function. In the swine the transformation into three metabolites has been shown (Änggard and Larsson, 1971) which have varying biological activities (Westwick, 1976). This study compares the actions of PGE_1 with those of 13,14-dihydro-PGE_1 (H_2-E_1), 15-keto-PGE_1 (K-E_1) and 15-keto-13,14-dihydro-PGE_1 (KH_2-E_1) in human platelets, human PMN and different rabbit and bovine arterial preparations.

PGE_1 and H_2-E_1 showed a comparable activity in inhibiting platelet function in collagen (2.5 μg/ml)-stimulated platelet-rich plasma. Inhibition of platelet function was accompanied by a dose-dependent reduction in thromboxane (TX)B_2 generation (n=5-6). The following IC_{50}-values [nM] for PGE_1/H_2-E_1 for the different parameters were evaluated: aggregation (22 / 35), ATP-secretion (12 / 25) and TXB_2 release (54 / 94). Both substances increased platelet cAMP levels 5-6 fold at 1 μM. Similar results were obtained in ADP (2.0 μg/ml)-stimulated platelets. K-E_1 and KH_2-E_1 were much less active (IC_{50} for collagen- and ADP-induced aggregation ≥ 10 μM).

In PMN, PAF (3 μM)-induced O_2^--generation and β-glucuronidase release were dose-dependently inhibited by PGE_1 and H_2-E_1, the IC_{50} for both effects being about 1 μM and 10 μM, respectively. Similiar results were obtained with FMLP (30 nM)-stimulated PMN. The inhibitory effects of both substances were also accompanied by a 6-7 fold increase in cAMP levels at 1 μM (n=4). K-E_1 and KH_2-E_1 inhibited O_2^--generation and β-glucuronidase release by about 30% at 10 μM.

In the rabbit the vascular effects of PGE_1 and its metabolites (10 μM) were dependent on the vascular region studied. Relaxations were expressed in % of precontraction ($PGF_{2\alpha}$, 10 μM or U 46.619, 1 μM). PGE_1 and H_2-E_1 had no effect on thoracic aorta, a mild relaxing effect on carotid artery (< 20 %) and caused an almost complete relaxation of mesenteric and iliac arteries (n=6-7). Interestingly the concentration-response curve for the relaxation of iliac artery for H_2-E_1 was shifted to the right indicating significantly lower potency than PGE_1 (n=6; $p < 0.05$). K-E_1 had no significant effect on any of the precontracted vessels tested. KH_2-E_1 was much less effective on iliac artery but showed a potent relaxing effect on the mesenteric artery. In 5-HT (1 μM)-precontracted bovine coronary arteries PGE_1 showed a potent relaxing effect (61±4 %, n=12). None of the metabolites had any significant effect (n=6).

These data demonstrate inhibition of human platelet and PMN function by H_2-E_1 at concentrations similar to those of PGE_1. The vasomotor activities of H_2-E_1 and PGE_1 vary considerably in different arterial preparations.

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91P EFFECTS OF PH CL28A IN TWO MODELS OF ACUTE INFLAMMATION INDUCED BY CARRAGEENAN IN RATS

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The azobenzene compound Ph CL28A is a derivative of sulphasalazine and like that drug it inhibits prostaglandin dehydrogenase (PGDH) and catabolism of PGE_2 and $PGF_{2\alpha}$ (Berry et al 1985; Bakhle & Pankhania 1987). However, Ph CL28A also potentiated PGI_2 output and inhibited leukotriene production in rat (Berry et al 1988; Pankhania & Bakhle 1990). Because these latter two properties could have anti-inflammatory effects, we assessed the response of two models of acute inflammation to Ph CL28A and to indomethacin treatment in vivo.

In the first model, paw oedema induced by carrageenan (1mg/paw) in rats was measured over 4h. Treatment with Ph CL28A (30mg/kg; i.p.) at the same time as carrageenan showed significant inhibition (55±10%; $n=5$; $p<0.05$) of the oedema 2h later. Inhibition of paw oedema by Ph CL28A over the whole 4h period, was dose dependent (10, 30 and 100mg/kg). Indomethacin (2mg/kg) given similarly, was equivalent to between 10mg/kg and 30mg/kg Ph CL28A. Lower amounts of Ph CL28A (2.5mg) and indomethacin (0.13 mg) were injected into the paw with the carrageenan. With this local treatment, Ph CL28A always potentiated, whereas indomethacin inhibited the oedema. As Ph CL28A is not a cyclo-oxygenase inhibitor (Berry et al 1985), its efficacy in the paw oedema model is most likely to be due to its potentiation of PGI_2 output (Rampart & Williams 1986; Bakhle & Pankhania 1987). The second model used carrageenan injection into the pleural cavity of rats; following this, intrapleural leucocyte numbers, TxB_2 and $6oxoPGF_{1\alpha}$ were increased for up to 3h. Treatment with Ph CL28A (30mg/kg.i.p.) decreased leucocyte accumulation by 50%, but did not change $6oxoPGF_{1\alpha}$ or TxB_2. Indomethacin (6.6mg/kg.ip) decreased both TxB_2 and $6oxoPGF_{1\alpha}$ but did not affect leucocyte accumulation. In this model, inhibition of LT synthesis is probably the more important feature of Ph CL28A activity.

We conclude that Ph CL28A did exhibit anti-inflammatory effects in two different models of acute inflammation and that these effects are compatible with its known actions on the cyclo-oxygenase and lipoxygenase pathways.

We thank Mr H Agback (Pharmacia) for a generous gift of Ph CL28A.

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92P CICAPROST POTENTIATES PLASMA EXUDATION INDUCED BY EP_3-SELECTIVE PGE ANALOGUES, IN THE ABSENCE OF OTHER INFLAMMATORY MEDIATORS

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We have recently demonstrated that potentiation of bradykinin (BK)-induced plasma exudation by PGE_2 in the skin of anaesthetised rabbits (measured by leakage of ^{125}I-albumin) may have two components (Armstrong *et al.*, in press). The high potency (1-10 ng/site) component may be mediated by an EP_3 receptor and appears to be unrelated to dilatation, yet accounts for 50% of the maximum potentiation achieved with PGE_2 (1000 ng). It is possible that BK-induced dilatation potentiates an EP_3 receptor-mediated, leukocyte-dependant mechanism, as shown for LTB_4 (Bray *et al*,1981). The effect of the potent vasodilator cicaprost (a PGI_2 analogue) was therefore examined on two leukocyte-dependant mediators of inflammation FMLP and LTB_4 (Wedmore & Williams, 1981), the EP_3 selective agonists, MB 28767 and sulprostone, and PGE_2 itself. MB 28767 has marked thromboxane (TP) agonist activity and was assessed after TP receptor block by GR 32191 (2 mg/kg i.v.).

Table 1 Potentiation of plasma exudation (µl of exudate) by cicaprost (0-1000 ng) in rabbit skin (n = 4, ± s.e.mean)

Intradermal injection of cicaprost with	Control rabbits 0	10 ng	1000 ng	Nitrogen mustard-treated rabbits 0	10 ng	1000 ng
FMLP (20 ng)	10.8 ± 0.8	16.7 ± 1.4	25.1 ± 2.3	10.9 ± 1.0	11.5 ± 0.5	13.6 ± 0.7
LTB_4 (10 ng)	10.7 ± 0.8	14.4 ± 0.7	20.6 ± 2.0	-	-	-
MB 28767 (1 ng)	9.9 ± 0.8	12.7 ± 1.3	20.2 ± 2.1	12.3 ± 1.2	10.4 ± 0.7	12.1 ± 0.6
Sulprostone (10 ng)	11.5 ± 0.8	14.1 ± 1.4	19.7 ± 1.4	11.5 ± 1.0	12.6 ± 1.0	14.9 ± 0.9
PGE_2 (1 ng)	13.4 ± 0.8	13.8 ± 0.8	18.7 ± 2.0	9.1 ± 0.9	9.3 ± 0.6	9.5 ± 0.6

Cicaprost (10-1000 ng) potentiated plasma exudation by the EP_3 agonists in the absence of any other inflammatory mediator (Table 1). In contrast the cicaprost (1000 ng) did not potentiate either butaprost (100 ng, 11.8 ± 1.2; 13.7 ± 1.8 µl) or 11-deoxy PGE_1 (100ng, 9.5 ± 1.0; 10.1 ± 1.1); BK-induced exudation is potentiated by this dose of the two EP_2 analogues. After pre-treatment of the rabbits with nitrogen mustard (1.75 mg/kg i.v. 4 days prior to experiment) to induce leukopeonia (Humphrey, 1955), the ability of cicaprost to potentiate plasma exudation by FMLP and the EP_3 agonists was markedly reduced. In contrast, BK-induced exudation was not impaired (23.9 ± 4.4 increased to 53.0 ± 8.1). This suggests that PGE_2 may exert part of its pro-inflammatory action via an EP_3 receptor-mediated, leukocyte-dependant mechanism.

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93P ANTISERUM TO LIPOCORTIN 1 REVERSES THE ACUTE ANTI-INFLAMMATORY ACTIONS OF DEXAMETHASONE IN THE RAT

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Lipocortin 1 (LC1, annexin 1) has been proposed to mediate some of the anti-inflammatory actions of corticosteroids (see Flower, 1988). Recombinant LC1 mimics some of their effects (Cirino et al, 1989) and the protein is induced *in vivo* by corticosteroids (Goulding et al, 1990). We report that a polyclonal antiserum to LC1 residues 1 - 188 (Carey et al, 1990) reverses the acute effect of dexamethasone in the rat carrageenin paw oedema model.

Carrageenin (2%, 0.1ml) was injected into the hind paw of male Wistar rats (150g) and the resulting oedema measured hourly using a hydroplethysmometer. Saline (0.05ml) containing dexamethasone phosphate (Dex) and serum as appropriate was preinjected 15 min prior to carrageenin. Oedema reached a maximum (0.94 ± 0.04ml, n=27) at 4h. Preinjection of 1μg Dex significantly inhibited the oedema ($P<0.001$) at all time points. For the first 2h, both control and LC1 antiserum (αLC) appeared to enhance the response, probably a result of mast cell degranulation: serum alone caused an acute oedema prevented by compound 48/80 pretreatment (data not shown). By 3h, αLC significantly reversed the effect of Dex; this was maintained up to 5h (Table 1).

Table 1: Reversal of dexamethasone inhibition by antisera

	% Control oedema, mean ± s.e.m. (n rats)				
	Dex (26)	+10μlαLC(9)	+20μlαLC(10)	+40μlαLC(10)	+40μlCon (5)
3h	41.3 ± 5.0	32.5 ± 6.3	83.8 ± 11.3[b]	75.0 ± 5.0[b]	63.1 ± 8.3
4h	41.5 ± 4.3	36.2 ± 4.3	74.5 ± 13.8[b]	81.9 ± 8.5[b]	48.6 ± 5.7
5h	45.7 ± 5.3	37.2 ± 4.3	78.7 ± 12.8[a]	86.2 ± 10.6[b]	56.1 ± 6.5

a $P<0.01$, b $P<0.001$ vs Dex, Student's t test.

In conclusion we demonstrate that antiserum to lipocortin 1 can reverse the acute anti-inflammatory action of dexamethasone *in vivo*. This suggests a physiological role for lipocortin 1 in the anti-inflammatory actions of corticosteroids.

RJF is supported by an endowment from Lilly.

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94P TOPICAL APPLICATION OF BETAMETHASONE-17-VALERATE CREAM INHIBITS OEDEMA FORMATION IN RAT SKIN

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The anti-inflammatory effects of systemic corticosteroids have been well documented in a variety of animal models, but little attention has been devoted to the action of topical steroids, despite their extensive use in the treatment of many inflammatory skin disorders. For this reason we have devised a model to investigate the comparative anti-inflammatory activity of both systemically and topically applied steroids.

For systemic administration, male Wistar rats (250g-350g) were given betamethasone sodium phosphate (0.1 or 1.0mg/kg) or saline s.c., either 1 or 3h prior to the experiment. For topical administration the rats were anaesthetised using a cocktail of 1 part Hypnorm and 1 part Hpnovel in 2 parts sterile water, the backs shaved and 2g 0.1% w/w betamethasone-17-valerate (Betnovate), or vehicle cream rubbed into the skin for 2-3min. Both topical treatments were compared to untreated controls. After an 18h interval a skin test was performed comprising of intradermal injections of 0.1ml: 5-HT (2.5×10^{-8}mol/site), PAF (5×10^{-8}mol/site) or 50% zymosan activated serum (ZAS), in the presence or absence of PGE_1 (3×10^{-10}mol/site). Oedema was expressed as a measure of [I^{125}]-labelled albumin extravasation as described by Williams, 1979.

In agreement with similar experiments, using systemic dexamethasone in the rabbit skin (Peers & Flower, 1988), betamethasone given s.c. caused a dose and time-dependent inhibition of oedema induced by all inflammatory stimuli tested. Pretreatment with betamethasone, 1mg/kg, for 3h produced a greater inhibition (range 77±10 - 47±11%) than either betamethasone, 1mg/kg, 1h pretreatment (43±10 - 0% inhibition) or, 0.1mg/kg, 3h pretreatment (34±19 - 0% inhibition). Topical betamethasone-17-valerate also inhibited the inflammatory responses induced by all stimuli (table 1).

Table 1: Effect of Topical Betamethasone-17-Valerate Cream Upon Oedema Formation

Oedema (ul) [mean ± s.e.mean (n= 6 - 9 rats per group)] *minimum value of $P < 0.01$

Agonist	PGE_1	5HT	5HT + PGE_1	PAF	PAF + PGE_1	ZAS	ZAS + PGE_1	Saline
Control	16.7±4.3	84.3±9.4	47.9±5.7	33.7±5.7	90.8±10.1	42.6±4.3	47.6±2.3	6.9±2.1
Steroid	7.9±1.8*	46.3±6.7*	32.1±7.8*	20.8±2.6*	51.7± 9.3*	25.8±3.8*	28.9±4.3*	3.4±0.9*
Inhibition %	58	45	33	38	43	39	39	51

The vehicle alone was without any net effect (3 experiments, 9 animals) except in the case of the oedema caused by PAF + PGE_1 when a mean inhibition of 23%, which reached significance at the 1% level, was achieved.

This broad spectrum of inhibition would suggest that the topical steroid is acting by affecting a fundamental feature of the inflammatory response common to all of the tested stimuli, perhaps at the endothelial cell level. Also the rat skin provides a useful model to investigate the anti-inflammatory effects and the mode of action of steroids given both topically and systemically.

AA is supported by British Pharmacological Society. We wish to thank Glaxo Group Research for kind gifts of Betnovate vehicle.
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95P SALMETEROL, BUT NOT SALBUTAMOL, HAS ANTI-INFLAMMATORY ACTIVITY IN GUINEA-PIG SKIN

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We have previously shown that the β_2-adrenoceptor agonists, salmeterol and salbutamol inhibit plasma protein extravasation in guinea-pig lung (Whelan and Johnson, 1990). The present report compares the inhibitory actions of salmeterol with those of salbutamol, on zymosan-induced polymorphonuclear (PMN)-cell accumulation and plasma protein extravasation (PPE) in guinea-pig skin, using the method previously described by Whelan (1989).

Intradermal zymosan (0.2mg.site^{-1}) caused both cell accumulation and PPE, measured 4h after injection. Salmeterol 10mg.kg^{-1} p.o. (30 min before zymosan), significantly ($p<0.05$) decreased PMN cell accumulation from a mean ± s.e.m. of 460.0 ± 82.0 (n=8) x10^3 to 190.0 ± 35.0 (n=8) x10^3 cells.site^{-1}, and PPE from a mean of 12.7 ± 2.8 (n=6) to 5.8 ± 1.3 (n=6) µl plasma.site^{-1}, reductions of 59 and 56% respectively. Lower doses of salmeterol (1.0 and 0.1mg.kg^{-1} p.o.) significantly reduced PPE but not PMN cell accumulation. In contrast, salbutamol (10 and 100mg.kg^{-1} p.o.) had no significant inhibitory effect on zymosan (0.2mg)-induced PMN cell accumulation or PPE.

Similarly intradermal administration of salmeterol (10^{-8}mol.site^{-1}) significantly ($p<0.05$) reduced PMN cell accumulation from 79.6 ± 13.3 (n=6) x10^3 to 39.0 ± 10.9 (n=6) x10^3 cells.site^{-1} and PPE from 15.2 ± 4.1 (n=6) to 7.3 ± 1.82 (n=6) µl plasma.site^{-1}. Intradermal injection of lower doses of salmeterol (10^{-9}mol.site^{-1}) had no significant effect on PMN cell accumulation or PPE. These inhibitory effects of salmeterol were blocked by prior administration of propranolol (1mg.kg^{-1}, s.c.). Intradermal injection of salbutamol (10^{-9}-10^{-7}mol.site^{-1}) had no significant effect on zymosan-induced PMN cell accumulation or PPE.

This study shows that salmeterol inhibits zymosan-induced inflammation in guinea-pig skin, by a mechanism involving β-adrenoceptors. The finding that salbutamol does not share this action suggests that such anti-inflammatory activity is a property of this long-acting, β-adrenoceptor agonist.

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96P CHARACTERISATION OF INFLAMMATORY RESPONSES INDUCED BY LIVE *E. COLI* IN RABBIT SKIN

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Neutrophil accumulation during the acute inflammatory response is an important defence against infection or injury. The suppression of the acute inflammatory response to microbes in skin increases the virulence of the local infection (Burke & Miles, 1958) while the local extravascular generation of prostaglandins and C5a is important in responses to microbial stimuli such as Bordetella pertussis organisms. Further, oedema responses to these stimuli were shown to be neutrophil-dependent (Wedmore & Williams, 1981). We have investigated the mechanisms involved in neutrophil accumulation and oedema formation induced by intradermally (i.d.) administered live Escherichia coli (E.coli) in the rabbit skin. Rabbit neutrophils were isolated from whole blood and labelled with ^{111}In. Neutrophil accumulation and oedema formation in the skin were simultaneously measured by local accumulation of intravenously injected ^{111}In-neutrophils and ^{125}I-albumin respectively (Nourshargh et al, 1989).

I.d. injections of live E.coli induced slow neutrophil accumulation and oedema formation over the 4hr in vivo test period. These responses were dependent on the number of organisms injected. Heat-killed bacteria induced greatly reduced responses. Bradykinin (10^{-10}moles/site) significantly ($p<0.01$,2xANOVA) potentiated the oedema seen in response to E.coli, this effect was maximal in 2hr old lesions. I.d. indomethacin (10^{-8}moles/site) inhibited E.coli induced oedema by 47.4±9.1% (n=6 rabbits). This response was unaffected by the selective PAF antagonist WEB 2086 or the formyl peptide receptor antagonist N-t-Boc-phe-leu-phe-leu-phe. In addition the leukotriene B_4 antagonist LY 255283 (10^{-7}moles/site), and an IL1 receptor antagonist (1.7x10^{-9}moles/site) (Eisenberg et al, 1990), which were both shown to be selective in our in vivo model, had no affect on oedema and neutrophil responses to E.coli. Local inhibition of protein biosynthesis with actinomycin D (ACD) (5x10^{-9}moles/site) inhibited E.coli induced neutrophil accumulation by 35.5±3.4% (n=7 rabbits, $p<0.01$) without affecting oedema responses. This concentration of ACD had no affect on the bacterial viability when tested in vitro. I.d. MoAb 60.3, an anti CD18 monoclonal antibody enhanced oedema responses to E.coli significantly in 4 out of 8 rabbits ($p<0.05$) and neutrophil accumulation in 3 out of 7 rabbits ($p<0.05$).

These results indicate that oedema formation to live E.coli involves the generation of vasodilator mediators, probably cyclooxygenase products in a modulatory role. Responses to live E.coli in vivo are very complex as both the bacteria and the host inflammatory cells can release mediators which act either directly on the neutrophil or on endothelial cells to cause leukocyte migration. Using specific antagonists, our results suggest that mediators other than LTB_4, FMLP, PAF or IL1 may be involved in the responses measured. Neutrophil infiltration, in response to E.coli is partially dependent on protein synthesis in the skin and/or in the bacteria, however mediators generated remain to be elucidated.

We thank Ciba Geigy Ltd, Basel, Switzerland for supporting this study, Synergen, USA for IL-1 receptor antagonist, Lilly, USA for LY 255283 and Boehringer Ingelheim, UK for WEB 2086.

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97P CLASSIFICATION OF THE 5-HT RECEPTOR MEDIATING CARDIO-EXCITATION IN THE SNAIL, *HELIX ASPERSA*

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5-Hydroxytryptamine (5-HT) has both intropic and chronotropic effects on molluscan hearts (Leake & Walker, 1980). A previous study has shown that the 5-HT receptor on the Helix heart recognised compounds that have been shown to interact with the 5-HT receptors in the vertebrate nervous system (Boyd et al., 1985). However, the receptor could not be clearly separated into either the 5-HT_1 or 5-HT_2 type. The aim of this study was to attempt to classify this excitatory 5-HT receptor, using more recent drug tools, into one of the existing categories of 5-HT receptor (Bradley et al., 1986).

Isolated whole snail hearts were mounted in organ baths, in physiological saline (Meech & Thomas, 1977) at room temperature, for isometric tension recording. Cumulative concentration-effect curves to the inotropic effect of 5-HT and test agonists were then constructed until a maximum effect was reached. Where the effects of antagonists were examined, these were added to the bath 30 minutes before the agonist. Experiments were controlled so as to monitor changes in tissue sensitivity and for the effects of drug vehicle were appropriate. Agonist EC_{50} values (concentration required to produce 50% of the maximum response) were determined for comparison with 5-HT values and pEC_{50} values (negative $\log_{10}$ of the EC_{50} values) calculated. Results are expressed as the mean ± s.e.m. of at least six experiments.

5-HT was the most potent agonist (pEC_{50} value of 5.7 ± 0.1). 5-Carboxamidotryptamine (5-CT) and α-methyl-5-hydroxytryptamine (α-Me-5-HT) both mimicked the effects of 5-HT but were less potent (pEC_{50} values of 4.9 ± 0.1 and 4.7 ± 0.1 respectively). Sumatriptan, 2-methyl-5-hydroxytryptamine (2-Me-5-HT), tryptamine and 8-hydroxy-dipropyl-aminotetralin (8-OH-DPAT) behaved like partial agonists, only reaching maximum response of between 45-65% as compared to 5-HT at 10^{-4}M. Methysergide, methyl ergometrine and ergotamine also behaved like partial agonists. The full rank order of potency for the agonists tested was 5-HT >methyl ergometrine = ergotamine = 5-CT > α-Me-5-HT = sumatriptan > methysergide = 2-Me-5-HT = tryptamine ≥ 8-OH-DPAT, but the potency range extended to only 40 fold weaker than 5-HT.

The cardio-excitatory actions of 5-HT were resistant to antagonism by ketanserin (10^{-6}M), methiothepin (10^{-6}M), ondansetron (10^{-6}), metoclopramide (10^{-6}) and ICS 205-930 (10^{-6}M and 10^{-4}M). No specific antagonist to the cardio-excitatory effect of 5-HT was found. The results suggest that the 5-HT receptor mediating cardio-excitation in the common snail heart is unique and does not fall into any of the categories yet described for vertebrate 5-HT receptors.

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98P PUTATIVE 5-HT_4-LIKE RECEPTORS IN PIGLET LEFT ATRIUM

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5-Hydroxytryptamine (5-HT) and benzamides, including renzapride, increase porcine heart rate. The tachycardia is blocked by 3α-tropanyl-1H-3-carboxylic acid (ICS205930) (Villalon et al. 1990) with a pK_B (M) of 6.9 on piglet right atria (Kaumann 1990)). The characteristics of porcine sinoatrial 5-HT receptors are similar but not identical to those of so called 5-HT_4 receptors coupled positively to adenylyl cyclase in rodent brain (Dumuis et al. 1989; Bockaert et al. 1990). Kaumann (1990) suggested that piglet sinoatrial 5-HT receptors strongly resemble human right atrial 5 - HT receptors (Kaumann et al. 1990 a), designated 5-HT_4-like receptors. To explore the existence of 5-HT_4-like receptors in another heart region we chose the left atrium, obtained from new-born piglets (2-9 days old) anaesthetised with halothane. Two to three left atrial strips were dissected and set up in modified Krebs solution at 37°C as described (Kaumann 1972) and paced to contract at 1 Hz. The solution contained (μM) (±)-propranolol 0.4, cocaine 6 and ascorbate 200. 5-HT caused positive inotropic effects with a pEC_{50} (M) of 6.8 ± 0.1 (x ± sem, n=11 piglets); the maximum effect of 5-HT was 27 ± 4% of the effect of 200 μM (-)-isoprenaline administered to terminate the experiment. ICS205930 0.3-10 μM caused surmountable competitive antagonism with a slope = 1.02 of a Schild plot and a pK_B = 6.7 ± 0.1 (M) (n=11 piglets). Renzapride 10 μM increased atrial force by 42 ± 10% of the maximum increase caused by 5-HT (n=4 piglets). Left atrial strips from 9 piglets were exposed 30 seconds to 30 μM 5-HT or 200 μM (-)-isoprenaline, freeze-clamped and cyclic AMP assayed as described (Kaumann et al. 1990 a). The cyclic AMP content was increased from 9.8 ± 1.0 pmol mg^{-1}(n=12 piglets) protein in untreated atria to 12.2 ± 1.9 pmol mg^{-1} (n=8 piglets) ($p<0.05$ vs control by paired t-test) by 5-HT and to 12.6 ± 1.5 pmol mg^{-1} (n=8 piglets) ($p<0.01$ vs control by paired t-test) by (-)- isoprenaline. The effects of 5-HT, renzapride and ICS205930 observed in piglet left atrium resemble those of human right atrium (Kaumann et al. 1990 a and 1990 b), consistent with the existence of 5-HT_4-like receptors.

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99P HAEMODYNAMIC CONSEQUENCES OF INTERFERENCE WITH THE RENIN-ANGIOTENSIN SYSTEM FOLLOWING MYOCARDIAL INFARCTION IN RATS

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Captopril, when given during 2 weeks after completion of the healing phase of myocardial infarction (MI), restores cardiac function in infarcted rats. The present study was designed to further characterize the effects of interventions in the renin-angiotensin system following MI.

Rats (under sodium pentobarbitone anaesthesia, 60 mg kg^{-1}, i.p.) were subjected to sham-surgery or to ligation of the left anterior descending coronary artery to induce MI. Following recovery for 3 weeks, MI-rats were assigned to treatment groups receiving saline, captopril (0.10 and 0.50 mg kg^{-1} h^{-1}), the non-sulfhydryl ACE-inhibitor benazepril, (0.02, 0.1 and 0.5 mg kg^{-1} h^{-1}), or the angiotensin II-b (AII-b) receptor antagonist, DuP 753, (15 mg kg^{-1} day^{-1}). All drugs were administered continuously from 3-5 weeks after MI using Alzet osmotic minipumps and all treatments resulted in inhibition of the renin-angiotensin system as assessed by the degree of rightward shift of the AI or AII pressor dose-response curves. At 4 weeks after MI an electromagnetic flow-probe was implanted on the ascending aorta to measure cardiac output (CO), and catheters were implanted in the abdominal aorta and thoracic vena cava to measure arterial (MAP) and central venous pressure (CVP) and in the abdominal vena cava for infusions (all surgery under anaesthesia as above). At 5 weeks after MI haemodynamics were measured in conscious animals at rest and following a maximal volume loading with 12 ml warm (37°C) Ringer's solution infused over 1 min. Results were subjected to analysis of variance.

Groups of MI-rats were comparable with respect to body weight (290-330 g) and infarct size (37-43% of the left ventricle). MI reduced both resting CO (sham 94±4 ml min^{-1}; MI 78±5 ml min^{-1}; mean ± s.e. mean; n=10 and n=12, respectively) and stimulated CO (CO_{vl}=118±4 and 107±5 ml min^{-1}, respectively). All 3 treatments resulted in reduction of MAP. Captopril (0.5 mg kg^{-1} h^{-1}; n=7) restored resting CO (97±6 ml min^{-1}) and CO_{vl} (138±10 ml min^{-1}). In contrast , neither benazepril nor DuP 753 affected resting or stimulated haemodynamics (benazepril 0.5 mg kg^{-1} h^{-1}: CO=76±10, CO_{vl}=104±18 ml min^{-1}, n=8; DuP 753: CO=78±5, CO_{vl}=118±9 ml min^{-1}, n=7).

In conclusion, following MI, the improvement of cardiac function with captopril is not mimicked by another ACE-inhibitor (benazepril), nor by an AII-b receptor antagonist. Mechanisms independent of diminished AII-receptor stimulation have to be considered.

100P ACTIONS OF PROPOFOL AND ENFLURANE ON SINGLE CALCIUM CHANNEL CURRENTS IN GUINEA-PIG VENTRICULAR MYOCYTES

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It is known that the harmful effects of certain anaesthetics on the heart arise at least in part from a suppression of 'L'-type calcium currents in ventricular muscle (e.g. Terrar & Victory, 1988; Puttick & Terrar, 1989). Effects of the anaesthetics propofol and enflurane were further investigated at the level of single Ca channels by using cell-attached patch-clamp recording to monitor channel activity.

Single cells were isolated from guinea-pig ventricular muscle and bathed in a high potassium (20 mM) solution to maintain resting potential at approximately -40 mV (Shibasaki, 1987). The pipette solution contained 110 mM $BaCl_2$ and 1 μM Bay K 8644 to increase the mean open lifetime and thus facilitate recording of single channel activity (e.g. Hess et al, 1986). Temperature was 34°C. Records were filtered at 300 Hz, digitized and analysed using the PAT software kindly provided by J. Dempster, University of Strathclyde. Calcium channels were identified by their characteristic slope conductance of 31 pS (ten cells), and were blocked by 2 μM nisoldipine.

When propofol (100 μM) was applied in the solution bathing the cells the proportion of time spent in the open state was reduced from 25.4±5.0% to 10.0±4.1% and the proportion of time spent in the closed state was increased from 69.7±6.7% to 83.1±1.1% while no change was detected in the open channel conductance (values derived from Gaussian curves fit to amplitude distributions for n=6 cells before and after propofol). In the case of enflurane (3%, assayed at 1.45 mM), comparable figures were: proportion of the time spent in the open state decreased from 22.1±5.0 to 7.3±4.0%; proportion of time spent in the closed state increased from 69.7±6.7% to 81.9±2.6%; again no change detected in the open channel conductance (n=4 cells). When transitions across a 50% threshold were detected, both anaesthetics decreased the mean open time (and decreased the time constant of a single exponential fitted to the open times), increased mean closed time (and increased the time constant of a single exponential fitted to the closed times), while the open channel probability was decreased (from 0.29+0.07 to 0.13+0.04% in the case of propofol, n=6, and from 0.24+0.05 to 0.09+0.04% in the case of enflurane, n=4).

The observations are consistent with the conclusion that both propofol and enflurane applied in the bathing solution can gain access to the region of Ca channels beneath a cell-attached patch electrode to decrease the probability of channel opening without altering channel conductance. Since the frequency response used was limited it is possible that fast components of channel opening and closing may have been missed, but it nevertheless appears that kinetics of opening and closing were altered by the anaesthetics to favour the closed state.

RMP is in receipt of an MRC Scholarship.

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101P INVESTIGATION OF RAT MYOCARDIAL PERIPHERAL TYPE BENZODIAZEPINE RECEPTORS

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Radioligand binding studies in sections of rat myocardium were performed using [^{3}H]PK 11195 [(1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline-carboxamide)] which binds specifically to peripheral type benzodiazepine receptors (Benavides et al., 1983). Three male Sprague-Dawley (250-350 g) rats were killed by stunning and cervical dislocation. Hearts were excised rapidly, rinsed in ice cold heparinized Krebs-Henseleit buffer and were then immediately frozen in isopentane (-35°C). Unfixed 10 μm cryostat sections from both right and left ventricular myocardium mounted on gelatine coated slides were incubated for 30 min at 25°C in a series of 12 solutions (n = 6 sections per solution) which contained 1 nM [^{3}H]PK 11195 and 0-20 nM unlabelled drug. All solutions were made up using 170 mM Tris-HCl buffer at pH 7.4. Radioligand binding was terminated by three 5-min washes in Tris-HCl buffer at 4°C. Sections were scraped off the slides and prepared for scintillation counting. Two sets of sections were obtained from each heart. Non-linear regression analysis of the bound and free ligand concentrations was used to calculate the receptor density (B_{max}), ligand affinity (K_d) and the degree of non-specific binding. Protein content was estimated to be 0.1 mg/section. Values of B_{max} and K_d were 3.10 $\pm$ 0.46 pmol/mg protein and 2.70 $\pm$ 0.47 nM, respectively. Our value of B_{max} is lower than those obtained previously using the refined membrane technique (Doble et al., 1985), but the values of K_d are almost identical. Radioligand binding studies using refined membrane preparations have the disadvantage of losing the majority of the membrane protein content during the refinement process (Wolff et al., 1989) and may account for the disparity between our results and those published previously. Estimates of non-specific binding from the fit were not significantly different from zero and the measured non-specific binding was less than 10% of maximal binding. This protocol may be suitable for quantitative autoradiographic studies of myocardial peripheral type benzodiazepine receptors using [^{3}H]PK 11195 which will enable spatial localisation as well as quantitation of these binding sites.

Supported by the Medical Research Council.

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102P CHRONIC β_1-BLOCKER TREATMENT DIFFERENTIALLY REGULATES β-ADRENOCEPTORS AND MUSCARINIC RECEPTORS, BUT DOES NOT AFFECT THE AMOUNT OF G_i-PROTEIN IN THE HUMAN RIGHT ATRIUM

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Chronic treatment of patients with coronary artery disease with β_1-adrenoceptor (AR) antagonists causes a subtype-selective increase in right atrial β_1-AR density without affecting β_2-AR (Michel et al., 1988), and simultaneously a decrease in right atrial muscarinic receptor (M_2-R) density and function (Motomura et al.,1990). Contractile responses of isolated electrically driven right atria to the rather β_1-AR selective agonist noradrenaline, however, were unchanged, while β_2-AR mediated positive inotropic effects caused by adrenaline (Hall et al., 1990) or procaterol (Motomura et al., 1990) were enhanced. To study possible mechanisms underlying this differential regulation of β_1-AR density, β_2-AR function and M_2-R density and function we assessed in 34 patients undergoing coronary artery bypass grafting whether chronic β_1-AR antagonist treatment might affect right atrial adenylate cyclase responses to stimulation with β_1- or β_2-AR selective agonists and/or the amount of right atrial G_i-protein.

Right atrial appendages were obtained from 34 patients undergoing coronary artery bypass grafting (NYHA class I-II). Sixteen of these patients were chronically treated with the β_1-AR antagonists metoprolol,bisoprolol or atenolol. Atrial β-AR density, the relative amount of atrial β_1- and β_2-AR, activation of atrial adenylate cyclase by noradrenaline and procaterol as well as atrial M_2R density were determined as recently described (Motomura et al., 1990).The amount of atrial G_i-protein was assessed by pertussis-toxin catalyzed ^{32}P-ADP-ribosylation.

In agreement with our recently published data, in the β_1-AR antagonist treated patients the density of right atrial β_1-AR, but not β_2-AR was significantly higher than in non-treated patients, while M_2-R density was decreased by about 25%. In atrial membranes from β_1-AR antagonist treated patients adenylate cyclase activation by the β_2-AR selective agonist procaterol was significantly enhanced, while that evoked by noradrenaline was not. Concomitantly, on isolated electrically driven right atria the positive inotropic effect of adrenaline, but not of noradrenaline, was significantly increased, thus confirming the data published by Hall et al. (1990). Pertussis-toxin catalyzed ^{32}P-ADP-ribosylation of atrial G_i-protein, however, was in both patient groups nearly identical.

We conclude that β_1-AR antagonist treatment causes transregulation of β-AR and M_2-R in the human right atrium. Possible changes in right atrial G_i-protein function, however, cannot be detected by pertussis-toxin catalyzed ADP-ribosylation.

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103P REDUCTION OF CARDIAC OUTWARD CURRENTS BY α-ADRENOCEPTOR STIMULATION: A SUBTYPE-SPECIFIC EFFECT?

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Functional and radioligand binding data suggest that α_1-adrenoceptors can be divided into α_{1A}- and α_{1B}-subtypes (Minnemann, 1988). In rat heart, some 23 % of the α_1-receptors are classified as α_{1A}-subtypes, the remaining are considered α_{1B}-subtypes (Gross et al., 1988). We have shown previously, that α-adrenoceptor stimulation with noradrenaline reduces outward currents in isolated cardiac myocytes (Ravens et al., 1989). Information as to which subtype is involved in producing this effect is still lacking. We have addressed this problem by studying the influence of the α_{1A}-selective antagonist 5-methyl-urapidil and of the irreversible α_{1B}-selective antagonist chloroethylclonidine on the changes in membrane currents produced by α-adrenoceptor stimulation. Single myocytes were isolated from the hearts of adult rats by enzymatic dispersion. For single-electrode voltage clamp studies, the myocytes were placed in a chamber (0.5 ml) on the stage of an inverted microscope. The chamber was superfused with oxygenated buffer at room temperature (20 - 22°C). Beta-adrenoceptors were blocked by propranolol (1 μM), phenylephrine (30 μM) was used as an α_1-agonist. Outward currents were measured in response to 250 ms long clamp steps to 0 mV from a holding potential of -40 mV in order to inactivate sodium currents. Calcium currents were blocked by addition of 0.1 mM Cd^{2+} to the superfusion medium. Peak transient outward current (I_p) and late outward current at the end of the clamp pulse (I_l) were analyzed. In time-matched controls, the currents decreased spontaneously to 91.3 ± 1.1 % and 92.8 ± 2.3 % of the value 5 min after Cd^{2+} exposure (mean ± S.E.M., n = 5) for I_p and I_l, respectively (clamp steps to +20 mV). Alpha-adrenoceptor stimulation with phenylephrine reduced I_p as well as I_l to a similar extent as reported recently for noradrenaline (Ravens et al., 1989), i.e. to 74.7 ± 1.8 % and 60.9 ± 3.5 % (n = 5) of control, respectively (clamp steps to +20 mV). After pretreatment of the cells with prazosin (0.3 μM), phenylephrine no longer reduced I_p and I_l. The phenylephrine-induced effects on I_p and I_l were both influenced by pretreatment with chloroethylclonidin (100 μM, 30 min preincubation at 37°C) and 5-methyl-urapidil (0.1 μM). Both antagonists abolished the effect of PE on I_p, whereas the effect on I_l was merely reduced. Our results suggest that both α_1-subtypes contribute to the reduction in outward currents by α_1-adrenergic stimulation in isolated rat myocytes.

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104P β-ADRENOCEPTOR DESENSITIZATION AND AGONIST-INDUCED ASTROGLIAL DIFFERENTIATION

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Astroglial cells prepared from postnatal rat brain can exhibit two distinct morphologies comprising either a flat epitheloid-like morphology (type 1) or a process-bearing (type 2) structure (Raff et al, 1983). Ligand-binding studies have shown that whilst type 1 astroglial possess substantial numbers of β-adrenoceptors, type 2 cells express less than 5% of those detected on the type 1 cells (McCarthy 1983). Administration of agents which raise cyclic AMP levels (eg β-agonists) can cause type 1 cells to convert rapidly (within 1 hour) to a process-bearing morphology (Facci et al, 1987) and this is accompanied by a reduction in β-adrenoceptor mediated cyclic AMP formation. The present study was undertaken to investigate the temporal relationship between β-agonist-induced morphology changes and β-adrenoceptor desensitization in primary rat type 1 astrocytes.

Primary cultures of astroglia (containing only type 1 cells) were prepared essentially as described by McCarthy & De Vellis (1980) from 8 day-old rat pups. ^{3}H-Cyclic AMP accumulation was measured as described by Ruck et al (1990) in astroglia after 14 days in culture. Morphological change (% stellation) was determined by counting the number of cells exhibiting process-bearing morphology per field of 50 cells. Isoprenaline (ISO; 1μM) induced an elevation of ^{3}H-Cyclic AMP which reached a maximum at 10 min (6.0 ± 0.9 fold over basal levels, n=4) and then fell rapidly towards basal levels over 50 min. Application of the incubation media from ISO-treated cells (1μM; added at time zero) to a second set of control cells (incubation continued for a further 10 min) indicated that a marked loss of ISO occurred during the 60 min incubation (n=3 experiments). However, the marked reduction in responsiveness to a second application of ISO (1μM) at various times after the primary stimulus indicated that desensitization also occurred. The development of the process-bearing morphology was a much slower process, progressing from 0% stellation at 10 min to 88± 5% stellation (n=4) at 60 min. A similar time-course was observed when forskolin (10μM) was used as the stellation agent (90 ± 6% stellation at 60 min, n=4), although elevated cyclic AMP levels (16.3 ± 4.3 fold, n=4) were maintained for up to 60 min with this agent. Following 24 h treatment with dibutyryl cyclic AMP (db-cAMP; 1μM) (which also induces stellation), cells reverted back to an epitheloid type 1 morphology. Under these conditions, cells were unresponsive to ISO (1μM; no cyclic AMP elevation or stellation) but could undergo morphological change in response to phorbol 12-myristate 13-acetate (300nM). These results suggest that the reduced cyclic AMP responsiveness to astrocyte β-adrenoceptor stimulation following ISO or db-cAMP treatment does not require maintenance of the process-bearing morphology.

We thank the MRC for financial support

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105P RENAL VASCULAR EFFECTS OF CYTOCHROME P450-DEPENDENT ARACHIDONATE METABOLITES

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Several cytochrome P450-dependent arachidonic acid (AA) metabolites, shown to be formed endogenously by the kidney, can affect renal ion transport and exhibit vasoactivity and, thereby, may play a role in coupling tubular and hemodynamic function. We studied the renovascular effects of epoxides (5,6-; 8,9-; 11,12- and 14,15-epoxyeicosatrienoic acids: EETs) and 20-hydroxyeicosatetraenoic acid (HETE), the major cytochrome P450-AA metabolites formed by the rat renal cortex and medulla, respectively (Takahashi et al, 1990).
Using the rabbit isolated kidney preparation perfused with Krebs' Henseleit solution (10 ml/min at 37°C) and preconstricted with phenylephrine (3 μM) to raise perfusion pressure (PP) from 40 mmHg to 110 mmHg, the EETs and 20-HETE were injected close-arterially. One, 5 and 10 μg 5,6-EET resulted in dose related decreases in PP of 10±5, 40±10 and 70±15 mmHg, respectively, (n=6). The 8,9-EET and the 11,12-EET also decreased PP but were less potent than 5,6-EET i.e. 10 μg of 8,9-EET reduced PP by 11±3 mmHg and 11,12-EET by 8±3 mmHg. In contrast, 14,15-EET resulted in moderate increases in PP. Bolus injections of 20-HETE at 1, 5 and 10 μg reduced PP by 3±1, 18±4 and 19±5 mmHg, respectively.
We tested the effect of cyclooxygenase inhibition on the renovascular responses to 5,6-EET and 20-HETE as both are substrates for cyclooxygenase (Oliw, 1984; Escalante et al, 1989). Indomethacin (2.9 μM) abolished the vasodilator response to 5,6-EET and 20-HETE without affecting responses to other EETs and acetylcholine. Incubation of 5,6-EET or 20-HETE with purified cyclooxygenase yielded vasoactive metabolites that had chromatographic (TLC and HPLC) properties similar to prostaglandins. Addition of indomethacin to the incubates prevented formation of these metabolites.
Therefore, of the cytochrome P450-arachidonate compounds tested, 5,6-EET and 20-HETE are the most potent vasodilators of the rabbit renal vasculature, effects that are dependent on cyclooxygenase conversion. This step of transcellular metabolism may represent a potential mechanism for coupling transport activity to changes in local blood flow.

The work was supported by the National Institutes of Health and the American Heart Association

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106P CYTOCHROME P450 ENZYMES (P450) IN THE RENAL VASODILATOR RESPONSE TO ARACHIDONIC ACID (AA) AND THE RELASE OF VASOACTIVE PRODUCTS

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In the isolated perfused kidney of the rat, AA causes cyclooxygenase-dependent vasoconstriction. When the vasoconstrictor response is abolished with indomethacin (2.8 μM) and vascular tone elevated with phenylephrine (5 x 10^{-7}M) a vasodilator response to AA is uncovered which was reduced by clotrimazole and 7-ethoxyresorufin (7-ER), inhibitors of P450 enzymes. These studies were extended by addressing vasodilator responses to AA in kidneys from rats in which P450 enzymes were induced or depleted. P450 enzyme were induced by treating 6 rats with a combination of 3-methylcholanthrene (3MC 80 mg/kg) and β-naphthoflavone (β-NF 80 mg/kg) s.c. for 3 days or dexamethasone (25 mg/kg) s.c. on alternate days for 7 days (n=6). Control rats received the appropriate vehicles (n=4). To deplete P450 enzymes rats were treated with stannous chloride (100 mg/kg/s.c.) for 4 days (n=6) or cobalt chloride (24 mg/kg i.p.) for 2 days (n=6). Control rats were treated with the respective vehicles. Indomethacin-treated phenylephrine preconstricted kidneys perfused with oxygenated Krebs buffer at 37°C were used to compare dose-response curves to AA (1-10μg). In some preparations a mixture of labelled and unlabelled AA (total 5 μg) was administered and the perfusate collected for extraction and separation of AA metabolites by HPLC. In other experiments rabbit aortic rings were used in a superfusion bioassay to detect vasoactive material released from the kidney following AA administration. The renal effluent was also collected for extraction and separation (HPLC) of eicosanoids and the fractions tested for relaxant activity on preconstricted rings of rabbit aorta.

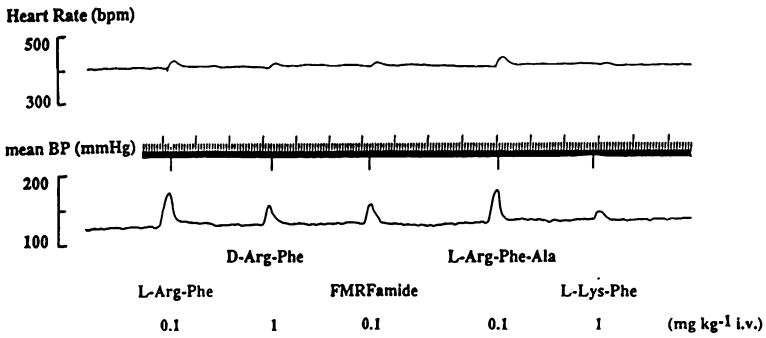
The induction of P450 enzymes with either 3MC/β-NF or dexamethasone enhanced the vasodilator responses to AA. Thus, in the 3MC/ -NF group 10 μg AA reduced renal perfusion pressure by 46±5mmHg compared to 33±2 mmHg in the control group, while in the dexamethasone-treated group the decrease in perfusion pressure was 52±7 mmHg compared to 39±4 mmHg for the vehicle control group ($p<0.05$). In contrast, depletion of P450 with either $CoCl_2$ or $SnCl_2$ reduced the vasodilator response to AA, 10 μg AA reducing perfusion pressure by 23±3 mmHg and 22±3 mmHg compared to 37±3 mmHg and 31±1 mmHg for the respective vehicle control ($p<0.05$).
Induction or depletion of P450 enzymes did not alter the response of acetylcholine (100 ng).
The administration of AA to the perfused kidney resulted in a dose-dependent release of vasorelaxant material detected by a superfusion bioassay. Addition of 7-ER (1 μM) to the perfusate reduced the renal vasodilator responses to AA and diminished the release of vasorelaxant material. The administration of labelled AA resulted in 4 consistent peaks of radioactivity following HPLC separation of the extracted eicosanoids.

Inhibition of P450 with 7-ER markedly reduced the radioactive peaks.
Bioassay of HPLC fractions on precontracted rabbit aortic rings revealed vasorelaxant activity in 3 fractions. These data support a role of P450 enzymes in the vasodilator effect of AA in the perfused kidney of the rat which generates specific metabolites, some of which possess vasorelaxant activity.

107P L-ARGINYL-L-PHENYLALANINE (Arg-Phe) IS A POTENT PRESSOR PEPTIDE IN THE ANAESTHETIZED RAT *IN VIVO*

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The neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2) increases mean arterial blood pressure (MAP) and heart rate (HR) in anaesthetized rats, and peptides immunohistochemically related to FMRFamide occur in areas of the rat brain concerned with autonomic control (Barnard & Dockray, 1984). Here we demonstrate that the dipeptide Arg-Phe mimicks the effects of FMRFamide on MAP and HR in the anaesthetized rat *in vivo*. Male Wistar rats (200-300 g), anaesthetized with sodium-thiopental (120 mg/kg i.p.), were cannulated for drug infusion and measurement of MAP and HR. When compared to FMRFamide, Arg-Phe or Arg-Phe-Ala produced a more pronounced increase in MAP and HR. Among more than 40 different peptides investigated, the following order of potency of the effective compounds was established: Arg-Phe = Arg-Phe-Ala > Met-Arg-Phe = FMRFamide > Arg-Trp = Arg-Tyr = D-Arg-L-Phe = Arg-Phe-OMe > Lys-Phe = Arg-Leu = Arg-Ile > Arg-Met. Interestingly, reversed dipeptides such as Phe-Arg or Leu-Arg were inactive, and Arg-Phe or FMRFamide (up to 30 mg/kg) did not cause any pressor response in pithed rats (n=4), indicating a central action. Fig. 1 shows a representative trace of the hemodynamic responses to i.v. bolus injections of some of the afore-mentioned peptides:



FMRFamide (0.1 mg/kg i.v.) and Arg-Phe (0.1 mg/kg i.v.) also increased plasma noradrenaline levels from 0.6 ± 0.2 nmol/l (control) to 1.3 ± 0.1 nmol/l (n=4; $P<0.05$) and 1.7 ± 0.2 nmol/l (n=4; $P<0.01$) respectively, but had no effect on plasma adrenaline levels. Thus, Arg-Phe may represent the active principle of FMRFamide activating the peripheral sympathetic nervous system by a central mechanism. **(Supported by a grant from Glaxo Group Research Ltd.)**

Barnard, C.S. and Dockray, G.J. (1984) *Reg. Peptides* **8**, 209-215.

108P ANTAGONISM OF THE HAEMODYNAMIC ACTIONS OF RAT ISLET AMYLOID POLYPEPTIDE BY HUMAN α-CALCITONIN GENE-RELATED PEPTIDE [8-37] IN CONSCIOUS RATS

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Islet amyloid polypeptide (IAPP) shows substantial homology with calcitonin gene-related peptide (CGRP) (Johnson et al., 1989). We have found that human α-CGRP [8-37] antagonizes the in vivo haemodynamic effects of human α-CGRP (Gardiner et al., 1990). Therefore, we investigated the effects of human α-CGRP [8-37] on the haemodynamic actions of rat amidated IAPP. Male, Long Evans rats (n=7, 350-450 g) were anaesthetized (sodium methohexitone, 60 mg kg^{-1} i.p. supplemented as required) and had pulsed Doppler probes implanted to monitor renal, mesenteric and hindquarter blood flow changes (Haywood et al., 1981). Seven-14 days later, animals were briefly re-anaesthetized (as above) and had intravascular catheters implanted. At least 24h later rat IAPP (150 nmol kg^{-1} h^{-1}) was infused i.v. (0.3 ml h^{-1}) continuously for 60 min; between the 20th and 40th min, human α-CGRP [8-37] was co-infused (1.5 μmol kg^{-1} h^{-1}). Infusion of rat IAPP alone for 20 min caused hypotension (-21±2 mm Hg, $P<0.05$, Friedman's test), tachycardia (126±12 beats min^{-1}, $P<0.05$) and renal and hindquarters vasodilatations (Δ conductance 17±6 and 61±10%, respectively, $P<0.05$), with no change in mesenteric vascular conductance (0±4%). After co-infusion of rat IAPP and human α–CGRP [8-37] for 20 min, the depressor effect of the former was converted into a slight rise in MAP (8±4 mm Hg, $P<0.05$), and the tachycardia was abolished (-4±10 beats min^{-1}). The renal and hinquarters vasodilatations were suppressed (Δ conductance -2±4 and -12±8%, respectively) while mesenteric vasoconstriction occurred (-19±4%, $P<0.05$). Following cessation of infusion of human α-CGRP [8-37] and continuation of the infusion of rat IAPP for a further 20 min the depressor, tachycardic and renal and hindquarters vasodilator effects of the latter re-appeared (-12±2 mm Hg, 91±13 beats min^{-1}, Δ conductance 23±8 and 38±10%, respectively, all $P<0.05$) and the mesenteric vasoconstriction waned (Δ conductance -7±7%).

The results are consistent with human α-CGRP [8-37] acting as a reversible antagonist of the cardiovascular actions of rat IAPP.

Gardiner, S.M., Compton, A.M., Kemp, P.A., Bennett, T., Bose, C., Foulkes, R. & Hughes, B. (1990) Biochem. Biophys. Res. Comm. (in press).
Haywood, J.R., Shaffer, R.A., Fastenow, C., Fink, G.D. & Brody, M.J. (1981) Am. J. Physiol., 241, H273-H278.
Johnson, K.H., O'Brien, T.D., Betsholtz, C. & Westermark, P. (1989) New Eng. J. Med., 321, 513-518.

109P HAEMODYNAMIC EFFECTS OF THE NON-PEPTIDE, ANGIOTENSIN II ANTAGONIST, DUP753, IN CONSCIOUS BRATTLEBORO RATS

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Recently, Wong et al. (1990) characterized DuP753 as a selective, non-peptide antagonist of the cardiovascular effects of angiotensin II. However, nothing is known about the in vivo regional haemodynamic actions of DuP753, so we investigated these in water-replete and water-deprived (14h), Brattleboro rats, since the latter show marked activation of the renin-angiotensin system.

Male, Brattleboro rats (350-400 g) were anaesthetized (sodium methohexitone 60 mg kg^{-1}) and instrumented for the measurement of renal, mesenteric and hindquarters blood flow (Haywood et al., 1981). At least 7 days later intravascular catheters were implanted under brief anaesthesia (as above) and experiments, involving administration of increasing doses of DuP753 at 15 min intervals (0.1-10 mg kg^{-1}), were begun at least 24h later. In water-replete (n=8) Brattleboro rats the lower doses of DuP753 had little effect. However, after a dose of 10 mg kg^{-1} there was (relative to pre-drug baseline) a small fall in mean arterial pressure (-8±4 mm Hg, P<0.05, Friedman's test), tachycardia (33±10 beats min^{-1}, P<0.05) and renal and mesenteric vasodilatations (Δ conductance 26±7 and 21±11%, respectively, both P<0.05); hindquarters conductance did not change (-3±9%). In water-deprived animals (n=7) there were dose-related hypotensions, tachycardias and renal, mesenteric and hindquarters vasodilatations. After DuP753 at 10 mg kg^{-1}, relative to pre-drug baseline, the depressor change was -46±4 mm Hg, the tachycardia 79±12 beats min^{-1}, and the increases in renal, mesenteric and hindquarters vascular conductances 45±11, 94±9 and 53±14%, respectively (all P<0.05). Captopril (2 mg kg^{-1}) had no significant, sustained effects under these conditions.

In a separate group of water-deprived Brattleboro rats (n=9) chronically instrumented for the measurement of cardiac output, DuP753 (10mg kg^{-1}) caused hypotension and tachycardia (-39±3 mm Hg and +69±11 beats min^{-1}, respectively, both P<0.05). However, there was a slight increase in cardiac output (7±2 ml min^{-1}, P<0.05).

These results are consistent with DuP753 acting as an effective antagonist of the haemodynamic actions of endogenous angiotensin II. The hypotensive effects of DuP753 in water-deprived Brattleboro rats are due to increases in total peripheral conductance unaccompanied by reduction of cardiac ouput.

Haywood, J.R., Shaffer, R.A., Fastenow, C., Fink, G.D., & Brody, M.J. (1981) Am. J. Physiol., 241, H273-H278.
Wong, P.C., Price, W. A., Chiu, A.T., Duncia, J.V., Carini, D.J., Wexler, R.R., Johnson, A.L. & Timmermans, P.B, M.W.M. (1990) Hypertension, 15, 459-468.

110P HAEMODYNAMIC EFFECTS OF PARAVENTRICULAR NUCLEUS INJECTIONS OF NORADRENALINE IN CONSCIOUS LONG EVANS AND BRATTLEBORO RATS

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The present study was designed to investigate the regional haemodynamic effects of noradrenaline (NA) injected bilaterally into the hypothalamic paraventricular nuclei (PVN) of conscious, unrestrained Long Evans (i.e. normal) and Brattleboro (i.e. vasopressin-deficient) rats.

Male Long Evans and Brattleboro rats (350-400g) anaesthetized with sodium methohexitone (60 mg kg^{-1}, i.p.) had two 23 g guide cannulae bilaterally implanted 2 mm above the PVN. Seven days later, the animals were re-anaesthetized (as above) and miniaturized pulsed Doppler probes were sutured around the left renal and superior mesenteric arteries and the distal abdominal aorta below the ileocaecal artery. Following 7 days recovery, animals were briefly re-anaesthetized (as above) and a catheter was inserted into the distal abdominal aorta via the caudal artery for the recording of heart rate (HR) and blood pressure. The experiments began on the next day. Mean arterial pressure (MAP), HR and mean Doppler shift (MDS) signals from the three probes were recorded continuously for a 30 min base-line period and for 60 min after bilateral PVN injections of NA. MDS is a reliable index of blood flow (Haywood et al., 1981). From the recordings of MAP and MDS, changes in regional vascular conductances were calculated.

In Long Evans rats (n=9), injection of NA (10 nmol in 0.3 µl) caused significant (P<0.05, Wilcoxon test) increases in MAP (+17 ± 2 mmHg), bradycardia (-64 ± 13 beats/min) and falls in mesenteric (-33 ± 4%) and hindquarters (-12 ± 4%) blood flows but no significant change in renal blood flow (-2 ± 2%). There were significant (P<0.05) falls in renal (-14 ± 2%), mesenteric (-42 ± 3%) and hindquarters (-25 ± 6%) vascular conductances. In Brattleboro rats (n=11), NA (10 nmol) had no significant effect on MAP or HR. However, there was a significant (P<0.05) increase in hindquarters blood flow (+15 ± 5%) and slight decreases in renal (-5 ± 2%) and mesenteric (-4 ± 1%) blood flows, although only the hindquarters vasodilatation (+20 ± 8%) was significant (P<0.05).

The present results indicate that the pressor response to bilateral injections of NA into the PVN in Long Evans rats was largely due to mesenteric vasoconstriction. (Since renal blood flow did not change it is likely the rise in vascular resistance was autoregulatory). Such effects are consistent with release of vasopressin from the posterior pituitary into the systemic circulation, especially since they were absent in vasopressin-deficient Brattleboro rats. In the latter the hindquarters vasodilatation following injection of NA into PVN was not associated with overt changes in motor behaviour and hence remains unexplained at present.

This work was supported by the Wellcome Trust. H.B. was in receipt of a Fellowship from the Canadian Heart Foundation

Haywood, J.R., Shaffer, R.A., Fastenow, C., Fink, G.D. & Brody, M.J. (1981) Am. J. Physiol. 241, H273-H278.

111P THE BENZODIAZEPINE INVERSE AGONIST FG7142 PREVENTS MODULATION OF AGONIST AND ANTAGONIST LIGAND BINDING TO CORTICAL β-ADRENOCEPTORS

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We have reported previously an increase in the density of β-adrenoceptors 7 days, but not 24h, after a single injection of the benzodiazepine partial inverse agonist, FG7142 (Stanford *et al.*, 1986). The present experiments investigated long-term effects of FG7142 on changes in binding of β-adrenoceptor ligands induced by Mg^{2+} or GTPγS *in vitro*.

Male CD1 mice (20-25g) were given a single injection of either FG7142 (80mg/kg i.p.) or vehicle (0.5% Tween 80 in distilled water). 7 days later, all animals were killed by cervical dislocation and the cerebral cortices removed. Displacement of antagonist ([^{3}H]dihydroalprenolol) binding by the agonist, isoprenaline, was measured in 50mM Tris buffer pH 7.6 ('TRIS'), Tris buffer with added Mg^{2+} (final concentration 5mM; 'TRIS-Mg^{2+}') and buffer with added GTPγS (final concentration 1μM; 'TRIS-GTP').

Mg^{2+} had no effect on displacement of bound [^{3}H]dihyroalprenolol by isoprenaline, but GTPγS caused an increase in the *Ki* for agonist displacement of this radioligand ((nM) control: 181±19; GTPγS: 339±50**). However both Mg^{2+} and GTPγS also reduced total, but not non-specific, binding of [^{3}H]dihydroalprenolol to membranes derived from vehicle-injected animals (Table 1). All these effects of Mg^{2+} and/or GTPγS were abolished by FG7142 pretreatment.

Table 1

	[^{3}H]dihydroalprenolol Binding					
	Total			Non-specific		
	TRIS	TRIS-Mg^{2+}	TRIS-GTPγS	TRIS	TRIS-Mg^{2+}	TRIS-GTPγS
Control	88±3	74±2*		33±2	32±3	
FG7142	85±3	76±3		40±3	35±4	
Control	95±5		84±6*	35±2		35±2
FG7142	94±5		92±5	38±3		37±3

Values show mean ± s.e.mean binding (cpm×10^{-2}/mg protein). n=7 (TRIS-Mg^{2+}); n=11 (TRIS-GTPγS). * : P < 0.05 ** P < 0.01 (cf Tween control; Wilcoxon's matched pair signed ranks test).

It is concluded that FG7142 disrupts processes involved in modulation of β-adrenoceptors in the brain. Moreover, the data are consistent with the possibility that [^{3}H]dihydroalprenolol also binds to non-β-adrenoceptor sites; Mg^{2+}- and GTP-dependent modulation of these putative non-β-receptors is also prevented by FG7142.

Stanford, S.C., Little, H.J., Nutt, D.J. & Taylor, S.C. (1986) *Eur. J. Pharmac.*, 134, 313-319.

112P CHLORMETHIAZOLE (CMZ) PROTECTS HIPPOCAMPAL NEURONES IN THE GERBIL FROM ISCHAEMIC DAMAGE

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Transient cerebral ischaemia produces a characteristic pattern of neuronal degeneration, with pyramidal neurones of the CA1 region of the hippocampus being particularly susceptible. This selective and delayed degeneration of CA1 pyramidal neurones is induced in the Mongolian gerbil by transient bilateral occlusion of the carotid arteries (Kirino, 1982), and provides a simple model to test putative neuroprotective compounds. We have used this model to examine the neuroprotective effects of chlormethiazole, a sedative, hypnotic and anticonvulsant drug.

Male Mongolian gerbils (60-80g) were anaesthestised with alphaxolone (45mg/kg^{-1}i.p.),and the carotid arteries occluded for 5 min. Drugs were administered as a single dose i.p. before or after operation. Body temperature was assessed with a rectal probe and maintained at 37°C with heated mats and lamps. Four days following ischaemia, hippocampal damage was assessed in Nissl-stained sections as described by Gill et al (1978). The extent of CA1 damage was expressed as a percentage of the whole CA1 region, to correct for variation in the level of sectioning through the hippocampus.

Following 5 min carotid artery occlusion, hippocampal CA1 neurones were almost completely lost, whilst CA3/CA4 neurones were unaffected (Table). Pretreatment with dizocilpine and ifenprodil protected against this degeneration.

Table — Hippocampal CA1 degeneration in transient ischaemia

Pre-ischaemia (30 min)			Post-ischaemia (60 min)		
Treatment	% Damage (±s.e.mean)		Treatment	% Damage (±s.e.mean)	
Control	94 ±2	(10)	Control	82 ±5	(24)
MK801 (3mgkg^{-1})	<5	(6)**	CMZ (50mgkg^{-1})	45 ±9	(11)*
Ifenprodil (4mgkg^{-1})	62 ±8	(7)*	CMZ (100 mgkg^{-1})	18 ±9	(12)**
CMZ (100mgkg^{-1})	<5	(7)**	Phenobarbitone (100mgkg^{-1})	78 ±9	(7)

Mann Whitney 'U' test: *p <0.05; **p<0.01; number of observations in brackets.

Chlormethiazole showed a marked protective action when administered either 30 min before or 60 min following ischaemia, while phenobarbitone was essentially without effect (Table).

Gill, R., Foster, A.C. & Woodruff, G.N. (1987) J.Neurosci. 7, 3343-3349.
Kirino, T. (1982) Brain Res. 239, 57-69.

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113P GABA$_B$ AUTORECEPTORS DIFFERENTIALLY DEPRESS INHIBITORY SYNAPTIC TRANSMISSION IN DORSAL STRIATUM OF RAT BRAIN SLICES

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Inhibition of glutamate-mediated synaptic transmission in dorsal striatum by baclofen and by 3-aminopropyl-methyl-phosphinic acid (SK&F 97541), a novel and more potent GABA$_B$ receptor agonist, has recently been shown in rat brain slices. This action on the presynaptic GABA$_B$ 'heteroceptor' was antagonised by 3-aminopropyl-diethoxymethyl-phosphinic acid (CGP 35348) and 3-aminopropyl-hexyl-phosphinic acid (3-APHPA; Seabrook et al. 1990). Here we examine the presynaptic GABA$_B$ 'autoreceptor' modulating GABA-mediated transmission in the rat striatum and compare its pharmacology to that of the heteroceptor.

Sagittal slices (350 µm; 3 mm lateral to midline) of rat forebrain were submerged in a recording chamber and superfused by a standard artificial cerebrospinal fluid at 36°C, to which drugs were applied. Intracellular recordings were made from neurones in dorsal striatum, and synaptic potentials were elicited by focal bipolar stimulation of the slice 200-500 µm from the recording electrode. Glutamate-mediated synaptic transmission was blocked by application of 6,7-dinitro-quinoxaline-2,3-dione (30 µM). Data is expressed as the mean ± SE.

Recordings were made from 15 neurones with a mean membrane potential of -78.5 ± 2.2 mV, and resting input resistance of 41.0 ± 4.3 MOhm. GABA-mediated synaptic potentials, identified by their sensitivity to bicuculline (30 µM), were depressed in amplitude by application of either SK&F 97541 (0.1-100 µM, n = 15) or (±)-baclofen (1-100 µM, n = 7). The dose-response relationship of the pooled data had a Hill slope of < 0.3 and did not show a clear maximal effect even at the highest agonist concentrations examined (100 µM), where depressions of 62.5 ± 5.8% with SK&F 97541 and 67.9 ± 7.2% with baclofen were observed. In 40% of cells multiphasic synaptic potentials were observed. A subset of these synaptic inputs exhibited a sensitivity to SK&F 97541 (EC_{50} = 90 nM, Hill slope of 1; data from 5 cells) that was comparable to the GABA$_B$ heteroceptor. The depression of this same population of GABA inputs by submaximal concentrations of SK&F 97541 (0.3 µM) was antagonised by CGP 35348 or 3-APHPA (both 100 µM).

It is concluded that there is more than one population of GABA nerve terminals innervating neurones in the dorsal striatum of the rat brain. One population exhibits GABA$_B$ receptors with a similar pharmacology to the striatal heteroceptor (Seabrook et al. 1990), whilst the other population does not appear to be directly affected by GABA$_B$ agonists.

Seabrook G.R., Howson W., Lacey M.G. (1990) Electrophysiological characterisation of potent agonists and antagonists at pre- and post-synaptic GABA$_B$ receptors on neurones in rat brain slices. Brit. J. Pharm. in press.

114P PHARMACOLOGICAL PROFILE OF F 2692, A NEW POTENTIAL ANXIOLYTIC COPMPOUND WITH WEAK AFFINITY FOR THE BENZODIAZEPINE BINDING SITES

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F 2692 (1-(3'-trifluoromethyl phenyl) 1,4-dihydro 3-amino 4-oxo 6-methyl pyridazine), a new potential anxiolytic, has been shown to have anxiolytic-like properties in animal tests similar to those of the benzodiazepines. Binding studies *in vitro* and *in vivo* have shown F 2692 to have very low affinity for the benzodiazepine binding sites (Assié et al, this meeting). The anticonvulsant properties against pentetrazol (125 mg/kg sc), myorelaxation in the rota-rod test, spontaneous locomotor activity, potentiation of chloral hydrate induced-sleep and the amnesic effects in the passive avoidance test are shown in table 1. F 2692 showed anticonvulsant, myorelaxant, sedative and amnesic properties at doses 10 to 20 times higher than diazepam.

Table 1 Pharmacological acute profile of F 2692 compared to diazepam

	Pentetrazol (rat)[a]	Rota-rod (rat)[a]	Rota-rod (mice)[a]	Locomotor activity (rat)[b]	Chloral hydrate induced sleep (rat)[b]	Passive avoidance (rat)[b]
F 2692	6.5	68	100	30	10	50
Diazepam	0.48	3.4	5.6	3	1	3

a : ED_{50} mg/kg ip ; b : minimal significant dose mg/kg ip.

After 21 days administration in rats, F 2692 (30 mg/kg ip) exhibited no tolerance to the sedative effect as determined by locomotor activity 30 min after the last administration (-22%, $p<0.05$ as compared to control ; -16%, $p<0.05$ acutely). Under the same experimental conditions, diazepam at the dose of 3 mg/kg ip no longer showed any significant sedative effect (+11% as compared to control ; -21%, $p<0.05$ acutely). After 21 days administration of F 2692 at the doses of 3, 10 and 30 mg/kg and 24 h after the last administration, the animals showed no significant difference to control animals in the elevated plus-maze. Under the same experimental conditions, diazepam given for 21 days at the doses of 0.3, 1 and 3 mg/kg ip produced a doses-dependent anxiogenic effect with a minimal significant dose of 1 mg/kg.
Thus F 2692 showed a pharmacological profile similar to benzodiazepines when tested acutely. However, after repeated administration there is no evidence for any tolerance or withdrawal effects.

Assié, M-B, Chopin, P. & Briley, M. (1990) This meeting.

115P F 2692, A NEW POTENTIAL ANXIOLYTIC WITH A BENZODIAZPINE-LIKE PROFILE BUT WEAK AFFINITY FOR THE BENZODIAZEPINE BINDING SITES

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Benzodiazepines have been the drugs of choice for the treatment of anxiety disorders for more than two decades. Problems of tolerance and withdrawal have however led to a certain retiscence in the use of these compounds. In the mean time, a number of compounds acting at the 5-HT_{1A} receptor and exhibiting anti-anxiety properties have been developed. Their effects, however, only appear after a week or more of treatment. Thus the choice in anxiolytic therapy is currently between an acute treatment with potential problems of dependency and a longer term treatment with a delayed onset of action.

The properties of a new potential anxiolytic compound, F 2692 (1-(3'-trifluoromethyl phenyl) 1,4-dihydro 3-amino 4-oxo 6-methyl pyridazine), suggest that it may offer an interesting alternative to existing therapies.

In the elevated plus-maze test in rats, F 2692 showed dose-dependent anxiolytic-like effects. Thirty min after administration at the doses of 1, 3, 10, 30 and 100 mg/kg ip the variation of the percent entries in open arms was 20, 56, 75, 94 and 115% respectively as compared to control animals. The minimal significant dose was 3 mg/kg ip. The acute effect was still present 4 hours after the administration of 30 mg/kg ip but was absent 24 h after administration. In the same experiments, diazepam exhibited similar anxiolytic-like effects at doses 10 times lower. In the mouse two compartment test, F 2692 administered ip 30 min before testing also showed anxiolytic-like effects from 0.01 mg/kg.

The anxiolytic-like effects of F 2692 are qualitatively similar to those of benzodiazepines. Flumazenil, the benzodiazepine antagonist, at 20 mg/kg ip reversed the anxiolytic-like effects of F 2692 administered 30 min before the test at the doses of 30 mg/kg ip in the elevated plus-maze in rats and 0.3 mg/kg ip in the two compartment test in mice.

Thus the behavioural profil of F 2692 appears to be closely related to benzodiazepines. *In vitro*, F 2692 inhibited [^{3}H]-flunitrazepam binding very weakly (IC_{50} = 19000 ± 5000 nM ; 14 ± 1 nM for diazepam) and was not changed after incubation of the compound with liver homogenates. Furthermore, *in vivo* F 2692 poorly inhibited [^{3}H]-flunitrazepam binding in mice whole brain (ID_{50} = 60mg/kg ip ; 0.3 mg/kg ip for diazepam).

Thus the new potential anxiolytic, F 2692, shows a behavioural anxiolytic-like profile similar to benzodiazepines in rats and mice. However the weak affinity of F 2692 for benzodiazepine binding sites *in vitro* and *in vivo* suggests that these effects are not mediated directly through benzodiazepine binding sites although F 2692 may well act indirectly through the GABA-benzodiazepine complex. This latter property may be related to the lack of tolerance and dependency reported after 21 days treatment of F 2692 (Chopin et al., this meeting).

Chopin, P., Stenger, A., Assié, M-B. & Briley, M. (1990) This meeting.

116P INTRACELLULAR AMINO ACID CONCENTRATIONS IN CULTURED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS DEPRIVED OF L-ARGININE OR TOTAL AMINO ACIDS

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Although the cationic amino acid L-arginine has been identified as the physiological precursor for endothelium-derived nitric oxide (EDNO; Palmer et al., 1988), it is still not clear whether its avalibility and transport into endothelial cells is rate limiting for EDNO synthesis. We have now addressed this issue in venous endothelium by determining the intracellular concentrations of arginine (and other amino acids) in cultured human umbilical vein endothelial cells (HUVEC) which have previously been shown to rapidly transport this amino acid via a saturable carrier system (Mann et al., 1989). In addition, we have examined the effects of total amino acid and L-arginine deprivation on intracellular arginine concentrations to explore whether changes in substrate availability modulates the transport and metabolism of L-arginine and thus EDNO synthesis in venous endothelial cells.

Endothelial cells isolated from human umbilical veins were grown to confluency in M199 culture medium containing Earl's salts and 10% newborn plus 10% foetal calf serum. Confluent monolayers of primary cultures were deprived of either all amino acids or L-arginine for periods of 1-24 h by replacing the standard culture media with an amino acid or L-arginine free medium supplemented with dialyzed serum. Amino acid concentrations in the cell extracts were determined by reverse phase HPLC following precolumn derivatization with *o*-phthaldialdehyde/mercaptoethanol at pH 9.

HUVEC cultured in standard medium had a total amino acid concentration of 59.9 ± 2.2 mM (n = 8) of which 86% were non-essential and 14% essential amino acids. Concentrations of L-arginine, L-citrulline and L-glutamine were 0.88 ± 0.10 mM, 0.30 ± 0.10 mM and 12.71 ± 1.27 mM, respectively (n = 4). Incubation in an amino acid free medium decreased the total essential amino acid pool by 35% and the non-essential pool by 60% after 24 h. By comparison arginine deprivation (1-24 h) had no significant effect on total essential amino acid concentrations and produced only a small reduction (13%) in the total non-essential pool after 24 h. In contrast to our previous findings in bovine aortic endothelial cells (Baydoun et al., 1990) intracellular arginine levels in HUVEC were maintained close to control values even after a 24 h incubation period in either total amino acid or L-arginine free medium. L-glutamine and L-citrulline levels were also maintained following L-arginine deprivation but reduced by 78% and 100% respectively after 24 h incubation in the total amino acid free medium. Whether these observed changes modify L-arginine transport into HUVEC now remains to be established.

Supported by the British Heart Foundation

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117P TRANSPORT OF L-ARGININE AND L-GLUTAMINE IS STIMULATED IN PORCINE AORTIC ENDOTHELIAL CELLS DEPRIVED OF L-ARGININE

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Endothelial cells (EC) contain high levels of L-arginine and other amino acids (Baydoun et al., 1990), and when cultured in an arginine-free medium for 24 h exhibit a reduced ability to release endothelium derived nitric oxide (EDNO) (Palmer et al., 1988). Furthermore, recent studies *in vivo* concluded that stimulated release of EDNO is sensitive to circulating levels of arginine (Aisaka et al., 1989). We have previously reported that EC rapidly transport arginine by a saturable cationic amino acid carrier, sensitive to inhibition by N^G-monomethylarginine, L-lysine, L-ornithine but not L-citrulline (Mann et al., 1990). We have now correlated amino acid transport with intracellular amino acid levels in porcine aortic endothelial cells (PAEC) cultured in the presence (DMEM + 20% serum) or absence (DMEM + dialysed serum) of L-arginine for 24 h. PAEC were grown to confluence on Biosilon microcarriers, packed into columns (5 x 10^6 cells) and perfused (0.4 ml/min) with a HEPES buffered Krebs solution. Amino acid uptake was assessed using a rapid tracer dilution technique (Mann et al., 1990) and intracellular concentrations were determined by reverse phase HPLC (Baydoun et al., 1990).

In control PAEC (400 μM arginine) high uptakes were measured for cationic and neutral amino acids including glutamine (Fig. 1). Deprivation of arginine for 24 h increased arginine uptake by 42 $\pm$ 8 % and decreased intracellular levels by 54 $\pm$ 5% (n = 8). Uptake of glutamine, a putative inhibitor of intracellular arginine biosynthesis (Hecker et al., 1990), was stimulated by 17 $\pm$ 5 %. This is of interest since glutamine levels are elevated signficantly in PAEC (> 53 %) following 24 h arginine deprivation. This study provides the first evidence that transport of arginine and certain other amino acids is up-regulated following arginine deprivation. It remains to be established whether increased arginine transport and changes in intracellular amino acid levels are involved in restoring the release of EDNO in arginine deprived endothelial cells.

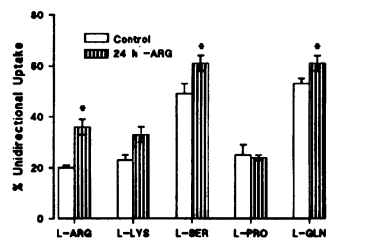


Fig 1. Effect of 24 h L-arginine deprivation on PAEC amino acid transport (n= 3-7, P < 0.05, unpaired t test)

Supported by the British Heart Foundation and SERC/CASE Wellcome Cooperative Award.

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118P DIPEPTIDES CONTAINING N^{ω}-NITRO-L-ARGININE INHIBIT EDRF BIOSYNTHESIS *IN VITRO* AND *IN VIVO*

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$N\omega$-Nitro-L-arginine (NO_2Arg) is a potent inhibitor of the biosynthesis of endothelium-derived relaxing factor (EDRF) both *in vitro* (Moore *et al.*, 1990) and *in vivo* (Hecker *et al.*, 1990). We have investigated the effects of three dipeptides containing NO_2Arg, NO_2Arg-L-phenylalanine (NO_2Arg-Phe), L-alanine-NO_2Arg (Ala-NO_2Arg) and NO_2Arg-L-arginine (NO_2Arg-Arg), on endothelium-dependent relaxations *in vitro* and *in vivo*. In anaesthetized rats, intravenous infusions of NO_2Arg-Phe, Ala-NO_2Arg or NO_2Arg-Arg (1-30 mg/kg) produced dose-dependent increases in mean arterial blood pressure (MAP), which were prevented by co-infusions of L-arginine (L-Arg) but not D-Arg (20 mg/kg/min for 15 min i.v.). Similarly, L-Ala-L-Arg or L-Arg-L-Arg (20 mg/kg/min for 15 min i.v.) prevented the increase in MAP caused by their NO_2Arg derivatives. NO_2Arg-Phe or Ala-NO_2Arg (10 mg/kg i.v.) also significantly inhibited the fall in MAP induced by acetylcholine (30 mg/kg/min for 3 min), and this effect was prevented by co-infusions of L-Arg. In contrast, NO_2Arg-Phe or Ala-NO_2Arg did not affect vasodilator responses to glyceryl trinitrate or iloprost. NO_2Arg, NO_2Arg-Phe or NO_2Arg-Arg (100 μM) inhibited the acetylcholine-induced relaxation of rabbit aortic strips by 72, 52 or 23% respectively. L-Arg but not D-Arg (1 mM; 3 consecutive 15 min incubations) reversed the effect of all three inhibitors. NO_2Arg-Phe (30 μM) also inhibited the bradykinin-induced release of EDRF from bovine aortic cultured endothelial cells (EC; 85-95% inhibition), and this inhibition was partially reversed (50% inhibition) by co-infusions of L-Arg (100 μM). Incubations of EC with L-Arg-L-Phe, L-Ala-L-Arg, L-Arg-L-Arg and their NO_2Arg derivatives revealed a rapid, time-dependent cleavage of the parent dipeptide but not of the NO_2Arg derivative, suggesting that NO_2Arg-Phe directly inhibits the biosynthesis of EDRF by these cells. Although our findings do not rule out NO_2Arg as the inhibitory principle derived from proteolytic cleavage *in vivo*, they point to a broader substrate specificity of the EDRF forming enzyme(s). **(Supported by a grant from Glaxo Group Research Ltd.)**

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119P SULFHYDRYL REAGENTS INHIBIT STIMULATED BUT NOT BASAL EDRF RELEASE FROM CULTURED ENDOTHELIAL CELLS

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Several recent publications indicate that endothelium-derived relaxing factor (EDRF) may not be identical with nitric oxide but with S-nitroso-L-cysteine. It was also noted that depletion of intracellular L-cysteine (L-Cys) and/or alkylation of intracellular sulfhydryl (SH) groups selectively inhibits the basal or flow-induced release of EDRF from cultured endothelial cells (Minor *et al.*, 1989). We have, therefore, investigated in more detail the effect of SH reagents on the release of EDRF from endothelial cells cultured from bovine aorta. Cells ($6\text{-}7 \times 10^7$) on microcarrier beads (2 ml) were perfused with warmed (37°C), oxygenated (95% O_2/5% CO_2) Krebs' solution at 5 ml/min, and the effluent superfused a de-endothelialized rabbit aortic ring which was preconstricted to 2-3 g of tension with U46619 (10 nM; $9_\alpha,11_\alpha$-methanoepoxy-prostaglandin $F_{2\alpha}$) for the detection and quantification of EDRF release. Basal EDRF release was defined as the degree of relaxation following infusion of superoxide dismutase (10 units/ml) through the column of cells (t.c.). Prostacyclin (PGI_2) release was measured by radioimmunoassay for 6-keto-prostaglandin $F_{2\alpha}$, and intracellular L-Cys and glutathione (GSH) concentrations were determined by HPLC analysis of their monobromobimane derivatives. Weak SH-alkylating agents such as ethacrynic acid (infused t.c. at 10 or 100 μM for 15 min) had no effect on either basal or stimulated EDRF release (n=3), and diamide (100 and 250 μM; n=3) or 1-chloro-2,4-dinitrobenzene (CDNB; 100 μM; n=3), compounds which oxidise or deplete intracellular GSH, were also ineffective. However, the strong SH-alkylating agent *N*-ethylmaleimide (NEM; 25 μM for 15 min) inhibited the stimulated release of EDRF induced by ADP (3 μg; from 36 ± 6 to 10 ± 4% relaxation; mean ± SEM; n=6; $P<0.01$), ionomycin (20 ng; from 40 ± 6 to 9 ± 4% relaxation; n=5; $P<0.01$) or poly-L-lysine (10 μg; from 26 ± 6 to 17 ± 2% relaxation; n=5; $P<0.05$) but had no effect on basal EDRF release (27 ± 4 and 31 ± 3% relaxation; n=8). The reactive disulfide 2,2-dithiodipyridine (DTDP; 25 μM for 15 min) exhibited the same pattern of inhibitory activity as NEM (n=3), whereas N^ω-nitro-L-arginine.OMe (100 μM), a potent inhibitor of EDRF biosynthesis, inhibited basal and stimulated (ADP; 3 μg) EDRF release to the same extent (from 29 ± 3 to 4 ± 2% and from 27 ± 1 to 5 ± 4% relaxation respectively; n=3; $P<0.01$). The intracellular levels of L-Cys and GSH were 0.36 ± 0.06 mM (n=15) and 2.69 ± 0.42 mM (n=20) respectively. Like diamide or CDNB, NEM caused a significant decrease in GSH (41 ± 5% of control; n=7; $P<0.001$) but not L-Cys, whereas DTDP had no effect on either thiol. Moreover, NEM significantly potentiated the basal release of PGI_2 (1961 ± 320% of control; n=7; $P<0.001$), probably due to a blockade of the reacylation of arachdonic acid by the SH reagent, but inhibited the stimulated release of PGI_2 (14 ± 5% of control; n=7; $P<0.01$). Thus, NEM selectively inhibits the stimulated release of EDRF from cultured endothelial cells, suggesting a fundamental difference between basal and stimulated EDRF release. For DTDP does not affect the intracellular level of L-Cys or GSH but inhibits the stimulated release of EDRF, an S-nitrosothiol is unlikely to be identical with the EDRF released upon cell stimulation. (**Supported by a grant from Glaxo Group Research Ltd.**)

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120P ISCHAEMIC CONDITIONS INHIBIT THE RELEASE OF EDRF FROM CULTURED ENDOTHELIAL CELLS

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Ischaemia-reperfusion of various vascular beds leads to an impairment of endothelial cell function, including a decrease in the release of EDRF (identified as nitric oxide, NO; Palmer et al., 1988) by mechanisms presently unknown. We have, therefore, examined whether ischaemic conditions *in vitro* affect the release of EDRF from endothelial cells cultured from bovine aorta (EC). The release of EDRF was detected by using a cascade of denuded rabbit aortic strips (RbAs) superfused at 5ml/min, half of which was with oxygenated Krebs' solution (95% O_2/5% CO_2; pH=7.45; pO_2 = 225 mmHg) and the other half was with the effluent from a column of EC (Gryglewski et al., 1986). EC were perfused at 2.5 ml/min with either oxygenated Krebs' solution or glucose-free Krebs' solution gassed with 95% N_2/5% CO_2 (pH = 7.25; pO_2 = 34 mmHg), herein defined as ischaemic conditions (IC).

Exposure of EC to IC for 20 min substantially attenuated the release of EDRF induced by ADP (1-8nmol) or bradykinin (BK, 5-10pmol), but did not interfere with the direct vasodilating actions of glyceryl trinitrate (GTN, 60pmol) on the RbAs (n=8). Hypoxic Krebs' solution containing glucose (pH = 7.28; pO_2 = 29 mmHg; n=4) or oxygenated Krebs' solution without glucose (pH = 7.40; pO_2 = 235 mmHg; n=4) did not inhibit the release of EDRF induced by ADP or BK. When EC previously exposed to IC were perfused with oxygenated Krebs' solution, the stimulated release of EDRF was only partially restored. Infusions of L-arginine (1 mM) through the EC column did not prevent the inhibitory effect of IC on stimulated EDRF release. However, there was a greater restoration of the stimulated release of EDRF upon subsequent reoxygenation (n=5) than that observed in the absence of L-arginine.

Thus, IC inhibit the release of EDRF by a mechanism probably related to perturbations in EC energy requirements. The ability of L-arginine to facilitate the recovery of EDRF release after IC suggests that IC or reoxygenation may interfere with the synthesis or utilization of L-arginine necessary for NO formation.

The William Harvey Research Institute is supported by a grant from Glaxo Group Research Ltd.

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121P PYOCYANIN MODULATES THE VASORELAXANT ACTION OF EDRF AND NO-DONORS ON ISOLATED STRIPS OF RABBIT AORTA

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Pyocyanin (PYO, 1-hydroxy-5-methyl-phenazinum betaine) is a redox pigment produced by Pseudomonas aeruginosa (Wilson et al., 1988) that reduces the biological activity of EDRF probably by scavenging nitric oxide (Warren et al., 1990). Here we show that PYO modulates the relaxations of rabbit aortic strips (RbAs) induced by NO-donors such as glyceryl trinitrate (GTN), S-nitroso-N-acetylpenicillamine (SNAP), sodium nitroprusside (NaNP), a metabolite of molsidomine (SIN-1) or sodium nitrite ($NaNO_2$). We also compared the effects of PYO with those of methylene blue (MeB) and oxyhaemoglobin (HbO_2). RbAs without endothelium were superfused in a cascade with warmed (37°C) and gassed (95%O_2-5%CO_2) Krebs' solution at a flow rate of 5ml/min. The strips were contracted with U46619 (15-30nM) or phenylephrine HCl (50nM). Doses (nmol) of GTN (0.03 or 0.1), SNAP (0.1 or 0.3), NaNP (0.1 or 0.3), SIN-1 (3) or $NaNO_2$ (1000) were given as bolus injections before and during infusions of PYO (1-10μM), MeB (1-10μM) or HbO_2 (1μM) (table 1).

	MeB (1μM) (n=8-12)	MeB (3μM) (n=4-16)	MeB (10μM) (n=8-15)	PYO (1μM) (n=8-23)	PYO (3μM) (n=10-18)	PYO (10μM) (n=8-12)	HbO_2 (1μM) (n= 4-8)
GTN	0.81± 0.1	0.76± 0.1	0.83± 0.2	0.99± 0.1	0.48± 0.2	0.50± 0.1	0.28± 0.09
SNAP	0.81± 0.3	0.54± 0.1	0.57± 0.1	0.69± 0.1	0.49± 0.2	0.35± 0.2	0.15± 0.03
SIN-1	0.67± 0.1	0.77± 0.2	0.71± 0.2	1.07± 0.2	0.68± 0.1	0.44± 0.2	0.30± 0.09
NaNP	1.40± 0.3	1.97± 0.6	2.52± 1.0	1.82± 0.6	3.62± 1.1	3.17± 0.7	0.56± 0.09
$NaNO_2$	1.15± 0.2	1.33± 0.2	1.88± 0.4	1.30± 0.3	1.35± 0.3	1.80± 0.6	0.32± 0.06

Results are expressed as the ratio of relaxations in the presence of PYO, MeB or HBO_2 to relaxations without PYO, MeB or HbO_2. Values represent mean ± SD

PYO (1-10μM) or MeB (1–10μM) potentiated the effects of NaNP or $NaNO_2$ but inhibited relaxations induced by GTN, SNAP or SIN-1. The effects of all NO-donors were inhibited by HbO_2. Thus, the pharmacological actions of PYO resemble those of MeB, rather than HbO_2. The potentiation of NaNP by PYO or MeB cannot be explained by scavenging NO, or by inhibition of guanylate cyclase. (The William Harvey Research Institute is supported in part by a grant from Glaxo Group Research Ltd.)

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122P L-ARGININE DEPENDENT ACCUMULATION OF CYCLIC GMP IN RAT AORTIC MEDIAL SMOOTH MUSCLE LAYER INCUBATED WITH LIPOPOLYSACCHARIDE

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We have recently shown that E.coli lipopolysaccharide (LPS) can activate the L-arginine/NO pathway in vascular tissue *in vitro*, *in vivo* and *ex vivo* resulting in hyporeactivity to various constrictor agents,via stimulation of guanylate cyclase (Fleming et al, 1990; Julou-Schaeffer et al, 1990; Gray et al, 1990). Although experiments in de-endothelialised tissue have suggested an extra-endothelial site of NO production, the remaining adventitial layer of vascular tissue contains resident monocytes, macrophages and lymphocytes all of which are potential sources of NO after LPS stimulation. The aim of the present study was to investigate, by measuring cellular cyclic GMP (cGMP) content and the effect of an inhibitor of NO production from L-arginine (L-N^{ω}-nitroarginine methyl ester, L-NAME), whether LPS can activate the L-arginine pathway in the medial smooth muscle layer of rat aorta. Thoracic aortae were rubbed to remove the endothelium and pre-incubated for 3h in minimal essential medium (MEM) or L-arginine/l-glutamine depleted MEM to facilitate the non-enzymatic separation of the adventitia. After adventitia removal the tissues were incubated for a further 5h in the presence or absence of LPS (1 μg ml^{-1}) in normal MEM, L-arg/L-glut depleted MEM or in MEM containing L-NAME (300μM), then rapidly frozen and stored at -20°C until determination of cGMP content by radioimmunoassay.

Table 1 [cGMP] fmol/μg DNA in the medial layer (N.S., not significant, **, P<0.01 vs control, Students T test)

	MEM (n=5)	MEM - L-arg/L-glut (n=4)	MEM + L-NAME (n=4)
LPS	33.20±2.86**	4.66±0.59N.S.	8.39±1.33N.S.
control	8.05±3.51	8.14±3.12	6.78±1.65

Incubation of the medial layer with LPS in normal MEM containing 600 μM L-arginine was associated a 4 fold increase in the cGMP content (Table 1). Cyclic GMP also increased 15 fold in intact tissue (not shown) taken from the same aortae in agreement with our earlier report (Fleming et al, 1990). In both groups this increase was inhibited when the tissue was incubated in L-arginine/L-glutamine deficient medium during pre-incubation and exposure to LPS, and when L-NAME was present during incubation.

These results strongly suggest the presence of a system capable of generating NO from L-arginine in vascular smooth muscle which can be activated by LPS and which is limited by the availability of L-arginine.

This work was supported by a grant (ST2J-0457-6) from the Commission of European Communities

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123P LPS-INDUCED ACTIVATION OF THE L-ARGININE/NO PATHWAY IN ENDOTHELIUM DENUDED ARTERIAL TISSUE IS TIME DEPENDENT AND REQUIRES *DE NOVO* PROTEIN SYNTHESIS

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Administration of *E. coli* endotoxin (LPS) to rats *in vivo* (Julou-Schaeffer et al., 1990) and incubation of arterial segments with LPS *in vitro*, results in depressed contractile responsiveness to noradrenaline (NA). This hyporeactivity is associated with increased tissue content of cyclic GMP and is subsequent to activation of the L-arginine/NO pathway in non-endothelial cells (Fleming et al., 1990). The aim of this study was to investigate the time course and the role of protein synthesis in LPS-induced hyporeactivity *in vitro*. Endothelium denuded aortic rings from male Wistar rats were incubated with LPS (100 ng ml^{-1}) for periods of between 30 min and 5h, or for 4h in the presence or absence of cycloheximide (100 μg ml^{-1}). Contractile responses were then obtained to NA (1 nM to 10 μM) as previously described (Fleming et al., 1990). L-arginine (1 mM), substrate for NO production, was added to the peak stable contraction followed by methylene blue (MB, 10 μM) an inhibitor of soluble guanylate cyclase. In some experimentscycloheximide was present throughout contraction studies. Developed tension is expressed as g per mg dried tissue. Sensitivity to NA was time dependently reduced from 3h of incubation onwards (pD2 values being 8.17±0.27 and 7.22±0.11 in rings incubated for 3h in the absence and presence of LPS respectively, $P<0.001$, n=6). A significant depression in the maximum contractile response (E_{max}) was not seen until 4h (E_{max} being 3.59±0.72 and 2.40±0.23 g mg-1 dried tissue, incubated in the absence and presence of LPS respectively, $P<0.05$, n=6) and was associated with the appearance of an L-arginine induced relaxation (48±8%, $P<0.001$, n=6). E_{max} was restored to control upon addition of MB (contraction being 2.34±0.21 to NA and 3.59±0.25 after addition of MB, $P<0.001$, n=6). LPS-induced hyporeactivity was prevented when cycloheximide was present throughout incubation and contraction studies and was reversed if cycloheximide was present during contractile studies only. In addition, vascular hyporeactivity reappeared 3 to 4h after cycloheximide was removed from the bathing medium.

The delayed appearance of hyporeactivity and its reversal by cycloheximide suggests that LPS-induced activation of the L-arginine/NO pathway in endothelium denuded arterial tissue requires de novo protein synthesis.

Supported by the Commission of European Communities (ST2J-0457-6)

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124P PRECONDITIONING OF THE ISCHAEMIC MYOCARDIUM: EFFECT OF N^{ω} NITRO-L-ARGININE METHYL ESTER (L-NAME)

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Short (5 min) periods of coronary artery occlusion protect the myocardium against the serious ventricular arrhythmias that result from more prolonged periods of ischaemia, a phenomenon known as 'preconditioning' (Komori et al., 1990). In a previous communication to the Society (Vegh et al., 1990) we showed that a major part of this protective effect of preconditioning is due to the release of substances from the heart that are derived from arachidonic acid. However, since a reduced incidence of ventricular fibrillation (vf) is still apparent during ischaemia even when the cyclooxygenase pathway is inhibited, the possibility exists that other endogenous myocardial protective substances are also involved. One possibility is nitric oxide. We have examined this in large (24 ± 1.5 kg; range 16 - 32 kg) mongrel open chest dogs anaesthetised with chloralose and subjected to two 5 min preconditioning occlusions of the left anterior descending coronary artery, with a 20 min reperfusion period between , followed 20 min later by a prolonged (25 min) occlusion. N^{ω} nitro-L-arginine methyl ester (L-NAME), an inhibitor of vascular nitric oxide production (Schulz et al. 1990) was given in a dose of 10 mg kg^{-1} as an intravenous injection, 10 min before the prolonged occlusion in an attempt to inhibit the production of nitric oxide via the L-arginine pathway. This dose of L-NAME substantially increased blood pressure (by a mean 26 ± 4 mmHg from a pre-injection level of 99 ± 6 mmHg) and resulted in a bradycardia (-19 ± 2 beats min^{-1} from a resting heart rate of 156 ± 7 beats min^{-1}) and in the appearance of occasional ventricular ectopic beats and transient ST-segment elevation, as assessed from epicardial leads.

Preconditioning markedly reduced the severity of the ventricular arrhythmias that occurred during the prolonged occlusion. This protective effect of preconditioning was still apparent in the presence of L-NAME; e.g. the incidence of ventricular fibrillation during occlusion was 2/10 (cf 4/10 in the control non-preconditioned dogs) and there was a marked reduction in the number of ventricular ectopic beats during the 25 min ischaemic period (133 ± 64 cf 445 ± 140; in the control group ($P < 0.01$) and 96 ± 22 in the preconditioned group without L-NAME). 75% of the dogs in the L-NAME group fibrillated during reperfusion (cf 100% in the control group and 60% in the preconditioned group without L-NAME).

We conclude that the protective effect of preconditioning on the ischaemic myocardium still occurs in the presence of an inhibitor of nitric oxide production and that there is at present no evidence that nitric oxide production participates to a major extent in the phenomenon of preconditioning.

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125P ISOPRENALINE RELAXATION OF RAT THORACIC AORTA IS ENDOTHELIUM-DEPENDENT, RELEASES NITRIC OXIDE AND RAISES CYCLIC GMP AND CYCLIC AMP

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Vasodilators have been classified into two distinct groups: those whose action requires the presence of an intact endothelium e.g. acetylcholine, and those independent of the presence of endothelium e.g. sodium nitroprusside. It is becoming apparent that a third group exists having both mechanisms e.g. calcitonin gene-related peptide. Isoprenaline has long been regarded as an "archetypal" endothelium-independent vasodilator (Furchgott & Vanhoutte, 1989). It probably acts by stimulating β-adrenoreceptors, activating adenylate cyclase and so increasing levels of cyclic AMP. We have investigated the relaxant effect of isoprenaline in rat thoracic aortic rings.

Rat thoracic aortic rings (approx. 4mm long) were suspended on wires (diam. 0.125mm) and equilibrated for 75 minutes in Krebs solution bubbled with 95% O_2/5% CO_2 at 37°C. Noradrenaline (10^{-7}M) was added and the contraction assessed for stability over a period of 15 minutes. Any tissues not exhibiting 80% relaxation of the noradrenaline-induced tone to acetylcholine (10^{-6}M) were discarded as having damaged endothelium. Some tissues had the endothelium removed by gentle abrasion. These showed no relaxation to acetylcholine. N^G-nitro-L-arginine (NO-L-Arg; 10^{-5}M), shown to inhibit the synthesis of nitric oxide (NO), or propranolol ($3x10^{-7}$M), a β-adrenoreceptor antagonist was added and 25 minutes allowed for equilibration before a dose/response curve to isoprenaline ($3x10^{-8}$-10^{-5}M) was constructed. Alternatively, a single concentration of isoprenaline (10^{-6}M), giving approximately 80% relaxation of the noradrenaline-induced contraction, was added and tissues removed and snap-frozen in liquid nitrogen at different time points to construct a time-course for cyclic nucleotide accumulation (scintillation proximity assay kit, Amersham).

Isoprenaline relaxed rat thoracic aortic rings only where the endothelium was intact (IC_{50} $2x10^{-7}$M). Propranolol ($3x10^{-7}$M) caused approximately a 10-fold parallel rightward shift in the isoprenaline dose/response curve with no diminution of the maximum response, indicating that this effect is mediated by a β-adrenoreceptor. NO-L-Arg (10^{-5}M) caused a rightward shift and a decrease in the maximum response in the dose/response curve to isoprenaline, implicating NO as the endothelium-derived relaxing factor. Cyclic nucleotide studies showed a 16-fold increase in cyclic GMP levels as well as a 4-fold increase in levels of cyclic AMP compared to control (control levels (mean ± s.e.m.) cyclic GMP 52 ± 9 fmol mg protein^{-1}, cyclic AMP 651 ± 38 fmol mg protein^{-1}).

In conclusion, although isoprenaline acts in an endothelium-independent fashion in some vascular tissues, in rat thoracic aorta it induces vasorelaxation by acting on the endothelium at a β-adrenoreceptor to release NO which stimulates cyclic GMP production. Whether the rise in cyclic AMP mediates the synthesis of NO or has any role in the relaxant response to isoprenaline remains unclear.

We thank Celltech Ltd for financial support. D.W.G. is an MRC student.

Furchgott, R.F. & Vanhoutte, P.M. (1989) FASEB J., **3**, 2007-2018

126P HUMAN α-CALCITONIN GENE-RELATED PEPTIDE (CGRP) RELAXES RAT THORACIC AORTA BY RELEASING NITRIC OXIDE, AND RAISES CYCLIC AMP AND CYCLIC GMP

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Calcitonin gene-related peptide (CGRP) relaxes rat thoracic aorta in an endothelium-dependent manner (Brain et al., 1985). The endothelium-derived relaxing factor (EDRF) for human α-CGRP may not be nitric oxide (NO), as it is for acetylcholine, as the second messenger accumulations are different, being cyclic GMP and cyclic AMP for acetylcholine and CGRP respectively (Griffith et al., 1985, Grace et al., 1987). However, recently we showed that human α-CGRP relaxation could be inhibited by N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine (NO-L-Arg), inhibitors of the synthesis of NO, and that this was reversed by L- but not D-arginine, consistent with an action mediated via NO and cyclic GMP (Gray & Marshall, 1990). The mechanism of action of human α-CGRP in rat aorta has been further investigated in the present experiments.

Rat thoracic aortic rings were suspended on wires in Krebs solution bubbled with 95% O_2/5% CO_2 at 37°C. Tissues not exhibiting 80% relaxation by acetylcholine (10^{-6}M) of the noradrenaline (10^{-7}M)-induced tone were discarded as having damaged endothelium. Haemoglobin (10^{-6}M) or methylene blue (10^{-5}M) was added before dose/response curves to acetylcholine ($3x10^{-9}$M-10^{-5}M), human α-CGRP ($3x10^{-9}$-$3x10^{-7}$M) and sodium nitroprusside ($3x10^{-9}$-10^{-6}M) were constructed. A time-course for cyclic nucleotide accumulation (scintillation proximity assay kit, Amersham) was obtained using concentrations of acetylcholine, human α-CGRP and sodium nitroprusside which gave about 80% relaxation.

Haemoglobin, known to bind NO, and methylene blue, a soluble guanylate cyclase inhibitor, increased the contraction to noradrenaline (10^{-7}M) by 30 and 36% respectively, consistent with a basal release of NO in rat thoracic aortic rings. Haemoglobin and methylene blue both inhibited the relaxant response to acetylcholine and human α-CGRP but not to sodium nitroprusside. In the cyclic nucleotide studies, acetylcholine ($3x10^{-8}$M) and sodium nitroprusside ($3x10^{-8}$M) gave a selective rise in cyclic GMP, between 14- and 16-fold above control levels (cyclic GMP 52 ± 9 fmol mg protein^{-1}, cyclic AMP 651± 38 fmol mg protein^{-1}; mean ± s.e.m.) at the optimum time point with no significant change in cyclic AMP levels while human α-CGRP ($3x10^{-7}$M) gave a smaller 10-fold increase in cyclic GMP levels as well as a 3-fold increase in levels of cyclic AMP at the optimum time-point.

It would appear from these data that human α-CGRP exerts its endothelium-dependent relaxant effects in rat thoracic aorta by releasing NO which stimulates soluble guanylate cyclase to raise cyclic GMP. It is unclear whether the increase in cyclic AMP is part of the transduction mechanism linking the CGRP receptor with vascular relaxation.

We thank Celltech Ltd for financial support. D.W.G. is an MRC student.

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127P ENDOTHELIUM-MEDIATED RELAXATION IS SELECTIVELY ALTERED BY THE FORMATION OF A NEO-INTIMA

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During neo-intima formation, an early and essential step in the development of atherosclerosis, endothelium-independent relaxations are preserved, whereas muscarinic endothelium-dependent nitric oxide (NO) biosynthesis and vascular relaxation are impaired. The present study was undertaken to determine the selectivity of these defects.

The neo-intima was induced by positioning a non-occlusive, flexible silicone collar around the left carotid artery of rabbits (Booth et al., 1989). The contralateral artery served as a control. It was identically manipulated, but not enclosed by a collar. Seven days later, rings were cut from the control and the left (central region of the collar) carotid artery, mounted in organ chambers and isometrically contracted with phenylephrine (0.35 μM) in the presence of indomethacin (3 μM). Subsequently, cumulative dose-relaxation curves to receptor mediated (acetylcholine, adenosine triphosphate (ATP), substance P) and non-receptor mediated (calcium ionophore A23187) inducers of endothelial NO biosynthesis were made. Concentrations producing half maximum relaxation (EC_{50}) were calculated. They are expressed as mean $\pm$ s.e.mean for six rabbits and were analysed with an analysis of variance.

When a neo-intima was present, a rightward shift of the dose-relaxation curve for acetylcholine was seen (EC_{50} 50 $\pm$ 9 nM for control, and 121 $\pm$ 33 nM for intima-bearing segments, $P < 0.01$). In contrast, the dose-relaxation curve for substance P (EC_{50} 0.19 $\pm$ 0.04 nM in control, and 0.37 $\pm$ 0.14 nM in intima-bearing segments) and for the calcium ionophore (EC_{50} 44 $\pm$ 12 nM in control, and 52 $\pm$ 7 nM in intima-bearing segments) were not altered in the presence of a neo-intima. The curve for ATP was even shifted to the left (EC_{50} 2.3 $\pm$ 0.9 μM in control, and 1.1 $\pm$ 0.2 μM in intima-bearing segments, $P < 0.05$).

These results indicate that in intima-bearing vessels endothelium-dependent relaxations are selectively altered. Furthermore, they suggest that the capacity to generate NO is initially preserved, and that the diffusion of NO is not impeded by the neo-intima. However, one of the first defects during the development of atherosclerosis might be localised at the level of the endothelial muscarinic receptors.

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128P ENDOTHELIUM-DEPENDENT HYPERPOLARIZATION AND RELAXATION TO ACETYLCHOLINE IN RABBIT ISOLATED CEREBRAL ARTERIES

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Stimulation of muscarinic receptors produces endothelium-dependent hyperpolarization in arterial smooth muscle (Bolton et al, 1984), which does not appear to be essential for the muscle relaxation which also follows the stimulation of these receptors. For example, smooth muscle hyperpolarization but not relaxation can be inhibited in the presence of either ouabain or a potassium-free Krebs solution (Feletou & Vanhoutte, 1988), whereas the relaxation but not the hyperpolarization can be blocked with either methylene blue or haemoglobin (Chen et al, 1988). These data can be explained by the release of a relaxing (EDRF) and a hyperpolarizing (EDHF) factor from endothelial cells. We have investigated the smooth muscle membrane response to acetylcholine (ACh) in cerebral arteries, their endothelium-dependence and role in relaxation.

In isolated segments of basilar artery under resting tension, acetylcholine did not alter smooth muscle tension until very high concentrations (>100μM) when it produced a small contraction. In the presence of sub-maximal contraction produced with either histamine or noradrenaline, endothelium and concentration-dependent relaxation was produced to ACh, 0.1-100μM. The EC_{50} values were 6.33 and 6.53 in the presence of contraction to noradrenaline (6.4mN, n=8) and histamine (11mN, n=63), larger than the values of between 7 and 8, found in peripheral arteries (e.g. Chen et al, 1988). Simultaneous intracellular recordings revealed membrane hyperpolarization which was initiated before (1-6 seconds) the onset of relaxation, was concentration and endothelium-dependent and not produced with concentrations of ACh below 1μM. In contrast, this concentration of ACh had already relaxed segments by 30%. Almost complete relaxation was produced with 100μM ACh, which was associated with a hyperpolarization of around 8mV. The relaxation to ACh was sustained and reproducible, whereas the hyperpolarization was relatively transient and desensitized with repeated applications of ACh. Hyperpolarization but not relaxation was also observed to ACh under resting tension.

These data show that higher concentrations of ACh are required for equivalent endothelium-dependent responses in cerebral compared to peripheral arteries, which may reflect differences in the release of vasoactive factors from the endothelium or in the sensitivity of the underlying smooth muscle cells.

This work was supported by the Wellcome Trust. CJG is a Nuffield Foundation Science Research Fellow.

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129P EFFECT OF L-N^G-NITROARGININE METHYL ESTERS (L-NAME) UPON BASAL BLOOD FLOW AND ENDOTHELIUM-DEPENDENT AND -INDEPENDENT VASODILATOR AGENTS IN THE DOG HINDLIMB

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L-N^G-nitroarginine methyl ester (L-NAME) has been reported to be a potent and stereospecific inhibitor of endothelium-derived relaxing factor (EDRF) in vitro (Moore et al., 1990). We have examined the effect of L-NAME on (i) basal blood flow, (ii) reactive hyperaemia (RH) following arterial occlusion and (iii) responses to endothelium-dependent (bradykinin (Bk), acetylcholine (ACh)) and -independent (sodium azide (Az), adenosine (Ad)) vasodilators in the skeletal muscle vasculature of the beagle dog. Animals were anaesthetised with pentobarbitone, pretreated with indomethacin (5mg/kg i.a.) and the hindlimbs were denervated and skinned. All major arteries to the right hindlimb were ligated, except the external iliac, through which blood flow was measured (Doppler) and drugs or vehicle (saline) were administered locally. During saline infusion (0.5ml/min i.a.) vasodilator responses to single, submaximal bolus doses of Bk (30-300ng), ACh (30-300ng), Az (3-30µg) and Ad (0.3-1mg), and RH following a 60s period of total iliac artery occlusion, were measured until reproducible and then repeated during infusion of L-NAME (0.1-3.0mg/min i.a.). Results are shown in Table 1.

Table 1 Effect of L-NAME on B.P., basal flow, peak RH and agonist-induced peak dilator responses in the dog hindlimb

Dose of	Blood Pressure	Responses expressed as % of pre-L-NAME values					
L-NAME (mg/min)	(mmHg)	Basal flow	Bk	ACh	Az	Ad	RH
saline	112±6	100	100	100	100	100	100
0.1	115±3	84±5	106±3	86±8	106±5	117±7	85±14
0.3	116±6	59±3*	78±11	61±10*	101±8	112±9	86±18
1.0	128±4*	51±7*	57±11*	55±8*	82±2*	91±7	86±18
3.0	140±5*	54±8*	59±10*	67±11*	94±15	100±15	92±11

Values represent mean ± s.e.m. (n=4). *Significantly different from pre-L-NAME values ($P<0.05$; paired t-test).

L-NAME dose-dependently reduced basal blood flow and inhibited dilator responses to Bk and ACh by about 45%. Responses to Az, Ad and RH were largely unaffected. L-NAME (1 and 3mg/min) significantly increased blood pressure without affecting heart rate. The effects of L-NAME were diminished by prior but not by subsequent infusion of L-arginine (100mg/min i.a.). These data suggest that EDRF plays some role in regulating basal blood flow and in mediating the vasodilator responses to Bk and ACh in the skeletal muscle vasculature of the dog hindlimb. The substantial component (~50%) of the peak vasodilator responses to Bk and ACh unaffected by L-NAME, may be independent of EDRF or mediated by an EDRF-dependent, but L-NAME-insensitive mechanism.

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130P ENHANCEMENT OF ARTERIAL ENDOTHELIAL BARRIER FUNCTION BY β-ADRENOCEPTOR AGONISTS

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Drugs that inhibit vascular permeability have therapeutic potential in the treatment of conditions involving endothelial barrier dysfunction such as atherosclerosis and inflammatory oedema. We have recently developed an in vitro technique which permits the effects of drugs to be examined on the permeability characteristics of cultured endothelial monolayers (Gudgeon & Martin, 1989). Using this technique, we have attempted to characterise the nature of the β-adrenoceptor which inhibits transfer of high molecular weight solutes across bovine aortic endothelial cell (BAEC) monolayers.

BAEC were grown to confluence on Costar Transwell membranes (3µm pore size). For experimentation, 100µl of trypan blue (4%)-labelled albumin in HEPES-buffered Krebs was added to the upper chamber, and transfer across the monolayer during a 90 min incubation period was measured colourimetrically at 590nm.

Unstimulated equilibration of albumin across endothelial monolayers was 9.8±1.0%, n=42, and this was increased by 3-5 fold by phorbol myristate acetate (PMA, 0.6µM). Noradrenaline, adrenaline and isoprenaline each inhibited completely PMA-stimulated transfer of albumin with EC_{50} values of 0.4, 0.8 and 0.6µM, respectively. The selective β_2-adrenoceptor agonist, salbutamol, was also a full agonist (EC_{50} 16.1 µM) but the selective β_1-adrenoceptor agonist, dobutamine (100µM), inhibited PMA-induced transfer of albumin by only 37.7±4.4%, n=12. The selective β_2-adrenoceptor blocker, ICI 118551 (5nM-5µM) produced parallel shifts in the concentration-response relationship to isoprenaline (0.1-1000µM), indicating a pA_2 value of 8.2. In contrast, the selective β_1-adrenoceptor blocker, atenolol (1-100µM), only weakly antagonised the effects of isoprenaline.

In conclusion, the rank order of potency of β-adrenoceptor agonists, and the relative antagonistic potencies of ICI 118551 and atenolol, suggest that bovine aortic endothelial cells contain β_2-adrenoceptors, activation of which leads to reduced transfer of macromolecular solutes.

This work was supported by the British Heart Foundation. J.F.L. has an SERC studentship.

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131P PHARMACOLOGICAL CHARACTERISATION OF RU 24969-INDUCED HYPERLOCOMOTION IN MICE

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RU 24969, a high affinity ligand for 5-HT1B and 5-HT1A receptors, produces marked locomotor activity which has been extensively characterised in rats, but not mice (Goodwin and Green, 1985; Tricklebank et al., 1986). This has now been studied by determining the effects of antagonists and 5-HT depletion on RU 24969-induced hyperlocomotion in mice.

Locomotion was measured using pairs of male C57/Bl/6 mice (20-30g) for 60 min after RU 24969 (i.p.) with a photocell activity monitoring system. 5-HT neurones were destroyed by intracerebroventricular (i.c.v.) injection of 5,7-dihydroxytryptamine (5,7-DHT; 75µg), or depleted with parachlorophenylalanine (PCPA; 200mg/kg i.p.) for 14 days. Controls received vehicle (4µl i.c.v.) or saline (0.25ml i.p.). Brain monoamine concentrations were determined by HPLC-ECD.

Table 1 Effects of various monoamine receptor antagonists on RU 24969-induced hyperlocomotion in mice

Antagonist (mg/kg i.p.)	Control	Drug-treated	Antagonist (mg/kg i.p.)	Control	Drug-treated
Pindolol (4)	5070±220	3217±272***	Metergoline (3)	4713±310	4325±372
Spiroxatrine (0.1)	4918±358	2265±134***	Prazosin (1)	5044±211	2496±290***
Ritanserin (0.1)	4614±508	4246±355	Idazoxan (1)	5331±437	4426±213
Ondansetron (1)	4961±263	5257±276	SCH 23390 (0.05)	4734±158	1100±232***
Methysergide (3)	5026±239	5370±243	BRL 34778 (0.03)	5162±266	1133±170***

Activity counts±s.e.mean, n=8-12. Antagonist dose given 30min prior to RU 24969 (7.5mg/kg i.p.). ***P<0.001 vs control.

The locomotor response to RU 24969 (1-30 mg/kg i.p.) was bell-shaped with a peak at 10mg/kg. It was accompanied by hunched posture, sniffing and grooming. The response was inhibited by 5-HT1, but not other 5-HT antagonists and was attenuated by prazosin (alpha1), SCH 23390 (D1), BRL 34778 (D2), but not idazoxan (alpha2) (Table 1). 5,7-DHT lesioning and repeated PCPA reduced brain 5-HT by 73% and 75%, respectively. Neither procedure altered the locomotor response to RU 24969 (3.75 or 7.5 mg/kg).

Thus, the data suggest that RU 24969 produces hyperlocomotion by activating 5-HT1 receptors. Since 5-HT1A agonists do not evoke behavioural excitation in mice (Goodwin and Green, 1985) and the effect of spiroxatrine was probably due to its known D2 inhibition (Leysen et al., 1977), they are likely to be of the 5-HT1B subtype. 5-HT depletion studies revealed that the receptors are postsynaptic and do not show denervation supersensitivity. In agreement with Tricklebank et al. (1986), we believe that catecholamine neurones are essential for the expression of this behavioural response.

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132P THE ACTION OF 5-CARBOXAMIDOTRYPTAMINE (5-CT) ON ADULT RAT FACIAL MOTONEURONES *IN VITRO* DIFFERENTIATES TWO DISTINCT DEPOLARIZING ACTIONS OF 5-HT

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Intracellular current-clamp recordings from adult rat facial motoneurones (FM's) in vitro have shown 5-HT and nor-adrenaline (NA) to evoke depolarizations mediated primarily by potassium channel closure. However, only the reversal potentials for NA (E_{NA}) were in close agreement with the predicted E_{K+} and these values for $E_{5\text{-}HT}$ were often more negative (Larkman and Kelly, 1990). We have further investigated the action of 5-HT using the single-electrode voltage clamp technique. FM's were voltage-clamped at or near to their resting potentials. (V_H =-63.6±7.5mV, n=11. mean ± SD). Hyperpolarizing step voltage commands evoked currents which displayed an initial peak followed by a time dependent relaxation which reached a steady-state within the voltage step (0.5 - 1.5 sec). The amplitude of the inward relaxation, which activated between -60 and -75mV, increased with hyperpolarization and underlies the characteristic inward rectification (IR) seen in current clamp records. Superfusion of 5-HT (50 to 200µM) evoked a reversible inward current (-0.2 to -1.4nA; n=11). In 5-HT, peak current-voltage plots indicated that a decrease in conductance was associated with the inward current. However the steady-state relationships suggested a conductance increase, especially at hyperpolarized potentials. Subtraction of control from 5-HT current-voltage plots gives a measure of the 5-HT sensitive current ($I_{5\text{-}HT}$) at different membrane potentials. Peak $I_{5\text{-}HT}$ appeared linear reversing at -96.3±17.4mV. The steady-state $I_{5\text{-}HT}$ showed similarity to the peak $I_{5\text{-}HT}$ close to V_H but then increased dramatically with hyperpolarization indicating a time and voltage dependent component to the total $I_{5\text{-}HT}$. The inward rectifier can be abolished by addition of Cs^+(2-5mM) to the aCSF. This treatment also blocked the 5-HT-evoked time and voltage dependent conductance increase leaving the inward $I_{5\text{-}HT}$ to be associated with only a conductance decrease.

5-CT, a general 5-HT_1 receptor agonist, depolarizes FM's. Under voltage clamp 5-CT evoked an inward current when V_H was within the activation range of IR and was associated only with an increase in conductance (n=3). If V_H was kept above the activation range of IR, 5-HT but not 5-CT, evoked an inward current. However hyperpolarizing voltage steps into the activation range of IR showed a clear increase in the steady- state current in the presence of 5-CT as well as 5-HT.

The results suggest that 5-HT evokes depolarization of FM's through a combination of mechanisms one involving closure of K^+ channels the other an enhancement of IR. Selective enhancement of IR by 5-CT suggests that two distinctly different 5-HT receptors are linked to the two different ionic mechanisms.

Larkman, P.M. and Kelly, J.S. (1990) Neurosci Letts Suppl. 38, S117

133P THE EFFECT OF 5-HT_1 RECEPTOR AGONISTS ON STIMULATED 5-HT OVERFLOW STUDIED USING FAST CYCLIC VOLTAMMETRY

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We have recently reported the use of FCV to measure electrically stimulated overflow of endogenous 5-hydroxytrytamine (5-HT) in untreated slices of rat brain (O'Connor & Kruk, 1990). Here we report the effect of putative 5-HT_1 receptor ligands on stimulated 5-HT overflow in somatodendriditic and axon terminal regions of the rat brain.

Rat brain slices (350μm) containing the dorsal raphe (DRN) or suprachiasmatic nuclei (SCN) were superfused with oxygenated ACSF at 32^0C. Stimulating electrodes were placed centrally in the DRN and SCN 80μm below the surface. 5-HT release was monitored with a carbon fibre microelectrode 100 to 200μm from the stimulating electrodes. 500ms trains of square waves (0.1ms; 50Hz; 20V) were applied every 5 minutes. Reproducible evoked release of 5-HT could be measured for more than 6h. Extracellular 5-HT concentrations of 40-60nM were evoked from each region. Table 1 shows the effects of putative 5-HT_1 receptor ligands on evoked 5-HT overflow.

Table 1 Effects of 5-HT_1 receptor ligands on evoked 5-HT overflow

		8-OHDPAT	RU 24969	5-CT	Buspirone
DRN	Maximum inhibition (%)	44.2 ± 4.3*	29.0 ± 5.8	47.2 ± 5.7	42.7 ± 7.8
	IC_{50} (x10^{-8}M)	2.5 ± 0.6	3.9 ± 1.9	2.1 ± 0.3	6.5 ± 3.5
SCN	Maximum inhibition (%)	24.2 ± 3.2	50.7 ± 6.0+	64.4 ± 6.5	24.0 ± 9.5
	IC_{50} (x10^{-8}M)	3.6 ± 1.8	1.4 ± 0.2	6.7 ± 1.6	14.0 ± 8.5

IC_{50} : concentration of drug required to reduce overflow to 50% maximum. n = 3-5 for all; * = $P<0.01$ compared to 8-OHDPAT (SCN); + = $P<0.05$ compared to RU 24969 (DRN).

8-OHDPAT, (a 5-HT_{1A} receptor agonist ; Sprouse & Aghajanian, 1986) had a greater inhibitory effect than the 5-HT_{1B} ligand, RU 24969 (Tricklebank et al., 1986) in the DRN while the converse applied to the SCN. The results are in agreement with the reported abundance of 5-HT_{1A} and $_{1B}$ receptors in the DRN and SCN respectively. None of the drugs behaved as a full agonist under these experimental conditions, as it is likely that endogenous 5-HT was activating autoreceptors in these experiments. Studies employing single pulse stimulation are in hand. The results presented here indicate that subtypes of 5-HT_1 receptors may have a role in the regulation of 5-HT release at somatodendritic and axon terminal sites in the rat brain.

Supported by Glaxo.

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134P ELECTROPHYSIOLOGICAL ACTIONS OF MDL 73005EF AT RAT HIPPOCAMPAL 5-HT_{1A} RECEPTORS

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MDL 73005EF is a new benzodioxane derivative, exhibiting high affinity and selectivity for 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors and potent anti-anxiety effects in rats (Moser *et al.*, 1990). The object of the present experiments was to determine the actions of MDL 73005EF on CA1 pyramidal neurones in rat hippocampus, where 5-HT_{1A} receptors are abundant (Pazos & Palacios, 1985).

The actions of 5-HT and MDL 73005EF on neuronal activity in the CA1 region of rat hippocampal slices in vitro (32°C) were recorded using intra- and extracellular recording techniques. Intracellular recordings using 4M KAc-filled microelectrodes demonstrated that 5-HT (1-30 μM) hyperpolarized the pyramidal neurones (≤ 10 mV) and reduced membrane resistance and action potential after-hyperpolarizations. A small depolarization sometimes followed the hyperpolarization. MDL 73005EF (1-30 μM) had no clear effects on membrane potential, membrane resistance or after-hyperpolarizations and thus did not mimic the actions of 5-HT. On the contrary, superfusion of the slices with 3 μM MDL 73005EF (10-60 min) antagonized the hyperpolarization induced by 30 μM 5-HT but not the reduction in spike after-hyperpolarization, the membrane depolarization or the hyperpolarization induced by the $GABA_B$-receptor agonist baclofen

Recordings using 3M KCl-filled microelectrodes revealed that 3 μM MDL 73005EF and 3 μM spiperone reduced the frequency and amplitude of spontaneous inhibitory (bicuculline-sensitive) post-synaptic potentials. Extracellular recordings of population action potentials showed that MDL 73005EF did not prevent the induction and maintenance of hippocampal long-term potentiation.

It is concluded that MDL 73005EF is an antagonist at 5-HT_{1A} receptors on hippocampal CA1 pyramidal neurones. This action, combined with the previously reported reduction in the firing of serotonin-containing dorsal raphe neurones (Sprouse *et al.*, 1990), could reduce central serotonergic tone and possibly explain the observed anti-anxiety effects of this compound.

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135P NAN-190 ANTAGONISES THE HYPERPOLARIZATION MEDIATED BY 5-HT_{1A} RECEPTORS ON RAT DORSAL RAPHÉ NEURONES

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NAN-190, (1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine), a compound structurally related to buspirone has been proposed as an antagonist at 5-HT_{1A} receptors (Glennon et al, 1988). Recent behavioural studies have suggested that NAN-190 may function either as an antagonist at post-synaptic $5HT_{1A}$ sites in the brain and an agonist at presynaptic somatodendritic $5HT_{1A}$ autoreceptors (Hjorth & Sharp, 1989) or as a partial agonist (Deans et al, 1989).

We have recorded intracellularly from dorsal raphe neurones (DRN's) in slices maintained in vitro to determine directly whether NAN-190 is an agonist at the autoreceptor on DRN's. Slices (350-400μm thick) containing the DR nucleus were placed at the gas-liquid interface of the slice chamber and were perfused with artificial cerebrospinal fluid (a.c.s.f.) at 35°C, of the following composition (mM): NaCl, 134; KCl 5; KH_2PO_4, 1.25; $MgSO_4.7H_2O$, 2; $CaCl_2$, 2; $NaHCO_3$, 16; D-glucose, 10. NAN-190, synthesized in this laboratory, was dissolved in an ethanol: H_2O mixture (20 : 80), the appropriate volume of this stock solution was dissolved in a.c.s.f. just prior to application to the slice.

Ten neurones in as many slices were studied, nine responded to bath application of 5-HT (100μM) with a hyperpolarization and fall in input resistance. Four of these cells responded similarly to 8-OHDPAT (10μM). The inhibition produced by 8-OHDPAT was reversed in a dose-dependent manner by NAN-190 (5-50μM, 3 expts.)

Application of NAN-190 (50μM) in the absence of any other drug to a further 4 DRN's caused a hyperpolarization and fall in input resistance (Rm) in 2 expts., a depolarization and increase in Rm in a third but no detectable effect in a fourth neurone. Exposure of the preparation to NAN-190 reduced the response to 5-HT (2 expts.) and 8-OHDPAT (1 expt.). After pretreatment of the preparation with the 5-HT- uptake inhibitor citalopram (10μM) NAN-190 exerted a powerful excitatory effect (1 expt.).

We conclude that NAN-190 acts as a partial agonist in the DR nucleus and speculate that when the tonic release of 5-HT, locally in the region of the impaled neurone, is raised the compound blocks the action of endogenous 5-HT and appears excitatory but when serotonergic tone is low its agonist actions are uncovered.

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136P EVIDENCE THAT PHARMACOLOGICAL ACTIVATION OF THE 5-HT_2 RECEPTOR INHIBITS NORADRENALINE RELEASE IN RAT HIPPOCAMPUS *IN VIVO*

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Several lines of evidence suggest that central noradrenergic neurones are under an inhibitory influence of the neurotransmitter 5-HT. For example, noradrenaline turnover, in rat brain, increases after electrolytic or chemical lesion of central 5-HT neurones, or inhibition of 5-HT synthesis (McRae-Degueurce *et al.*, 1985). In addition, in vivo electrophysiological studies have shown that 5-HT_2 receptor agonists, including the phenethylamines DOM and DOB, inhibit the firing rate of noradrenergic neurones in the locus coeruleus (Rasmussen & Aghajanian, 1986; Gorea & Adrien, 1988). Here we report the effect of the putative 5-HT_2 receptor agonist, DOI, on noradrenaline release measured in rat hippocampus in vivo using microdialysis.

A short-loop microdialysis probe was stereotaxically implanted into the ventral hippocampus of the chloral hydrate anaesthetized rat and was continuously perfused with artificial CSF (1.2 μl/min), containing the noradrenaline uptake inhibitor, desipramine (5 μM). Dialysates were collected every 20 min and analysed for noradrenaline by HPLC-EC. Following a baseline period of 2-3 h drugs were administered subcutaneously, antagonists being injected 40 min before DOI.

DOI (0.02-2mg/kg) induced a dose-dependent decrease of noradrenaline in hippocampal dialysates with the maximum effect (at t=60 min) being about 50% of pre-drug levels and long-lasting (>120 min). Pretreatment with the 5-HT_2/5-HT_{1C} antagonist, ritanserin (0.4 mg/kg), completely blocked the effect of DOI (0.5 mg/kg). The 5-HT_2/D_2 antagonist, spiperone (0.2 and 1 mg/kg) also blocked, dose-dependently, the effect of DOI (0.5 mg/kg). Sulpiride (50 mg/kg), a D_2 antagonist, did not alter the response to DOI (0.5 mg/kg).

These results show that DOI decreases noradrenaline in rat hippocampal dialysates, in a ritanserin- and spiperone-sensitive manner. Our data suggest that in vivo noradrenaline release is inhibited by 5-HT_2 receptor activation, this probably being associated with a decrease in noradrenergic neuronal activity (Rasmussen & Aghajanian 1986; Gorea & Adrien, 1988).

C. Done is an MRC student funded by SmithKline Beecham.

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137P EFFECTS OF CUMULATIVE DOSES OF THE 5-HT_2 AGONIST DOI, INTO THE IVTH VENTRICLE, ON AUTONOMIC OUTFLOW IN ANAESTHETIZED CATS

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Investigation of the role of central $5HT_{1A}$ and 5-HT_2 receptors in cardiovascular control indicates that these receptors have opposing actions on sympathetic outflow, 5-HT_{1A} receptor activation causes sympathoinhibition (see Ramage, 1990) whilst i.v. administration of the 5-HT_2 receptor agonist DOI, causes sympathoexcitation (McCall & Harris, 1988). As both i.v. and IVth ventricular administration of 5-HT_{1A} agonists produce a similar pattern of sympathoinhibition, it was concluded that the 5-HT_{1A} receptors primarily responsible for this effect are located in the brainstem (Shepheard *et al.*, 1989). The possibilty that 5-HT_2 receptors at the level of the brainstem are involved in producing the sympathoexcitatory effects of DOI are investigated in this study.

Cats anaesthetized with α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (6 mg kg^{-1}) i.v. were artificially ventilated after neuromuscular blockade with vecuronium bromide (200 μg k^{-1}). Simultaneous recordings were made of cardiac (CNA), splanchnic (SNA), renal (RNA) and phrenic nerve activity (PNA), blood pressure, heart rate, femoral arterial conductance (FAC) tracheal and intragastric pressures. Cumulative doses of DOI (80-320 nmol kg^{-1}) were given into the IVth ventricle by microinjection (20 μl over 1 min).

DOI caused an increase in mean blood pressure of 14 ± 2 mmHg, a decrease in FAC of -22 ± $7x10^{-3}$ ml $mmHg^{-1}$ min^{-1} and an increase in tracheal pressure of 2.4 ± 0.2 cmH_20. These changes were associated with no change in sympathetic nerve activity. However, a control infusion of noradrenaline (18 μg ml; i.v.) producing a similar rise in blood pressure as that caused by DOI caused an approximately 50% decrease in sympathetic nerve activity. This indicates that a baroreceptor reflex, elicited by the rise in blood pressure caused by DOI, could be masking the sympathoexcitatory action of DOI. The rise in blood pressure and the decrease in femoral arterial conductance were only partially reversed, whilst the rise in tracheal pressure was completely reversed, by the peripherally acting 5-HT_2 antagonist BW501C67 (2 mg kg^{-1}; i.v.; Mawson & Whittington, 1970).

These results indicate that activation of 5-HT_2 receptors at the level of the brainstem causes sympathoexcitation.

This work was supported by the Wellcome Trust.

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138P 2-METHYL-5-HT IS A PARTIAL AGONIST AT 5-HT_3 RECEPTORS IN N1E-115 NEUROBLASTOMA CELLS

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Both physiological and ligand-binding studies have demonstrated the presence of high concentrations of 5-HT_3 receptors on N1E-115 mouse neuroblastoma cells (Neijt *et al*, 1988; Lambert *et al*, 1989; Lummis *et al*, 1990). We have characterised these receptors using whole cell voltage clamp and radiolabelled binding studies with the specific 5-HT_3 ligand [^{3}H]GR65630. Application of 5-HT to N1E-115 cells clamped at -60mV causes a rapid, transient inward current with an EC_{50} (concentration which yields 50% maximum response) of 4.1 ± 0.6 x 10^{-6}M (n=6), similar to values previously reported (Lambert *et al*, 1989). The 5-HT_3 receptor antagonists BRL43694 and GR67330 block the response to 1.0 x 10^{-5}M 5-HT at 1.0 x 10^{-9}M and 1.0 x 10^{-10}M respectively.

2-Methyl-5-HT and the novel 5-HT_3 receptor agonist, m-chlorophenylbyguanide (mCPBG) (Kilpatrick *et al*, 1990), elicit similar inward currents and have a similar reversal potential to 5-HT (approximately 0mV). The EC_{50}s of 2-methyl-5-HT (1.7 ± 0.3 x 10^{-5}M) and 5-HT compare well with their IC_{50}s (that concentration which inhibits 50% specific [^{3}H]GR65630 binding) of 2.0 ± 0.5 x 10^{-6}M for 2-methyl-5-HT and 7.2 ± 0.6 x 10^{-7}M for 5-HT (n=3). However, mCPBG is about 1000 times less potent than expected from the binding data (EC_{50}: 1.4 ± 0.2 x 10^{-6}M;IC_{50}:4.8 ± 0.8 x 10^{-10}M). The application of 5-HT and mCPBG to the same cell elicit currents of similar maximum amplitude, whilst 2-methyl-5-HT evokes a current of 19.9 ± 2.4% (n=9) of that evoked by 5-HT. Dose-response curves to 5-HT, 2-methyl-5-HT and 5-HT in the presence of 2-methyl-5-HT (1.0 x 10^{-4}M) performed in the same cell, show that in this cell line 2-methyl-5-HT acts as a partial agonist at the 5-HT_3 receptor.

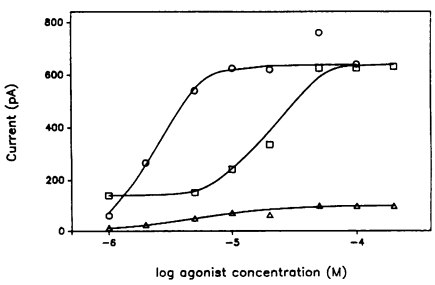


Figure 1: Dose-response curve in the same cell for 5-HT (o); 2-methyl-5-HT (Δ) and 5-HT in the presence of 1.0 x 10^{-4}M 2-methyl-5-HT ( ).

Thus the N1E-115 5-HT_3 receptors show some unexpected characteristics. The novel 5-HT_3 agonist mCPBG is much less potent than expected from binding studies and 2-methyl-5-HT, the selective 5-HT_3 agonist, acts as a partial agonist.

M.-I. Sepulveda is a Glaxo funded fellow.

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139P KETAMINE POTENTIATES 5-HT$_3$ RECEPTOR-MEDIATED MEMBRANE CURRENTS RECORDED FROM ADULT RABBIT NODOSE GANGLION NEURONES

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The dissociative anaesthetic ketamine exerts pharmacological actions which include voltage-dependent blockade of nicotinic cholinoceptor and N-methyl-D-asparate (NMDA) receptor-ion channel complexes (Maleque et al., 1981; MacDonald et al., 1987), and the suppression of monoamine uptake systems (Azzaro & Smith, 1977). In this study, the influence of ketamine upon 5-HT$_3$ receptor mediated currents recorded from voltage-clamped adult rabbit nodose ganglion neurones (Malone et al., 1990) was examined.

Patch electrode techniques, in conjuction with either List Electronics L/M EPC-7 or Axoclamp 2A recording systems, were employed to voltage-clamp isolated nodose ganglion neurones maintained in culture for 1-7 days. Recordings were made at a holding potential of -60mV and 17-23°C. Quantitative data are given as the mean ± s.e.m.

Bath applied ketamine, at concentrations of 3, 10 and 30μM, reversibly potentiated the amplitude of submaximal inward currents evoked by pressure applied 5-HT (10μM) to 110.5 ± 2.0 (n = 4), 170.0 ± 8.3 (n = 8) and 196.4 ± 7.7% (n = 9) of control respectively. The duration of 5-HT-induced currents was also enhanced, such that the total charge passed during a response increased to 158.0 ± 5.3 (n = 4), 326.8 ± 19. 4 (n = 8) and 385.0 ± 32.4% (n = 9) of control in the presence of 3, 10 and 30μM ketamine respectively. Within this concentration range, potentiation was well maintained throughout the period of ketamine application (ca. 5 min) and occurred in the absence of any change in the reversal potential of the 5-HT-induced current. The response to higher concentrations of ketamine (100-300μM) was complex, consisting of an initial potentiation, followed by a decline in current amplitude toward or below, control values, and terminating in a further potentiation during wash-off of the drug. Ketamine (10μM) also potentiated currents evoked by 5-HT (1μM) and the 5-HT$_3$ receptor selective agonists 2-methyl-5-HT (2μM) and 1-phenylbiguanide (30μM) applied by rapid superfusion, rather than pressure ejection. Responses to pressure applied 5-HT were unaffected by the NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (30μM; n = 3), but were slightly depressed to (93.3 ± 3.2% of control n = 3) by the NMDA channel blocker MK-801 (30μM). The 5-HT uptake inhibitor citalopram (0.01 - 1.0μM) did not mimic the action of ketamine (n = 3), nor in preliminary experiments did the inclusion of citalopram (1μM) or paroxetine (30nM) in the perfusate block the potentiation to ketamine (30μM).

In conclusion, these results demonstrate potentiation of 5-HT$_3$ receptor mediated currents in rabbit nodose ganglion neurones by ketamine via a mechanism not obviously related to the inhibition of 5-HT uptake.

Supported by the Wellcome Trust (JJL) and a Dundee University Research Grant (JAP).

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140P CHARACTERIZATION OF THE 5-HT RECEPTOR SUBTYPES INVOLVED IN THE PRODUCTION OF BACK MUSCLE CONTRACTIONS: A SPINAL MOTOR BEHAVIOUR IN THE RAT

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We have recently shown that intrathecal (i.t.) injection of 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT), a non-selective 5-hydroxytryptamine (5-HT) agonist, causes brief caudo-rostral contractions of back muscles (BMC) in conscious rats (Fone *et al.*, 1989). The present study further characterizes the 5-HT receptor subtype/s involved in the production of BMC.

In adult male Wistar rats (280-350 g) with indwelling i.t. cannula (Fone *et al.*, 1989) the number of BMC and wet-dog shakes (WDS) were counted separately for 30 min following i.t. (10 μl + 15 μl saline) injection of either 5-HT (0.5 to 200 μg, n=6 given 30 min after the 5-HT uptake inhibitor fluoxetine, 5 mg kg^{-1} i.p.), 5-MeODMT (2 to 100 μg, n=8) or agonists with high affinity for 5-HT$_1$-like receptors 8-hydroxy-2-(di-N-propylamino)tetralin (8-OHDPAT, 0.1-100 μg, n=9), 1-(3-chlorophenyl)piperazine hydrochloride (mCPP, 10-100 μg, n = 5) or 5-carboxamidotryptamine (5-CT, 0.1-100 μg, n=6), selective 5-HT$_2$ receptor agonists 2,5-dimethoxy-α,4-dimethyl-benzene ethamine hydrochloride (DOM, 2-25 μg, n=7) or (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 1-50 μg, n=6) or the 5-HT$_3$ agonist 2-methyl-5-HT (2-Me-5-HT, 1-100 μg, n=7). Following these dose-response studies a selected dose of each agonist was re-examined after pretreatment with a selective 5HT$_2$ receptor antagonist. The behavioural response to fluoxetine (1-20 mg kg^{-1} i.p) was also examined in 6 rats with no i.t. cannula.

Systemic administration of fluoxetine produced dose-related BMC (reaching 27 ± 3 mean ± s.e.mean in 30 min with 20 mgkg^{-1}, $P<0.001$ from saline, Dunnett t test following ANOVA) which lasted for 20-30 min and were re-initiated in rats given high i.t. doses of 5-HT 30 min later (24 ± 11 with 50 μg, $P<0.05$ from fluoxetine + saline i.t.) without producing WDS. 5-MeODMT, DOI and DOM all produced dose-related BMC (ED_{50} values being 10.6, 8.5 and 11.0 μg, respectively) and WDS which were significantly attenuated by pretreatment with either ritanserin (1 mgkg^{-1} i.p.) or ketanserin (0.16 mgkg^{-1} i.p.), while in contrast mCPP, 8-OHDPAT, and 2-Me-5-HT did not induce either behaviour. 5-CT was the most potent agonist at eliciting WDS (from 10 ± 3 in 30 min with 1 μg to 91 ± 27 with 100 μg) yet it failed to evoke any BMC.

The present results demonstrate that BMC is another behaviour elicited by 5-HT which like WDS is mediated by 5HT$_2$ receptor activation. The finding that i.t. 5-CT elicits WDS but not BMC suggests i) that 5-CT acts on 5-HT$_2$ receptors *in vivo* and ii) is consistent with previous findings (Fone *et al.*, 1989) that i.t. pretreatment with a serotonergic neurotoxin only potentiates 5-MeODMT-induced BMC, and not WDS, suggesting that the 5-HT$_2$ receptors producing WDS and BMC have different locations within the CNS.

This work was funded by Action Research for the Crippled Child.

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141P EFFECT OF SELECTIVE 5-HT AGONISTS AND A THYROTROPHIN-RELEASING HORMONE ANALOGUE (RX77368) ON SINGLE SPINAL MOTONEURONES IN ANAETHETISED RATS

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Intrathecal injection of 5-hydroxytryptamine (5-HT) and 5-HT_2 agonists produces a behavioural syndrome including back muscle contractions (BMC) in the rat which are facilitated by pretreatment with the thyrotrophin-releasing hormone (TRH) analogue RX77368 [Fone *et al*, 1989]. Although electrophysiological studies show spinal motoneurones are excited by TRH and 5-HT this excitation is not blocked by 5-HT_2 antagonists [Connel & Wallis, 1989]. The present iontophoretic study examined the effect of 5-HT_2 agonists and a TRH analogue on spinal motoneurones.

Male Wistar rats were maintained under urethane anaesthesia (1.4mg/kg i.p.) and a dorsal laminectomy performed to record extracellular activity from motoneurones in the lumbar spinal cord using 7-barelled glass microelectrodes from which drugs were iontophoretically applied.

In all motoneurones 5-HT (5 - 80nA) and 2,5-dimethoxy-α,4-dimethylbenzene ethamine hydrochloride (DOM, 20 - 120nA) increased glutamate evoked neuronal firing (> 20% control) in relation to the magnitude of the ejection current applied. However, in some cells the excitation induced by 5-HT (6/9 cells) and DOM (2/8) was preceeded by a transient short-term inhibition of firing. The 5-HT_{1A} agonist 8-hydroxy-2-(di-N-propylamino)tetralin (8-OHDPAT, 10 - 100nA; n=5) and the 5-HT_1-like receptor agonist 1-(3-chlorophenyl)piperazine (mCPP, 10 - 75nA; n=3) failed to affect neuronal firing. The TRH analogue RX77368 (60nA) caused a sustained (up to 30min) increase in firing frequency (6/7 motoneurones), however, 3/7 cells displayed a short term inhibition of firing prior to excitation and 1 cell failed to respond. The second and subsequent iontophoretic applications of RX77368 produced smaller amplitude excitations than that elicited by the first application of RX77368 but the excitatory responses to 5-HT or DOM were unaffected in those cells desensitized to RX77368.

The excitation elicited by 5-HT and the lack of effect of 8-OHDPAT and mCPP is in agreement with previous data [Connel & Wallis, 1989] on spinal motoneurones. The present finding that the 5-HT_2 selective agonist DOM excited all motoneurones examined, suggests that activation of 5-HT_2 receptors results in the excitation of motoneurones. In support of this suggestion Elliot & Wallis (1990) have shown a ketanserin-sensitive 5-HT-induced excitation of spinal motoneurones. The TRH analogue RX77368 excited motoneurones but tachyphalaxis developed as previously reported for TRH [White 1985]. The excitatory effect of 5-HT and DOM remain unaffected after desensitization to RX77368 suggesting that TRH facilitates spinal motoneurone excitability via a separate TRH receptor. These present iontophoretic studies support behavioural data indicating BMC are elicited by an action on 5-HT_2 receptors located in the ventral horn of the spinal cord possibly on motoneurones.

We acknowledge Action Research for the Crippled Child for financial assistance and Reckitt and Coleman for RX77368.

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142P QUANTITATIVE AUTORADIOGRAPHIC DISTRIBUTION OF 5-HT_3 RECEPTORS IN RAT, FERRET AND DOG BRAIN

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$5HT_3$ antagonists are potent antiemetics, anxiolytics and antipsychotics (Costall et al., 1988) but their precise sites of action are unknown. We have used quantitative receptor autoradiography to map $5HT_3$ receptors in rat, ferret and dog brain using [^{3}H]quipazine ([^{3}H]QUIP; 0.8-1.2 nM + 0.1 μM paroxetine) and [^{3}H]GR65630 ([^{3}H]GR; 0.2-0.5 nM). The binding assays were performed in 50 mM Tris.Krebs for 1 h at 23°C. Non-specific binding was defined with either 1 μM (S)-zacopride (ZAC) or 50 μM metoclopramide (MET) and was high for [^{3}H]QUIP binding when defined with MET.

Table 1. Quantitative autoradiographic localization of $5HT_3$ receptors in the mammalian hind-brain

Brain Regions	Specific Radioligand Binding (amol/mm^2; means ± SEM, n=3-5)					
	Rat Brain		Ferret Brain		Dog Brain	
	[^{3}H]QUIP (+ ZAC)	[^{3}H]GR (+ ZAC)	[^{3}H]QUIP (+ MET)	[^{3}H]GR (+ MET)	[^{3}H]QUIP (+ MET)	[^{3}H]GR (+ MET)
Nuc. Solitary Tract (NTS)	6236 ± 412	4706 ± 324	1704 ± 217	4216 ± 289	1076 ± 286	4997 ± 122
Area Postrema (AP)	3109 ± 825	1441 ± 130	860 ± 149	1868 ± 267	330 ± 99	1299 ± 192
Dors. Motor Nuc. Vagus (DMX)	2898 ± 346	2114 ± 140	781 ± 151	1433 ± 225	587 ± 227	1622 ± 316
Spinal Trigeminal Nuc. (Sp5N)	1239 ± 178	728 ± 96	940 ± 164	2169 ± 293	Not detected	

The discrete localization of $5HT_3$ receptors in the vagal complex (NTS/AP/DMX) appears to correlate with the antiemetic sites for $5HT_3$ antagonists since cisplatin-induced emesis in the ferret and dog is antagonised by local injections of these drugs or by vagotomy (see Pratt et al., 1990). The relatively high density of $5HT_3$ sites in the rat basomedial amygdala & v.dentate gyrus of hippocampus (~1596 & ~580 amol/mm^2 with [^{3}H]QUIP and [^{3}H]GR respectively), correlate with data showing that injection of these drugs in the rat amygdala elicits anxiolytic effects (Costall et al., 1988). The amygdala may also mediate the inhibitory effects of $5HT_3$ antagonists on the withdrawal from drugs of abuse (Costall et al., 1988). The high level of $5HT_3$ sites in the rat entorhinal, piriform cortex (958-1118 & 405-450 amol/mm^2 with [^{3}H]QUIP and [^{3}H]GR respectively) and the Sp5N (Table 1) may represent sites for modification of cognitive and sensory functions respectively (Costall et al, 1988). The significance of the apparent lack of $5HT_3$ sites in the dog Sp5N is unknown at present. However, these data suggest that $5HT_3$ receptors are highly concentrated in the mammalian vagal complex (NTS in particular) and limbic areas and they may represent loci of action of $5HT_3$ antagonists.

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143P EFFECT OF CHRONIC TREATMENT WITH 5-HT$_3$ RECEPTOR ANTAGONISTS ON MONOAMINE CONCENTRATIONS IN VARIOUS REGIONS OF THE RAT BRAIN

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5-Hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonists may represent a new class of antipsychotic agents as they inhibit the hyperactivity resulting from raised mesolimbic dopamine levels but do not inhibit amphetamine-induced stereotypy nor induce catalepsy (Costall *et al.*, 1987a; 1987b). This has been interpreted as a selective interaction with mesolimbic, rather than nigro-striatal, dopamine systems. In contrast, Sorensen *et al.* (1989) have demonstrated that chronic, but not acute, treatment with MDL 73147EF (1-H-indole-3-carboxylic acid,trans-octahydro-3-oxo-2,6-methano-2H-quinolizin-8-yl ester methylsulphonate) inhibits dopaminergic cell firing in both the ventral tegmental area and the substantia nigra. The present study has examined the effect of acute and chronic treatment with 5-HT$_3$ receptor antagonists on the concentrations of monoamine transmitters and their metabolites in various regions of the rat brain.

Male Sprague-Dawley rats (160-180 g) received either saline, 1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate methylsulphonate (MDL 72222EF; 3 mg kg^{-1}), MDL 73147EF (1 or 3 mg kg^{-1}) or ondansetron (0.1 mg kg^{-1}) s.c. as a single dose or twice a day for 15 days. Rats were killed 1 h after the final treatment, their brains rapidly removed and the frontal cortex, cortex (i.e. remaining cortex), caudate-putamen, nucleus accumbens and hippocampus dissected out. The brain areas were stored at -80°C until assayed for dopamine (DA), 5-HT, noradrenaline, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA). Data were analysed with analysis of variance which, if significant, was followed by Fisher's Protected Least Significant Difference test.

The only significant changes following acute treatment were an increase in 5-HIAA/5-HT ratio in the cortex after MDL 73147EF and a decrease in DOPAC in the frontal cortex following MDL 72222EF and MDL 73147EF (1 mg kg^{-1}). The principal effects of chronic treatment are shown in Table 1. The only other statistically significant effects of chronic treatment were in the nucleus accumbens, where MDL 73147EF (3 mg kg^{-1}) increased the 5-HIAA/5-HT ratio and MDL 72222EF increased noradrenaline concentrations.

Table 1. Effects of MDL 73147EF and ondansetron on transmitter and metabolite concentrations in the frontal cortex and caudate-putamen. All values (except ratios) are in ng g^{-1} ± s.e.mean for n = 6. Asterisks indicate $P < 0.05$ compared to saline treated controls.

	Frontal cortex: 5-HT	5-HIAA/5-HT	DOPAC	HVA	Caudate: 5-HIAA/5-HT	DA	DOPAC	HVA
Saline	697±55	0.61±0.05	49±7	64±6	1.38±0.06	8455±313	2213±169	1187±54
MDL 73147EF 1 mg kg^{-1}	519±36*	0.86±0.04*	36±6	57±4	1.28±0.06	7648±352	1800±129	1093±46
MDL 73147EF 3 mg kg^{-1}	620±32	0.75±0.03*	29±3*	50±3*	1.24±0.03	7049±258*	1587±142*	1164±44
Ondansetron 0.1 mg kg^{-1}	562±24*	0.72±0.02	34±5	48±3*	1.19±0.04*	7457±331*	1460±120*	990±39*

Chronic treatment with the structurally unrelated 5-HT$_3$ receptor antagonists MDL 73147EF and ondansetron led to a significant decreases in DA, DOPAC and HVA concentrations in the frontal cortex and the caudate-putamen. As both transmitter and metabolite levels were reduced these results suggest that dopaminergic function was reduced, possibly as a consequence of decreased neuronal activity (Sorensen *et al.*, 1989). These results also support the finding of Sorensen *et al.* (1989) that both the mesolimbic and nigro-striatal systems are affected by chronic treatment with 5-HT$_3$ receptor antagonists.

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144P EFFECTS OF 5-HT$_3$ RECEPTOR ANTAGONISTS ON BEHAVIOURAL INDICES OF MORPHINE REWARD AND WITHDRAWAL

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Recently, Carboni et al (1988) reported that the 5-HT$_3$ receptor antagonists MDL72222 and ICS205-930 attenuated morphine-induced place preference, a measure of the appetitive effects of this drug (Mucha and Iversen, 1984). Furthermore, Costall et al (1990) have shown that another 5-HT$_3$ antagonist, ondansetron, produced behavioural changes consistent with an alleviation of certain symptoms associated with post-dependence withdrawal; however, opiate withdrawal was not assessed. The purpose of the present study was a) to examine the effects of ondansetron and MDL72222 against morphine withdrawal; and b) to re-examine these antagonists against morphine place conditioning using the unbiased protocol. The advantage of this latter technique is that the test stimulus is paired equally with either test compartment and both appetitive and aversive responses may be assessed in the same experiment (Mucha and Iversen, 1984).

Male Wistar rats (200-350 g) were used throughout. Morphine place conditioning was essentially as described by Mucha and Iversen (1984) with 4 x 45 min pairings of drug and vehicle to respective compartments. In separate experiments, rats were made physically dependent upon morphine by the subcutaneous implantation of a 75 mg morphine base pellet. Withdrawal was precipitated by injection of naloxone (N) (0.5 mg/kg sc) 72 h and 96 h later. Somatic signs of withdrawal, e.g. wet dog shakes (WDS), weight loss were assessed for 30 min following naloxone injection. Place conditioning to naloxone-precipitated withdrawal was as described by Mucha (1987). 5-HT$_3$ receptor antagonists were administered s.c. 30 min before morphine conditioning (place conditioning experiment) or naloxone injection (withdrawal experiment).

Both ondansetron and MDL72222 (0.01-1 mg/kg) produced a dose-related antagonism of the place conditioning induced by 1.5 mg/kg s.c. morphine (e.g. veh/morphine +289 ± 40 sec; MDL 1/morphine +59 ± 82 sec; $p < 0.05$). Neither ondansetron (0.01-1 mg/kg) or MDL72222 (1-3 mg/kg) affected any somatic signs of morphine withdrawal except weight loss (e.g. veh -14 ± 1 g; MDL 3 -9 ± 1 g; $p < 0.01$). Naloxone-precipitated withdrawal (0.002-1.5 mg/kg) was associated with a dose-dependent place aversion: the effect at the 0.05 mg/kg dose level was attenuated by both ondansetron (0.1-1 mg/kg) and MDL72222 (1 mg/kg) (e.g. veh/N -370 ± 140 sec; MDL 1/N -40 ± 116 sec). These findings confirm 5-HT$_3$ receptor antagonists produce effects consistent with blockade of morphine reinforcement but may alleviate only certain responses associated with opioid withdrawal.

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145P DIFFERENTIAL EFFECTS OF 5-HT_{1A}, 5-HT_{1B} AND 5-HT_{1C} RECEPTOR AGONISTS ON NON-OPIOID DEFEAT ANALGESIA IN MALE MICE

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Recent evidence has implicated 5-HT_{1A} receptor mechanisms in the mediation of non-opioid analgesia in defeated male mice (e.g.Rodgers & Shepherd, 1990). The reaction is potently blocked by buspirone, gepirone, ipsapirone and 8-OH-DPAT; furthermore, this effect is stereoselective and can be antagonized by (-) pindolol. We now report that this particular form of adaptive pain inhibition is dose-dependently inhibited by the 5-HT_{1A} partial agonist, MDL 73005EF (Moser et al., 1990), but not by either the 5-HT_{1B} agonist, CGS12066B (Neale et al., 1987), or the 5-HT_{1C} agonist, MK-212 (Conn & Sanders-Bush, 1987). Group-housed adult male DBA/2 mice (Bantin & Kingman, Hull) were maintained under a reversed LD cycle in a temperature-controlled room (24±1°), and all testing was conducted under dim red light during the dark phase of the cycle. Tail-flick latencies (temperature adjusted to give 2-3 sec baselines) were determined before and after defeat. For defeat experience, intruder mice were placed into the home cage of an aggressive resident conspecific and removed immediately upon display of species-typical defeat behaviour. Compounds were dissolved in physiological saline and injected i.p. either 20 (MDL 73005EF, CGS12066B) or 30 (MK-212) min before testing. Doses used were as follows: MDL 73005EF (0.05-1.0 mg/kg), CGS12066B (0.5-10.0 mg/kg), MK-212 (0.1-3.0 mg/kg). Data were analyzed by ANOVA and appropriate follow-up tests.

TABLE 1 Effects of MDL 73005EF (0.05-1.0 mg/kg) on defeat analgesia in male mice. Data are presented as Mean ±(SE) tail-flick latencies (sec). Pre=baseline, Post=post-defeat. * $p < 0.001$ vs baseline. # $p < 0.05$ and ## $p < 0.005$ vs vehicle control.

	Vehicle	0.05	0.25	0.50	1.0 mg/kg
Pre	2.47(0.07)	2.60(0.18)	2.57(0.14)	2.78(0.11)	2.81(0.10)
Post	5.55(0.42)	4.70(0.42)	3.91(0.39)	4.04(0.26)	3.32(0.21)
	*	*#	*##	*##	##

Table 1 shows that defeat analgesia was dose-dependently blocked by MDL 73005EF [$F_{4,50} = 7.4$, $p < 0.01$], with complete inhibition evident at the highest dose tested. This effect was observed in the absence of intrinsic effects of the compound on tail-flick latencies per se [$F_{4,50} = 1.63$, NS]. In direct contrast (data not shown), CGS12066B and MK-212 were devoid of activity on both tests [tail-flick assay: $F_{4,35}=0.74$, $F_{4,41}=1.19$, NS; defeat analgesia: $F_{4,40}=0.13$, NS, $F_{4,41}=0.83$, NS]. Together, present data further confirm the efficacy of 5-HT_{1A} receptor agonists in the model and suggest that 5-HT_{1B} and 5-HT_{1C} sites are not involved in this particular form of non-opioid analgesia.

This work was supported by the Yorkshire Regional Health Authority. We thank Merrell Dow (Strasbourg) and Merck Sharp & Dohme (Harlow) for the generous supply of MDL 73005EF and MK-212, respectively.

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146P EVIDENCE FOR AN INVOLVEMENT OF 5-HYDROXYTRYPTAMINERGIC NEURONES IN BEHAVIOURAL REINFORCEMENT

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There is evidence that the ascending 5-hydroxytryptaminergic (5HTergic) pathways are involved in regulating the intake of certain foodstuffs, notably carbohydrates; recently it has been proposed that 5HTergic transmission may exert a limiting influence on the reinforcing property of such foods (see Samanin, 1989). In the present experiment we used a quantitative behavioural paradigm (Morley et al., 1987) to assess the effect of destruction of central 5HT-containing neurones on the effectiveness of a sucrose reinforcer in maintaining operant behaviour.

Nine female Wistar rats received injections of 5,7-dihydroxytryptamine (4 µg base in 2 µl 0.9% NaCl) into the dorsal and median raphe nuclei; 12 rats received sham injections. Then the rats were reduced to 80% of their free-feeding body weights and were trained to press a lever for a sucrose reinforcer (0.6 M, 50 µl), using the following variable-interval (VI) schedules, exposure to each schedule being for at least 30 daily 30-min sessions: VI 10-s, VI 20-s, VI 40-s, VI 80-s, VI 160-s, VI 640-s, VI 10-s. At the end of the experiment the concentrations of 5HT and 5-hydroxyindoleacetic acid (5HIAA) in the neocortex, hippocampus and hypothalamus were determined by high-performance liquid chromatography with electrochemical detection.

Response rates in the last 5 sessions of exposure to each schedule were used to derive hyperbolic functions relating response rate (R) to reinforcement frequency (r) ($R = R_{max}.r/[K_H+r]$; Herrnstein, 1970; Morley et al., 1987). The mean values of R_{max} (the theoretical maximum response rate) did not differ significantly between the two groups (responses min^{-1}: lesioned, 84.7 ± 9.5; control, 95.1 ± 8.0; t-test, $P > 0.1$). The mean value of K_H (the reinforcement frequency needed to obtain the half-maximum response rate) was significantly lower in the lesioned than in the control group (reinforcers h^{-1}: lesioned, 62.5 ± 12.5; control, 152.2 ± 21.2; $P < 0.01$). The levels of 5HT and 5HIAA in the neocortex and hippocampus were less than 20%, and in the hypothalamus less than 40%, of those of the control group ($P < 0.001$ in each case).

Since the value of K_H is determined by motivational variables, and is not influenced by changes in motor capacity (see Morley et al., 1987), the present results are consistent with the suggestion that destruction of the ascending 5HTergic pathways results in potentiation of the reinforcing property of sucrose.

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147P LEVAMISOLE-ACTIVATED SINGLE CHANNEL CURRENTS IN ISOLATED MUSCLE VESICLES FROM *ASCARIS SUUM*

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A variety of anthelmintic drugs, including levamisole, are thought to be nicotinic agonists acting on parasite muscle (Harrow and Gration, 1985). Acetylcholine has been shown to open non-selective cation channels (mean open time 4.9ms) in isolated muscle vesicles in the nematode parasite *Ascaris suum* (Pennington & Martin, 1990). The aim of this study was to examine the properties of levamisole activated channels in this preparation. Levamisole (2-30μM) activated non-selective cation channels (conductance 20-45pS, mean open time 2.3ms). The open times exhibited a degree of voltage sensitivity, decreasing with depolarization. At the higher concentrations there was evidence of a voltage sensitive channel block (Figure 1) and desensitization.

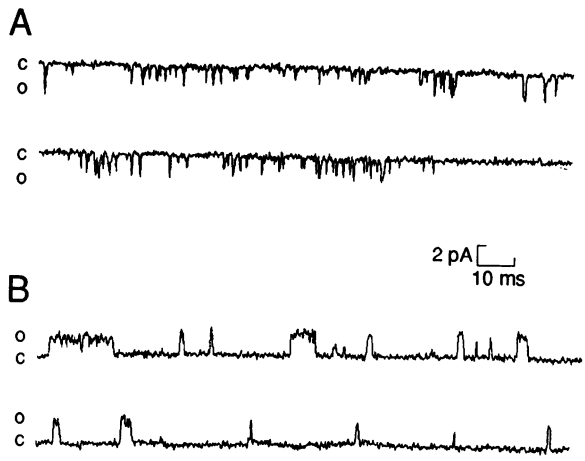


Figure 1: Levamisole (30μM) activated channels.
Isolated inside-out patch from muscle vesicle (for methods see Pennington & Martin, 1990).
c: closed state, o: open state.
A: -75mV membrane potential, mean open time 0.69ms.
B: +75mV membrane potential, mean open time 1.9ms.

Work supported by Merck, Sharp and Dohme.

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148P SELECTIVE INDUCTION OF NICOTINE RECEPTOR SUBUNIT GENE EXPRESSION FOLLOWING CHRONIC NICOTINE ADMINISTRATION

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Chronic nicotine administration has been shown to result in tolerance to nicotine, and also to induce an anomalous increase in the levels of high affinity nicotine binding sites. In this study, we have sought to determine whether this increased binding capacity is associated with enhanced expression of nicotinic acetylcholine receptor (nAChR) subunit genes.

Rats were infused subcutaneously with nicotine (10mg/kg/day) or saline (24μl/day) via osmotic minipumps, for 7 days. The levels of 3H-nicotine binding were then assayed on cryostat sections of rat brain by liquid scintillation counting and autoradiography, while the levels of nAChR subunit mRNAs in individual brain regions were determined by in-situ hybridisation histochemistry as previously described (Morris et al, 1990), using ^{35}S-labelled 45 base oligonucleotides (10^9dpm/μg). Hybridisation was performed at 42°C in buffer containing 4 x standard saline citrate (SSC) and 50% formamide, and washing was in 1 x SSC at 55°C.

Animals treated with nicotine showed enhanced levels of ^{3}H-nicotine binding (30% increase in specific binding to sections, using 12nM ^{3}H-(-)-nicotine) relative to saline-treated animals. In the medial habenula, nicotine treatment increased the levels of $\alpha 3$, $\beta 2$ and $\beta 4$ subunit mRNAs, but did not affect the levels of $\beta 3$ subunit mRNA. These results suggest that, in analogy to neuromuscular junction nAChRs, up-regulation of CNS nAChRs is associated with increased expression of the majority of the genes encoding nAChR subunits. However, some mRNAs encoding potential nAChR subunits show unchanged levels after chronic nicotine treatment. This suggests that there is some selectivity in the induction of increased nAChR subunit gene expression, although the reasons for this remain to be determined.

BJM holds an Ian Henderson postdoctoral fellowship from the Mental Health Foundation.

Morris, B.J., Hicks, A.A., Wisden, W., Hunt, S.P. and Barnard, E.A. (1990). Mol. Brain Res. 7, 305-315.

149P A PHARMACOLOGICAL STUDY OF THE M_4 MUSCARINIC RECEPTOR MEDIATING INHIBITION OF THE VOLTAGE-GATED Ca-CURRENT IN NEUROBLASTOMA x GLIOMA HYBRID (NG108-15) CELLS.

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Activation of muscarinic receptors in NG108-15 cells (probably m4 type; Peralta *et al.*, 1987) reduces the voltage-gated Ca current (Higashida *et al.*, 1990; Caulfield & Brown, 1990). We have studied the effect of some selective muscarinic antagonists on this response, using the whole cell patch-clamp technique (Caulfield & Brown, 1990).

Pirenzepine (30nM) shifted agonist dose-response curves (ACh + neostigmine 1μM, muscarine or oxotremorine-M) to the right in a parallel fashion (4.1 & 10-fold, n=2) but atropine (1nM) and pirenzepine (100nM & 300nM) shifted the curves and reduced maximum agonist responses. This anomalous behaviour at higher antagonist concentrations may have resulted from slow antagonist dissociation from receptors in a situation where there are few spare receptors (Rang, 1965). Therefore, antagonist occupancy (p) was estimated by taking agonist concentrations producing equal responses on control curves (x) and in the presence of antagonist (x') and fitting the function $x/(x+K)=(x'/(x'+K)(1-p)$, where K is agonist K_D (Rang, 1965), giving pK_B values (as p=[antagonist]/([antagonist]+K_B)) of 7.74 $\pm$ 0.29 (n=7) for pirenzepine and 9.30 (n=2) for atropine. Methoctramine (30nM-1μM) and himbacine (10nM-400nM) shifted agonist curves to the right in a parallel fashion, possibly because of more rapid dissociation kinetics. pK_B values estimated from dose-ratios (DR), where K_B=[antagonist]/(DR-1), were 7.63 $\pm$ 0.23 (n=4) for methoctramine and 8.83 $\pm$ 0.13 (n=3) for himbacine.

Like m1 receptors, these m4 receptors show high sensitivity to pirenzepine (K_B ~18nM) and low sensitivity to methoctramine (K_B ~23nM) compared to m2 or m3 receptors and are best distinguished from m1 receptors by the high potency of himbacine (K_B ~1.5nM; cf. Lazareno *et al.*, 1990).

This study was supported by the Medical Research Council.

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150P M-CURRENT INHIBITION IS PREFERENTIALLY MEDIATED BY THE GENOMIC M_3 RECEPTOR IN DNA-TRANSFECTED NG108-15 CELLS

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Several muscarinic receptor genotypes have been identified. We have tried to find out which of these most effectively inhibit the M-current when expressed in DNA-transfected neuroblastoma x glioma (NG108-15) cells. NG108-15 cells expressing m_1 (BM8, porcine brain m_1 cDNA), m_2 (HM105, porcine heart m_2 cDNA), m_3 (RM3-309, rat genomic m_3 cDNA) and m_4 (RM4-312, rat genomic m_4 cDNA) receptors (Fukuda et al, 1988) were compared with control cells and vector-transfected cells (containing native M_4 receptors; Michel et al, 1989). Cells were differentiated with PGE_1 and IBMX for 3 days. Membrane currents were recorded at 35 °C using whole-cell patch-clamp techniques with 3-6 MΩ electrodes containing (mM) K acetate, 90; KCl, 20; HEPES, 40; $MgCl_2$, 3; EGTA, 3 and $CaCl_2$, 1. Cells were superfused with a bicarbonate buffered modified Krebs' solution containing tetrodotoxin (0.5 μM). M-currents were recorded by clamping the cells at -30mV, then stepping to -60mV and measuring the deactivation relaxations. Muscarine and acetylcholine (ACh) were applied to the superfusate and cumulative concentration-response curves constructed using 4-16 cells at each concentration.

The M-current in m_2/m_4-, vector-transfected and control cells were unaffected by muscarine (μ 300μM) or ACh (μ 3mM). However, M-currents in m_3-transfected cells were inhibited by both muscarine (IC_{50} 2.4 μM; C.L. 2.3-2.7 μM) and ACh (11.4 μM; C.L. 9.4-13.8 μM). Inhibition was complete at high agonist concentrations, with no desensitisation over 15 mins. m_1-Transfected cells were also inhibited but only partly. IC_{50} values for the fraction of current inhibited were: muscarine, 8.6 μM (C.L. 3.1-23.6 μM) and ACh 6.4 μM (C.L. 2.8-14.6 μM). Inhibition of the M-current normally recovered within <5 min washing but became irreversible with GTP-γ-S (500 μM) in the pipette medium. Inhibition was unaffected by pretreatment with Pertussis toxin (0.5-1.0 μg/ml for 4-24 hours).

These results are not inconsistant with the idea that a Pertussis-insensitive G-protein is involved in the transduction of the muscarinic inhibition of the M-current and that the receptor which best mediates this is the m_3- type. This is not due to a greater number of receptors, since ^{3}H-QMB binding to m_1-transfected cells exceeded that to m_3-transfected cells (Fukuda, et al, 1988). m_3-Receptors are also the most effective in stimulating phosphoinositide (PI) turnover and increasing intracellular calcium (Bujo et al, 1988; Neher et al, 1988). This suggests a close relationship between M-current inhibition and increased PI turnover.

Supported by the MRC. We are grateful to Professors S. Numa and K. Fukuda for the transfected cells.

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151P CHOLINERGIC MARKERS IN NEOCORTEX AND HIPPOCAMPUS IN HUNTINGTON'S DISEASE

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Dementia in Alzheimer's disease and Parkinson's disease is associated with alterations of the subcortico-cortical cholinergic systems (Lange *et al.*, 1989). Huntington's disease (HD) is also characterized by progressive dementia. While there is a substantial loss of acetylcholine containing neurones in the striatum of patients with HD, the status of cholinergic markers in neocortex and hippocampus is less clear. We have examined choline acetyltransferase (CAT) activity and muscarinic cholinergic receptor binding in the temporal cortex and hippocampus in HD.

Brains were obtained from 10 patients with histopathologically confirmed HD and from 10 matched controls without neurological or psychiatric disorders. CAT activity was measured by the formation of [^{14}C]-acetylcholine from [^{14}C]-acetylcoenzyme A and choline. Using washed membranes saturation analysis was performed for [^{3}H]-quinuclidinylbenzilate binding (QNB; total number of muscarinic receptors; concentrations 9.4 pM - 0.3 nM) and [^{3}H]-pirenzepine binding (PZ; M-1 receptors; concentrations 0.5 - 64 nM). Non-specific binding was defined by 1µM atropine.

Table 1. CAT activity (nmol/h/mg protein) and B_{max} values for muscarinic receptor binding (fmol/mg protein), means ± s.e.mean

	TEMPORAL CORTEX			HIPPOCAMPUS		
	CAT	QNB	PZ	CAT	QNB	PZ
CONTROLS	4.6 ± 0.3	851 ± 29	593 ± 23	13.1 ± 1.0	375 ± 11	229 ± 16
HUNTINGTON	4.4 ± 0.3	819 ± 22	576 ± 15	8.7 ± 0.7 *	289 ± 14 *	216 ± 15

*) Significantly different from controls ($P < 0.05$, Mann-Whitney U test)

In HD no changes in CAT activity or densities of muscarinic receptors, measured by specific binding of [^{3}H]-QNB and [^{3}H]-PZ, were observed in the temporal cortex. By contrast, both CAT activity and the concentration of the total number of muscarinic receptors were reduced in the hippocampus. Alterations of K_D values were not seen. The results suggest that in HD the septo-hippocampal cholinergic pathway is damaged while the innominato-cortical pathway remains intact.

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152P THE ANTIEMETIC ACTIONS OF FENTANYL IN THE FERRET

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Opioids have a complex influence on emetic mechanisms in animals and man. The acute administration of morphine can induce emesis yet when administered as a pretreatment it is an effective antiemetic against subsequent challenge with morphine and other emetic stimuli (Costello & Borison, 1977). Here we investigate the antiemetic actions of fentanyl, a selective µ-receptor agonist, in the ferret.

Albino or fitch ferrets of either sex (0.8-1.4 kg) were challenged with either apomorphine (0.25 mg/kg s.c.), morphine (0.5 mg/kg s.c.), copper sulphate (100 mg/kg intragastric) or cisplatin (10 mg/kg i.v.) Animals receiving cisplatin had chronic jugular vein cannulations to enable i.v. injection. Fentanyl (fent.,10-80 µg/kg) and naloxone (nal., 0.1 mg/kg) or vehicle (NaCl 0.9 %w/v) were administered as 15 min and 20 min pretreatments respectively prior to the administration of apomorphine, morphine and copper sulphate. Animals receiving cisplatin were injected subcutaneously with fentanyl and/or naloxone or vehicle 45 min post administration of cisplatin.

Table 1. The effect of naloxone to antagonise the antiemetic effects of fentanyl against apomorphine (APO), morphine (MOR), copper sulphate (CU) and cisplatin (CP) induced emesis in the ferret during 30, 30, 30, and 240 min observation times respectively. If an animal failed to either retch or vomit then the time to onset of emesis was taken as the respective observation time. Doses are indicated in 'brackets' and represent the dose in mg/kg.

Treatment	Onset (min)	Episodes	Retches	Vomits	Treatment	Onset (min)	Episodes	Retches	Vomits
APO (cont 0.25)	1.4±0.2	5.3±1.0	32.0±5.4	3.5±0.8	Cu (cont 100)	5.3±0.7	12.2±1.6	60.0±7.9	8.0±0.8
+ fent (0.02)	30±0.0*	0±0*	0±0*	0±0*	+ fent (0.08)	30±0*	0±0*	0±0*	0±0*
+ nal (0.1)	1.4±0.3	14.2±3.3*	68.1±16.4	9.2±2.1*	+ nal (0.1)	4.0±0.8	18.8±2.7*	118.8±21.7*	14.4±1.8*
+ fent (0.02)+nal (0.1)	1.5±0.1†	7.0±0.9†	39.5±5.1†	5.3±1.0†	+ fent (0.08)+nal (0.1)	5.0±0.4†	13.8±8.4†	47.0±8.4†	4.5±1.2†
MOR (cont 0.5)	3.0±0.3	8.0±1.8	30.0±9.3	3.7±0.9	CP (cont 10.0)	69.6±5.7	13.8±1.8	78.3±11.5	6.8±1.3
+ fent (0.01)	22.8±4.9	0.8±0.7**	1.8±1.2**	0±0**	+ fent(0.08)	229.4±10.6**	0.5±0.5**	2.5±2.5**	0±0*
+ fent (0.02)	10.6±6.6	1.3±0.5*	6.5±2.4*	0±0*	+ nal (0.1)	70.7±11.1	17.3±3.8	100.3±23.5	11.3±3.5
+ fent (0.04)	30±0*	0±0*	0±0*	0±0*	+ fent (0.08)+nal (0.1)	80.7±17.1†	5.0±0.8†	24.5±1.8†	2.5±1.0†
+ nal (0.1)	24.9±5.1*	0.8±0.8*	3.6±3.6*	0.4±0.4*					

Results represent the means±s.e.m. of 4 to 8 determinations. Significant differences relative to control (cont) are indicated as *$p<0.05$; **$p<0.01$; significant antagonism by naloxone of the effects of fentanyl is indicated as †$p<0.05$ (Mann-Whitney U test).

Fentanyl antagonised the emetic effects of all four emetogens tested. Naloxone as a single pretreatment potentiated the emesis to all emetogens except morphine. Naloxone also had the capacity to antagonise the antiemetic effects of fentanyl.

Fentanyl is thus revealed to be a potent 'broad spectrum' antiemetic agent and may be used to further investigate the complex opioid control of emetic mechanisms in the ferret.

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153P L-687,384 IS A POTENT, SELECTIVE LIGAND AT THE CENTRAL SIGMA RECOGNITION SITE

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Martin *et al* (1976) have postulated that the hallucinogenic effects of SKF 10,047 (N-allylnormetazocine) are mediated via σ receptor activation. σ recognition sites can be labelled using the radioligand [^{3}H]ditolylguanidine (DTG) but (+)-SKF 10,047 shows only modest affinity for this site (Weber *et al.*, 1986). We report here the characterisation of a new ligand, L-687,384 (1-benzylspiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine]) with high affinity and selectivity for the central σ recognition site.

In vitro radioligand binding assays were carried out on crude membranes as described in Table 1. *In vivo* occupation of the sigma recognition site was assessed 60 min after s.c. administration of drug using the radioligand [^{3}H](+)-SKF 10,047 by the method of Ferris *et al.*, (1988). Non-specific binding was determined by injection of haloperidol (3mg/kg).

Table 1

Recognition Site	[^{3}H]Ligand [nM]	Tissue	Non-specific [μM]	(+)-SKF 10,047	DTG	Haloperidol	L-687,384
						pIC_{50}	
σ	DTG [5]	Guinea-pig Brain	Haloperidol [10]	5.8	7.4	8.5	8.4
NMDA Channel	MK-801 [1]	Rat Cortex	TCP [100]	6.5	<5.0	<5.0	<5.0
D_2	Sulpiride [10]	Rat Striatum	Haloperidol [10]	-	<5.0	8.8	6.0
α_1	Prazosin [1]	Rat Brain	Phentolamine [10]	<5.0	<5.0	-	5.8
α_2	Rauwolscine [5]	Rat Brain	Phentolamine [10]	<5.0	<5.0	-	5.3
5-HT_{1A}	8-OH-DPAT [1]	Rat Frontal Cortex	5-HT [10]	<5.0	<5.0	-	<5.0

pIC_{50} is $-\log_{10}$ of the concentration of drug required to reduce specific binding by 50%.
Data are the mean of 2-3 separate experiments calculated from displacement of at least 5 concentrations of drug in duplicate.

L-687,384 was found to be a potent and specific displacer of [^{3}H]DTG from the σ recognition site (Table 1). *In vivo* [^{3}H](+)-SKF 10,047 was displaced from the σ recognition site in mouse brain by both (+)-SKF 10,047 (ED_{50} 0.25mg/kg) and L-687,384 (ED_{50} 0.08mg/kg). L-687,384 is a useful tool to explore the functional significance of the σ recognition site in the brain.

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154P DO MINOXIDIL SULPHATE AND CROMAKALIM OPEN PHARMACOLOGICALLY SIMILAR K^+-CHANNELS IN RAT ISOLATED AORTA?

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Minoxidil sulphate (MxS), the active metabolite of minoxidil (Johnson et al., 1982) is a smooth muscle relaxant with K^+-channel opening properties (Meisheri et al., 1988, Newgreen et al., 1990). The present study examines the $^{42}K^+/^{86}Rb^+$ effluxes induced by MxS and the K^+-channel opener cromakalim (CRK), with respect to their concentration dependency, cross-desensitization and sensitivity to glibenclamide, in rat isolated aorta. Vascular rings were loaded with $^{42}K^+$ and $^{86}Rb^+$ and the rate of tracer efflux determined (k_K or k_{Rb}), using a perfusion assay system, as previously described (Quast and Baumlin, 1988).

Both MxS (0.3-100μM) and CRK (0.1-100μM) produced concentration-dependent increases in the efflux of both $^{42}K^+$ and $^{86}Rb^+$ from rat aorta, with EC_{50} values for $^{42}K^+$ efflux of 1.8 ± 0.3μM and 0.7 ± 0.3μM and maximum increases in efflux of 4.4 ± 0.2 and 0.5 ± 0.05 ($10^{-2}.min^{-1}$), for CRK and MxS respectively (n=5-12). The ratio of $^{86}Rb^+$:$^{42}K^+$ efflux was concentration-dependently increased by CRK from 0.15 (at CRK 0.1μM) to 0.48 (at CRK 100μM), whereas after treatment with MxS the ratio remained constant (≈ 0.35). In cross-desensitization experiments, exposure to MxS (1 and 30μM) for 20min inhibited subsequent CRK (1μM)-induced peak increases in $^{42}K^+/^{86}Rb^+$ efflux to 77 ± 5/73 ± 8 and 56 ± 5/59 ± 6 % of control values (results with 1 and 30μM MxS, respectively). Conversely, preincubation with CRK (0.3μM) inhibited MxS-induced $^{42}K^+$ and $^{86}Rb^+$ efflux by up to 50%. Glibenclamide (50-500nM) inhibited the $^{42}K^+/^{86}Rb^+$ effluxes stimulated by MxS (10μM) and CRK (1μM) in a concentration-dependent manner (in the presence of 0.1μM glibenclamide the $^{42}K^+/^{86}Rb^+$ efflux induced MxS and CRK was inhibited to 40 ± 5/40 ± 4 % and 41 ± 4/35 ± 4% of control values, respectively n=3-5).

It is concluded that in rat aorta, MxS and CRK induce concentration-dependent increases in the efflux of both $^{42}K^+$ and $^{86}Rb^+$. In addition, induced $^{42}K^+$ and $^{86}Rb^+$ effluxes were inhibited equally by preincubation with glibenclamide. This, coupled with the observation that MxS desensitized tissues to subsequent exposure to CRK, and vice versa, strongly suggests that considerable overlap exists between the K^+-channels opened by MxS and those opened by low concentrations of CRK in rat aorta.

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155P CROMAKALIM-INDUCED POTASSIUM CURRENTS IN SINGLE DISPERSED SMOOTH MUSCLE CELLS OF RABBIT ARTERY AND VEIN

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Cromakalim(CKM) may vasodilate due to hyperpolarization of the membrane and partial suppression of voltage-dependent Ca current; the opening of Ca-dependent K-channels in guinea-pig or rat vessels may contribute to the hyperpolarization (Nakao et al.,1988, Okabe et al.,1990). However, Beech & Bolton (1989) suggested that CKM acted on the delayed rectifier K current in rabbit portal vein, and recently Standen et al. (1989) reported the existence of ATP-sensitive K-channels in rabbit or rat mesenteric artery smooth muscle cells, which were activated by CKM and inhibited by glibenclamide(GLIB). In the present study we have examined the properties of the CKM-induced K current and the existence of an ATP-sensitive K-channel in smooth muscle cells of rabbit portal vein and mesenteric artery using patch-clamp technique. CKM(>1μM) produced an outward current which was dose-dependently inhibited by GLIB(>0.1μM), with or without ATP (6mM cyanide with 14mM 2-deoxy-D-glucose) in the pipette. CKM(>0.3μM) augmented and GLIB(>1μM) inhibited the delayed rectifier K current (I_{dk}). Both CKM and GLIB affected the activation rather than inactivation process of I_{dk}. When the pipette solution contained EGTA(3mM), two types of K-channels with different conductances (55pS and 7.5pS) were seen in excised-patches. Application of phencyclidine(0.1mM) to the external membrane surface blocked the 7.5pS K-channel without affecting the 55pS K-channel. Tetraethylammonium(5mM) blocked the 55pS K-channel and reduced the current amplitude through the 7.5pS K-channel. Charybdotoxin(0.1μM) inhibited only the 55pS K-channel. Both external and internal applications of CKM increased and GLIB decreased the 7.5pS K-channel activity without obvious effects on the 55pS K-channel activity. CKM(20μM) increased the mean open time and open probability, and reduced the slow component of the mean closed time of the 7.5pS K-channel. GLIB(30μM) reversed the CKM-induced increase in mean open time and open probability, and increased the fast component of the mean closed time of the 7.5pS K-channel. Application of ATP(1-5mM) to the internal membrane surface had no effect on the 7.5pS K-channel.

CKM produced a similar whole-cell current whether ATP was present or not in the pipette and increased the probability of opening of a 7.5pS K-channel. We have not found channels sensitive to the application of ATP on the internal membrane surface. The results suggest that the CKM-induced current seen in these cells is due to the opening of a 7.5pS K-channel which is insensitive to ATP but sensitive to GLIB.

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156P VASODILATOR ACTIVITY OF MINOXIDIL SULPHATE: COMPARISON WITH CROMAKALIM

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Cromakalim and minoxidil sulphate are thought to belong to a class of vasodilators collectively known as potassium channel openers. Thus, these compounds share the ability to increase the $^{42}K^{+}$ efflux rate coefficient and produce membrane hyperpolarisation to a value close to the calculated potassium equilibrium potential (Hamilton et al., 1986, Meisheri et al., 1989). The purpose of the present study was to compare the actions of cromakalim and minoxidil sulphate in a variety of isolated vascular smooth muscle preparations.

Cromakalim and minoxidil sulphate were compared in spontaneously active longitudinal sections of guinea-pig and rat portal vein and in noradrenaline (NA) pre-contracted rings of rabbit thoracic aorta, rat mesenteric artery and rat aorta. Responses were recorded isometrically under a resting tension of 1g.

In the guinea-pig portal vein cromakalim and minoxidil sulphate inhibited spontaneous mechanical activity (IC50 values 74 ± 40nM and 37 ± 9nM respectively). Glibenclamide (0.1-10μM) inhibited the actions of cromakalim in an apparently competitive manner in this preparation. In contrast, glibenclamide antagonised the action of minoxidil sulphate non-competitively.
Cromakalim and minoxidil sulphate also inhibited spontaneous mechanical activity in the rat portal vein (IC50 values 0.14 ± 0.02μM and 0.71 ± 0.02μM respectively) and relaxed the NA (30nM) pre-contracted rat aorta (IC50 values 0.29 ± 0.11μM and 0.54 ± 0.08μM respectively).
Cromakalim relaxed the NA pre-contracted rat mesenteric artery (0.3μM NA) and rabbit thoracic aorta (1μM NA) with IC50 values of 0.21 ± 0.05μM and 1.3 ± 0.4μM respectively. In contrast minoxidil sulphate was without effect in these tissues.
In summary, the potassium channel openers cromakalim and minoxidil sulphate show different profiles of activity in a variety of vascular preparations. This may indicate that these compounds interact with different potassium channel sub-types. The non-competitive interaction between minoxidil sulphate and glibenclamide may support this conclusion.

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157P EFFECTS OF MICROAPPLICATION OF PINACIDIL IN RAT PIAL ARTERIOLES *IN SITU*

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Pinacidil (PIN) induced relaxation of rat isolated basilar artery has been shown to be blocked by the ATP-dependent K^+ channel blocker glibenclamide (GLIB) (Parsons et al. 1990). In cat pial arterioles in situ PIN induced potent vasodilatation, at lower concentrations as compared to isolated arteries (Wahl 1989). The aim of the present study was therefore to assess the effects of PIN in rat pial arterioles in situ and to investigate the antagonistic actions of GLIB.

Twelve male Sprague-Dawley rats (220-320g) were anaesthetized with inactin (90-100 mg kg^{-1} i.v.). After tracheostomy, rats were inserted with femoral arterial and venous cannulas, immobilized with pancuronium bromide (1mg kg^{-1} h^{-1}) and ventilated. After a left parietal craniotomy, the dura was incised and deflected across the bone edges. Microapplication was performed as described previously (Wahl et al. 1987). Vessel reactivity was assessed by microapplication of normal artificial cerebrospinal fluid (CSF) (pH 7.25) or acidic CSF (pH 6.86). Only vessels which dilated to acidic CSF were used. PIN (0.10nM to 100nM) was then infused by microapplication. Effects of co-administration of GLIB 100nM and 1µM were then assessed on PIN (3nM to 100nM) induced dilatation.

Rectal temperature was 36.9 ± 0.1°C, mean arterial blood pressure was 99.6 ± 2.3 mmHg, and arterial pCO_2, pO_2 (both mmHg) and pH were 36.4 ± 0.6, 133 ± 11 and 7.38 ± 0.02 respectively. The resting diameter of arterioles was 21-55um. Artificial CSF had no significant effects on vessel diameter whereas acidic CSF induced vasodilatation (29.0 ± 4.5%; n=20). PIN 3nM to 100nM induced concentration dependent vasodilatation with a mean (± SEM) maximal effect of 29.6 ± 3.3% at 10nM (n = 20). Responses to PIN and acidic CSF were not dependent on the resting vessel calibe. GLIB 100nM and 1µM had no significant effect on resting diameter or on acidic CSF dilatation (n=5). In contrast, GLIB produced inhibition of PIN induced dilatation:

PIN	control	time matched control	GLIB 100nM	GLIB 1µM
3nM	14.8 ± 2.5%	12.2 ± 6.3%	12.7 ± 5.6%	n.d.
10nM	23.6 ± 3.2%	30.3 ± 6.1%	17.2 ± 2.9%	5.7 ± 4.0%[a,b]
100nM	n.d.	n.d.	n.d.	27.0 ± 2.3%

Data are mean ± SEM of 4-14 determinations. a: $p < 0.05$ from control. b: $p < 0.05$ from time matched control: n.d. not determined

The present study demonstrates that PIN produces dilatation of rat pial arterioles over a similar concentration range as in cats, indicating a higher sensitivity of pinacidil to cerebral arterioles in situ as compared to isolated cerebral arteries. However, the inhibitory actions of GLIB suggest activation of a similar mechanism.

A.A.P. was supported by the Royal Society and the Alexander von Humboldt Foundation

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158P IS KETANSERIN A CALCIUM ANTAGONIST?

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Ketanserin is a 5-HT_2 receptor antagonist which also blocks α-adrenoceptors at much higher concentrations. It has been consistently observed in this laboratory that Ketanserin possesses calcium antagonist actions. This communication reports such observations. Ring segments from male rabbits (2.80 ± 0.12g) were set up from the thoracic aorta (TA), central ear artery (CEA), renal (R.A),Saphenous artery (S.A) and femoral artery (FA) (Bevan & Osher, 1972). All preparations were treated with phentolamine (1µM) and propranolol (1µM) to block alpha- and beta-adrenoceptors respectively. Ketanserin (0.01µM -10µM) relaxed vascular segments contracted with K+ (64mM) in a concentration-related manner. The IC_{50} values of Ketanserin are 0.67±0.02µM; 0.34±0.09µM; 0.32±0.05µM; 0.60±0.08µM and 0.67±0.06µM (n =6) in the R.A., F.A., C.E.A., S.A. and T.A. respectively. There was no significant difference in these values (P>0.05). The maximal relaxant effect of Ketanserin in these vessels are 85±5.0%, 80.0±4.0%, 88±2.3%, 80±8.0 and 45.0±5.0% (n = 6) respectively in the R.A., F.A., C.E.A., S.A., and T.A.. The maximal effect in the TA was significantly lower than those in the other tissues (P>0.05).

It was important to study the effect of Ketanserin on calcium induced constrictions of these vessels. Ketanserin at the concentrations that reduced calcium entry also produced rightward shifts of the calcium concentration-effect curves in these vessels with K_B values of 8.60±4.0nM (n = 5) and 9.60±3.0nM (n = 6) in the R.A. and C.E.A. respectively. Calcium induced constrictions of the T.A. was not influenced by Ketanserin.

For comparative purposes the effect of diltiazem (0.01µM - 10µM) was studied on these vessels. In K+-depolarized medium, diltiazem caused concentration-dependent relaxation of the R.A., C.E.A., S.A., F.A., and T.A. with the following IC_{50} values respectively (0.5±0.01µM; 0.2±0.06µM), 0.60±0.03µM; 0.5±0.05µM, and 0.60±0.06µM, (n = 5). These was no significant difference in the IC_{50} values of diltiazem and Ketanserin in these vessels (P>0.05). The maximal relaxant effect of diltiazem was 95±0.6; 85±8.5; 85±6.2; 80±4.5 and 80±1.5% respectively in the R.A., C.E.A., S.A., F.A., and T.A. There was a significant difference (P>0.05) between the maximal effect of diltiazem and Ketanserin in the T.A. Diltiazem gave K_B values of 4.0±1,5 and 5.0±2.2 against Ca^{++} in the C.E.A. and T.A.

These results suggest that Ketanserin has both calcium entry blocking and receptor blocking properties. Inability of Ketanserin to block Ca^{++}-induced constrictions remains to be fully investigated.

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159P LIDAMIDINE DOES NOT REVERSE T84 HUMAN COLONIC EPITHELIAL CELL LINE SHORT CIRCUIT CURRENT RESPONSES TO VASOACTIVE INTESTINAL PEPTIDE

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Intact sheets of human colonic mucosa set up in Ussing chambers respond to noradrenaline with a decrease in short circuit current (S.C.C.) (Burleigh 1990). The effect is probably directly on the epithelial cell as it is not reduced by tetrodotoxin (unpublished observations). However, an anti-secretory effect for clonidine could not be shown in the T_{84} human colonic epithelial cell line, for the single concentration (10^{-5}M) tested, even though anti-secretory effects could be shown in this preparation for somatostatin (Dharmsathaphorn *et al.*, 1984). The purpose of the present investigation was to determine whether the above report was representative of alpha-2 agonist action on T_{84} cells. T_{84} cells were grown to confluence, trypsinised and plated onto collagenised permeable supports (1.98 cm^2). Seven days were allowed to permit development of tight junctions with maximum electrical resistance. The supports were inserted into a modified Ussing chamber for recording of S.C.C. Resting S.C.C., potential difference (P.D.) and conductance (G) were 5.6 ± 0.8 μA, -2.5 ± 0.4 mV and 3.2 ± 0.8 millisiemens respectively (n = 29). These values were recorded 25 min after the preparations were set up. Basolateral addition of vasoactive intestinal peptide (VIP, 10^{-9} - 10^{-8} M) produced a large well-maintained increase in S.C.C. (5.6 ± 0.9 → 47.3 ± 3.2 μA, n = 24). After 15 min the effect had reached a plateau level and various potential anti-secretagogues were added. Changes in S.C.C. were recorded after a contact time of 10 min (5 min for somatostatin).

Treatment		before addition (μA)	after addition (μA)	n	
Control		43 ± 6	38 ± 5	10	* P > 0.5 compared to control
NA	10^{-6}M	55	48	2	
Lidamidine	10^{-5}M	50 ± 7	45 ± 8*	4	
	10^{-4}M	48 ± 5	43 ± 5*	8	
Somatostatin	10^{-5}M	42	27	2	

The T_{84} cell line with its low basal S.C.C. and ability to secrete Cl^- in response to a number of secretagogues displays the characteristics of colonic crypt cells rather than surface absorptive cells. Therefore a possible explanation for the ineffectiveness of alpha-2 agonists in this preparation is that these compounds are pro-absorptive and do not owe their anti-secretory actions to inhibition of chloride ion secretion. This is in contrast to the actions of somatostatin which reduced the secretory response to VIP as previously reported (Dharmsathaphorn, 1984).

Acknowledgements: To the Royal Society and the Nuffield Foundation for provision of travel grants to visit the laboratories of Dr. K. Dharmsathaphorn

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160P MECHANISM OF CARBACHOL AND HIGH K^+ CONTRACTIONS OF GUINEA-PIG ILEUM CLOSE TO FREEZING POINT

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Carbachol may cause contraction in smooth muscle by increasing the intracellular Ca^{2+} via Ca^{2+} influx through receptor-operated cation channels or voltage-dependent Ca^{2+} channels, causing IP_3-induced Ca^{2+} release from the sarcoplasmic reticulum (Bolton, 1979) or by increasing the Ca^{2+} sensitivity of the contractile proteins (Nishimura et al, 1988). The present study compared the properties of contractions produced by carbachol and high K^+-containing solutions at temperatures close to freezing point to examine which of these mechanisms were still functional.

Strips of guinea pig ileum were suspended in a narrow tube under 0.5g initial tension in bicarbonate-buffered physiological salt solution continuously bubbled with 95% O_2:5% CO_2 and perfused at 1.1 ml/min. The bath temperature could be adjusted and maintained to within 0.5°C. Contractions to carbachol were blocked by atropine and carbachol sensitivity was less at 3°C compared with 37°C although the amplitude of the maximum contraction was not significantly different (n=7; paired Student's t-test). The maximum contractile response to 140mM K^+-containing solution was significantly reduced at 3°C ($p<0.05$; n=9) with or without 5×10^{-6}M atropine. The contribution of Ca^{2+} influx to the responses seen at 37°C and 3°C was assessed by perfusing the muscle with the Ca^{2+} channel antagonists, nifedipine (5×10^{-7}M) or verapamil (10^{-5}M), by perfusion of 140mM K^+ (Table 1) or by applying Ca^{2+}-free solution (1mM EGTA).

Table 1. Tension measurements (expressed as % control response at the same temperature)

	5×10^{-7}M Nifedipine		10^{-5}M Verapamil		140mM K^+	
	37°C	3°C	37°C	3°C	37°C	3°C
140mM K^+	1.9±1.1	0	1.6±0.9	2.2±1.4	-	-
10^{-4}M carbachol	17.3±1.9**++	25.9±9.5*+	11.2±2.4*+	8.8±1.0***+	25.7±1.9***	47.0±7.8*o

Results are mean±S.E.M. (n=4 to 6) *$p<0.05$,**$p<0.005$,***$p<0.001$ compared with control response at same temperature;+$p<0.05$,++$p<0.005$ compared with 140mM K^+ response at same temperature;o$p<0.05$ compared with response in 140mM K^+ at 37°C using paired Student's t-test.

The contraction to 10^{-4}M carbachol at 3°C was 11.9±3.8% in Ca^{2+}-free and 25.1±3.3% in high K^+ Ca^{2+}-free solution. These results demonstrate that a large part of the contraction to 10^{-4}M carbachol at 3°C, as at 37°C, appears to be mediated by Ca^{2+} entry through voltage-dependent Ca^{2+} channels. However, the persistence of a response to carbachol in high K^+ Ca^{2+}-free solution suggests the involvement of Ca^{2+} store release or increased Ca^{2+} sensitivity even at temperatures close to freezing point.

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161P STUDIES ON THE INTERACTION BETWEEN GUANETHIDINE AND POTASSIUM CHANNEL BLOCKERS IN RAT ANOCOCCYGEUS

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Stutzin et al., (1983) showed that the adrenergic neurone blocking action of guanethidine in guinea-pig vas deferens could be antagonised by tetraethylammonium (TEA) and by apamin. They concluded that guanethidine opened neuronal K-channels leading to hyperpolarisation and a reduction in transmitter output. In the present study we have examined the effects of TEA, 4-aminopyridine (4-AP), apamin and glibenclamide on the adrenergic neurone blockade produced by guanethidine in rat anococcygeus.

Paired anococcygeus muscles were removed from male Sprague-Dawley rats (300-400g) and set up under isometric conditions in 20ml organ baths containing bicarbonate-buffered physiological salt solution (PSS) at 37°C. Isometric mechanical changes were displayed and evaluated using a Maclab recording system (W.P. Instruments). Preparations were stimulated using stimulus trains of duration 10 sec and frequency 1min^{-1}. Stimulus pulse width and amplitude were 0.1 msec and 40V, respectively; pulse frequency was varied.

In normal PSS, field stimulation (0.5-16Hz) produced a frequency dependent series of tonic contractions. Addition of TEA (5mM) potentiated the frequency-response relationship, especially at lower stimulus frequencies. Similar results were obtained with 4-AP (1mM) and glibenclamide (1μM) whereas apamin (60nM) was without effect. Addition of guanethidine (15-30μM) to the PSS produced an increase in tone which usually declined in the continuing presence of guanethidine. Contractions to all stimulus frequencies were rapidly abolished revealing relaxations of the raised tone. Exposure of tissues previously pretreated with guanethidine to TEA (5mM) or 4-AP (1mM) rapidly and completely restored the contractile frequency-response relationship whereas apamin and glibenclamide were each without effect.

The ability of both TEA and 4-AP to reverse completely the effects of guanethidine suggests that this effect cannot be explained solely by functional antagonism. Instead the data suggest that guanethidine could exert its effects by opening neuronal K-channels, in support of which are chemical similarities between guanethidine and the guanidine-type smooth muscle K-channel openers (Edwards & Weston, 1990). However, the possibility (Drukarch et al., 1989) that TEA and 4-AP might displace guanethidine from its site of action cannot be discounted and further experiments are in progress to clarify this possibility.

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162P THE ROLES OF EXTRACELLULAR Ca^{2+} AND INTRACELLULAR Ca^{2+} STORES IN THE CONTRACTION OF SMOOTH MUSCLE

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Analysis of the phases of smooth muscle contraction in response to drugs is complex because diffusion blurs the distinction between phases. We have therefore used thin strips (approx. 5mm by 0.3mm) of different guinea-pig smooth muscles, mounted in Locke's solution at room temperature for tension recording (Harvard force transducer). Where bath Ca^{2+} was removed, Locke's solution without $CaCl_2$ but with added EGTA (4 mmol/l) was used. Ryanodine (100μmol/l, 30 min) was used to deplete Ca^{2+} stores (Kanmura *et al.*, 1988).

In ileum longitudinal smooth muscle, carbachol (0.1-100 μmol/l) elicited a biphasic contraction (peaks at 10 sec & 3 min). Removal of bath Ca^{2+} attenuated the first and abolished the second phase of the response to carbachol (100 μmol/l). Ryanodine reduced both phases. No response was apparent after treatment with ryanodine and removal of bath Ca^{2+}. It is concluded that the first phase of contraction involves both extracellular Ca^{2+} and intracellular Ca^{2+} stores and that the second phase of contraction is dependent on extracellular Ca^{2+} but may also be dependent on the first phase of contraction.

In ileum circular smooth muscle, carbachol (1-100 μmol/l) elicited only a transient response (approx. 1 min). Removal of bath calcium abolished the response to carbachol (100 μmol/l) and treatment with ryanodine attenuated it. It is concluded that the response is dependent on extracellular Ca^{2+}, but also partly dependent on the intracellular Ca^{2+} store.

In trachealis, carbachol (0.1-100 μmol/l) elicited a biphasic response (peak at 4 min declining to a plateau). Removal of bath Ca^{2+} attenuated the first phase and abolished the second phase. Ryanodine slowed tension development without affecting ultimate tone. It is concluded that the first phase of contraction involves both extracellular Ca^{2+} and intracellular Ca^{2+} stores, and that the second phase is dependent on extracellular Ca^{2+} and ultimately independent of intracellular Ca^{2+} stores.

M.J.Allen is in receipt of a Fisons studentship.

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163P 5-HT RECEPTORS IN HUMAN ILEUM AND COLON

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The mechanism by which certain substituted benzamides, including metoclopramide, cisapride, renzapride and zacopride, enhance gastrointestinal motility remains unclear (King & Sanger 1988). Recently, however, it has been disclosed that the benzamides agonize a novel 5-hydroxytryptamine (5-HT) receptor (putative 5-HT_4) in the alimentary tract of guinea-pigs and rats (Craig & Clarke 1990; Baxter & Clarke 1990) and this property may ultimately explain their motility stimulating action. Thus, the present study was conducted to examine the mode of action of 5-HT and certain substituted benzamides in human ileum and colon.

Three macroscopically normal specimens of human ileum, and one of colon, were obtained from patients undergoing resection for carcinoma. The mucosa was carefully removed and strips of muscle (15 x 3 mm) were cut in the appropriate axes to obtain preparations for measurement of tension development in longitudinal or circular muscle. All muscle strips were mounted in tissue baths, under 1g tension, bathed with Tyrode's solution at 37°C and aerated constantly with 95% O_2/5% CO_2.

In longitudinal muscle preparations from ileum (8 strips from 2 patients), 5-HT (1-100 nM) caused neither contraction nor relaxation. However, the same preparations contracted readily when exposed to KCl (50 mM), or carbachol (3 μM). Ileal circular muscle also responded to KCl and carbachol, but unlike longitudinal muscle, responded to 5-HT (0.3-10 μM, 12 strips from 3 patients) with concentration-dependent contractions. Contractile reponses to 5-HT were mimicked by α-methyl-5-HT but not by cisapride, (S)-zacopride or renzapride at concentrations up to 3, 30, and 10 μM respectively. In addition, contractions to 5-HT were resistant to both atropine (0.1 μM) and tetrodotoxin (0.1μM) but inhibited by methysergide (1 μM), ketanserin (1 μM) and cisapride (3 μM). 5-HT did not relax preparations of ileal circular muscle contracted with carbachol (1 μM). In the colon both the longitudinal and circular muscle responded with powerful contractions to KCl (50 mM), $PGF_{2\alpha}$ (1 μM), and AII (1-1000 nM), but 5-HT (0.01-100 μM) was ineffective (4 strips from 1 patient). In a single circular strip of colon, 5-HT (0.1-100 μM) caused inhibition of spontaneous activity and a slight lowering of basal tension, however, when tested on preparations contracted with $PGF_{2\alpha}$ (1 μM) no relaxation was observed. In the same preparations, isoprenaline (0.1 μM) caused complete relaxation.

In conclusion, 5-HT contracts circular muscle of human ileum via a postjunctional receptor which exhibits more in common with the 5-HT_2 receptor than the putative 5-HT_4 receptor subtype. No evidence was obtained for the existence of 5-HT receptors in human colon.

This work was supported by NIH Grant NS 24871

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164P ACTIONS OF PINACIDIL ON RAT ILEUM

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Potassium (K) channel opening agents relax various types of smooth muscle including gastrointestinal muscle (Davies et al., 1990) as do calcium channel blocking agents. Both types of agent have been reported to relax smooth muscle by reducing intracellular calcium concentration (Robertson & Steinberg, 1990). In the present work using isolated rat ileum a comparison was made between the actions of pinacidil (PIN), as an example of a K channel opening agent and verapamil (VERAP), a calcium channel blocker.

Concentration-response curves to K (4 - 16 mM) were constructed in segments of rat mid-ileum suspended in Krebs' solution (37°C, 95% O_2/5% CO_2) under 1g tension. An approximately 50% maximal contraction was elicited with 10 mM K^+. Tissues were then incubated with either PIN (50 - 500 μM) or VERAP (50 - 200 nM) for 30 mins (n = 5 for each concentration). Contractions elicited by both high and low concentrations of K were inhibited by PIN and VERAP, the EC_{50} response to K being inhibited by PIN with an apparent IC_{50} value of 150 ± 85 μM: the comparable value for VERAP was 84 ± 8 nM (mean ± 95% confidence limits).

In a second series of experiments, sustained contractions to 10 mM K (approximately 50% max) were elicited. At the plateau of the response cumulative concentration-response curves to PIN (1 - 130 μM) and VERAP (2.5 - 2,600 nM) were constructed (n = 5 for each inhibitor). Both PIN and VERAP fully relaxed the K contraction. The IC_{50} value for PIN was 6.9 ± 3.8 μM and for VERAP was 38 ± 6 nM (mean ± 95% confidence limits). Thus a slight difference between the IC_{50} values for VERAP was obtained using the two methods, whereas PIN appeared considerably more potent when added to an established K contraction than when incubated with the tissue before addition of K. PIN relaxed the K contraction more rapidly than VERAP. The time taken to achieve the maximum response at each concentration of PIN was approximately 1.9 mins whereas approximately 4.1 mins were required with VERAP.

These results show that the action of the K channel opener PIN can be qualitatively distinguished from that of VERAP, the potency of PIN but not VERAP being substantially affected by the experimental protocol used. Further experiments are in progress to ascertain whether other K channel openers exhibit similar characteristics.

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165P IONIC REQUIREMENTS OF [^{125}I]IODO-Tyr^{11}-SOMATOSTATIN-14 BINDING SITES IN HUMAN GASTRIC MUCOSA AND RAT CEREBRAL CORTEX

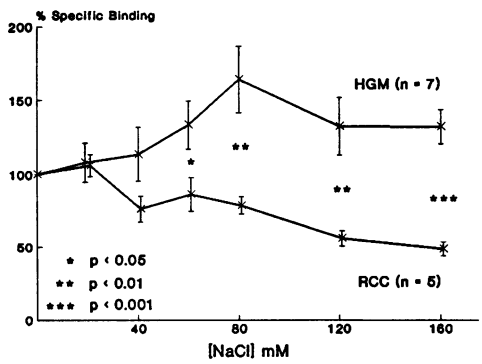
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Investigation of the ionic requirements of somatostatin (SS) binding sites in rat brain (Reubi & Maurer, 1986, Srikant et al, 1990) have revealed that bivalent cations generally increase binding, whereas monovalent cations decrease binding. The aim of the present investigation is to determine the ionic requirements of SS binding sites in human gastric mucosa (HGM) and rat cerebral cortex (RCC).

Normal HGM was obtained at operation from seven patients with resectable gastric carcinoma. Rat cerebral cortex was dissected from five female Wistar rats killed by cervical dislocation. Crude membrane preparations (20ug protein) were incubated with 2nM [^{125}I]iodo-Tyr^{11}-somatostatin-14 (^{125}I-SS) in 50mM Hepes.KOH buffer (pH 7.4) containing 1% BSA and 0.01% bacitracin in a final volume of 100ul. Either NaCl (20-120mM) or $MgCl_2$ (1.25-10mM) were added to the incubation mixtures. Non-specific binding was determined by the addition of 5uM SS-14. Results were expressed as a percentage of the binding in the absence of salts.

In the concentration range 60-160mM NaCl, specific ^{125}I-SS binding to RCC membranes was decreased by up to 60%. In contrast, the addition of NaCl increased ^{125}I-SS binding to HGM membrane preparations in a dose dependent manner. Maximal increase in specific binding occurred at 80mM NaCl (170$\pm$22% (mean$\pm$ SEM, n=7), see Figure 1). Specific ^{125}I-SS binding to RCC membranes was significantly increased by the addition of $MgCl_2$ whereas this had no influence upon binding to HGM membranes. Further studies using salts in combination revealed that elevated NaCl concentration did not increase ^{125}I-SS binding to HGM membranes in the presence of 5mM $MgCl_2$. However, the decrease in binding to RCC membranes observed in the presence of 60-160mM NaCl persisted despite the addition of 5mM $MgCl_2$.

In conclusion, the ionic requirements for ^{125}I-SS binding to membrane preparations from RCC and HGM appear to be quite different. Reubi and Maurer (1986) defined SS receptors in the rat cortex according to their affinity for SS analogues. Binding to the high affinity SS_1 site was enhanced by Mg^{2+} but reduced by 120mM NaCl, whereas the SS_2 site (defined as binding in the presence of excess SMS-201-995) was unchanged by the presence of Mg^{2+} and enhanced by the addition of NaCl. Previous investigations have suggested that the SS-14 binding site in HGM may have a lower affinity than that in RCC (Miller et al, 1989). These findings suggest that the SS-14 binding site in HGM may be similar to the SS_2 binding site of Reubi and Maurer (1986).



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166P CATABOLISM OF PLATELET ACTIVATING FACTOR (PAF) BY HUMAN COLON MUCOSA

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PAF is a phospholipid having pro-inflammatory and pro-ischaemic properties. It is formed by the human colon mucosa in larger amounts in ulcerative colitis than in normal mucosa (Eliakim et al, 1988). Mechanisms for PAF and lyso PAF catabolism have not been described for human intestinal tissue. PAF (1-alkyl-2-acetyl glycerophosphocholine) can be catabolised by removal of the 2-acetyl group giving lyso PAF. This can then be (1) acylated to 1-alkyl-2-acyl glycerophosphocholine (alkyl acyl GPC) or (2) phospholipases can act on the head group of lyso PAF to give neutral lipids. These possibilities were studied in supernatants of homogenates of normal human colon mucosa prepared by centrifuging at 1000g for 15min (4°C). Aliquots (200μg protein) were incubated with PAF labelled in the 1-alkyl position ([^{3}H]alkyl PAF) 0.2μCi, or 1-0-[^{3}H]alkyl-2-lyso glycerophosphocholine ([^{3}H]lyso PAF), 500nCi or 1-0-alkyl glycero-3-phospho N-methyl (^{14}C)choline ([^{14}C]lyso PAF) 0.04μCi at 37°C for up to 90min in 0.05M Tris-HCl buffer containing 5mM calcium (pH8). After extracting with chloroform:methanol the radioactive products were separated by silica gel TLC of the organic phase in chloroform:methanol:acetic acid:water (50:25:8:2.5) and aqueous phase in methanol:0.9% NaCl:NH_4OH (50:50:5). Results are products formed as a percentage of total radioactivity on TLC plate (mean $\pm$ SD). The presence of an acetylhydrolase was confirmed in 90min incubation of colon mucosal enzymes; with [^{3}H]alkyl PAF as substrate 26.4 $\pm$ 5.4% was unchanged and 9.5 $\pm$ 3.6% alkyl lyso PAF was formed. Acylation of lyso PAF occurred as 16.0 $\pm$ 7.0% alkyl acyl GPC was formed. In addition, 35.6 $\pm$ 19.2% NL and 11.8 $\pm$ 4.0% unknown product at Rf 0.8 were formed (n=3). Moreover, addition of an acetylhydrolase inhibitor phenyl methyl sulphonyl fluoride (10mM) substantially reduced lyso PAF, alkyl acyl GPC and NL formation from [^{3}H]alkyl PAF. That lyso PAF was an intermediate was also suggested by time course experiments and because with [^{3}H]alkyl lyso PAF as substrate, 24.4 $\pm$ 9.2% was unchanged and 28.9 $\pm$ 4.1% alkyl acyl GPC, 22.8 $\pm$ 11.1% NL and 16.8 $\pm$ 4.7% unknown peak were formed (n=4 mean $\pm$ SD). The possibility that the NL could be formed from lyso PAF by a phospholipase acting at the head group was examined, using [^{14}C]choline labelled lyso PAF as substrate the time dependent and Ca^{2+} dependent production of an aqueous product co-chromatographing with choline was found. In 7 out of 20 preparations a phosphohydrolase inhibitor sodium vanadate (1mM) decreased NL but not alkyl acyl GPC using [^{3}H]lyso PAF as substrate. In Ca^{2+} free EDTA(.5mM) Tris, NL formation from [^{3}H]lyso PAF or [^{3}H]PAF and choline from [^{14}C]choline lyso PAF was reduced. However, alkyl acyl GPC formation from [^{3}H]PAF or [^{3}H]lyso PAF was not reduced. This work shows that in human colon mucosa PAF appears to be de-acetylated to lyso PAF which is then 2-acylated by a Ca^{2+} independent mechanism or hydrolysed at the head group by a Ca^{2+} dependent phospholipase which may be phospholipase D.
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167P β-ADRENOCEPTORS MEDIATING RELAXATION IN RAT SMALL INTESTINE ARE ATYPICAL IN NATURE

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Evidence exists for the presence of a population of atypical β-adrenoceptors in the gastrointestinal tract with similarities to the atypical, or β_3-, adrenoceptors of adipocytes (for review see Zaagsma & Nahorski, 1990). The atypical β-adrenoceptors are characterized by a low affinity for classical β-adrenoceptor antagonists, e.g. propranolol, and by the high potency of a novel class of β-adrenoceptor agonists, e.g. BRL 37344 (Arch *et al.*, 1984). It has also been reported that cyanopindolol is a competitive and relatively potent antagonist at atypical β-adrenoceptors in the gut (Blue *et al.*, 1989, McLaughlin & MacDonald, 1990). The present study was carried out to examine the characteristics of β-adrenoceptors mediating relaxation in rat small intestine.

Longitudinal smooth muscle strips of rat jejunum were suspended in Krebs solution at 37°C for isotonic recording. The Krebs solution contained cocaine (3 μM), ascorbic acid (30 μM) and EDTA (30 μM) and was gassed with 95/5% O2/CO2 . Strips were contracted with a submaximal concentration of potassium chloride (40 mM). Cumulative concentration response curves to agonists were carried out and response expressed as % relaxation.

Catecholamines and BRL 37344 produced relaxation of the KCl-contracted strips with pD_2 values of (mean ± s.e.) 6.9 ± 0.1 (isoprenaline), 5.9 ± 0.2 (noradrenaline), 5.5 ± 0.15 (adrenaline) and 6.7 ± 0.3 (BRL 37344) giving an order of potency of isoprenaline (1.0) > BRL 37344 (0.63) > noradrenaline (0.1) > adrenaline (0.04) (n = 4-6). In the presence of both prazosin (1 μM) and propranolol (1 μM) only small dextral shifts of the concentration response curves were observed (isoprenaline, 6.1 fold shift, $P < 0.001$; BRL 37344, 1.5, $P > 0.5$; noradrenaline, 3.3, $P < 0.05$; adrenaline, 2.7, $P < 0.05$), giving an order of potency of BRL 37344 (2.5) > isoprenaline (1.0) > noradrenaline (0.2) > adrenaline (0.1) (n = 7-10). In the presence of prazosin (1 μM) and propranolol (1 μM), cyanopindolol (0.1, 0.3, 1.0, 3 & 10 μM) produced a concentration dependent rightward shift of the concentration response curve to adrenaline with a Schild slope not significantly different from unity (0.89 ± 0.17, 95% C.L., n = 23) and a pA_2 value (mean ± s.e. mean) of 7.00 ± 0.06 (n = 23).

The resistance of relaxant responses to propranolol, the relatively high potency of BRL 37344 compared to catecholamines and the competitive antagonism of relaxant responses to adrenaline by cyanopindolol suggest that β-adrenoceptors in rat small intestine are atypical in nature.

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168P A SPIROLACTAM CONFORMATIONALLY-CONSTRAINED ANALOGUE OF PHYSALAEMIN WHICH IS A PEPTIDASE-RESISTANT, SELECTIVE NEUROKININ NK-1 RECEPTOR ANTAGONIST

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Introduction of the (S)-spirolactam bicyclic conformational constraint into substance P (SP) analogues confers potent NK-1 antagonist activity (for example GR71251, [D-Pro^9[Spiro-γ-lactam]Leu^{10},Trp^{11}]SP(1-11), Hagan et al.,1990; Ward et al., 1990). We now report the effect of this modification in the non-mammalian tachykinin, physalaemin (PHYS).

[D-Pro^9[Spiro-γ-Lactam]Leu^{10},Trp^{11}]PHYS(1-11) (GR82334) is a reversible, competitive antagonist at NK-1 receptors *in vitro*. It potently antagonised SPmethylester (SPOMe)-induced contractions of guinea-pig ileum longitudinal muscle (GPI; in the presence of atropine, 1μM) and spiral-strips of guinea-pig trachea (GPT; in the presence of the peptidase inhibitors, phosphoramidon, 1μM and bestatin, 100μM) but was much weaker at antagonising NK-2 receptor-mediated contractions of rat colon muscularis mucosae (RCM) or NK-3 receptor-mediated contractions of rat everted portal vein (RPV) (Table 1).

Table 1 Neurokinin Receptor Antagonist Activity of GR82334

Tissue Preparation	Agonist	Receptor Type	mean pK_B	s.e.mean	n
GPI	SPOMe	NK-1	7.64	0.01	17
GPT	SPOMe	NK-1	7.23	0.10	16
RCM	neurokinin A	NK-2	no significant effect at 10μM		
RPV	neurokinin B	NK-3	no significant effect at 10μM		

In contrast to SP or GR71251 (Hagan et al.,1990), at concentrations up to 30μM, GR82334 did not release histamine from rat peritoneal mast cells *in vitro*. Unlike GR71251, which was extensively degraded, GR82334 (50μM) was not significantly metabolised (100% recovery by HPLC) after 1h incubation at 37°C with rat liver S9 homogenates (600μg protein; 0.1M Tris-HCl buffer pH 7.4). In pentobarbitone-anaesthetised, artificially ventilated guinea-pigs, GR82334 (3μmolkg^{-1} given subcutaneously), produced a long lasting antagonism (>3h) of the increase in tracheal insufflation pressure-induced by the NK-1 agonist SPOMe (2-160nmolkg^{-1} iv).

In conclusion, the replacement of the SP sequence in GR71251 by that of PHYS in the spirolactam-containing analogue, GR82334, appears to confer metabolic stability and loss of histamine-releasing potential whilst maintaining NK-1 antagonist potency and selectivity.

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169P CONTRACTILITY OF RAT ISOLATED SMALL INTESTINE

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Rats have frequently been used as experimental models in endocrine and neuroendocrine but rarely in gastrointestinal pharmacology. As a basis for studies of endocrine and neuroendocrine effects on gastrointestinal motility we have now studied contractions of rat isolated small intestinal longitudinal muscle evoked by electrical field stimulation (EFS) of the intrinsic innervation and by ACh, 5-HT, $PGF_{2\alpha}$ and substance P.

Segments of ileum (1-2 cm long) were taken from freshly-killed 200-250g female CFY rats and suspended (counterbalanced by 0.5 g) in isolated organ baths containing Krebs solution (pH 7.4, 37°C) for isotonic recording of longitudinal muscle contractility. Intrinsic nerves were activated by 15s trains of EFS with 1 ms pulses at 1-8 Hz, supramaximal voltage. All contractions were measured as percentages of maximal contractions evoked by 1μM ACh in each tissue after an initial equilibration period. Dose-related contractions were evoked by 5-HT (5-200nM), $PGF_{2\alpha}$ (5-400nM) and substance P (1.5-600nM), maximal contractions being 32±5%, 43±5% and 51±3% respectively of the 1μM ACh - evoked response. EFS-evoked contractions increased with frequency up to 79±7% of maximum at 8Hz.

Atropine prevented ACh (1μM)-evoked contractions and virtually abolished contractions elicited by 1.5-30nM substance P ($P<0.05$, n=8) but did not significantly alter contractions evoked by higher concentrations of the peptide or by $PGF_{2\alpha}$ (8-200nM). The EFS-evoked contractions were substantially atropine-resistant ($P>0.05$, n=8). The substance P antagonist ([D-arg, D-pro, D-trp, leu]-substance P, 1.3 μM) inhibited 1.5nM substance P-evoked contractions ($p<0.05$, n=8) but did not alter responses to higher concentrations. 5-HT (5-1,000 nM)-evoked contractions were virtually abolished by methysergide (210nM) ($P<0.05$, n=8) but unaffected by MDL 72.222 (240nM) (n=8). Ketanserin (170nM) only antagonised contractions elicited by 5-HT in concentrations greater than 100nM ($p<0.05$, n=8).

Thus, in this tissue there was evidence of functional muscarinic cholinoceptors, prostaglandin $F_{2\alpha}$ receptors, substance P receptors and 5-HT receptors. Substance P-evoked responses were partially cholinergically-mediated. 5-HT receptors were of the $5\text{-}HT_1$ -like and $5\text{-}HT_2$ subtypes. EFS-evoked contractions were predominantly non-cholinergic.

170P PHARMACOLOGICAL CHARACTERISATION OF THE RECEPTORS MEDIATING THE CONTRACTILE RESPONSE TO BRADYKININ IN THE GUINEA-PIG ILEUM AND RAT UTERUS

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Bradykinin (BK) receptors have been divided into at least two subtypes, B_1 and B_2, (Steranka et al., 1988) and preliminary evidence suggests that the B_2 receptor may be subdivided further (Field et al., 1988). The aim of this study was to characterise more fully the B_2 receptors mediating the contractile response to BK in the guinea-pig ileum and rat uterus.

Guinea-pig ileum longitudinal muscle (GPILM) from male Dunkin-Hartley guinea-pigs (250-350g) or rat uterus (RUT) from female RH rats (Glaxo-bred, 120-200g, in oestrous) were set up under a resting tension of 1g. The GPILM was bathed in Krebs-Henseleit solution (mM: NaCl 118, $NaHCO_3$ 29, glucose 11.1, KCl 4.7, $MgSO_4$ 4, $CaCl_2$ 2.6, KH_2PO_4 1.2; pH 7.4, 37°C) and the RUT in de Jalon's solution (mM: NaCl 154, KCl 2.8, $NaHCO_3$ 6, glucose 2.8, $CaCl_2$ 0.5, pH 7.4, 28°C). Atropine (1μM), mepyramine (1μM) and indomethacin (3μM) were also present. In the GPILM, the contractile response to BK (0.3 to 100nM) was potentiated (8.1±1.4-fold leftward shift, n=8) by the ACE inhibitor captopril (1μM), whereas the kininase I inhibitor, MERGETPA (10μM, n=8) and the enkephalinase inhibitor, phosphoramidon (1μM, n=4), had no effect. The calcium channel blocker nifedipine (1μM, n=4) completely abolished the BK response. In the GPILM, BK antagonists were added in the presence of captopril and MERGETPA. In the RUT, BK (0.1 to 30 nM) produced a biphasic response which consisted of an initial rapid phasic contraction followed, at higher concentrations, by a slow tonic contraction. Nifedipine (1μM) selectively blocked the phasic contraction (11.6±1.3-fold rightward shift, n=11), but had no effect on the tonic contraction. Phosphoramidon (1μM) produced a potentiation of the response to BK (8.7±3.9-fold leftward shift, n=8), whereas captopril (1μM, n=4) and MERGETPA (10μM, n=4) had no effect. In the RUT, antagonist studies were performed in the presence of phosphoramidon, captopril, MERGETPA and nifedipine. The pK_B values and Schild slopes (95% C.I.; n=4) for standard antagonists are given in the table.

	GPILM		RUT	
Antagonist	pK_B	Slope	pK_B	Slope
D-Phe^7BK	5.4 (5.3-5.5)	1.1 (0.9-1.2)	5.3 (5.2-5.4)	1.2 (1.1-1.3)
$Thi^{5,8}$D-Phe^7BK	5.5 (5.4-5.6)	0.9 (0.6-1.2)	6.3 (6.2-6.4)	1.1 (1.0-1.4)
D-$ArgHyp^3$D-Phe^7BK	5.5 (5.4-5.7)	0.9 (0.7-1.0)	6.8 (6.7-6.9)	1.0 (0.9-1.2)
D-$ArgHyp^3Thi^{5,8}$D-Phe^7BK	5.8 (5.7-5.9)	1.0 (0.9-1.1)	6.9 (6.8-7.0)	1.1 (0.8-1.3)

There are clear differences in the pK_B values for the antagonists in the GPILM and RUT. Whether this indicates the presence of B_2 receptor subtypes in these preparations or simply reflects a species difference is unclear at present. The finding that the tonic contraction in the RUT is not blocked by nifedipine suggests that B_2 receptors are coupled to different effector systems in the two preparations.

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171P HOE140: A NOVEL BRADYKININ ANALOGUE THAT IS A POTENT ANTAGONIST AT BOTH B_2 AND B_3 RECEPTORS *IN VITRO*

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A bradykinin analogue DArg[Hyp^{3}Thi^{5}DTic^{7}Oic^8]Bk (HOE140, Tic:-1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid; Oic:-(3aS,7aS)-octahydroindol-2-carboxylic acid) has recently been claimed (Breipohl et al 1990) as the most potent bradykinin antagonist presently known. We have compared the antagonistic potency and selectivity of HOE-140 with a number of other bradykinin B_2 antagonists against responses to bradykinin in the guinea pig ileum (GPI), the oestrogen-primed rat uterus (RU), NG108-15 cells and peripheral nociceptors in a spinal cord/tail preparation maintained in vitro. In addition we present evidence that HOE140 is also a potent antagonist at the proposed B_3 receptor in the guinea pig trachea (GPT) (Farmer et al 1989).

The GPI, RU and GPT were removed from adult animals, suspended in organ baths and bathed in physiological solution. Contractile responses to bradykinin were recorded isometrically, in the case of the GPT in preparations lacking an intact epithelium as described previously (Farmer et al 1989). NG108-15 cells were equilibrated with ^{45}Ca and the increase in the rate of efflux in response to bradykinin determined (Snell et al 1990). Bradykinin-induced activation of peripheral nociceptors was measured in a neonatal rat spinal cord/tail preparation maintained in vitro (Dray et al 1988). Comparison of antagonist potencies were expressed as the IC_{50} concentrations obtained by measuring the effect of 3 or more concentrations of antagonist against the response produced by a submaximal concentration (EC_{50} concentration) of bradykinin.

The rank order potency in the RU, NG108-15 cells and cord/tail was HOE140 (0.5- 16nM) > DArgHyp^{3}Thi^{5,8}DPhe^{7}Bk (16-45nM) ' DArgHyp^{3}DPhe^{7}Bk (NPC567, 30-43nM) > DArgHyp^{2}Thi^{5,8}DPhe^{7}Bk (150nM - 1µM) > Thi^{5,8}DPhe^{7}Bk (350-500nM). However in the guinea pig ileum all antagonists were 10-50 times less potent and the rank ordering was HOE140 > DArgHyp^{3}Thi^{5,8}DPhe^{7}Bk > Thi^{5,8}DPhe^{7}Bk > NPC567 > DArgHyp^{2}Thi^{5,8}DPhe^{7}Bk. pA_2 analysis of HOE140 on the GPI and RU gave values of 8.82±0.06 and 9.74±0.08 respectively. Schild analysis was consistent with competitive antagonism in the RU (slope 1.09±0.06) but not in the GPI (slope 1.68±0.08). In the GPT, NPC567 was inactive at 10µM but HOE140 antagonised Bk with an IC_{50} of <100nM.

These data confirm that HOE140 is the most potent bradykinin antagonist at B_2 receptors hitherto described with similar apparent potency on the RU, NG108-15 cells and nociceptive neurones. The difference in activity of the antagonists studied in the GPI is consistent with the presence of a different bradykinin receptor in this tissue. In addition, HOE140 is also an antagonist at the B_3 receptor as proposed by Farmer et al (1989).

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172P THROMBOXANE RECEPTOR BLOCKADE AND THE ISOLATED HUMAN MYOMETRIUM

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The human uterus *in vitro* has been shown to contract to the thromboxane mimetic U46619 (11,9-epoxymethano prostaglandin H_2) (Clayton et al., 1986). In this study BM13.505 (Stegmeier et al., 1986), EP092 (Armstrong et al., 1985) and GR32191 (Lumley et al., 1989) and BAY u3405 (McKenniff et al., 1990) were compared for thromboxane receptor antagonist activity on myometrium from non-pregnant and pregnant subjects. Strips (n = 6-7) of human myometrium were obtained from pre-menopausal patients at hysterectomy or during Caesarean section and set up as previously described (Massele & Senior, 1981).

All thromboxane receptor antagonists tested caused a progressive parallel shift to the right of the dose-response curve to U46619 with increasing concentration of antagonist and the pA_2 values are shown in the table below.

Thromboxane antagonist	Dose range	pA_2 Non-pregnant	pA_2 Pregnant
BM13.505	(10^{-6}M-10^{-8}M)	7.1	6.8
EP092	(10^{-6}M-10^{-8}M)	8.4	8.7
GR32191	(10^{-6}M-10^{-7}M)	7.0	7.2
BAY u3405	(10^{-6}-10^{-9}M)	9.3	8.1

It has previously been suggested that BAY u3405 has no thromboxane sub-type selectivity (McKenniff et al., 1990) which may explain the higher pA_2 value seen with this compound.

Thromboxane may be implicated in several pathological disorders involving uterine hypercontractility such as dysmenorrhoea and also in pregnancy-induced hypertension and it is possible that thromboxane antagonists could be useful clinically for the treatment of such disorders.

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173P THE EFFECTS OF CROMAKALIM ON MEMBRANE POTENTIAL AND TENSION IN ISOLATED BOVINE TRACHEALIS IN Ca-FREE AND Ca-CONTAINING PHYSIOLOGICAL SALT SOLUTIONS

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The reported effects of cromakalim (CK) in smooth muscle focus mainly on the relaxant nature of this compound. However, we have recently described a novel contractile response to CK in rabbit aortic strips in Ca-free solution (Bray et al., 1989; Duty & Weston, 1990). In the present study, to further analyse this response we have examined both the electrophysiological and mechanical effects of cromakalim in bovine tracheal smooth muscle in Ca-free solution.

For tension measurements, strips of bovine tracheal smooth muscle were mounted for isometric recording in a MOPS-buffered physiological salt solution (PSS). Following a 15 min equilibration in Ca-free PSS, concentration effect curves to either CK (0.3 -10μM) or vehicle were constructed. Electrophysiological determinations of membrane potential in similar strips of trachealis muscle were obtained by cell sampling techniques. The effects of CK (10μM) were examined in both Ca-containing and Ca-free PSS. In the studies using Ca-free PSS, tension was simultaneously recorded from an adjacent strip of muscle.

CK (0.3 -10μM), produced a concentration-dependent increase in tension in tissues bathed in Ca-free PSS. No increase in tension was observed in vehicle controls. In Ca containing PSS, the resting membrane potential of tracheal smooth muscle was -62.5±1.4mV (n=20 cells; 4 tissues). CK (10μM) produced a maximum hyperpolarisation of 22.5±2.6mV (n=4 cells; 4 tissues) which was sustained throughout the duration of CK contact. In tissues bathed in Ca-free PSS the membrane potential was depolarised to a resting level of -47.4±2.0mV (n=11 cells; 6 tissues). Under these conditions, CK (10μM) produced a maximum hyperpolarisation of 24.4±0.9mV (n=3 cells; 3 tissues) which, after a period of about 15 min, declined gradually over the remaining period of contact with CK. During this period when the hyperpolarisation was fading, tension developed slowly in the adjacent muscle strip. A correlation coefficient of approximately 0.76 was determined between the gradual decrease in membrane potential and the increase in tension developed over this prolonged contact period with CK in Ca-free PSS.

Clearly, CK produces membrane hyperpolarisation under Ca-free conditions which, unlike the response in Ca-containing PSS, is not sustained. Whether there is a causal relationship between the decline in hyperpolarisation and the development of tension remains to be determined. However, it is proposed that the contractile response to CK, revealed in a Ca-free solution, may reflect this reversal of membrane potential in addition to the previously implicated factors such as intracellular Ca release (Duty & Weston, 1990). Possible links between these two events are subject to future investigation.

This work was funded by a SERC Case Award with Roche Products Ltd.

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174P COMPARISON OF THE EFFECTS OF THE PAF ANTAGONISTS RP 59227 AND WEB 2086 IN ANIMAL MODELS OF AIRWAY HYPERREACTIVITY

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One of the characteristic, well documented features of bronchial asthma is the accompanying non-specific airway hyperreactivity. The precise mechanism underlying the development of the hyper-reactivity is not known though several mediators including PAF (Cuss et al., 1986; Barnes & Chung, 1987) have been implicated. PAF also induces sustained bronchial hyperreactivity in man (Cuss et al., 1986). We have examined the effects of the PAF antagonists RP 59227 ((+)-N-(3-benzoyl-phenyl)-3-(3-pyridyl)-1H,3H-pyrrolo [1,2-C]thiazole-7-carboxamide) and WEB 2086 in three animal models of airway hyperreactivity.

Hyperreactivity was assessed by measuring bronchoconstrictor responses (change in pulmonary inflation pressure, PIP) to bombesin (100 - 300 ng kg^{-1} i.v.) before and after intravenous infusion of PAF (600 ng kg^{-1} h^{-1}) or vehicle (0.1 % BSA) to anaesthetized (pentobarbitone sodium, 60 mg kg^{-1}), artificially ventilated guinea pigs. Alternatively, conscious guinea pigs were exposed to an aerosol of PAF (500 μg ml^{-1} for 5 min) or ovalbumin (OA, 300 μg ml^{-1} for 3 min) in previously sensitized animals. After 24 h animals were anaesthetized as before and the reactivity to bombesin (100 - 500 ng kg^{-1}, i.v.) or histamine (1 - 100 μg kg^{-1}, i.v.) was assessed in comparison with animals which had not been challenged. To examine their effects on hyperreactivity, RP 59227 or WEB 2086 were given either as an i.v. bolus injection 10 min prior to PAF infusion or p.o. 60 min before exposure to aerosolized PAF or OA. Hyperreactivity was assessed by comparing PIP before and after PAF infusion or by comparing dose response curves to spasmogen in untreated versus challenged animals.

Exposure to PAF but not vehicle (i.v.) increased the response to bombesin by 179 ± 17 % (n = 6). PAF and antigen aerosol significantly augmented responses to bombesin (2 fold leftward shift, 53% increased maximum response, n = 11, PAF-exposed) or to histamine (2.5 fold leftward shift, 53 % increased maximum response, n = 9, OA-exposed). No increase in reactivity to bombesin was seen following antigen exposure (n = 10). Vehicle controls (0.1 % BSA or saline) did not induce hyperreactivity. RP 59227 and WEB 2086 (100 μg kg^{-1} i.v. or 10 mg kg^{-1} p.o.) respectively abolished or markedly attenuated PAF (i.v. and inhalation)-induced hyperreactivity. Neither compound protected against OA-induced hyperreactivity.

From the data obtained in these animal models it is concluded that both compounds inhibited PAF but not antigen-induced hyperreactivity. The data suggest that endogenous PAF contributes little to antigen-induced hyperreactivity in this species.

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175P PHARMACOLOGICAL MODULATION OF PAF-INDUCED PLATELET ACCUMULATION IN THE PULMONARY CIRCULATION OF THE RABBIT

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Platelet activating factor (PAF), a phospholipid generated by a range of inflammatory cells, has been implicated in the development of shock and the adult respiratory distress syndrome. A prominent feature of these conditions is the accumulation of platelets within the pulmonary vasculature. The present study reports findings on the pharmacological modulation of PAF-induced intrathoracic platelet accumulation in the rabbit *in vivo*.

We have recently described a non-invasive technique for the automated, continuous, external imaging of ^{111}Indium-labelled platelets in rabbits (May *et al.*, 1990). Test drugs were administered i.v. one minute prior to the i.v. bolus injection of PAF (100 $n$gkg^{-1}) to assess their effects on ^{111}In-labelled platelet responses.

The PAF receptor antagonists SRI 63-441 (1 mgkg^{-1}) and brotizolam (1 mgkg^{-1}) significantly inhibited PAF-induced ^{111}In-labelled platelet accumulation in the rabbit ($p<0.05$). Inhibition was selective, since this dose of SRI 63-441 and brotizolam did not diminish the response to ADP of a similar magnitude. The effect of SRI 63-441 was dose-related over the range 0.01-1 mgkg^{-1}. When administered alone into rabbits, the anticoagulant heparin (500 I.U.kg^{-1}) had no effect on ^{111}In-labelled platelet movement. This dose of heparin significantly inhibited the PAF-induced platelet response ($p<0.05$). The stable prostacyclin analogue iloprost (10 $u$gkg^{-1}) exerted significant inhibition of PAF-induced ^{111}In-labelled intrathoracic platelet accumulation ($p<0.05$), which was dose related over the range 0.01-100 $u$gkg^{-1}. Both non-steroidal anti-inflammatory drugs, acetyl salicylic acid (1 mgkg^{-1}) and indomethacin (1 mgkg^{-1}) produced significant enhancement of PAF-induced ^{111}In-labelled platelet accumulation (72% and 66% respectively) ($p<0.05$). Dazmegrel (10 mgkg^{-1}), a thromboxane synthetase inhibitor, did not significantly inhibit the *in vivo* platelet response to PAF.

In summary, PAF induces accumulation of platelets within the pulmonary vasculature of the rabbit independent of the release of TXA_2.

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176P SALMETEROL INHIBITS GRANULOCYTE ACCUMULATION IN GUINEA-PIG LUNG

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Previous studies have shown that salmeterol inhibits histamine-stimulated protein extravasation in guinea-pig lung and zymosan-induced granulocyte accumulation and increased vascular permeability in guinea-pig skin (Whelan and Johnson, 1990a; 1990b). The present report describes the inhibitory actions of salmeterol on lipopolysaccharide (LPS)-induced neutrophil infiltration and platelet activating factor (PAF)-induced eosinophil accumulation in guinea-pig lung.

Guinea-pigs were treated with salmeterol or vehicle by inhalation, as described previously (Whelan and Johnson, 1990a). After 30 min, animals were placed in a plexiglas chamber and exposed to an aerosol of LPS (100µg.ml^{-1}) or PAF (100µg.ml^{-1}) for 10 min. Four hours after LPS, or 24h after PAF, guinea-pigs were killed (Expiral) and the lungs lavaged twice with 10ml phosphate-buffered saline, containing heparin (10U.ml^{-1}). Total and differential leukocyte counts were performed on aliquots of the pooled bronchoalveolar lavage fluid (BALF). From these data, granulocyte numbers .ml^{-1} BALF were calculated.

LPS increased the neutrophil content of BALF from 3.8 ± 1.2 (mean ± s.e.m., n=6) to 141.3 ± 33.3 x10^3 cells.ml^{-1} 4h after challenge. LPS had no significant effect on the numbers of other cells found in the BALF. Salmeterol, at an aerosol concentration of 0.1mg.ml^{-1}, reduced LPS-induced neutrophil accumulation to 46.8 ± 9.1 (n=6) x10^3 cells.ml^{-1}. A lower dose of salmeterol (0.01mg.ml^{-1}) had no significant effect. Twenty-four hours after administration of PAF (100µg.ml^{-1}), the eosinophil content of BALF increased from 53.3 ± 12.5 (n=6) to 97.7 ± 18.8 (n=9) x10^3 cells.ml^{-1}. This treatment had no effect on other cells in BALF. Salmeterol administration (0.1 and 1.0mg.ml^{-1}) prevented PAF-induced eosinophil accumulation in BALF (eosinophil count 59.2 ± 19.8 and 46.8 ± 13.7 x10^3 (n=6) cells.ml^{-1} respectively).

The above experiments demonstrate that salmeterol at bronchodilator doses (Ball et al., 1987), inhibits LPS-induced neutrophil infiltration and PAF-induced eosinophil accumulation in guinea-pig lung. These findings provide further evidence to support the proposal (Whelan & Johnson, 1990a, 1990b) that salmeterol may exhibit useful anti-inflammatory effects in man.

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177P ANTIGEN CHALLENGE INDUCES AIRWAY HYPERRESPONSIVENESS FOLLOWING TRANSFER OF EOSINOPHILS FROM SENSITISED GUINEA-PIGS

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There is increasing evidence that the eosinophil (Eos) is an important effector cell in the pathogenesis of bronchial asthma. However, it remains to be demonstrated that antigen challenge can induce Eos activation and the subsequent development of airway hyperresponsiveness (AHR) in-vivo. In these experiments, we have transfered Eos from sensitised animals to naive animals and investigated the modification in airway responsiveness following antigen challenge.

Male Hartley guinea pigs were exposed to an aerosol of 2% ovalbumin (OA) for 30 min on day 0 and 2 and used between day 21-35. Control animals were exposed to an aerosol of saline alone. Pulmonary Eos were obtained by bronchoalveolar lavage (BAL) and purified on a metrizamide gradient (18-24%).

Recipient animals were anaesthetised with sodium pentobarbitone (40 mg/kg), the trachea cannulated and the animal mechanically ventilated. Airflow and transpulmonary pressure were measured and total lung resistance (R_L) calculated using a computorised pulmonary mechanical analyser (PMS, London, UK). Airway responsiveness was assessed by i.v. administration of 5HT and the dose required to increase R_L by 100% calculated (PD100).

Thirty min after the last 5HT injection, 1 x 10^6 cells containing 71±3% Eos were injected directly to the lungs of recipient animals. Fifteen min later, 200µg of OA was injected i.t. AHR to 5HT was then assessed 3 hr later. BAL was then performed and levels of eosinophil peroxidase (EPO) determined in the supernatant.

OA challenge of animals that received Eos from sensitised animals induced a small increase in RL which resolved within 15 minutes. OA challenge of control animals failed to induce any significant bronchoconstriction. 3 hr after OA challenge of animals that had received sensitised Eos there was a marked enhancement of the bronchoconstrictor response to 5HT (Pre PD100 1.45 ± 0.27 µg/kg, Post 0.48 ± 0.16 µg/kg, $P< 0.05$). In contrast, OA challenge of animals that received Eos from non-immunised guinea pigs was not associated with any change in airway responsiveness (Pre PD100 1.13 ± 0.09, Post 1.23 ± 0.04 µg/ml). Antigen induced AHR was associated with an elevation of EPO levels in the BAL (43.8 ± 25 mU/ml) compared to control animals (1.2 ± 1.2 mU/ml).

Our data supports the hypothesis that Eos activation can induce AHR. However, the precise mechanisms underlying this phenomenon remain to be determined.

178P THROMBOXANE ACCOUNTS FOR BRONCHOCONSTRICTION BUT NOT FOR PLATELET SEQUESTRATION NOR INCREASED ALBUMIN EXCHANGES INDUCED BY fMLP IN THE GUINEA-PIG LUNG

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The granulocyte secretagogue fMLP, injected i.v. to anaesthetized guinea-pigs, induces bronchoconstriction (BC), hypotension, polymorphonuclear leukocyte (PMN) sequestration and an increase of trans-endothelial albumin exchanges. We recently observed that fMLP induces a transient platelet (PL) entrapment within the lungs, a typical exemple of cell cross talk "in vivo" since platelets are not directly activated by fMLP. The fMLP-induced release of thromboxane A_2 (TXA_2), from guinea-pig lungs (Boukili et al., 1986), might account for BC and for lung PL sequestration and contribute to the increase of trans-endothelial albumin exchanges through its effect on vascular pressure. In this study, BC was evaluated from the pulmonary insuflation pressure. Using radioactive tracers, we evaluated the variations of the lung contents in the radioactivity of labelled PL, red blood cells (RBC) and extravascular albumin (ALBev), which correspond to platelet aggregation and to variations of lung blood volume and of trans-endothelial albumin exchanges, respectively (Bureau et al., 1989). In all experiments fMLP was injected i.v. at the dose of 10 µg/kg.

Trimetoquinol, an antagonist of TXA_2-PGH_2 receptors (200 µg/kg i.v. 3 min before fMLP challenge) suppressed BC and reduced PL entrapment (- 74.6 ± 28.4 %, SE n=4, 1 min after fMLP injection); it attenuated the increase in ALBev (- 48.3 ± 3.3 %), but not the drop in RBC lung contents. However, Trimetoquinol was non specific, since it also suppressed histamine-induced BC. Its protective effect against fMLP-induced increase of lung vascular albumin exchange might also be explained by its β2 agonist properties.

Ridogrel is a specific dual TXA_2 synthetase inhibitor / TXA_2-PGH_2 receptor antagonist (de Clerck et al., 1989) free from anti-histaminic effects. At 5 mg/kg i.v. 15 minutes before fMLP, Ridogrel suppressed BC and reduced PL entrapment (- 70.4 ±13.9 %, n=6, 1 min after fMLP injection) with no significant effect on fMLP-induced variation of ALBev and RBC lung contents. Paradoxically, the anti-cyclooxygenase drug aspirin at high or low doses (50 or 5 mg/kg i.v.) before fMLP challenge, suppressed BC but failed to prevent the lung PL entrapment. Consequently, we hypothesised that "in vivo" fMLP-induced platelet activation is not TXA_2 dependent and that its inhibition by Ridogrel might be due to the rechanelling of arachidonic acid metabolism toward the synthesis of protective prostaglandins. This hypothesis is consistent with our observation that ridogrel and the TXA_2 synthetase inhibitor dazoxiben were significantly more effective against arachidonate-induced thrombocytopenia than the TXA_2-PGH_2 receptors antagonist sulotroban. The use of Ridogrel as a TXA_2-PGH_2 receptor antagonist in aspirin treated animals confirmed this hypothesis since fMLP induced again a transient lung platelet sequestration whereas bronchoconstriction was largelly reduced. In the same time, fMLP-induced reduction in RBC content and increase in ALBev were not modified.

As a conclusion, we obtained evidences that TXA_2 is an essential mediator of bronchoconstriction during fMLP-induced chock in guinea-pigs, and that in contrast, platelet sequestration, capillary albumin exchanges and blood volume reduction within the lung are TXA_2-independent. The platelet sequestration was inhibited by Ridogrel via the rechanelling of arachidonate to protective prostaglandins.

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de Clerck et al (1989) Thromb. Haemost **61**, 35-43

179P EFFECTS OF POLYARGININE ON BRONCHIAL MUCOSAL PERMEABILITY AND ITS RELATIONSHIP TO SMOOTH MUSCLE CONTRACTION IN ISOVOLUMIC BRONCHIAL SEGMENTS

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Damage to the airways mucosa by arginine-rich proteins of the eosinophil specific granule have been incriminated in the pathogenesis of bronchial hyperresponsiveness. Such injury could remove a diffusion barrier in the mucosa or inhibit the release of endogenous relaxing factors. We have used polyarginine (PA) as a model of the eosinophil granule proteins in order to investigate these possibilities. Mucosal permeability to albumin was measured using sheets of bovine bronchial mucosa mounted in modified Ussing chambers (Herbert et al., 1990). The effects of PA on smooth muscle contraction to acetylcholine (ACh; 0.1-10mM), KCl and field stimulation (EFS; 20Hz, 60V, 0.5ms) was assessed by measuring intralumenal pressure changes in fluid-filled isovolumetric segments of bovine bronchus (5mm internal diameter).

Exposure of bronchial mucosal sheets to 1mg ml^{-1} PA for 3h resulted in an increase in the the net basolateral-apical flux of albumin from 2.32 ± 0.23 to 3.79 ± 0.18 fmol cm^{-2} min^{-1} (n=6, P<0.05). However, it was ineffective at a concentration of 0.5 mg ml^{-1}. In the bronchial segment preparation, adventitial addition of 10mM ACh elicited an increase in pressure of 33.5 ± 9.9 cm H_2O compared with 6.12 ± 0.95 when it was added lumenally (n=4). Exposure of the exterior of the segment to 1mg ml^{-1} PA for 60min had no effect on the responses to ACh added adventitially, EFS or KCl. However, when added intralumenally, PA specifically augmented the responses to lumenal additions of ACh and KCl as shown below. Data are expressed as a percentage of the response elicited by adventitial administration of the same dose of ACh or KCl. Asterisks indicate significant differences (P<0.05-0.001) in 4 experiments.

	Percentage of the corresponding adventitial response when doses given lumenally			
	KCl	ACh 0.1mM	ACh 1.0mM	ACh 10mM
control	4.6 ± 1.1	12.4 ± 2.1	20.5 ± 4.6	20.5 ± 4.6
+PA (1mg ml^{-1})	26.9 ± 0.8*	78.4 ± 6.1*	45.8 ± 4.9*	58.9 ± 8.4*

Similar results were obtained in the segment preparation using a concentration 100μg ml^{-1} and a contact time of 2h. However, no effect was seen with PA at 10μg ml^{-1}. Histological examination of segments demonstrated a concentration-related disruption of epithelial integrity in PA-treated tissues. These results demonstrate that lumenal application of PA effectively augments the responses to agonists presented in the same way. However, this effect does not involve a change in the inherent sensitivity of the smooth muscle. A high concentration of PA was able to enhance the macromolecule permeability of the bronchial mucosa, suggesting that PA, and by inference eosinophil granule proteins, cause loss of a barrier to paracellular movement of solutes in the epithelium.

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180P THE ACTIONS OF PLATELET ACTIVATING FACTOR ON THE TRACHEAL VASCULATURE OF ANAESTHETIZED SHEEP

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Platelet activating factor (PAF) may be an important inflammatory mediator in the airways. It dilates the tracheobronchial circulation but its mode of action is unknown; it could act directly or by the release of further inflammatory mediators.

Eleven sheep (27-32 kg) were anaesthetized with pentobarbitone sodium (initially 20 $mg.kg^{-1}$), paralysed with gallamine triethiodide (initially 1.3 $mg.kg^{-1}$) and artificially ventilated through a low cervical tracheostomy. The cervical trachea was exposed and the associated vasculature isolated and perfused with the sheep's own blood (Webber *et al.*, 1988) An electromagnetic flow probe was inserted into the perfusion circuit to measure arterial inflow. Drugs were administered by injections directly into the tracheal arterial inflow. The magnitude of each response (mean ± s.e. mean) was assessed by the maximum change in vascular resistance expressed as the percentage change from control. Tracheal smooth muscle tone was assessed by measuring changes in the external diameter of the trachea. PAF (2 pmol to 2 nmol; n= 3 to 8) produced a dose-dependent decrease in tracheal vascular resistance (-0.9±0.9% to -43.2±7.9%). Lyso-PAF (0.2 nmol; n=4) did not change vascular resistance. Tracheal smooth muscle tone was not altered by PAF. The fall in vascular resistance produced with PAF (0.1 or 0.2 nmol) was unaffected by nedocromil sodium (1 mg i.a.; n=5), a cyclooxygenase inhibitor indomethacin (5 $mg.kg^{-1}$ i.v.; n=5), a leucotriene antagonist FPL55712 (2 $mg.kg^{-1}$ i.v.; n=5), or the histamine H_1 and H_2 antagonists mepyramine (2 $mg.kg^{-1}$ i.v.) with cimetidine (5 $mg.kg^{-1}$ i.v.; n=5). The PAF antagonist WEB 2086 (300 $\mu g.kg^{-1}$ i.v.; n=4) significantly reduced the vasodilation produced with PAF (before: -40.8±1.0%, after -16.5±5.9%; p<0.05).

Thus in this model PAF is a potent vasodilator of the tracheal circulation but has no action on tracheal smooth muscle. The vasodilation is mediated by specific PAF-receptors but is not due to the release of prostanoids, leucotrienes or histamine.

Acknowledgements: D.R. Corfield and S.E. Webber were supported by the Asthma Research Council.

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181P TWO LARGE CONDUCTANCE K^+ CHANNELS IN BOVINE TRACHEAL MYOCYTES

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Unitary current recordings were made from inside-out membrane patches from enzymatically dissociated bovine tracheal myocytes under recording conditions of symmetrical high [K^+] (140mM). Numerous large conductance, K^+-selective channels were seen which were of two types with differing properties. One type was sensitive to the [Ca^{2+}] at the cytosolic surface of the patch and had a slope conductance of 249 ± 18pS between +20 and +40mV. The other channel had a slope conductance of 268 ± 14pS between +20 and +40mV and showed a voltage-dependence which was insensitive to changes in [Ca^{2+}] at the cytosolic surface between zero and 10μM. The channels were also distinguished by their response to K^+-channel blocking agents. Only the Ca^{2+}-independent channels were sensitive to 1mM TEA and 10mM Cs^+ applied to the cytosolic surface, while only the Ca^{2+}-dependent channels were blocked by 100μM quinidine. Both channels were sensitive to Ba^{2+} although its effects on the two channel types were clearly different. 1mM Ba^{2+} increased the P_{open} of Ca^{2+}-dependent channels at negative potentials and decreased their P_{open} at positive potentials which resulted in inversion of the voltage-dependence of the currents. Unitary currents carried by the Ca^{2+}-independent channel were decreased in amplitude by 10mM Ba^{2+} only at positive potentials which indicates voltage-dependent block of the channel by Ba^{2+}. Neither channel was affected by 1mM 4-aminopyridine. Of 70 patches examined, 44 showed Ca^{2+}-dependent channels while in the remaining patches the channels were Ca^{2+}-independent. Most patches contained multiple channels but these were generally of one type, suggesting clustering of channels of each type. The large Ca^{2+}-dependent channels seen in the present investigation closely resemble the maxi K^+-channel (B_K) which has been widely reported in tracheal (McCann and Welsh, 1986) and other smooth muscle types. The Ca^{2+}-independent channels appear similar to the slow potential-sensitive K^+-channels described in rabbit jejunal smooth muscle by Benham and Bolton (1983).

The work was supported by grants from the National Asthma Campaign, and the Wellcome Trust.

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182P FURTHER STUDIES ON THE MECHANISM OF ACTION OF ISOPRENALINE IN BOVINE TRACHEAL SMOOTH MUSCLE

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It is generally accepted that isoprenaline (ISO) causes relaxation of smooth muscle tissues via elevation of intracellular cAMP levels $[cAMP]_i$, (eg see Rinard et al., 1983). In some tissues the relaxant effects of ISO are also associated with K channel opening (as evidenced by membrane hyperpolarisation and increased K efflux). However, it is not clear if this action is independent of its ability to raise $[cAMP]_i$. Indeed, recent electrophysiological studies have shown that ISO and cAMP can directly promote K channel opening in tracheal smooth muscle (Kume et al., 1989). In the present experiments we examined whether K channel opening contributes to the relaxant action of ISO.

Strips of bovine tracheal smooth muscle were mounted on a manifold for ^{42}K efflux studies (Bray et al., 1990) or in a tissue bath for the recording of either isometric tension or membrane potential. For the relaxant experiments the tissues were precontracted with 25mM or 100mM KCl. ISO (<μM), forskolin (<3μM), and the non-selective phosphodiesterase inhibitor isobutylmethylxanthine (IBMX, 0.1mM) attenuated contractile responses evoked by 25mM KCl but not 100mM KCl. Glibenclamide (1μM), inhibited relaxant responses evoked by high concentrations of ISO (0.16 to 1μM) and forskolin (0.3 to 10μM). The resting membrane potential of bovine tracheal smooth muscle cells was 60.0 ± 2.1 mV (n=7). ISO (0.1 to 1μM) produced marked hyperpolarisation (ranging from -6 to -20mV, n=9), although responsiveness varied between tissues. The basal ^{42}K efflux rate coefficient was 0.27 ± 0.02% per min and ISO (5μM) alone, IBMX (0.1mM) alone and IBMX (0.1mM) plus ISO (5μM) increased efflux to 0.46 ± 0.03, 0.38 ± 0.04 and 0.47 ± 0.04% per min, respectively (n=5). Glibenclamide (1μM) abolished the response to ISO (5μM). Forskolin (1μM) alone had no effect on ^{42}K efflux but in the presence of IBMX, forskolin (1μM) produced a small rise in ^{42}K exchange (n=5).

In the present study ISO caused relaxation, membrane hyperpolarisation and increased K efflux. Some of these effects were inhibited by glibenclamide. These results indicate that the relaxant action of ISO may be associated with K channel opening. Furthermore, it is possible that the K channel involved may be regulated by intracellular cAMP levels since both forskolin and IBMX showed differential inhibitory effects against contractions evoked by 25 or 100mM KCl and IBMX potentiated the effects of forskolin on ^{42}K efflux.

We acknowledge support from SmithKline Beecham (JL) and from Glaxo Group Research (DJT).

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183P QUINPRENALINE, A LONG ACTING β_2-ADRENOCEPTOR AGONIST *IN VITRO* - A COMPARISON WITH SALMETEROL

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Salmeterol is a potent β_2-adrenoceptor agonist with a remarkably long duration of action both in vitro and in vivo. It has been suggested that this persistent activity results from binding of the aralkyloxyalkyl side-chain to a specific 'exo-site' adjacent to the β_2-adrenoceptor (Bradshaw et al, 1987). Quinprenaline, a compound without such an extended side-chain, has also been reported to exhibit a prolonged duration of action at β_2-adrenoceptors in vitro (Scriabine et al, 1968). We have now compared the in vitro potency, duration of action and β_2-adrenoceptor binding characteristics of quinprenaline with those of salmeterol and the short-acting ß-agonist, salbutamol.

β_2-adrenoceptor agonist potency and duration of action were determined on the guinea-pig superfused, electrically-stimulated trachea (ESGPT) as described by Coleman and Nials (1989). Binding affinities and reversibility were measured in rat lung membranes, using [^{125}I]-iodopindolol (^{125}IPIN) as the radioligand (Barovksy and Brooker 1980).

Infusions of all three agonists caused concentration-related inhibitory activity on ESGPT. Salmeterol was the most potent, with an equipotent concentration (EPC, isoprenaline = 1) of 0.44 (95% confidence limits, 0.17-0.71, n=14), compared with 3.0(1.5-6.5, n=8) and 34.7 (9.9-120.9, n=6) for salbutamol and quinprenaline respectively. After termination of infusion, responses to salbutamol declined rapidly, with an Rt_{50} value (time to 50% recovery from an EC_{50} concentration) of 11 min. In contrast, responses to quinprenaline declined only slowly (Rt_{50} >150 min), and those to salmeterol persisted for the duration of the experiment (> 420 min). The inhibitory effects of both salmeterol and quinprenaline, persisting after washout, were rapidly reversed by sotalol (10µM), but on stopping the sotalol infusion, responses to both agonists were reasserted. Rat lung β_2-adrenoceptor binding affinities (pKi, ±s.e.m., n) were: salmeterol, 7.5 (±0.1, n=9) salbutamol, 5.5 (±0.1, n=4) and quinprenaline, 4.8 (±0.2, n=3). After 30 min pre-exposure to concentrations of the ß-agonists calculated to cause 90% occupancy of binding sites, membranes were diluted with buffer containing 100pM ^{125}IPIN, and rates of agonist dissociation measured at intervals up to 60 min. Both salbutamol and quinprenaline showed complete dissociation (i.e. no inhibition of ^{125}IPIN binding) in less than 20 min, whereas there was no dissociation with salmeterol within 60 min.

Quinprenaline is a long-acting β_2-adrenoceptor agonist in vitro. Its rapid reversal by sotalol indicates that, like salmeterol, this does not result from irreversible binding to ß-adrenoceptors. However, ligand binding data suggest that quinprenaline achieves its duration of action through a different mechanism to that of salmeterol.

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184P D,L-BUTHIONINE SULPHOXIMINE, A GLUTATHIONE-DEPLETING AGENT, POTENTIATES ALLYLAMINE-INDUCED CYTOTOXICITY IN RAT AORTIC SMOOTH MUSCLE CELL CULTURES

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Allylamine (AA) administration to some laboratory animals produces vascular lesions mimicking certain features of atherosclerosis. The toxicity of AA is attributed to its metabolism to the reactive aldehyde acrolein, by a semicarbazide-sensitive amine oxidase (SSAO) found in vascular smooth muscle cells (VSMC) since SSAO inhibitors block AA-induced cytotoxicity in vivo and in aortic VSMC cultures (Hysmith & Boor, 1988; Ramos et al., 1988). After AA administration, rats excrete acrolein in urine as a derivative conjugated with reduced glutathione (GSH) (Boor et al 1987). The possibility that cellular GSH protects against AA damage to VSMC was studied here by use of buthionine sulphoximine (BSO), an inhibitor of GSH synthesis via glutamylcysteine synthetase.

Primary cultures of adult rat aortic VSMC, grown to confluence in 75cm^2 flasks containing Medium 199 and 10% heat-inactivated foetal calf serum, were passaged by trypsinization, and reseeded into six-well Multidishes. At confluence, all cells to be exposed to BSO received 72h pretreatment with the drug (50µM) in culture medium. Then, all wells received 5ml fresh medium containing either (a) 50µM AA (b) 50µM BSO (c) 50µM AA + BSO, or (d) no drugs (controls). Samples (1ml) of medium were removed from wells at various times for determination of lactate dehydrogenase (LDH) release from damaged cells by following NADH oxidation (at 340nm). Background LDH in the medium (from calf serum) was assayed from "cell free" wells.

Table 1 LDH activity (ΔA_{340} x 10^3/min/ml medium)

	3h	6h	23h	47h
Cell-free medium	41 ± 7	29 ± 6	41 ± 16	42 ± 12
Control cultures	79 ± 20	57 ± 3	67 ± 14	80 ± 12
BSO-treated	77 ± 17	62 ± 5	99 ± 21	103 ± 16
AA-treated	111 ± 31	79 ± 9	367 ± 82^{a}	371 ± 70^{a}
AA + BSO	123 ± 42	259 ± 46^{ab}	842 ± 210^{ab}	595 ± 35^{ab}

Mean ± S.E.M., n = 6-13 wells from 4 separate experiments. $^{a}P < 0.005$ vs control; $^{b}P < 0.05$ vs AA alone.

AA caused LDH release (above controls) by 23h onwards. Significantly greater effects than AA alone were seen with AA + BSO from 6h onwards, whereas BSO alone did not increase LDH from control levels. Thus, BSO appears to enhance AA cytotoxicity, suggesting that GSH levels in VSMC may determine the rate and extent of cell damage produced by this experimental vascular toxin.

Equipment (Smith-Kline 1982 Foundation) and running expenses (Royal Society) are gratefully acknowledged.
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185P PROSTACYCLIN RELEASE IN RESPONSE TO ANGIOTENSIN II IS COMPROMISED IN EXPERIMENTAL DIABETES

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Diabetes mellitus is accompanied by a variety of debilitating complications of which the vasculopathies are the largest single cause of death. Impaired function of the vascular endothelium is a potential cause of both micro and macroangiopathies. This study investigated the release of the vasoactive eicosanoid prostacyclin from the lungs of control and diabetic rats and related any change seen with flux through the sorbitol pathway by studying the effects of an aldose reductase inhibitor. Age matched male Wistar rats were divided into 3 groups. Diabetes was induced in 2 groups by IP administration of streptozotocin (50 mg/kg in normal saline); the third group (control) received saline only. Diabetes was confirmed after 48 hr by blood glucose levels in excess of 15 mmol/l. The control and one diabetic group recieved normal rat diet ad libitum; the second diabetic group received normal diet supplemented with the aldose reductase inhibitor ponalrestat (300 mg/kg diet). After 4 weeks diabetes the animals were anaesthetised with Na pentobarbitone (60 mg/kg i.p.) and the lungs removed and suspended in a heated chamber. The lungs were perfused with Krebs-Henseleit physiological saline via the pulmonary artery at 5 ml/min and were allowed to equilibrate for 30 min. Then angiotensin II (Ang II) or arachidonic acid (AA) were administered as 5 min infusions. The perfusate was collected over a period of 15 min. Prostacyclin in the perfusate was measured by radioimmunoassay of its stable breakdown product, $PGF_{1\alpha}$. Basal (unstimulated) release of $PGF_{1\alpha}$ was 0.334 ± 0.072 pg/ml in control rats but was reduced in diabetic and ponalrestat treated diabetic rats (0.237 ± 0.051 and 0.260 ± 0.102 ng/ml respectively) though this was not statistically significant. Arachidonic acid stimulated release of $PGF_{1\alpha}$ from control and diabetic groups were similar to each other (40.01 ± 6.87 and 37.61 ± 7.72 ng/ml respectively); release from the ponalrestat treated group was reduced (21.83 ± 6.21 ng/ml). Ang II stimulated release of $PGF_{1\alpha}$ from diabetic rats (9.43 ± 1.49 ng/ml) and ponalrestat treated rats (9.99 ± 1.49 ng/ml) were significantly reduced compared to controls (18.09 ± 2.54 ng/ml). Subsequent infusion of AA after a 20 min recovery period elicited a similarly reduced response from all the diabetic animals. These results indicate that Ang II-induced release of prostacyclin by rat lungs is reduced in diabetes, but that this effect is not related to hyperglycaemic stimulation of flux through the polyol pathway.

This study was supported by the British Heart Foundation.

186P AMINOPEPTIDASE INHIBITION SELECTIVELY INCREASES THE POTENCY OF SOME ANGIOTENSIN RECEPTOR AGONISTS AND ANTAGONISTS IN RABBIT ISOLATED AORTA

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The rabbit aorta has been used extensively to determine the potencies of various peptide antagonists against angiotensin II and III (AII and AIII)-induced contractions (e.g., Trachte & Peach, 1983). However, the extent to which endogenous enzymatic degradation may influence agonist or antagonist potency has rarely been considered. We now report the effect of peptidase inhibition on both the contractile response to AII and AIII, and on the potency of a peptide (Ile^7-AIII) and a non-peptide (DuP753 ; Wong et al., 1990) angiotensin receptor antagonist in this tissue.

The thoracic aorta was removed from male, New Zealand White rabbits. Helical strips (1.5-2cm) were cut on a perspex rod and suspended under a resting tension of 0.5g in Krebs solution at 37 °C, containing indomethacin (30μM) and ascorbic acid (100μM) and gassed with 95% O_2 / 5% CO_2. Cumulative concentration-response curves to AII (0.1-100nM) or AIII (1nM-10μM) were repeatedly constructed until reproducible. AIII was approximately 40-times weaker than AII. The geometric mean concentration (95% confidence limits) of AII or AIII in the test curve, required to produce 50% of each control maximum, was 3.8 (2.9-4.9)nM and 160 (104-244)nM, respectively (n=19-20). However, the two agonists caused similar maximum increases in contractile tension. The aminopeptidase inhibitor, amastatin (10μM, n=5-7) did not affect the potency of AII, but displaced the AIII curve approximately 14-fold to the left. The carboxypeptidase-N inhibitor, MERGETPA (D-L-mercaptomethyl-3-guanidino-ethylpropanoic acid ; 10μM, n=4-5) did not affect the potency of AII or AIII. The angiotensin receptor antagonists, DuP753 (0.03, 0.1 and 0.3μM) and Ile^7-AIII (0.1, 0.3 and 1μM) caused parallel, concentration-related, rightward displacements of the AII concentration-response curve. The mean ± s.e.m. pA_2 and slope (SL) for DuP753 was 8.15 ± 0.11 (SL = 1.02 ± 0.1, n=4) and for Ile^7-AIII was 6.98 ± 0.11 (SL =1.35 ± 0.07, n=4), after a 45 min antagonist incubation period. The potency of DuP753 was unaltered in the presence of amastatin (pA_2 = 8.14 ± 0.05, SL = 0.98 ± 0.07, n=4) ; however that of Ile^7-AIII was increased (P=0.001), approximately 13-fold (pA_2 = 8.1 ± 0.15, SL = 1.15 ± 0.06, n=4). In the absence of amastatin, DuP753 and Ile^7-AIII caused concentration-related, rightward displacements of the AIII concentration-response curve. Interestingly, the maximum response to AIII was 40% greater in the presence of DuP753 compared with time-matched controls. The apparent pA_2 for DuP753 against AIII was 7.94 ± 0.15 (SL = 1.16 ± 0.05 , n=4) and for Ile^7-AIII was 7.16 ± 0.18 (SL = 1.05 ± 0.05, n=4). In the presence of amastatin, the potency of DuP753 against AIII was not significantly changed (pA_2 = 8.08 ± 0.08, SL = 1.08 ± 0.05, n=4), whilst that of Ile^7-AIII was again increased (P=0.024), approximately 6-fold (pA_2 = 7.94 ± 0.03, SL = 1.17 ± 0.03, n=4).

In conclusion, endogenous aminopeptidase activity can profoundly influence estimates of peptide agonist and antagonist potency in the rabbit aorta. Future studies using this tissue to investigate the possible presence of angiotensin receptor subtypes, based on a rank order of angiotensin agonist and/or antagonist potency, should therefore include an aminopeptidase inhibitor in the assay system.

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187P ANGIOTENSIN II SELECTIVELY POTENTIATES THE α_1-ADRENOCEPTOR NERVE-MEDIATED RESPONSE IN A BLOOD VESSEL EXHIBITING CO-TRANSMISSION

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The effects of angiotensin II and III on contractile responses to sympathetic nerve stimulation in the rabbit and guinea pig vas deferens have been reported (Trachte, 1988; Ellis & Burnstock, 1989) and show that the results depend on the angiotensin used as well as the parameters of stimulation. The present study examined the effects of angiotensin II on the isolated ileocolic artery of the rabbit, which, like the vas deferens, has been shown to exhibit NA-ATP co-transmission (Bulloch & Starke, 1990). In the ileocolic artery, long trains of electrical stimulation (over 20sec) produce biphasic contractile responses; the first phase being almost exclusively purinergic; whilst the second phase, although still predominantly purinergic, also had a large α_1-adrenergic component (Bulloch & Starke, 1990).

Ring segments of rabbit ileocolic artery were placed in physiological salt solution maintained at 37°C and gassed with 95%O_2 :5%CO_2. Isometric contractile responses to electrical field stimulation (4-32 Hz, 25-40 V, 0.1 msec pulse width, for 1 sec and 20 sec) were recorded under a resting tension of 0.5 g wt.

Nerve mediated responses of the rabbit ileocolic artery can be blocked by a combination of mATP and prazosin (Bulloch & Starke, 1990). Antagonist (prazosin 0.3μM) and desensitising drugs (α,β-methylene ATP (mATP) 3 μM) were used to separate the purinergic and adrenergic contributions involved in the sympathetic vasopressor responses produced by electrical field stimulation in the ileocolic artery of the rabbit. These antagonist drugs were applied either alone or in various combinations and sequences. Angiotensin II (AII) (0.1μM) produced a transient vasoconstriction. The effect of AII (0.1μM) on the sympathetic nerve mediated response was studied in conditions of α_1-adrenoceptor blockade (n=5), P_{2x}-purinoceptor desensitisation (n=6), or in the absence of antagonists (n=5). On its own and under conditions of P_{2x}-purinoceptor desensitisation, A II (0.1μM) potentiated nerve mediated responses at 16Hz and 32Hz for 1 sec as well as the second component of the biphasic response at 5 Hz 20sec and 10Hz 20sec, and the first component at 10Hz 20sec. However in the presence of prazosin (0.3μM) the subsequent addition of AII (0.1μM) did not significantly affect the nerve mediated responses at any of the parameters studied. Contractile responses produced by the addition of NA (0.03μM - 30μM) or the α_2-adrenoceptor agonist UK 14304 (0.03μM - 30μM) were potentiated by AII (0.1μM). The subsequent addition of prazosin (0.1μM) but not rauwolscine (0.1μM) significantly attenuated the responses to both NA and UK 14304.

From these studies it is concluded that A II has a selective potentiating effect on α_1-adrenoceptor mediated responses in this tissue.

J.M.B. holds an Intermediate Fellowship from the British Heart Foundation.

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188P THE EFFECT OF HUMAN α-CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN HUMAN CEREBRAL ARTERIES

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The calcitonin gene-related peptides (CGRP) are vasodilators in many vascular beds in many species. Recent work has found some selectivity *in vivo* for the cerebral vasculature in animals and in Man (Gardiner et al, 1989; Macdonald et al, 1989) and CGRP has been tried in reversing vasospasm following sub-arachnoid haemorrhage (Johnston et al, 1990). The present experiments have compared human α-CGRP with sodium nitroprusside and nifedipine in the large posterior communicating artery (PCA), and in the smaller pial vessels, branches of the middle cerebral artery.

Macroscopically normal branches of cerebral arteries were removed at post-mortem from individuals none of whom died from a cerebrovascular condition. Rings (approx. 3mm long) were set up in Krebs solution at 37°C and bubbled with 95% O_2 5% CO_2. In preliminary experiments acetylcholine (10^{-6}M) did not relax rings pre-contracted with either noradrenaline (NA; $3x10^{-7}$M) or 5-hydroxytryptamine (5-HT; $3x10^{-7}$M) suggesting there was no endothelium present. All subsequent preparations were rubbed to ensure the loss of any residual endothelium.

When rings of pial arteries or PCA were contracted with NA, the most potent relaxant was human α-CGRP (10^{-9}-$3x10^{-7}$M; EC_{50} approx. $3x10^{-9}$M), with nifedipine (10^{-9}-10^{-6}M) and nitroprusside (10^{-9}-10^{-6}M) at least 5 and 8 times less potent respectively. When rings were contracted with 5-HT, human α-CGRP (EC_{50} $1.7x10^{-8}$M) was 2-3 times more potent than the other relaxants in PCA but in the pial arteries CGRP (EC_{50} $1.2x10^{-8}$M) was about equipotent with nifedipine and both were more potent than nitroprusside. All the relaxants were full agonists. The antagonist, human α-$CGRP_{(8-37)}$ (10^{-5}M), shifted the CGRP curve to the right nearly one log unit without altering the curve for sodium nitroprusside. Glibenclamide (10^{-5}M), a blocker of the ATP-sensitive K^+-channel, did not alter the relaxation of arterial rings to either human α-CGRP, sodium nitroprusside or nifedipine. Tone was also evoked with K^+ ($6x10^{-2}$M) and here human α-CGRP was inactive over the concentration range examined, with nifedipine the most potent in both types of human cerebral artery.

These results show that human α-CGRP is a potent endothelial-independent relaxant of human cerebral arteries and was usually more potent than the nitro-vasodilator sodium nitroprusside and the calcium antagonist nifedipine. The CGRP receptor appears to be of a type with a relatively low affinity for the antagonist, human α-$CGRP_{(8-37)}$. In these vessels the mechanism linking receptor activation by CGRP to relaxation does not appear to involve activation of ATP-dependent K^+-channels (Nelson et al, 1990).

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189P SUBTYPES OF THE CALCITONIN GENE-RELATED PEPTIDE (CGRP) RECEPTOR IN VASCULAR TISSUES

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Calcitonin gene-related peptide (CGRP) is a vasodilator with two mechanisms of action, being endothelium-independent in the pig coronary artery but endothelium-dependent in the rat thoracic aorta. It has recently been reported that human α-CGRP$_{(8-37)}$ is an antagonist of CGRP receptors (Chiba et al., 1989). We investigated whether human α-CGRP$_{(8-37)}$ could differentiate between the receptors responsible for endothelium-dependent and endothelium-independent vasorelaxation.

Rings of pig anterior interventricular artery (approx. 4mm long, internal diameter 1mm) were denuded of endothelium by gentle abrasion of the intimal surface. They were suspended on wires and equilibrated for 75 minutes in Krebs solution bubbled with 95% O2/5% CO_2 at 37°C. Acetylcholine ($2x10^{-7}$M) was added and the contraction assessed for stability over a period of 30 minutes. Any tissues relaxing to bradykinin (10^{-6}M) were discarded as having functional endothelium remaining. Human α-CGRP$_{(8-37)}$ ($3x10^{-7}$-10^{-5}M) was added and 15 minutes allowed for equilibration before a dose/response curve to human α-CGRP (10^{-9}M-10^{-6}M) was constructed. The dose/response curve to human α-CGRP (IC_{50} $5x10^{-9}$M, concentration required to give a half-maximal response) was shifted rightwards in a parallel manner with no decrease in the maximum response by $3x10^{-7}$, 10^{-6}, $3x10^{-6}$ and 10^{-5}M human α-CGRP$_{(8-37)}$ the dose ratios being 1.4, 3.6, 15 and 43 respectively. A Schild plot gave values of pA2 equalling 6.7 ± 0.5 and slope equalling 1.0 ± 0.1 (mean ± s.e.m., correlation coefficient 0.99). Human α-CGRP$_{(8-37)}$ had no relaxant effect on its own up to 10^{-5}M. Thus human α-CGRP$_{(8-37)}$ appears to be a competitive antagonist for CGRP in the pig coronary artery.

Rat thoracic aortic rings were set up in a similar fashion to the pig coronary artery, being careful in this tissue to avoid damage to the endothelium. Human α-CGRP ($3x10^{-9}$-$3x10^{-7}$M) caused endothelium-dependent vasorelaxation (IC_{50} $3x10^{-8}$M) of noradrenaline (10^{-7}M)-induced tone. Human α-CGRP$_{(8-37)}$ caused a rightward non-parallel shift of the α-CGRP curve and decreased the maximum response, 10^{-5}M virtually abolishing the relaxant effect of α-CGRP. Human α-CGRP$_{(8-37)}$ had no relaxant effect on its own at these concentrations. Therefore, in rat thoracic aorta, human α-CGRP$_{(8-37)}$ seems to be a non-competitive antagonist of human α-CGRP.

From these data it would appear that there exists two different sub-types of the receptor for human α-CGRP in the pig coronary artery and the rat thoracic aorta respectively, and that human α-CGRP$_{(8-37)}$ can be used to differentiate between these two forms. In these two tissues it appears as if different sub-types of CGRP receptors are linked to the endothelium-dependent and independent mechanisms of vasorelaxation.

We thank Celltech Ltd for financial support. D.W.G. is an MRC student.

Chiba et al., (1989) Am. J. Physiol. E331-E335

190P HUMAN α-CALCITONIN GENE-RELATED PEPTIDE (CGRP) RELAXES PIG CORONARY ARTERY IN AN ENDOTHELIUM-INDEPENDENT MANNER AND ELEVATES CYCLIC AMP LEVELS

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Calcitonin gene-related peptide (CGRP) has two mechanisms of inducing vasorelaxation. In the rat thoracic aorta it induces vasorelaxation in an endothelium-dependent manner, whereas in the perfused rat mesentery the relaxant reponse is independent of the endothelium. In this study we investigated the endothelium dependence and signal transduction of human α-CGRP in rings of pig coronary artery.

Rings from between the top of the pig left anterior descending coronary artery and the left conus artery (4mm long, internal diameter approx. 3mm, LAD) or from lower down the anterior interventricular artery (4mm long, internal diameter approx. 1mm, AIA) were suspended on wires and equilibrated for 75 minutes in Krebs solution bubbled with 95% O_2/5% CO_2 at 37°C. Some rings of either type were abraded gently to remove the endothelium. Tissues were constricted with U46619 (10^{-8}M) or acetylcholine ($2x10^{-7}$M) for LAD and AIA respectively. Dose/response curves to human α-CGRP, isoprenaline or sodium nitroprusside were then constructed. Alternatively, a concentration of these drugs giving 80% relaxation was used for second messenger accumulation studies (scintillation proximity assay, Amersham).

In LAD with endothelium present, U46619 induced a contractile response of 2.7 ± 0.2g. Removing the endothelium increased this to 3.7 ± 0.3g, but did not affect the human α-CGRP-induced vasorelaxation (IC_{50} $4x10^{-9}$M). Pretreatment with N^G-nitro-L-arginine (NO-L-Arg; 10^{-5}M) in rings with endothelium increased the tone to 5.2 ± 0.3g. NO-L-Arg had no such effect in rings denuded of endothelium. In neither preparation did NO-L-Arg have any effect on the relaxation induced by human α-CGRP. Repeat curves to human α-CGRP showed tachyphylaxis by a rightward shift and a decrease in the maximum response. In the absence of endothelium, human α-CGRP ($3x10^{-8}$M) induced a selective 6-fold increase in cyclic AMP from basal levels of 580 ± 73 fmol mg protein^{-1} (mean ± s.e.m.) at the optimum time-point without affecting levels of cGMP (96 ± 12 fmol mg protein^{-1}). Isoprenaline ($3x10^{-8}$M) also gave a selective rise in cyclic AMP levels (6-fold), but did not show any signs of tachyphylaxis.

In AIA rings without endothelium, acetylcholine induced a contractile response of 3.4 ± 0.5g. In a similar fashion to the responses in LAD, human α-CGRP induced vasorelaxation (IC_{50} $4x10^{-9}$M) which was totally independent of the endothelium. In contrast to LAD, repeat curves to human α-CGRP showed no sign of tachyphylaxis.

In conclusion, human α-CGRP induces vasorelaxation in the left anterior descending coronary artery which is independent of the presence of endothelium and may be associated with a rise in cyclic AMP. The size of the vessels did not affect the potency of human α-CGRP, but whereas the larger vessels showed tachyphylaxsis, the smaller coronary vessels did not. It is not known if this decreased response in the larger vessels reflects a change in the receptor and/or transduction mechanism e.g. cyclic AMP.

We thank Celltech Ltd for financial support. D.W.G. is an MRC student.

191P THE INTERACTION OF ARGININE-VASOPRESSIN AND CORTICOTROPHIN RELEASING FACTOR -41 ON HUMAN UMBILICAL ARTERIES

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As part of an investigation into pregnancy-induced hypertension (PIH), the vasoactive effects of corticotrophin releasing factor - 41 (CRF-41) and arginine vasopressin (AVP) and their possible interaction on human umbilical arteries have been investigated.

Umbilical arteries were dissected from cords collected from normotensive women. The vessels were suspended within 1 hour of delivery in a 20 ml organ bath of Krebs solution (pH 7.4) at 37°C. Vessels were exposed to CRF-41 over concentrations 1 x 10^{-10}M for 10 minutes at each concentration. One of the 12 vessels showed a dose-dependent contractile response. Six out of 12 were not affected by any concentration of CRF-41. Five out of 12 showed inconsistent contractile responses, frequently only at one dose in the low to middle ranges.

Using the same system, AVP induced a dose-dependent contractile response over a range of 1 x 10^{-9} to 1 x 10^{-5}M with an ED_{50} of 1.35 x 10^{-7}M and a maximum response at 1 x 10^{-5}M. The presence of 1 x 10^{-7}M AVP together with increasing doses of CRF-41 did not alter the effect of the latter. However, the presence of a constant concentration of 1 x 10^{-7}M CRF-41 increased the AVP-evoked contractions with a shift of the dose response curve to the left ($P<0.05$; ED_{50} 2.65 x 10^{-8}M) without significantly altering the maximum response.

CRF-41 rises throughout pregnancy and is higher in PIH (Campbell et al, 1987). Although it has only a slight, if any, effect on umbilical artery, it enhances the contractile response of this tissue toward AVP.

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192P CALCIUM-DEPENDENT AND -INDEPENDENT MECHANISMS OF SUSTAINED CONTRACTION IN VASCULAR SMOOTH MUSCLE OF RAT TAIL ARTERY

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Serotonin (5HT), noradrenaline (NA) and high potassium (KCl) induce an increase in cytosolic calcium ($[Ca^{2+}]_i$) and sustained vasoconstriction in the rat tail artery (Thorin-Trescases *et al.*, 1990). In order to clarify the calcium-dependence of sustained contraction, we examined the effects of a calcium entry blocker verapamil (VP) and of a calcium chelator EGTA on perfusion pressure and $[Ca^{2+}]_i$ of the perfused tail artery.

One cm of tail artery from male Wistar rats (12 month old, n =7 to 9) was perfused for 90 min with 5 µM of Fura 2 AM, a fluorescent calcium indicator. We measured the change in perfusion pressure (ΔP% = the amplitude of the contraction in presence of antagonists as a percentage of the initial contracted state) and in $[Ca^{2+}]_i$ (ΔR% = the ratio of the fluorescence at the excitation wavelenghs of 340 and 380 nm in the presence of antagonists as a percentage of the ratio of the initial state).

In the absence of external calcium (free calcium and 0.3 mM EGTA), 5HT (1 µM), NA (3 µM) and KCl (60 mM) induced a transient increase in ΔP% of 58.6 ± 6.7; 35.3 ± 11.0 and 2.6 ± 1.6 % respectively and a transient increase in ΔR% of 61.0 ± 6.0; 41.6 ± 3.8 and 9.2 ± 4.3 %. When added at the plateau of the contractions, VP (10 µM) and EGTA (6.25 mM) produced a decrease in ΔP% and in ΔR% (see Table 1).

Table 1: Decrease in ΔP% and in ΔR% induced by VP and EGTA (mean ± sem).

	5HT (3 µM)		NA(3 µM)	
	ΔP%	ΔR%	ΔP%	ΔR%
VP (n = 9)	-61.4 ± 7.2	-37.8 ± 5.9	-41.7 ± 5.7	-23.8 ± 3.3
EGTA (n = 7)	-46.7± 18.5	-216.6 ± 39.3	-27.6 ± 10.6	-120.3 ± 20.4

The transient contraction observed in absence of external calcium is due to a mobilization of intracellular calcium. The results observed with VP showed that contraction is partly dependent on extracellular calcium. Sustained contraction can be maintained although the increase in $[Ca^{2+}]_i$ is abolished with EGTA. In conclusion, calcium- dependent and -independent mechanisms are involved in sustained contractions.

Thorin-Trescases, N., Oster, L., Atkinson, J. & Capdeville, C. (1990) *Eur. J. Pharmacol.* **179**, 469-471.

193P EFFECT OF AGEING ON THE CYTOSOLIC FREE CALCIUM-VASOCONSTRICTION RELATIONSHIP IN THE TAIL ARTERY OF WISTAR AND FISCHER RATS

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Although ageing is associated with a loss of contractility (Docherty, 1988), the effects of ageing on cytosolic free calcium ($[Ca^{2+}]_i$) and vascular reactivity have received scant attention. We have studied the relationship between $[Ca^{2+}]_i$ and vascular reactivity in the perfused tail artery of old rats.

The caudal artery was dissected from 24 month old male Wistar rats (545.3 ± 11.4 g, n = 14) and Fischer F 344 rats (376.5 ± 8.9 g, n = 6) and from young Wistar and Fischer F 344 rats 2 month old (n = 7). The segment of artery was perfused for 90 min with 5 µM of Fura 2 AM, a fluorescent calcium indicator (Thorin-Trescases *et al.*, 1990). Increases in perfusion pressure (ΔP, mmHg) and in $[Ca^{2+}]_i$ (ΔR % = the ratio of the fluorescence at the excitation wavelenghs of 340 and 380 nm as a percentage of the ratio in pre-contracted state) induced by noradrenaline (NA, 1 µM), serotonin (5HT, 1 µM) and high potassium (KCl, 60 mM) were measured.

Table 1 shows that in old Wistar rats, the decrease in reactivity is associated with a reduction in $[Ca^{2+}]_i$. However, in Fischer rats, ageing produces a loss in reactivity without any change in $[Ca^{2+}]_i$.

Table 1: Effects of ageing on vascular reactivity (ΔP, mm Hg) and $[Ca^{2+}]_i$ (ΔR %) in Wistar and Fischer rats (mean ± sem).

	ΔP mm Hg			ΔR %		
	NA	5HT	KCl	NA	5HT	KCl
Young Wistar	31.2 ± 4.8	104.3 ± 16.4	37.8 ± 4.8	14.3 ± 1.8	16.5 ± 1.1	25.2 ± 0.9
Old Wistar	9.1 ± 2.4 *	58.4 ± 8.4 *	32.7 ± 6.3	9.5 ± 1.0 *	12.1 ± 0.8 *	16.0 ± 1.4 *
Young Fischer	46.6 ± 9.7	102.6 ± 28.9	40.0 ± 12.4	16.2 ± 4.4	21.9 ± 8.3	19.5 ± 6.2
Old Fischer	3.3 ± 1.9 *	52.7 ± 12.3	0.0 ± 0.0 *	16.2 ± 5.2	21.2 ± 5.6	10.9 ± 5.4

*: $P < 0.05$, old Wistar vs young Wistar rats and old Fischer vs young Fischer rats (t test).

In conclusion, with the Fura 2 technique, we reveal two different phenomena which occur in ageing: 1) an impairment of excitation-contraction coupling as observed in Wistar rats, and 2) a decrease in calcium sensitivity of contractile elements with no change in $[Ca^{2+}]_i$ as seen in Fischer rats.

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194P EFFECTS OF SK&F 96365 ON TENSION DEVELOPMENT AND INTRACELLULAR Ca CONCENTRATION IN THE RABBIT MAIN PULMONARY ARTERY

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The contraction of vascular muscle induced by agonists such as noradrenaline, results primarily from a rise in intracellular Ca^{2+}, caused by Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} influx through voltage-gated and receptor-operated, transmembrane channels. Recently, compounds have been described, which show some specificity in blocking receptor-gated Ca^{2+} influx in smooth muscle and other cell types. We have examined the effects of one of these, SK&F 96365 (Benham *et al*, 1989), on tension development and intracellular Ca^{2+} concentration in isolated strips of rabbit pulmonary artery, during stimulation with phenylephrine (PE; 3 µM), caffeine (20 mM) or depolarization with elevated extracellular potassium (50 or 120 mM). The drug caused a concentration-dependent block of responses to each of these stimuli, with half-maximal inhibition occurring at around 10 µM in each case. Precise evaluation of these effects was, however, complicated by the prolonged time course of blockade, with the onset taking up to 1 hr and recovery even longer.

In an attempt to eliminate the components of the PE-induced contracture due to cellular Ca^{2+} release and influx through voltage-gated channels, nifedipine (2 µM) was added to tissues during continuous exposure to PE. Removal of extracellular Ca^{2+}, with 1 mM EGTA added, then relaxed the tissue completely, with its readdition inducing 80 ± 3% (mean ± s.e.m.; n=4) of the contracture observed in PE alone. This contracture reflected Ca^{2+} influx, which was independent of voltage-gated Ca channels, and was thus presumably mediated mainly by Ca^{2+} influx through receptor-operated channels. This component of the PE-induced contracture was inhibited by 59 ± 3% (n=4) by SK&F 96365 at 16 µM, an effect similar to that seen with full contractures to PE, high K^+ and caffeine.

The non-specific nature of the inhibition by SK&F 96365 could be explained if, rather than blocking transmembrane movements of Ca^{2+}, the drug had a direct action on the contractile proteins. To determine if this was possible, we measured the effects of SK&F 96365 on rises in intracellular Ca^{2+} concentration mediated by PE and high K^+, using the fluorescent, Ca^{2+}-sensitive indicator, fura-2. Stimulation of pulmonary muscle strips with PE caused a sustained increase in intracellular Ca^{2+} concentration, which was slowly depressed by the addition of SK&F 96365 (16 µM). A similar effect was observed when high K^+ solution was used to stimulate the tissue, and in both cases the onset and recovery from blockade followed a similar time course to that observed with measurements of tension.

The results do not support a specific, receptor-operated channel-blocking action for SK&F 96365 in the rabbit pulmonary artery, although its actions are associated with a reduction of the intracellular Ca^{2+} concentration.

We thank Dr C.D. Benham of Smith Kline & Beecham for the gift of SK&F 96365.

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195P VASODILATOR ACTIONS OF AMLODIPINE ON HUMAN SUBCUTANEOUS RESISTANCE VESSELS

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Amlodipine is a calcium antagonist with slow onset and long duration of action as a hypotensive agent (Burges *et al.*, 1985). However, there is little information about its effects in isolated human blood vessels. The aim of this study was to investigate the action of amlodipine against agonist-induced contractions in human subcutaneous resistance vessels.

Subcutaneous arteries (internal diameter 143-493μm,n=42) were obtained from tissue resected at surgery and mounted as ring segments in an isometric myograph bathed in physiological saline solution (PSS), bubbled with 95% oxygen;5% carbon dioxide. The vessels were allowed to equilibrate and then set at a normalised internal circumference as previously described by Mulvany and Halpern (1977). Viability was assessed by exposing arteries to 118mM potassium solution (KPSS) and noradrenaline (10μm). Vessels which failed to produce more than 100mmHg effective active pressure for either of these responses were excluded. Cumulative dose response relationships were obtained to noradrenaline (NA), the α_1 agonist phenylephrine (PE) and the α_2 agonist BHT-933 (BHT) (1nM to 30μM) alone and in the presence of amlodipine (1μM, 3h incubation). Experiments with endothelin (ET-1) and neuropeptide Y (NPY) were done with single maximal constrictor concentrations in separate vessels with and without amlodipine (1μM, 3h) due to the marked tachyphylaxis observed with these agents. We have also confirmed that the effect of amlodipine is very persistent and resistant to repeated washouts.

Table 1: Effect of Amlodipine (A) on responses to agonists

		NA	PE	BHT	ET-1	NPY
Emax	-A	90.8±2.9	82.2±3.9	63.2±4.8	137±9.49	92±4.01
	+A	38.7±4.0 *	30.2±6.4 *	24.4±4.6 *	117±9.26	17±2.08 *
pD2	-A	6.2±0.03	5.6±0.06	5.6±0.05		
	+A	6.4±0.06	5.7±0.08	5.9±0.06		

Values are means ± s.e. means. *P<0.001, t-test comparison with controls. Emax is expressed as percentage of the maximal response to 118mM KPSS.

NPY induced contraction (300nM) was most readily relaxed by amlodipine but it had little effect on ET-1 induced contraction (100nM). Amlodipine produced a downward shift of the dose response curve to the alpha (α) agonists in the order BHT > PE > NA. Thus amlodipine has been shown to produce noncompetitive relaxation of human resistance vessels. As amlodipine was very effective against NPY and relatively ineffective against ET-1 this suggests that the contractile response to NPY is more dependent on extracellular calcium (Pernow *et al.*,1987). It should be noted that α_2 receptor activation is also thought to be largely dependent on calcium influx (Lee & Debernadis, 1990). Our findings also suggest that the prolonged action of amlodipine can be explained by pharmacodynamic as well as pharmacokinetic factors.

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196P ANTAGONISM OF α_1-ADRENOCEPTOR SUBTYPES BY NIGULDIPINE AND 5-METHYL-URAPIDIL IN RAT KIDNEY

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Blue et al. (1990) have reported that vasoconstrictor responses to noradrenaline (NA) in the isolated perfused rat kidney appear to be mediated via alpha$_{1A}$-adrenoceptors (α_{1A}-ARs). The present study examines the actions of the highly selective α_{1A}-AR ligands, niguldipine (NIG) and 5-methyl-urapidil (5-MU).

Kidneys were perfused with Krebs' solution containing cocaine (30μM), corticosterone (30μM), (±)-propranolol (1μM) and indomethacin (10μM) as described previously (Bond et al., 1989). Vasoconstrictor responses to infusions of NA or 5-hydroxytryptamine (5-HT) were measured before and in the presence of antagonists (preperfused for 1h). In several experiments, a third curve to NA was obtained 1h after the second curve. In other experiments, kidneys were pretreated by perfusion with chloroethylclonidine (CEC; 100μM for 20 min, followed by washout for 40 min) prior to construction of the first NA concentration-effect curve.

(+)-NIG (0.03-0.3nM) potently, but unsurmountably antagonized vasoconstrictor responses to NA, being about 100-times more potent than (-)-NIG. Antagonism by (+)-NIG was specific as vasoconstrictor responses to 5-HT were not affected by (+)-NIG at 3nM. The effect of (+)-NIG was not due to antagonism of dihydropyridine-sensitive calcium (Ca^{++}) channels as responses to NA were largely resistant (<5-fold shift) to nifedipine, nitrendipine, isradipine, nimodipine, amlodipine and diltiazem (all at 1μM). Furthermore, nitrendipine (1μM) failed to prevent (+)-NIG (0.1nM) from inhibiting NA. In contrast, coperfusion of prazosin (50nM) afforded partial protection from (+)-NIG, suggesting an interaction of (+)-NIG with α_{1A}-ARs.

5-MU (0.03-3μM) evoked dextral shifts in the concentration-effect curves to NA which consisted of two phases. The first phase was unsurmountably antagonized by 5-MU and was sensitive to inhibition by CEC (100μM) and nitrendipine (1μM). In the presence of nitrendipine (1μM), 5-MU (3-300nM) acted as a competitive antagonist toward NA. Schild regression analysis yielded a slope not significantly different from 1 (slope = 1.07; 95% confidence limits: 0.94-1.21) and pA_2 value of 9.17 upon constrainst of the slope to 1.

The results with 5-MU indicate that both α_{1A}- and α_{1B}-ARs mediate responses to NA in rat kidney. α_{1B}-AR-mediated responses are sensitive to CEC and nitrendipine whereas α_{1A}-AR-mediated responses are resistant to CEC and classical Ca^{++} channel antagonists. Protection experiments with prazosin indicate that (+)-NIG interacts at the level of the α_{1A}-AR. However, unsurmountable antagonism of NA by (+)-NIG limits its utility as a singular probe for α_{1A}-ARs. Resistance of NA to inhibition by classical Ca^{++} channel antagonists, in the face of a critical dependence of NA upon extracellular Ca^{++} (Blue et al., 1990), suggests that α_{1A}-ARs are coupled to a novel Ca^{++} channel. The only documented pharmacology of this channel is that it is inhibited by cadmium (Blue et al., 1990).

This work was supported by NIH Grant GM 39621.

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197P CHARACTERIZATION OF THE ACTION OF L-660,863 (3- (3-AMINO-1, 2, 4-OXADIAZOL-5-YL) QUINUCLIDINE) AT MUSCARINIC RECEPTORS *IN VITRO*

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L-660,863 exhibits enhanced potency and efficacy at M_1 muscarinic receptors with respect to arecoline (Freedman and Saunders, 1989). However, measurements of affinities and subsequent estimations of the selectivity of L-660,863 for muscarinic receptor subtypes have not been reported.

L-660,863 exhibited a greater potency with respect to carbachol in all assays (Eglen et al., 1990; Table 1). The differences in potencies that was seen with both agonists were probably attributable to variation in the receptor reserves. In terms of affinity (-log K_A, Furchgott, 1966; Table 1) L-660,863, but not carbachol, was preferential for the M_2 receptor.

Table 1 Comparison of Agonist Activity of Carbachol and L-660,863 at Muscarinic Receptors In Vitro

		-Log EC_{50}		-Log K_A		E_r	
Subtype	Preparation	Carbachol	L-660,863	Carbachol	L-660,863	Carbachol	L-660,863
M_1	Canine saphenous vein	4.35	6.43	4.51	6.53	0	0
M_2	Guinea-pig left atria	6.80	8.16	4.80	7.55	100	4
M_2	Guinea-pig right atria	7.24	8.78				
M_2	Guinea-pig uterus	6.27	7.41				
M_3	Guinea-pig ileum[a]	6.60	7.77	4.66	6.24	87	33
M_3	Guinea-pig ileum[b]	4.70	6.30				
M_3	Guinea-pig trachea	6.78	7.81				
M_3	Guinea-pig urinary bladder	6.18	7.43				
M_3	Rat aorta (relaxation)	6.12	7.56				
M_3	Rat anococcygeus	5.69	6.60				

[a]contraction; [b] PI hydrolysis. Values are mean, s.e. mean <10%, n=4-10. E_r (effective receptor reserve) defined as K_A/EC_{50}.

In radioligand binding studies the -log K_i values, (M_1, 6.2; M_2, 7.2; M_3, 6.4) were similar to those estimated in functional studies (Table 1). The displacement at M_2 and M_3, but not M_1 sites, was best described using a two-site model, i.e. M_2 R_H 8.0 R_L 6.5; M_3 R_H 6.6, R_L 5.6. L-660,863, in conclusion, is a potent muscarinic agonist with some degree of M_2 selectivity. These data also show that high potency does not necessarily correlate with high intrinsic efficacy.

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198P EVIDENCE THAT THE ADENOSINE RECEPTOR MEDIATING RELAXATION IN DOG LATERAL SAPHENOUS VEIN AND GUINEA-PIG AORTA IS OF THE A_{2b} SUBTYPE

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Subdivision of the A_2 adenosine receptor into A_{2a} and A_{2b} has been proposed (Daly, 1985). The xanthine antagonist PD115199 (N-[2-(dimethylamino)ethyl]-N-methyl-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)benzenesulfonamide) (Bruns et al., 1987b) and the agonists, 2-phenylaminoadenosine (CV1808) and 2-[p-(2-carboxyethyl)-phenethylamino]-5'-N-ethylcarboxamidoadenosine (CGS-21680) appear to have higher affinities for the A_{2a} than the A_{2b} receptor (Bruns et al., 1987b; Jarvis et al., 1989) and this study reports the potencies of these selective A_{2a} compounds in two functional assays, isolated dog saphenous vein (DSV) and guinea pig aorta (GPA). Ring segments, from DSV and GPA were suspended in Krebs solution, containing dipyridamole (5μM) bubbled with 95%O_2/5%CO_2 at 37°C, under resting tensions of 1g and 2g respectively. Changes in tone were measured isometrically. Half maximal contractions induced by phenylephrine were relaxed by 5'-(N-ethyl)carboxamidoadenosine CV1808 and CGS21680. Using adenosine as the agonist, the antagonistic effects of PD115199, the A_1 selective antagonist DPCPX (8-cyclopentyl-1,3-dipropylxanthine) and the non-selective xanthine 8-parasulphophenyl-theophylline (8-PST) were investigated.

Table 1

	Dog saphenous vein	Guinea-pig aorta	Rat striatal membranes (Jarvis et al., 1989; Bruns et al., 1987a)
Agonist	ED_{30} (nM)	ED_{30} (nM)	IC_{50} (nM)
NECA	340	312	15
CV-1808	23500	12,500	130
CGS 21680	> 20,000	> 20,000	18
Antagonist	Ki (nM)	Ki (nM)	
8-PST	2930	4560	4320
PD115199	151	275	16
DPCPX	213	234	350

The rank order of potency of adenosine analogues was similar in DSV and GPA, but different from that reported at the A_{2a} receptor in striatal membranes (Table 1). The antagonist PD115199 exhibited lower affinity in DSV and GPA than in striatal membranes. This study demonstrates that the adenosine receptor responsible for vascular relaxation in the DSV and the GPA differs from the A_{2a} subtype and shows properties closer to the A_{2b} subtype.

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199P VASORELAXANT PROPERTIES OF A GROUP OF STRUCTURALLY DIVERSE POTASSIUM CHANNEL OPENERS IN RABBIT ISOLATED BASILAR ARTERY

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The relaxant properties of the chemically dissimilar potassium channel openers (KCOs) lemakalim (the active enantiomer of cromakalim), pinacidil, minoxidil sulphate ($MxSO_4$), and diazoxide in certain peripheral blood vessels are well established (for review see Weston, 1989). However, the effects of these agents on isolated cerebral arteries have not been extensively investigated.

Basilar arteries were removed from pentobarbitone-overdosed (300mg/kg) male New Zealand White or half-lop rabbits. Arterial rings were mounted on parallel L-shaped fine wires, in normal Krebs' solution (at 37°C aerated with 95% O_2/5% CO_2), for the recording of isometric tension. To evaluate the importance of the endothelium to the relaxant effects of KCOs arteries were perfused with either Krebs' solution (control) or 0.1% v/v Triton-X at a rate of 0.5ml/min for 1min, prior to mounting. Pretreatment with Triton-X did not significantly alter the contractile response to 40mM KCl but abolished the endothelium-dependent relaxant response to acetylcholine (10μM) in arterial rings precontracted with 5-HT (30μM).

All four KCOs evoked concentration-related relaxation of both endothelium-intact (+E) and endothelium-denuded (-E) arterial rings contracted with 20mM KCl. These agents had little relaxant effect on tissues precontracted with 80mM KCl, with the exception of pinacidil which caused relaxation at high concentrations (>10μM). The concentrations required to elicit half maximal relaxation (mean values IC50; μM) and the maximal relaxant effect (E_{Max}; % initial spasm) are summarised in table 1 (n>4). There was no significant difference in IC50 values or E_{Max} values between +E and -E groups (Student's t-test, unpaired analysis).

Table 1;

	20mM KCl (+E)		80mM KCl (+E)		20mM KCl (-E)		80mM KCl (-E)	
	IC_{50}	E_{Max}	IC_{50}	E_{Max}	IC_{50}	E_{Max}	IC_{50}	E_{Max}
Lemakalim	0.06	91.7	>3	14.3	0.15	89.5	Not tested	-
Pinacidil	0.25	80.7	>100	42.5	0.40	80.0	Not tested	-
MxSO4	0.63	86.9	>100	17.2	0.82	81.1	Not tested	-
Diazoxide	18.0	90.0	>100	9.8	21.1	85.2	Not tested	-

In summary, KCOs relax rabbit basilar arteries precontracted with 20mM KCl, but not with 80mM KCl. This effect is endothelium-independent. The differential sensitivity of low/high KCl contractions to these agents is suggestive of an action at potassium channels. The sensitivity of rabbit isolated basilar arteries to KCOs is similar to that of peripheral arteries such as rat aorta and portal vein.

D.J.T. is supported by a Glaxo Group Research Studentship.

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200P GLIBENCLAMIDE FAILS TO REVERSE RELAXATIONS INDUCED BY CALCITONIN GENE RELATED PEPTIDE (CGRP) IN HUMAN MAMMARY ARTERY AND SAPHENOUS VEIN

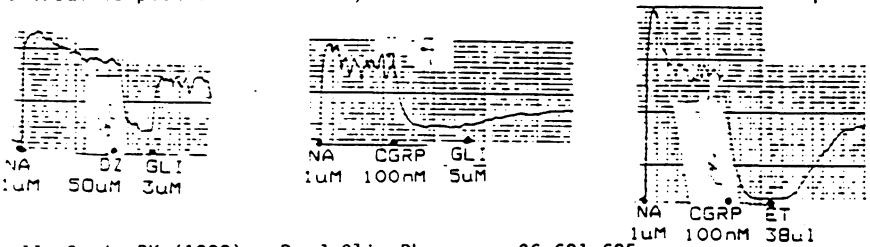
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Glibenclamide, which blocks activation of ATP-sensitive K^+ channels by diazoxide and cromakalim (Standen et al., 1989) has been reported to reverse partially the relaxation of rabbit mesenteric artery to single doses of αCGRP. We wished to determine by dose-responsing with CGRP on intact human artery whether pre-treatment with glibenclamide would lead to a biphasic dose-response curve suggesting that CGRP activates different cell signalling pathways depending on its concentration. As a prior experiment, we have tested the effect of βCGRP, and its reversal by glibenclamide, on isolated human internal mammary artery (HIMA) and saphenous vein (HSV) obtained at coronary artery bypass grafting. Vessels were gently denuded of endothelium and 2mm transverse strips were suspended at 37.5°C in Krebs' solution gassed with 95% O_2, 5% CO_2. Tension was recorded isometrically. Vessels were precontracted by noradrenaline 1 μM. HIMA was relaxed by CGRP, the time to peak response being 10-20 minutes, followed by spontaneous decay over 30-60 min. The EC_{50} for this response was 20 nM, and the maximum relaxation was 40% of that caused by sodium nitroprusside 10^{-4}M (n = 3-7 patients). As in previous reports (Marshall et al., 1988) CGRP upto 10^{-7}M had no effect on HSV strips precontracted with noradrenaline (n = 6). In tissues from 6 patients studied, glibenclamide (1-10 μM) failed to cause any reversal of a submaximal effect of CGRP (Fig 1). This contrasted with the expected reversal by the same doses of glibenclamide of equivalent relaxations caused by the ATP-dependent K^+ channel opener, diazoxide.

The failure of glibencamide to cause reversal of βCGRP induced relaxation in HIMA precluded further study of the effect of glibenclamide pretreatment on the dose response curve. Further studies are required to establish whether glibenclamide reversal is peculiar to αCGRP, or restricted to certain tissues or species.

Figure 1

Effect of glibenclamide (GLI) on relaxation by Diazoxide (DZ) or CGRP of HIMA precontracted by noradrenaline (NA). (ET=ethanol)



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201P EFFECT OF 12(S)HETE AND LEUKOTRIENES ON THE TONE OF THE DOG ISOLATED CORONARY ARTERY

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We have previously reported that 12(S) hydroxyeicosatetraenoic acid (HETE) induced concentration dependent relaxation of the dog isolated coronary artery (Kraneveld and Trevethick, 1990). In this study we have compared the relaxant activity of 12(S) HETE with other lipoxygenase products and assessed the effects of various compounds on 12(S) HETE induced relaxation. Rings of the left anterior descending coronary artery were isolated and mounted for isometric tension recording as described previously (Kraneveld and Trevethick, 1990). Preparations were contracted with an EC_{50} concentration of either U46619 (2.5×10^{-8}M) or KCl (2×10^{-2}M). When tension had plateaued cumulative concentration effect curves to 12(S) HETE, leukotriene (LT) B_4 (10^{-8}–10^{-6}M) and LTC_4 or LTD_4 (10^{-8}–10^{-5}M) were constructed. Compounds studied for their effects on 12(S) HETE induced relaxation were pre-incubated with the tissue for 30 min prior to adding U46619.

Only 12(S) HETE and LTC_4 induced relaxation of U46619 induced tone, EC_{50} values being $14.7 \pm 1.2 \times 10^{-8}$M (mean ± s.e.m., n = 7) and $14.1 \pm 4.4 \times 10^{-8}$M (mean ± s.e.m, n = 4) respectively. In a further four preparations LTC_4 did not induce any relaxation. 12(S) HETE also induced concentration dependent relaxation of KCl tone, EC_{50}: $15.0 \pm 3.2 \times 10^{-8}$M (mean ± s.e.m., n = 3).

Table 1 **Effect of compounds on 12(S) HETE induced relaxation (values are mean ± s.e.m.)**

	$EC_{50} \times 10^{-8}$M				
	Aspirin 10^{-4}M	A64077 5×10^{-5}M	Propranolol 10^{-6}M	8-PT 2×10^{-5}M	FPL55712 10^{-5}M
No treatment	16 ± 1 (n = 3)	13 ± 2 (n = 3)	15 ± 4 (n = 3)	15 ± 2 (n = 3)	15 ± 2 (n = 2)
Treatment	15 ± 1 (n = 3)	12 ± 4 (n = 3)	15 ± 2 (n = 3)	14 ± 3 (n = 3)	14 ± 1 (n = 2)

8PT = 8 phenyltheophylline, A64077 [N-(1benzo(b)thien-2-ylethyl)-N-hydroxyurea]-LT synthesis inhibitor.

The relaxant effect of 12(S) HETE does not involve: (1) antagonism of thromboxane receptors as relaxation was evident with KCl tone, (2) enhanced prostaglandin or leukotriene synthesis or (3) activation of ß adrenoceptors, adenosine receptors or FPL55712-sensitive leukotriene receptors. Thus 12(S) HETE may induce relaxation by acting on a specific receptor.

A.D. Kraneveld and M.A. Trevethick (1990) Br. J. Pharmac., 99, 46P.

202P INTERACTION BETWEEN CICLETANINE AND THE VASCULAR EICOSANOID SYSTEM IN HUMAN RESISTANCE ARTERIES

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Cicletanine is an antihypertensive agent and at high concentrations acts like a diuretic (Bippi & Guinot, 1988). It has a novel chemical structure containing a furopyridine group (Chabrier *et al.*, 1988). The mechanisms responsible for cicletanine's hypotensive efficacy are unknown although many hypotheses have been put forward including an action on potassium channels or an interaction with the vascular eicosanoid system, specifically prostacyclin (Castro *et al.*, 1989). The aim of this study is to investigate the proposed interaction between cicletanine and the eicosanoid system and to contrast these findings with results obtained from different classes of drugs: namely a guanylate cyclase activator (sodium nitroprusside),a potassium channel opener (cromakalim) and a thiazide diuretic (hydrochlorothiazide).

Human subcutaneous resistance arteries (internal diameter 150-740µm mean 380µm) were dissected free and mounted on the Mulvany microvascular myograph (Mulvany & Halpern, 1977) for the measurement of isometric tension. The arteries were normalised to a standing resting tension in aerated physiological saline solution (PSS) maintained at 37°C. After an hour the vessels were tested for viability using depolarising K+ in PSS and noradrenaline; those vessels failing to produce a tension equivalent to 90mmHg were discarded. Each experiment involved the construction of two concentration response curves to one of the drugs. One was constructed after thirty minutes incubation with indomethacin (20µM) and the other after a period of thirty minutes exposure to vehicle. The order the two relaxation curves were constructed were varied for each experiment. At the end of the experiment the vessels were again challenged with depolarising K+ in a final test for viability; vessels which failed to produce a tension equivalent to at least 80% of the original tension produced by depolarising K+ were discarded and the experiment repeated using different vessels. The experiment was repeated with cicletanine using the alternative cyclo-oxygenase inhibitor mefenamic acid (100µM). Tranylcypromine,100µM,(a prostacyclin synthetase inhibitor) was also used in conjunction with cicletanine.

All the drugs utilised produced vasorelaxation of human subcutaneous resistance arteries pre-constricted with noradrenaline 10µM. The relaxation produced by sodium nitroprusside and hydrochlorothiazide was unaffected by incubation with indomethacin whereas cicletanine and cromakalim produced less relaxation in the presence of indomethacin. The relaxant response to cicletanine was reduced by 51% at 30µM ($P<0.0001$) and the response to cromakalim by 22% at 30µM ($P<0.02$). Maximal cicletanine-induced relaxation was reduced by 55% at 30µM ($P<0.0005$) in the presence of mefenamic acid, and by 43% at 30µM ($P<0.01$) in the presence of tranylcypromine.

The results of this study indicate that the vascular eicosanoid system may be involved in the mechanism of the hypotensive action of cicletanine. It also shows that, although cicletanine may have diuretic properties at high concentrations, it does not in this model at least act like a thiazide diuretic. The potassium channel opener,cromakalim,also demonstrated a reduction of response in the presence of indomethacin which suggests involvement of the vascular eicosanoid system in its mechanism of action.

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203P AMPLIFYING INTERACTIONS BETWEEN 5-HT_1-LIKE, 5-HT_2 AND THROMBOXANE A_2 RECEPTORS IN VASCULAR SMOOTH MUSCLE

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5-HT and thromboxane A2 (TxA_2) administered directly or released from aggregating platelets act synergistically to produce vascular contraction (De Clerck and Van Neuten, 1983), but the nature of this 'amplification' phenomenon remains ill-defined. In view of the possible relevance of this interaction to vasospastic disease, we have examined the interplay between these vasoconstrictor agents using rings of rabbit femoral artery, a vessel in which smooth muscle contraction is mediated by 5-HT_1-like and 5-HT_2 as well as TxA_2 receptors.

Rings of femoral artery (2.0mm) were obtained from male New Zealand White Rabbits (2.4-3.0kg) killed by pentobarbitone sodium (≥80mg/kg i.v.). The endothelium was removed by gently rolling each ring around a thin wire before mounting between tungsten wire hooks (250μM diameter) in organ baths containing Krebs solution (+0.3μM Prazosin) aerated with 95% O_2/5%CO_2 at 37°C. Tissues were treated with Pargyline (500μM for 30mins.) before constructing cumulative concentration-effect curves to agonists.

Evidence for the presence of 5-HT_1-like, 5-HT_2 and TxA_2 receptor types was obtained using selective receptor agonists and antagonists. Although the response curve to 5-HT was apparently monophasic ($p[A_{50}]$=7.55±0.07, max. c.f. KCl 80mM = 156± 7%), shifts produced by ketanserin (0.01-1.0μM) were biphasic suggesting a mixed population of 5-HT_1-like and 5-HT_2 receptors. This was confirmed using the selective agonist sumatriptan (GR43175) which showed that 5-HT_1-like receptors accounted for ~15% of the maximum response to 5-HT. To study independently 5-HT_1-like/TxA_2 and 5-HT_2/TxA_2 receptor interactions, tissues were treated with phenoxybenzamine (0.15μM, 30min) or benextramine tetrahydrochloride (3.0μM, 30min) respectively. Contraction was then induced with a concentration of the TxA_2 mimetic U46619 corresponding to 10, 30 or 50% of the U46619 maximum and, at steady state, a concentration-effect curve to either sumatriptan or α -Me-5-HT (AMT) was constructed. 5-HT_2 receptor-mediated responses to AMT demonstrated threshold synergism i.e.curves were potentiated (left-shifted) but not augmented (maximum unchanged at ~150% c.f. KCl) in the presence of U46619. The mid-point location ($p[A_{50}]$) at each level of U46619 contracture was: 0%, 6.82±0.04; 10%, 7.12±0.05; 30%, 7.24±0.09; 50%, 7.16±0.07. This type of interaction is expected when receptor-effect coupling for different receptors occurs in parallel except for a final common transducer which is positively co-operative (Leff, 1987). Indeed simulations based on this model described the experimental data well. In contrast, the 5-HT_1-like/TxA_2 receptor interaction did not accord with threshold synergism and could not be described by the model. In this case, the curve to sumatriptan was both potentiated and augmented, the $p[A_{50}]$ / maximum (% KCl 80mM) at each level of U46619 contracture being: 0%, 6.56±0.09 / 21±3; 10%, 6.52±0.03 / 45±6; 30%, 6.81±0.16 / 74±8; 50%, 6.85±0.05 / 67±8. Evidently TxA_2 profoundly alters the efficiency of 5-HT_1-like receptor occupancy-effect coupling, increasing the sumatriptan maximum from 15% to 50% of the AMT maximum. Although 5-HT_1-like receptor mediated responses in some isolated tissues may appear to make a minimal contribution to the total effects of 5-HT (e.g. the human coronary artery (Connor et al (1989)), our results suggest that when 5-HT and TxA_2 are co-released from aggregating platelets 5-HT_1-like receptors may indeed play an important role in mediating the contractile effects of 5-HT.

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204P REGULATION OF HEPATOCYTE SECOND MESSENGER SYSTEMS BY HEPATOCYTE GROWTH FACTOR

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The ability of the liver to regenerate after injury is a remarkable example of controlled cell growth. This process is controlled by the complex interactions of a number of factors of neuronal, hepatic and extra-hepatic origin. Such factors act either to stimulate (e.g. noradrenaline and epidermal growth factor) or inhibit (e.g. transforming growth factor-β) liver cell growth. The most exciting new development in this field has been the discovery and recent cloning of a novel hepatocyte growth factor: HGF (Nakamura, T. et al, 1989). HGF is the most potent mitogen for parenchymal cells in culture. To date, little is known about the HGF receptor or the mechanisms through which HGF induces hepatocytes to proliferate. In the present study, in an attempt to characterise the HGF receptor mechanism, we have examined the effects of human recombinant HGF on various second messenger systems in hepatocytes. Experiments were performed using either freshly isolated rat hepatocytes or hepatocytes which had been maintained in culture for 24 to 48 hours. HGF-induced DNA synthesis was monitored as incorporation of [^{3}H]-thymidine and we examined the effects of HGF on the activity of adenylate cyclase, levels of inositol (1,4,5) trisphosphate (IP_3) and whole cell protein phosphorylation using methods as described (Palmer et al, 1988; Bushfield et al, 1990).

HGF induced the concentration-dependent stimulation of DNA synthesis in cultured hepatocytes with an $E.C._{50}$ of ~6 ng/ml. HGF had no significant effect on the stimulation of adenylate cyclase by forskolin (100μM) or glucagon (10^{-9} to 10^{-6}M) although HGF caused a concentration-dependent ($E.C._{50}$ = 20ng/ml) inhibition of basal adenylate cyclase activity in hepatocyte plasma membranes. Incubation (15 min, 37°C) of freshly isolated hepatocytes with HGF (12 ng/ml) resulted in an increase in the phosphorylation of an unidentified protein of ~44,000 Mr. HGF had no effect on the intracellular level of IP_3 under conditions where vasopressin induced a greater than five-fold increase in IP_3 levels. Three main signalling systems have been implicated in the growth factor-induced stimulation of mitogenesis: (1) inhibition of adenylate cyclase, (2) activation of phosphoinositide hydrolysis and (3) activation of tyrosine kinases (reviewed by Pouyssegur, 1990). These results indicate that the activation of protein phosphorylation may be an important mechanism underlying the induction of hepatocyte growth by HGF, which is a complete mitogen for hepatocytes.

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205P THE EFFECT OF GUANINE-NUCLEOTIDES ON AGONIST INTERACTIONS WITH ANGIOTENSIN II RECEPTOR SUBTYPES

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The binding of agonists to the angiotensin II (AII) receptor is sensitive to guanine nucleotides suggesting that AII receptors couple to G-proteins (De Lean et al., 1984). Recent studies have indicated that the AII binding sites correspond to two subtypes denoted as AII_A and AII_B (Chiu et al., 1989). The present study investigated the effects of guanine nucleotides on agonist interactions with these two binding sites.

Rat liver or bovine cerebellum was homogenised, centrifuged and resuspended in 50 mM Tris 5 mM EDTA buffer. After centrifugation, membranes were washed in 50 mM Tris buffer and stored frozen until required. Assays were conducted in 50 mM Tris, 1 mM EDTA, 0.1% BSA, 1 mM Bacitracin buffer (90 mins at 22°C) and terminated by filtration over glass fibre filters. In studies with bovine cerebellum, 3 mM dithiothreitol was included in the buffer. NSB was defined by 1 uM AII. All results are the mean of 3 - 4 experiments (S.E.M. < 8% of mean).

The AII_A selective ligand EXP655 (Chiu et al., 1989) did not inhibit [^{3}H]AII (0.3 nM) binding in rat liver at concentrations up to 10 uM whereas it produced complete inhibition of binding in bovine cerebellum (pKi=7.0 nH=1.1). In contrast the AII_B selective ligand DuP 753 (Chiu et al., 1989) did not inhibit [^{3}H]AII binding in bovine cerebellum at concentrations up to 10 uM whereas it produced complete inhibition of binding in rat liver (pKi=8.1 nH=1.0). These data indicate that rat liver and bovine cerebellum contain homogenous populations of the AII_B and AII_A receptors, respectively. In rat liver, 10 mM $MgCl_2$ increased specific [^{3}H]AII binding by 389% and this effect could be reversed by guanosine 5'-O-[3-thiotriphosphate] (GTP S) with a pIC_{50} of 7.0. Full inhibition (94.8%) of $MgCl_2$-stimulated binding by GTP S required the presence of 100 mM NaCl. In bovine cerebellum $MgCl_2$ increased [^{3}H]AII binding by 140%. Neither 0.1 mM GTP S, nor 1 mM 5'-guanylylimidodiphosphate (GppNHp), had any affect on either basal, or magnesium-stimulated, levels of [^{3}H]AII binding.

AII_A and AII_B binding sites were also labelled using the antagonist radioligand [^{125}I]sar^1ile^8-AII in the presence of $MgCl_2$ and NaCl. In rat liver, agonist ligands (AII, AIII and A(3-8)) identified both high (H) and low (L) affinity sites (eg, AIII pKi_H = 9.1 pKi_L = 7.5, % high affinity site = 40%). In the presence of 0.1 mM GppNHp only the low affinity site (pKi=7.5) was identified. In bovine cerebellum agonist ligands identified a single population of binding sites. Their pKi values were not affected by 0.1 mM GppNHp.

These data demonstrate that the two AII binding sites differ in their susceptibility to GTP analogues and may suggest that while the AII_B site interacts with G-proteins the AII_A site does not.

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206P MUSCARINIC RECEPTOR STIMULATION PRODUCES AN INOSITOL PHOSPHATE RESPONSE IN BOVINE AIRWAY MUCOSA

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Cholinergic receptor stimulation of airway submucosal glands results in mucus secretion (Shimura et al 1990). Pharmacomechanical coupling in a range of secretory tissues is dependent upon hydrolysis of phosphatidylinositol 4,5 bisphosphate to release the intracellular second messengers inositol 1,4,5 trisphosphate and diacylglycerol, which can release intracellular calcium and activate protein kinase C respectively. The aim of this study was to examine the effects of muscarinic receptor stimulation on inositol phospholipid hydrolysis in bovine airway mucosa.

Slices of mucosal tissue were prepared from bovine trachea by removing epithelial cells by gentle rubbing and then dissecting mucosa clear from underlying trachealis muscle and connective tissue. The resultant tissue was then chopped twice (300um) using a Mcilwain tissue chopper. Total [^{3}H]-inositol phosphate formation was then measured over a period of 45 min. as previously described for tracheal smooth muscle slices (Hall & Hill 1988). Histological examination of tissue before chopping revealed approximately 50% to be composed of glandular elements, the rest being predominantly an acellular matrix of collagen and elastin.

Carbachol (CCh) produced dose dependent [^{3}H]-inositol phosphate formation (EC_{50} 47 $\pm$ 17 uM, n=12). 1mM CCh produced a 7.7 $\pm$ 0.8 fold increase in [^{3}H]-inositol phosphate formation compared to basal (n=12). This response was inhibited by atropine (50nM, K_A 1.5 x $10^9 M^{-1}$, n=5) suggesting the involvement of muscarinic receptors. No increase in incorporation of [^{3}H]-inositol into membrane phospholipids was noted, in contrast to previous observations in bovine tracheal smooth muscle slices (Hall & Hill 1988). NaF (20mM), an activator of the G protein linked to phospholipase C (Hall et al 1990) also produced [^{3}H]-inositol phosphate formation, the response being 5.4 $\pm$ 1.4 fold over basal (n=3), indicating the involvement of a G protein in this response in bovine mucosa.

These results demonstrate that muscarinic receptor stimulation induces an inositol phosphate response in bovine mucosal tissue and that this response is mediated through a G protein.

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207P ACETYLCHOLINE AND ANGIOTENSIN II STIMULATE BREAKDOWN OF A COMMON POOL OF PHOSPHOINOSITIDES IN CULTURED BOVINE ZONA FASICULATA/RETICULARIS CELLS

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Zona fasciculata / reticularis cells from the bovine adrenal cortex were prepared and maintained in primary culture for 72 hours (Bird *et al*, 1990). The cells were labelled with [^{3}H]inositol for the last 6 hours, including, where necessary, 1mM Mn^{2+} for the full 6 hours, or 10^{-4}M acetylcholine (ACh) for the last 1 hour only. Cells were washed, and subsequently challenged with 10^{-7}M angiotensin II (AII) for 15 mins in the presence of 10 mM Li^+, and the phosphoinositide-associated radioactivity determined. Cells labelled under these conditions do not reach isotopic equilibrium (achieved after 42 hours labelling). Therefore the specific activity of the cytosolic inositol pool is greater than that of the phosphoinositide pool, and increased turnover of phosphoinositides is reflected by increased [^{3}H]inositol incorporation.

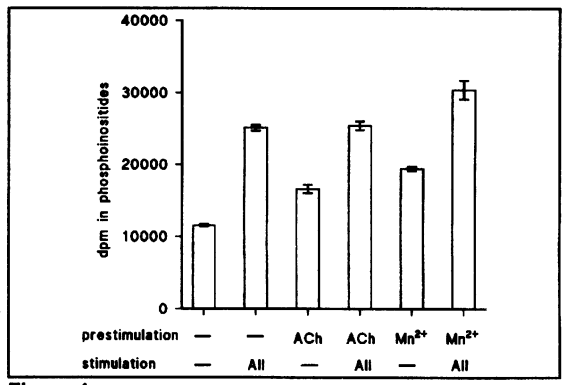


Figure 1

Although ACh prestimulation increased the specific activity of the phosphoinositide pool (see figure 1), it did not alter the level of [^{3}H]inositol incorporation subsequently stimulated by AII. In contrast, Mn^{2+} prestimulation increased phosphoinositide labelling, but the incremental increase in response to AII was equal to that seen in control cells.

In cells prelabelled to steady state with [^{3}H]inositol for 42 hours, AII and ACh cause dose-dependent increases in the formation of water-soluble inositol phosphates (Walker *et al*, 1990; Bird *et al*,1990). When given in combination, the effects of ACh (10^{-6}M, 10^{-4}M) and AII (10^{-9}M) were additive, whereas the maximum inositol phosphate production observed in response to 10^{-7} M AII could not be further increased by ACh.

We conclude that activation of ACh and AII receptors leads to the breakdown and resynthesis of a common hormone sensitive pool of inositol lipid which is distinct from the hormone insensitive pool labelled in the presence of Mn^{2+}.

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208P TRANSIENCE AND RECOVERY OF THE RESPONSE TO IP_3 IN SAPONIN-PERMEABILISED GUINEA-PIG ILEUM LONGITUDINAL SMOOTH MUSCLE: A ROLE FOR GTP?

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GTP has been shown to cause Ca^{2+} release from homogenised smooth muscle endoplasmic reticulum in the presence of polyethylene glycol, whereas in intact cells, GTP has been postulated to cause a movement of Ca^{2+} between different intracellular pools (Ghosh *et al.*, 1989). We have therefore studied the effects of GTP on tension development in saponin-permeabilised strips of guinea-pig ileum longitudinal smooth muscle (Allen & Carpenter, 1989).

The response of permeabilised strips to IP_3 is transient (approx. 1 min). A second dose of IP_3 given after the first response had decayed, without washing out the first dose of IP_3, causes no further response. If, however, the first dose is washed out and the second dose applied 5 min later, a response is obtained. This could imply that the transience is due not to the breakdown of IP_3, but to depletion of the IP_3-releasable Ca^{2+} pool.

GTP (1-1000 μmol/l) produces no response *per se* but increased the rate of recovery of the ability of the tissues to respond to a second dose of IP_3. It is possible, then, that GTP helps to mediate the refilling of the IP_3-releasable Ca^{2+} pool.

Although GTP was without a direct effect, GTPgammaS (1-100 μmol/l) caused a concentration-dependent contraction of saponin-permeabilised tissues. This may indicate the existence of a G-protein mediating Ca^{2+} release from the endoplasmic reticulum.

M.J.Allen is in receipt of a Fisons studentship.

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209P THE INOTROPIC POTENCY OF FLOSEQUINAN IS CONSISTENT WITH ITS PDE III INHIBITORY ACTION

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Flosequinan, (BTS 49465) (F) is currently in clinical development for the treatment of hypertension and congestive heart failure. Although the exact mechanism of action of F is still unclear we have recently suggested the involvement of phosphodiesterase type III (PDE III) inhibition in its inotropic actions (Gristwood et al. 1990). This evidence came from in vitro pharmacological studies with guinea pig ventricle.

We have now investigated the effect of F along with its major metabolite, BTS 53554 and SK&F 94120, a well known PDE III inhibitor (Reeves et al, 1987), on a purified PDE III enzyme preparation and compared the enzyme inhibitory potencies with the respective inotropic activities on guinea pig ventricles in vitro.

PDE III was isolated from guinea pig ventricle essentially according to Reeves et al (1987), except that the chromatographic separation was performed using a Mono Q column (Pharmacia). Enzyme purity was confirmed using selective PDE inhibitors. Activity was measured by the method of Thompson & Strada (1984) at 0.25 µM substrate concentration. Concentration inhibition curves were obtained for each compound. Inotropic activity was assessed using guinea pig right ventricle strip preparations electrically stimulated (1 Hz) placed under 1 g of resting tension in oxygenated Krebs solution at 37° C.

BTS 53554, F and SK&F 94120 produced concentration dependent inhibition of PDE III activity with IC_{50} values of 133, 57 and 7.5 µM respectively. All compounds also caused concentration dependent increases in force of contraction of guinea pig ventricle with EC_{50} (concentration causing 50% increase in force) values of 75, 29 and 4 µM respectively (n = 7). The ratios of the PDE inhibitory and inotropic activities for BTS 53554, F and SK&F 94120 were thus 1.8, 2.0 and 1.9.

The finding that the ratios for F and its major metabolite are very similar to that obtained for a known selective PDE III inhibitor leads us to conclude that the PDE III inhibitory potencies of these are sufficient to account for their positive inotropic activity.

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210P EFFECTS OF CYCLIC NUCLEOTIDES ON PROLIFERATION OF RAT AORTIC SMOOTH MUSCLE CELLS IN CULTURE

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Abnormal proliferation of vascular smooth muscle cells is a key event in the development of atherosclerosis. Elevation of cyclic AMP content inhibits proliferation of vascular smooth muscle in culture (Tertov et al, 1986). More recently, sodium nitroprusside, which acts through formation of nitric oxide and elevation of cyclic GMP content, has been shown to inhibit vascular smooth muscle proliferation (Garg & Hassid, 1989). In this latter study, it was proposed the EDRF, recently identified as nitric oxide, might act as an endogenous regulator of smooth muscle proliferation.

The object of this study was to examine the effects of elevating cyclic AMP and cyclic GMP content on proliferation of rat cultured aortic smooth muscle cells (RASM).

RASM were isolated by treatment with collagenase (0.125%) and elastase (0.025%) and seeded at a density of 12.5^4 cells/cm² in 9.6cm² plates in Dulbecco's modified Eagle's medium containing 5% each of foetal calf and newborn calf serum. Cells between passage number 4 and 15 were used. Drugs were added twice daily. After 4 days growth, cells were detached and counted by haemocytometry.

The membrane permeant analogues, dibutyryl cyclic AMP (1mM) and 8 bromo cyclic GMP (1mM) reduced cell numbers by 66±2% (n=6) and 31±4% (n=6), respectively. Forskolin (0.1-30µM), a direct activator of adenylate cyclase, reduced cell numbers in a concentration-dependent manner: the maximum reduction obtained was 65±2% (n=6). Rolipram (30µM), a specific cyclic AMP phosphodiesterase inhibitor, reduced cell numbers by itself by 37±2% (n=6), and potentiated the forskolin (1µM) - induced reduction from 32±4% to 57±4% (n=6). The nitrovasodilator, sodium nitroprusside (0.1-1mM) which acts through elevation of cyclic GMP, reduced cell numbers in a concentration-dependent manner. Proliferation was inhibited completely at 1mM, but this was associated with detachment of cells. M & B 22948 (30µM), a specific cyclic GMP phosphodiesterase inhibitor reduced cell numbers by itself by 33±2% (n=6), but did not potentiate the reduction induced by sodium nitroprusside (0.1-1mM). Haemoglobin (20µM) had no effect on cell numbers by itself and had no effect on the reduction induced by sodium nitroprusside (0.1-1mM).

In conclusion, elevation of cyclic AMP content inhibits rat aortic smooth muscle proliferation with no signs of cytotoxicity. Although elevation of cyclic GMP content can inhibit proliferation, it is likely that the inhibition induced by sodium nitroprusside resulted from a cytotoxic action. Whether or not endothelium-derived nitric oxide (EDRF) can inhibit smooth muscle growth in the arterial wall without a cytotoxic action remains to be determined.

This work was supported by the British Heart Foundation.

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211P INHIBITORY EFFECTS OF PGE ANALOGUES ON CICAPROST-INDUCED ELEVATION OF CYCLIC AMP IN HUMAN WASHED PLATELETS

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It has been suggested that potentiation of aggregation in human platelet suspensions by PGE_2 is mediated by a discrete PGE (EP-) receptor (Andersen *et al.*, 1980) and that this receptor may be negatively coupled to adenylate cyclase (Ashby, 1988). Our recent experiments on the potentiation of the PAF aggregation in human PRP by PGE analogues indicated that an EP_3-receptor may be involved, but there were problems associated with the very high sensitivity of the IP-receptor-adenylate cyclase system (Jones & Wilson, 1990). In this study we have raised the cyclic AMP level in human washed platelets using the specific IP-receptor agonist cicaprost and measured the abilities of PGE analogues to inhibit the rise. We reasoned that any weak cyclic AMP-elevating activity of the PGE analogues would be of lesser importance in this system than in the functional system. Time-expired (5 day-old) human platelets were suspended in Tris-EDTA buffer at 37°C in the presence of the TP-receptor antagonist GR 32191 (500 nM). After incubation with the PGE analogue for 2 min, cicaprost (10 nM) was added for 1min; this concentration of cicaprost increases cyclic AMP levels to 6 - 8 times basal (protein binding assay).

Table 1 Inhibition of cyclic AMP production in human washed platelets (mean ± s.e.mean, n = 4 - 7)

PGE analogue	Cyclic AMP remaining (% of control rise) in presence of PGE analogue concentration (nM) 2.5	7.5	25	75	750
Sulprostone	58.8 ± 9.9	34.2 ± 4.9	26.4 ± 6.0	20.8 ± 5.2	18.8 ± 6.0
16,16-Dimethyl PGE_2	46.9 ± 1.7	38.2 ± 4.4	26.4 ± 5.1	23.7 ± 5.6	20.8 ± 6.4
MB 28767	66.8 ± 11.1	53.1 ± 6.7	35.1 ± 7.5	30.3 ± 7..0	20.8 ± 5.5

It is apparent that this biochemical system provides a highly sensitive assay of the inhibitory actions of the PGE analogues, with half-maximal inhibition being produced at concentrations of about 2.5, <2.5 and 5 nM of sulprostone, 16,16-dimethyl PGE_2 and MB 28767 respectively. The aggregation studies conducted with PAF on human PRP showed sulprostone and 16,16-dimethyl PGE_2 to be 10 - 20 times more potent than MB 28767. This difference may reflect either the greater binding of the more lipophilic MB 28767 to plasma proteins or a certain degree of IP-receptor agonism operating in the functional assay. More comprehensive structure-activity studies are in progress to characterise the EP-receptor on the human platelet.

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212P THE D_1-LIKE DOPAMINE RECEPTOR OF THE COCKROACH SALIVARY GLAND IS COUPLED TO PHOSPHOLIPASE C IN ADDITION TO ADENYLYL CYCLASE

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A D_1-like dopamine receptor has been characterized on the cockroach salivary gland which appears to be coupled to at least two second messengers (Evans & Green, 1990). One, known to be cyclic-AMP (Grewe & Kebabian, 1982), subserving the secretory response to dopamine (Gray *et al.*, 1984) and the other, as yet unknown, subserving the electrical response. We have carried out biochemical assays to determine (i) whether inositol-1,4,5-trisphosphate (IP_3) and protein kinase C (PKC) were present in the acinar cells and (ii) the effect of dopamine on the levels of these two substances. The protocol for the IP_3 assay was as described by Palmer *et al.* (1989). Salivary glands (3/tube) were incubated at room temperature in physiological solution (see Evans & Green, 1990) in the absence and presence of dopamine (10^{-6}M) ± the D_1 dopamine receptor antagonist SCH23390 ($5x10^{-5}$M). Incubations were stopped at 5 and 30 s by addition of 50 µl ice cold 10% $HClO_4$ and immediate homogenization. PKC levels were determined by use of a specific [^{3}H]-phorbol dibutyrate (PDBu) binding assay (Leach *et al.*, 1983). Two groups of 20 glands were pre-incubated in physiological solution in the absence and presence of dopamine (10^{-6}M), respectively, for 5 min, homogenized in 25 vol of buffer (Leach *et al.*, 1983), centrifuged (100,000 g, 1 hr, 4°C) then assayed for particulate and cytosolic PKC. Both IP_3 and PKC were present in the acinar cells at rest. In the presence of dopamine (10^{-6}M) the levels of IP_3 were increased 2.3 and 2.1 fold over control levels after 5 (n = 2) and 30 s (n = 2), respectively. These increases were blocked in the presence of SCH23390 ($5x10^{-5}$M). The distribution of PKC at rest was (values = mean ± s.e.mean, n = 6-12) 56.2 ± 2.1% in the particulate and 43.8 ± 3.1% in the cytosolic fraction. After 5 min stimulation with 10^{-6}M dopamine, total [^{3}H]-PDBu levels were 29% of that in the unstimulated glands. The distribution of PKC in the stimulated cells had also changed to 76.4 ± 4.2% particulate and 23.6 ± 9.0% cytosolic. Thus dopamine can induce translocation and down-regulation of PKC. It therefore seems quite possible that activation of phospholipase C may underlie the electrical response of the gland to dopamine. The inhibition of this response by LiCl (10mM), an inhibitor of the phosphoinositide cycle, and potentiation by the G-protein activator NaF (500 µM) supports this conclusion.

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213P cAMP-DEPENDENT PROTEIN KINASE ACTIVITY IN BASOLATERAL MEMBRANE VESICLES ISOLATED FROM HUMAN PLACENTA

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Chloride transport in certain epithelial cells is stimulated via cAMP dependent protein kinase (A kinase) directly at the brush borders and possibly indirectly via modulation of ion transport in basolateral membranes. ß-Adrenoceptor stimulated chloride transport is defective in many epithelial cell types derived from cystic fibrosis (CF) patients and this may be due to defective regulation by the A kinase of the CF gene product, the 'cystic fibrosis transmembrane regulator' (CFTR) whose expression in CF cells has now been reported (Rich et al, 1990). In this study we have sought to establish the presence of A kinase activity in basolateral membrane vesicles (BLMV) isolated from human placenta, as part of an overall strategy to investigate the role of the kinase in regulation of the function of this epithelial cell type.

BLMV were isolated from human placenta within 0.5 hr of delivery by a modification of the method of Kelly et al (1983). 'CF' placentas were those obtained from mothers whose children were diagnosed on the basis of the sweat test. The purity of individual preparations was assessed by electron microscopy (E.M.) and confirmed by the presence of adenylate cyclase and of specific ß-adrenoceptor binding. Brush border membrane vesicles were available for comparison purposes and displayed characteristic scanning E.M. profiles and a lack of adenylate cyclase activity. The activity of A kinase was measured using an assay based on transfer of the γ-phosphate of γ-32[P]ATP to the synthetic substrate kemptide and was expressed as pmol phosphate transferred/min/mg protein. Basal activities differed considerably between preparations and some results are therefore reported as fold stimulation over basal. Significant differences (w.r. to basal activity) were determined using the unpaired Student's t test. Results are means ± s.e.m. of 4 experiments each performed in duplicate unless otherwise stated. Basal kinase activity, (96.7 ± 40 pmol/min/mg) measured in the presence of 2 mM $MgCl_2$, was stimulated 2.07 fold by 1 µM cAMP ($p < 0.05$) and this effect was abolished in the presence of protein kinase A inhibitor type II (PKI), resulting in activity not significantly different from basal. PKI alone did not alter basal activity. $CaCl_2$ (2mM) did not significantly lower cAMP-stimulated, or basal activity. In separate experiments MgCl2 produced a dose-dependent increase in kinase activity over a range of 1-14 mM, in the presence or absence of 1 µM cAMP. Comparisons of kinase activities in BLMV isolated from normal and CF placentas (n=3 in both cases) did not show significant differences in basal or stimulated activities between groups (while 1 µM cAMP increased mean activities, this increase was not significant in either group).

These results demonstrate that cAMP dependent protein kinase activity is present in BLMV isolated from human placental tissue. However clear assessment of the functionality of the enzyme in CF BLMV will depend on the availability of larger numbers of tissues.

This work was supported by the Cystic Fibrosis Association of Ireland.

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214P USING HIGH RESOLUTION COMPUTER GRAPHICS TO ANALYSE DRUG INTERACTIONS

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Interpretation of the effects of co-administering pairs of drugs can be made easier by plotting two-dimensional contour graphs of the combined dose-response curves (isobols) by hand (Rushton & Steinberg, 1967) or by computer (Hughes et al, in press).

In our first attempts to construct and analyse isobols by computer, we used software designed for personal computers. We have now extended the method to software which is more widely available, more powerful, and which can be used on most types of computer (VAX, IBM mainframes etc) (Hughes et al, 1990). As a result, three dimensional surfaces of the effects of combined drug treatments can be plotted using high resolution colour graphics, and compared with the theoretical surface to be expected if the interactions were merely additive; this theoretical surface is then used as a base from which the deviations of the observed effects are calculated and displayed by the computer, again as a three-dimensional surface. Dose combinations which act in an antagonistic or synergistic manner are easily identified from such displays by simple inspection. The surfaces incorporate all the statistical information available from the experimental data.

We now present results of the combined administration of clenbuterol and chlordiazepoxide to mice, which in some dose combinations induces backward walking (Steinberg et al, 1989), and show how the methods described can be applied to relatively small amounts of irregularly distributed experimental data. The three-dimensional surface models can be presented to advantage using the latest laser colour wax printer technology.

We thank Professor R. Needham for help and advice.

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215P PA2CALC: AN ACCURATE METHOD OF CALCULATING pA_2 VALUES

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The affininty of a competetive antagonist for its receptor is often measured by a pA_2 value (Arunlakshana & Schild, 1959). In several biological models the response is biphasic ie., two receptors are involved. The computer program PA2CALC uses all of the data points to determine the EC_{50} values, with errors, and allows mono- or bi-phasic systems to be analysed with more accuracy than conventional approaches. The final part of the program performs linear regression on -log [antagonist] vs -log (dose ratio - 1) to yield the pA_2 value, the intercept on the x-axis. The whole procedure is automatic and only takes a short time to run.

The agonist concentration [A] vs response (R) curve can be described by a one or two receptor model eg.

$$R = \frac{\alpha_1 [A]^{n_1}}{k_1^{n_1} + [A]^{n_1}} + \frac{\alpha_2 [A]^{n_2}}{k_2^{n_2} + [A]^{n_2}} \quad \text{for two receptors}$$

Where k is the EC_{50} value, α the maximum response and n the curve parameter. The numerical procedure uses routines from the pharmacokinetic program MODFIT (Allen, 1990).

The response data to 5-HT were obtained in guinea pig ileum as described by Sanger (1987). Increasing concentrations of 5-HT were added every 15 min with 45s contact time. Contraction heights were expressed as a percentage of a previously determined, maximum ACh-evoked concentration.

A plot of the experimental concentration-response data and the computed line is shown in Figure 1. Each point has five observations, shown as mean and ± s.e.mean. The program is fast and easy to use and has been tested extensively in our laboratories for the determination of pA_2 values.

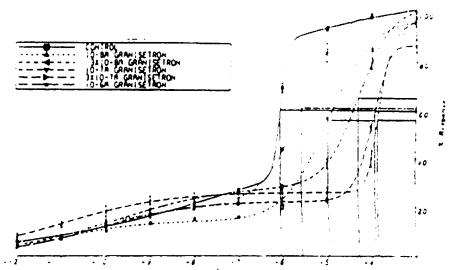


Figure 1. Concentration-response curve to 5HT using Granisetron (Guinea-pig ileum (LMMP)). The curves are computed lines of best fit through the data using a bi-phasic model.

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216P COMPUTER-ASSISTED LEARNING (CAL) PROGRAMS IN DRUG DISPOSITION - REPLACEMENT OF LECTURES

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We have developed a suite of CAL lessons in drug disposition and assessed their place in the curriculum when used as a reinforcement of lectures (Aarons et al., 1988). In the session 1989-90, lectures to second year medical students, concerning concepts in drug disposition, were reduced to 2h from 5h in 1988-89. Both cohorts received 4 lectures on the handling of type substances. CAL is now mainly used in an initial teaching mode in one 3h practical session plus student free time. Our aim was to assess for any adverse consequences of this change. Views of students in the 1989-90 session were sought by questionnaire, each question requiring an answer on a 5 point ordinal scale. Students' knowledge was measured by comparing multiple choice (MCQ) examination results between the two sessions.

33% of the 181 students responded to the questionnaire. They looked at 60% (median) of the lessons that they were asked to view. Students significantly preferred private reading (4, median) and lectures (3) to CAL (3, $P < 0.001$ and $P < 0.05$ respectively, Wilcoxon test). Overall, students recommended a similar balance of CAL to lectures (3, on a scale of less (1) to more (5) CAL). Interestingly, Biological Science students (n=21), taking part in a similar exercise, preferred CAL (4) to private reading (2.5, $P < 0.05$).

The pharmacology examinations consisted of 140 MCQs in 2.5h. The class results of the whole examinations in 1988-89 and 1989-90 were 55.4 ± 1.1% (mean ± s.e.m., n=187) and 53.1 ± 1.0 (n=179) respectively which were not different from each other ($P > 0.05$, Mann-Whitney U-test). The predicted results for 1988-89 (58.1 ± 1.2%) and for 1989-90 (57.4 ± 1.1%), based on a large sub-set of the questions which had been standardised by use in previous years, were not different ($P > 0.05$). This data suggests that both the whole examinations and the abilities of the students in pharmacology overall were not different between the two years. The results in the drug disposition part of the examinations were not different ($P > 0.05$) between 1988-89 (62.9 ± 1.3%) and 1989-90 (62.3 ± 1.1%).

We conclude that the increased use of CAL as replacement rather than reinforcement of lectures has not met with reduction in their knowledge as assessed by examination or overwhelming adverse student comments.

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217P MOLECULAR NEUROANATOMY OF MAO-A AND MAO-B IN HUMAN BRAIN: CELLULAR COMPARTMENTATION AND DISTRIBUTION OF PREFERRED NATURAL SUBSTRATES

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The enzyme monoamine oxidase (MAO) deaminates amine transmitters and xenobiotic amines, the preferred natural substrates in human brain being noradrenaline, dopamine and serotonin (MAO-A) and dopamine as well as several trace amines including octopamine, tryptamine, phenethylamine (MAO-B). We have investigated whether the cellular compartmentation of the two enzymes in human brainstem nuclei is in accordance with the distribution of these substrates. In human brain, MAO-A is thought to be present predominantly in neurons and MAO-B in glia as well as neurons. We used tritiated high-affinity ligands (the selective inhibitors Ro 41-1049 and Ro 19-6327) to reveal the distribution and abundance of MAO-A and MAO-B respectively by quantitative enzyme radioautography. The distribution of enzyme transcripts was revealed by in situ hybridization histochemistry using ^{35}S-labelled oligonucleotide probe sequences from the least conserved regions of the human MAO-A and MAO-B genes (Bach et al., 1988).

MAO-A and its mRNA were expressed in noradrenergic neurons of the locus coeruleus while MAO-B and its mRNA were expressed in serotoninergic neurons of the raphé and not vice versa. Although the substantia nigra contained both enzymes (B > A), neither transcript could be detected in the dopaminergic A9 neurons. Surprisingly, neither MAO-A nor MAO-B mRNAs could be detected in astrocytes. Since alternative splicing of the 15 exons in the MAO-B gene (Grimsby et al., 1990) could lead to the synthesis of isoforms of the enzyme (a neuronal and a glial?), further experiments using least conserved alternative exon sequences are required.

We conclude that the cellular compartmentation of the enzymes in human brain is not entirely in accordance with the distribution of their preferred natural substrates. Whereas MAO-A in noradrenergic neurons probably serves to inactive the neurotransmitter, the role of MAO-B in serotoninergic neurons might be to protect these cells from accumulating, as false transmitters, dopamine and trace amines (its preferred natural substrates). Astrocytic MAO-B (at the pial surface and around blood vessels) as well as MAO-B in ventricular ependymal cells (including most circumventricular organs), in addition to its protective role preventing trace amines from entering neurons to act as false transmitters, probably acts as a barrier to the transfer of trace amines at the blood-brain and CSF-brain interface.

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218P α-ADRENOCEPTOR BINDING SITES IN POST-MORTEM HUMAN BRAIN

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α-Adrenoceptors may be classified pharmacologically into α_1 and α_2 (McGrath et al., 1989). Both can be further divided into A- and B-subtypes according to their differing affinity for WB4101 (α_1) and oxymetazoline (α_2) (Petrash & Bylund, 1986; Minneman et al., 1988). Few studies have investigated the distribution of these receptors in human brain. We have therefore characterized and quantified α-adrenoceptor binding sites in membranes prepared from post-mortem human brain (5 male subjects, mean age ± SD, 57 ± 7 yrs, dying by myocardial infarction, mean post-mortem delay 51 ± 18 h).

Saturation binding of [^{3}H]prazosin (0.06-1 nM) and [^{3}H]rauwolscine (0.125-4 nM) was performed to determine the distribution of α_1 and α_2 adrenoceptors respectively (Table 1). Specific binding was defined by 100 μM phentolamine. [^{3}H]Prazosin bound with high affinity (K_D range 0.04-0.14 nM) as did [^{3}H]rauwolscine (K_D range 0.90-2.10 nM). The density of both α_1 and α_2 adrenoceptors was highest in hippocampus, hypothalamus and cortex and lowest in basal ganglia. The proportion (% of total α_1) of α_{1A} sites (defined by 10 nM WB4101) was highest in hypothalamus (72%) and lowest in caudate (41%). The proportion of α_{2A} sites (defined by 10 nM oxymetazoline) was highest in hypothalamus (92%) and lowest in caudate (52%).

Table 1. B_{max} of α-adrenoceptor binding sites (fmol/mg protein, mean ± SD, n=5)

Brain region	α_1-adrenoceptors			α_2-adrenoceptors		
	Total	α_{1A}	% α_{1A}	Total	α_{2A}	% α_{2A}
Caudate	41 ± 4	17 ± 3	41 ± 7	106 ± 7	55 ± 6	52 ± 4
Temporal cortex (Ba. 21/22)	138 ± 33	75 ± 15	55 ± 7	257 ± 7	211 ± 12	82 ± 6
Hypothalamus	158 ± 36	113 ± 24	72 ± 7	250 ± 20	230 ± 20	92 ± 3
Hippocampus	188 ± 36	124 ± 26	66 ± 2	383 ± 29	288 ± 29	75 ± 4

The proportion of α_{1A}- and α_{2A}-subtypes varies throughout the human brain.

We thank the Sir Jules Thorn Charitable Trust for financial support.

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219P A COMPARISON OF VARIOUS ANTIDEPRESSANTS AND ECS ON PRE- AND POST-SYNAPTIC α_2-ADRENOCEPTOR FUNCTION: EVIDENCE FOR RAPID ATTENUATION BY SIBUTRAMINE HCl

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Central pre- and postsynaptic alpha2-adrenoceptor function, respectively assessed using clonidine-induced hypoactivity and mydriasis in conscious mice (Heal et al., 1989a), is attenuated by prolonged administration of electroconvulsive shock (ECS) or antidepressants which enhance noradrenergic function (Heal et al., 1990). Sibutramine HCl is a monoamine reuptake inhibitor which down-regulates central beta-adrenoceptors within 3 days (Heal et al., 1989b). We have now determined whether this antidepressant can also rapidly desensitise pre- and postsynaptic alpha2-adrenoceptors.

Male C57/Bl/6Ola (Olac, Bicester) weighing 20-30g were used. The following drugs were dissolved in saline and injected at 3mg/kg i.p. daily for 3 days:- sibutramine HCl (SIB), amitriptyline (AMI), desipramine (DMI), zimeldine (ZIM), tranylcypromine (TCP) and mianserin (MIAN). ECS (200V, 2s) was given to halothane anaesthetised mice on 3 consecutive days. Responses to clonidine (0.1mg/kg i.p.) were tested 24h after the final treatment. Pupil diameter was measured before and 10 min after clonidine. Hypoactivity was then immediately rated from 0-3 on 5 behavioural parameters at 10 min intervals for the following 50 min. Both procedures are described by Heal et al. (1989b).

Clonidine produced a mean increase in pupil diameter of 0.17 ± 0.01 mm (n=34) and a total hypoactivity score of 23.4 ± 0.5 (n=34). As shown in Table 1, sibutramine was the only treatment which significantly reduced hypoactivity and mydriasis after 3 days of administration, although ECS x 3 did markedly attenuate the latter response.

Table 1 Comparison of the reversal of clonidine responses by 3 days treatment with various antidepressants and ECS

Response	SIB	AMI	DMI	ZIM	TCP	MIAN	ECS
Hypoactivity	15 ± 3*	0 ± 3	0 ± 3	-3 ± 2	1 ± 4	-4 ± 3	5 ± 6
Mydriasis	24 ± 7*	16 ± 8	1 ± 10	5 ± 9	11 ± 6	-4 ± 8	27 ± 6**

Results are % reversal of appropriate control response ± s.e. mean. *P<0.05, **P<0.01 (n = 8 - 10).

Since sibutramine alone rapidly reduced pre- and postsynaptic alpha2-adrenoceptor function, the data provide additional evidence to suggest that this antidepressant may have rapid clinical effect. Furthermore, unlike beta-adrenoceptor down-regulation where desipramine also has fast actions (Heal et al., 1989b), this comparison of antidepressant drugs showed that early alpha2-adrenoceptor desensitisation was exclusive to sibutramine.

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220P COMPARISON OF THE DEPRESSANT EFFECT OF CLONIDINE ON PERIPHERAL AND CENTRAL SYNAPTIC TRANSMISSION *IN VITRO*

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Clonidine has been reported to depress a long duration ventral root reflex (DR-VRP) of immature (1 to 5 day old) rat spinal cord preparations (Savola et al, 1990) in accordance with the proposal that analgesic effects can be mediated via spinal α_2-adrenoceptors (Sagen and Proudfit, 1984). In the present experiments the depressant action of clonidine on spinal transmission has been compared with that on transmission through the superior cervical ganglion (SCG) of the mature (body weight 150 to 250 g) rat. The isolated desheathed ganglia and hemisected spinal cords were superfused with medium containing 1 mM Mg^{2+} and 1.5 mM Ca^{2+} at 25°C. Depressant effects were measured as the reduction in peak amplitude of the postganglionic response (latency 21.1 ±1.8 msec, N=4) or area of the DR-VRP integrated over 30 sec, following supramaximal electrical stimulation of preganglionic nerve (at 0.033 Hz) or dorsal root (L4 or 5) (at 0.011 Hz). The maximal depressant effect of clonidine on four spinal preparations (produced by 0.1 to 1 µM) was to 18.8% ±3.8 s.e.mean of the control (100%) area of the DR-VRP (EC_{50}=20.4 nM ±2.0 s.e. mean , N=4). The corresponding values obtained with four ganglion preparations were maximal depression 60.0% ±5.6 s.e. mean and EC_{50} 15.2 nM ±3.3 s.e. mean. These EC_{50} values from spinal and ganglion preparations are not significantly different (P>0.05 by Student's t two tailed test).

The depressant effect of clonidine on the spinal preparations was very slowly reversible following introduction of drug-free medium (time to half recovery >60 min) and the depressant effects of clonidine on both spinal and ganglionic preparations were reversed completely within a few minutes following application of the α_2-adrenoceptor antagonist idazoxan (1 µM).

The present EC_{50} value for depression of transmission in SCG corresponds with the concentrations of clonidine which have been reported to depress cholinergic transmission in the guinea-pig ileum (Drew 1978) and to produce hyperpolarizing responses in the SCG (Brown and Caulfield, 1979). The similarity between spinal and ganglionic EC_{50} values in the present experiments suggests that the sites which mediate analgesic actions of clonidine at the spinal level are similar to those mediating presynaptic inhibitory actions in the autonomic nervous system.

RHE was supported by The Medical Research Council, The Taberner Trust and The Wellcome Trust.

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221P COMPARATIVE BINDING STUDIES OF [^{3}H]IDAZOXAN AND [^{3}H]RX821002 IN THE RAT BRAIN

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Idazoxan (IDX) and its 2-methoxy analogue, RX821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline), are both potent and selective α2-adrenoceptor antagonists (Stillings et al, 1985). However, recent reports have indicated that [3H]-IDX binds to additional sites that are not adrenoceptors and have thus been termed non-adrenoceptor idazoxan binding sites or NAIBS (Michel & Insel, 1989). In contrast, RX821002 has minimal affinity for NAIBS and higher affinity for α2-adrenoceptors (Langin et al, 1989). We have now compared the binding characteristics of these two ligands in the rat brain in parallel studies. Membranes together with the required concentrations of [3H]-ligand and displacing drug were incubated (Tris/HCl buffer, pH7.4) in triplicate to equilibrium (30min) in a final volume of 1ml at 25°C. Bound and free radioactivity were then separated by filtration through Whatman GF/B filters. Specific binding of [3H]-IDX and [3H]-RX821002 was defined in the presence of 1mM and 10μM phentolamine respectively.

Over the concentration range 0.1 to 60nM, [3H]-IDX and [3H]-RX821002 binding was saturable (respective Bmax values of 333.0 ± 7.9 and 263.9 ± 10.3 fmol/mg protein, n=3) and of high affinity (respective Kd values of 9.6 ± 0.7nM and 7.4 ± 0.9nM). In competition studies a variety of compounds active at α-adrenoceptors displaced 3nM [3H]-RX821002 binding monophasically, consistent with a single site interaction. The rank order of potency observed was RX821002 > RX811059 (2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline) > efaroxan > IDX > clonidine > (-) adrenaline > yohimbine > RX801023 (6-fluoro-2-(2-(1,4-benzodioxanyl)-2-imidazoline) > prazosin, confirming the specificity of RX821002 for α2-adrenoceptors. In contrast, whilst [3H]-RX821002 binding was fully displaced, 23.7% ± 1.1% of specific [3H]-IDX binding remained in the presence of 100μM (-) adrenaline reflecting binding to NAIBS. Additionally, RX821002, RX811059, efaroxan and yohimbine displaced [3H]-IDX binding biphasically, each revealing a similar proportion of high affinity sites (70 - 80%) corresponding to α2-adrenoceptor binding. The best selectivity profile for α2-receptors compared with NAIBS was displayed by RX811059 (pKi α2 = 8.64 ± 0.12, pKi NAIBS = 4.2 ± 0.13, n=4) whilst selectivity towards NAIBS was displayed by RX801023 (pKi NAIBS = 8.06 ± 0.33, pKi α2 = 6.73 ± 0.23, n=5).

In conclusion, [3H]-RX821002 is a superior ligand to [3H]-IDX for the labelling of α2-adrenoceptors in the rat brain since it displays minimal affinity for NAIBS and is of higher affinity for α_2-adrenoceptors. The selectivity of RX811059 and RX801023 for α2-adrenoceptors and NAIBS also warrants further investigation.

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222P β-ADRENOCEPTOR BINDING SITES IN OLFACTORY BULBECTOMISED RATS

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Bilateral olfactory bulbectomy (OB) in the rat produces a number of behavioural changes, including enhanced locomotor activity in a novel environment, which are normalised by chronic, but not acute, administration of a range of antidepressant drugs but not other psychoactive drugs (Leonard & Tuite, 1981). Since the most consistent property of antidepressant drugs is the ability to reduce the number of cortical β-adrenoceptors, we have in this study examined brain β-adrenoceptor binding sites in OB rats.

Sprague-Dawley male rats (weight 250-280 g) were anaesthetised with 250 mg/kg tribromoethanol and the olfactory bulbs removed bilaterally or the dura over the bulbs pierced but the bulbs left intact (sham-operated). The rats were housed 4 to a cage (2 OB and 2 sham) and regularly handled. Seven, 14, and 21 days later, locomotor activity was measured by the number of line crossings in 3 min in a brightly illuminated open field (divided into 10 cm squares). Total β- and β_1-adrenoceptor binding was determined in frontal and occipital cortex, hippocampus and striatum by saturation binding of [^{3}H]CGP 12177 (defined with 200 μM (-)isoprenaline and 100 nM CGP 20712A respectively, De Paermentier et al., 1989).

Table 1. Bmax of [^{3}H]CGP 12177 binding (fmol/mg protein, mean ± SD) and locomotor activity (mean ± SD)

		7 days		14 days		21 days	
		total β-	β_1-	total β-	β_1-	total β-	β_1-
Frontal cortex (n=5-6)	Sham	81 ± 11	40 ± 5	80 ± 8	57 ± 2	78 ± 9	51 ± 8
	OB	79 ± 6	39 ± 6	68 ± 6*	44 ± 6*	82 ± 9	56 ± 6
Occipital cortex (n=5-6)	Sham	79 ± 14	39 ± 8	80 ± 19	60 ± 15	71 ± 12	61 ± 20
	OB	88 ± 17	45 ± 8	67 ± 8	44 ± 4*	78 ± 12	53 ± 8
Locomotor activity (n=10-12)	Sham	113 ± 41		85 ± 24		107 ± 29	
	OB	168 ± 39*		188 ± 32*		210 ± 131	

Seven days after bulbectomy, total β- and β_1-adrenoceptors did not differ significantly between sham and OB rats in the brain regions studied. At 14 days, binding was lower in the cortical areas of OB rats, significantly so for total β- and β_1-adrenoceptors in frontal cortex (Table 1, $p < 0.05$, Mann Whitney test). At 21 days, β-adrenoceptor binding did not differ significantly. Kd values did not differ between sham and OB rats. Locomotor activity was significantly greater in OB rats at each time point.

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223P PHARMACOLOGICALLY DIFFERENT ACTIONS OF DOPAMINE IN THE MOUSE SUBSTANTIA NIGRA

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The electrophysiology and pharmacology of neurones in the substantia nigra have been studied extensively in the rat (Aghajanian & Bunney, 1977; Grace & Onn,1989), but to a far lesser extent in the mouse (Sanghera *et al.*, 1984). This study examines the actions of dopamine on substantia nigra neurones in a slice preparation of mouse brain.

Conventional extracellular microelectrode recordings were made from transverse mid-brain slices (300 μm thick from 16-35g male BKTO mice) superfused with an artificial cerebrospinal fluid at 31 ± 1°C. Substantia nigra *pars compacta* (SNC) and substantia nigra *pars reticulata* (SNR) neurones were identified by the shape and width of their action potentials and the position of the recording electrode. Values are mean ± s.d.

Results were obtained from 41 putative dopamine cells in the SNC. Their mean firing rate was 2.6 ± 0.6 Hz. Superfusions of dopamine for 1 min produced dose-dependent inhibitions of all these cells (mean pIC_{50} = 5.0 ± 0.5). A Schild Plot was constructed for the D_2 receptor antagonist (-)sulpiride (0.1 to 3.0 μM, 30 min, n=12). This had a slope of 0.6, not significantly different from unity, and a pA_2 value of 7.9 (95% confidence limits of 7.4 & 8.3). SNR cells (n=17) had narrower spikes and generally fired faster (11.5 ± 6.6 Hz) than SNC cells. A sub-maximal dose of dopamine (10 μM) increased the firing rate of 16 SNR cells (+3.8 ± 3.1 Hz). On two of these cells, the dopamine-induced excitation was followed by a complete inhibition of firing. (-)Sulpiride (1-3 μM, 30 min) did not antagonise the excitation of SNR cells by dopamine (10-100 μM, n = 4).

As in the rat (Pinnock *et al,* 1979), the inhibitory action of dopamine on mouse SNC neurones appeared to be mediated via D_2 receptors. The excitatory action of dopamine on mouse SNR cells (Ruffieux & Schultz, 1980), however, appeared not to involve D_2 receptors.

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224P PHARMACOKINETIC STUDY OF THE DOPAMINE AGONIST, BROMOCRIPTINE, FOLLOWING SUBCUTANEOUS ADMINISTRATION IN THE RAT

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Bromocriptine (BCR) has been in clinical use for several years, mainly for the treatment of hyperprolactinaemia (Fluckiger *et al*, 1982) and Parkinson's disease. Previous studies investigating the potential teratogenicity of BCR, indicated that high doses of the drug (up to 100 mg/kg subcut.) had not effect on pregnancy or fetal development in the rat (Narburgh *et al*, 1990). In the present study, absorption and distribution of BCR were investigated after parenteral administration of the drug in the rat.

Female Wistar rats (approx. 200g) were injected subcutaneously with BCR (50 mg/kg dissolved in 20% ethanol). Blood was obtained from anaesthetised rats at specified time intervals after dosing. Unchanged BCR concentrations were determined in serum, by minor modifications of a specific HPLC method (Larsen *et al*,1979).

The results demonstrated that serum levels of unchanged BCR remained low (<100 ng/ml) until 3 hours post-dose. From 3 hours post-dose, the serum levels of drug increased rapidly to reach a peak (810.60 ± 171.57 ng/ml) at 6 hours post-dose. The rate constant of absorption was 1.41 $hours^{-1}$. Elimination of the drug appeared to be biphasic (from 6-10 hours post-dose and 10-24 hours post-dose). The biphasic elimination suggests that the drug molecules distribute into two compartments. The rate constants of transfer of the BCR were 0.53 $hours^{-1}$ from the central to a peripheral compartment (K_{12}) and 3.33 $hours^{-1}$ from the peripheral compartment back into the central compartment (K_{21}). Volume of distribution of BCR in a 200g rat was 70.44 cm^3, which further indicated localisation of drug within peripheral tissues.

In vitro studies to investigate the potential de velopmental toxicity of BCR have previously been carried out. 9.5 day old rat embryos were explanted from the dam and cultured for 48 hours in 75% normal rat serum with varying concentrations of BCR in the serum (New, 1978). BCR was embryotoxic at a concentration of > 5ug/ml for the duration of the culture period (Narburgh, Turner and Freeman - unpublished data).

Exposure to BCR induced dysmorphogenesis in developing rat embryos *in vitro*. However, this embryotoxicity was not reflected *in vivo*. *In vivo* teratogenicity as a result of BCR administration may manifest itself at much higher and prolonged concentration of circulating drug, such as that used in the whole embryo culture studies. It is believed that the reported absence of BCR-induced teratogenicity after subcut.administration of high doses of drug to pregnant rats may be parti ally due to the effect of maternal detoxifying systems.

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225P LONG TERM EFFECTS ON THE BINDING OF DOPAMINERGIC RECEPTOR LIGANDS ELICITED BY THE 8-α-ERGOLINE, LISURIDE

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Lisuride exhibits a complex pattern of in vitro binding to aminergic receptors. In autoradiographic studies the compound binds principally to serotonin 1a receptors in extra-striatal areas and to an additional site(s) in striatum most potently displaced by serotonin. Despite this, the most prominent activity of lisuride in vivo is dopamine agonistic. The current experiments were made to investigate further the interactions of lisuride with aminergic binding sites in rat striatum.

Washed membranes from rat striatum were used for the determination of displacement IC50 of lisuride using the following tritiated ligands: N-0437 (0.5nM; D_2 agonist); spiperone (0.5nM; D_2 antagonist); Sch 23390 (1nM; D_1 antagonist); ketanserin (1nM; 5-HT_2 antagonist). Membranes prepared as above were preincubated (approx 3mg/tube in 1.6ml, 30min, 37°C) with lisuride concentrations spanning the IC50s. After dilution (1:100) with assay buffer, the mixtures were maintained at 37°C. At differing time points (0min, 3hr, 24hr and 48hr) aliquots were centrifuged (45000g, 15min), resuspended and used for the determination of total and nonspecific binding (each in triplicate) in 30min assays at 37°C. Control membranes were preincubated only with assay buffer.

Results showed that the binding of the four chosen ligands to control membranes remained remarkably stable during 48hr incubations. After 30min preincubation with lisuride (1.4 times and 14 times IC50), N0437 binding was inhibited (respectively 40% and 80%) throughout the time course (48hr) of the experiment, without changing the K_D for the ligand. Effects of lisuride in spiperone binding, although evident at time 0, decayed to control levels by 3hr. In the ketanserin and Sch 23390 binding assays, lisuride preincubation (up to 29 times IC50) failed to influence binding.

These observations indicate that lisuride induces long term or irreversible changes in the N0437 recognition site (presumably the D_2 receptor agonist conformation) in rat striatum. The relative lack of effect on spiperone binding suggests that lisuride is not irreversibly occupying the D_2 receptor, and the lack of effect of preincubation with other D_2 agonists (eg PHNO) and lack of K_D change shows that the binding changes are not only the consequence of agonist occupation of the receptor and that it has not undergone a conformational change. One possibility is that the phenomenon represents differing activities of lisuride and N0437 at D_3 receptors. An alternative, taking account of the potency of serotonin in displacing lisuride from its striatal binding sites, is that lisuride is binding to a serotonergic site which regulates the availability of D_2 receptors.

226P PUNCTATE KAINIC ACID LESIONS OF THE CEREBRAL CORTEX ABOLISH THE ANTICONVULSANT ACTION OF INTRASTRIATAL LY 171555

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Bilateral intrastriatal microinjections of the dopamine D-2 receptor agonist LY 171555 in conscious rats, prevent the limbic motor seizures induced by systemic pilocarpine (Turski et al., 1988), provided the indwelling cannulae have not caused extensive damage to the overlying cerebral cortex (Al-Tajir & Starr, unpublished observations). To test the possibility that an intact corticostriatal projection is essential for LY 171555's anticonvulsant effect, rats were equipped with superficial guide cannulae and given discrete kainic acid lesions (1μg in 0.5μl) of the primary motor cortex. Two weeks later LY 171555 (1μg in 1μl) or saline vehicle was injected into the anterior striata and seizure susceptibility determined with a normally convulsant dose of pilocarpine (600mg/kg i.p.) injected 15 min. later (Table 1).

Table 1 *Effect of cortical lesions on seizure protection by LY 171555*

	Stereotaxic treatment		Convulsion
Group	Cortex	Striatum	frequency
1	None	Saline	10/18
2	Kainate	Saline	4/7
3	Kainate	None	7/9
4	None	LY 171555	1/14
5	Kainate	LY 171555	7/7

Lesioning the primary motor area of the cerebral cortex in both hemispheres did not alter the convulsion frequency to pilocarpine ($p>0.05$ for groups 2 & 3 versus group 1, Table 1). By contrast, the protective effect of intrastriatal D-2 receptor stimulation was completely abolished in the cortical-lesioned rats ($p<0.001$ for group 4 versus group 5, Table 1). Since the primary motorcortex projects topographically to the underlying region of the anterior striatum, these findings suggest that the corticostriatal connection is implicated in the anticonvulsant action of D-2 agonist drugs.

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227P STUDIES ON THE EFFECTS OF TRAZODONE AND HALOPERIDOL ON FOOD REINFORCED OPERANT CONDITIONING

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We are studying the effects of trazodone and haloperidol on operant conditioned behaviours in order to elucidate the neurochemical basis of their actions. Male rats, aged 35 days, which had been reared under reverse light conditions to facilitate learning, were food deprived to 85-90% of their free body weight. For food reinforced operant conditioning, the rats were trained to selectively lever press on a fixed interval schedule (FI30sec), with one lever having a light cue. Ten days later conditioned rats were tested at 11.00 & 15.00h and at 0800h the following day without food reinforcement (extinction mode). They were then reconditioned over a period of days and retested as before, but with food reward (conditioned mode). Drugs were administered intraperitoneally 1h before testing as follows: trazodone (100mg/kg, n=4) or its vehicle (37% PEG 200); haloperidol (0.1mg/kg, n=4) or vehicle (0.9% NaCl solution). Doses chosen were maximal for conditioned avoidance response (CAR) operant conditioning (Patel 1983) and were administered in volumes of 1.0 ml/kg.

Both haloperidol and trazodone reduced significantly ($P<0.002$) the frequency of responding in conditioned rats at 11.00 & 15.00h but in both cases conditioned behaviour re-emerged the following morning. The saline vehicle was without effect but, in preliminary studies (n=2) PEG appeared to amplify the response at all times studied. When given in the extinction mode, haloperidol was without effect but trazodone produced a further significant ($P<0.01$) reduction in the frequency of responding.

The results demonstrate important differences between the effects of the two drugs and suggest that the mechanisms underlying actions of trazodone differ from those inducing extinction. Our previous in vitro studies have shown that the electrically stimulated release of noradrenaline and dopamine is significantly reduced in rat brain corticostriatal slices taken from animals trained in CAR (Patel & Turnbull, 1989). Further studies are underway to determine whether food reinforced behaviour elicits similar neurochemical changes and to what extent such changes are influenced by pharmacological modification of the behavioural pattern in vivo.

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228P THE EFFECT OF ENDOTHELIN-1 ON LOCOMOTOR ACTIVITY IN RATS: INTERACTION WITH CENTRAL DOPAMINE SYSTEMS

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The occurrence of endothelin-1 (ET-1), as well as its mRNA and binding sites, in various regions of the brain (Jones *et al.*, 1989; Lee *et al.*, 1990), suggests that it might act as a neuropeptide. We have studied the effect of intracerebroventricular (i.c.v.) injections of ET-1 on locomotor activity.

Male wistar rats of 125-150 g were anesthetized with Hypnorm (fentanyl and fluanison) and a polyethylene cannula was implanted in the right lateral brain ventricle. Five days after the operation the rats were injected i.c.v. with ET-1 (dissolved in 10 µl/kg 0.9% saline), followed 10 min later by subcutaneous (s.c.) or intraperitoneal (i.p.) injections of dopaminergic drugs (in 1 ml/kg vehicle). Locomotor activity was then measured for one hour with automated activity cages (Opto-varimex mini, Columbus). Each rat was used only once. Data is expressed as mean cumulative activity counts ± s.e.mean for n = 8-12 and analysed using analysis of variance.

After being placed in the novel environment of the activity cage, saline-treated rats displayed an initial high level of locomotor activity, which then decreased to reach a low baseline activity after approximately 45 min. Rats treated with either 0.4 mg/kg apomorphine (s.c.) or 1 mg/kg amphetamine (i.p.) showed similarly high activity in the first 20 min, but maintained this high level during the remaining period of measurement. Rats treated with 5 mg/kg quinpirole (i.p.) tended to show lower activity than controls in the first 20 min but maintained this level so that scores were higher than controls in subsequent time periods.

Activity counts of rats injected i.c.v. with 0.08, 0.4, 2 or 10 pmol/kg ET-1 were not significantly different from those of saline-treated controls during any time period. In contrast, 2 pmol/kg ET-1 potentiated the effect of apomorphine during the first 20 min by 47% (apomorphine 2080±253; apomorphine plus ET-1 2712±309; $P < 0.05$). Activity in subsequent time periods was not affected by ET-1. When 2 pmol/kg ET-1 was combined with quinpirole treatment, during the first 20 min activity counts were 59% higher than those of rats receiving quinpirole alone (quinpirole 661±60; quinpirole plus ET-1 1052±138; $P < 0.05$). No significant differences were observed during the 20-40 min or 40-60 min periods. Both 0.4 and 10 pmol/kg ET-1 were ineffective on the response to quinpirole treatment, suggesting a bell-shaped dose-response curve for ET-1. Pretreatment with 2 pmol/kg i.c.v. ET-1 did not affect the locomotor activity of rats treated with 1 mg/kg amphetamine (first 20 min: amphetamine 1822±200; amphetamine plus ET-1 1648±189).

These results suggest that central injections of ET-1 can potentiate the effect of dopamine D2 receptor activation on locomotor activity in rats. This effect could be mediated by increased sensitivity of postsynaptic dopamine D2 receptors or stimulation of receptor transduction mechanisms. However, the lack of an effect of ET-1 alone (i.e. interacting with endogenous dopamine release) or in combination with the dopamine releasing agent amphetamine, suggests that it may also have other, opposing, central actions, e.g. on dopamine release. A similar dual action of ET-1 has been suggested for peripheral adrenergic neuroeffector systems (Tabuchi *et al.*, 1990; Yang *et al.*, 1990).

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229P TIC-LIKE BEHAVIOURS INDUCED BY TRH-AMIDE: DIFFERENTIAL EFFECTS OF RITANSERIN AND HALOPERIDOL

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Intrathecal thyrotropin releasing hormone (TRH) and its analogues induce wet-dog-shakes and forepaw-licking in rats which are attenuated by the 5-HT2 antagonist ritanserin (Fone et al., 1989). We have here injected TRH-amide by the intracerebroventricular (ICV) route in mice and observed a range of movements which were each antagonised to a different extent by ritanserin and haloperidol.

Male MF1 mice (20-25g N=6/treatment) received TRH-amide in saline icv (Brittain & Handley, 1967) and were observed (blind to treatment) for the ensuing 30 min. Rapid blinking, straub-tail, tail-tremor and unilateral tremor of forepaw when raised (forepaw tremor) were scored (0-6, 0 = Control intensity for blinking); head-shakes and bursts of scratching were recorded as N in 30 min. All were dose-dependent over the dose-range tested (0.2-20µg) Blinking appeared to be virtually continuous at 20µg but to occur in bursts after lower doses. Tail-tremor and scratching were absent at 0.2µg while rapid blinking (mean score 2) and head-shakes were still prominent. At present, we cannot exclude that the fore-paw tremor may be associated with licking for the forepaw as observed in rats (Fone et al., 1989).

Ritanserin (1 mg/kg) and haloperidol (0.5 mg/kg) ip 30 min before 1µg TRH-amide icv caused the following percentage reductions:-

TABLE 1	Eye-Blinking	Straub-Tail	Tail Tremor	Fore-paw Tremor	Scratch Bursts	Head-Shakes
Ritanserin	66*	50*	100*	66*	49.5*	81.6*
Haloperidol	0	100*	100*	0	81.5*	41.2*

(* = $P < 0.05$)

Thus each agent had different effects on individual movements and the pattern of antagonism by ritanserin was different from that produced by haloperidol. This suggests that 5-HT and dopamine may be differentially involved in modulating these movements when induced by TRH-amide.

We thank the Tourette Syndrome Association of America for financial support.

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230P LACK OF EFFECT OF TWO 5-HT ANTAGONISTS ON MORPHINE-STIMULATED DA-RELEASE IN RAT NUCLEUS ACCUMBENS

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Behavioural as well as biochemical studies have indicated that 5-HT_3 receptors exert a modulatory action on dopamine (DA) function in the mesolimbic part of the rat brain (Costall et al., 1987, Carboni et al., 1989). For example, morphine at doses which are rewarding in rats, increases DA release preferentially in the nucleus accumbens (NA), see Carboni et al., 1989, an effect which has been shown to be attenuated by the 5-HT_3 antagonist ICS 205-930 (Carboni et al., 1987, Imperato et al., 1989). In the present study we have tested the effect of ICS 205-930 and another specific 5HT_3 antagonist, granisetron (BRL 43694) on morphine-induced stimulation of DA release from NA using microdialysis.

A concentric dialysis probe (3mm) was implanted in the rat NA under chloral hydrate anaesthesia. Experiments were carried out on freely moving rats 24 hours after surgery. The dialysis probe was perfused with artificial CSF at 2µl/min and dialysates were collected every 20 min. Content of DA in the perfusates was analyzed by HPLC-EC.

Systemic administration of morphine (1 mg/kg s.c.) increased DA levels in dialysates from NA over control values. The maximal effect (212% of control values) occurred 60-80 min after morphine injection. The total effect during the 3h experimental time course was 177±19% compared to saline injected control rats (mean±s.e.m.,n=3-4 rats;$p<0.01$). Injection of naloxone (1 mg/kg, s.c.) 20 min prior to the morphine administration completely blocked the increase in DA levels in the dialysates seen after morphine alone ($p<0.01$, n=3-4 rats). Naloxone given alone had no effects on the DA concentration. Rats pretreated with granisetron (0.1 mg/kg, s.c.) 40 min prior to morphine showed a very similar total increase of DA levels (171 ± 20%, n= 3-4) to those rats given morphine alone. Another 5HT_3 antagonist ICS 205 930 (0.1mg/kg, s.c.) was also ineffective in preventing the morphine-induced increase of DA levels in dialysates from NA

In summary we have shown that morphine increases DA release in NA an effect which is fully blocked by naloxone. However, we are unable to attenuate this increase by pretreatment with ICS 205-930 or granisetron at the doses used. Further studies to explain the discrepancy are in progress.

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231P BASAL DOPAMINE-RELATED ELECTROCHEMICAL SIGNALS IN RAT STRIATUM MEASURED WITH FAST CYCLIC VOLTAMMETRY

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Fast Cyclic Voltammetry (FCV) has hitherto been used to measure electrically stimulated release of dopamine (DA) in vivo (Millar et al, 1985), the lower limit of detection being above that needed to measure basal DA efflux. However, recent improvements in the FCV methodology (Hafizi et al, 1990) have pushed the DA detection limit below 50 nM and thus prompted attempts to measure unstimulated DA levels.

Experiments were conducted in male Sprague-Dawley rats (200-300g) anaesthetised with chloral hydrate (400 mg/kg ip). Carbon fibre microelectrodes (Armstrong James & Millar, 1979) were implanted into the striatum as previously described (Millar et al, 1985) while the reference (Ag/AgCl) and stainless steel auxiliary electrodes were inserted into the neck muscle. Striatal electrochemistry was monitored using FCV (scan range: -1.0 to +1.4 V vs Ag/AgCl, scan rate: 300 V/s) with measurements every 250 ms.

Upon electrode implantation into the striatum a peak was detected at +496 mV vs Ag/AgCl, a potential similar to that of DA oxidised following release by electrical stimulation (+519 mV). The peak was anatomically specific, being 5 and 3 times larger in the striatum than in cerebellum and overlying cortex respectively. The signal was stable over the course of the experiment (2 h). When compared with post-experiment calibration curves, the endogenous peak corresponded to an extracellular DA concentration of 461 $\pm$ 41 nM, assuming DA to be the sole constituent of the signal. This is approximately an order of magnitude higher than values reported from dialysis studies (eg 54 nM: Zetterström et al, 1983) and thus strongly argues that other compounds contribute to the in vivo peak.

This is supported by the pharmacological data. although the peak was decreased by gamma butyrolactone (400 mg/kg ip) and increased by haloperidol (1 mg/kg ip), the uptake blockers D-amphetamine (2.5 mg/kg ip) and GBR 12909 (10 mg/kg ip) had no effect. Ro 4-1284 (1 mg/kg ip) elevated the peak height although the drug depletes striatal DA (Shore, 1976). This pharmacology is inconsistent with DA being the sole component of the peak. It is concluded that other compounds, including acidic and basic DA metabolites, contribute to the in vivo signal. Studies with Nafion-coated electrodes may help to resolve the signal further.

This work was supported by the Royal Society.

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232P A FAST CYCLIC VOLTAMMETRIC STUDY OF PRESYNAPTIC RECEPTORS MEDIATING DOPAMINE RELEASE IN NUCLEUS ACCUMBENS AND STRIATUM

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The site of action of neuroleptic drugs is thought to centre on the mesolimbic system, and in particular the nucleus accumbens (Acc) (Mathysse, 1974). However, extrapyramidal side effects (EPS) are thought to result from neuroleptic actions in the striatum (Str). Atypical neuroleptics, however, have reduced capacity to produce EPS which may result from their selectivity for the mesolimbic system (Matz et al, 1974). We have compared the receptors mediating the release of dopamine (DA) in these two areas in order to determine whether any differences are apparent.

Fast cyclic voltammetry (FCV) can be used to measure electrically evoked endogenous DA release from brain slices in vitro (Bull et al, 1990). We have used this method to determine the actions of agonists and antagonists on DA release and so characterize the receptor involved.

Brain slices containing either Acc or Str were superfused and DA release evoked with single electrical pulses. Cumulative agonist concentration response curves (CRC) for inhibition of dopamine release were performed. After equilibration with an antagonist a second agonist CRC was performed. Antagonist potencies were estimated from concentration ratios at the EC50 level.

The substance released by electrical stimulation was verified as DA voltammetrically and biochemically. The only difference between Acc and Str was that desipramine (a noradrenaline and 5-HT uptake blocker) increased release in Acc but not Str, an effect we attribute to the greater density of noradrenergic terminals in the Acc or to an effect of despiramine on dopamine uptake at the higher concentration.

The D2 receptor agonist quinpirole inhibited DA release, whilst the D1 receptor agonist SKF38393 had no effect. The quinpirole-induced inhibition was antagonized by haloperidol, sulpiride, metoclopramide and clozapine with pA2 values of 8.6, 7.9, 7.2 and 7.1 respectively in the Acc and 9.1, 7.5, 8.0 and 6.8 respectively in the Str. The selective D1 antagonist, Sch23390, had no effect.

FCV is an effective method for investigating DA release from brain slices. The potencies of the antagonists in the two brain areas agree closely with each other and compare favourably with published values. We conclude that presynaptic receptors in the Acc and the Str do not differ. Thus if the atypical neuroleptic profile of such drugs as clozapine are due to a difference in D2 receptors in limbic and striatal areas, this difference must occur in the post- rather than the pre-synaptic receptor.

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233P NAFION-COATED MICRO BIOSENSORS WITH DIFFERENTIAL PULSE VOLTAMMETRY SELECTIVELY MEASURE *IN VIVO* RELEASE OF CORTICAL DOPAMINE AND 5-HT SIMULTANEOUSLY

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Electrically pretreated carbon fibre micro electrode (mCFE) combined with the electrochemical technique of differential pulse voltammetry (DPV) have been used for a number of years to monitor *in vivo* and *in situ* changes in the extracellular concentration of ascorbic acid, as well as that of the metabolites of dopamine (DA) and serotonin (5-HT) (Crespi, 1990).

We have recently prepared a mCFE which specifically electrically and chemically pretreated and then coated with Nafion (a negatively charged polymer which repels acids such as 3,4-dihydroxyphenylacetic acid (DOPAC)) allows the direct selective detection of the oxidation of extracellular levels of 5-HT *in vivo* (peak B at +240mV) (Crespi *et al.*, 1988)

We now show that this Nafion mCFE, which can detect the oxidation of DA *in vitro*, (peak A at +70/90mV) stereotaxically implanted in the frontal cortex (FC) of anaesthetised (chloral hydrate 500mg/kg i.p.) adult (300g) male rats can also detect the stimulated release of DA (peak A at +70/90mV).

Injection of potassium chloride (0.1mM, 1µl n=4) into the FC close to the Nafion-mCFE while increasing the size of peak B from basal levels (corresponding to 5nM 5-HT from *in vitro* calibration) up to 23nA (25nM) was also followed by the appearance of peak A which lasted only one voltammetric scan, (consecutive scans being performed at 10 min intervals) but reached a maximum level of 4nA corresponding to an *in vitro* concentration of 320nM DA.

Similarly, local injection of amphetamine was followed by the appearance of cortical peak A which reached a maximum level of 3nA (corresponding to an *in vitro* concentration of 240nM DA) which correlates with the levels of DA obtained after amphetamine treatment in experiments performed with the *in vivo* microdialysis method (Zetterstrom *et al.*, 1983). In addition, local injection of DA (0.1mM 1µl, n=4) but not that of the same dose of DOPAC was followed by the appearance of peak A. The maximum size of the signal was aproximately 25nA (200nM DA).

It is concluded that Nafion biosensors can detect the simultaneous cortical release of DA and 5-HT *in vivo*.

We thank the MRC for financial support and AVCO-Textron USA for the gift of carbon fibres.

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234P EFFECTS OF CERANAPRIL (SQ29,852) ON *EX VIVO* CATECHOLAMINE AND INDOLEAMINE LEVELS IN THE RAT

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It has been previously shown that ACE inhibitors, including the novel compound ceranapril, can potently improve cogitive performance in animal models (Costall et al., 1989). In the present study we assess the ability of ceranapril to alter *ex vivo* neurochemical levels in various rat brain nuclei.

Female Lister Hooded rats (230-270g) were injected i.p. with either ceranapril (10 µg/kg) or 0.9% saline (controls). After 40 min the animals were killed by cervical dislocation and the brains removed. Brain areas were dissected over ice, homogenised, and assayed by HPLC with electrochemical detection for levels of noradrenaline (NA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptophan (5HTP), 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA).The results are summarised in Table 1.

Table 1. Effect of ceranapril (10 µg/kg i.p.)(C) on *ex vivo* neurochemical levels in the rat brain.

BRAIN AREA		NA	DA	DOPAC	HVA	5HTP	5HT	5HIAA
Nucleus accumbens	S	1424±221	1863±375	358±47	72±27	45±3	497±31	334±37
	C	1652±176	831±93	90±22**	n.d.	40±7	540±66	353±42
Amygdala	S	416±56	407±84	34±12	n.d.	61±10	433±33	212±4
	C	450±18	682±40*	93±10*	21±13	52±9	625±47	352±50
Septum	S	704±177	714±84	168±41	20±13	53±5	280±19	190±35
	C	693±112	1373±524	268±48	101±29*	58±8	275±50	180±53
Tub. Olfactorium	S	314±38	2682±429	361±49	167±15	58±6	630±65	228±18
	C	387±35	3530±281	443±35	204±13	44±2*	641±41	266±29

Values are pg/mg tissue wet weight, Mean±SEM, n=5 per group. *P<0.05. **P<0.01,significantly different from saline-treated (S) animals (Student's t test). n.d., not detected.
In other brain areas (entorhinal and frontal cortex, hippocampus and striatum) no significant effects were detected.

The results appear to indicate a relatively selective effect of ceranapril on rat brain dopaminergic systems.

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235P MECHANISMS UNDERLYING THE *IN VIVO* RELEASE OF STRIATAL DOPAMINE INDUCED BY NOMIFENSINE AND METHYLPHENIDATE

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Behavioural studies have delineated the psychostimulant actions of amphetamine (reserpine insensitive) from those of nomifensine and methylphenidate (reserpine sensitive) (MacMillan, 1983) We have previously used in vivo brain microdialysis to examine the neurochemical basis of this division by studying the mechanisms underlying amphetamine-induced dopamine release in the striatum (Butcher et al, 1988). In the present study we have extended this investigation by examining dopamine release induced by behaviourally relevant doses of nomifensine and methylphenidate using an identical experimental protocol.

Microdialysis probes (Sandberg et al, 1986) were implanted into the striata of halothane anaesthetised, male Han-Wistar rats (250 - 350g). Probes were perfused with modified Krebs-Ringer bicarbonate at 1.25µl/min and the catecholamine content of dialysates (20 min fractions) collected after a 60 min equilibrium period was determined by high performance liquid chromatography combined with electrochemical detection (Butcher et al, 1988). Nomifensine (20mg/kg) or methylphenidate (50mg/kg) were given as intraperitoneal injections. Where appropriate animals were given reserpine (5mg/kg; i.p. injection) 180 min prior to injection of the psychostimulant. TTX (2µM) and Ca^{2+}-free buffer (containing 20mM EGTA) were infused directly into the striatum via the dialysis probe for 60 min prior to drug injection.

Nomifensine induced an increase in the dialysate content of dopamine (0.12 $\pm$ 0.05 pmol/20min to 0.38 $\pm$ 0.07pmol/min; 312% of basal efflux). A similar increase was induced by injection of methylphenidate (0.09pmol/20min to 0.39pmol/20min; 433% of basal efflux). Maximal increases were observed 40-60 min post-injection and, in contrast to amphetamine, no alterations in the dialysate content of DOPAC were observed.

The amount of dopamine released over the 60min period following drug injection was then calculated; control values = 0.53pmol/60 min for nomifensine, 0.53pmol/60 min for methylphenidate. Pretreatment with reserpine caused a dramatic reduction in dopamine efflux induced by both nomifensine and methylphenidate (16-19% of control values; $p < 0.01$). Perfusion of TTX or Ca^{2+}-free buffer produced less marked reductions in the amount of dopamine release by the psychostimulants; TTX, 36-40% of control, $p < 0.05$; Ca^{2+}-free, 40-43% of control, $p < 0.05$.

These data suggest that both nomifensine and methylphenidate increase the dialysate concentration of dopamine via a reserpine sensitive mechanism likely to involve exocytotic transmitter release. The most likely interpretation of this finding is that both compounds, at the dose tested, act solely to prevent reuptake of transmitter released into the synaptic cleft by normal physiological processes. This profile of reserpine, TTX and Ca^{2+} sensitivity is in marked contrast to that observed with amphetamine (Butcher et al, 1988).

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236P DOPAMINE AUTORECEPTOR CHARACTERISATION IN THE AMYGDALA *IN VITRO* USING FAST CYCLIC VOLTAMMETRY

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The technique of in vitro fast cyclic voltammetry has recently been used to characterise D_2 autoreceptors in the corpus striatum and nucleus accumbens (Bull et al, 1989 and Palij et al, 1990). We have now applied this technique to measuring endogenous dopamine release in the amygdala in vitro. Brain slices 350 µm thick containing the left amygdaloid complex were prepared from Wistar-derived rats. The slices were maintained at 32°C and superfused with artificial cerebrospinal fluid. Dopamine release was monitored with a carbon fibre microelectrode located 75 µm below the surface of the slice. Release was evoked by a parallel bipolar electrode placed 200-400 µm from the recording electrode.

A single 0.1 ms electrical pulse (20V) usually failed to evoke detectable dopamine release, but trains of 2 or 3 pulses, 100 ms apart, successfully did so. However, the dopamine thus released produced auto-inhibition, as shown by the increase which occurred on adding a D_2 antagonist, sulpiride (3×10^{-6}M), to the medium. This could be eliminated by using two 0.1 ms pulses, only 2 ms apart, to evoke dopamine release. We have termed this "pseudo-single-pulse" stimulation, and it was used throughout the study (applied once every 3 min) to evoke dopamine release from the amygdala.

The substance detected was identified as being predominantly or exclusively dopamine of neuronal origin from its electrochemical characteristics, its abolition by tetrodotoxin (10^{-7}M) or by calcium-free medium, its enhancement by the dopamine uptake blocker, GBR12909 (10^{-6}M), and its inhibition by RO4-1284, a depleter of monoamines (Shore, 1976). The release was also inhibited concentration-dependently by quinpirole (3×10^{-8} to 3×10^{-7}M), a selective D_2 agonist. This inhibition could itself be competitively antagonised by the D_2 receptor antagonists sulpiride, clozapine and metoclopramide. Using Schild analyses pA_2 values were found to be 7.6, 6.3 and 7.5 (confidence limits 6.9-7.5, 6.1-6.3 and 7.3-7.7 respectively all n=6) respectively, with slopes not significantly different from 1. These results are almost identical to those obtained in the striatum and accumbens (Bull et al, 1989 and Palij et al, 1990) and to those reported in binding studies (Richelson, 1988).

These results demonstrate that the terminals of dopaminergic neurones in the amygdala possess release-modulating autoreceptors, and that these receptors are of the D_2 type and indistinguishable from those in the striatum and nucleus accumbens.

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237P EFFECTS OF LONG-TERM TREATMENT WITH L-DOPA PLUS CARBIDOPA ON FOETAL DOPAMINE GRAFTS IN 6-OHDA-LESIONED RATS

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The concurrent treatment with L-DOPA may inhibit the survival and/or function of foetal ventral mesencephalic cells implanted into the 6-OHDA lesioned striatum. We have shown that administration of L-DOPA and carbidopa for 6 weeks to rats with unilateral 6-OHDA lesions and foetal dopamine grafts does not inhibit the survival or function of such grafts (*Blunt et al 1990), but have not previously examined effects of longer term treatment or glial responses to the grafts. We now report the effects of treatment with L-DOPA (200mg/kg/24h) plus carbidopa (25mg/kg 24h) for upto 30 weeks in rats with a unilateral 6-OHDA lesion receiving a graft of foetal ventral mesencephalic cells or a sham graft.

When tested 6 weeks and 30 weeks after foetal or sham graft surgery, animals receiving sham grafts continued to show high contralateral rotation scores in response to apomorphine. Animals receving foetal grafts showed a significant decrease in contralateral rotation ($p<0.01$) Following administration of amphetamine, animals with sham grafts still had high ipsilateral turning scores at 6 weeks and 30 weeks after sham graft surgery. Animals receiving foetal grafts showed complete behavioural recovery ($p<0.001$). Tyrosine hydroxylase immunhistochemistry revealed surviving grafts in all animals with foetal grafts, with no significant difference betweeen the mean cell numbers found in the 2 groups (Table 1). The mean density of glial fibrillary-acidic protein-postive cells was significantly higher in the striata of animals receiving foetal grafts ($F(1,18)=19.8$,**$p<0.001$, ANOVA).Table 1 Within the grafts themselves, the density of glial cells was higher in animals receiving treatment with L-DOPA and carbidopa ($\$p<0.001$). However, there was no evidence that the higher densities of glial cells found in the grafts of animals receiving L-DOPA was associated with a reduction of TH cell number or fibre outgrowth. This study shows that long term treatment with L-DOPA and carbidopa permits the survival and function of rat foetal DA grafts in this animal model of Parkinson's disease, and that the grafts can function well as late as 30 weeks after implantation. However, such long-term treatment is associated with an increase in glial cell density within the grafts and striatum.

Table 1 Densities of glial cells and number of TH-positive cells in striata and grafts

	GLIAL DENSITY		TH CELL NUMBER	
	Intact striatum	les/graft striatum	graft	graft (mean±sem)
6-OHDA lesion plus sham graft	60.6±4.1	61±4.9	-	-
6-OHDA lesion plus sham graft plus L-DOPA and carbidopa	66.8±5.9	66.5±5.0	-	-
6-OHDA lesion plus foetal graft	63.8±5.3	**91.7±4.6	73.2±6.9	979±228
6-OHDA lesion plus foetal graft plus L-DOPA and carbidopa	66.1±3.3	**96.8±5.3	$151±16.7	818±480

*SB Blunt, P Jenner, CD Marsden (1990) Neuroscience, in press.

238P FURTHER STUDIES OF 5-HT AGONISTS AND ANTAGONISTS ON MOTONEURONES AND NEURONES IN THE BRAINSTEM

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Davies et al. (1988a,b) and Roberts et al. (1988) reported the effects of a range of 5HT receptor agonists and antagonists on motoneurones and neurones in the medullary brainstem of the anaesthetised rat. Some brainstem neurones were excited by 5HT acting at a receptor blocked by ketanserin and not affected by several 5HT 1-like receptor agonists. Other brainstem cells were depressed by 5HT. These responses were not antagonised by ketanserin but were mimicked by DPAT (8-hydroxy-2-(di-n-propyamino)tetralin) and 5CT (5-carboxamidotryptamine). Motoneurones were depolarised and antidromic invasion facilitated by 5HT. This effect was resistant to ketanserin and cyanopindolol but was readily blocked by methysergide. 5CT was a potent agonist on motoneurones but DPAT was inneffective. All the effects of 5HT were resistant to 5HT 3 receptor antagonists. These data suggest that brainstem excitation may be mediated by 5HT 2 receptors but the receptors mediating brainstem depression and motoneurone facilitation are not sufficiently defined by these experiments to suggest more than a resemblence to the 5HT 1-like group of receptors.

These data have been extended using identical techniques of microiontophoresis of drugs from multibarrelled microelectrodes onto cells of the fluothane anaesthetised rat. The 5HT 1 receptor selective agonist sumatriptan had few and weak effects upon motoneurones and in the brainstem was only a weak agonist mimicking both excitatory and depressant effects of 5HT when high iontophoretic currents were applied. The relatively non-selective agonist mCPP (1-(3-chlorophenyl)piperazine) was not an agonist on motoneurones and mimicked the excitatory effects but not the depressant effects of 5HT on brainstem neurones. Three putative antagonists of 5HT 1-like receptors: mesulergine, methiothepin and cyanopindolol did not antagonise selectively the effects of 5HT on motoneurones, nor antagonise the depressant effects of 5HT in the brainstem.

These additional data suggest that the effects of 5HT in the brainstem and on motoneurones seem not to be via 5HT 1C receptors nor upon any rodent receptor on which sumatriptan is potent. It remains unclear whether these data imply the existence of a novel 5HT receptor mediating depression in the CNS; whether the receptor antagonists are insufficiently selective to block only the depressant receptor or whether some other technical constraint associated with in vivo iontophoresis prevents firm identification of the receptors responsible.

Thanks are expressed to P.P.A.Humphrey (Glaxo) for supplying sumatriptan.

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239P EFFECTS OF RESERPINE AND FLESINOXAN ON THE Ca^{++}-INDEPENDENT RELEASE OF [^{3}H]5-HT FROM RAT SPINAL CORD SLICES *IN VITRO*

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It has been suggested that a proportion of the [^{3}H]5HT, released by elevated K^+ stimulation from rat spinal cord slices in vitro, can take place in the absence of calcium ions (Roberts & Livingston, 1990). In this study we have investigated the possible mechanisms involved in this Ca^{++}-independent [^{3}H]5HT release from rat spinal cord slices.

Slices, 0.4 x 0.4 x 0.4 mm, were prepared from the spinal cords of adult female Porton rats (body weight 200-250g) with a McIlwain tissue chopper, incubated for 10 min in 10^{-6}M [^{3}H]5HT with 0.5 mM pargyline in a physiological salts solution (PSS) containing 0.75 mM Ca^{++}. Following incubation the slices were thoroughly washed and placed in superfusion chambers and perfused with PSS at 0.5 ml.min^{-1} at 37^{o}C. Pargyline was removed from the perfusing medium during the stimulation studies. Control experiments in the presence of 0.75 mM Ca^{++} indicated that the release of [^{3}H]5HT was dose dependent in a range of 15-55 mM K^+ stimulating pulses. Omission of Ca^{++} from, and inclusion of 0.1 mM EGTA in, the medium reduced the release of [^{3}H]5HT by 55mM K^+ to about 30% of control values, but in other control experiments using [^{3}H] noradrenaline, release was completely abolished.

Spinal cord slices from animals which had received 2.5, 3.5 or 5 mg.kg^{-1} reserpine i.p. 24 hr. earlier showed a marked (60%) reduction of [^{3}H]5HT release in the presence of 0.75mM Ca^{++} in response to 55mM K^+ stimulation, whereas in the absence of Ca^{++} there was no difference in release compared to untreated animals.

In other experiments flesinoxan, a 5HT uptake inhibitor, was included in the medium at concentration of 50 or 100nM and there was a clear stimulation of basal release of [^{3}H]5HT, but a considerable reduction in evoked release which was more marked in the Ca^{++}-free situation.

The results with reserpine would suggest that the [^{3}H]5HT released in the zero calcium experiments came from a non-vesicular site, possibly cytoplasmic or glial. The results with flesinoxan could suggest that the release seen in zero calcium may be associated with a reversal of the 5HT uptake system, although further experiments would be required to substantiate this.

In summary it would appear that although [^{3}HT]5HT release can take place in calcium-free medium this may represent an unphysiological action, possibly associated with interference with the 5HT uptake system.

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240P POSSIBLE EXPLANATION FOR THE VARIATION OF INHIBITION CONSTANTS FOR BLOCKADE OF [^{3}H]5-HT UPTAKE

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Marked differences in the inhibitory affinity constant (K_I) for inhibition of [^{3}H]-5-hydroxytryptamine uptake by antidepressants have been noted (see Cheetham et al, 1990). These authors found that increasing tissue concentration decreased the K_I values of various antidepressants. They proposed that this may reflect non-specific binding decreasing the effective concentration of the drug. Alternatively this effect on K_I values may reflect differential drug effects on the kinetic components of 5-HT uptake.

It has been proposed that the initial phase of monoamine uptake represents carrier binding whereas the latter phase represents carrier recycling and monoamine translocation (Wood & Wyllie, 1982). The carrier binding phase is sodium independent whereas translocation requires sodium (Wood, 1987). In agreement with this, the inhibitory potency (IC_{50}) of imipramine for blockade of [^{3}H]-5-HT uptake is reduced from 249nM ± 20 (± SEM, n=4) in normal Krebs to 545nM ± 51 in low sodium (54mM) Krebs. Similarly the IC_{50} of imipramine is reduced from 200nM ± 24 (n=3) after a 6 min incubation to 1600 ± 230 after only a 1 min incubation. Interestingly the inhibitory potency of panuramine (Blurton et al, 1984) was not affected by lowering the sodium concentration (IC_{50} in normal Krebs 87.3 ± 19, 97.1nM ± 10 low sodium Krebs).

These variations in the inhibitory potencies of antidepressant drugs may reflect their differential effects on these two kinetic phases of monoamine uptake. Thus tricyclic antidepressants such as imipramine have a preferential effect on the second phase or translocation component. Panuramine probably blocks the initial binding of 5-HT to the carrier. Methodological differences e.g. incubation time, tissue concentration etc. will therefore result in different estimates of inhibitory potency.

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241P THE LOCALISATION OF 5-HT_{1A} AND 5-HT UPTAKE BINDING SITES IN THE RAT FOREBRAIN

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Although autoradiographic and binding studies have demonstrated the presence of 5-HT_{1A} sites in rat cortex and hippocampus, the synaptic localization of these sites is not known (Pazos and Palacios, 1985). A loss of 5-HT_{1A} binding sites has, however, been observed post-mortem in frontal cortex obtained from Alzheimer's patients (Middlemiss et al, 1986). In the present study we have investigated whether 5-HT_{1A} and uptake binding sites are located on cholinergic and serotonergic nerve terminals in rat cortex and hippocampus.

Cholinergic neurones in the nucleus basalis were lesioned by direct injection of ibotenic acid (25 nmoles; AP -0.9; ML ±2.6; DV -6.8mm). Rats were sacrificed after 7 days for the determination of choline acetyltransferase activity (ChAT, Fonnum, 1975) or for binding studies. Serotonergic neurones were destroyed by i.c.v. injection of 5,7-dihydroxytryptamine (150µg; animals pretreated for 30 min with 25mg/kg desmethylimipramine). Brain tissue was prepared for analysis of 5-HT levels or binding studies 14 days later. 5-HT_{1A} binding sites were labelled with [^{3}H]8-OHDPAT (0.5nM), using 10µM 5-HT to define specific binding and the 5-HT uptake sites using [^{3}H]citalopram (0.4nM), with 10µM citalopram used to define specific binding. Whole brain membranes were prepared from frontal cortex and hippocampus of individual animals (n = 5 or 6 for each group).

Following ibotenate injection ChAT activity was reduced by 49.44±2.43% (n = 6) in the frontal cortex but was unaltered (98.69±5.13%) in the hippocampus suggesting that the cholinergic innervation of the cortex had been selectively destroyed. No significant alteration in the number of binding sites was seen for [^{3}H]8-OHDPAT (Bmax [pmol/mg protein]; cortex, control = 0.45±0.02, lesion = 0.52±0.03; hippocampus, control = 1.83±0.06, lesion = 1.85±0.14) or [^{3}H]citalopram (Bmax [pmol/mg protein]; cortex, control = 1.54±0.10, lesion = 1.33±0.11; hippocampus, control = 1.34±0.25, lesion = 1.37±0.31). Destruction of serotonergic fibres led to 77±2% and 78±5% reductions in cortical and hippocampal levels of 5-HT respectively. [^{3}H]Citalopram binding was reduced by 45% in frontal cortex and was almost undetectable in the hippocampus of lesioned animals (Bmax [pmol/mg protein]; cortex, control = 1.80±0.2, lesion = 1.00±0.16; $P < 0.05$). No alterations were observed in [^{3}H]8-OHDPAT binding (Bmax [pmol/mg protein]; cortex, control = 0.68±0.04, lesion = 0.61±0.07; hippocampus, control = 1.96±0.12, lesion = 1.64±0.12). For both lesions there was no significant alteration in the affinity of either ligand for its binding site.

These data suggest that the majority of [^{3}H]citalopram binding sites are located on the terminals of serotonergic neurones in rat cortex and hippocampus. Binding of [^{3}H]8-OHDPAT was not altered following lesions of the cholinergic and serotonergic innervation of the frontal cortex.

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242P REPEATED ANTIDEPRESSANT DRUG AND ELECTROCONVULSIVE SHOCK ADMINISTRATION DOES NOT ALTER [^{3}H]PAROXETINE BINDING TO RAT FRONTAL CORTICAL MEMBRANES

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Repeated administration of several classes of antidepressant drug has been reported to decrease the number of rat cortical [3H]imipramine binding sites (Brunello et al., 1987). However, [3H]imipramine is now considered to be a less than ideal radioligand for labelling 5-HT uptake sites as it labels a heterogeneous population of sites in brain tissue, only one of which is related to the 5-HT uptake site (Marcusson et al., 1986). Therefore, using the more potent and selective 5-HT uptake inhibitor paroxetine as a radioligand, we have examined the effects of acute and chronic administration of several antidepressant drugs and electroconvulsive shocks (ECS) on 5-HT uptake sites in rat frontal cortex.

Male CD rats (100-120g) were administered antidepressant drugs (Table 1) or saline orally once daily for 1 or 14 days or anaesthetised with halothane and given ECS (200v, 2s) either once or 5 times over a 10 day period. Frontal cortices were removed 24h after the final treatment. [3H]Paroxetine binding (8 concentrations, 5-320pM) was performed on freshly prepared membranes (1mg wet weight of tissue/tube). Non-specific binding was defined by citalopram (1µM). Binding parameters (Kd and Bmax) were determined using weighted non-linear least-squares curve fitting.

Table 1 Effect of repeated administration of antidepressant drugs and ECS on [3H]paroxetine binding

	Bmax		Bmax		Bmax
Pooled Control (n=40)	672±17	Zimeldine (10)	680±27	Nomifensine (10)	692±29
Sibutramine (3)	655±27	Tranylcypromine (10)	636±14		
Amitriptyline (10)	670±30	Citalopram (10)	647±30	Halothane control	694±40
Desipramine (10)	664±18	Mianserin (10)	723±28	ECS	674±20

Bmax values (fmol/mg protein) ± s.e.mean (n=10). Dose (mg/kg) in parentheses.

[3H]Paroxetine binding was of high affinity and fitted well to a single site binding model. Kd and Bmax values obtained compared well with previous reports (Habert et al., 1985; Gleiter and Nutt, 1988). Neither acute nor chronic administration of a wide variety of antidepressant drugs or ECS altered the number or affinity of [3H]paroxetine binding sites (Table 1). Thus we confirm and extend the findings of Graham et al. (1987) and Gleiter and Nutt (1988) that repeated antidepressant drug and ECS administration does not alter 5-HT uptake sites labelled with [3H]paroxetine.

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243P BMY 7378 BLOCKS 8-OH-DPAT INDUCED REDUCTIONS IN LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT

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Previously we have shown that the selective $5HT_{1A}$ agonist, 8-OH-DPAT, reduces glucose utilization in certain limbic and cortical areas in the rat brain in vivo (Grasby et al, 1989). BMY 7378 also has a high affinity and selectivity for brain $5HT_{1A}$ receptors but, in the rat, antagonizes the inhibition of forskolin-stimulated hippocampal adenylate cyclase (Yocca et al, 1987), the 5HT behavioural syndrome (Sharp et al, 1990) and the suppression of hippocampal pyramidal neurone firing (Chaput & de Montigny, 1988) induced by 8-OH-DPAT. In this study we have used BMY 7378 in an attempt to pharmacologically characterize 8-OH-DPAT-induced reductions in cerebral glucose utilization. Groups of male Sprague Dawley rats (250g-350g) received subcutaneously either BMY 7378 (5 mg/kg) or saline 30 min before subcutaneous injection of either 8-OH-DPAT (250 µg/kg) or saline; 5 min later 40 µCi of [^{14}C]-2-deoxyglucose was injected intravenously for 2-deoxyglucose quantitative autoradiography. BMY 7378 significantly attenuated 8-OH-DPAT-induced reductions of glucose utilization in the hippocampus, dentate gyrus, entorhinal, piriform and cingulate cortices (Table 1). Interestingly, BMY 7378 alone markedly increased glucose utilization in the lateral habenular nucleus and moderately reduced glucose utilization in hippocampal structures.

TABLE 1. EFFECT OF 8-OH-DPAT AND BMY 7378 ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT

Structure	Saline/Saline (n=6)	Saline/8-OH-DPAT (n=6)	BMY 7378/8-OH-DPAT (n=5)	BMY 7378/Saline (n=5)
Hippocampus	55±2	36±2*	46±3*†	45±2*†
Dentate Gyrus	66±1	39±1*	50±3*†	50±3*†
Entorhinal Cortex	64±2	49±2*	61±2†	61±3†
Piriform Cortex	67±2	48±1*	59±4†	59±3†
Cingulate Cortex	90±5	66±2*	88±7†	78±1
Lateral Habenular	105±4	86±4	161±1*†	148±6*†

(Mean ± s.e.mean, µmol/100g/min), *P<0.05 vs saline/saline; †P<0.05 vs saline/8-OH-DPAT: ANOVA with Scheffe correction.

These findings suggest that 8-OH-DPAT-induced reductions in rat regional cerebral glucose utilization are mediated by the $5\text{-}HT_{1A}$ receptor and may provide an in vivo measure of central $5HT_{1A}$ receptor function in the rat. With appropriate pharmacological tools and imaging techniques such an approach could potentially be developed for use in man.

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244P STRUCTURE-ACTIVITY RELATIONSHIPS OF ARYLPIPERAZINE DERIVATIVES (NAN-190 CONGENERS) BINDING AT $5\text{-}HT_{1A}$ RECEPTOR SITES

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1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)]butylpiperazine (NAN-190) a buspirone-related compound has one of the highest binding affinities at the $5HT_{1A}$ receptor (K_i ca. 1nM)(Glennon et al, 1988). In a search for yet more potent compounds as candidates for potential radio-imaging ligands for this class of receptor using SPET, a series of congeners were synthesised and their K_i values measured at $5HT_{1A}$ receptor sites in rat cerebrocortical membranes using [^{3}H]8-OHDPAT (0.25nM) in a standard filtration assay (Peroutka, 1986).

The main emphasis was placed on varying the substituent in the 2-position of the aromatic ring (i.e. electron donating, withdrawing, hydrogen bonding and non-bonding effects of substituents were examined) as the pi-orbital electronic interaction of the aromatic ring at the receptor protein binding site makes an important contribution to the binding energy of the ligand (Hacksell et al, 1990). Positional isomerism was also investigated.

A clear trend emerged from this study and is summarized as follows:

1. Binding is optimal when electron donating groups (i.e. hydroxyl and methoxyl) are substituted in the aromatic ring. The 2-halophenylphthalimidobutylpiperazines bind with decreasing affinity in the following order I>Br>Cl>F consistent with increasing net electron withdrawal from the aromatic ring.

2. Methoxyl groups in other positions at the aromatic ring results in decreased binding affinity. This is more pronounced in the 4- than in the 3-position suggesting the binding cleft can accommodate a methoxyl group in the 3-position.

3. Hydrogen bonding of substituents in the 2-position is not an important contributor to the binding energy.

In conclusion methoxyl or hydroxyl substituents at the 2-position of the aromatic ring give the highest binding affinities, K_i values 1nM and 2nM respectively for inhibition of [^{3}H]8-OHDPAT binding for this class of arylpiperazine derivatives.

Supported by grants from the Scottish Hospital Endowments Research Trust and the Wellcome Trust.
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245P TOLERANCE TO ACTIONS OF THE 5-HT$_{1A}$ PARTIAL AGONIST IPSAPIRONE IN A BEHAVIOURAL MODEL OF ANXIETY IN RATS

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This study assessed:- (i) Actions of ipsapirone in a two-chambered light/dark "conflict" model of anxiety, and (ii) Effects of chronic ipsapirone treatment. Female rats were treated (15 days) with saline or ipsapirone (20 mg/kg/day). On day 16 they received saline or ipsapirone (3 doses tested), 30 min before placement in the dark side of the apparatus. Behaviour was videotaped (8 min/rat). Transitions between chambers and time spent in the dark were recorded. The experimental groups (n = 9/group) and data [group mean ±S.E.s] are summarised in Table 1.

Table 1

Pretreatment	Day 16 "Test" Treatment	Transitions	Duration in Dark(secs)
Saline	Saline	13.8 (0.8)	357 (15)
Saline	Ipsapirone (1.25 mg/kg)	10.7 (0.7)	394 (12)
Saline	Ipsapirone (2.5 mg/kg)	8.8 (0.7)	416 (11)
Saline	Ipsapirone (5.0 mg/kg)	9.0 (1.2)	421 (8)
Ipsapirone	Ipsapirone (1.25 mg/kg)	14.6 (0.6)	369 (13)
Ipsapirone	Ipsapirone (2.5 mg/kg)	12.7 (0.7)	360 (15)
Ipsapirone	Ipsapirone (5.0 mg/kg)	14.3 (1.7)	382 (15)

In saline-pretreated subjects ipsapirone increased time in the dark ($p < 0.05$ at 2.5 and 5 mg/kg relative to controls). No such increase was seen in ipsapirone-pretreated animals. ANOVA revealed a significant effect of pretreatment type (saline/ipsapirone), showing that tolerance developed to ipsapirone's action in increasing time in the dark. In saline-pretreated subjects ipsapirone decreased transitions ($p < 0.05$ at 2.5 and 5 mg/kg relative to controls). No such decrease was seen in animals pretreated with ipsapirone. ANOVA again revealed a significant effect of pretreatment type, showing that tolerance also developed to ipsapirone's action in reducing transitions. These data can be interpreted in two ways. Either ipsapirone had a paradoxical "anxiogenic" action (increasing time in the dark and reducing transitions), or ipsapirone acted as a "sedative", (increasing time in the dark chamber in which the animal was first placed, and reducing transitions). It is currently impossible to decide between these interpretations. However, tolerance clearly developed to ipsapirone, extending previous studies which have demonstrated that tolerance develops to many of ipsapirone's actions (Goudie et al. 1989; 1990).

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246P 5-HT$_{1D}$ AND 5-HT$_{1E}$ BINDING SITES IN POST-MORTEM HUMAN BRAIN

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The division of 5-HT$_1$ binding sites into 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ subtypes is generally accepted. However in the presence of drugs which fully occupy these sites, residual [^{3}H]5-HT binding is displaced by 5-carboxamidotryptamine (5-CT) with high and low affinity components that have been termed 5-HT$_{1D}$ and 5-HT$_{1E}$ sites respectively (Heuring & Peroutka, 1987; Leonhardt et al., 1989). In this study we have examined the distribution of 5-HT$_{1D}$ and 5-HT$_{1E}$ sites in human brain tissue obtained at post-mortem examination from 9 males and 2 females (mean age ± sem, 49 ± 4 years, post-mortem delay 52 ± 5 h).

Saturation binding of [^{3}H]5-HT (0.5-32 nM, specific binding defined with 10 μM 5-HT) was determined concurrently under three conditions: (1) no competing drug to determine total 5-HT$_1$ binding (2) in the presence of 500 nM (-)pindolol and 100 nM mesulergine to determine 5-HT$_{1D}$/5-HT$_{1E}$ binding (3) in the presence of 100 nM 5-CT and 100 nM mesulergine to determine 5-HT$_{1E}$ binding. 5-HT$_{1D}$ sites were determined by difference between conditions 2 and 3. The distribution of 5-HT$_{1D}$ and 5-HT$_{1E}$ sites (fmol/mg protein, means ± sem, n=4-5, and expressed as a percentage of total 5-HT$_1$ sites) is shown in Table 1.

Table 1. The distribution of 5-HT$_{1D}$ and 5-HT$_{1E}$ binding sites in post-mortem human brain.

Brain region	Total 5-HT$_1$ sites	5-HT$_{1D}$	% 5-HT$_1$	5-HT$_{1E}$	% 5-HT$_1$	5-HT$_{1D/1E}$ as % 5-HT$_1$
G. pallidus	445 ± 38	331 ± 30	75 ± 3	92 ± 14	20 ± 2	95
Hippocampus	333 ± 64	72 ± 20	21 ± 4	53 ± 7	17 ± 2	38
Caudate	236 ± 43	53 ± 10	24 ± 4	169 ± 39	70 ± 3	94
Putamen	247 ± 22	46 ± 13	18 ± 5	158 ± 17	64 ± 4	82
Amygdala	264 ± 16	71 ± 14	26 ± 5	110 ± 6	42 ± 1	68
Frontal cortex	331 ± 20	78 ± 9	24 ± 3	140 ± 13	42 ± 3	66

5-HT$_{1D}$/5-HT$_{1E}$ sites were the predominant 5-HT$_1$ subtypes, except in hippocampus. The proportion of 5-HT$_{1D}$ sites was high in globus pallidus (75%) but relatively low and uniform (18-26%) in other areas. The proportion of 5-HT$_{1E}$ sites was highest in caudate (70%) and putamen (64%), intermediate in amygdala and frontal cortex (42%) and lowest in globus pallidus (20%) and hippocampus (17%).

We thank the Wellcome Trust for financial support.

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247P CENTRAL AND PERIPHERAL 5-HT_2 RECEPTORS ARE INVOLVED IN THE SYMPATHO-EXCITATORY AND HYPERTENSIVE EFFECT OF QUIPAZINE IN ANAESTHETIZED CATS

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Quipazine produces an hypertensive response due to the stimulation of peripheral 5-HT_2 receptors in anaesthetized rats ; no evidence was found for a central sympatho-excitatory effect (Vayssettes-Courchay et al, 1990). In contrast, DOI, a potent 5-HT_2 agonist, induces an increase in blood pressure and in sympathetic nerve activity in anaesthetized cats (Mac Call and Harris, 1988). Our purpose was to examine the effect of the 5-HT_2 agonist quipazine on the central and peripheral cardiovascular regulation in the cat. The experiments were performed in pentobarbital anaesthetized cats, artificially ventilated. Mean blood pressure (MBP) heart rate (HR) and splanchnic nerve activity (SNA) were recorded. The drugs were administred intravenously or applied on the rostral ventrolateral medulla (RVLM).

Quipazine (0.5mg/kg i.v.) induced long lasting hypertension and activation of SNA (49 + 3 mmHg ; 249 + 30% ; n = 6) in intact as well as in baroreceptor-denervated cats. This effect was prevented by the 5-HT_2 antagonist ritanserin (0.5mg/kg i.v.). Bilateral application of quipazine (25ug/side) on RVLM elicited an increase both in MBP and SNA (51 + 5mmHg ; 204 + 26% ; n = 7). In this case, nerve activation appeared faster than after intravenous injection an preceeded the hypertension (Figure 1). Application of the 5-HT_2 antagonists LY53857 (10ug/side) and cyproheptadine (10ug/side) had no effect on MBP and SNA but reversed or prevented the actions of locally applied quipazine. Pretreatment with prazosin (0.1mg/kg i.v.) abolished the hypertension but not the sympathoexcitatory effect of quipazine. After application of LY53857 on RVLM (10ug/side), intravenous administration of quipazine 0.5mg/kg failed to increase SNA but induced an hypertension, of smaller intensity and shorter duration than in non treated animals (24 + 9mmHg, n = 5), (Figure 1).

These results indicate that the hypertension elicited by quipazine is partly due to activation of peripheral vascular 5-HT_2 receptors and partly due to sympathetic stimulation following the activation of central 5HT_2 receptors located in the RVLM.

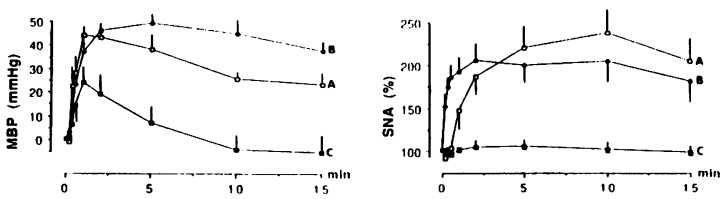


Figure 1 : Effect of quipazine on MBP and SNA, A : i.v. administration 0.5mg/kg ; B : application on the RVLM 25ug/side ; C: i.v. administration 0.5mg/kg after application of LY53857 10ug/side on the RVLM.

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248P CENTRAL CARDIOVASCULAR EFFECTS OF NEUROPEPTIDE Y AND RILMENIDINE IN THE CONSCIOUS, SPONTANEOUSLY HYPERTENSIVE RAT

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The central hypotensive effects of neuropeptide Y (NPY) and oxazoline derivative rilmenidine have been examined in conscious spontaneously hypertensive rats (SHR). NPY, which is found colocalised with catecholamines in the brain stem is putatively involved in central cardiovascular control. Rilmenidine, a centrally active α_2-adrenoceptor agonist, produces hypotension without significant sedative effects (Fillastre et al. 1988).
Experiments were carried out on male SHR rats (300-400gms) which had intracisternal and femoral arterial cannulae implanted (brietal sodium anaesthesia, 60mg.kg^{-1} i.p.). Three doses of NPY (1.7, 5 and 15 $\mu g.kg^{-1}$) and rilmenidine (25, 75 and 225$\mu g.kg^{-1}$) were administered intracisternally in separate animals and compared to control groups (5µl saline).

Both drugs produced a significant and dose-dependent fall in blood pressure (NPY: -15, -20 and -27mmHg respectively; rilmenidine: -19, -30 and -54mmHg respectively) which was maximal at 45 mins post-injection. Both drugs also elicited a significant bradycardia. The vehicle, saline was without significant haemodynamic effect. Submaximal doses of NPY and rilmenidine co-administered to investigate possible interaction between NPY and α_2-adrenoceptors (Table) revealed a negative interaction on blood pressure. This was also reflected in heart rate responses. These results may suggest an interaction between brainstem NPY receptors and α_2-adrenoceptors. Alternatively, rilmenidine may elicit its hypotensive effects via the recently discovered medullary imidazoline receptor (Bricca et al. 1988) in which case the interaction may involve NPY and imidazoline receptors.

Blood Pressure mmHg — TIME (Mins)

Treatment	n	-15	0	15	30	45	60	90	120
Saline	6	165±5	168±6	168±4	167±6	167±3	166±4	168±3	164±4
NPY (1.7µg.kg^{-1})	6	166±7	168±6	160±7	153±8	154±8	156±8	164±7	163±8
Rilmenidine (25µg.kg^{-1})	8	169±4	173±4	155±5*	158±5*	154±5*	156±4*	164±4	165±5
NPY + Rilmenidine	9	177±5	179±4	164±3*	162±4*	162±2*	164±2*	169±5	172±5

Values are mean ± SEM. * P<0.05. Significant difference from saline control group using ANOVA with Bonferroni correction.

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This work was supported by the British Heart Foundation.

249P ELECTROPHYSIOLOGICAL EVIDENCE FOR SELECTIVE 5-HT RECEPTOR ANTAGONISM BY RP 62203

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The naphtosultam derivative, RP 62203, binds potently to the rat brain 5-HT_2 receptor with a K_i=0.26 nM while showing 300 fold lower affinity for 5-HT_{1A} receptors and no detectable binding to 5-HT_3 receptors (Doble et al., 1990). In addition, in behavioural models, RP 62203 reduces DOI-induced head twitches (Piot et al., 1990). In the present study we chose to further investigate the effects of RP 62203 in the raphe nuclei, which form the main serotonergic cell group in the mammalian brain and for which the effects of 5-HT have been extensively reported.

Single unit extracellular recordings of raphe neuronal firing were carried out in chloral hydrate anaesthetized rats. Neurones showing an increase in firing rate on microiontophoretic application of 5-HT were used to study the effects of RP 62203 and two other recently developed 5-HT_2 antagonists, ritanserin and ICI 169,369.

RP 62203 potently depressed 5HT-evoked excitations at a dose of 0.5 $mg.kg^{-1}$ i.p. with an onset of action of approximately 10-20 minutes and with no recovery from the effects within the 2 hour recording period. When glutamate was cycled with 5-HT, no effect of RP 62203 on the glutamate-evoked firing accompanied the block of 5-HT-evoked firing at a cumulative dose of 4.0 $mg.kg^{-1}$ i.p.. In contrast, at doses as high as 8.0 $mg.kg^{-1}$ i.p., RP 62203 did not block 5-HT-evoked depressions of neuronal firing (attributed to a 5-HT_{1A} receptor). Ritanserin showed comparable activity, depressing 5-HT excitations at a dose of 1.0 $mg.kg^{-1}$ i.p. but higher doses of ICI 169,369 (4.0 $mg.kg^{-1}$ i.p.) were required to obtain an equivalent effect.

These results provide further evidence of the potency of RP 62203 as a 5-HT_2 antagonist. In addition, its selectivity for the 5-HT_2 receptor suggests that it could be a useful tool for investigating the relative contribution of 5-HT receptor subtypes to neuronal transmission at specific synapses.

ICI 169,369 was kindly supplied by I.C.I. Pharmaceuticals.

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250P EVIDENCE THAT THE 5-HT_2 RECEPTOR AGONIST(±)DOI HAS MEMBRANE STABILISING PROPERTIES

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(±)2,5-Dimethoxy-4-iodophenylisopropylamine (DOI) is reported to be a selective agonist at 5-HT2 receptors (McKenna et al., 1989). Recently, Garrett et al. (1990) claimed that DOI inhibited raphe 5-HT neurone firing via an atypical 5-HT2 receptor. However, these authors failed to consider whether DOI had membrane stabilising properties. We have, therefore, examined the local anaesthetic activity of DOI in comparison with established membrane stabilising drugs.

Compound action potentials were recorded from isolated frog sciatic nerve trunks which were placed in a perspex chamber resting on electrodes for stimulating and recording action potentials. The desheathed part of the nerve rested on a perspex block onto which, drops of frog Ringer solution (pH 7), containing drugs where appropriate, were added. During stimulation of the nerve by maximal (0.3-0.5 V) rectangular pulses of 0.1 ms duration, monophasic action potentials were amplified and recorded on a digital storage oscilloscope.

Following a 30 min stabilisation period, cumulative addition of DOI (15 μM to 4 mM) resulted in a concentration-dependent reduction in action potential amplitude (EC50 = 1.2 mM, 95% confidence limits 0.8-1.5 mM). Similarly, action potential amplitude was decreased by lignocaine (EC50 = 0.5 mM, 95% confidence limits 0.2-0.7 mM), (±)propranolol (EC50 = 0.9 mM, 95% confidence limits 0.4-1.3 mM) and disopyramide (EC50 = 4.3 mM, 95 % confidence limits 1.9-6.6 mM). The effect of DOI on action potential amplitude was not affected by the presence of ketanserin (1 μM), a 5-HT2 receptor antagonist (DOI EC50 = 1.4 mM in the presence of ketanserin, 95% confidence limits 0.6-2.3 mM).

These results demonstrate that DOI decreases action potential amplitude in the frog sciatic nerve by a non-5HT2 receptor mechanism. The potency of DOI was similar to that of lignocaine, propranolol and greater than that of disopyramide, well known membrane stabilising drugs. DOI conforms to the general structural requirements for local anaesthetic type activity (i.e. lipophilic centre linked via an intermediate chain to a hydrophilic centre; Strother et al., 1977). Therefore it is probable that some of the previously reported effects of DOI on neuronal firing (e.g. Garrett et al., 1990) were due to membrane stabilising, rather than agonist activity at an atypical 5-HT2 receptor.

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251P DIFFERENT ANAESTHETICS DO NOT ALTER THE EFFECTS OF ELECTROCONVULSIVE SHOCK ON 5-HT_2 RECEPTORS IN THE RAT BRAIN

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It is well established that repeated electroconvulsive shock (ECS) increases rat cortical 5-HT2 receptor number (inter al. Green et al., 1983). In these studies, ECS has either been given to conscious or halothane anaesthetised animals. However, Nicoll and Madison (1982) have shown that neuronal activity is altered not only by anaesthesia, but also the type employed. We have, therefore, determined the effects of repeated ECS on 5-HT2 receptor number in frontal cortex and hippocampus using a variety of anaesthetic procedures.

Male Wistar rats (100-125g, Charles River) were given either a single ECS (200V, 2s) or 5 ECS over 10 days under halothane, chlorhexitone (45mg/kg i.p.) or chloral hydrate (600mg/kg p.o.). Control rats received the appropriate anaesthetic, but no shocks. Frontal cortices and hippocampi were removed 24h after the last treatment. 5-HT2 binding parameters were determined using [3H]ketanserin (0.1-5nM), with specific binding defined by 5μM methysergide (Leysen et al., 1982).

The single ECS treatments had no effect on 5-HT2 binding parameters (data not shown). However, the number (Bmax) of 5-HT2 sites in the frontal cortex and hippocampus were significantly increased by 11-13% and 30-46% respectively following repeated ECS, regardless of the anaesthetic employed (Table 1). Kd values were not altered by any treatment.

Table 1 The effects of repeated ECS on 5-HT2 receptor number in rat frontal cortex and hippocampus

ANAESTHETIC	FRONTAL CORTEX		HIPPOCAMPUS	
	CONTROL	ECS	CONTROL	ECS
Halothane	396±11 (18)	443±18 (17)*	71±9 (10)	104±8 (10)*
Chlorhexitone	396±9 (17)	439±12 (17)**	77±6 (6)	99±8 (6)*
Chloral Hydrate	342±12 (17)	386±14 (12)*	91±7 (8)	120±9 (4)*

Mean [3H]ketanserin specifically bound (fmol/mg protein) ± s.e. mean (n). *P<0.05, **P<0.01 vs control Student's t-test.

In conclusion, these data confirm that cortical 5-HT2 receptor number is increased by repeated ECS and further show that this effect is independent of the anaesthetic co-administered. The results further indicate that the therapeutic effects of ECS may also involve hippocampal 5-HT2 receptors.

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252P DISTRIBUTION OF [^{3}H]-(s)-ZACOPRIDE BINDING SITES IN POST-MORTEM SAMPLES OF HUMAN BRAINSTEM

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Previous studies have identified 5-HT_3 (5-hydroxytryptamine$_3$) receptor recognition sites in the brainstem of various species including man (for review see Pratt et al., 1990). In the present studies we describe the topographical distribution of 5-HT_3 receptor recognition sites in the human brainstem using the 5-HT_3 receptor radioligand [^{3}H]-(S)-zacopride (Barnes et al., 1990).

Human brainstems were obtained at autopsy (1 male, 2 female, 65-75 years old) from patients who had died within 48 hrs from a non-neurological disorder. 20 μm sections were cut using a cryotome and thaw-mounted onto gelatin coated slides. For the [^{3}H]-(S)-zacopride binding assay, slide mounted brainstem sections were pre-incubated in Hepes/Krebs buffer (Hepes 50.0; NaCl 118.0; KCl 4.75; KH_2PO_4 1.2; $MgSO_4$ 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 25.0; glucose 11.0 mM; final pH 7.4) for 30 min at 37°C before being incubated in Hepes/Krebs buffer containing [^{3}H]-(S)-zacopride (1.0 nM) in the absence (total binding) or presence (non-specific binding) of granisetron (1.0 μM) for 30 min at 37°C. The sections were washed (ice-cold Hepes/Krebs 2 x 30 sec, ice-cold distilled water 1 sec) before being dried and apposed to tritium sensitive film for 6 weeks along with tritium standards (tissue equivalent values for intact grey matter, fmol mg^{-1}, Amersham). After being developed the autoradiographs were quantitated using densitometry.

Specific [^{3}H]-(S)-zacopride binding (defined by granisetron, 1.0 μM) was located within the dorsal vagal complex (dorso-medial nucleus tractus solitarius, 322.8±10.6; remainder of nucleus tractus solitarius, 78.7±11.7; area postrema, 123.1±21; dorsal motor nucleus of the vagus nerve, 43.8±9.2; fmol mg^{-1}, mean ±S.E.M., n = 3) and spinal trigeminal nerve complex (66.5±10.5 fmol mg^{-1}, mean ±S.E.M., n = 3), whilst no other specific binding was detected in the remainder of the brainstem. Non-specific binding was homogenously distributed, being only slightly higher than background levels.

The present studies confirm the identification of 5-HT_3 receptor recognition sites within the dorsal vagal complex of the human brainstem; these sites may provide the anti-emetic site of action for 5-HT_3 receptor antagonists.

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253P ANXIOLYTIC-LIKE ACTIVITY OF THE SELECTIVE 5-HT$_3$ RECEPTOR ANTAGONIST, BRL 46470A, IN THE RAT ELEVATED X-MAZE TEST

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The 5-HT$_3$ receptor antagonists as a class exhibit an impressive profile of anxiolytic activity in a range of rodent (mouse light/dark box, rat social interaction) and primate (human threat test in marmoset) models (Costall et al, 1988). The present study investigated the effects of the potent and selective 5-HT$_3$ receptor antagonist, BRL 46470A (Thomas et al, 1990), in the rat elevated X-maze test of anxiety.

The X-maze test was based on that of Handley & Mithani (1984). Groups of male Wistar CFY rats (250-300g, n=11-12) were treated orally with either vehicle (1% methyl cellulose in water), BRL 46470A (0.0001-0.1 mg/kg) or diazepam (1.25mg/kg). 30 minutes later the rats were placed on the X-maze and the percentage of open/total entries and time, and actual entries and time on the end (i.e. outer half) of the open arm (EOOA) assessed over a 10 minute observation period. Statistical comparisons were made using Kruskal Wallis followed by Mann Whitney U test (2-tailed: *P<0.05, **P<0.01).

The results obtained (expressed as median values) are shown in the table below.

Treatment	Dose (mg/kg)	% open/total Entries	% open/total Time	EOOA Entries	EOOA Time(s)	Total Entries
Vehicle	-	23	7	2	12	19
BRL 46470A	0.0001	33	12*	3.5*	26.5**	18.5
	0.001	31.5	13*	4	37.5**	18.5
	0.01	29*	13**	4*	30**	21
	0.1	32**	11*	4	28**	20
Diazepam	1.25	33.5**	14**	6**	40**	23

At all doses tested (0.0001-0.1mg/kg po) BRL 46470A selectively enhanced open arm exploration thereby indicating potential anxiolytic activity. This effect was most pronounced in terms of time spent on the EOOA where significant (P<0.01) 121-213% increases above control values were observed. There was no indication of a decline in efficacy of BRL 46470A at the highest dose (0.1mg/kg po) tested. The magnitude of the anxiolytic activity produced by BRL 46470A was comparable to that of diazepam (1.25mg/kg po).

The present findings accord with a previous report of anxiolytic activity of 5-HT$_3$ receptor antagonists in the X-maze test (Costall et al, 1989). This and other studies in the rat social interaction test (Kennett & Blackburn, 1990) clearly illustrate that BRL 46470A has anxiolytic efficacy over a wide dose range.

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254P EFFECTS OF SOCIAL ENVIRONMENT ON THE BEHAVIOURAL RESPONSES BY MICE TO THE 5-HT$_3$ RECEPTOR ANTAGONIST, GRANISETRON (BRL 43694)

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The 5-HT$_3$ receptor antagonists have wide ranging psychopharmacological actions, which include anxiolytic potential in some though not all of the preclinical tests (Costall et al., 1989; File & Johnston, 1989; Tricklebank, 1989). This suggests that 5-HT$_3$ receptors in the central nervous system may play an important role in the control of behaviour. The present experiments examine effects of subchronic administration of the 5-HT$_3$ receptor antagonist, granisetron (BRL 43694), on the behavioural responses shown by mice to different social circumstances.

Granisetron was given in drinking fluid at 40 ug/l (10 ug/kg body weight daily) for 7-10 days to adult male and female CD1 mice. Control mice received tap water. Effects of the treatments on behaviour were examined in three different test situations. Behaviour shown by each of the mice during encounters for a 7 min period with an unfamiliar partner was recorded by ethological procedures, using the check list of acts and postures described by Dixon (1986).

In resident males that had been pair-housed, treatment with granisetron significantly increased the duration (s., mean $\pm$ S.E.) of the act "attend" (Control 12.4 $\pm$ 1.8, Treated 18.9 $\pm$ 2.2, $P < 0.05$) and of social investigation involving contact (Control 41.3 $\pm$ 6.7, Treated 61.3 $\pm$ 8.5, $P<0.05$), but granisetron had no significant effect at this dose level on behaviour in group-housed mice acting as intruders in the resident-intruder paradigm. The effects of granisetron in group-housed female mice encountering male partners were to significantly reduce the duration of flight (Control 31.4 $\pm$ 10.5, Treated 14.3 $\pm$ 4.8, $P < 0.05$) and increase social investigation with contact (Control 56.3 + 4.8, Treated 82.2 + 10.0, $P < 0.05$).

It is suggested that the reduction of flight in female mice and increase of social investigation in mice of both sexes after sub-chronic administration of granisetron may arise from its anxiolytic actions. These actions are more evident in some than in other social circumstances, and the paradigm in which female mice are exposed to an approach-avoidance conflict by the presence of an unfamiliar male may be particularly sensitive as a test for anxiolytic effects.

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255P EFFECTS OF THE 5-HT_3 ANTAGONIST ICS 205-930 ON BENZODIAZEPINE WITHDRAWAL AND BODYWEIGHT IN RATS

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We have previously reported that the 5-HT_3 antagonist, ondansetron, alleviates benzodiazepine (BZ) withdrawal in rats, when withdrawal is assessed with weight loss and food intake indices (Leathley et al., 1990). To assess possible effects of the 5-HT_3 antagonist ICS 205-930 on BZ withdrawal, ten groups of female rats (n = 10/group) were treated b.i.d. (1100, 1700 hrs) for 21 days with either chlordiazepoxide (C) or saline (S) at 2 ml/kg. The dose of chlordiazepoxide was escalated during 16 days of chronic treatment by 2 mg/kg/day from 10 to 40 mg/kg, followed by 5 days of treatment with 40 mg/kg. The study was run in two phases, an initial chronic treatment phase was followed by a withdrawal phase. During the seven days of "withdrawal assessment" rats were treated b.i.d. with either saline, chlordiazepoxide or ICS 205-930 (at 1.0, 0.1, 0.01, or 0.001 mg/kg). Groups are coded in terms of their initial and "withdrawal" treatments (e.g. group C-S was treated with chlordiazepoxide followed by saline during withdrawal). In groups pretreated with chlordiazepoxide, bodyweight fell during withdrawal by a maximum, recorded around 60-80 hrs into withdrawal, of 6.0% (C-S), 5.0% (C-I 1.0), 6.0% (C-I 0.1), 5.3% (C-I 0.01) and 4.9% (C-I 0.001). Weight loss in group C-S differed significantly from S-S controls ($p < 0.05$, Tukey test after ANOVA), whereas weight loss in all four ICS 205-930 treated groups did not differ from weight loss in group C-S. Thus ICS 205-930 did not alleviate the effect of BZ withdrawal on bodyweight. The effects of ICS 205-930 on 24 hr food intake showed a similar pattern to the bodyweight data (i.e. withdrawal-induced anorexia which was not alleviated by ICS 205-930). In saline pre-treated groups bodyweights continued to rise during the "withdrawal" phase of the study. By the end of the study bodyweights during "withdrawal" had risen by 3.0% (S-S control group), 1.4% (S-I 1.0), 2.5% (S-I 0.1), 3.4% (S-I 0.01) and 3.0% (S-I 0.001). At none of the doses of ICS 205-930 was the rise in bodyweight of saline pretreated animals significantly different from controls ($p > 0.05$, Tukey test after ANOVA). Thus ICS 205-930 did not itself cause weight gain. Ondansetron has also been found not to stimulate growth (Leathley et al., 1990).

The 5-HT_3 antagonist ICS 205-930 did not alleviate BZ withdrawal signs in the same paradigm in which ondansetron was effective. The negative findings with ICS 205-930 may be attributable to either:- (i) The use of an "ineffective" dosing regime (incorrect doses, too low a frequency of drug administration etc.); or (ii) A difference between the actions of ondansetron and ICS 205-930 in this paradigm, related perhaps to antagonist actions that ICS 205-930 has at the $5HT_4$ receptor (Dumuis et al. 1988).

Acknowledgement: Supported by a grant from The Nuffield Foundation.

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256P THE ANXIOLYTIC-LIKE ACTIONS OF THE SELECTIVE CCK-B RECEPTOR ANTAGONIST CI-988

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The neuropeptide cholecystokinin (CCK) has recently been implicated to play a role in anxiety (Hughes et al., 1990). However, the receptor type involved in mediating the action of CCK remains unclear. In the present report, we have examined the effect of a highly selective CCK-B receptor antagonist PD 134308 (CI-988), in three experimental models of anxiety: the mouse light/dark (L/D) box, the rat social interaction (SI) test and the rat elevated X-maze.

Male To mice (25-30g; Bantin and Kingman, U.K.) and Hooded Lister rats (250-300g; Olac, U.K.) were used. The effect of CI-988 in the three models was examined during the 5 min observation period. When administered i.p. 40 min before test CI-988, dose-dependently increased (1) the time spent on the illuminated side of the L/D box, (2) increased the duration of active SI between pairs of rats under high light/unfamiliar conditions and (3) increased the time spent on the end halves of open arms of the X-maze (Table 1). However, CI-988 up to 30mg/kg had no effect on locomotor activity in the rat.

Table 1. The anxiolytic-like actions of CI-988.

			CI-988 (mg/kg, i.p.)			
Test	0	0.01	0.1	1.0	10.0	CDP
L/D box	81±7	114±15	133±15*	130±10*	166±22*	152±16*
SI	36±2	52±3*	57±2*	62±2*		69±6*
X-maze	9±3	21±3	31±4*	30±6*	27±4*	34±4*

Results are the mean time ± SEM of at least 10 animals. CDP 5mg/kg, i.p. (3mg/kg in the SI test). *$P<0.05$ ANOVA followed by Dunnett's t-test.

In conclusion, CI-988 shows potent activity in three animal models predicitive of anxiolytic activity. In these models, it is as effective as CDP. However, unlike CDP it is devoid of sedative properties. Thus, CI-988 is a promising candidate for the treatment of anxiety in man.

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257P NEUROCHEMICAL CONSEQUENCES OF RAT SOCIAL INTERACTION

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Previous studies have associated the neurotransmitter 5-hydroxytryptamine (5-HT) with the expression of anxiety in pre-clinical models (see Iversen, 1984). In the present studies we assess the effects of an 'anxiogenic' environment (social interaction test using unfamiliar rats under conditions of high illumination) on indoleamine and catecholamine metabolism in various brain nuclei of the rat.

Two groups (5 pairs/group) of male hooded-Lister rats (250-300g, Bradford bred) were assessed for social interaction as has been described in detail previously (Costall et al., 1990). One group of rats was allowed to habituate to the social interaction test apparatus (under low light conditions) and their partner on 3 consecutive days (10 min/day, familiar, low light, FL), whilst the other group was assessed for social interaction only on the third day under aversive high light conditions (unfamiliar, high light, UH). Immediately following the social interaction test animals were killed and various brain nuclei analysed for indoleamine and catecholamine neurochemicals by HPLC-ECD.

Pairs of familiar rats performed more social interaction (under low light conditions) than unfamiliar pairs under high light conditions (FL 81±5, UH 47±5 sec/10 min in social interaction, mean ±S.E.M., 5 pairs, P<0.01 Student's t test). Furthermore the UH social interaction test induced changes in the levels of indoleamine and/or catecholamine neurochemicals in various regions of the brain (Table 1).

Table 1. Effect of the social interaction on neurochemical levels of indoleamine and catecholamine.

Area/test		5-HT	5-HIAA	DA	DOPAC	NA
Nucleus accumbens	FL	707±24	349±12	6226±268	764±13	381±95
	UH	725±34	457±32**	6377±273	920±50**	431±83
Entorhinal cortex	FL	596±26	116±6	53±4	ND	303±10
	UH	580±21	146±4**	54±3	ND	278±12
Amygdala	FL	796±23	253±10	379±27	40±4	516±15
	UH	747±25	356±20***	711±77**	92±11***	454±17*

pg mg^{-1} wet weight, mean ±S.E.M., n = 8-10. *P<0.05, **P<0.01, ***P<0.001 (Student's t test) (5-HIAA, 5-hydroxyindoleacetic acid; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NA, noradrenaline). ND, levels below the limit of detection.

Thus there are neurochemical differences between the brains of animals tested in the social interaction test under aversive and non-aversive conditions. The disturbances in the biochemistry of indoleamines and catecholamines in an aversive situation would further link these neurotransmitters with anxiety.

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258P HETEROGENEOUS PROFILES OF PROTEIN KINASE C ACTIVATION

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Depolarisation-induced calcium influx in anterior pituitary prisms and in GH_3 cells are both mediated by a nimodipine-sensitive process and are modulated in two ways by activation of protein kinase C (PKC) (Johnson *et al.*, 1989). Phorbol 12,13-dibutyrate (PDBu) activation of PKC leads to an increase in K^+-induced $^{45}Ca^{2+}$ influx in anterior pituitary pieces, but a decrease in GH_3 cells. These actions are also seen with 4ß-phorbol 12,13-didecanoate (4ß-PDD) but not 4α-PDD. The two opposing profiles may represent the actions of different forms of PKC on 'L'-channels in the two preparations. A number of diterpenes have been described which differentially display certain aspects of phorbol-like actions, for example, inflammation/tumour promotion/ornithine decarboxylase induction (Dunn & Blumberg, 1983). The present experiments investigate the actions of a range of such analogues in the Ca^{2+} influx models.

$^{45}Ca^{2+}$ influx measurements into anterior pituitary prisms and GH_3 cells were as previously described (MacEwan and Mitchell, 1990). In pituitary prisms, the marked facilitation of influx caused by (10-1000nM) PDBu and 4ß-PDD (~+180% at 1000nM) was mimicked, but to a lesser degree, by 1,2-dioctanoyl-sn-glycerol (1-100μM) (DOG), mezerein, phorbol 12-retinoate 13-acetate (PRA), phorbol 12-myristate 13-acetate 4-0-methyl ether (MPMA) and to a small degree by 12-deoxyphorbol 13-isobutyrate (DPB) (all at 10-1000nM). In GH_3 cells, PDBu and 4ß-PDD reduced K^+-induced $^{45}Ca^{2+}$ influx by up to 50% and a similar effect, of greater magnitude, was displayed by arachidonic acid (AA) (MacEwan and Mitchell, 1990). In contrast, DOG, PRA, MPMA and DPB were inactive. Surprisingly, the effect of mezerein in GH_3 cells was a facilitation of K^+-induced $^{45}Ca^{2+}$ influx, apparently identical to that in pituitary prisms. The effects of diterpenes were not altered by differing times of pre-incubation nor by VO_4^{3-} (1mM) or substitution of Na^+ by N-methyl-d-glucamine. The results are consistent with the idea that at least two forms of PKC can exert qualitatively different influences over 'L'-type Ca^{2+} channel function. The two complex profiles could be explained if different relative amounts of two distinct forms of PKC were involved in the two models. Whilst PDBu and 4ß-PDD were non-selective activators, mezerein and AA may be selective activators of the PKC forms that facilitate or attenuate $^{45}Ca^{2+}$ influx respectively. The other analogues may show partial selectively for the facilitatory effect. Although other explanations of these profiles may well be possible, selective antagonism by H7, but not staurosporine, of the effect of PDBu in GH_3 cells but not in pituitary prisms (Johnson *et.al.*, 1989) supports our hypothesis.

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259P CHRONIC EXPOSURE OF NG108-15 CELLS TO AGENTS THAT INHIBIT ADENYLATE CYCLASE INCREASES THE SENSITIVITY OF $G_i\alpha$ TO FLUORIDE AND Gpp(NH)p

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Chronic exposure of NG108-15 hybrid cells to opiate or cholinergic agonists (both of which inhibit adenylate cyclase) results in increased basal and agonist-stimulated adenylate cyclase activity. Previous studies with these cells have lead to the suggestion that this effect of inhibitory agonists may contribute to opiate addiction. In the present study, we have measured the abundance and activities of Gsα and Giα to examine further the mechanism(s) that underlie this altered responsiveness of these cells.

NG108-15 cells were exposed for 24 h to 1μM DADLE ([D-Ala^2,D-Leu^2]-enkephalin or 1 μM carbachol or both. Adenylate cyclase activity was measured in cell homogenates (Kelly et al. 1990). The abundance of Giα was determined by ADP-ribosylation with pertussis toxin, and the abundance of Gsα by immunoblots with anti-Gsα antibody (Milligan and Unson, 1989).

Results showed that the abundance of Gsα was unaltered by pre-treatment of cells with both DADLE and carbachol. Furthermore, NaF-dependent activation of adenylate cyclase was unchanged. In contrast, the capacity of NaF or Gpp(NH)p to inhibit iloprost-stimulated adenylate cyclase activity was increased significantly ($p < 0.01$) following pre-treatment with DADLE, carbachol or both. In addition, these agents mediated homologous desensitization of their individual responses. ADP-ribosylation of membrane protein (Giα and Goα) were unaltered by these pre-treatment. We considered the possibility that the effects of DADLE and carbachol might be mediated by activation of protein kinase C in these cells. However, addition of staurosporine during the pre-treatment of cells was not accompanied by a reduction in the elevated levels of iloprost-stimulated or basal adenylate cyclase activity.

In conclusion, pre-treatment of NG108-15 cells results in increased basal and iloprost-stimulated adenylate cyclase activity, without altered sensitivity to NaF. In addition, the abundance of Gsα remains constant. In contrast, this treatment of the cells results in homologous desensitization of agonist-mediated inhibition of adenylate cyclase, and an increased capacity of both NaF and Gpp(NH)p to inhibit the enzyme. Total ADP-ribosylation of membrane proteins is unaltered. There is clearly a striking similarity between the effects of exposure of these cells to pertussis toxin (Kelly et al. 1990) and the effects of prolonged exposure of the cells to inhibitory agonists.

This work was supported by a grant from the Wellcome Trust.

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260P THE EFFECTS OF ASCORBIC ACID AND CAPTOPRIL ON MPTP NEUROTOXICITY IN RAT FOETAL NEURONAL CELL CULTURE

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One of the proposed mechanisms of action of 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP) neurotoxicity involves the generation of free radicals and antioxidants have been shown to afford partial protection against MPTP-induced neurotoxicity in mice (Perry et al., 1985; Yong et al., 1986). In the present study we investigate the ability of ascorbic acid and captopril to modify the neurotoxic effect of MPTP using a rat neuronal cell culture.

Rat foetuses (crown to rump length = 13-14 mm) were taken at 15 days gestation and the brain area from the mesencephalon containing the substantia nigra was dissected out in cold phosphate buffer solution using aseptic techniques. 0.15 ml of a dissociated cell suspension in serum supplement medium was plated in each 96 Corning cell well and incubated at 37°C, 100% humidity , 5% CO_2 for 18-20 h. The medium was then replaced with serum free medium containing insulin 5 μg/ml, progesterone 20 nM, putrescine 100 μM, selenium 30 nM, transferrin 100 μg/ml, L-glutamine 2 mM, penicillin 100 units/ml and streptomycin 100 μg/ml in equal parts of Ham F12 solution and Dulbecco's Modification of Eagle medium and incubated for 48 h. MPTP and ascorbic acid or captopril were added to the feeding medium and incubated for a further 24 h. The live and dead cells were identified using ethidium bromide/acridine orange staining and fluorescent microscopy (table 1).

Table 1. The effects of ascorbic acid and captopril to modify the neurotoxicity of MPTP in rat foetal neuronal cell culture.

Treatment	% Live Cells	Treatment	% Live Cells
Control	86.8±2.3	Control	87.7±1.9
MPTP 500 μM	56.7±3.8*	MPTP 500 μM	53.0±3.7*
MPTP 500 μM plus		MPTP 500 μM plus	
Ascorbic acid 1.0 μM	57.7±2.9*	Captopril 0.1 μM	55.0±2.8*
10 μM	52.3±3.3*	1.0 μM	54.6±3.8*
100 μM	48.8±3.2*	10 μM	50.3±3.9*

Each value is the mean ± S.E.M. of 4 determinations. Significant reductions compared to the respective control values are indicated as * $P<0.01$ (One-way Anova followed by Dunnett's t-test).

Ascorbic acid and captopril, an angiotensin converting enzyme inhibitor which has known antioxidant properties (Bagchi et al., 1989), failed to protect the mesencephalic neuronal culture against MPTP-induced neurotoxicity. Therefore, in the present study, no evidence was obtained to support the hypothesis that antioxidants can prevent the toxic action of MPTP in cell culture.

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261P APPARENT ANXIOLYTIC ACTIVITY OF THE CORTICOSTERONE SYNTHESIS INHIBITOR, METYRAPONE

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Pellow et al. (1985) showed that rats confined to the open arms of an elevated X-maze (EXM) had significantly higher plasma corticosterone levels compared to rats restricted to the closed arms and home-cage controls. We now report on two experiments in which we have investigated the ability of metyrapone, a corticosteroid synthesis inhibitor (De Nicola & Dahl, 1971), to modify the behaviour of animals placed on the EXM.

The EXM was based on that of Handley & Mithani, 1984. Groups of male Wistar CFY rats (250-300g; n=11-12) were administered either vehicle (1% methyl cellulose in water), chlordiazepoxide (CDP, 5mg/kg) or metyrapone (25-150 mg/kg), intraperitoneally, 60 minutes before placement on the EXM. The animals were observed for 3 minutes and the percentage of time spent on and entries into the open arms were calculated. Statistical comparisons were made using Kruskal-Wallis followed by Mann-Whitney U test (2 tailed; *P<0.05; **P<0.01). Results are expressed as median values.

	Experiment 1			Experiment 2		
		% open/total			% open/total	
	Dose (mg/kg)	Entries	Time	Dose (mg/kg)	Entries	Time
Vehicle	-	29.5	17.5	-	31.5	17.5
CDP	5	46.0**	46.0**	5	48.5**	43.5**
Metyrapone	25	33.0	18.5	75	47.5*	29.5*
Metyrapone	100	40.5	28.0*	150	44.5*	29.5*

Metyrapone (75,100 and 150 mg/kg) produced a significant (P<0.05) increase in the percentage of time spent on the open arm of the EXM; however, a significant increase (P<0.05) in the percentage of open arm entries was only observed at 75 and 150 mg/kg. Metyrapone had no effect on the total number of entries at any dose tested. The increase in open arm exploration induced by metyrapone was less than that of CDP (5mg/kg).

These results demonstrate that metyrapone has an apparent anxiolytic action; whether this effect can be ascribed to an inhibition of corticosterone synthesis is yet to be established.

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262P VISUALIZATION OF G PROTEIN mRNAs AND SECOND MESSENGER ENZYMES BY COMPARATIVE *IN SITU* HYBRIDIZATION AND LIGAND BINDING IN POST-MORTEM SAMPLES OF HUMAN HIPPOCAMPUS

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Manipulation of second messenger enzymes may represent a valuable target for pharmacological manipulation (Worley *et al*, 1981). Relatively little is known of the distribution of such systems in the human. Using quantitative autoradiography and *in situ* hybridisation, we have visualised and localised second messenger enzymes and G protein mRNA signals in medial temporal lobe from six control human post-mortem samples. For autoradiography, fresh frozen cryosections were incubated under appropriate conditions of temperature and pH with 100 nM ^{3}H forskolin (activated adenylate cyclase), 2.5 nM ^{3}H phorbol-12,13-dibutyrate (protein kinase C) and 5 nM ^{3}H Gpp(NH)p (GTP binding protein). For *in situ* hybridisation, ^{35}S-labelled oligonucleotide probes complementary to G proteins sα and oα were incubated with pretreated cryosections (Harrison & Pearson, 1990). In all cases sections were developed against hyperfilm. Forskolin binding showed a somewhat homogeneous distribution but with higher levels in dentate gyrus and CA_1. Protein kinase C activity was localised primarily to CA_1/CA_2 and GTP binding to CA_3. Gsα mRNA was concentrated over dentate and CA_1, whereas Goα mRNA was concentrated over dentate and CA_3.

These studies confirm the usefulness of autoradiography and *in situ* hybridisation in post-mortem studies and localise second messenger enzymes and RNA message in sites of putative pathological interest (e.g. in Alzheimer's Disease and schizophrenia).

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263P THE STABILITY OF G-PROTEIN mRNA IN POST-MORTEM SAMPLES OF HUMAN BRAIN OF NORMAL SUBJECTS AND PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE

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The measurement of messenger RNA (mRNA) levels is being utilised increasingly in the investigation of various diseases of the human central nervous system most notably using in situ hybridization. This necessitates the use of postmortem tissue and the stability of the mRNA is a major concern.

We have analysed RNA purified from the frontal cortex of six individuals with a neuropathologically-confirmed diagnosis of dementia of the Alzheimer type (DAT) and from six individuals with no neuropathological abnormality. The interval from death to freezing ranged from 2 to 24 hours (9.3 ± 6.3 hours mean ± SD). RNA was purified using the acid guanidinium phenol method (1). Rat brain was prepared in parallel to the human samples and extracts processed on all electrophoresis gels to act as an internal control. Northern blotting, utilising oligonucleotide probes, was then used to measure the levels of mRNA encoding the G-protein subunits, $G\alpha_S$, $G\beta_1$ and $G_{\beta 2}$. Each blot was reprobed to show 28S ribosomal RNA (rRNA) to assess RNA degradation.

All control and DAT RNA samples displayed degradation as assessed by band smearing of the G-protein mRNAs. No band smearing was apparent in any rat brain sample. The amounts of mRNA for both G-protein species as assessed using densitometry, showed great variability between individuals even in the control group, the coefficients of variation (SD % of mean) ranging from 83% ($G_{\beta 2}$) to 157% ($G\alpha_S$). Subsequent reprobing with an oligonucleotide complimentary to 28S rRNA showed a number of major, clearly definable degradation products in the human RNA which were never present in the rat brain control samples. The extent of 28S rRNA degradation was inversely related to the quantity of G-protein mRNA present.

In experiments in rat tissue, we assessed the extent to which postmortem freezing delay contributes to degradation of G-protein mRNA. Rats were sacrificed and the tissue maintained at 4°C for up to 72 hours before the brain was homogenised and the RNA extracted and purified. Minimal degradation of G-protein mRNA was observed even at 72 hours postmortem although there was a time-dependent increase in 28S rRNA degradation products.

These data emphasise the need for rigorous assessment of mRNA degradation when this material is being studied in the human CNS at postmortem.

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264P AGONIST-INDUCED STIMULATION OF $[Ca^{2}]_I$ IN NIE-115 CELLS: USE OF MANGANESE TO QUENCH EXTRACELLULAR FURA-2

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Histamine and bradykinin have been shown to cause a transient increase in $[Ca^{2+}]_i$, in populations of undifferentiated N1E-115 cells (Iredale et al., 1990). However, measurement of $[Ca^{2+}]_i$ in this cell line is complicated by a significant extracellular leakage of fura-2. One possible way to overcome this involves the use of Mn^{2+} to quench the fluorescence of fura-2 extracellularly. Mn^{2+} binds to fura-2 more avidly than Ca^{2+} and under resting conditions N1E-115 cells are relatively impermeable to Mn^{2+}. However, experiments in other cells have shown that Ca^{2+} and Mn^{2+} entry can be stimulated by activation of a number of calcium-mobilising receptors (Hallam et al., 1988). In this communication we report that a number of calcium-mobilising agonists do not stimulate Mn^{2+} entry into N1E-115 cells and that the presence of extracellular Mn^{2+} can markedly improve calibration of the agonist $[Ca^{2+}]_i$ signal.

Measurement of agonist-stimulated $[Ca^{2+}]_i$ in undifferentiated N1E-115 cells was as described previously (Iredale et al., 1990). Inclusion of Mn^{2+} ions (100μM) in normal Ca^{2+}-containing medium produced a relatively stable base-line signal (120 ± 25nM, n=10), which contrasted with the elevated, gradually increasing baseline signal (203 ± 57nM, n=31) obtained in the absence of Mn^{2+}. To monitor the effects of different agonists on Mn^{2+} influx, measurements were also made in nominally calcium-free medium containing Mn^{2+} (100μM)at an excitation wavelength of 360nm (which results in an emission signal independent of Ca^{2+} but quenched by Mn^{2+}). Ionomycin (20μM) caused a rapid fall in the 360nm signal indicating an influx of Mn^{2+} (n=3). However addition of histamine (300μM), bradykinin (100nM) and ATP (1mM) all showed transient changes in the 340nm signal (indicating a rise in $[Ca^{2+}]_i$) but had no significant effect on the 360nm signal (n=3). These results suggest that inclusion of Mn^{2+} (100μM) in the extracellular medium may be useful in the study of agonist-induced increases in $[Ca^{2+}]_i$ in N1E-115 cells.

PAI holds a SERC/CASE studentship with Boots Pharmaceuticals.

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265P IMMUNOCYTOCHEMISTRY OF C-FOS PROTEIN INDUCED BY CISPLATIN: FUNCTIONAL MAPPING OF BRAINSTEM EMETIC PATHWAYS

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The immediate-early gene c-*fos* can be expressed in neurones as a result of neurotransmitter-receptor interaction. It has been suggested that immunochemical detection of Fos protein produced by c-*fos* gene activation may be used to identify metabolically active pathways in the brain. We have used Fos protein immunochemistry to investigate the pathways involved in emesis induced by the cytotoxic drug cisplatin. In addition, we have examined the effect of the 5-HT_3 receptor antagonist granisetron (BRL 43694) and the effect of unilateral vagus nerve section on cisplatin-induced Fos protein production in the ferret brainstem.

17 adult ferrets were used, 4 of which had unilateral cervical vagus nerve lesions 9 days before the study. At time 0 mins animals received either saline or granisetron (5mg/kg) s.c. followed 30mins later by saline or cisplatin (10mg/kg) i.p. At 225 mins animals received an overdose of pentobarbitone and were perfused with fixative. Brains were removed and processed for Fos immunochemistry using an antiserum raised against a beta-gal-Fos fusional protein (Medac).

All animals given cisplatin in the absence of granisetron (4 lesioned and 4 unlesioned) displayed emetic behaviour. Treatment with granisetron abolished cisplatin-induced retching and vomiting (n=4 unlesioned). Control animals treated with saline alone or granisetron followed by saline did not retch or vomit (n=5). In control animals basal levels of Fos staining in the area postrema (AP) and the nucleus of the solitary tract (NTS) were very low or undetectable. In all 4 unlesioned animals receiving saline followed by cisplatin, heavy Fos staining was observed in both the AP and the NTS. In the 4 animals with unilateral cervical vagus nerve lesions (all of which received saline followed by cisplatin) Fos staining in the ipsilateral NTS was greatly reduced but the contralateral NTS and AP still showed heavy Fos staining. Granisetron given before cisplatin (n=4) markedly reduced Fos staining in the NTS but did not affect AP Fos staining.

This study demonstrates that c-*fos* expression is induced in brainstem nuclei known to be involved in emesis by treatment with cisplatin in vivo. Unilateral vagotomy greatly reduces Fos immunostaining in the ipsilateral NTS. Granisetron markedly reduces Fos staining in the NTS suggesting that its effect too may be mediated by an action on the vagus, either via 5-HT_3 receptors on vagal fibres in the gut or on vagal afferent terminals in the NTS. Neither treatment affected cisplatin-induced c-*fos* expression in the AP. This may indicate a direct action of cisplatin on the AP which is independent of vagal afferent firing but its relevance to the emetic activity of cisplatin is unknown.

266P PHARMACOLOGY OF THE DC POTENTIAL CHANGES INDUCED BY HYPOXIA/HYPOGLYCAEMIA IN THE GERBIL HIPPOCAMPAL SLICE

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Exposure of the gerbil hippocampal slice to hypoxia/hypoglycaemia induces a transient depolarization of the slice which is followed by a large, maintained hyperpolarization (Rogers et al., 1990). The pharmacology of these hypoxia/hypoglycaemia-induced potential changes has been investigated.

Transverse hippocampal slices (500μm) were prepared from the brains of male, Mongolian gerbils (65-85g). Individual slices were placed on an inclined plane and superfused at 30^{o}C with artificial cerebrospinal fluid (aCSF), composition (mM): NaCl 124, KCl 1.9, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, glucose 11, equilibrated with 95% O_2/5% CO_2. $MgSO_4$ (2mM) was present in the aCSF only during the dissection, and on wash-out of magnesium spontaneous synaptic activity was recorded from the slice. Hypoglycaemic/hypoxic solutions were obtained by equilibrating aCSF containing 2mM glucose/9mM sucrose with 95% N_2/5% CO_2. Slices were exposed to hypoxia/hypoglycaemia for 20min and then superfused again with oxygenated/9mM glucose aCSF. Drugs were present in the aCSF for 20 to 30min before, during and after the hypoxic/hypoglycaemic episode. Membrane polarization changes of the CA1 pyramidal cell layer were recorded extracellularly using the grease gap method of Blake et al. (1988). Data was only collected from slices that elicited a response to KCl (10mM) greater than 0.5mV.

Tetrodotoxin (TTX 1μM; 30min) had no effect on the hypoxia/hypoglycaemia-induced potential changes (control depolarization: 0.44±0.10mV, n=6; TTX 0.36±0.13mV, n=4; control hyperpolarization: 1.44±0.26mV; TTX 1.28±0.53mV). In the presence of the excitatory amino acid antagonists, 6,7-dinitroquinoxaline-2,3-dione (DNQX 10μM; 30min) or MK801 (10μM; 30min) the hypoxia/hypoglycaemia-induced depolarization was slightly, but not significantly, reduced (control: 0.54±0.23mV, n=6; MK801 0.24±0.04mV, n=5; DNQX 0.20±0.03mV, n=5). The hypoxia/hypoglycaemia-induced hyperpolarization was not reduced (control: 1.04±0.37mV; MK801 1.09±0.46mV; DNQX 1.64±0.84mV). The enhancement in the presence of DNQX was not significant. DNQX (10μM) and TTX (1μM) blocked the spontaneous synaptic activity recorded in magnesium-free aCSF. The effect of a range of agents having potassium channel blocking activity on the hypoxia-induced hyperpolarization was also examined. The ATP-sensitive potassium channel blocker, glibenclamide (1μM; 30min) had no effect on the hypoxia/hypoglycaemia induced-hyperpolarization (control: 1.44±0.26mV, n=6; glibenclamide 1.66±0.32mV, n=5). Tetraethylammonium (TEA 10mM; 20min) caused a significant reduction in the hyperpolarization (control 1.44±0.26mV; TEA 0.61±0.16mV, n=7) as did d-tubocurarine (100μM; 20min; 0.75±0.10mV, n=6) or replacement of the calcium in the aCSF with barium 2mM (0.41±0.17mV, n=6).

These findings suggest that the hypoxia/hypoglycaemia-induced potential changes do not result from synaptic activation, but are most probably the result of metabolic changes within the hypoxic/hypoglycaemic slice. Activation of a potassium conductance, possibly a calcium-dependent potassium conductance, appears to underlie the hypoxia/hypoglycaemia-induced hyperpolarization.

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267P STRAIN DIFFERENCES IN SENSITIVITY TO THE EFFECTS OF BENZODIAZEPINE AGONISTS ON BODY TEMPERATURE IN MICE

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We have previously shown that the benzodiazepine full agonist loprazolam induces hypothermia in TO mice whereas the partial agonist Ro 17-1812 (Potier et al., 1988) does not (Jackson & Nutt, 1990). Since variations in the sensitivity of different strains of mice to the effects of benzodiazepine full agonists and inverse agonists on seizure threshold have been reported (File et al., 1985; Nutt & Lister, 1988) we have repeated our studies in BALB/c, DBA/2 (Bantin & Kingman), CBA/Ca and CBATHY101 (University of Bristol) mice. Body temperatures (male mice, 10-12 weeks old) were measured every 15 min using a rectal probe (inserted 2 cm) and digital thermometer (BAT-12). Drugs were suspended in 0.9% saline (containing Tween 80) and administered i.p. Treatment group means (n=8) were compared using ANOVA and Dunnett's test. Loprazolam (1, 10 mg/kg) significantly decreased body temperature in all 5 strains, although this effect was more marked in the CBA/Cas and CBATHY101s. In contrast, Ro 17-1812 (1, 10 mg/kg) was hypothermic only in CBA/Cas and CBATHY101s, and, at the highest dose, in DBA/2 mice.

Treatment (mg/kg)	TO	BALB/c	CBA/Ca	CBATHY101	DBA/2
Vehicle	37.8±0.1	37.2±0.2	38.0±0.2	37.8±0.1	38.3±0.2
Loprazolam 1	36.3±0.1*	35.8±0.2*	35.1±0.3*	35.4±0.3*	36.9±0.2*
Loprazolam 10	36.1±0.3*	35.3±0.2*	35.1±0.3*	35.3±0.2*	36.4±0.1*
Ro 17-1812 1	37.1±0.2	36.9±0.2	36.6±0.1*	36.9±0.1*	37.3±0.2
Ro 17-1812 10	37.5±0.1	36.8±0.1	36.7±0.1*	37.1±0.2*	36.5±0.5*

Results represent body temperatures °C (means ± s.e.mean) 30 min after injection; *P<0.05-v-vehicle-treated group

These findings demonstrate strain differences in the effects of benzodiazepines on body temperature. Furthermore, the fact that strain differences were more clear-cut with Ro 17-1812 suggests that the use of partial agonists may be of value in future studies on genetic variation in benzodiazepine receptor sensitivity.

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268P BIPHASIC BROWN FAT TEMPERATURE RESPONSES TO ELECTRICAL STIMULATION OF THE VENTROMEDIAL HYPOTHALAMUS IN RATS

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Unilateral electrical stimulation (30s train of monophasic square-wave pulses, 50Hz, 60μA, 0.5ms) of the ventromedial hypothalamus (VMH) of the rat results in a biphasic response in interscapular brown adipose tissue (IBAT) temperature (Perkins et al, 1981). We have found that prolonged stimulation (2min or longer) at a lower frequency (15Hz) and current strength (7.0μA) can exaggerate these responses, particularly the initial decrease in IBAT temperature (Phase 1), which is sustained for as long as the stimulus is applied. The increase in temperature (Phase 2) occurs only when the stimulus is removed. Similar biphasic responses can be obtained by direct stimulation of the sympathetic nerves.

Bilateral lesions of the lateral hypothalamus (LH) are known to cause a monophasic rise in IBAT temperature, which can be sustained for up to 180min (Corbett et al, 1988). In our experiments unilateral LH lesions caused a rapid rise (1.65±0.12°C; n=6) in IBAT temperature followed by a decrease to a stable, but elevated baseline level. Following the LH lesion, Phase 1 of the IBAT response to ipsilateral VMH stimulation was abolished and IBAT temperature increased immediately the stimulator was switched on. Electrical stimulation of the contralateral VMH (i.e. with intact LH) produced a typical biphasic response.

Attempts to mimic, or inhibit Phase 1 responses with central (icv) injections of a variety of adrenergic, serotonergic, GABAminergic and cholinergic agonists and antagonists were unsuccessful. However, it was found that icv injection of dopamine (10μg) produced a fall (-0.75±0.08°C; n=6) in IBAT temperature, similar in duration to Phase 1 of the response to a 5min electrical stimulation of the VMH; this effect was blocked by the D_2 antagonist pimozide (5μg, icv). Furthermore, pre-treatment with pimozide (5μg) was found to abolish the Phase 1 IBAT response to electrical stimulation of the VMH.

These results suggest that the Phase 1 IBAT temperature response to VMH stimulation requires an intact LH, and involves a central dopaminergic (D_2) pathway.

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269P PYROGENICITY OF INTERLEUKIN-1 FRAGMENT (VQGEESNDK) IN RATS AND ITS EFFECT ON PGE_2 RELEASE FROM BRAIN TISSUE *IN VITRO*

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The complexity of the biological effects of IL-1β suggests that different regions of the 17.5KDa molecule may be responsible for its diverse biological functions. Fragment 163-171 from human IL-1β (VQGEESNDK, C163-171) is immunostimulatory but not pyrogenic in mice (Antoni et al., 1986). The aims of this study were to compare the effects of C163-171 and IL-1β on colonic temperature in rats which are more sensitive to pyrogens than mice, and to determine their effects on PGE_2 release from rat brain tissue in vitro as this prostaglandin is thought to mediate the febrile response to pyrogens.

Male Sprague Dawley rats (150-300g) were injected i.v. (tail vein) or i.c.v. using cannulae chronically implanted into the third cerebral ventricle under equithesin anaesthesia (4ml/kg i.p.) at least 7 days earlier. Colonic temperature was measured in conscious, hand held rats before administration of putative pyrogens, and intermittently for the following 3 h at an ambient temperature of 25 ± 1°C. PGE_2 release from rat brain tissue in vitro (10mg wet weight/ml) was determined by radioimmunoassay after a 20 min pre-incubation period and a 20 min contact time with pyrogens (Redford & Dascombe, 1989).

Murine IL-1β (5μg/kg i.v., n=6) produced a maximum increase in colonic temperature of 0.68 ± 0.18°C (mean ± s.e. mean) 120 min after injection ($P<0.05$ compared to saline 1ml/kg i.v.) which was blocked by 15 min pretreatment with flurbiprofen (100μg i.v.). C163-171 (500μg/kg i.v., n=5) caused a maximum increase in colonic temperature of 0.62 ± 0.09°C 60 min after injection. C163-171 (5 and 50μg/kg i.v., n=6) had no effect on colonic temperature. Central injection of murine IL-1β (10ng, i.c.v., n=6) produced a maximum rise in temperature of 2.06 ± 0.18°C 120 min after injection ($P<0.05$ compared to water for injection, paired t-test). C163-171 100ng (n=7) and 10μg (n=5) i.c.v. had no effect on colonic temperature, but 1μg produced a rise in temperature of 0.50 ± 0.11°C 20 min after injection (n=7, $P<0.05$). Murine IL-1β (0-100ng/ml) caused a dose dependent increase in PGE_2 release from rat brain tissue in vitro when compared to time matched controls (control: 224 ± 52pg/ml; 10ng: 314 ± 76pg/ml‡; 100ng: 413 ± 104pg/ml‡, n=8, ‡$P<0.05$). C163-171 (100ng-1,00μg/ml) had no effect on PGE_2 release into the supernatant.

These results indicate that C163-171 is much less potent than IL-1β as a pyrogen in the rat. C163-171 fails to stimulate PGE_2 release from rat brain tissue in vitro. These observations indicate that this region of IL-1β is not responsible for the pyrogenic actions of IL-1β.

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270P AUTORADIOGRAPHICAL COMPARISON OF THE BINDING OF [^{3}H]TRH, [^{3}H]RX77368 AND [^{3}H]METHYL-TRH IN RAT BRAIN

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The tripeptide thyrotropin releasing hormone (TRH) has a variety of neural actions, in the CNS, which are believed to be mediated via specific receptors (Sharif 1985). Clinically, TRH is potentially useful for the treatment of motor neurone disease, Alzheimers's disease, stroke and spino-cerebellar disease. RX77368 (dimethyl-proline-TRH) is a biologically stable analogue with enhanced *in-vivo* neuropharmacological potency (Metcalf 1983) but with lower potency and affinity for TRH receptors in cultured GH_3 cells (McDermott et al. 1990). This study compares, autoradiographically, the distribution of binding of tritiated RX77368, TRH and a high affinity analogue methyl-histidine-TRH (MeTRH) in rat brain sections.

Brain area	TRH	MeTRH	RX77368
100% (fmol/mg tissue) (bregma -13.3mm)	11.5	28.5	7.3
N.Sol.Tract.	**100%**	**85**	**67**
Cranial.N.XIIth	98	96	100%
Cranial.N.Xth	92	100%	88
N.Ambiguous	60	65	48
Parv.Cell.Retic.N.	39	31	63
Cerebell.Purk.Layer	**28**	**16**	**61**
Cerebell.Molec.Layer	12	8	21
100% (fmol/mg tissue) (bregma -5.6mm)	22.7	44.8	8.7
Pre-rhinal Cort.	**100%**	**94**	**59**
Vent.Dentate Gyrus	**72**	**100%**	**55**
Post-med.Cort.Amyg.N.	**69**	**84**	**33**
Sup.Colliculus (grey)	57	60	37
Central Grey	37	28	63
Retrosplenial Cort.	**<10**	**15**	**100%**

Data expressed as mean % (3 sections) specific binding in region of maximal density.

To overcome differences in affinity and specific activity, the relative distributions of the 3 ligands are shown, in Table 1, as % of maximal bound. Although distributions are similar, there are clearly differences in relative densities in several areas (highlighted). Of particular interest is the finding that RX77368, but not TRH or MeTRH, binds prominently to cell layers in the retrosplenial cortex, an area which is closely implicated with mechanisms of learning and memory. Along with metabolic stability, these differences in binding distribution may contribute to the improved therapeutic profile of RX77368 (Guiloff et al. 1987). Furthermore, all three ligands bind to the Purkinje cell layer in cerebellum, a finding which has not previously been emphasised but may be of major importance in motor function.

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271P DIFFERENCES IN THE EFFECT OF PUTATIVE ACTIVATORS OF PROTEIN KINASE C ON SECRETION OF PITUITARY HORMONES

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Secretion of pituitary hormones *in vitro* can be induced by activation of protein kinase C (PKC) (Johnson and Mitchell, 1989). Using several putative activators and inhibitors of PKC, we investigated the possibility that PKC-mediated regulation of hormone secretion may be differentially organised in distinct pituitary cell types. Anterior pituitary glands were removed from long term (4 weeks) ovariectomised cob wistar rats and hemisected. Release of luteinizing hormone (LH) and growth hormone (GH), *in vitro*, was measured as previously described (Johnson & Mitchell,1989). Pituitary LH release progressively increased over 3 consecutive hourly incubations with 300nM phorbol 12,13-dibutyrate (PDBu), reaching a level 192±21% (n=16) of basal secretion in the third hour. In contrast, GH was rapidly released by PDBu with a maximal response in the first hour of 558±95% (n=16) of basal secretion. PDBu-induced release of both LH and GH was unaffected by the PKC inhibitor H7(30μM) but was attenuated in the presence of staurosporine (300nM). Selective inhibition by staurosporine but not H7 of certain PKC actions has been described previously in the regulation of L-type calcium channels by PKC (Johnson *et al.*,1989). In the present experiments, 200μM 1,2-dioctanoyl *sn*-glycerol (DOG) induced a small release of LH but not GH. Luteinizing hormone release induced by DOG was inhibited by staurosporine but was unaffected by H7. Release of LH could also be evoked by 300μM arachidonic acid (AA), but this effect was unaffected by PKC inhibitors. Release of GH was reduced by AA, an effect which is also unaltered by PKC inhibitors.

The release of LH and GH from pituitary tissue obtained from ovariectomized rats can be influenced by activation of PKC. However, the lack of effect of H7 on LH release shown here contrasts with those effects observed on PDBu induced LH release from tissue obtained from intact pro-oestrous rats (Johnson & Mitchell,1989.). It appears that AA can exert stimulatory (LH) or inhibitory (GH) effects on hormone secretion by means other than activation of PKC. This is consistent with evidence suggesting a role for AA metabolites in the control of LH and GH release (Naor *et al*, 1983; Schweitzer *et al*, 1990).

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272P EFFECT OF THE CRF ANTAGONIST, ALPHA-HELICAL CRF_{9-41}, ON THE DEPRESSOR ACTIVITY OF CORTICOTROPHIN RELEASING FACTOR IN ANAESTHETISED RATS

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Corticotropin releasing factor (CRF) is the principal hypothalamic regulator of adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary. In addition to this action, systemic administration of CRF has been found to produce vasodilation and a long lasting depressor response (Lenz *et al.*, 1985; Gardiner *et al.*, 1988). Similar hypotensive activity has also been described for the structurally homologous non-mammalian peptides sauvagine (SVG) and urotensin I (UI) (Lenz *et al.*, 1985). Although all three peptides are equipotent as ACTH secretagogues, SVG and UI are ten fold more potent than CRF as vasodilator agents (Lederis *et al.*, 1983). Alpha-helical CRF_{9-41} is an N-terminal deleted analogue of these peptides which employs the amino acids from each sequence that maximise the alpha-helix-forming potential of the peptide. This analogue has been found to be a competitive antagonist of CRF stimulated ACTH release *in vitro* and *in vivo* (Rivier *et al.*, 1984). To further characterise the hypotensive properties of CRF, the ability of this antagonist to block the ACTH release and depressor responses produced by *i.v.* administration of CRF has been tested.

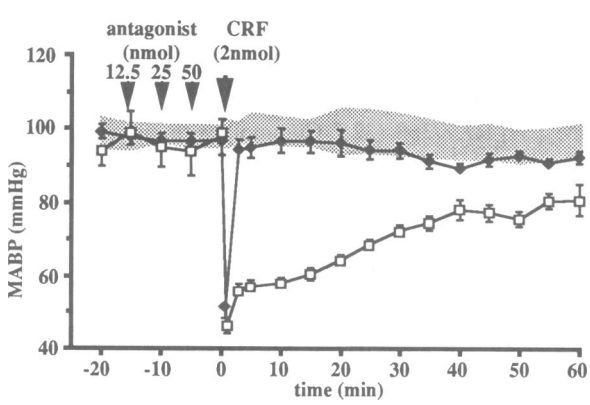


Figure 1 *Effect on blood pressure of CRF administered alone (□) and after pre-treatment with CRF antagonist (◆)*

Female Wistar rats, anaesthetised with ketamine and midazolam, were administered *i.v.* alpha-helical CRF_{9-41} in increasing doses at -15, -10 and -5 min (Figure 1), followed by 2 nmol of rCRF at 0 min. Blood pressure was measured from the right femoral artery, and blood samples (250 μl) were withdrawn from this arterial catheter at -16, -1, 1, 3, 10, 30 and 60 min for the measurement of plasma levels of ACTH and CRF. Each blood sample was replaced by an equivalent volume of 0.9% saline. BP and ACTH responses were compared with rats receiving CRF alone and controls which were administered only vehicle.

Consistent with published data CRF produced a marked fall in BP which did not return to control values during the 60 min study period. The antagonist alone had no effect on BP, nor did it alter the initial rapid fall in BP after CRF administration, however it completely prevented the long-lasting hypotension (Figure 1). In comparison, the antagonist only reduced the ACTH response by 29% and 43% at 10 and 30 min respectively. Based on the differing ability of the antagonist to block the initial fall in BP (vasodilation), the prolonged depressor response, and stimulated ACTH release it is concluded that these three responses to CRF are mediated by different types of CRF receptors.

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273P PRAZOSIN IS NOT A MELATONIN ANTAGONIST: BINDING AND ELECTROPHYSIOLOGICAL STUDIES

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Radioligand binding and *in vitro* autoradiograpic studies have revealed binding sites for the pineal hormone melatonin (5-methoxy-N-acetyltryptamine) in the brain, including the suprachiasmatic nucleus (SCN), a circadian rhythm pacemaker (Morgan & Williams, 1989). We have shown that hamster SCN cells are electrophysiologically responsive to melatonin (Mason & Rusak, 1990). The α_1-adrenoceptor antagonist prazosin has been reported to inhibit binding of 2-[^{125}I]iodomelatonin in Syrian hamster brain (Niles *et al*, 1987; Pickering & Niles, 1990). The present study examined (i) the effects of prazosin on 2-[^{125}I]iodomelatonin binding to chick brain membranes and (ii) the modulation by prazosin of SCN cell responses to melatonin.

We have reported that 2-[^{125}I]iodomelatonin binds specifically to a single high affinity site in chick brain membranes with an equilibrium dissociation constant (K_D)=69pM and a maximal number of binding sites (B_{max})=25fmol/mg protein (Chong & Sugden, 1990). Competition experiments indicated that only melatonin and closely related analogues inhibited binding. Prazosin inhibited binding slightly (19%) only at a very high concentration (10^{-4}M). Extracellular recordings were made from SCN neurones in Syrian hamster hypothalamic slices maintained *in vitro* (Mason & Brooks, 1988; Mason & Rusak, 1990) and the effects of pressure-ejected melatonin (2mM melatonin in 150mM NaCl) on spontaneous neuronal discharge activity were observed. Melatonin-responsive SCN neurones were only recorded during the late projected day/early projected night (Mason & Rusak, 1990); melatonin suppressed firing in 11/12 neurones and activated the remaining neurone in a pressure(dose)-dependent manner. Superfusion of prazosin (10^{-8}M-10^{-4}M) alone had no effect on spontaneous firing. In the presence of prazosin the response to melatonin was not altered in 10/12 neurones; melatonin-induced suppressions was enhanced (10-15%) in 2/12 neurones tested. These findings provide evidence that prazosin is not an inhibitor at the high affinity 2-[^{125}I]iodomelatonin binding site and does not act as a melatonin antagonist in the SCN at the concentrations tested.

Supported in part by the Royal Society, US AFOSR, NSERC (Canada) & MRC (Canada).

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274P STRUCTURE-AFFINITY RELATIONSHIPS OF MELATONIN ANALOGUES FOR 2-[^{125}I]IODOMELATONIN BINDING SITES IN WHOLE CHICK BRAIN MEMBRANES

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Melatonin (5-methoxy N-acetyltryptamine) is produced rhythmically by the pineal gland, with elevated levels occuring only at night. Melatonin regulates seasonal changes in the reproductive axis in photoperiodic species (Tamarkin et al., 1985), entrains circadian rhythms in some species of bird, reptile and mammal (Armstrong, 1989) and modulates various aspects of retinal physiology (Besharse et al., 1988). High affinity melatonin binding sites have been identified in the brain of various species, including human, using radioreceptor and autoradiographic techniques (Morgan and Williams, 1989). High affinity melatonin binding sites in whole chick brain membranes were identified using 2-[^{125}I]iodomelatonin, and the pharmacological characteristics of these sites were studied using various recently synthesized melatonin analogues.

Specific binding of 2-[^{125}I]iodomelatonin accounted for 85-95% of total binding, was rapid, saturable, stable, of high affinity and reversible. Saturation studies demonstrated that 2-[^{125}I]iodomelatonin binds to a single class of sites with an equilibrium dissociation constant (K_d) of 69 ± 3.5pM (mean ± s.e.mean) and a total binding capacity (B_{max}) of 24.8 ± 0.6fmol/mg protein (n = 4). The K_d obtained from kinetic analysis (47 ± 2.5pM, n = 3) was in close agreement with that obtained in saturation experiments.

Specific binding was inhibited by melatonin (K_i = 1.02 ± 0.1nM) and several recently synthesized, closely-related analogues. Substitution of a halogen atom at the 2-position of the indole ring dramatically increased affinity (2-iodomelatonin, K_i = 62 ± 6pM; 2-chloromelatonin, K_i = 24 ± 2pM, n = 3). Analogues in which the carbon chain of the N-acyl group was increased by 1 or 2 atoms showed an increase in affinity compared to melatonin (5-methoxy N-propionyltryptamine, K_i = 110 ± 10pM; 5-methoxy N-butanoyltryptamine, K_i = 45 ± 8pM, n = 3); larger N-acyl groups led to a progressive reduction in affinity. Halogen substitution at the 6-position (6-fluoro, 6-chloro) increased affinity 3 and 2-fold respectively. 6-Hydroxymelatonin, the major metabolite of melatonin showed a reduction in affinity (K_i = 6.3 ± 0.4nM, n = 3). Of the substitutions on the 5-position of the indole ring, 5-methoxy was clearly preferred; analogues with 5-H, 5-OH, 5-benzyloxy and 5-methyl substitutions were much weaker than melatonin itself. Future studies on additional analogues will continue to define the structural features of the molecule required for receptor interaction and may lead to the design of potent melatonin agonists and antagonists.

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275P ELECTROCHEMICAL MONITORING OF HYDROGEN PEROXIDE OXIDATION BY CARBON FIBRE MICRO ELECTRODES

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The development of micro-biosensors for selective and sensitive analysis of electrochemically active compounds is one of the main fields of the modern scientific approach to the study of biological mechanisms.

We have recently prepared a specifically electrically and chemically pretreated carbon fibre microelectrode (CFE, diameter 10-30 µm) which,allows the electrochemical measurement of neurotransmitters such as dopamine *in vitro* and serotonin *in vitro* and *in vivo* when used with differential pulse voltammetry (DPV), a slow scanning electrochemical method, (Crespi *et al*., 1988). Another pretreatment for the same basic biosensor (CFE) has permitted the simultaneous detection of the oxidation of dopamine and serotonin metabolites as well as that of neuropeptides (i.e., somatostatin) (Crespi, 1990).

We now show that these specifically pretreated CFE can detect the selective oxidation of peroxide *in vitro* either with DPV or with the fast scanning cyclic voltammetric method (FCV) as applied by the Millar voltammeter, (Bull *et al.*, 1990). The oxidation signal (R) of peroxide is obtained at +1150mV or at +980mV with these two methods respectively in a phosphate buffered saline solution (PBS) at pH7.4, and appears to be concentration dependant.

These new biosensors should allow the assessment of the methodology for future studies of putative toxic compounds and the direct analysis of their capability to generate peroxide as well as to determine the capability of drugs to neutralise the generation of free radicals using *in vitro* cell preparations and cell culture techniques.

We thank the MRC for financial support and AVCO-Textron USA for the gift of carbon fibres.

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276P CORRELATION BETWEEN STIMULATION OF ADENYLATE CYCLASE ACTIVITY AND GTP MODULATION OF BINDING AFFINITY FOR A RANGE OF DOPAMINE D_1 RECEPTOR AGONISTS

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Dopamine D_1 receptors are thought to act via G_s protein coupling to stimulation of the adenylate cyclase effector system. In radioligand binding studies, the affinity of agonist binding to D_1 receptors can be modulated by GTP (De Keyser et al 1988). We have used a range of novel D_1 agonists to investigate whether there is a correlation between stimulation of adenylate cyclase activity and GTP modulation of agonist affinity.

Stimulation of adenylate cyclase activity was assessed in rat striatal slices by a modification of previous methods (Herdon 1988) using RIA measurement of cyclic AMP accumulation. Competition for ^{3}H-SCH 23390 binding to D_1 receptors was assessed by the method of De Keyser et al (1988). Dopamine (100 uM), SK&F 38393, SK&F 81297 and SK&F 80723 (all 1 or 10 uM) each stimulated cyclic AMP accumulation to approximately the same maximal extent; their effects were blocked by SCH 23390 (1uM). In contrast, neither SK&F 83959 or SK&F 83565 (both 10uM) had any effect on cyclic AMP accumulation (Table 1). In radioligand binding studies, all compounds showed high affinity for D_1 receptors. However, whilst K_i values of dopamine, SK&F 38393, SK&F 81297 and SK&F 80723 were modulated by GTP, those of SK&F 83959 and SK&F 83565 remained unaffected (Table 1).

Table 1		Dopamine	SK&F 38393	SK&F 81297	SK&F 80723	SK&F 83959	SK&F 83565
Maximal cyclic AMP accumulation (%basal)		177±20$^+$	165±17$^+$	145±9$^+$	170±19$^+$	104±10	108±9
K_i (nM) for ^{3}H-SCH23390 binding	Control	99±52(59%) 2900±1000(41%)	20±9	2.7±0.7	3.4±1.4	0.9±0.1	3.2±0.9
	+GTP (100 uM)	3500±1200*	66±18*	6.4±1.6*	3.3±1.0(60%) 18±2*(40%)	0.7±0.1	3.2±0.8

Results are means±SEM of 3-6 experiments. Values in parentheses are % of each site where data were fitted to a two-site model (using the LIGAND computer program). + $p<0.05$ vs basal; * $p<0.05$ vs control.

These results demonstrate a clear correlation between stimulation of adenylate cyclase activity and GTP modulation of binding affinity for the agents tested. The lack of GTP modulation or adenylate cyclase stimulation for SK&F 83959 or SK&F 83565 could indicate these compounds posess antagonist properties; however, the fact that all the compounds tested here induce D_1 receptor-mediated grooming behaviour (Rogers, Hunter & Hill, presented at 20th annual meeting Soc. Neurosci.) argues against this. Taken together therefore, these findings suggest some effects of D_1 receptors may be independent of the adenylate cyclase system and possibly of any G protein-coupled system (Waddington & O'Boyle 1989).

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277P ELECTROPHYSIOLOGICAL EFFECTS OF ANGIOTENSIN PEPTIDES ON RAT ISOLATED NODOSE GANGLION

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Angiotensin II (AII) binding sites have previously been identified on rat vagal afferent terminals in the nucleus tractus solitarius and over cell bodies in the nodose ganglion (Lewis et al., 1986; Allen et al., 1988). We have recently reported that these AII binding sites are indeed functional receptors, since AII evoked concentration-dependent depolarisation of the rat isolated nodose ganglion (Widdop et al., 1990).

In the present study, we have examined whether or not other angiotensin peptides cause similar excitatory responses in the nodose ganglion preparation. Nodose ganglia were removed from male Sprague-Dawley rats (220-280g) and the nodose ganglion was placed in one bath compartment and this was isolated from the attached vagal nerve trunk in a second bath compartment by a silicone grease seal. Angiotensin peptides were superfused only over the nodose ganglion-containing compartment and the d.c. potential between the two compartments was recorded by calomel electrodes connected to the nodose ganglion preparation via agar-KCl bridges.

AII caused concentration-dependent depolarisation of the nodose ganglion and this was blocked by the AII receptor antagonist, saralasin (Widdop et al., 1990). AI evoked similar excitatory responses ($-\log EC_{50}$ = 7.31 ± 0.22, n=4) which were significantly attenuated in the presence of 1 μM enalaprilat ($-\log EC_{50}$ = 6.53 ± 0.10, n = 4, $P < 0.05$). AIII also caused concentration-dependent depolarisation ($-\log EC_{50}$ = 6.34 ± 0.10, n=5), although this peptide was significantly less potent than AII ($-\log EC_{50}$ = 7.46 ± 0.15, n = 5, $P < 0.05$) when tested in the same preparations. In contrast, the N-terminal heptapeptide fragment of AII, A(1-7), failed to evoke any effect over the concentration range 100 nM-10 μM (n=13).

These results demonstrate that the rat isolated nodose ganglion is a convenient model in which to examine direct neuronal responses to angiotensin peptides.

This work was supported by the National Health and Medical Research Council of Australia.

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278P CENTRAL EFFECTS OF ACE INHIBITORS ON ANGIOTENSIN I-INDUCED DIPSOGENICITY AND AGAINST A SCOPOLAMINE-INDUCED MEMORY DEFICIT IN THE RAT

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The angiotensin converting enzyme inhibitors (ACEi), SQ29852 (SQ), captopril (CAPT) and HOE288 (HOE) have been reported to improve cognition in the rodent (Costall et al, 1989; Usinger et al, 1988), but there is still some controversy concerning the ability of these agents to penetrate the CNS. We have examined the ability of peripherally (ip) administered ACEi, to attenuate a dipsogenic response induced by angiotensin I (AI). We have also compared the ability of HOE, administered into the lateral ventricles (icv), to attenuate the AI-induced dipsogenicity with its ability to improve memory in the radial maze.

All experiments used male Lister-hooded rats (300-350g), implanted with guide cannulae allowing icv injection. In the dipsogenic studies, animals were treated with AI (30pmole in 2μl icv) and were immediately allowed access to water. The volume of water (ml) consumed over a 20min period was recorded. Animals were pretreated with either SQ (0.005 or 50mg/kg ip), CAPT (10 or 30mg/kg ip), HOE (3mg/kg ip or 10nmol icv) or saline (1ml/kg ip or 2μl icv), 30-60min prior to AI. In the 12-arm radial maze, animals were trained to retrieve food from 3 arms. Each animal received 4 trials per day and the number of errors (entries into unbaited arms) was recorded. Drug treatment commenced when rats achieved a criterion performance of no more than 12 errors/day. The treatments were: HOE (10nmole icv, 30min pretest) or saline (2μl icv), administered with scop (0.1mg/kg sc, 20min pretest) or saline (1ml/kg sc).

Peripheral administration of SQ, CAPT or HOE failed to modify the AI dipsogenicity. However, icv HOE (10nmol), significantly reduced the dipsogenic response to AI (see Table 1, *$p<0.05$ vs AI control). In the radial maze, 10nmol icv HOE significantly attenuated the deficit induced by scop. Total errors/day were: saline 6.4±1.5; scop 33.0±1.6 ($P<0.01$ vs saline); HOE 5.1±1.2; scop plus HOE 26.5±2.8 ($P<0.05$ vs scop).

Table 1: Effect of ACEi on AI-induced dipsogenicity (mean±s.e.m. vol of water consumed/20min)

SQ (mg/kg ip)	ml	CAPT (mg/kg ip)	ml	HOE (mg/kg ip)	ml	HOE (nmol icv)	ml
0	7.3±1.4	0	7.9±1.7	0	8.6±0.9	0	5.4±1.2
0.005	7.3±2.9	10	8.4±0.9	3	10.9±0.9	10	0.7±0.2*
50	5.3±0.9	30	6.6±0.8				

In conclusion, we show that peripheral administration of SQ, HOE and CAPT fails to attenuate the AI-induced dipsogenic effect, suggesting that they poorly penetrate into the CNS. However, the observation that icv administration of HOE can partially reverse a scopolamine-induced memory deficit implies a central action. The inability of peripherally administered ACEi to attenuate AI-induced dipsogenic response at doses reported to produce cognitive effects remains to be explained.

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279P EFFECT OF PEPTIDASE INHIBITORS ON THE POTENCY OF ANGIOTENSIN ANALOGUES IN THE SUPERIOR CERVICAL GANGLION PREPARATION OF THE RAT

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Early evidence for the existence of angiotensin II (AII) receptor subtypes has been based on differences in the relative potencies of AII and AIII (Peach et al, 1977) and on differences in the ability of peptide antagonists such as [Ile7]AIII to antagonise various AII responses (Campbell and Schmitt, 1979). However, such selectivity may result from variations in peptide metabolism. The aim of the present study was to investigate the effects of two peptidase inhibitors, bacitracin (BCT) and amastatin (AM) on depolarisations to AII and AIII in the rat isolated, superior cervical ganglion. In addition, we have investigated the influence of AM on the antagonist potency of [Ile7]AIII against AII responses in this preparation.

Superior cervical ganglia from male LH rats (300-350g) were desheathed and arranged in two-compartment chambers. The ganglion cell body was separated from the post-ganglionic trunk by a grease barrier and both regions were perfused with Krebs buffer at 37°C. Potential differences (mV) across the grease barrier were recorded using Ag/AgCl electrodes. To avoid tachyphylaxis to AII and AIII, increasing agonist concentrations (<1.0μM) were applied serially, every 20 min. Maximum responses, particularly to AIII, could not always be achieved and therefore to assess agonist potency, the log concentration of agonist, required to produce a depolarisation of 300μV (pEC_{300}) was calculated for each curve. A response of 300 μV always lay on the linear part of the dose-response curve. In the antagonist experiments, AII curves were established (±AM), prior to and following incubation with one of three concentrations of [Ile7]AIII. AII and AIII produced concentration-dependent depolarisations with pEC_{300} values of 7.17±0.14 (n=4) and 6.46±0.08 (n=5) respectively. In the presence of BCT (1mM), the potency of both agonists was increased (pEC_{300} values: 8.10±0.09 for AII, 7.33±0.09 for AIII, n=4-8, P<0.05 vs AII or AIII alone). In the presence of BCT, AM (10μM) failed to modify the potency of AII (pEC_{300} for AII + AM = 8.11±0.23 vs 8.14±0.27 for AII alone, P>0.05, n=4). In contrast, the potency of AIII was increased (pEC_{300} for AIII + AM = 8.09±0.13 vs 7.45±0.12 for AIII alone, P<0.05, n=4). Antagonism of the AII responses by [Ile7]AIII resulted in dose-dependent non-parallel rightward shifts of the AII control curve, with a suppression of the maximum response. Concentrations of 0.03, 0.1 and 0.3μM [Ile7]AIII reduced the maximum AII response to 84.1±8.9%, 78.4±7.0% and 66.6±2.9% respectively. AM potentiated this effect. Thus, at concentrations of 0.01, 0.03 and 0.1μM [Ile7]AIII, maximum responses achieved were 78.7±3.85%, 63.2±2.85% (P<0.05 vs 0.03μM [Ile7]AIII, no AM) and 47.6±6.1% (P<0.05 vs 0.1μM [Ile7]AIII, no AM).

In conclusion, the activity of peptidase enzymes in the ganglia can markedly influence the potency of peptide angiotensin analogues. Similar influences in other tissues may contribute to the differences in relative potency of AII and AIII as well as differences in peptide antagonist potencies reported by others.

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280P CROMAKALIM REVERSES ISCHAEMIC DEPRESSION OF SKELETAL MUSCLE CONTRACTIONS IN THE RAT HIND LIMB

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There is significant evidence that cromakalim (Hamilton et al., 1986), and other drugs which enhance K+ conductance of vascular smooth muscle, can increase resting skeletal muscle blood flow in an animal model of chronic occlusive arterial disease (Angersbach & Nicholson, 1988). Despite this, evidence for improvement in performance of skeletal muscle by K+ channel openers has not been forthcoming. The present experiments have been performed to determine the ability of cromakalim to produce beneficial and selective effects on limb blood flow and exercising muscle performance following acute partial ischaemia in the rat hind limb. This utilises an in-vivo model of peripheral ischaemia as described by Downing et al (1986) in which evoked contractions of the extensor digitorum longus muscle (EDL) are used to enhance and to assess the effects of ischaemia on muscle function.

In intraval - anaesthetised female Wistar rats, ischaemia was induced by occluding the femoral arteries for 45 s in every min for 15 min. Iliac blood flow was assessed by an ultrasonic doppler technique by placement of flow probes (Valpey Fisher) on each iliac artery from which the flow velocity signal (doppler shift) and electronically derived mean flow velocity (directly proportional to blood flow) was recorded. Blood pressure was measured from a catheter inserted into a carotid artery and a jugular vein was cannulated for injection of drugs. The sciatic nerves in each limb were sectioned and electrically stimulated simultaneously for 30 s every 5 min (5Hz,0.1ms,5v). Two periods of ischaemia were performed, each involving three bursts of 5 Hz stimulation; a control ischaemia and a drug infused ischaemic period. The twitch height at the end of the third 5Hz stimulation was taken as the measured response to ischaemia. Cromakalim 1 to 100μg/kg, or its vehicle, was given by bolus i.v. injection to groups of 6 to 10 rats, one dose per rat, immediately prior to the second ischaemia period.

A reduction in mechanical activity of the EDL muscle of 45.6±4.5% was seen to occur during an initial period of ischaemia. This did not occur to the same degree (P<0.05) when this was repeated in the same animal following i.v. cromakalim 100μg/kg (24.5±3.5%) in comparison with its vehicle (44.9±6.1%). A similar pattern was seen for other doses of cromakalim e.g. 30μg/kg, at which a moderate increase in iliac blood flow (39±8.9%) and reduction in blood pressure (18±5.8 mmHg) was seen to accompany the protective effect on the muscle. The precise mechanism and site of action of cromakalim was unclear since the effect on the muscle also occurred at 1μg/kg (24.6±4.1% reduction) which itself did not increase iliac blood flow nor lowered blood pressure.

The possibility remains that cromakalim affected the distribution of blood flow within the ischaemic limb or exerted a direct and possibly selective action on skeletal muscle to produce a beneficial effect.

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281P MIDAZOLAM INDUCES MUSCLE TENSION AND INHIBITION OF TWITCHES BY DIFFERENT PROCESSES

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Midazolam and other benzodiazepines enhance twitch and tetanic contraction at relatively low concentrations, followed by inhibition at higher concentrations in indirectly stimulated rat diaphragm preparations (Van Wilgenburg *et al.*, 1990). The latter effect is also seen in curarized directly stimulated muscle preparations (Eigenhuis *et al.*, 1990). A transient stimulus independent muscle tension is induced by midazolam at bath concentrations of 100 µM and higher.
In the present study the effects of midazolam on twitch and muscle tension are compared under conditions of enhanced depolarization with high K^+ and increased electric field with high Ca^{2+}. Calcium antagonists, the membrane active drugs lidocaine and diltiazem, ruthenium red and dantrolene are used in addition.

Isolated rat diaphragm preparations *in vitro*, were stimulated directly (0.1 Hz) between a surgical steel wire attached to the tendon and to pins onto which the costal margin was impaled. Phrenic nerves were stimulated with bipolar platinum electrodes using rectangular waves (0.5 msec) at supramaximal voltages. Midazolam was given in increasing concentrations ranging from 10 to 450 µM. Bath concentrations of K^+ (9.5 mM), Ca^{2+} (9.0 mM), dantrolene (15 µM), lidocaine (190 µM), diltiazem (45 µM), ruthenium red (10 µM), verapamil (31 µM) and nifedipine (30 µM) were established 15 min before the application of midazolam. All values were related to the pretreatment twitch.

The inhibitory effect on indirectly evoked twitches is preceded by an increase (175%) of the twitches at midazolam concentrations ranging from 10 to 100 µM. The latter effect is not significant in pancuronium treated directly stimulated preparations. A transient increase of the muscle tension is induced stimulus independently at midazolam concentrations of 100 µM and higher. High K^+ reduces the twitch amplitude to 68% at all midazolam concentrations tested, but has no significant effect on the muscle tension. Neither the membrane active drugs diltiazem and lidocaine, nor the excitation-contraction uncoupler dantrolene reduces the stimulus independent contraction. In high Ca^{2+} solution the effect of midazolam on both the twitch and the muscle tension is potentiated by 120% and 176% respectively. These effects are strongly antagonized by the calcium antagonists and also by lidocaine, indicating that both a direct transport through Ca^{2+} channels and an indirect effect on the Na^+-Ca^{2+} exchange is affected by midazolam. The effect of calcium is also inhibited by ruthenium red, suggesting that Ca^{2+} taken up by the mitochondria potentiates the midazolam effect.

In conclusion, the results indicate that the inhibitory effect, which is preceded by an increase of the twitches in indirectly stimulated preparations, can be explained by a depolarizing effect of midazolam. The depolarization is not a necessary condition for the induction of the stimulus independent muscle tension caused by midazolam.

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282P COMPARATIVE EFFECTS OF PANCURONIUM AND VECURONIUM ON LEAKAGE OF LACTATE DEHYDROGENASE IN RAT ISOLATED DIAPHRAGM, LIVER KIDNEY AND HEART

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Previous reports have indicated that pancuronium is a powerful neuromuscular blocking agent, more powerful than vecuronium, in blocking neuromuscular transmission and in inhibiting muscle twitch or tetanic contractions, in both animal and human species (Hilgenberg,1983; Carpenter & Mulroy,1983; Adams,Jones & Wali, 1985). Furthermore,recently,it has been shown that pancuronium was more effective than vecuronium,in causing or enhancing the leakage of lactate dehydrogenase (LDH) from rat isolated hepatocytes (Makinde & Wali,1988).

In the present investigation,we have extended the previous studies, by comparing the effects of pancuronium and vecuronium on leakage of intracellular LDH from rat isolated diaphragm,liver,kidney and heart.

Adult,male,Sprague-Dawley rats,weighing 200-300g,were killed by a blow to the head and bled. The tissues were dissected and placed in separate tissue culture media (Klaunig,Goldplatt,Hinton,Lipsky,Chacko & Trump, 1981). The concentration of LDH released spontaneously (control),in the presence of pancuronium(0.34-43.16 µM) or vecuronium(0.32-41.48 µM), and by a detergent (Triton 1%),to totally lyse the cells, was measured, in Units per ml, using spectrophotometric determination at 340 nm.

The results showed that both pancuronium and vecuronium, in low concentrations(0.32 µM, 0.34 µM)(which is close to a clinical dose of 0.1 $mg.kg^{-1}$), did not significantly enhance the LDH release from all four different types of the rat tissues. For example, in the heart,the control LDH was 270±13 $U.ml^{-1}$, and in the presence of pancuronium 0.34 µM was 300±11, means±s.e.,n=6, $P > 0.05$). However, in high concentrations, e.g. 9.59 µM or more,the effects of the two muscle relaxants were significant ($P < 0.05$ & $P < 0.01$), in all types of tissues studied.

In conclusion, pancuronium and vecuronium, in low concentrations did not significantly enhance the release of LDH from all four different types of rat tissues. In high concentrations both pancuronium and vecuronium significantly enhanced leakage of LDH from these tissues, pancuronium was more effective than vecuronium.

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283P THE EFFECT OF CHRONIC ADMINISTRATION OF MK-801 UPON LOCAL CEREBRAL GLUCOSE USE AND EXCITATORY AMINO ACID RECEPTORS

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Acute administration of MK-801 causes significant changes in local cerebral glucose use (LCGU) (Kurumaji et al. 1989). Little information exists of the consequences of chronic MK-801 administration despite the importance of such data in the design of therapeutic strategies. We have investigated the effect of chronic MK-801 treatment on both LCGU and ligand binding to the NMDA receptor complex.

Adult, male Sprague-Dawley rats (370-460g) received MK-801 (0.5mg/kg, i.p. twice daily) for 14 days. LCGU was measured using [^{14}C]-2-deoxyglucose autoradiography 17 hours after the last drug administration. Adjacent sections from those used for LCGU measurement were used in ligand binding studies. These sections were preincubated for 1 hour at 4°C, followed by 15 min at 30°C in an appropriate buffer: 50mM Tris/HCl, pH 7.2 for NMDA-sensitive [^{3}H]glutamate, pH 7.4 for [^{3}H]MK-801 and 50mM Tris/citrate, pH 7.4 strychnine-insensitive [^{3}H]glycine, pH 7.0 for [^{3}H]kainate binding. Incubations were with 150nM [^{3}H]glutamate (+5 µM quisqualate + 100µM 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid) at 4°C for 10min, 30nM [^{3}H]MK-801 at 23°C for 20min, 100nM [^{3}H]glycine at 4°C for 20min, and 50nM [^{3}H]kainate at 4°C for 30min. Levels of non-specific binding were determined in the presence of 150µM NMDA, 100µM MK-801, 1mM glycine and 50µM kainate respectively. Resulting autoradiograms were quantified by image analysis.

Chronic administration of MK-801 caused significant changes of LCGU only in 4 of the 74 brain areas; decreases in glucose use in frontal cortex (-10%), nucleus accumbens (-17%), layer 4 of parietal cortex (-13%) and subthalamic nucleus (-14%). Binding study of NMDA receptor complex as well as [^{3}H]kainate binding, showed no change.

	LCGU		[^{3}H]GLUTAMATE		[^{3}H]MK-801		[^{3}H]GLYCINE	
Structure	Chronic Vehicle	Chronic MK-801	Chronic Vehicle	Chronic MK-801	Chronic Vehicle	Chronic MK-801	Chronic Vehicle	Chronic MK-801
Frontal Cortex	118±3	106±2*	96±14	93±8	237±13	231±12	45±7	55±14
Nucleus Accumbens	95±3	79±2*	55±8	52± 8	146±12	150±11	45± 5	56±11
Parietal Cortex	112±3	97±3**	86±9	91±14	199±17	214± 6	50±13	62±13
Subthalamic Nuc.	85±2	73±3*	26±5	30± 4	46± 6	39± 4	22± 5	21± 7

LCGU: µmol/100g/min. Ligand binding data are expressed as pmol/g tissue. Mean ± SEM, n=5-6. *P<0.05, **P<0.005 Student's t-test.

These minimal changes in LCGU are in contrast with dramatic alteration after acute treatment. Attenuation of the effect of acute MK-801 treatment after chronic administration may not be explained simply by down-regulation of NMDA receptor complex.

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284P 2,3-DIHYDROXY-6-NITRO-SULFAMOYL-BENZ(F)-QUINOXALINE (NBQX) AS AN EXCITATORY AMINO ACID ANTAGONIST ON NEONATAL RAT HEMISECTED SPINAL CORDS

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It is well known that 6,7-dinitro- and 6-cyano-7-nitro-quinoxalinediones, DNQX and CNQX, only weakly differentiate between the actions of amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and of kainate on spinal neurones in vivo (Honore et al., 1988) and cortical neurones in vitro (Fletcher et al., 1988). These and other similar findings have led to the hypothesis that depolarisation by these two agonists is mediated via the same receptor subtype. Using NBQX, recently discovered to displace AMPA binding 30 fold more potently than that of kainate (Sheardown et al., 1990), we have examined this hypothesis on spinal neurones in vitro.

Briefly, the spinal cords were dissected from freshly killed 2-7 day old rats, hemisected and placed in a two compartment Perspex chamber so that a greased barrier fitted transversely across the thoraco-lumbar cord. DC potentials along the caudo-cranial axis of the cord could thus be recorded in response to superfusion of the lumbar cord by excitatory amino acids which were added in 5 ml aliquots.

Dose-response curves to AMPA (3-20uM) and kainate (3-30uM) were produced in the presence and absence of 100nM-3.16uM NBQX. The curves were shifted to the right in a more or less parallel manner and to a similar extent for both agonists with pA_2 values (+S.E.) of 6.7+0.2 and 6.8+0.2 respectively, a result indicating that AMPA and kainate are likely to depolarise via the same receptor subtype.

It did, however, appear that the lower end of the kainate dose-response curve was more resistant to NBQX. This was investigated in more detail by examining the effect of 3.16uM NBQX on 316nM to 3uM doses of kainate and AMPA. Although the amplitudes of responses at such low concentrations are small and hence subject to variation due to background fluctuations, it was nevertheless clear that responses to kainate were markedly less sensitive to antagonism by NBQX than those to AMPA. This result indicates that these low doses of kainate and AMPA act at pharmacologically distinct receptors.

In conclusion, the depolarising effects of AMPA and kainate on the spinal cord of neonatal rats in vitro can be attributed to their actions on pharmacologically defined receptor subtypes which have their parallel in the high and low affinity AMPA and kainate binding sites described from biochemical studies (Honore et al., 1986).

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285P AN AUTORADIOGRAPHIC COMPARISON OF D-[^{3}H]ASPARTATE AND [^{3}H]CYCLOHEXYLADENOSINE BINDING IN THE RAT VISUAL SYSTEM AFTER ORBITAL ENUCLEATION

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D-[^{3}H]Aspartate binding to sodium-dependent high-affinity uptake sites has been used to locate glutamate terminals in the CNS in studies of human neurological disorders, and in animal lesion models. A recent autoradiographic study suggests that D-aspartate binding is not restricted to presynaptic neuronal uptake sites (Greenamyre et al. 1990). Glutamate is the major excitatory neurotransmitter of primary retinal projections, which are 97% crossed at the optic chiasma of the hooded rat. [^{3}H]Cyclohexyladenosine ([^{3}H]CHA) binds to adenosine A_1 receptors located on presynaptic terminals of excitatory projections from retinal ganglion cells (Goodman et al. 1983). In the present study, we have used quantitative autoradiography to measure D-[^{3}H]aspartate and [^{3}H]CHA binding following unilateral orbital enucleation in the rat.

Adult, male, black-hooded PVG rats (250-350g) underwent unilateral orbital enucleation under 2% halothane anaesthesia. 10 days post-enucleation, D-[^{3}H]aspartate and [^{3}H]CHA autoradiography was carried out. Adjacent brain sections (20µm) were incubated in either 50mM Tris-acetate buffer (pH 7.4) containing 250nM D-[^{3}H]aspartate and 150mM sodium acetate, or in 170mM Tris-HCl buffer (pH 7.4) containing 3nM [^{3}H]CHA and 0.5 IU/ml adenosine deaminase. Non-specific binding was measured in the presence of 250µM D,L-threo-hydroxyaspartic acid (D-[^{3}H]aspartate), or 3 µM (-)-N^6-phenylisopropyladenosine ([^{3}H]CHA). Resulting autoradiograms were quantified by image analysis, with reference to [^{3}H] microscales (Amersham).

Results

D-[^{3}H]Aspartate and [^{3}H]CHA Binding 10 days Post-enucleation.

	D-[^{3}H]Aspartate (n=5)		[^{3}H]CHA (n=6)	
	Non-deprived	Deprived	Non-deprived	Deprived
Visual Areas:				
Superior Colliculus (Superficial Layer)	139.8 ± 5.6	151.4 ± 4.8	75.5 ± 6.1	39.7 ± 2.3*
Dorsal Lateral Geniculate Body	151.0 ± 14.7	146.7 ± 6.8	61.0 ± 2.3	49.8 ± 3.3*
Visual Cortex: Layer IV	205.6 ± 9.6	219.9 ± 13.4	70.7 ± 6.5	66.8 ± 4.8
Non-Visual Areas:				
Dentate Gyrus	231.1 ± 5.6	237.2 ± 46.3	62.8 ± 5.9	64.2 ± 6.7
Retrosplenial Cortex	173.9 ± 14.4	191.5 ± 34.1	44.5 ± 4.4	43.7 ± 4.9

Values are mean ± SEM ligand bound (pmol/g tissue). Statistical differences between visually-deprived (contralateral to orbital enucleation) and non-deprived hemispheres were evaluated using Student's paired t-test. *P<0.01. There were no significant inter-hemispheric asymmetries in sham-operated animals.

D-[^{3}H]aspartate binding in the visual system was unaffected by enucleation. In contrast, [^{3}H]CHA binding was significantly reduced in the primary target sites of the lesioned glutamatergic retinal efferents. The results suggest that D-[^{3}H]aspartate binding is not restricted to presynaptic sites on glutamatergic retinal efferents, and that [^{3}H]CHA binding to adenosine A_1 receptors is a more specific marker of glutamate terminals in this neuronal system.

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286P HETEROGENEITY OF BINDING SITES FOR [^{3}H]CPP ON RAT AND HUMAN BRAIN SYNAPTIC PLASMA MEMBRANES

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The NMDA receptor complex comprises multiple functional components, each with a discrete binding site. The competitive NMDA antagonist [^{3}H]CPP (3-((+)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate) is a highly specific probe for labelling NMDA sites in ligand binding studies (Olverman et al., 1986). It appears that NMDA may interact with two separate binding sites or sub-states of the receptor (Monaghan et al., 1988). In this study we have investigated the binding of [^{3}H]CPP to synaptic plasma membranes from both rat forebrain and human frontal cortex. Incubations were performed in 50 mM Tris-acetate buffer (pH 7.7) for 30 min at room temperature followed by microcentrifugation. Non-specific binding was defined using 1 mM cold CPP. Saturation analysis (2-3000 nM CPP) revealed apparent heterogeneity of binding with a two site model providing the best fit with LIGAND (Table 1).

Table 1 [^{3}H]CPP binding to rat and human brain synaptic membranes

	K_{D1} (nM)	K_{D2} (µM)	B_{max1} (pmol/mg protein)	B_{max2} (pmol/mg protein)
Rat forebrain (n=4)	80.6 ± 6.5	4.26 ± 0.94	0.72 ± 0.07	8.89 ± 1.66
Human frontal cortex (n=3)	85.7 ± 10.5	5.26 ± 0.95	0.30 ± 0.07	11.84 ± 0.70

The pharmacological specificity of binding of 20 nM [^{3}H]CPP was examined and yielded rank orders of potency as follows: CPP> (-)AP5> cis-2,4-methanoglutamate=L-glutamate> (+)AP5> NMDA> quinolinate. Hill slopes were all consistently significantly less than unity, indicating likely site heterogeneity, which may reflect proposed agonist and antagonist-preferring states of the NMDA receptor (Cotman & Monaghan, 1989).

This research is supported by Research into Ageing and the Wessex Medical Trust.

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287P MEMBRANE AND ACTION POTENTIAL RESPONSES EVOKED BY EXCITATORY AMINO ACIDS IN THE RAT THALAMUS, *IN VIVO*

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In certain brain regions, e.g. the caudate nucleus (Herrling et al., 1983), excitatory amino acids (EAA's) acting at N-methyl-D-aspartate (NMDA) receptors can elicit a burst firing pattern of action potentials; non-NMDA receptor agonists elicit responses with a more regular firing pattern. It is probable that the burst firing pattern is a product of the interaction between the intrinsic membrane properties of these neurones and the unconventional voltage-dependency of NMDA receptor-mediated responses. Since EAA receptors mediate sensory synaptic input to thalamic relay nuclei (Eaton & Salt, 1990; Salt & Eaton, 1989) and the firing pattern of these neurones is likely to be an important determinant in the appreciation of sensory stimuli, we have recorded the firing patterns of thalamic neurones evoked by iontophoretic application of EAA's, in vivo.

Intracellular recordings were made in the thalamus of urethane (1.2g/Kg I.P.) aneasthetised rats using micro-pipettes filled with 1.6M K Citrate. EAA's and their antagonists were iontophoretically applied from a multi-barrel glass electrode cemented alongside the recording pipette (Herrling et al., 1983). Both N-methyl-D,L-aspartate (NMA) and non-NMDA receptor agonists evoked a membrane depolarisation and a repetitive, regular pattern of action potential firing on all of 34 thalamic neurones tested. Burst firing patterns were never seen. Spike interval histograms constructed from the responses to EAA's show a distribution of spike interval epochs consistent with a regular firing pattern for all EAA's studied. Since NMDA receptor channel-block by Mg^{2+} is responsible for the unconventional voltage-dependency of NMDA receptor-mediated responses and thus, feasibly, the burst firing pattern seen in other brain regions, Mg^{2+} was tested for its ability to convert regular NMA-evoked thalamic firing patterns into burst firing patterns. Mg^{2+} selectively antagonised the responses evoked by NMA on all of five neurones but never revealed NMA-evoked burst firing patterns. The NMDA receptor antagonist, CPP, selectively antagonised the responses to NMA on all of 17 neurones tested.

In certain circumstances the intrinsic membrane properties of thalamic neurones are specifically geared to produce tonic firing patterns to depolarising stimuli (Steriade & Llinas, 1988). It is possible that the intrinsic membrane properties dominate the overall firing pattern, taking precedence over the characteristics of NMDA receptor-mediated depolarisation in the determination of the thalamic firing pattern; in other regions of the brain this may not be the case to the same extent. This may be important in preserving the integrity of sensory transmission through the thalamus, particularly in view of the participation of NMDA receptors in high frequency afferent input to the thalamus (Eaton & Salt, 1990; Salt & Eaton, 1989).

Supported by the MRC.

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288P NMDA INHIBITION OF PHOSPHOINOSITIDE METABOLISM IN RAT STRIATAL SLICES

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NMDA inhibits agonist-induced phosphoinositide hydrolysis in several regions of rat brain and the suggestion has been made that this inhibition may simply reflect a cytotoxic action of NMDA (Schmidt et al, 1987; Godfrey et al, 1988). In rat hippocampal slices NMDA causes a Ca^{2+}-independent inhibition of carbachol-induced accumulation of [^{3}H]-inositol phosphates ([^{3}H]-IP), which is not reversed by extensive washing (Morrisett et al, 1990). However, in rat striatum a specific biochemical response to NMDA-receptor activation has been proposed to play a key role in an interaction with dopamine to control the activity of the GABAergic projection neurones (Halpain et al, 1990). Since this implies a non-cytotoxic action of NMDA, we have examined the effect of NMDA-pretreatment on [^{3}H]-inositol incorporation into inositol phospholipids, an ATP-dependent process, and on basal and agonist-induced [^{3}H]-IP accumulation in vibratome-cut slices from rat striatum.

Measurements were made on 3 mm diameter discs of rat striatum punched from 300 μm thick vibratome-cut slices. The discs were preincubated in Krebs-Henseleit medium for 60 min at 37°C and then exposed to NMDA for 5 or 15 min, followed by washing for 30 min. The discs were then labelled with [^{3}H]-inositol for 30 min, washed, and finally treated with agonist, usually for 15 min, in the presence of 10 mM Li^+. The medium was bubbled with O_2/CO_2 (95:5) throughout. Incubations were normally terminated by addition of $CHCl_3$/methanol/HCl. [^{3}H]-Inositol incorporation into total inositol phospholipids and [^{3}H]-IP accumulation were determined by standard methods (Godfrey et al, 1988).

Treatment of striatal discs with 100 μM NMDA for 15 min, followed by 30 min washing, caused a 45 ± 2% (n=7) inhibition of [^{3}H]-inositol incorporation into phospholipids and a 48 ± 2% (7) inhibition of basal [^{3}H]-IP accumulation. The [^{3}H]-IP response to 0.25 mM carbachol was almost abolished by the NMDA pretreatment (94 ± 1% inhibition), as was that to 10 μM quisqualate (96 ± 1% inhibition), but the response to noradrenaline was much less affected (34 ± 5% inhibition). The IC_{50} for NMDA, *circa* 30 μM, was similar to that when NMDA was added concurrently with carbachol. The inhibitory effect of pretreatment with NMDA on [^{3}H]-inositol incorporation and basal [^{3}H]-IP formation was abolished when the tissue was exposed to NMDA in a Ca^{2+}-free medium, containing 1 mM EGTA, and the inhibition of the response to carbachol (18 ± 6% inhibition, 3 measurements) was greatly reduced. Reduction of the pretreatment time with 100 μM NMDA to 5 min, the conditions employed by Halpain et al (1990), still resulted in a 32 ± 5% (4) decrease in [^{3}H]-inositol incorporation, a 23 ± 3% (4) reduction in basal [^{3}H]-IP formation and an 87 ± 2% (4) inhibition of the response to carbachol. The simplest interpretation of these data is that the inhibitory action of NMDA is due to a cytotoxic action on a specific set of striatal cells, which is mediated by Ca^{2+} entry.

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289P ACTION OF L-GLUTAMATE ON NEONATAL RAT HEMISECTED SPINAL CORDS: ROLE OF EXCITATORY AMINO ACID RECEPTORS AND OF TRANSPORT PROCESSES

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On rat cortical slices in vitro and on rat spinal neurones in vivo, the excitatory actions of L-glutamate are not substantially reduced by antagonists such as D-2-amino-5-phosphonopentanoate (D-AP5), ketamine and quinoxalinediones which block responses to N-methyl-D-aspartate (NMDA), quisqualate, alpha-amino-3-hyroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate appropriately (Davies et al., 1988). The selective and potent AMPA antagonist, 2,3-dihydroxy-6-nitro-sulfamoyl-benz(F)quinoxaline (NBQX; Sheardown et al., 1990) was not, however, available at that time. Electrogenic transport systems are alternative means by which the depolarising action of L-glutamate may be effected (see Nicholls and Attwell, 1990) but such transport systems also limit its action in intact tissue (Garthwaite, 1985).

We used the hemisected spinal cord of neonatal rats to investigate this. Briefly spinal cords were dissected from freshly killed 3-8 day old rats, hemisected and placed in a two compartment Perspex chamber so that a greased barrier fitted across the thoraco-lumbar cord. DC potentials along the caudo-cranial axis of the cord could thus be recorded in response to superfusion of the lumbar cord by excitatory amino acids.

Dose-response curves for L-glutamate (100uM-1.6mM) were prepared by addition to 5ml aliquots of the superfusing artificial CSF. D-AP5 and NBQX had little effect on responses to L-glutamate. Thus D-AP5 25uM caused a shift to the right with an estimated dose-ratio of 2 whereas the same dose of D-AP5 produced a dose-ratio of 30 for depolarisations produced by NMDA. NBQX 3.16uM produced no discernible effect on dose-response curves for L-glutamate whereas those for AMPA and kainate were shifted markedly to the right (see Zeman and Lodge, 1990). This result indicates, as suggested by many earlier workers, that NMDA and AMPA receptors make only a minor contribution to the depolarising action of L-glutamate.

To investigate the role of sodium-dependent uptake processes, we examined the effect of changing the $[Na^+]$ in the superfusate from 143mM to 25mM by substituting choline chloride for sodium chloride. This resulted in relatively minor changes in responses to NMDA and AMPA, but doubled the amplitude of the response to 800uM L-glutamate and shifted the upper part of the dose-response curve for L-glutamate to the left. This result is consistent with the idea that sodium-dependent uptake processes limit diffusion of L-glutamate within the tissue but does not indicate the contribution of electrogenic uptake processes to its depolarising action in this preparation.

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290P 2,3-DIHYDROXY-6-NITRO-SULFAMOYL-BENZ(F)-QUINOXALINE (NBQX) AS AN EXCITATORY AMINO ACID ANTAGONIST ON THE RAT CORTICAL WEDGE PREPARATION

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Despite the long-standing division based on electrophysiological and biochemical studies of postsynaptic ionotropic glutamate receptors into N-methyl-D-aspartate (NMDA), kainate and alpha-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA; formerly known as quisqualate) subtypes, there is still considerable debate as to which of the non-NMDA receptors mediate the depolarising actions of several acidic amino acids (Watkins et al., 1990). In order to investigate this, we have tested the effect of the new quinoxalinedione, NBQX, which is 30-fold more potent at displacing AMPA than kainate binding to rat brain membranes (Sheardown et al., 1990), on responses of slices of rat neocortex to amino acids (Harrison & Simmonds, 1985).

Wedge-shaped 500um slices of rat neocortex were placed in a two compartment chamber so that test compounds could be administered to the grey matter side and DC recordings made across the grease seal separating this from the white matter. Superfusion of AMPA, kainate and quisqualate to the grey matter was used to construct dose-response curves in the presence and absence of NBQX.

NBQX, 100nM-10uM, displaced the curves to the right in a more or less parallel manner with a markedly greater effect on AMPA and quisqualate than on kainate, so much so that the calculated pA2 values (mean $\pm$ SD) were 7.1 $\pm$ 0.2, 7.0 $\pm$ 0.1 and 5.6 $\pm$ 0.2 respectively. 10uM NBQX was without effect on responses to NMDA. At 316nM, responses to AMPA 40uM and quisqualate 40uM were reduced by about 50% whereas that to kainate 5uM was unchanged. This 316nM dose of NBQX was therefore used in an attempt to distinguish between actions of other amino acids at NBQX-sensitive or insensitive receptors. Responses to willardiine, bromo-willardiine, and beta-oxalylamino-L-alanine (BOAA; L-ODAP) were reduced by about 50% whereas those of domoate and L-cysteate were unaffected, a result suggesting actions mediated by the AMPA and kainate receptors for the former and latter groups of compounds respectively. Effects of L-homocysteate, L-aspartate, L-homocysteinesulphinate, L-cysteinesulphinate and quinolinate were reduced by less than 10%. Responses to L- and D- glutamate, D-aspartate, D-homocysteate and beta-methyl-amino-L-alanine (BMAA) were reduced between 10 and 20%.

Such results indicate that there are two subtypes of non-NMDA receptor mediating depolarising responses of excitatory amino acids consistent with their binding to AMPA and to kainate sites in radioligand studies.

A.J.P. is an AFRC Scholar.

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291P EFFECTS OF LEAD, ZINC, AND OTHER DIVALENT CATIONS ON RESPONSES OF CORTICAL SLICES TO EXCITATORY AMINO ACIDS

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It is well established that divalent cations such as magnesium, nickel and cobalt block channels activated by N-methyl-D-aspartate (NMDA) and not those activated by alpha-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), quisqualate or kainate (see Mayer et al., 1989). It has, however, been recently shown that lead and zinc also selectively block NMDA channels on neurones in culture (Alkondon et al., 1990; Mayer et al., 1989). We have therefore compared the effects of lead and zinc with other divalent cations on a more intact system, namely the cortical wedge preparation (Harrison and Simmonds, 1985).

Wedge-shaped 500um slices of rat neocortex were placed in a two compartment chamber so that test compounds could be administered to the grey matter side and DC recordings made across the grease seal separating this from the white matter. Control responses were obtained to 40uM NMDA, 40uM AMPA and 10uM kainate before superfusing the slices with increasing concentrations of metal ions in magnesium-free artificial CSF.

Magnesium, cobalt and nickel selectively antagonised NMDA with IC50 values of approximately 600, 400 and 175 uM respectively, effects which were readily reversible on return to control medium. Lead and zinc, however, were less selective and their effects were not reversible. Lead and zinc, 1-100uM, dose-dependently reduced responses to NMDA, AMPA, quisqualate and kainate. Such reductions were also increased by length of exposure time to lead and zinc, and in all cases responses continued to decline even after returning to the control medium. Thus, after one hour of exposure to 100uM lead or zinc, responses to NMDA were reduced by 41±4% and 47±5% and those to quisqualate by 46±6% and 35±10% respectively. Cadmium, 50uM, reduced responses to NMDA by 40±8% with no significant effect on those to quisqualate; there was, however, a further reduction of this selective NMDA antagonism after returning to control medium.

In conclusion, the selective effects of lead and zinc on the NMDA receptor-channel complex observed with neurones in culture cannot be easily demonstrated in the more intact slice preparation, perhaps because of some direct neurotoxic effect?

K.H.K.L. is a supported by the Dale and Rushton Funds and A.J.P. is an AFRC Scholar.

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292P EFFECTS OF HOMOCYSTEINE ON EXTRACELLULAR LEVELS OF AMINO ACIDS IN RAT HIPPOCAMPUS

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Injection of homocysteine, an intermediate of methionine metabolism, induces convulsions in experimental animals (see Allen et al, 1986). These data are relevant to the human genetic disorder of homocysteinurea, in which patients experience convulsive episodes and have greatly elevated plasma and brain levels of homocysteine (Mudd and Levy, 1983). The neurochemical basis of homocysteine induced seizure activity is, however, not clearly defined.

We have used in vivo brain microdialysis to investigate the effect of homocysteine on extracellular levels of amino acids in the rat hippocampus with simultaneous monitoring of electroencephalogram (EEG) activity. Han-Wistar rats (250-350g; male) were anaesthetised with either Hypnorm (n = 6) or urethane (n = 6). Dialysis probes with tungsten electrode attached for EEG recordings (Sandberg et al, 1986) were implanted into the hippocampus and were perfused with modified Krebs-Ringer bicarbonate buffer at 2.5µl/min. Dialysates were collected in 15 min fractions after a 120 min equilibrium period and amino acid content was determined by high performance liquid chromatography with fluorescence detection. Homocysteine thiolactone (1200mg/kg) was administered as an intraperitoneal injection.

Alterations in EEG activity were not observed following drug injection in urethane anaesthetised animals. In contrast, 5/6 of the animals anaesthetised with Hypnorm exhibited a "desynchronisation" of EEG activity following homocysteine injection. In 2 animals, high amplitude epileptic spikes were also observed beginning 20-40 min after drug administration. In one animal, a more complex pattern of seizure activity with periods of high frequency, high amplitude spikes interspersed with periods of electrical silence was noted.

Alterations in the extracellular levels of amino acids in hippocampus were, however, noted in both groups. Aspartate levels were significantly elevated above basal levels with a maximum increase in efflux (255% ± 38% of basal efflux; $p < 0.05$) occurring 60 min following drug administration. Extracellular levels of glutamate, glutamine, and valine were not altered significantly. In animals exhibiting seizure activity (n = 3) taurine and alanine levels also rose (300-500% of basal efflux). GABA levels were reliably detected in 6/12 animals used in this study. In these animals homocysteine injection reduced dialysate GABA content below detection limit within 60 min (representing a reduction of at least 50%).

These data demonstrate that homocysteine alters the extracellular concentration of both excitatory (aspartate) and inhibitory (GABA) transmitters in rat hippocampus. The mechanism underlying these alterations is most likely to be an inhibition of pyridoxal phosphate dependent enzymes involved in the catabolism of these substances (see Allan et al, 1986).

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293P INHIBITION OF G-PROTEIN ACTIVITY IN THE RAT CORTICAL WEDGE PREPARATION MODIFIES ANTICONVULSANT EFFECTS OF GABA AND ADENOSINE

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The cortical wedge preparation has been used as a pharmacological tool to evaluate potential anticonvulsant properties of drugs. More recently, studies using this model have indicated that investigations into the role of endogenous neurotransmitters and second messengers in epilepsy may also be meaningful (O'Shaughnessy et al, 1988; McCrohan et al, 1990). In this study we demonstrate that modulations of G-protein sensitivity using pertussis toxin in vivo can be maintained in vitro in the wedge preparation. Such tissues show altered sensitivity to the endogenous anticonvulsant neurotransmitters, GABA and adenosine.

Sprague-Dawley rats were given pertussis toxin (4 µg icv). 3-5 days later the brains were removed and cortical wedges mounted in a two-compartment chamber using grease-seal techniques. Spontaneous epileptiform activity was induced by removal of magnesium. Slices taken from control rats were treated similarly. All slices were exposed to the GABA agonists muscimol (GABA-A), GABA and baclofen (GABA-B) and adenosine agonists, adenosine, 2-chloroadenosine (2-CHA) and L-phenylisopropyladenosine (L-PIA) at EC_{50} concentrations for 60 min. Effects on burst and afterpotential frequency were determined. Results are from 4-6 slices.

Pertussis toxin slightly reduced the inhibitory effects of 316 µM GABA and 100 nM muscimol, but completely abolished effects of 1 µM baclofen on both burst and afterpotential frequency ($P<0.0083$ unpaired t-test followed by a Bonferroni correction). Pertussis toxin had no effect on responses to 31.6 µM adenosine and 3.16 µM 2-CHA. There was, however, a small inhibition of 0.1 uM L-PIA-induced attenuation of afterpotential frequency: L-PIA reduced afterpotential frequency in untreated slices by 51.7 ± 2.7%, and in pertussis toxin treated slices by 29.2 ± 4.4%.

Anticonvulsant actions of GABA and adenosine are mediated through GABA-A, GABA-B and A1 adenosine receptors. Such effects are reduced by pertussis toxin and can be evaluated in vitro. These results suggest that modulation of specific G-proteins may be important in epilepsy.

CLB is an SERC scholar.
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294P UNILATERAL QUINOLINIC ACID LESION OF THE MOUSE STRIATUM: A QUANTITATIVE *IN VIVO* ASSAY FOR THE DETECTION OF PUTATIVE NEUROPROTECTIVE AGENTS

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Quinolinic acid (QUIN) is an endogenous tryptophan derivative which causes neurodegeneration by an agonist action on the N-methyl-D-aspartate (NMDA) receptor, and has been put forward as a possible factor in a number of neurodegenerative diseases (Schwarcz et al 1983).
Recent studies have demonstrated that the delayed neurodegeneration following an infusion of QUIN in rat striatum can be antagonised by the non-competitive NMDA receptor antagonist MK-801 and the selective strychnine-insensitive glycine receptor antagonist 7-Cl-kynurenic acid (7-Cl-KYNA) (Foster et al 1988, 1989). These workers revealed striatal damage using histological methods and the neurochemical parameters of choline acetyl transferase (ChAT) and glutamate decarboxylase (GAD) activity, as markers for cholinergic and GABA-ergic neurones, respectively.

The present studies were designed to investigate behaviourally, the consequences of infusing QUIN unilaterally into mouse striatum in order to produce a quantitative, in vivo model for the detection of putative neuroprotective agents. Ipsilateral rotation following amphetamine was assessed seven days after QUIN infusion. This provided a behavioural measure of the functional integrity of the lesioned striatum (Ungerstedt 1971). The ability of MK-801 and 7-Cl-KYNA to attenuate the rotational asymmetry following amphetamine was investigated.
Male CD-1 mice (28-30g) were anaesthetised using avertine and QUIN prepared in isotonic phosphate buffered saline (pH 7.4) was infused unilaterally into the striatum using standard stereotaxic techniques (1.0ul infused over a 2min period).
Seven days following surgery animals were injected with amphetamine sulphate (5.0 mg/kg i.p.) and the number of ipsilateral rotations exhibited by the animals over the following 60 min period were recorded manually by an observer who was 'blind' to the animal's pretreatment.

Inital dose response studies revealed that QUIN 75nmol produced a high and reproducible level of ipsilateral rotation following amphetamine treatment (e.g. 655.2±90.9/60min n=10) and this dose was selected for the antagonism studies. Animals which received an intra-striatal infusion of isotonic phosphate-buffered saline failed to show asymmetry following amphetamine treatment.
MK-801 (0.1-1.0mg/kg i.p. 40 min pretreat. n=7-10) and 7-Cl-KYNA (1.0-25.0 nmol co-injected with QUIN n=6-10) produced dose dependant statistically significant reductions in the number of ipsilateral rotations following amphetamine when compared to relevant control groups (e.g. MK-801 0.5mg/kg=50% reduction $p<0.05$, 7-Cl-KYNA 5 nmol= 78% reduction $p<0.001$, ANOVA followed by 't' test)

In conclusion, this study provides functional evidence that antagonism of the strychnine-insensitive glycine receptor may be a useful target for novel neuroprotective agents and it is suggested that the use of this rotational model in the mouse may provide a behavioural assay to detect such compounds.

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295P MOLECULAR NEUROANATOMY OF $GABA_A$ RECEPTORS: DIFFERENTIAL CO-LOCALIZATION OF 10 SUBUNIT ISOFORMS IN ADJACENT SECTIONS OF RAT BRAIN, SPINAL CORD AND DORSAL ROOT GANGLION

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Although a total of 11 $GABA_A$ receptor subunit isoforms ($\alpha_{1-3,5,6}$; β_{1-3}; γ_1, γ_2; δ) from rat brain have been cloned to date (see Olsen & Tobin, 1990; Ymer et al., 1990), the subunit composition of the native receptor(s) and its stoichiometry are not known. The physiological role of receptor diversity and its pharmacological implications continue to be the subject of much speculation. Since the demonstration of the cellular co-localization of receptor subunit isoforms is a prerequisite for their participation in receptor composites, we studied their mRNA distribution in adjacent sections of rat brain, spinal cord and ganglion by in situ hybridization histochemistry using ^{35}S-labelled 60-mer oligonucleotide probes. Least conserved sequences from the M_3-M_4 regions were chosen in order to prevent cross-hybridization.

In the brain, α_1, β_2 and γ_2 subunits are the most ubiquitous and co-localize in several regions (olfactory bulb mitral cells, cerebral cortex layers II-VI, pallidum, hippocampal formation, thalamus, substantia nigra reticular, colliculi, cerebellar Purkinje and granule cells, deep cerebellar and brainstem nuclei – interpositus, vestibular, facial, motor trigeminal). Other subunit mRNAs, in contrast, have a more restricted and unique distribution: olfactory bulb granule cells – $\beta_3 > \alpha_2 > \alpha_3, \alpha_5, \gamma_2, \delta$; caudate putamen – $\beta_3 >> \alpha_2, \gamma_2, \delta > \alpha_3, \beta_1, \beta_2$; hippocampus – α_3 poorly expressed, α_6, δ absent; septum, hypothalamus and medial amygdala – γ_1; dentate gyrus – α_3 poorly expressed, α_6 absent; thalamus – α_2, α_5, α_6, β_3 absent; substantia nigra compacta – α_3 ; cerebellar Bergmann glia – α_2, β_1; deep cerebellar and brainstem nuclei – α_5, α_6, β_1, δ absent. Finally, of all the subunit isoforms studied to date α_6 is the most restricted (to cerebellar granule cells). We also found evidence for receptor diversity in: the spinal cord, where the subunit isoforms α_2, β_3 and γ_2 are colocalized in motoneurons; and spinal ganglion where large sensory neurons strongly expressed i.a. the isoforms α_2 and γ_2.

We conclude that in situ hybridization histochemistry is able to identify neuronal populations in the CNS co-expressing subunit-specific mRNAs of rat $GABA_A$ receptors. It remains to be seen whether the translation of these transcript composites, after protein trafficking, leads to their insertion in neuronal membranes to form native receptors (immunocytochemical evidence using antibodies to isoform-specific oligopeptides). It will then require electrophysiological evidence to demonstrate that the appropriate pharmacology of recombinant receptor composites can be found in patch-clamped neurons in slices of the brain regions identified in this study.

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296P UPTAKE SYSTEMS SPECIFIC FOR GABA OR GLUTAMATE COEXIST ON THE SAME NERVE TERMINAL IN RAT CEREBRAL CORTEX

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Modulation of transmitter release often occurs through presynaptic receptors located on the releasing terminals. Here we show, however, that in rat cerebral cortex γ-aminobutyric acid (GABA) and glutamate (GLU) may interact modulating each other release without apparent involvement of the respective receptor types or subtypes.

Rat brain cortex synaptosomes were prepared and superfused as previously described (Raiteri et al., 1974). Synaptosomes were then exposed to GABA and the release of endogenous GLU was determined by means of HPLC analysis, after precolumn derivatization with o-phtalaldehyde. The spontaneous outflow of GLU was increased (30 - 90 %) by GABA (10 - 300 µM), reaching a plateau at 100 µM GABA (EC_{50} = 16.1 µM). Bicuculline or picrotoxin did not antagonize the effect of GABA. Muscimol or (-)baclofen (100 µM) did not increase significantly GLU efflux. The enhancement of GLU release elicited by GABA was counteracted by GABA uptake inhibitors such as SKF 100330A (Yunger et al., 1984) or nipecotic acid. Moreover, the effects of GABA diminished by lowering the Na^+ content of the superfusion medium and disappeared at 46 mM Na^+.

Conversely, when rat brain cortex synaptosomes prelabeled with ^3H-GABA were exposed to GLU in superfusion a concentration-dependent (10 - 1000 µM) increase (25 - 60 %) of the spontaneous ^3H-GABA release could be observed. A plateau was reached at 300 µM GLU (EC_{50} = 17.8 µM). N-methyl-D-aspartate, kainic or quisqualic acid, up to 300 µM, did not increase significantly the release of ^3H-GABA but D-aspartate (100 - 300 µM) did. The enhancing effect of GLU was antagonized by D-threo-hydroxy-aspartate, a blocker of the GLU uptake (Johnston et al., 1980) and prevented by omitting Na^+ ions in the superfusion medium.

It appears, therefore, that some glutamatergic terminals in rat cerebral cortex possess a carrier for the high affinity uptake of GABA whereas some GABAergic nerve endings possess a GLU transporter. Another possibility is that GABA and GLU coexist in some axon terminals.

Supported by Italian M.U.R.S.T. (40% and 60%) and Italian C.N.R.

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297P HETEROGENEITY IN THE INFLUENCE OF ALPHAXALONE ON $GABA_A$ RECEPTOR COMPLEXES

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A number of pregnane steroids, including alphaxalone, influence the operation of $GABA_A$ receptor complexes in a barbiturate-like manner (Peters *et al.*, 1988). Interestingly, the anaesthetic potency of alphaxalone differs markedly in male/female rats (Fink *et al.*, 1982) raising the possibility that its interaction with particular $GABA_A$ complexes may differ in male/female rats.

We have measured allosteric facilitation of [^{3}H] GABA binding to membranes from rat cerebellum, hippocampus and frontal cortex; regions relatively enriched in mRNA's for α_1, α_2 and α_3 - subunits respectively (Sequier *et al.*, 1988). Frozen membranes were washed four times in 50mM Tris HCl, 5mM EDTA, pH 7.4 and binding of 5nM [^{3}H] GABA in the presence of 200mMKCl was determined using a centrifugation assay with non-specific binding defined by 30μM GABA. Alphaxalone (0.03 - 10μM) increased [^{3}H] GABA binding in each region but with distinct differences in potency and magnitude of effect. In cerebellum from both male and female rats, alphaxalone caused 123 ± 12% and 115 ± 8% increase in binding at 10μM. The response of hippocampal membranes was similar (135 ± 9%) in male but not female rats where the response did not even reach threshold until 3μM. Frontal cortex membranes from female rats showed a typical 114 ± 10% increment at 10μM, but those from male rats showed a markedly greater (197± 9%) response. Interestingly, directed expression of α_3, rather than α_1 or α_2 subunit-containing complexes in a kidney cell line yields the greatest effect of another pregnane steroid on [^{3}H] flunitrazepam binding (Lan *et al.*, 1990). Gonadectomy (3 weeks previously) indicated that different $GABA_A$ complexes are influenced by both current steroidal status and previously determined sex differences. The characteristics of the alphaxalone responses may be determined by the level of expression of genes for particular subunits. In hippocampus, for example, membranes from ovariectomised rats showed a typical response of 127 ± 8% at 10μM, suggesting that circulating ovarian steroids in intact animals had been responsible for the lesser effect there. In contrast, the distinctive male/female responses in frontal cortex membranes were little affected by gonadectomy. The processes regulating the receptor elements that determine this effect of alphaxalone are clearly very complex.

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298P MODIFICATION OF THE $GABA_A$ RECEPTOR COMPLEX BY HANDLING AND ELEVATED PLUS-MAZE EXPOSURE

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We have recently reported that the handling procedures involved in chronic i.p. treatment in rats abolish the acute anxiolytic effect of diazepam (DZP) as measured in the elevated plus-maze test of anxiety (Brett and Pratt, 1990). Work by Biggio's group has demonstrated differences in $GABA_A$ receptor binding between rats habituated to the handling procedures preceding decapitation and unhandled rats (Corda and Biggio, 1986). The present experiments were therefore designed to investigate whether the handling procedures involved in our chronic experimental protocol effected a change at the $GABA_A$ receptor complex which might explain the inability of DZP to produce an acute effect in handled rats.

Male Long-Evans rats were either injected daily with saline (i.p.) for 28 days ('handled') or left unhandled except for routine cage cleaning ('unhandled'). On day 29 the rats were sacrificed, the brain removed and cortex and hippocampus stored frozen at -20°C. The GABA shift was determined in well-washed membranes by taking the ratio of the IC_{50}s of DZP to displace 1nM [^{3}H]-flumazenil (FZL) in the absence and in the presence of GABA (100μM). In cortex, the GABA shift was significantly increased in the handled animals (unhandled 2.93 ± 0.08; handled 3.43 ± 0.11; $P<0.01$). This was due to a significantly lower IC_{50} in the presence of GABA in the handled animals than in the unhandled group (unhandled 13.8 ± 0.03 nM; handled 11.8 ± 0.6 nM; $P<0.05$). The IC_{50} in the absence of GABA was not changed, nor was the specific [^{3}H]-FZL binding in the absence of diazepam. No differences were observed between the groups for any parameter in hippocampal membranes. 28 days handling appears therefore not to alter the benzodiazepine (BZ) receptor itself, but in cortex to increase the coupling between the BZ and GABA receptors. Alternatively, the increased GABA shift may reflect an alteration in $GABA_A$ binding itself.

However, similar investigations on the cortex of handled and unhandled male PVG rats which had been exposed to a 15 min test in the elevated plus-maze immediately prior to sacrifice failed to demonstrate any significant difference in specific [^{3}H]FZL binding, GABA shift (unhandled 2.22 ± 0.20; handled 2.15 ± 0.12) or IC_{50}s for DZP displacement of [^{3}H]FZL. This forms preliminary evidence that the difference in binding characteristics between handled and unhandled rats is abolished or mitigated by the stress of the behavioural test. It may, however, be that after such an exposure subtle regional changes persist which might be difficult to determine in homogenate binding, but may be apparent using receptor autoradiography.

We thank the Wellcome Trust for financial support.

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299P CHARACTERISTICS OF THE GABA RECEPTOR COMPLEX IN CULTURED CORTICAL NEURONES AFTER REPEATED MIDAZOLAM EXPOSURE

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There is no clear consensus as to whether components of the $GABA_A$ receptor complex are altered in tolerance to and dependence on benzodiazepines (BZs). In this investigation we have examined the binding characteristics of the $GABA_A$ receptor complex of neurones in primary culture after chronic BZ exposure.

In initial experiments, the binding characteristics of the $GABA_A$ receptor complex were investigated in homogenates of cultured embryonic rat cortical cells using a modification of Segal (1983) and compared to values obtained from neonate and adult tissue. In saturation experiments, Scatchard analysis of ^{3}H-flunitrazepam binding, using clonazepam (2μM) to define non-specific sites, revealed similar apparent affinities in cultured cells, neonate and adult tissue (K_D: 3.22±0.14, 3.26±0.24 and 3.74±0.28nM respectively) However, the number of binding sites increased during ontogenic development (B_{max} : 0.10±0.01, 0.23±0.02 and 0.98±0.13 pmol/mg protein, respectively).

Cortical cells were maintained in culture for 7 days and then exposed to midazolam (10^{-7}M). Control cultures were exposed to fresh medium. Subsequently, the medium (with or without midazolam) was changed every 48 hours and after 7 days exposure the cells were homogenised for radioligand binding. In a parallel study, adult male Sprague Dawley rats (250-280g) were treated with midazolam (1mg/kg i.p.) or vehicle (distilled water) twice daily for 7 days. Cortex from these animals was also homogenised for radioligand binding. The GABA shift was determined in well-washed membranes by taking the ratio of the IC_{50}s of diazepam to displace 1nM ^{3}H-flumazenil in the absence and in the presence of GABA (100μM). Neither exposure of cortical cells to midazolam (10^{-7}M) for 7 days, nor treatment of adult rats with midazolam (1mg/kg b.d.) for 7 days resulted in a change in the GABA shift values as compared to vehicle treated controls (cultures (n=5): control 2.5±0.2; midazolam 2.3±0.1; adult (n=5-6): control 4.1±0.3; midazolam 4.8±0.4).

These studies demonstrate that the binding characteristics of rat embryonic cortical cells in culture are consistent with the presence of central type BZ receptors which are coupled to $GABA_A$ receptors since GABA positively modulates BZ binding. Exposure to a mild chronic midazolam dosage regime did not appear to alter the coupling between the $GABA_A$ and the BZ receptors. However, further studies could, profitably examine different treatment regimes and different components of the $GABA_A$ receptor complex.

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300P GABA-MEDIATED GIANT DEPOLARIZING SYNAPTIC POTENTIALS INDUCED BY ZINC IN ADULT RAT HIPPOCAMPAL PYRAMIDAL NEURONES

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Zinc is concentrated within synaptic vesicles in the nerve terminals of mossy fibres synapsing with CA3 neurones in the hippocampus. Stimulation of this pathway can cause the release of Zn (Assaf & Chung, 1984), but the physiological and pharmacological action of Zn on postsynaptic neurones in intact brain slices remains unclear.

Intracellular recordings were obtained from pyramidal neurones in 400µm thick adult rat hippocampal slices maintained at 30°C in a standard Krebs solution. Bath application of 50-300µM Zn for 3 - 6min to neurones at the resting membrane potential (-68±10mV, mean±SD, n=52), induced the appearance of giant spontaneous depolarizations in a dose-dependent manner. These intracellular events consisted of a large and long-lasting depolarization (20-40mV amplitude, 1-5s duration) associated with increased input conductance. Spontaneous depolarizations recurred at an interval of approximately 2 min (117±62s, n=52) and disappeared within 2-4 min on washout of Zn. Excitatory and inhibitory postsynaptic potentials (EPSP-IPSP) were evoked by stimulation of the Schaffer collateral-commissural pathway when recording from CA1 neurones, and by stimulation of mossy fibres when recording from CA3 neurones. In the presence of Zn, orthodromic stimulation now evoked a large depolarizing synaptic potential in both CA1 and CA3 cells, which eventually occluded the original EPSP-IPSP complex. Spontaneous and evoked depolarizing events induced by Zn were blocked by bath-applied tetrodotoxin (1µM).

The reversal potential for the Zn-induced depolarization was -53±7mV (n=4) or -28±3mV (n=3) with K acetate- or KCl-filled microelectrodes, respectively, which was similar to the reversal potential for ionophoretically-applied GABA responses obtained in the same cells. Zn (50-300µM) did not block bath- or ionophoretically-applied GABA responses recorded from adult hippocampal neurones. Furthermore, Zn-induced depolarizations were enhanced by pentobarbitone (100µM) and reversibly blocked by the $GABA_A$ receptor antagonist, bicuculline (10µM, n=8). The induction of these large depolarizing synaptic potentials, which are apparently mediated by $GABA_A$ receptor activation, suggests that Zn may have a physiological and/or pathological role in hippocampal synaptic transmission.

Supported by the MRC and the Wellcome Trust.

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301P DIFFERENT ACTIONS OF PENTOBARBITAL AND BENZODIAZEPINES ON GABA-RESPONSES ON VAGAL NERVES, DORSAL ROOTS AND SPINAL SYNAPSES *IN VITRO*

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In vitro both barbiturates (BARBs) and benzodiazepines (BZDs) have been shown to potentiate the GABA-response (Simmonds, 1981), but the way of potentiation is different (Study & Barker, 1981). The present study investigated the effects of pentobarbital (PB) and diazepam (DIAZ) on picrotoxin (PTX) or bicuculline (BIC) antagonized GABA-responses on vagal nerves, dorsal roots and spinal synaptics.

Desheathed cervical vagus from male Wistar rats (170-270 g) and dorsal roots or intact hemicords from neonate rats (2-5 days) were superfused, 1 ml/min at 25 °C. D.C.-polarity was continuously recorded. Synaptic potentials were evoked by electrical stimulation of a dorsal root. The dorsal root-dorsal root potential (DR-DRP) was recorded from an adjacent dorsal root; the dorsal root-ventral root potential (DR-VRP) was recorded from the segmental ventral root.

On both vagal nerves and dorsal roots GABA (3-30 μM) induced depolarizations, which showed pronounced desensitization at doses higher than 30 μM. 30 μM GABA was used as a working maximum. On the vagus GABA (30 μM) produced a response of 0.26 mV $\pm$ 0.02 (38), with an EC_{50} of 7.62 μM $\pm$ 0.36 (36). On the dorsal root the maximal response was 1.22 mV $\pm$ 0.17 (14), with an EC_{50} of 6.14 μM $\pm$ 0.41 (14). Table 1 shows the effects of PTX (vagus: 17 μM, dorsal root: 3 μM), BIC (vagus and dorsal root: 10 μM), PB (vagus and dorsal root: 50 μM) and DIAZ (vagus and dorsal root: 10 μM). PTX (7.5-20 μM) reduced the the DR-DRP (n=10) and potentiated the DR-VRP (n=11). PB (50-100 μM, n=3), in contrast to DIAZ (10 μM, n=3), reduced the PTX effect on both DR-DRP and DR-VRP.

Table 1: Logshifts of the GABA dose-response curve.

	PTX	PTX/PB	PTX/DIAZ	BIC	BIC/PB	BIC/DIAZ
vagus	1.03 $\pm$ 0.05 (10)	-0.03 $\pm$ 0.03 (3)	1.19 $\pm$ 0.08 (4)	1.00 $\pm$ 0.07 (9)	0.41 $\pm$ 0.10 (9)	1.05 $\pm$ 0.07 (3)
dorsal root	0.71 $\pm$ 0.11 (8)	0.41 $\pm$ 0.04 (5)	0.68 $\pm$ 0.05 (3)	0.71 $\pm$ 0.04 (6)	0.44 $\pm$ 0.04 (3)	0.91 $\pm$ 0.14 (3)

All results are expressed as mean $\pm$ s.e.mean and number of experiments.

The different effects of DIAZ and PB may be explained by differences in coupling to the $GABA_A$-receptor, between binding sites for BZDs (Corda et al., 1989; Saano et al., 1989) and BARBs (Evans, 1979).

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302P AN ETHOPHARMACOLOGICAL STUDY OF THE EFFECTS IN MICE OF THE BENZODIAZEPINE RECEPTOR INVERSE AGONIST, DMCM

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Inverse agonists at the benzodiazepine receptor site, which inhibit transmission at the $GABA_A$-supramolecular complex, induce pro-convulsant and anxiogenic effects (File & Baldwin, 1987; Pellow & File, 1984). The present study, using ethopharmacological procedures, examines the behavioural effects in mice of the inverse agonist, methyl-6, 7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM) when given at subconvulsant dose levels.

The DMCM (Research Biochemicals) was dissolved in 0.01 N HCl and made up to volume with 0.85% NaCl immediately before use. It was given at two dose levels (0.4 mg/kg and 1 mg/kg, i.p.) to pair-housed adult male CD1 mice. Control mice each received an equivalent volume of solvent (i.p.). At 35 minutes after injection, behaviour of each animal during a 7 minute encounter with an unfamiliar partner in a neutral cage was recorded by ethological procedures.

In dominant mice, DMCM at 0.4 mg/kg significantly decreased aggressive behaviour (Duration, s., mean $\pm$ S.E., Control 54.6 $\pm$ 7.8, Treated 30.1 $\pm$ 4.8, $P < 0.05$) and increased the duration of flight relative to aggression received (Ratio of flight/aggression, Control 0.7 $\pm$ 0.1, Treated 2.6 $\pm$ 0.3, $P < 0.05$). There was a small but not significant reduction of aggression in dominant mice, given DMCM at 1 mg/kg. No other behavioural changes were detectable in dominant mice, and DMCM had no effect on the behaviour of subordinate animals.

Thus, in mice, DMCM at a low dose level appears to specifically influence agonistic behaviour, decreasing aggression and increasing the magnitude of the flight response relative to aggression received. This may correspond to increased fearfulness, a shift of behaviour from "fight" to fear-induced "flight". Reduction of aggressive behaviour has also been shown to occur in rodents after treatment with low doses of benzodiazepine partial inverse agonists, such as FG 7142 and beta-CCE (Thiebot et al, 1988) and more studies of the relationship between these anti-aggressive effects and the anxiogenic and pro-convulsant actions of benzodiazepine inverse agonists are clearly needed.

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303P THE EFFECT OF CHRONIC LITHIUM, AMITRIPTYLINE AND ELECTROCONVULSIVE SHOCK ON DIHYDROPYRIDINE BINDING TO RAT BRAIN MEMBRANE PREPARATIONS

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Verapamil is an antagonist at 'L'-type voltage-sensitive calcium channels (L-VSCC's) and is reported to be clinically effective in the treatment of manic-depressive illness (Dubovsky et al., 1986). Chronic administration of verapamil to rats down-regulates the L-VSCC in brain (Panza et al., 1985), as measured by binding of dihydropyridines (DHP). If the clinical effect of verapamil is due to such down-regulation then perhaps other treatments with a similar clinical profile also down-regulate this calcium channel. This study measured DHP binding with (+)-[^{3}H]-PN 200-110 in rat cortex and hippocampus following chronic administration of lithium, ECS and amitriptyline. Male Sprague-Dawley rats (200-230 g) were treated with lithium, amitriptyline and ECS as indicated in Table 1. Preparation of rat brain membranes and DHP binding were carried out as previously described with minor modifications (Glossman & Ferry, 1985). Results are shown in Table 1, each value being the mean (± s.e.mean) of 6 independent experiments.

TABLE 1: CORTICAL AND HIPPOCAMPAL DIHYDROPYRIDINE BINDING	Bmax (fmol/mg protein)	Kd (n M)	Bmax (fmol/mg protein)	Kd (n M)
	CORTEX		HIPPOCAMPUS	
Control 1. - saline, 14 days i.p. once daily	220 ± 15.7	0.31 ± 0.06	274 ± 10.4	0.35 ± 0.04
Amitriptyline -10 mg/kg, 14 days i.p. once daily,	238 ± 9.2	0.32 ± 0.06	300 ± 16.3	0.29 ± 0.07
Control 2. -lithium free diet for 28 days	189 ± 4.5	0.28 ± 0.08	250 ± 11.3	0.27 ± 0.04
Lithium -0.1% lithium carbonate diet for 28 days	191 ± 5.2	0.29 ± 0.09	257 ± 4.3	0.33 ± 0.06
Control 3. - halothane anaesthesia x 5	189 ± 12.3	0.23 ± 0.05	238 ± 22.1	0.22 ± 0.05
ECS Treated- ECS under halothane anaesthesia x 5	187 ± 6.1	0.23 ± 0.05	217 ± 16.1	0.23 ± 0.04

All three treatments had no significant effect on DHP binding. This study confirms earlier findings that ECS does not affect DHP binding (Gleiter & Nutt, 1989). The findings of this study suggest that down-regulation of the L-VSCC's is not a common action of treatments which are effective in manic-depressive illness.

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304P AUTOMATION OF THE RAT ELEVATED X-MAZE TEST OF ANXIETY

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The rat elevated X-maze is a valuable model for the detection of potential anxiolytic and anxiogenic agents (Handley & Mithani, 1984; Pellow et al., 1985). It has been demonstrated to detect compounds with widely different mechanisms of action. However, this test does have the disadvantages of being very labour intensive and only allows a small number of measures to be assessed at one time. Here, we describe an automated system developed to overcome these drawbacks of the model.

A standard X-maze was divided into 9 zones, being the two halves of each arm and the centre of the maze. Infrared sensors were mounted underneath each zone which were interfaced to an IBM compatible PC. The system allows up to 4 mazes to be connected in this way. Software was developed to detect which zone the animal is in and to use this information to calculate the number of zone entries and the time spent in each zone.

Male Hooded rats (250-300g, Olac, U.K.) were transferred to the experimental room the evening before the test. The anxiolytic chlordiazepoxide (CDP; suspended in 1% w/v carboxymethylcellulose) and the anxiogenic FG 7142 (dissolved in 0.9% NaCl) were administered i.p. 40 and 30 min before test respectively. The test was started with the animal positioned in the centre of the maze facing an open arm. The following measures were recorded for 5 min: (1) time on open arms (TOA), (2) time on ends of open arms (TEOA), (3) entries into open arms (EOA) and (4) entries into ends of open arms (EEOA).

	CDP (mg/kg)					FG 7142 (mg/kg)			
	0	0.3	1	3	10	0	10	20	30
TOA	29±6	43±10	40±7	70±8*	89±19**	51±8	21±6**	9±4**	7±2**
TEOA	9±3	19±7	18±5	37±6**	49±10**	21±4	6±3**	1±1**	0**
EOA	5±1	7±2	6±1	9±1*	9±11*	6±1	3±1**	2±1**	2±0**
EEOA	1±0	4±1	3±1	5±1**	6±1**	3±1	1±0**	0**	0**

Data is shown as the mean ±SEM of at least 10 animals per group. $^{*}P<0.05$, $^{**}P<0.01$ compared with controls (ANOVA followed by Dunnett's t-test). Opposite effects were observed in the closed arms (data not shown).

In conclusion, the automated X-maze system described here is able to detect both anxiolytic and anxiogenic actions. It has the advantages of allowing up to 4 mazes to be run simultaneously and can measure a large number of parameters in each maze. Thus, the automated system should prove to be a useful tool in future anxiety studies.

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305P HANDLING STRESS: BENZODIAZEPINE BINDING AND BEHAVIOUR IN THE ELEVATED PLUS-MAZE TEST OF ANXIETY IN THE RAT

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In previously unhandled rats the stress of handling decreased $GABA_A$ binding in the cortex; this was counteracted by habituating rats to handling (Biggio 1983); flumazenil blocked this effect of stress indicating that it was mediated by the benzodiazepine (BDZ) receptor. We therefore compared BDZ binding in extensively washed cortical membranes from previously unhandled rats (UNH) and those habituated to handling for 21 days (H-HAB), based on the method of Giorgi et al., (1989). Biggio's group has suggested that handling stress leads to the release of an inverse agonist for the BDZ receptor and therefore UNH rats might be expected to display behaviour indicating increased anxiety compared with H-HAB rats. We therefore compared UNH and H-HAB rats in elevated plus-maze, both undrugged and after sub-anxiolytic doses of diazepam; all rats were tested 30 min after transport to a novel room and i.p. injection.

Table 1. [^{3}H] flunitrazepam binding (fmols/mg protein) and Elevated plus-maze (% number of open arm entries)

	[^{3}H] flunitrazepam binding (fmols/mg protein)		Elevated plus-maze (% number of open arm entries)		
	UNH	H-HAB		UNH	H-HAB
Control	123.2 ± 9.7	189.1* ± 21.2	Control	14.45 ±2.42	32.57* ±3.61
Diazepam^{+} (30 μM)	168.8** ± 8.0	197.9 ± 23.5	diazepam 0.125 mg/kg	22.89 ±4.02	22.61 ±3.80

** P<0.01, * P<0.05 compared with control UNH group

$^{+}$ 30 min. pre-incubation followed by 9 washes

Table 1 shows that the stress of handling decreased [^{3}H]-Flunitrazepam binding only in UNH rats which agrees with the finding of Mennini et al., (1988) that handling stress results in internalisation of benzodiazepine receptors. In 3 out of 4 experiments, the UNH rats were **more** anxious than the H-HAB rats in the plus-maze, see table 1. However, in one experiment where the UNH rats had high scores in the plus maze (38.4% entries onto the open arms) the effects of handling were to reduce scores on the open arms to 28.1%. Thus handling may have bi-directional effects, depending on the baseline response level. The direction of the effects of a sub-anxiolytic dose of diazepam (0.125mg/kg) was also different according to the baseline response, increasing scores if they were low and decreasing scores if they were high (analysis of variance, handling x diazepam interaction, P<0.05). However, in general, the direction of the effects of handling habituation to increase open arm scores in the plus maze is consistent with the increased binding reported here.

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306P ANXIOLYTIC AND SEDATIVE PROPERTIES OF CHLORMETHIAZOLE IN THE RAT

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Chlormethiazole (CMZ) is used clinically as a sedative hypnotic and anticonvulsant and may also have anxiolytic properties (Haslam, 1976). CMZ interacts with the Cl^- ionophore of the $GABA_A$-receptor (Harrison and Simmonds, 1983; Cross et al., 1989). This study investigated the effects of CMZ in rats tested in locomotor activity monitors and in the elevated plus-maze test of anxiety. The benzodiazepine (BDZ)-receptor antagonist, flumazenil and the $GABA_A$-receptor antagonist, picrotoxin were used to examine the mechanism of action of CMZ.

Naive male hooded Lister rats (170-350g) were used. In the plus-maze CMZ (15 mg/kg i.p.) significantly increased the percentage of time spent on the open arms indicating anxiolytic-like activity (vehicle=30.5±6.5,n=7; CMZ=57.9±6.2,n=7; t=3.1,p<0.01). Administration of CMZ (20 or 40 mg/kg) twice daily for 5 days produced no tolerance to CMZ's anxiolytic effect (% time on open arms: vehicle=28.5±4.5,n=7; chronic CMZ 20mg/kg=57.0±5.8,n=7; t=3.9,p<0.01). Flumazenil 4 or 10 mg/kg i.p. did not reverse the effect of CMZ.

In the locomotor activity monitors, acute CMZ (20 & 40 mg/kg i.p.) significantly reduced the number of photobeam breaks in a 10 min test (vehicle=6119±332,n=7; CMZ 20mg/kg=1173±293,n=7; CMZ 40mg/kg=103±51,n=8; ANOVA p<0.01). There was no tolerance to this effect after administration of CMZ (20 or 40 mg/kg) for 5 days (vehicle=4909±327; CMZ 20mg/kg=1542±240; CMZ 40mg/kg=92±33; ANOVA p<0.01). Flumazenil (6 mg/kg) and picrotoxin (0.5-4 mg/kg) did not reverse the sedative effect of CMZ.

CMZ has anxiolytic and sedative properties in rats which persist after 5 days treatment. Results with flumazenil and picrotoxin suggest that CMZ's effects are not directly mediated via the BDZ- or picrotoxinin-binding sites on the $GABA_A$ receptor.

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307P BEHAVIOURAL EFFECT OF ACTIVATION OF NEUROKININ RECEPTORS IN THE MEDIAN RAPHÉ NUCLEUS OF THE RAT

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Both neurokinin (NK)-like immunoreactivity (Ljungdahl et al, 1978) and NK binding sites (Saffroy et al, 1988) have been reported within the raphe nuclei of the rat. Injection of senktide, an NK-3 receptor-selective agonist, into the median raphe nucleus (MRN) increases locomotor activity (LMA) (Paris & Lorens, 1989). However, the effects of selective NK-1 and NK-2 agonists have not been investigated in this model. The aim of the present study was to determine which NK receptor subtype(s) are associated with the increase in LMA, using peptidase-resistant and receptor-selective NK-1 (GR73632; Hagan et al, 1989), NK-2 (GR64349; Hagan et al, 1989) and NK-3 agonists (senktide).

Male Random Hooded rats (200-250g, Glaxo) were surgically implanted with chronic indwelling stainless-steel guide cannulae (23 guage), aimed at the MRN. One week following surgery, animals were habituated to the test cages (1h), then vehicle or peptides under test were infused into the MRN (1 μl in 60s) and LMA recorded for 1h by means of infra-red photocell beams. In separate studies, after MRN infusion rats were placed in a novel open field arena (60x 60x 30cm high) and behaviours recorded for 30min by an observer blind to the drug treatments. The doses of agonists used were : GR73632, 0.01-1000pmol; GR64349, 0.001-30pmol and senktide, 0.001-100pmol.

Agonists selective for all three receptors were found to increase LMA. The NK-1 agonist, GR73632, only increased LMA at high doses (100-1000pmol). The NK-2 agonist, GR64349, increased LMA over a narrow dose-range (0.01-10pmol). For both of these agonists, the level of stimulated activity was less than that observed with the NK-3 agonist, senktide, which produced a marked increase in LMA (0.1-100pmol) : vehicle treatment, 779 ± 188 (n=11); 100pmol senktide, 2785 ± 234 (n=8) counts/60min; $p<0.001$). In the open field, no obvious behaviours were noted after treatment with the NK-1 or NK-2 agonists. In contrast, senktide caused grooming, yawning, chewing and straub-tail.

These data suggest that NK-3 receptors are the predominant NK receptor subtype involved in NK-induced LMA in the MRN. This is in agreement with autoradiographical studies which have shown a high-density of NK-3 receptor binding sites in this brain region (Saffroy et al, 1988).

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308P CALCIUM DEPENDENCE OF RESPONSES TO ENDOTHELIN-1 AND NORADRENALINE IN RAT ISOLATED SUPERIOR MESENTERIC ARTERIAL BED DEVOID OF ENDOTHELIUM

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Since the initial characterization of endothelin-1 (ET-1; Yanagisawa *et al.*, 1988), conflicting reports have appeared concerning the role of extracellular Ca^{2+} (Ca^{2+}_o) in ET-1-induced vasoconstriction in vascular preparations. This study aimed to examine the role of Ca^{2+}_o in the pressor response to ET-1 in the rat isolated superior mesenteric bed using nifedipine. The effects of this Ca^{2+} channel blocker on responses to the peptide were compared with those on vasoconstriction induced by noradrenaline (NA).

Male Wistar rats (200-350 g) were anaesthetized (60 mg kg^{-1} sodium pentobarbitone) and heparinized (1000 u kg^{-1}). The superior mesenteric arterial bed was removed and perfused at 2 ml min^{-1} with Krebs-Henseleit solution containing 10 μM indomethacin as described by Randall *et al.* (1989). The endothelium was removed by perfusion with 0.3% CHAPS (3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propane sulphonate; Sigma) in distilled water for 90 s (Hiley *et al.*, 1987) before determination of the dose/response curve for either ET-1 (Peptide Institute, Osaka, Japan) or NA (Sigma). Ca^{2+}-free medium was obtained by omitting Ca^{2+} (normally 2.5 mM) from the perfusing medium and adding 0.2 mM EGTA (ethyleneglycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid; Sigma); 60 min equilibration was allowed after changing to Ca^{2+}-free medium. Nifedipine (Bayer UK) was dissolved in 30% ethanol, 5% polyethylene glycol, 5% glycerol (v/v/v) in water to give a 1 mM solution which was diluted with perfusion medium to give the required concentrations. Dose/response curves were analysed by fitting to a logistic equation (Randall *et al.*, 1989).

ET-1 (1-1000 pmol) or NA (0.3-94 nmol) had dose-dependent pressor effects in a Ca^{2+}-containing medium. Neither induced pressor responses in the Ca^{2+}-free medium. Addition of Ca^{2+} to tissues previously in a Ca^{2+}-free medium, restored the responses to ET-1. 1 μM nifedipine significantly reduced the maximum pressor responses (R_{max}) elicited by either agonist (Table 1). 0.2 μM nifedipine also reduced the R_{max} to NA and both concentrations significantly decreased the ED_{50} of NA.

Table 1. Effect of nifedipine on the pressor responses to ET-1 and NA

	Endothelin-1			Noradrenaline		
Nifedipine	ED_{50} (pmol)	R_{max} (mmHg)	*n*	ED_{50} (nmol)	R_{max} (mmHg)	*n*
Control	171 ± 71	64.5 ± 13.2	15	2.57 ± 0.22	48.5 ± 1.2	8
0.2 μM	87 ± 17	40.7 ± 6.7	12	1.82 ± 0.28*	28.9 ± 1.4***	4
1.0 μM	97 ± 23	14.3 ± 1.8**	11	0.82 ± 0.19***	11.5 ± 0.5***	5

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ relative to control (Student's unpaired t-test).

Overall, these results show that the vasoconstrictor responses to ET-1, and to NA, in the rat mesenteric arterial bed are largely dependent on Ca^{2+}_o in the perfusion medium of the preparation. Furthermore, it appears that significant quantities of Ca^{2+} enter through dihydropyridine-sensitive channels since the responses to both agonists were affected in a similar manner by nifedipine.

We thank the MRC for a Research Studentship for SAD and for a project grant. ET-1 was the kind gift of the Marion Merrell Dow Research Institute, Strasbourg.

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Yanagisawa M., Kurihara H., Kimura S., Tomobe Y., Kobayashi M., Mitsui Y., Yazaki Y., Goto K. & Masaki T., 1988, *Nature,* **332,** 411-415.

309P EFFECTS OF ENDOTHELIN ON TRACHEAL SUBMUCOSAL GLAND SECRETION AND EPITHELIAL ALBUMIN TRANSPORT

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Endothelin (ET) is a potent vasoconstrictor peptide which was first isolated from the conditioned medium of cultured aortic endothelial cells. ET potently contracts vascular and bronchial smooth muscle, but its effects on airway submucosal gland secretion and epithelial function are unknown. We have examined the effect of ET on baseline and stimulated (methacholine and phenylephrine) mucus volume, lysozyme (as a marker for submucosal gland serous cell secretion) and albumin (active epithelial transport; Webber & Widdicombe, 1989) outputs, and on potential difference (epithelial integrity) across the ferret trachea in-vitro. Air-filled tracheas mounted in organ baths were bathed on the submucosal side with Krebs buffer containing a combination of BSA (4mg/ml) and fluorescent BSA (40μg/ml). Secretions were carried by gravity and mucociliary transport to the lower or laryngeal cannula and periodically withdrawn for analysis of volume, lysozyme and albumin (fluorescence). All drugs were added to the submucosal buffer.

ET (0.01-100nM) had no significant effect on baseline mucus volume, lysozyme or albumin outputs, but produced a concentration-dependent increase in negativity of PD (-0.02±0.16 to -3.2±0.05mV, n=8, mean±s.e.m). ET (0.01-100nM) produced concentration-dependent reductions in methacholine (-2.8±12.1 to -49.7±6.7%, n=8) and phenylephrine (-13.1±16.2 to -60.2±8.7%, n=8) induced mucus volume outputs, and in methacholine (-15.1±6.8 to -44.9±9.1%) and phenylephrine (-7.5±16.2 to -73.7±7.7%) induced lysozyme outputs. ET also produced a less potent reduction in methacholine (-6.9±12.4 to -22.4±7.5%) and phenylephrine (-1.0±6.9 to -39.1±16.9 %) induced albumin outputs. The PD during methacholine and phenylephrine-induced secretion was made significantly more negative by ET (-0.01±0.07 to -0.5±0.08mV and -0.13±0.05 to -3.1±0.7mV, respectively).

Thus, ET has no effect on baseline submucosal gland secretion and epithelial albumin transport, but makes baseline PD more negative. ET inhibits methacholine and phenylephrine-induced submucosal gland secretion (including serous cell secretion), and to a lesser extent epithelial albumin transport.

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310P DIFFERENCE IN BLOOD PRESSURE RESPONSIVENESS TO ENDOTHELIN 1 (ET-1) BETWEEN CONSCIOUS RATS OF THE MILAN HYPERTENSIVE (MHS) AND NORMOTENSIVE (MNS) STRAINS

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Endothelin might be involved in the development or maintenance of hypertension, since patients with essential hypertension had elevated plasma level of ET-like immunoreactivity (Saito et al., 1990), and SHR rats showed larger hypotensive and smaller hypertensive responses than normotensive controls (Wright & Fozard, 1990). The purpose of this study was to compare the sensitivity of MHS and MNS rats to intravenous ET-1 administration.

Male, 10-12 wk old animals were used 24 h after catheter implantation. Mean arterial pressure (MAP) was recorded from femoral artery. Drugs were administered by bolus injection (femoral vein). Pressure changes are Δ mm Hg.

Administration of ET-1 elicited a dose-dependent biphasic effect in both strains (Table 1). At the lowest dose, ET-1 hypotensive effect was larger in MNS. Conversely, MHS were more sensitive to hypertension at all doses tested.

Table 1 - Sensitivity of MHS and MNS rats to ET-1-induced hypotension (-Δ mm Hg) and hypertension (+Δ mm Hg)

	MHS				MNS			
Dose (pmol Kg^{-1})	basal MAP	-Δ	+Δ	n	basal MAP	-Δ	+Δ	n
200	*125 ± 3.5	-3.6 ± 3.6*	31 ± 5.3*	5	102 ± 1.9	-18 ± 2.6	+ 15 ± 2.3	6
400	*128 ± 5.2	-23 ± 4.3	45 ± 2.2*	7	104 ± 3.0	-21 ± 3.1	+ 25 ± 4.4	5
800	*121 ± 1.8	-32 ± 5.7	51 ± 7.9*	5	106 ± 2.9	-36 ± 5.5	+ 34 ±5.7	4

* $p < 0.05$ versus MNS, by 2-way ANOVA.

Similar differences in strain responsiveness were found with nitroprussiate (NP) and angiotensin I. NP produced larger hypotension in MNS than MHS: ED25 (μg kg^{-1}) were 14.8 (12.5-19.3) and 23.2 (20.5-26.0) respectively. Maximum hypertensive effect of angiotensin I was achieved with 125 pmol kg^{-1} in MHS (Δ50.4 ± 9.4) and 250 pmol kg^{-1} in MNS (48.5 ± 2.7). Thus, MHS responsiveness to ET-1 is opposite to that described for SHR, suggesting that the peptide plays a different role in the two strains. This agrees with previous evidence showing that pressor mechanisms in MHS and SHR are different (Bianchi et al., 1990).

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311P PHARMACOLOGICAL ACTIONS OF ENDOTHELIN-1 ON MYOFIBROBLASTS *IN VITRO*

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Endothelin-1 (ET-1) is a peptide which was isolated from porcine cultured endothelial cells and displays potent vasocontrictor activity as shown by numerous in vivo and vitro studies (Yanagisawa et al.,1988). The mechanism(s) of action of ET-1 is being studied extensively in tissues containing predominantly smooth muscle cells. However, the activity of this peptide has not been examined in tissues populated with contractile fibroblast-like cells termed 'myofibroblasts'. In this communication we investigated the effects of ET-1 and its calcium ion requirements using myofibroblasts from the rat testicular capsule (Gorgas and Bock, 1974).

Male Wistar rats (250-350g) were killed by cervical dislocation and the testicular capsules were removed. The capsules were placed in an aerated (95%O_2 and 5%CO_2) Krebs-Henseleit solution and strips measuring 3x20mm were prepared. The preparations were arranged in a superfusion system and allowed to equilibrate for 2 hours, using a flow rate of 2mlmin^{-1} maintained at 37°C. Dose response curves were constructed with ET-1 (1-320pmoles, n=7) and in the presence of calcium antagonists verapamil ($1x10^{-6}$-$1x10^{-4}$M, n=8), nitrendipine ($1x10^{-7}$-$1x10^{-5}$M, n=8), diltiazem ($1x10^{-6}$-$1x10^{-4}$M, n=8), and flunarizine ($1x10^{-6}$-$1x10^{-4}$M, n=8): and Bay K8644 ($1x10^{-9}$-$1x10^{-7}$M, n=8), a calcium channel agonist. ET-1 was dissolved in phosphate buffered saline (PBS).

ET-1 produced dose dependent and reversible contractions of the testicular capsule myofibroblasts. The magnitude of the ET-1 responses ranged from 10mg at 1 pmole to 60mg at 320pmoles, whilst the duration of the responses ranged from 1 to 30 minutes at the respective doses used. No contractions or relaxations were observed with the PBS. In the antagonism studies verapamil, nitrendipine, diltiazem, flunarizine all inhibited ($p<0.05$ to $p<0.01$) in a dose dependent manner the ET-1 induced contractions. Bay K8644 had no effects on the ET-1 induced responses.

In conclusion, these findings show that a) myofibroblasts are sensitive to ET-1 and b) the responses are partially dependent on extracellular calcium ion influx. These findings are similar to those reported in smooth muscle preparations. Furthermore, the pharmacological sensitivities of 'naturally' occurring myofibroblasts to ET-1 are similar to those in induced granulomatous tissues (this meeting).

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312P STIMULATION OF CALCIUM INFLUX IN A7r5 SMOOTH MUSCLE CELLS ACTIVATED BY KCL OR ENDOTHELIN-1

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A7r5 aortic smooth muscle cells respond to a variety of agonists, including vasopressin and 5-HT, with an elevation in cytosolic free Ca^{2+} ($[Ca^{2+}]i$) as a consequence of both Ca^{2+} influx to the cells and Ca^{2+} mobilisation from internal stores. Such changes in $[Ca^{2+}]i$ are presumed to result from the generation of $Ins(1,4,5)P_3$ and $Ins(1,3,4,5)P_4$. In addition, A7r5 cells have L-type voltage operated calcium channels (VOCs) (Van Renterghem et al., 1988) which would allow Ca^{2+} entry to the cells under conditions of depolarisation. Here we have investigated changes in $[Ca^{2+}]i$ in A7r5 cells stimulated with depolarising potassium or with endothelin-1 (ET-1) an agonist proposed to act specifically through activition of VOCs (Yanagisawa et al. 1988).

A7r5 cells grown on glass coverslips, were loaded with fura-2 by incubation with 2μM fura-2-AM (30 min at 37°C) in serum free DMEM containing 20 mM Hepes and 0.1% bovine albumin. After loading, cells were kept at room temperature in the dark until use. Each coverslip was rinsed with 3 ml of Hepes (10 mM) buffered tyrodes (pH 7.4) and placed on the base of a plastic microbath containing 100μl of buffer at 23 ± 2°C. Fura-2 fluorescence (505 nm emission) was collected from confluent patches of 10-20 cells using a Nikon diaphot microscope (x20 obj.) coupled to a Spex dual excitation (340 nm and 380 nm) fluorimeter. $[Ca^{2+}]i$ was calculated as originally described by Grynkiewicz et al.(1985). Addition of 20 mM KCl to A7r5 cells caused an instantaneous increase in $[Ca^{2+}]i$ from 100nM to 352 ± 12nM (mean ± SE n = 22) followed by a maintained (175-250 nM) response. This response to 20 mM KCl was completely blocked or reversed by EGTA (1-2 mM) or nifedipine (0.1-1μM) whilst lemakalim (1-30μM) was ineffective. Readdition of $CaCl_2$ in excess of any EGTA present during the KCl response returned $[Ca^{2+}]i$ to over 200nM and induced rapid spiking. Similarly addition of 10nM ET-1 to A7r5 cells evoked $[Ca^{2+}]i$ spiking to around 300-400nM that was completely blocked or reversed by nifedipine (1μM) or EGTA (1mM). However 100-300nM ET-1 caused some Ca^{2+} mobilisation from internal stores.

These results suggest that A7r5 cells have L-type VOCs that can enable a 2-4 fold increase in $[Ca^{2+}]i$, but do not have ATP-dependent potassium channels. ET-1 either directly or indirectly can activate these VOCs to raise $[Ca^{2+}]i$ and initiate spiking activity although the underlying biochemical mechanisms for this effect of ET-1 remain unknown.

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313P THE ACTIONS OF ENDOTHELIN-3 IN THE RAT PULMONARY VESSELS

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The endothelins are a group of physiologically active peptides. However, the pharmacological properties of endothelin-3 (ET-3) in the rat pulmonary circulation are not well defined. In vitro, ET-3 causes a concentration-dependent constriction of pulmonary artery rings, (Rodman et al,1989) but in vivo, low doses of ET-3 elicit vasodilation in the rat isolated perfused mesentery (Warner et al,1989). The vasodilation in this preparation is inhibited by the endothelium-derived relaxing factor synthesis inhibitor L-N^G-monomethyl-L-arginine (L-NMMA) indicating the involvement of EDRF release by ET-3 in this response (Fukuda et al,1990). In this study we have examined the actions of ET-3 in both the isolated blood perfused rat lung and also in isolated rat pulmonary artery rings. For the isolated lung experiments male Wistar rats (300-350g) were anaesthetised, exsanguinated, and their lungs perfused with blood at 38°C at a constant 18ml/min and ventilated with either normoxic (21%O_2, 5%CO_2, 74%N_2), or hypoxic (3%O_2, 5%CO_2, 92%N_2) gas. For the in vitro experiments, rat large pulmonary arteries were cut into rings and mounted over two parallel tungsten wires; one being fixed the other attached to a force-displacement transducer. After a 60min equilibration period drug(s) were added cumulatively. In isolated lungs, at baseline perfusion pressures, ET-3 caused a dose-dependent increase in pulmonary artery pressure (PpA). Pretreatment with 10nM ET-3 significantly slowed the response time to maximal PpA generated by hypoxic vasoconstriction (HPV). The relaxation by 10nM ET-3 in hypoxia constricted lungs was 100±8% compared to 65±8% in $PGF_{2\alpha}$ constricted lungs. This relaxation of the HPV response was unaffected by indomethacin (1μM) but was inhibited by L-NMMA (30μM) pretreatment, the relaxation being attenuated to 63±4% of the HPV response. In vitro, ET-3 caused a concentration-dependent relaxation of artery rings preconstricted with $PGF_{2\alpha}$ (EC_{50} = 8.8±1nM). The maximal relaxation seen with ET-3 was 31±6% at 0.1μM. The response at this concentration was biphasic resulting in a large and sustained contraction (121±5% of initial $PGF_{2\alpha}$ response). Pretreatment with L-NMMA (100μM) attenuated the vasodilation to ET-3, the maximal relaxation being 66±4% at 0.1μM. ET-3 was without a vasodilator action in vessel rings denuded of endothelium, the constrictor action remaining.
These results indicate that ET-3 is a potent vasodilator of both large arteries and resistance vessels, being more potent in the latter. The inhibition of this relaxation in both vessel types by L-NMMA indicates the vasodilator effect of ET-3 is associated with the release of EDRF.

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314P HYPOXIA CAUSES AN UNUSUAL ENDOTHELIUM-INDEPENDENT CONTRACTION IN THE ATHEROSCLEROTIC RABBIT AORTA

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Hypoxia causes relaxations partly dependent on the endothelium (E) in isolated rabbit aortas (Coburn et al., 1986 ; Pohl & Busse, 1989). Atherosclerotic lesions profoundly alter vascular responsiveness of arterial tissues (Verbeuren et al., 1986). The present investigation was designed to compare the effects of hypoxia on aortas obtained from control and hypercholesterolemic rabbits.

The studies were performed on aortas of White New Zealand rabbits fed either a control or a cholesterol rich (0.3%) diet for 20 weeks ; some data were obtained on aortas of hyperlipidaemic (WHHL) rabbits. Rings of the aortas were mounted in organ chambers filled with Krebs-Ringer solution for isometric tension recording. Rapid switching from normoxic (95% O_2-5% CO_2) to hypoxic (95% N_2-5% CO_2) conditions could be achieved. The experiments were performed in parallel on control and atherosclerotic aortas.

During a contraction induced with noradrenaline (NA ; 0.3 μM) hypoxia relaxes control aortas with E (by 92.1 ± 1.4% ; n = 6 ; Figure 1) and to a slightly lesser extend those without E (by 82.2 ± 4.9% ; n = 6). In atherosclerotic aortas with E hypoxia causes a transient enhancement of the contraction to NA (by 41.5 ± 15.4% ; n = 6) followed by a relaxation (by 44.6 ± 17.6% ; n = 6 ; Figure 1) ; E removal does not alter these responses. Similar results are observed during contractions to 5-hydroxytryptamine (5-HT), endothelin and prostaglandin $F_{2\alpha}$. In the absence of vasoconstrictor agents, hypoxia causes a transient contraction (0.96 ± 0.30 g ; n = 5) in atherosclerotic but not in control aortas. In aortas from WHHL rabbits, hypoxia transiently potentiates the contractions to NA and 5-HT. The hypoxia-induced contractions in the atherosclerotic aortas were not inhibited by adrenergic or thromboxane receptor antagonists (phentolamine, 1 μM and BM 13505, 10 μM), by cyclooxygenase and lipoxygenase inhibitors (indomethacin, 10 μM and nordihydroguairetic acid, 10 μM), by scavengers of oxygen derived radicals (superoxide dismutase, 150 U / ml and catalase, 1 200 U / ml) or by a Ca^{2+} entry blocker (verapamil, 1 μM).

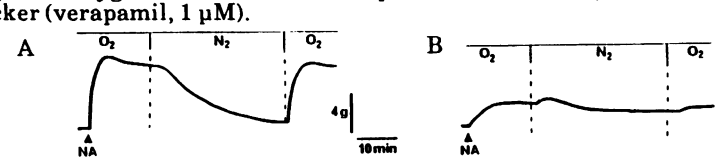


Figure 1 : Hypoxia relaxes a control aorta (A) and causes a transient contraction followed by a relaxation in an atherosclerotic aorta (B). Note that the initial contraction to NA (0.3μM) is smaller in (B).

Our data suggest that the hypoxic relaxation noted in control rabbit aortas is partly E-dependent. Such an E-dependent relaxation is absent in the atherosclerotic aortas where hypoxia causes a transient contraction, the nature of which remains to be determined. One possible explanation is that hypoxia liberates a constrictor substance in the wall of the atherosclerotic artery.

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315P EFFECT OF METHYLENE BLUE (MB) UPON ENDOTHELIUM-DEPENDENT AND -INDEPENDENT VASODILATOR RESPONSES IN THE DOG HINDLIMB

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Basal blood flow and endothelium-dependent vasodilator responses to bradykinin (Bk) and acetylcholine (ACh) in the canine skinned hindlimb can be selectively reduced by L-N^G-nitroarginine methyl ester (L-NAME), suggesting the involvement of endothelium-derived relaxing factor, EDRF (White et al; 1990). To study this possibility further, we have examined the effects of MB, a reported inhibitor of guanylate cyclase (Gruetter et al., 1981) on endothelium-dependent (Bk, ACh) and -independent (sodium azide (Az), adenosine (Ad)) vasodilator responses in the skinned, denervated hindlimb of the beagle dog pretreated with indomethacin (5mg/kg i.a.) (White et al; 1990). During a saline infusion (0.5ml/min i.a.) single submaximal bolus doses of Bk (100ng), ACh (100-300ng), Az (10μg) and Ad (1mg) were administered locally into the external iliac artery until responses were reproducible and then repeated during infusion of MB (1,3 and 10mg/min i.a.). Results are shown in Table 1.

Table 1 Effect of MB on basal iliac artery blood flow and agonist-induced peak vasodilator responses in the dog hindlimb.

Dose of MB (mg/min)	Blood Pressure (mmHg)	Responses expressed as % of pre-MB values				
		Basal Flow	Bk	ACh	Az	Ad
Saline	121±8	100	100	100	100	100
1	124±9	94±5	97±10	133±29	83±4*	87±9
3	127±11	88±5	92±12	122±29	65±7*	86±14
10	129±14	105±3	77±13	88±23	39±8*	69±12

Values represent mean ± s.e.m. (n=4). *Significantly different from pre-MB values ($p<0.05$; paired t-test)

MB (1 to 10mg/min) had no significant effect on blood pressure, basal iliac artery blood flow, or on the vasodilator responses to Bk, ACh or Ad, but dose-dependently reduced the response to the nitrovasodilator, Az, possibly through inhibition of guanylate cyclase in the skeletal muscle vasculature. However, the finding that the L-NAME-sensitive (EDRF) component of basal flow and Bk and ACh responses was unaffected by MB suggest that (i) the effect of MB on Az responses may be unrelated to the inhibition of guanylate cyclase, or (ii) EDRF responses in the canine hindlimb may not be mediated via guanylate cyclase stimulation.

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316P ACETYLCHOLINE, BUT NOT SUBSTANCE P AND BRADYKININ, PRODUCES ENDOTHELIUM-DEPENDENT RELAXATION OF RABBIT ISOLATED FEMORAL ARTERY

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Vascular endothelial cells produce endothelium-dependent relaxing factor (EDRF), the continuous generation and endogenous release of which is thought to regulate basal vascular tone (Moncada et al., 1989). EDRF can also be released in response to stimulation of pharmacological receptors with appropriate agonists (Angus & Cocks, 1989). Acetylcholine (ACh) has been reported to evoke EDRF-mediated relaxation in the rabbit isolated femoral artery (Vedernikov & Hellstrand, 1989). However, no data concerning the effects of other agonists on EDRF responses has been reported for this tissue. We have therefore compared the effects of ACh with those of the calcium ionophore (A23187), bradykinin (BK), substance P (SP), calcitonin gene-related peptide (CGRP) and 5-HT in rabbit femoral artery rings.

Isolated femoral artery rings (2-3mm) taken from New Zealand White rabbits (2-3kg) were either left intact (+E) or rubbed gently to remove the endothelium (-E) and then immersed in Krebs solution (containing 3μM indomethacin), gassed with 5% CO_2 in O_2 and maintained at 37°C. After elevating resting tone with either phenylephrine (Phe, 10μM) or the thromboxane A_2 mimetic, U46619 (U-19, 0.1μM), the effects of ACh (0.01-100μM), A23187 (0.001-3μM), BK (0.01-10μM), SP (0.001-10μM), CGRP (0.1-1000nM) and 5-HT (0.1-100nM; examined in the presence of ketanserin (1μM)) were studied. All agonists were compared with ACh in separate rings (n=4-8).

Both ACh and A23187 produced concentration-dependent relaxation in +E rings, preconstricted with either Phe or U-19, but were inactive in -E rings. For example, in U-19-pretreated tissues, ACh and A23187 produced maximal relaxations (mean ± s.e.mean) of 89 ± 4% (n=14) and 82 ± 7% (n=8) and with an EC_{50} (the concentration of agonist producing 50% relaxation; geometric mean (95% confidence intervals)) of 0.22 (0.11-0.43)μM and 1.04 (0.56-1.91)μM, respectively. In contrast, BK, SP, CGRP and 5-HT did not relax +E rings preconstricted with either Phe or U-19, with 5-HT and BK further increasing the tone of the tissues. BK and SP also failed to cause relaxation when tested in the presence of the peptidase inhibitors bestatin (100μM) and phosphoramidon (1μM). Responses to ACh and A23187 appeared to be mediated via EDRF release, since these responses were reduced by the EDRF synthesis inhibitors, L-N^G-monomethyl arginine (1-100μM) and L-N^G-nitroarginine (0.1-10μM). Furthermore, this inhibition was reduced by prior incubation with L-arginine (100μM).

In conclusion, the endothelium of rabbit femoral artery responds to ACh to release EDRF. In contrast, receptors for BK, SP, CGRP and 5-HT which mediate EDRF release would appear to be absent in this tissue.

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317P RENAL CORTICAL BLOOD FLOW IS MODULATED BY EDRF

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Endothelium-derived relaxing factor (EDRF), characterized as nitric oxide (NO), is released by the endothelium and contributes to the control of blood pressure. However, relatively little is known about its role in the control of renal vascular resistance *in vivo* (Tolins *et al.*, 1990). N^G-monomethyl-L-arginine (MeArg) and N^ω-nitro-L-arginine (NO_2Arg), two inhibitors of EDRF synthesis, were used to elucidate the role of basal and stimulated release of EDRF in the renal cortical vasculature.

Male Wistar rats (195-462 g), anaesthetised with sodium-thiopental (120 mg kg^{-1}), were cannulated for drug infusion and the measurement of mean arterial blood pressure (MAP) and heart rate (HR). The kidney was exposed via a mid-line laparotomy and renal cortical blood flow (RCBF) expressed as perfusion units (PU) was continuously monitored using a standard laser Doppler flow probe placed on the ventral surface of the capsule of the kidney. All animals received indomethacin (5 mg kg^{-1}).

MeArg (1 or 3 mg kg^{-1} min^{-1}) significantly decreased RCBF by 29 + 7% or 35 + 5%, respectively. This decrease was significantly reversed by a co-infusion of a three fold excess of L-arginine (L-Arg; n=5-6; $p<0.05$). Similarly, NO_2Arg (30 or 300 μg kg^{-1} min^{-1}) attenuated RCBF by 21 + 4% or 53 + 4%, respectively, an effect which was reversed by a 100 fold excess of L-Arg (n=4-5). Most importantly, the low dose of NO_2Arg, while having no pressor effect, considerably reduced RCBF, indicating that basal release of EDRF is relatively more important for the maintenance of renal cortical blood flow than for the regulation of blood vessel tone in other vascular beds. In addition, both MeArg (3 mg kg^{-1} min^{-1}) and NO_2Arg (300 μg kg^{-1} min^{-1}) inhibit endothelium-dependent (acetylcholine 10 μg kg^{-1} min^{-1}) increases in RCBF in an L-Arg reversible manner, but not endothelium-independent (dopamine 10 μg kg^{-1} min^{-1}) increases.

Thus, MeArg and NO_2Arg inhibit basal and acetylcholine-stimulated release of EDRF in the renal cortical vasculature suggesting that endogenous EDRF formation plays an important role in the local regulation of renal cortical blood flow.

This work was supported by a grant from Glaxo Group Research Limited.

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318P INHIBITORY EFFECTS OF INTERLEUKIN-8 ON NO-MEDIATED BIOLOGICAL ACTIVITIES

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Interleukin-8 (IL-8), a novel cytokine activating polymorphonuclear neutrophils (PMN), has been shown to trigger chemotaxis, shape change, degranulation and the respiratory burst (Baggiolini et al., 1989). PMN can in addition generate NO (Palmer et al., 1987), which is, however, destroyed by simultaneously generated superoxide anions (Gryglewski et al., 1986). In order to get further insight into this interaction we have investigated the effect of IL-8 on the biological activity of endothelium-derived relaxing factor (EDRF)/NO released from rat peritoneal PMN and rat aortic endothelium. Peritoneal PMN were harvested from male Wistar rats 12 hours after i.p. injection of oyster glycogen. Release of EDRF/NO was determined by inhibition of platelet aggregation (Rimele et al., 1988). Addition of 4-10 x 10^5 cells to washed human platelets inhibited thrombin (60-120 mU ml^{-1})-induced aggregation by 70±17%. Preincubation of PMN with 100 nM IL-8 for 1 min caused reduction of their antiaggregatory activity to 16±13%. The effect of IL-8, which did not affect platelet aggregation directly, was transient. Thus, preincubation of PMN with 100 nM IL-8 for more than 8 min did not significantly affect their antiaggregatory activity (56±29%) as compared to untreated cells. Superoxide dismutase (330 U ml^{-1}) protected the antiaggregatory activity of PMN from the IL-8 mediated inhibition.

Rat aortic strips with intact endothelium precontracted with 100 nM phenylephrine responded to carbachol with concentration-dependent relaxations. Preincubation of the vascular tissues with 50 nM IL-8 for 1 min reduced the carbachol (50-100 nM)-induced relaxations by 15-67%. Restoration of the carbachol-induced relaxations was observed immediately after washing with IL-8 free Krebs-Henseleit buffer.

The results suggest that IL-8 at the concentrations used induces decrease of EDRF/NO activity probably as a consequence of release of superoxide anions from rat neutrophils and endothelial cells (Baggiolini el al., 1989; Gryglewski et al., 1986). The altered ratio EDRF/superoxide anions released by neutrophils and endothelial cells might be important for the expression of proinflammatory effects of IL-8.

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319P EFFECTS OF N^G-NITRO-L-ARGININE METHYL ESTER (L-NAME) ON REGIONAL HAEMODYNAMICS IN CONSCIOUS, DIABETIC WISTAR RATS

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Since there is evidence for endothelial dysfunction in diabetes mellitus we investigated the effects of inhibiting nitric oxide production with L-NAME (Moore et al., 1990) in conscious, unrestrained, Wistar rats made diabetic with streptozotocin (STZ). Twenty eight days following i.p. injections of STZ (70 mg kg^{-1}) or saline, regional haemodynamics were measured in conscious Wistar rats chronically instrumented with pulsed Doppler flow probes and intravascular catheters. All surgery was carried out under sodium methohexitone anaesthesia (60 mg kg^{-1} i.p.). Flow probes were implanted at least 2 weeks and catheters at least 1 day before measurements were made. The results are summarized in the Table.

Table 1: Basal haemodynamics and the effects of L-NAME (values are mean ± s.e. mean; control n=9, STZ n=11).

	Resting values		Change after L-NAME	
	Control	STZ	Control	STZ
Heart rate (b min^{-1})	337±10	291±13*	-91±13	-55±4*
Mean arterial blood pressure (mm Hg)	114±4	109±3	39±3	31±3*
Vascular conductance (arbitrary units)				
Renal	80±6	105±10*	-34±5	-39±10
Mesenteric	75±7	114±16*	-41±6	-55±8
Hindquarters	37±3	26±4*	-17±2	-10±2*

[*P<0.05 STZ versus control, Mann Whitney U Test]

Since the changes in renal and mesenteric vascular conductances following L-NAME were similar in control and STZ-treated rats, it is unlikely the resting renal and mesenteric vasodilatations in the latter were due to increased production of and/or sensitivity to nitric oxide. However, the resting hindquarters vasoconstriction and diminished response to L-NAME are consistent with decreased production of and/or sensitivity to nitric oxide in that vascular bed in STZ-treated rats.

Acknowledgement: R.J. Kiff is supported by Wellcome Research Laboratories.

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320P REVERSAL OF ENDOTOXIN-INDUCED IMPAIRMENT OF RESPONSIVENESS TO SYMPATHETIC STIMULATION BY L-NITROARGININE METHYL ESTER AND VASOPRESSIN IN PITHED RATS

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There is evidence that increased nitric oxide production from L-arginine mediates endotoxin-induced impairment of vascular responsiveness to noradrenaline (Fleming et al, 1990). In the present work we have examined the effect of endotoxin on pressor responses to noradrenaline (NA) and to electrical stimulation of the spinal sympathetic outflow (ESS) in pithed rats. We have compared the ability of nitro-L-arginine methyl ester (L-NAME) and vasopressin to modify the endotoxin-induced impairment of responsiveness. Rats were pithed under ether anaesthesia and ventilated artificially (100% O_2; 48 strokes/min, 1ml/100g). Rectal temperature was maintained at 37 ± 0.5°C. Blood pressure was recorded from a common carotid artery and drugs were injected via the jugular veins. *E. coli* endotoxin (Difco O55:B5, 250 ug $kg^{-1}h^{-1}$) or 0.9% saline were infused i.v. after a 20 min equilibration. Dose-response curves to NA or responses to ESS (1-10 Hz, in the presence of gallamine, 10mg kg^{-1} h^{-1}) were commenced at 1h. Infusion of endotoxin produced a fall in mean arterial blood pressure which was maximal at 1h (control 55 ± 2.2 mmHg (n = 14); endotoxin 45 ± 2.0 mmHg, (n=13); P<0.01). L-NAME (4mg kg^{-1}, 15 min before ESS or injection of NA) produced an increase in blood pressure of 15 mmHg in either control or endotoxin-treated rats, which was prevented by infusion of L-arginine (50 mg kg^{-1} min^{-1}). The endotoxin-induced fall was prevented by vasopressin coinfusion (0.64 i.u.$kg^{-1}h^{-1}$) which in control animals increased blood pressure by 23 mmHg. ESS produced frequency dependent increases in blood pressure, which were depressed by endotoxin (e.g. increase in pressure in response to 3Hz, mmHg, control 30 ± 2; endotoxin 8 ± 2, P<0.01). L-NAME or vasopressin each restored responsiveness. However, each agent augmented responsiveness in control rats (e.g. response to 3 Hz was increased by L-NAME to 44 ± 6 and by vasopressin to 56 ± 5 mmHg), this augmentation explaining the restoration of responsiveness in endotoxin-treated animals (Two way ANOVA, interaction, n.s.). A similar pattern was seen with dose response curves to NA. Thus, either L-NAME or vasopressin restored responsiveness to NA in endotoxin-treated rats, which appeared to be explained by the augmented responsiveness seen in controls (e.g. E_{max50} ,μg kg^{-1}, control 3.5 ± 1; endotoxin 20 ± 1.8, L-NAME 0.94 ± 0.2; endotoxin + L-NAME 2.8 ± 0.9; effect of endotoxin P<0.05; effect of L-NAME P<0.01; interaction n.s.). Although endotoxin-induced impairment of vascular reactivity may be mediated by increased nitric oxide production, the effect of L-NAME in restoring responsiveness is mimicked by vasopressin, at least in the pithed rat. Both agents may work by increasing the availability of Ca^{2+} to the vascular contractile apparatus.

M.O. Guc is supported by the Turkish High-Education Council and Hacettepe University. The work is supported by an EEC grant.

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321P CHARACTERIZATION OF ENDOTHELIUM-DEPENDENT RELAXATIONS INDUCED BY NEBIVOLOL IN CANINE CORONARY ARTERIES

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The effects of nebivolol, a competitive, preferential $beta_1$-adrenoceptor antagonist was investigated using isolated canine coronary rings. Rings (4-5mm) of canine left anterior descending coronary arteries (LAD). were dissected free of connective tissue and suspended for isometric tension recording, in 25 ml organ chambers containing modified Krebs-Ringer bicarbonate at 37°C bubbled continuously with a carbogen mixture of 95% O_2 and 5% CO_2. In some rings, the endothelium was removed mechanically. Presence or absence of a functional endothelium was verified by the response to acetylcholine $3x10^{-7}$M. The rings were stretched to their optimal resting tension (8 gms) and were contracted with $4.0x10^{-6}$M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Nebivolol $1x10^{-6}$M to $1x10^{-5}$M produced concentration-dependent relaxations which were significantly greater ($P<0.05$) with a functional endothelium (relaxation to $1x10^{-5}$M nebivolol was 70% with endothelium versus 20% without). In contrast to its $beta_1$-adrenoceptor blocking activity, in which d-nebivolol is over 100 times more potent than l-nebivolol (Pauwels et al., 1988), l-nebivolol was more potent than d-nebivolol at producing the endothelium-dependent relaxations (<0.05). The responses to nebivolol were not affected by incubation with the following: indomethacin ($1x10^{-5}$M), phentolamine ($5x10^{-6}$M), propranolol ($3x10^{-6}$M), alprenolol ($1x10^{-5}$M), or methysergide ($3x10^{-6}$M), indicating the response was not mediated by a cyclooxygenase metabolite or by alpha- or beta-adrenoceptors or 5-HT_1, or 5-HT_2 receptors. The endothelium-dependent responses to nebivolol were inhibited by nitro-L-arginine ($3x10^{-5}$M) and methylene blue ($1x10^{-5}$M); suggesting the involvement of EDRF(s).

Subthreshold concentrations of nebivolol ($3x10^{-7}$M) for the endohelium-dependent relaxations potentiated the endothelium-dependent relaxations to adenosine 5'-diphosphate (ADP), causing a parallel leftward shift of 3 to 5 fold in the concentration-response curve to ADP. These effects of nebivolol, may partially explain its more favorable hemodynamic profile compared with other beta-adrenoceptor antagonists (Van de Water et al., 1988 a, b); in particular why l-nebivolol potentiates the anti-hypertensive effects of d-nebivolol and other beta-adrenoceptor antagonists at concentrations which occupy only a negligible percentage of beta-adrenoceptors.

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322P LYSOPHOSPHATIDYLCHOLINE AND ENDOTHELIUM-DEPENDENT RELAXATIONS IN THE RABBIT AORTA

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Oxidised low density lipoproteins (OXLDL) inhibits endothelium-dependent relaxations mediated by nitric oxide (EDNO) in the rabbit aorta (Jacobs *et al.*, 1990). It has been proposed that a lipid factor, possibly lysophosphatidylcholine (LPC), which is generated during the oxidative modification of LDL, is the inhibitory component of OXLDL (Kugiyama *et al.*, 1990). Although LPC (synthetic 1-palmitoyl LPC) inhibits relaxations, the inhibitory properties of OXLDL do not correlate with their LPC content (Plane *et al.*, 1990). Paradoxically, LPC also evokes endothelium-dependent relaxations (Saito *et al.*, 1988). In this study, we have further examined the effects of LPC in the rabbit aorta.

Isolated thoracic aortic rings from 6-month old New Zealand white rabbits were mounted in organ baths as previously described (Jacobs *et al.*, 1990). In some experiments the endothelium was removed. Tissues were precontracted with phenylephrine and relaxed to cumulative doses of ACh, glyceryl trinitrate (GTN) or LPC. In experiments investigating the inhibitory effects of LPC, LPC was added to the baths immediately before precontraction.

LPC (10μM) inhibited maximal relaxation to ACh by 94.1±5.9% (n=4). This effect was partially reversible (10.0±2.0%, n=4). Endothelium-dependent relaxations evoked by A23187 were also inhibited. In tissues with endothelium intact, LPC (10μM) caused a reversible rightward shift in the dose response curve to GTN (approximately 10-fold). In contrast, LPC (10μM) had no effect on GTN-evoked relaxations in rings denuded of endothelium. In precontracted endothelium intact rings, LPC (0.1μM-50μM) caused dose dependent relaxations which were inhibited by N^{ω}-Nitro-L-Arginine (100μM). At concentrations greater than 50μM, LPC removed the endothelium as confirmed by *en face* silver staining. No relaxations were observed in endothelium denuded rings.

These results indicate that LPC causes a large inhibition of endothelium-dependent relaxations whereas it has a small effect on GTN-evoked relaxations only when the endothelium was present. This suggests that the site of action of LPC is at the endothelium and not the soluble guanylate cyclase of vascular smooth muscle. In contrast to its inhibitory effects, LPC also causes the release of EDNO, although the mechanism is unknown. In conclusion, LPC causes paradoxical effects in the rabbit aorta which have not been reported for OXLDL (Jacobs *et al.*, 1990).

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323P EVIDENCE THAT LOCALLY GENERATED NITRIC OXIDE HAS A MODULATORY ROLE IN THE CONTROL OF MICROVASCULAR BLOOD FLOW IN RAT DORSAL SKIN

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We have examined the possibility that nitric oxide (NO) modulates unstimulated blood flow and vasoconstrictor responses to endothelin-1 (ET-1) and phenylephrine (PE) by using a selective inhibitor of NO generation; L-N^G-nitro arginine methyl ester (L-NAME; Gardiner et al., 1990). Local blood flow was measured in response to intradermal (id) injections of agents (100μl volumes), over 15 min, using a multiple site xenon clearance technique (Williams 1976), modified for use in the anaesthetised male Wistar rat (150-250g).

The effect of L-NAME was investigated by comparing sites injected id with Tyrode solution ± L-NAME. L-NAME, but not the enantiomer D-NAME, inhibited blood flow (L-NAME 100nmol, 34.5±3.2% decrease n=5; mean ± s.e.mean; D-NAME 100nmol, 5.9±4.6% decrease n=5). Further, L-arginine (600μmol/kg iv) significantly attenuated the inhibitory effect of L-NAME (saline iv; L-NAME 37.5±1.6% decrease in blood flow n=6: L-arginine iv; 15.8±5.9% decrease n=8 $p<0.05$). L-NAME also inhibited blood flow when the id injection volume was reduced to 20μl and a second inhibitor of NO generation had similar inhibitory effects.

The effect of L-NAME (100nmol) on the constrictor responses induced by ET-1 and PE; is shown in table 1. Results (mean ± s.e.mean) are calculated from % decrease in blood flow compared with that at Tyrode sites in each rat.

Table 1.

Agents	Control	+L-NAME	n	p (Modified t-test)
Tyrode	0.0	33.8±3.1	27	---
ET-1 (1pmol)	23.6±5.7	43.0±3.3	11	<0.05
ET-1 (3pmol)	41.9±2.7	76.4±4.6	6	<0.001
ET-1 (10pmol)	80.3±4.4	83.8±4.1	3	ns
PE (0.1nmol)	30.9±4.7	69.5±3.6	9	<0.001
PE (1nmol)	70.7±6.5	85.5±2.9	9	<0.05
PE (10nmol)	85.0±3.4	87.0±3.9	9	ns

In conclusion L-NAME inhibited blood flow and potentiated the vasoconstrictor effects of submaximal doses of endothelin and phenylephrine. This suggests that locally generated NO could play an important role in maintaining blood flow and in modulating microvascular responses to vasoactive agents in the cutaneous microvasculature.

EL holds a BHF Ph.D studentship.

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324P MODIFIED LOW DENSITY LIPOPROTEINS IMPAIR INHIBITION OF PLATELET AGGREGATION INDUCED BY ENDOTHELIAL CELLS

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Endothelium-derived relaxing factor (EDRF) is a potent inhibitor of platelet aggregation and has been identified as nitric oxide (NO) or a closely related compound. Low density lipoproteins (LDLs) inhibit endothelium-dependent vasodilation (Andrews et al., 1987; Kugiyama et al., 1990). However, the effect of modified LDLs on inhibition of platelet aggregation by endothelial cells (ECs) has not been studied. We have now investigated the effects of native LDL (N-LDL), acetylated LDL (Ac-LDL) and oxidatively modified LDL (Ox-LDL) on endothelial cell-dependent inhibition of platelet aggregation mediated by EDRF.

Human washed platelets treated with indomethacin (10^{-5} M) were incubated for 1 min in an aggregometer cuvette and then mixed with indomethacin-treated bovine aortic ECs on beads treated with LDLs. After 1 min incubation platelet aggregation was induced by thrombin (20-40 mU ml^{-1}).

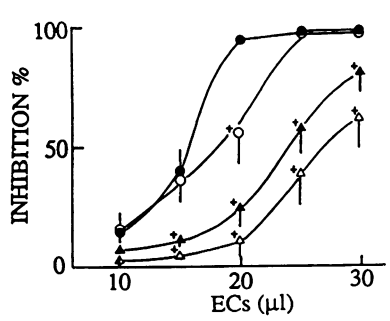


Fig 1: Platelet inhibitory effect of ECs incubated for 40 h with 100 µg ml^{-1} of N-LDL (○), Ac-LDL (▲) or Ox-LDL (△).
+, $p < 0.05$ compared to control (●).

Incubation of platelets with ECs resulted in a cell-dependent inhibition of platelet aggregation. The pharmacological profile of the inhibitory factor was identical to that of NO, eg potentiated by superoxide dismutase and inhibited by oxyhaemoglobin and NO synthesis inhibitors (Salvemini et al., 1990). It was not impaired by the presence of up to 200 µg/ml of N-LDL, Ac-LDL or Ox-LDL. However, preincubation of ECs with these LDLs (50-200 µg ml^{-1}) for 5-40 h irreversibly impaired the inhibition of platelet aggregation in a time- and dose-dependent manner (Fig 1). Ox-LDL was the most potent followed by Ac-LDL. These results show that treatment of ECs with LDLs impair their ability to release an NO-like factor as measured by inhibition of platelet aggregation. Furthermore, the modification of N-LDL to Ox-LDL or Ac-LDL accelerates this impairment.

The authors thank Miss Elizabeth Wood for culturing the endothelial cell. This work was partly supported by a grant from Glaxo Group Research Limited.

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325P INDEPENDENT REGULATION OF THE RELEASE OF PROSTACYCLIN AND EDRF FROM BOVINE AORTIC ENDOTHELIAL CELLS

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Both endothelium derived relaxing factor (EDRF) and prostacyclin (PGI_2) are released from endothelial cells following activation of phospholipase-C linked receptors. The function of other endothelial receptors linked to the activation of adenylate cyclase (AC) is not yet defined. We have examined the effect of receptor-linked or direct activation of AC on PGI_2 and EDRF release.

Bovine aortic endothelial cells (BAEC) were obtained from the Institute of Aging Cell Repository (cat no. AG4762), and cultured as previously described (McEwan et al, 1989). For assay of bradykinin(BK)-stimulated EDRF release, BAEC were cultured to confluence on microcarrier beads, and superfusate from a column of cell-covered beads dripped on to rings of de-endothelialized rat aorta. Cells were grown to confluence in 37mm wells and BK-stimulated PGI_2 release was measured by RIA of 6-oxo-$PGF_{1\alpha}$ in the culture medium after 5 min exposure to BK 1μM. Unstimulated production of 6-oxo-$PGF_{1\alpha}$ was 28pg/min/well and BK 1μM stimulated production to 416(±111)pg/min/well (n=6). After pre-incubation with isoprenaline 1μM for 30min, BK-stimulated levels were 230(±51)pg/min/well and after pre-incubation with forskolin 5μM this was reduced to 73(±49)pg/min/well. Perfusion of the endothelial cells with 1μM isoprenaline did not inhibit subsequent BK-stimulated EDRF release.

In further experiments we examined the effect of forskolin on basal and BK-stimulated intracellular free ionised calcium ($[Ca^{2+}]_i$) of BAEC. $[Ca^{2+}]_i$ was measured in confluent monolayers of cells grown on glass coverslips using the fluorescent indicator Fura 2 (Hallam and Pearson 1986). Mean resting calcium of control BAEC was 93nM which rose transiently to 960±108nM (n=3) after exposure to BK 100nM. Pre-incubation with forskolin 5 μM resulted in a higher resting calcium, 198nM, but reduced the stimulated level to 634±64nM, a 50% reduction in the response to BK 100nM.

These results provide the first demonstration of the independent regulation of the release of PGI_2 and EDRF from endothelial cells, and suggest that endothelial receptors coupled to adenylate cyclase may have a role to inhibit the release of PGI_2 from these cells.

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326P TREATMENT WITH THE CONVERTING ENZYME INHIBITOR, PERINDOPRIL, PROTECTS ENDOTHELIAL FUNCTION IN A RAT MODEL OF CALCIUM OVERLOAD

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We have previously reported that (i) vascular calcium overload induced by treatment with vitamin D_3 and nicotine decreases endothelium-dependent relaxation of precontracted aortic rings (Henrion et al., 1990) and (ii) treatment with perindopril improves aortic distensibility in vivo (Chillon et al., unpublished results). In this experiment, we investigated whether perindopril would improve endothelial function in the vascular calcium overload model. Young Wistar rats received vitamin D_3 (300,000IU/kg, i.m) and nicotine (2 x 25 mg/kg, p.o.). A subgroup received perindopril (1 mg/kg, i.p. per day). Sixteen days later, aortic rings were precontracted with noradrenaline, then vasorelaxation was induced by either carbachol (endothelium-dependent) or sodium nitroprusside (endothelium-independent). Vitamin D_3 plus nicotine treatment led to calcium overload in the aorta (420 ± 56 μmol/g dry weight versus controls 10 ± 1, n=6, $P < 0,05$) and this was accompanied by a marked decrease in the potency of carbachol. Perindopril had no effect on calcium overload but restored the effect of carbachol. Vasorelaxation produced by sodium nitroprusside was unaltered.

		Carbachol		Sodium nitroprusside	
	Perindopril	IC_{50}	maximum	IC_{50}	maximum
		nM	10^{-5}M (%)	nM	10^{-5}M (%)
controls (7)	-	0.25 ± 0.01	90 ± 2	2.29 ± 1.07	99 ± 1
controls (5)	+	0.33 ± 0.10	87 ± 3	8.68 ± 2.31	97 ± 1
Vitamine D3 + nicotine (7)	-	335 ± 21*	61 ± 10*	4.36 ± 1.66	96 ± 3
Vitamine D3 + nicotine (5)	+	0.48 ± 0.11	92 ± 5	31.3 ± 7.1*	100 ± 1

*: $P < 0.05$ with controls not receiving perindopril.

The converting enzyme inhibitor perindopril protects the endothelium against the effects of calcium overload.

Henrion, D., Chillon, J.M., Capdeville-Atkinson, C. & Atkinson, J. (1990), Fund. Clin. Pharmacol. (in press).

327P INTERACTION OF ANGIOTENSIN II AND ENDOTHELIUM IN THE CONTROL OF NORADRENALINE OVERFLOW AND VASOCONSTRICTION IN THE RAT TAIL ARTERY

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Although angiotensin II (AII) enhances the vasoconstrictor response to sympathetic nerve stimulation (Ziogas & Story, 1987), potentiation of field stimulation-induced vasoconstriction by AII in the rat tail artery is not accompanied by an increase in noradrenaline (NA) overflow (Thorin *et al*, 1990). The aim of this work was to investigate the involvement of endothelium in this phenomenon.

Tail arteries were taken from male Wistar rats (n = 6, 400-450g) anesthetized with pentobarbitone (50 mg/kg, i.p.). Two segments were cannulated and the endothelium removed from one. They were perfused/superfused at a constant flow rate (2 ml/min) with oxygenated Krebs plus cocaine (4µM). Field stimulation-induced vasoconstriction (60 V, 0.2 msec., 30 sec., 1Hz) was performed with or without AII (10^{-7}M) and NA overflow was assessed by HPLC-ECD. In a separate series of arteries (n = 6), exogenous NA (10^{-6}M) was perfused for 30 sec. with or without AII (10^{-7}M) and with or without endothelium.

Removal of endothelium produced a rise in basal perfusion pressure (11.2±0.9 versus 7.0±0.8 mmHg, $P < 0.05$) and an increase in spontaneous NA overflow (0.9±0.2 versus 0.3±0.1 pg/30sec., $P < 0.05$) and potentiated field stimulation-induced vasoconstriction (100±7 versus 57±6 mmHg, $P < 0.05$) and NA overflow (6.3±0.7 vs 3.3±0.5 pg/30sec., $P < 0.05$). In arteries with endothelium, AII potentiated vasoconstriction with no change in NA overflow (84±7 mm Hg, $P < 0.05$, and 3.4±0.3 pg/30sec.). Upon removal of endothelium, AII had no significant effect on the vasoconstrictor response but increased NA overflow (132±14 mmHg, and 10.7±1.7 pg/30sec., $P < 0.05$). AII potentiated the vasoconstriction induced by exogenous NA in control arteries but not in rubbed arteries (% of potentiation: +250±80% versus +29±18%, $P < 0.05$).

In addition to a presynaptic AII receptor controlling NA release, our results suggest the existence of (i) an endothelial negative feedback control of NA release, and (ii) release by AII of an endothelial vasoconstrictor factor.

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328P INHIBITION OF COUGH BY INHALED MORPHINE, DEXTROMETHORPHAN, CODEINE AND DALDA (H-Tyr-D-Arg-Phe-Lys-NH_2): EVIDENCE FOR A PERIPHERAL ACTION

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Opiates are generally considered to exert their antitussive action centrally through inhibition of the cough centre in the medulla. However, evidence now supports the presence of opiate receptors on peripheral sensory nerve endings through which some of the effects of antitussive agents may be mediated (Karlsson et al., 1990). In this study measurements were made of the abilities of i.p. or inhaled morphine sulphate, codeine phosphate, dextromethorphan hydrobromide and DALDA, a dermorphin analogue with selectivity for peripheral µ-receptors (Schiller et al., 1989), to inhibit citric acid-induced cough in conscious guinea pigs (Boura et al., 1970). The number of coughs were counted over a 5 min period beginning 3 min after commencing nebulised inhaled citric acid. Inhaled drugs were given simultaneously with citric acid, but i.p. administration was 30 min previously. Estimated ID_{50}'s for inhibition of the number of coughs for i.p. morphine and dextromethorphan were 1.0 and 2.4 mg/kg respectively (n=4-6). In comparison, ID_{50}'s were approximately 2.2 and 12 µg/kg by the aerosol route (n=4-5). Inhaled codeine (≈72 µg/kg) significantly inhibited coughing by 62 ± 23% (n=5) whereas 3 mg/kg i.p. (n=7) was required to produce an equivalent antitussive effect. Inhalation of DALDA (≈7.2 µg/kg, n=4-7) also significantly inhibited coughing by 59 ± 22%. The antitussive effect of inhaled morphine (≈7.2 µg/kg) was significantly inhibited 30 min after 3 mg/kg i.p. of either naloxone HCl or naloxone methylbromide. These results provide evidence for a peripheral site of action since: 1) opiates were considerably more potent by inhalation than i.p.; and 2) both DALDA and naloxone methylbromide would not be expected to act centrally in this test situation. The aerosol route of administration could afford considerable advantages in terms of the avoidance of central adverse effects.

Acknowledgements: We gratefully acknowledge P.W. Schiller of Institut de Recherches Cliniques de Montreal, Canada, for the gift of DALDA.

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329P EFFECTS OF FATTY ACID CHAIN LENGTH ON THE ACTIONS OF SOME ACYL CARNITINE ESTERS IN THE ISOLATED RAT HEART

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Acyl carnitines accumulate in the ischaemic myocardium and are thought to be detrimental to the heart. Esterification of acyl carnitines alters their activity such that the ethyl and isopropyl esters of palmitoyl carnitine are coronary vasodilators in the isolated rat heart while the parent compound, palmitoyl carnitine is a vasoconstrictor. The effects of altering the saturated fatty acid chain length (C8-C18) on the activity of the isopropyl esters of acyl carnitine have now been studied in the same preparation.

Rat hearts were perfused at a constant flow of $10ml.min^{-1}$ using a Krebs-Henseleit solution containing 3.2mM K^+. Developed tension, heart rate, and perfusion pressure were monitored. Drugs were administered as bolus injections into the perfusion stream in volumes not exceeding 30ul. All acyl carnitine esters examined caused coronary dilation as indicated by a fall in perfusion pressure. This fall in perfusion pressure was proportional to fatty acid chain length such that EC_{50} vasodilator doses of the C18,C16,C14 and C12 derivatives were 0.5±0.05, 0.6±0.04, 1.3±0.02, and 6.5±0.61nmoles respectively (n=4). With the C10 and C8 derivatives maximum vasodilator responses could not be assessed due to solubility limitations, but the EC_{50} values were in excess of 30 and 100nmoles respectively. Therefore there was approximately a one thousand fold difference in the potency of these acyl carnitine esters in producing coronary dilation. In addition to the drop in perfusion pressure, the duration of the vasodilator response was also dependent on, and proportional to, fatty acid chain length. With the shorter chain length fatty acid substituants (C10 & C8) vasodilation was also associated with a transient depression of developed tension, this was not seen with the longer chain fatty acid compounds. For comparison, 1nmole of the C18 derivative reduced perfusion pressure by 30±3mmHg without affecting developed tension, while 100nmoles of the C10 derivative reduced perfusion pressure by 35±5mmHg and depressed developed tension by 50%. This fall in developed tension was dose related, reached its peak before the peak of the vasodilator response, and recovered rapidly and completely before the perfusion pressure returned to its basal level.From these and our earlier results it is clear that acyl carnitines and their derivatives possess an interesting pharmacological profile which requires further characterisation.

Supported by The British Heart Foundation

330P NON-ADRENERGIC, NON-CHOLINERGIC INHIBITORY NERVES OF HUMAN INTERNAL ANAL SPHINCTER ARE ANTAGONISED BY L-N^G-NITRO-ARGININE

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Non-adrenergic, non-cholinergic (NANC) relaxations of rat anococcygeus muscle may involve the release of endogenous nitric oxide (Hobbs & Gibson, 1990). Evidence for such a role for NO was partly based on inhibition of NANC relaxations by L-N^G-nitro-arginine (L-NOARG) an inhibitor of NO synthesis and reversal of this inhibition by L-arginine (L-ARG). Electrical field stimulation (EFS) of human internal anal sphincter muscle (IAS) produces a NANC inhibitory response which is unaffected by α-chymotrypsin in concentrations that antagonised relaxations to vasoactive intestinal peptide (Burleigh, 1983). To determine whether NANC inhibitory transmission in the human IAS involves an NO-like substance L-NOARG and L-NOARG plus L-ARG were tested against relaxations of IAS to NANC nerve stimulation.

Results have been obtained using 17 muscle strips from 4 specimens. Some sphincter muscle strips were incubated with noradrenaline (0.5 μM) plus ascorbic acid (100 μM) to maintain an elevated and constant level of muscle tone. EFS (1 msec, 50 v, at 1-10 Hz for 30 s) caused frequency dependent relaxations of muscle strips. L-NOARG reduced these relaxations by 90.5 $\pm$ 3.4% at 2 μM (n=4) and by 92.8 $\pm$ 7.8% at 10 μM (n=5). At the lower concentration of L-NOARG reversal by L-ARG (100 μM) was complete (107.3 $\pm$ 3.7% recovery, n = 4). At the higher concentration of L-NOARG reversal by L-ARG (100 μM)was partial (35.0 $\pm$ 15.5% recovery, n=5). Those muscle strips not incubated with noradrenaline were affected in a similar fashion by L-NOARG and L-NOARG + L-ARG. Relaxations to sodium nitroprusside (NaNP, 0.4 μM) were unaffected by L-NOARG (2 μM n=2, 10 μM n =1). In tissue that was not stored at 4°C L-NOARG caused an increase in muscle tone, suggesting that there was some tonic inhibitory nerve activity present in fresh tissue.

Through activation of soluble guanylate cyclase and subsequent elevation of cGMP EDRF which has now been identified as NO, relaxes smooth muscle. NO is synthesised from L-ARG by a calcium dependent mechanism and the reaction can be inhibited by certain analogues of L-ARG such as L-NOARG. This inhibition may be reversed by L-ARG (Moncada *et al.*, 1989). The existence of this system no longer appears to be restricted to vascular tissue, thus Hobbs and Gibson (1990) have shown that NO may function as an inhibitory neurotransmitter in the rat anococcygeus muscle. The present study demonstrates the presence of the L-ARG/NO neurotransmission process in human tissue. The absence of inhibition of relaxations of IAS to NaNP indicates a presynaptic site of action for L-NOARG and its reversal by L-ARG. It is possible that NO may mediate NANC relaxation of IAS.

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331P NITRIC OXIDE AS A MEDIATOR OF NON-ADRENERGIC, NON-CHOLINERGIC RELAXATION IN GUINEA-PIG PULMONARY ARTERY

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A non-adrenergic non-cholinergic inhibitory nerve (i-NANC) system has been demonstrated in many organs, including guinea-pig (GP) main pulmonary artery (PA) (Kubota et al, 1988). Calcitonin gene-related peptide (CGRP) has been demonstrated to be a possible transmitter of the i-NANC response in GP main PA (Maggi et al, 1990). In a preliminary study we found that while CGRP (0.1 μM) induced 73 ± 5% relaxation of stem PA, but only a 11 ± 2% relaxation of branch PA rings from the same group of arteries (n=8). This result suggests that the transmitter of i-NANC in the GP main pulmonary artery may be different from its branches. We sought to investigate the i-NANC relaxation in the branch PA of GP and determine whether nitric oxide (NO) may be involved in the i-NANC relaxation.

The two branches of pulmonary artery were dissected from male Dunkin-Hartley guinea-pig, cut into rings of 2 mm in lenghth and mounted in 2-ml organ bathes by two stainless holders under an initial tension of 700 mg. One holder was connected to a force transducer and changes in isometric tension recorded. Field stimulation was applied via a pair of platinum electrodes positioned on either end of the vessel rings, using a Grass S88 stimulator.

Field stimulation (50v, 0.2 ms duration, 1-32 Hz) induced a frequency-related relaxation of the U44069 precontracted vessel rings. This relaxation was reduced by 19 ± 3% in the presence of 1 μM atropine and propranolol and abolished by 1 μM tetrodotoxin, indicating that it is neural in origin and the main component of the relaxation is i-NANC in nature. L-N^G-monomethyl arginine (L-NMMA) caused a concentration-dependent inhibition of the i-NANC relaxation by 21 ± 5% (n=6) and 54 ± 4% (n=5) at concentrations of 30 and 100 μM respectively ($p < 0.05$, compared with control group). The inhibitory effect of 100 μM L-NMMA was completely reversed by 300 μM L-arginine, but was unaffected by 300 μM D-arginine.

Our results suggest that the i-NANC vasodilator nerves are present in the branches of the guinea-pig pulmonary artery and that endogenous NO is involved in mediating the i-NANC relaxation in these vessels.

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332P INVESTIGATION OF THE EFFECT OF OVALBUMIN SENSITISATION ON MUSCARINIC M_2 RECEPTORS ON GUINEA-PIG PULMONARY NERVES

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Autoinhibitory muscarinic M_2 receptors inhibit acetylcholine (ACh) release from pulmonary cholinergic nerves (Fryer & Maclagan 1984). A defect of this inhibitory feedback mechanism has been suggested as a possible explanation for the hyperresponsiveness to vagal nerve stimulation, observed in guinea-pigs sensitised to ovalbumin (McCaig 1987). The present study investigated this possibility.

Three groups of male DH guinea-pigs (250-300g) were used: untreated (UT), saline treated (ST) and ovalbumin treated (OT). OT were sensitised to ovalbumin (OA) by parenteral administration on days 1 and 8 (McCaig 1987), followed by aerosol exposure (0.1% OA) for 60s on day 14 and 30s on days 16-17. On days 17 or 18, 24 hours after the last aerosol exposure, animals were killed by cervical dislocation and tracheas removed with vagi and recurrent laryngeal nerves intact for *in vitro* experiments. Contractions were recorded as changes in intraluminal pressure. The effect of OA sensitisation on the response of the post-junctional M_3 receptors to ACh, applied exogenously and released by pre-ganglionic nerve stimulation (PGS), were investigated. Autoinhibitory M_2 receptor function was tested by studying; (1) the facilitatory effect of the selective, muscarinic M_2 antagonist, methoctramine (MET) on PGS-induced contractions, (2) the inhibition, by ACh, of PGS-induced contractions and (3) the antagonism of this effect by methoctramine. A single dose of OA (10μg/ml), given to all tissues at the end of the experiment, confirmed sensitisation.

Group	Basal (%AChmax)	PGS-induced contraction + MET(3×10^{-8}M) (%Basal) (1)	% inhibition by ACh (3×10^{-6}M) (2)	(+ MET 10^{-7}M) (3)	MET-induced change (2-3)
UT	45 ± 5	114 ± 2	35 ± 9	21 ± 10	14 ± 2
ST	45 ± 5	110 ± 4	33 ± 5	10 ± 10	22 ± 6
OT	48 ± 5	112 ± 2	36 ± 5	15 ± 7	21 ± 4

There was no significant difference in the contractile response of tracheas from either group, to ACh applied exogenously or released by PGS. Methoctramine caused a significant, concentration dependent facilitation of PGS -induced contractions in all tracheas (column 1 $p<0.05$), while ACh caused a concentration dependent inhibition of PGS-induced contractions (column 2). This inhibition was not a consequence of the concomitant increase in basal tone, at low concentrations of ACh ($<10^{-5}$M). Methoctramine 10^{-7}M significantly ($p<0.05$) and non-competatively antagonised the inhibitory effect of ACh on PGS-induced contractions, in all three groups (column 3). Therefore we were unable to show significant alteration in autoinhibitory M_2 receptor function, following ovalbumin sensitisation. This is in contrast to recent results with parainfluenza virus type 1 infection in guinea-pigs (Fryer & Jacoby 1990).

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333P COMPARISON OF THE EFFECTS OF CROMAKALIM ON VAGALLY-MEDIATED BRONCHOCONSTRICTION IN TRACHEA ISOLATED FROM NORMAL AND ALBUMIN-SENSITIVE GUINEA-PIGS

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The K^+-channel activator cromakalim relaxes airway smooth muscle and may have a role in the treatment of bronchial asthma. Cromakalim attenuates vagally-mediated bronchoconstriction in isolated guinea-pig trachea (McCaig & De Jonckheere, 1989). and the effects of cromakalim on vagal bronchoconstriction in trachea isolated from normal and albumin-sensitized guinea-pigs, are now compared.

Tracheae, with attendant right vagus and recurrent laryngeal nerves were obtained from 3 groups of male guinea-pigs : 1) albumin-sensitized (i. p. plus i.m. injections of albumin followed 2 weeks later by daily exposure to nebulised 4% albumin for 20±1% days, n=12); 2) sham-sensitized (as 1) but with saline vehicle only, n=6); and 3) no treatment (n=14). Intraluminal pressure (ILP) was recorded from the closed, Krebs-filled trachea as described previously (Blackman & McCaig, 1983). The vagus nerve was stimulated (40V, 1ms pulses for 5s at 1-50Hz at 90s intervals) and frequency response curves were obtained in the absence then presence of increasing concentrations of cromakalim (2×10^{-7}M - 10^{-5}M). Six tissues from each of groups 1 and 3 were not exposed to cromakalim and served as time-matched controls.

Stimulation of the vagus nerve evoked frequency-dependent increases in ILP which varied widely in amplitude between preparations but were consistent in any one preparation and were unchanged over time in time-matched controls. Initial frequency-response curves (expressed as percentage maximum response) were superimposable in the 3 groups of tissues. Vagal response amplitude was decreased by cromakalim in all groups, resulting in a concentration-dependent right-ward shift of the frequency-response curve and reduced maximum responses. The degree of attenuation varied between approximately 20% at 2×10^{-7}M, cromakalim and approximately 80% at 10^{-5}M. Responses at lower frequencies of stimulation (1 or 2Hz), however, were more susceptible to attenuation by cromakalim. 10^{-5}M cromakalim, for example, reduced responses at 1Hz by 98± 1% (mean ± s.e. mean, n=8), 92± 4% (n=5) and 97±2% (n=5) in untreated, sham-sensitized and albumin-sensitized tissues respectively, whilst at 20Hz the corresponding reductions were 70±7%, 54±13% and 78±12%.

These results indicate that cromakalim is effective in combatting vagal bronchoconstriction in both normal and albumin-sensitized guinea-pig trachea. The effect of cromakalim in normal trachea is largely prejunctional but since sensitization alters the electrical properties of the trachealis muscle (McCaig, 1987), postjunctional effects may contribute to attenuation in sensitized trachea. The results reinforce the notion that cromakalim may be particularly effective in asthma where there is a strong vagal component to the bronchoconstriction.

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334P INDUCTION OF TRACHEAL PLASMA PROTEIN EXTRAVASATION IN THE CONSCIOUS GUINEA-PIG BY OZONE: ROLE OF NEUROPEPTIDES

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Exposure of guinea-pigs to ozone (O_3) induces bronchial hyperreactivity (BHR) to a number of inhaled spasmogens, for example, histamine and particularly to peptides such as substance P (Yeadon and Payne, 1989; Yeadon, et al, 1990). It has been suggested that airways plasma protein extravasation (PPE) could contribute to BHR in asthma (Kowalski, et al, 1989). We have therefore examined O_3-induced PPE in guinea-pig airways. Adult, male guinea-pigs were used. As a marker of PPE, Evans blue dye was injected (30mg/kg) into an ear vein 10 min before exposure to either air or O_3 (3ppm) for 30 or 120 min. Following cervical dislocation the trachea was removed, weighed and dye extracted in formamide at 25°C (48h) and quantified against standards at 620nm, expressed as ng/mg tissue, mean±sem, n=6, in parentheses. Exposure to O_3 (30 min) did not increase PPE above basal (41±5 vs 51±7), but after 2hr, tracheal PPE was raised (105±14; $p<0.01$). Mepyramine (2mg/kg i.p.) 30 min prior to O_3 (2hr) did not inhibit the increase in PPE (97±7). The anti-oxidant ascorbic acid prevents BHR induced by O_3 (30 min) but not by O_3 (120 min) (Yeadon and Payne, 1989). Animals were treated with ascorbic acid (1g/kg i.p.) 30 min prior to, and 60 min into a 2hr exposure to O_3. This prevented the O_3-induced increase in tracheal PPE (25±3; $p<0.01$) but did not prevent BHR to nebulised histamine in anaesthetised, pump-ventilated guinea-pigs. Opioid drugs are known to reduce neuropeptide (NP) release from stimulated C-fibre afferents in the airways (Barnes, 1987). To investigate the contribution of O_3-induced release of NPs in PPE, animals were treated with fentanyl (30µg/kg, s.c.) 30 mins prior to O_3 (2hr) which reduced tracheal PPE (56±12; $p<0.05$). Animals were also treated with neutral endopeptidase inhibitors phosphoramidon and thiorphan (1 and 5mg/kg, i.v.) and exposed to O_3 for 30 min. Tracheal PPE was enhanced to the same level as for 2hr O_3 exposure (91±12; $p<0.01$). These studies suggest that O_3 produces PPE in the airways through oxidant-induced C-fibre stimulation and release of neuropeptides, rather than via histamine release, and further that the upper airway oedema which results does not contribute significantly to subsequent BHR.

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335P REVERSAL OF INDUCED AIRWAY HYPERREACTIVITY BY POTASSIUM CHANNEL OPENERS: PCO 400 AND CROMAKALIM

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Low doses of cromakalim (C) have symptomatic benefit in nocturnal asthma (Williams et al., 1990), but it has not been established whether inhibition of airway hyperreactivity or spasmolytic activity accounts for clinical efficacy. Two potassium channel openers C and PCO 400 (P) (Quast et al., 1990) have been studied in order to resolve this issue.

Guinea-pigs (450-600 g) were anaesthetised with phenobarbitone (100 mg/kg i.p.) and pentobarbitone (30 mg/kg i.p.), paralysed with gallamine (10 mg/kg i.m.) and ventilated (1 Hz, 8 ml/kg) via a tracheal cannula. Airway resistance (R_L cm H_2O/l/sec) and compliance (C_{dyn} ml H_2O/l/sec) were calculated from measurement of tracheal airflow and transpulmonary pressure using a digital electronic pulmonary monitoring system (PMS, Mumed Ltd., U.K.). Airway hyperreactivity was induced by three intravenous injections of preformed immune complexes (IC), (0.1 ml bovine gamma globulin (BGG) + anti BGG serum) at 10 minute intervals. Airway responses (maximal R_L cm H_2O/l/sec ± sem) to intravenous injection of histamine (H, 1.8 µg/kg) were monitored before, and after, administration of IC. Subsequently, P or C were administered as bolus intravenous injections (0.1 mg/kg) and maximal R_L in response to intravenous injection of H monitored at 10 minutes intervals thereafter. (TABLE: * p< 0.05, ** p<0.01, *** p<0.001, n=10)

	NORMOREACTIVE				HYPERREACTIVE			
TIME	0'	10'	20'	30'	0'	10'	20'	30'
VEHICLE	325 (57)	309 (51)	455 (63)	527 (55)	675 (62)	611 (52)	575 (54)	547(50)
PCO 400	310 (58)	191 (26)	343 (45)	526 (37)	654 (94)	208 (84)***	289 (92)***	263 (95)*
CROMAKALIM	239 (41)	212 (62)	293 (49)	369 (54)	772 (88)	456 (110)**	505 (90)*	468 (77)

Both P and C caused acute, sustained inhibition of the increased bronchoconstrictor response to H in hyperreactive animals: whilst these concentrations did not affect responses to H in normoreactive animals. The inhibitory effect of P and C in hyperreactive animals was abolished by glibenclamide (30 mg/kg i.d).

The ability of P or C to reverse established airway hyperreactivity may determine efficacy in asthma. No effect of P or C on airway eosinophilia has been detected (Chapman et al., 1990), so that effects upon airway hyperreactivity may reflect actions on nerve or smooth muscle. Reversal of airway hyperreactivity by potassium channel openers may be a novel approach to the resolution of airway obstruction in asthma.

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336P EFFECTS OF BRL 38227 ON NEURALLY-MEDIATED RESPONSES IN GUINEA-PIG ISOLATED BRONCHUS

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In the anaesthetised guinea-pig, cromakalim caused a greater suppression of neurally-mediated bronchoconstriction than of responses evoked by exogenously added transmitter, suggesting that cromakalim may act at a pre-junctional site to inhibit inhibit transmitter release by nerves in the airways (Ichinose and Barnes, 1990). The present study was undertaken to investigate the effects of the active enantiomer of cromakalim, BRL 38227 (Buckingham et al, 1986) on cholinergic- and non-cholinergic, non-adrenergic excitatory (NANCe) nerve-mediated responses in the guinea-pig isolated bronchus.

Rings of guinea-pig main bronchus were mounted between platinum electrodes in Krebs solution containing indomethacin (2.8 µM). Electrical field stimulation (EFS: 60V, 0.5 ms pulse duration, 10 Hz, for 10 s) evoked a tetrodotoxin(3 µM)-sensitive, biphasic contractile response. This comprised a rapid, transient, atropine-sensitive (cholinergic) component, succeeded by a slowly developing, atropine-resistant (NANCe) component. These responses were equivalent in magnitude to the respective contractions evoked by exogenously applied acetylcholine (ACh; 6 µM) and substance P (SP; 1 µM), a putative transmitter in the NANCe pathway (Barnes, 1986).

BRL 38227 (0.3-3 µM) caused a concentration-dependent inhibition of both NANCe and cholinergic nerve-mediated responses to EFS. Maximal suppression of responses to EFS was obtained at 3 µM. The potassium channel activators pinacidil (Bray et al, 1987) and RP 52891 (Lewis and Raeburn, 1990) exhibited very similar profiles of activity to BRL 38227, but were less potent at causing suppression of responses to EFS. At concentrations up to 100 µM, BRL 38226, the (+)-enantiomer of BRL 38227, failed to cause suppression of responses to EFS. Prior exposure of tissues to glibenclamide (3 µM for 30 minutes) attenuated the suppressant effect of BRL 38227 (3 µM) on the cholinergic and NANCe-components of the response to EFS.

By contrast with its effects on NANCe responses to field stimulation, BRL 38227 (3 µM) had no significant effect on contractions evoked by SP (1 µM). Responses to exogenously applied ACh (6 µM) were slightly inhibited, but this was to a lesser extent than the suppression of cholinergic-nerve mediated contractions caused by BRL 38227 (3 µM).

The present data suggest that BRL 38227 inhibits the release of transmitter from cholinergic and NANCe nerves in guinea-pig bronchus in vitro in a stereo-specific manner. This shows good agreement with previous findings when cromakalim was tested in guinea-pigs in vivo (Ichinose and Barnes, 1990). The similar profiles of activity of pinacidil and RP 52891 suggest that the actions of BRL 38227 may involve the activation of potassium channels. The antagonism of BRL 38227 by glibenclamide suggests that these may be ATP-dependent potassium channels (Ashcroft, 1988).

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337P EVIDENCE FOR AUTOREGULATION OF ACETYLCHOLINE RELEASE IN THE CANINE LUNG *IN VIVO* BY PREJUNCTIONAL MUSCARINIC M_2 RECEPTORS

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A negative feedback loop regulating acetylcholine (ACh) release through prejunctional muscarinic receptors has been demonstrated in the canine lung *in vitro* (Ito & Yoshitomi 1988, Deckers *et al* 1989). Similar autoregulation has been described in cat and guinea pig *in vivo* where the prejunctional muscarinic receptor involved is thought to be of the M_2 subtype (Fryer & Maclagan 1984, Blaber *et al* 1985). In order to characterise the receptor in the dog, the effect of the M_2 selective antagonist methoctramine (Melchiorre *et al* 1987) has been investigated *in vivo* and compared to data obtained in the anaesthetised cat.

Submaximal bronchoconstriction and bradycardia were induced using i.v. ACh, 5 - 20 µg/kg, and stimulation of the right cervical vagus (V.S.), 5 - 30 Hz, 0.5 ms, 15 V, 10 s train, in chloralose (100 mg/kg) anaesthetised cats and pentobarbitone (35 mg/kg) anaesthetised dogs. The effect of cumulative i.v. doses of methoctramine on these responses was determined allowing a 10 min interval between dosing and the challenge sequence. Doses which potentiated the lung vagal challenge and those which reduced the responses to 50% of the control values (ID_{50}) are shown below.

		Lung ID_{50}		Heart ID_{50}		Potentiation of lung vagal response	
	n	ACh	V.S.	ACh	V.S.	Dose range	Peak increase
CAT	3	>3 mg/kg	3 mg/kg	11.0 µg/kg	18.6 µg/kg	10 -1000 µg/kg	122 ±31 % (100 µg/kg)
DOG	3	>1 mg/kg	560 µg/kg	4.2 µg/kg	18.7 µg/kg	3 - 100 µg/kg	90 ±26 % (30 µg/kg)

Methoctramine potently antagonised bradycardia induced by ACh and V.S. in both species consistent with its selective action at the M_2 receptor. At these M_2 inhibitory doses on the heart, vagally induced bronchoconstriction was potentiated in both cat and dog. Higher doses of the antagonist inhibited vagally induced bronchoconstriction, although bronchoconstrictor responses to ACh were not blocked at any of the doses tested, commensurate with the low potency of methoctramine at M_3 smooth muscle receptors (Melchiorre *et al* 1987).

Potentiation of nerve induced bronchoconstriction by M_2 selective doses of methoctramine in the anaesthetised cat confirm previous studies showing the lung prejunctional receptor to be of the M_2 subtype in this species. The data generated in the dog are qualitatively similar to those seen in the cat and suggest a comparable negative feedback system mediated by prejunctional M_2 receptors in the canine lung. Inhibition of vagally induced bronchoconstriction in the absence of antagonism of ACh bronchoconstrictor responses may indicate blockade of ganglionic muscarinic M_1 or nicotinic receptors (Watson *et al* 1990) at doses below those producing antagonism of M_3 receptors on airway smooth muscle.

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338P EFFECTS OF STANDARD LABORATORY DIETS ON FATTY ACID COMPOSITION AND SUSCEPTIBILITY TO ISCHAEMIA-INDUCED ARRHYTHMIAS IN RATS

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30 min of acute coronary artery ligation (CAL) in the anaesthetised male Wistar rat resulted in reproducible arrhythmias over an eight year period in our laboratory. Mean incidences of ventricular fibrillation (VF) ranged from 45-73% and mean total ventricular premature beats (VPBs) ranged from 1001-1532 (n = 12-30/group). Recently a marked decrease in arrhythmias was observed and the incidence of VF and VPBs fell to 20% and 502 respectively ($p<0.05$). Approximately two months earlier, our animal suppliers (Interfauna) had switched their animal feed from Labsure CRMX pellets (LS) to Grain Harvester 693 pellets (GH). No other changes were associated with the decline in arrhythmias. Controlled studies were therefore performed to assess the possible effects of diet on arrhythmia severity and fatty acid composition of adipose and cardiac tissue.

Two days after mating female rats were split into two groups. One group and their offspring were continued on GH pellets whilst the other group and offspring were fed on LS diet. When the male offspring were 10 weeks old, CAL was performed under pentobarbitone anaesthesia (Clark *et al.*, 1980). The table summarises the incidences of VF and mortalities and the numbers of single VPBs and VPBs contributing to salvos and ventricular tachycardia (VT) seen in each group.

Table Arrhythmias during the first 30 min of coronary artery ligation

Diet	n	Single	Salvo	VT	Total	% VF	% Death	
LS	14	148±26	67±16	1218±276	1432±291	64	29	
GH	15	95±20	35±12	*553±166	*683±175	*20	13	* $p<0.05$

Rats fed LS diet developed twice as many VPBs and 3 times as many fibrillated compared with those fed GH diet. Adipose tissue from animals fed GH diet contained a significantly lower ($p<0.05$) percentage of monounsaturated fatty acids (35.9±2.5% vs 40.2±1.9%) and a higher percentage of polyunsaturated fatty acids (31.7±4.4 vs 27.2±2.3; $p<0.05$). Increased cardiac phospholipid n-3 polyunsaturated fatty acid composition was also observed.

These results suggest that diet influences both susceptibility to ischaemia-induced arrhythmias and the fatty acid composition of adipose tissue and cardiac phospholipids. The latter may have functional consequences. It is therefore recommended that scientists using rats for the study of cardiovascular phenomena keep checks on the dietary history of animals supplied by animal breeders.

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339P ANTIARRHYTHMIC AND CLASS III ELECTROPHYSIOLOGICAL ACTIONS OF THE 2,3,16-TRIOL METABOLITE OF ORG 7797, A CLASS Ic ANTIARRHYTHMIC AGENT

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Org 7797 is a steroidal class Ic antiarrhythmic agent (Winslow *et al.*, 1989a; 1989b). Human liver homogenates convert Org 7797 to the 2,3,16-triol derivative (Org 20781). Org 20781 has therefore been compared with Org 7797 for antiarrhythmic and electrophysiological effects.

In pentobarbitone-anaesthetised rats, both compounds (0.5 mg/kg) given i.v. 15 min prior to coronary artery ligation reduced the expected number of ventricular arrhythmias seen during the first 30 min of ligation (Table 1). At 1 mg/kg both compounds abolished the development of ventricular fibrillation (VF).

Table 1 Arrhythmias induced by acute coronary artery ligation

Treatment	mg/kg	n	Number of Ventricular Premature beats				% VF	% Death	
			Single	Salvo	VT	Total			
0.9% Saline		15	154±29	120±49	827±358	1101±391	47	33	
Org 7797	0.5	8	136±61	34±17	220±156	390±87	13	13	
	1.0	8	38±25	*5±40	*80±76	*127±100	*0	0	
Org 20781	0.5	10	169±154	*8±6	*21±21	*198±180	40	40	
	1.0	10	67±27	*14±6	148±112	*229±137	*0	0	* $p<0.05$

In isolated guinea-pig papillary muscle driven at 0.3, 1 and 2 Hz, Org 7797 (0.5-5 μM) decreased the maximum rate of rise (Vmax) of cellular transmembrane action potentials in a concentration- and frequency-dependent manner whilst action potential duration (APD) was unchanged except for a slight prolongation at 5 μM and 2 Hz. In contrast, Org 20781 (2 μM and above) additionally prolonged both APD and the effective refractory period (ERP), effects which became more pronounced as stimulation frequency increased. EC_{25} values are given in Table 2. These results suggest that the major metabolite of Org 7797 also has pronounced antiarrhythmic actions which can partly be explained by prolongation of the cardiac action potential.

Table 2 EC_{25} values with 95% confidence limits (μM) for electrophysiological effects

	Vmax (1 Hz)	APD_{90} (1 Hz)	ERP (2 Hz)
Org 7797	1.1 (0.8-1.5)	-	-
Org 20781	3.6 (2.4-5.1)	2.7 (0.4-8.3)	1.4 (0.3-2.4)

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340P INVESTIGATION OF THE ADENOSINE RECEPTORS MEDIATING BRADYCARDIA AND VASODILATION IN THE DOG

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P_1-purinoceptors have been divided into A_1 and A_2 sub-types. At the A_1 receptor N^6-substituted analogues such as N^6-cyclopentyladenosine (CPA) and R-N^6-phenylisopropyladenosine (R-PIA) are generally more potent as agonists than 5'-N-ethylcarboxamide adenosine (NECA) and 2-phenylamino adenosine (PAA). 8-cyclopentyl-1,3-dipropyl xanthine (CPX) is an A_1 selective antagonist. At A_2 receptors the order of agonist potency is NECA>PAA>R-PIA=CPA. A_2 receptors have been further sub-divided into A_{2a} which has high affinity for PAA and for the antagonist N-(2-(dimethylamino)ethyl)-N-methyl-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-IH-purin-8-yl)benzenesulphonamide (PD115199) and A_{2b} which has low affinity for these compounds (Bruns et al, 1986, 1987). In this study we have investigated the adenosine receptors that mediate bradycardia and vasodilation in the anaesthetised dog.

Female beagle dogs were anaesthetised (pentobarbitone), the left carotid artery was catheterised into the left ventricle for administration of agonists. The right hind-limb was denervated and blood perfused at constant flow. The vagi were sectioned and xamoteral (1mg/kg) administered to maintain a stable high heart rate. Nitrobenzylthioinosine (0.5mg/kg) was administered to prevent purine uptake.

The order of agonist potency for decreasing heart rate (HR) was NECA>Adenosine>CPA>R-PIA>PAA, whilst for a decrease in hind-limb perfusion pressure (HLPP) it was NECA>PAA>adenosine>CPA=R-PIA. Antagonist potency was assessed by the dose required to cause a 2 fold rightward shift of the adenosine dose response curve (DR2). Theophylline had slightly higher potency on HR (DR2 = 0.7mg/kg) than on HLPP (DR2 = 3.4mg/kg). Surprisingly, CPX was only slightly more potent against HR responses (DR2 = 0.4mg/kg) than against HLPP (0.9mg/kg). PD115199 was 40 fold selective as an antagonist of HLPP responses (DR = 0.034mg/kg) compared with HR (DR = 1.37mg/kg).

The order of agonist potency on HLPP is consistent with an A_{2a} receptor. The low affinity of CPX and the unusual order of agonist potency on the HR response indicates that it is not mediated via a conventional A_1 receptor. A similar conclusion was reached by Belloni et al (1983). The low potency of PAA and of PD115199 indicate that the canine cardiac receptor may resemble the A_{2b} sub-type.

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341P PHARMACOLOGICAL AND BIOCHEMICAL EFFECTS OF ORG 9935: A CARDIOTONIC AGENT WITH POSITIVE INOTROPIC, Ca-SENSITISING AND VASCULAR RELAXANT PROPERTIES

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We recently reported the cardiac and vascular effects of a new cardiotonic agent Org 30029 (Cottney et al., 1990). In this study we have examined the in vitro effects of Org 9935 (4,5-dihydro-6-(5,6-dimethoxy benzo [b] thien-2-yl)-5-methyl-3(2H)-pyridazinone), an analogue of Org 30029. Inotropic, chronotropic and vascular relaxant activities were measured as described by Cottney et al. (1990). EC_{50} values were determined using either Org 9935- or isoprenaline-induced maximum responses for aorta and cardiac muscle, respectively. Cyclic nucleotide levels of rabbit aortic rings were measured after incubation in oxygenated Krebs-Henseleit solution at 37°C in the presence of Org 9935 for 30 min. PDE isoenzymes were separated from rabbit cardiac ventricle and assayed as in Cottney et al. (1990). Ca^{2+}-sensitising activity was examined in rat Triton X-100 treated trabeculae as described by Cottney et al. (1990). Skinned trabeculae were contracted to 30% of maximal response to Ca^{2+} (Cmax) and Ca^{2+} responses in the presence of Org 9935 (10-300 µM) were expressed as % of Cmax and EC_{25} determined. Org 9935 (5-1000 µM) produced relaxant (EC_{50}: 0.5 (0.3-0.6) µM), positive inotropic (EC_{50}: 84±26 µM) and modest positive chronotropic (maximal 18% increase from a control value of 192±8 beats min^{-1}) responses in rabbit isolated aortic rings, papillary muscles and spontaneously beating right atria, respectively. The maximum positive inotropic effect was not different from that produced by isoprenaline whilst the maximum positive chronotropic action was significantly less (20-30% of isoprenaline maximum). Org 9935 elicited a complete relaxation of the phenylephrine (0.3 µM) contracted aorta. In rat skinned trabeculae, Org 9935 potentiated the response to a submaximally activating concentration of free Ca^{2+} and had an EC_{25} of 49±9.7 µM. At a subthreshold inotropic concentration, Org 9935 (0.3 µM) potentiated the positive inotropic and cyclic AMP elevating effects of isoprenaline, producing a 2.6 fold parallel leftward shift in the isoprenaline dose-response curve. Org 9935 elevated both cyclic AMP and cyclic GMP levels in aortic rings producing increases of 84% and 246%, respectively at 300 µM. Cyclic AMP activity of rabbit ventricular PDE III was potently inhibited by Org 9935 (IC_{50}: 0.15±0.07 µM) whilst weak effects on PDE I, II and IV were observed.

Org 9935 produces marked positive inotropism and vascular relaxation but weak positive chronotropy. Potentiation of Ca^{2+} responses in skinned trabeculae and isoprenaline in intact papillary muscles suggests the involvement of both cyclic AMP and Ca^{2+}-sensitisation of contractile proteins in the cardiac actions of Org 9935. Furthermore unlike Org 30029, which is a dual PDE III/PDE IV inhibitor, Org 9935 showed high selectivity for inhibiting PDE III.

Cottney, J., Logan, R., Marshall, R.J., Nicholson, C.D., Shahid, M. and Walker, G. (1990) Cardiovasc. Drugs Revs., 8, 179-202.

342P CARDIOVASCULAR ACTIVITY OF PHOSPHODIESTERASE INHIBITORS IN THE CONSCIOUS DOG

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Phosphodiesterase (PDE) inhibitors have been closely studied with regard to stimulating the failing heart. Less emphasis has been placed on the vasodilator and hypotensive activity of such drugs. We have examined the cardiovascular activity of the specific PDE-III inhibitor milrinone (M) (Alousi 1983), the PDE-I specific M&B 22948 (M&B) and the non-specific 3-isobutyl-1-methyl xanthine (IBMX) (Lugnier et al. 1986) when given intravenously to conscious dogs. Seven male beagles, four with renal hypertension and three normotensives with implanted Konigsberg left ventricular pressure transducers, were used. Blood pressure was measured via direct carotid loop puncture (Parkinson, 1978) while drugs were infused via the cephalic vein. Left ventricular pressure (LVP) was differentiated to give dP/dt max for rate of contraction and relaxation. Left ventricular end diastolic pressure (LVEDP) was also measured. Data are expressed as overall means of readings taken every five minutes during a one hour control period and during 30 minutes of drug infusion.

In normotensive dogs IBMX 50 µg/kg/min increased heart rate (HR) (68 to 113 bpm) and dP/dt max (3403 to 6123 mmHg/sec) and decreased LVEDP (-0.9 to-2.74 mmHg) but did not modify systolic (SBP)(148 to 143 mmHg) or diastolic (DBP)(84 to 86 mmHg) blood pressure. In contrast, M&B 30 µg/kg/min increased HR only slightly (65 to 74 bpm), did not change dP/dt max (3304 to 3245 mmHg/sec) but transiently decreased both SBP (151 to 131 mmHg) and DBP (85 to 71 mmHg) and LVEDP (-0.38 to -2.83 mmHg). M 30 µg/kg/min increased HR (71 to 91 bpm) and dP/dt max (3865 to 7731 mmHg/sec) and decreased SBP (150 to 129 mmHg) more than DBP (86 to 75 mmHg). LVEDP was reduced from 0.44 to -6.17 mmHg. Ventricular relaxation (-dP/dt max) was unaltered with IBMX and M but reduced with M&B (-3579 to -2902 mmHg/sec).

In renal hypertensive dogs similar effects were found. With IBMX, HR was increased (79 to 131 bpm) but SBP(170 to 163 mmHg) and DBP (103 to 105 mmHg) were little affected. M&B reduced both SBP(179 to 145 mmHg) and DBP (110 to 88 mmHg) but did not change heart rate (82 to 85 bpm). M increased heart rate (77 to 127 bpm) and decreased both SBP (171 to 137 mmHg) and DBP (103 to 89 mmHg). M&B is interesting because the hypotension is unaccompanied by changes in heart rate or contractility and is quite unlike milrinone or IBMX which give increases in both. This would be a preferred profile for treatment of hypertension. The absence of cardiac changes in response to the hypotensive effect of M&B may be due to cardiac effects of cGMP, the myocardial function of which remains speculative (Linden & Brooker 1979).

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343P THE INFLUENCE OF VERAPAMIL ON THE EXTRACELLULAR POTASSIUM CONCENTRATIONS OF THE GUINEA-PIG HEART UNDER ISCHAEMIC CONDITIONS

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An important event of myocardial ischemia is the rise of extracellular potassium concentrations ($[K_e]$). The Ca entry blocker verapamil has been claimed to possess antiischemic properties. Aim of this study was to investigate the effect of verapamil on $[K_e]$ and to evaluate whether such actions are related to cardiodepressant properties.

Isolated guinea-pig hearts were perfused according to Langendorff at constant flow with Tyrode-solution (Na 149, K 5.4, Ca 1.8, Mg 1.1, glucose 11 mM; 37°C). Left ventricular pressure (LVP) and the area under the LVP curve (AUC) were determined. $[K_e]$ were registered by potassium-sensitive minielectrodes in the wall of the left ventricle. After a first conditioning ischemia (5 min) the hearts were reperfused (10 min). After a second ischemia (5 min) and another reperfusion period (50 min) a third ischemia (5 min) followed. Verapamil (10^{-8}-10^{-5} M) was added 40 min before the third ischemia. In some tests verapamil was replaced by 30mM $MgCl_2$. AUC was determined for the first minute and $[K_e]$ at the end of the first minute of each ischemia. Values for the third ischemia were expressed as mean % ± S.D. (n>5) of the second (untreated) ischemia.

Verapamil reduced the LVP before ischemia with an ED_{50} of 2.9×10^{-8} M. The AUC of the third ischemia amounted to 113.5 ± 28.3 %. Verapamil decreased the AUC of the third ischemia with an ED_{50} of 3.3×10^{-8} M. The $[K_e]$ of the third ischemia amounted to 102.4 ± 18.5 % of the second ischemia. Verapamil diminished $[K_e]$ with an ED_{50} of 9×10^{-8} M. 10^{-5} M verapamil abolished LVP and consequently AUC , but it only reduced $[K_e]$ to 41.8 ± 13.5 %. 30 mM $MgCl_2$ also lead to complete cessation of contractility and reduced $[K_e]$ to 25.2 % ± 14.4. This result was not significantly different (P>0.05) from the one after 1×10^{-5} M verapamil.

The nearly identical ED_{50}'s for the reduction of contractile force before and during ischemia show that verapamil acts also negative inotropic during ischemia. The reduction of the increase in $[K_e]$ during the initial minute of ischemia is closely related to this cardiodepressant action of verapamil, as evidenced by comparable ED_{50}'s. Two possible mechanism of action of verapamil on ischemic $[K_e]$ are proposed: 1) Verapamil reduces the intracellular calcium concentration via blockade of the slow channel and causes a diminished conductance of the calcium induced K channel and in this way a reduced K-efflux. 2) Because of its cardiodepressant action during ischemia verapamil saves ATP, which is now available for the Na/K-ATPase. This could lead to a raised K-influx relative to ischemic controls. At very high verapamil concentrations with full blockade of the Ca-channel and cessation of contractility a residual net efflux of K could be observed.

344P NON-PLANAR, BUT NOT PLANAR, PREGNANES POTENTIATE TONE OF PRE-CONTRACTED HUMAN RESISTANCE ARTERIES

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Metabolites and semi-synthetic derivatives of progesterone inhibit Na/K ATPase. Such substances exhibit negative (planar) or positive (cis, non-planar) inotropism depending on structure. Using a myograph, we have investigated the action of chlormadinone acetate (CMA, planar), JT153 (cis) and ouabain (cardiac-glycoside) on pre-contracted resistance arteries from normotensive patients.

Arteries (n=22) were submaximally activated by noradrenaline and changes in tension were measured at 4 minute intervals for 20 minutes (control). The experiment was repeated in the presence of cumulatively increasing concentrations of ouabain (n=9) or CMA (n=6) or JT153 (n=7) added sequentially at 4 minutes intervals.

TENSION (10^{-5}N/mm)

	1nM	10nM	100nM	1uM	10uM
Control	- 7 ± 2	-11 ± 3	-13± 3	-14 ± 3	-13 ± 7
Ouabain	- 6 ± 6	10 ± 8*	32±13*	-	61 ±15**
CMA	2 ± 3	0 ± 2	- 4± 4	- 6 ± 3	- 6 ± 4
JT153	-18 ± 5	-21 ± 5	- 3± 8	26 ±11*	42 ±13**

* p<0.02; ** p<0.001 on comparison with control.

Although the planar pregnane did not potentiate tone of pre-contracted human resistance arteries, the non-planar pregnane induced potentiation similar to that induced by ouabain. Such compounds could be in part responsible for endogenous cardiac-glycoside activity and raised peripheral vascular resistance found in certain forms of hypertension.

345P TONIC AND FREQUENCY-DEPENDENT V_{max} BLOCK INDUCED BY NAFENODONE IN GUINEA-PIG VENTRICULAR MUSCLE FIBRES

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Nafenodone (NF) is a new antidepressant drug which inhibits the maximum rate of depolarization (V_{max}) in isolated atrial and ventricular muscle fibres being classified as a class I antiarrhythmic drug (Delpón et al., 1990a). In the present experiments the tonic and frequency-dependent V_{max} block induced by NF has been analyzed.

The experiments were performed in guinea-pig papillary muscles excised from the right ventricle. V_{max} values obtained by electronic differentiation were used as an indirect estimative index of the magnitude of the fast inward Na current.

NF, 10^{-6} M and 10^{-5} M, had no effect on resting membrane potential or action potential duration by producing a 2.3 ± 1.3% and 5.0 ± 0.5% inhibition, respectively, of the V_{max} of the first action potential of a train of stimuli (tonic block). NF also produced a frequency-dependent V_{max} block which increased not only with the concentration of the drug but also by decreasing the interstimulus interval from 2 to 0.5 s. In papillary muscles driven at 0.5, 1 and 2 Hz the magnitude of the frequency-dependent V_{max} block induced by NF 10^{-5} M was of 5.5 ± 0.6%, 14.6 ± 0.2% and 24.4 ± 4.3%, the onset rate constants being 0.414 ± 0.04 AP^{-1}, 0.203 ± 0.02 AP^{-1} and 0.152 ± 0.03 AP^{-1}, respectively. NF also prolonged the time constant of recovery of frequency-dependent V_{max} block from 34.0 ± 5.9 ms to 2.3 ± 0.4 s this effect being independent of the drug concentration. Furthermore, NF 10^{-5} M shifted the membrane responsiveness curve along the voltage axis in the hyperpolarizing direction by 5.0 ± 0.9 mV.

These results demonstrated that NF, like imipramine (Delpón et al., 1990b) produced a frequency- and voltage-dependent inhibition of the fast Na channels similar to that exhibited by other class Ia, or intermediate kinetic, antiarrhythmic drugs.

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346P EFFECTS OF PROPAFENONE ON CALCIUM CURRENT IN GUINEA-PIG VENTRICULAR CELLS

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Propafenone (P) is a class Ic antiarrhythmic drug with demonstrated effectiveness in suppressing chronic ventricular arrhythmias (Funck-Brentano et al., 1990). It also decreases the V_{max} of the slow action potentials elicited in guinea-pig ventricular muscles (Tamargo & Delgado, 1985; Delgado et al., 1985) which suggested that P exhibits also Ca antagonist effects that can be involved in its clinical effectiveness. Therefore, the effects of P on the Ca current (I_{Ca}) were studied in guinea-pig ventricular cells using the patch-clamp technique.

Guinea-pig single ventricular myocytes were enzymatically isolated as previously described by Isengerg & Klockner (1982). Measurement of membrane currents was performed at room temperature by the tight-seal whole-cell patch-clamp technique.

When depolarizing pulses (150 ms steps from a holding potential of -40 mV to +10 mV at 0.03 Hz) were imposed P produced a dose-dependent inhibition of the ICa which gradually decreased and reached steady-state level within 5 min. The average of ICa decrease produced by P, 10^{-6} M, 5 x 10^{-6} M, 10^{-5} M and 5 x 10^{-5} M was 30.4 ± 5.9% (n=6), 52.8 ± 4.1% (n=13), 62.5 ± 8.5% (n=3) and 93.7 ± 5.7% (n=7), respectively. Half inhibition dose (IC_{50}) for the peak I_{Ca} was 4 x 10^{-6} M. In cells treated with P in concentrations ≤ 10^{-5} M, the I_{Ca} recovered to approximately 80% of the control after withdrawal of the drug. After repetitive stimulation P produced a use-dependent blockade which increased with the frequency of stimulation from a holding potential of -40 mV to +10 mV for 150 ms. P not only reduced the peak magnitude of the I_{Ca} but also hastened its decay. The process was well described by a double exponential fit. The fast time constant was similar in the presence and absence of 5 x 10^{-6} M P (5.5 ± 0.1 vs 5.1 ± 0.3 ms, respectively), but the slow time constant was significantly shortened by P (41.3 ± 1.6 vs 26.9 ± 1.9 ms, respectively. $P < 0.01$), In addition, 5 x 10^{-6} M P caused a 7.3 mV hiperpolarizing shift (n=5) in the steady-state inactivation curve. The effect of P on the time course for recovery from apparent inactivation of I_{Ca} was also studied. Under control conditions the recovery of I_{Ca} can be fitted by a single exponential (time constant = 502 ms) while in the presence of 5 x 10^{-6} M P the recovery process was slowed being better described by a double exponential (time constants = 1.1 s and 8 s).

In conclusion, in single guinea-pig ventricular myocytes P produced a dose- and use-dependent decrease of I_{Ca} that can explain some of its antiarrhythmic properties.

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347P ACTIONS OF DODECYLAMINE ON NA:CA EXCHANGE AND OTHER IONIC PATHWAYS IN GUINEA-PIG CARDIAC MUSCLE

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The possible selectivity of dodecylamine as an inhibitor of Na:Ca exchange (Bielefield et al, 1986; Terrar & White, 1989), as opposed to other ionic pathways, was investigated in cardiac preparations.

Single cells were isolated from guinea-pig ventricular muscle. Transmembrane currents were measured under voltage-clamp conditions (holding potential -40 mV, 36°C, 2.5 mM Ca). Cell shortening was measured by an optical technique (using computer analysis of a digitized video image). In additional experiments tension was recorded isometrically in isolated atria and in ventricular strips.

Three measures which are at least in part related to Na:Ca exchange were investigated: inward 'tail' currents following 20 ms depolarizations to 0 mV; relaxations in the presence of 10 mM caffeine to suppress Ca uptake into the sarcoplasmic reticulum; and contractions at positive potentials following a prepulse to 0 mV to inactivate 'L-type' Ca channels (see Terrar & White, 1989). Dodecylamine reduced the amplitude of tail currents by 19±6% (2 μM, n=9) and 31±11% (8 μM, n=5), increased the half-times for relaxations in the presence of caffeine from 43±3 to 65±6 ms (2 μM), and reduced the contractions at positive potentials by 38±6% (2 μM, n=3) and by 62±20% (8 μM, n=3). Peak inward Ca currents evoked by a 200 ms depolarization to 0 mV were also measured. Dodecylamine reduced the amplitude of these currents by 27±9% (2 μM, n=9) and 47±8% (8 μM, n=7), an effect which was not decreased by intracellular application of the Ca chelator BAPTA (n=3). Time-dependent potassium currents (measured as outward 'tails' upon repolarization following 200 ms depolarizations in the voltage range -20 to +60 mV) were reduced by dodecylamine; following repolarization from +40 mV reductions were 30±12% (2 μM, n=5) and 63+9% (8 μM, n=6). In four atrial preparations and six ventricular strips, negative inotropic effects were seen at concentrations above approximately 30 μM, whereas at lower doses either no effect or a small positive inotropic effect was seen.

It is concluded that at none of the doses tested did dodecylamine exhibit total selectivity for the Na:Ca exchange process. The results from multicellular preparations, however, suggest that at low doses effects of dodecylamine tending to cause a positive inotropic effect may equal or predominate over those effects tending to produce a negative inotropic effect. The balance between these two classes of effects appears to change with increasing dodecylamine concentraction.

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348P FREE AND BOUND PLASMA DIGOXIN AFTER DIGOXIN-SPECIFIC FAB FRAGMENTS IN THE RABBIT

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In spite of the established clinical usage of digoxin-specific Fab fragments (DSFab) in treating cardiac glycoside toxicity (Antman et al, 1990), some aspects of their effects on hapten disposition are unclear. For example, recent reports (Hursting et al, 1987; Sinclair et al, 1989) indicated that although free active plasma digoxin was rapidly reduced by DSFab administration, free concentrations significantly rose 12 h later. It is conceivable that this 'rebound' phenomenon could be associated with a recurrence of clinical toxicity.

We propose to use a rabbit model in which we can measure the plasma concentrations of free and bound digoxin as well as DSFab, so that the rebound effect and the stoichiometry between digoxin and DSFab can be studied.

Rabbits (female, New Zealand-White, 3.2-5.1 kg) were given [^{3}H]digoxin (15μg kg^{-1}, 12.5μCi kg^{-1}, 19μM kg^{-1} i.v.) followed by DSFab (2mg kg^{-1}, 38μM kg^{-1} i.v.). Free (separated by ultrafiltration, molecular cut-off 5 kDaltons) and total [^{3}H]digoxin were quantitated by liquid scintillation counting. DSFab were measured using an enzyme-linked immunosorbent assay (Timsina & Hewick, 1990). The pharmacokinetic parameters were obtained by standard procedures (Timsina & Hewick, 1990). Student's paired t-test was used to detect significant differences ($P<0.01$).

DSFab injection caused an immediate 3 to 4 fold rise in total digoxin which was associated with a decrease in free drug to 30% of the initial concentration. However, within 30 min free digoxin had started to rise and by 60 min reached 60% of pretreatment levels. The reason for this rebound is unclear although a possible explanation is that the significant amounts of DSFab are cleared from plasma before sufficient digoxin has diffused from the tissues. To maintain a minimum concentration of free digoxin a considerable molar excess of antibody fragments was required and a DSFab: total digoxin ratio of about 5 was needed, since when this fell below 4 the rebound occured. Calculated during the initial 24 h period, the pharmacokinetic parameters (Means ± s.e.mean, n=5) for DSFab were distribution half-life ($t^1/2\alpha$), 0.3 ± 0.1 h; elimination half-life ($t^1/2\beta$), 3.2 ± 0.6 h; volume of distribution at steady-state (Vd_{SS}), 184.7 ± 18.0 ml kg^{-1} and systemic clearance (Cl) 56.9 ± 2.1 ml kg^{-1} h^{-1}. Measured during the 2 to 5 day period, the control total digoxin pharmacokinetic parameters of $t^1/2\beta$, 37.7 ± 2.9 h; Vd_{SS}, 2.8 ± 0.2 L kg^{-1}; Cl, 57.1 ± 3.9 ml kg^{-1} h^{-1} were significantly ($P<0.01$) reduced by 40, 90 and 75% respectively after DSFab administration. The prolonged elevation in total digoxin, which was still apparent at 100 h ($P<0.05$), is surprising in the view the short $t^1/2\beta$ of DSFab. It appears that further distribution and elimination after the initial DSFab-induced redistribution of digoxin is slower than would be anticipated.

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349P ELECTROPHYSIOLOGICAL ACTIONS OF SR 33557 IN COMPARISON TO NIFEDIPINE, VERAPAMIL, DILTIAZEM AND AMIODARONE IN THE DOG

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We have compared the electrophysiological actions of the calcium antagonist, SR 33557, (Polster et al. 1990) to nifedipine (Nif), verapamil (Ver), diltiazem (Dil) and amiodarone (Am). We have used a visual technique of multi-parameter analysis displayed in the form of a star with hexagons representing the pre- and post-drug effects. Mongrel dogs were anaesthetised with chloralose and electrodes implanted into a)the right atrium (for sino-atrial node stimulation and electrocardiogram reception) and b)the right ventricle (His bundle electrocardiogram and retrograde ventricular stimulation). Six indices were measured as a percent of pre-drug values; sinus cycle length (SCL), AH interval (AH), Wenckebach cycle length (WCL), ventricular, AV node and atrial effective refractory periods (VERP, AVNERP, AERP respectively). Drugs were given iv at two doses (at 0 and 1h); SR 33557 (2x50 ug/kg), Dil (2x300 ug/kg), Ver (43 and 100 ug/kg), Nif (2x25 ug/kg), Am (2x5 mg/kg).

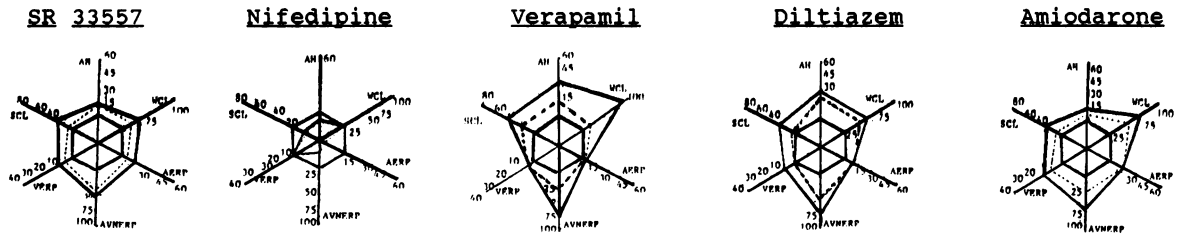


Figure 1. Graphical representation of electrophysiological actions (n=5 in each group).

These results suggest SR 33557 possesses an electrophysiological profile that shares some similarity to that of Am in that SCL, AERP and AVNERP were prolonged to similar extents, although Am tended to have a greater effect on WCL. Both Dil and Ver had greater effects on AVNERP and on WCL at higher doses. Nif tended to reduced all indices measured. Thus this graphical approach a) can give an immediate "overview" of electrophysiological effects and b) suggests that SR 33557 may be beneficial against certain types of arrhythmias.

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350P PREVENTION OF CALCIUM OVERLOAD IN RAT HEART BY SR 33557, A NOVEL CALCIUM ENTRY BLOCKER

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Administration of a large dose (40 mg/kg s.c.) of isoproterenol (ISO) to rats produces myocardial lesions (Rona *et al.*, 1959) accompanied by a massive Ca^{++} overload (Fleckenstein, 1983). This study has investigated the effects of SR 33557, a novel Ca^{++} entry blocker (Nokin *et al.*, 1989; Schmid *et al.*, 1989) on tissue Ca^{++} content, beta-adrenoceptors and L-type Ca^{++} channel characteristics (B_{max} and K_d). Saturation isotherms and Scatchard analysis were peformed on tissue homogenates using ^{125}I-cyanopindolol (^{125}I-CYP), ^{3}H-PN200-110 and ^{3}H-SR 33557. Rats were sacrified 8 hours after ISO administration shown to produce maximal Ca^{++} overload in preliminary studies.

ISO induced a 9-fold increase in the Ca^{++} content of the rat heart. The number of beta-adrenergic and Ca^{++} channel binding sites were reduced by 27 and 23 % respectively. K_d values were not modified. Intravenous injection of SR 33557 (0.5 to 10 mg/kg) 30 min before ISO induced a dose-related decrease in Ca^{++} content, the 5 mg/kg dose reducing the Ca^{++} overload by 50 percent. At this dose, SR 33557 had no effect on the number of beta-adrenergic binding sites but prevented the decrease in Ca^{++} channels number.

Table 1. Effect of SR 33557 on Ca^{++} content and binding sites

	Control	+ ISO	+ ISO + SR 33557 (5 mg/kg iv)
Ca^{++} content (nmol/kg w.w.)	5.7 ± 0.1	34.9 ± 4.3 **	15.4 ± 16 **
B_{max} (^{125}I CYP)	141 ± 10	110 ± 7 *	121 ± 4
B_{max} (^{3}H PN200-110)	222 ± 4	172 ± 11 *	241 ± 14
B_{max} (^{3}H SR 33557)	327 ± 17	262 ± 17 *	310 ± 31

Binding capacity (B_{max}) is given in fmol/mg protein. Results shown as mean ± S.E.M. (n = 6). * $p < 0.05$; ** $p < 0.01$ *versus* control

In conclusion, SR 33557 is able to protect the heart from Ca^{++} overload induced by sympathetic overstimulation which down-regulates the beta-adrenergic receptors and Ca^{++} channels. This down-regulation is reduced by SR 33557.

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351P REGULATION OF MYOCARDIAL CONTRACTILE PERFORMANCE BY SODIUM NITROPRUSSIDE IN THE ISOLATED PERFUSED HEART OF THE FERRET

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Shah and colleagues (1990) have shown that the endocardial endothelium (EE) can modulate the contraction of mammalian papillary muscles via the release of two factors, a contraction-abbreviating factor with properties indistinguishable from those of endothelium-derived relaxing factor (EDRF) and a contraction-prolonging factor, the identity of which is unknown. In the present study we have investigated the effect of the nitric oxide donor, sodium nitroprusside (SNP) on the regulation of myocardial contraction in isolated whole heart preparations.

Ferrets (0.5-1.0 kg) were anaesthetised with sodium pentobarbitone (120 mg/kg i.p.). The hearts were rapidly excised, mounted and perfused by the Langendorff technique with Krebs Henseleit solution containing (in mM) CaCl 1.25, and acebutolol 0.01 and indomethacin 0.01, and gassed with 95% O_2/5% CO_2 at 37°C. Left ventricular pressure was recorded via a fluid-filled latex balloon. SNP 10^{-5} M was infused for 15 min into either the coronary circulation via the aortic root cannula or directly into the left ventricular cavity. Samples of left ventricular myocardium were rapidly excised and immersed in liquid nitrogen at the end of the SNP infusion for later assay of cyclic GMP.

The intracoronary infusion of SNP produced an abbreviation of myocardial contraction. The peak left ventricular pressure decreased by 5.7% and there was a reduction in the duration and rate of development of left ventricular pressure by 4.2 and 5.6% respectively. This was accompanied by a 4.13 fold increase in cyclic GMP levels above baseline measurements (9.94±2.06 fmol/mg wet weight (SEM); n=6). Infusion of SNP into the left ventricle however had no effect on left ventricular contraction or intracellular cyclic GMP levels.

These results show that SNP (and thus nitric oxide) while able to modulate myocardial contraction when infused via the coronary circulation, has no effect on contractile performance when infused over the surface of the left ventricular cavity. In view of the short half-life of EDRF and its inactivation by red cells, these findings make it unlikely therefore that EDRF released from the EE will have a major effect on myocardial contractility. Whether EDRF released from the endothelium of the coronary circulation can influence myocardial contractile performance however remains unknown at present.

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352P EVIDENCE FOR M_2 CHOLINOCEPTOR HETEROGENEITY IN GUINEA-PIG ATRIA AND VENTRICLES

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Mammalian heart muscarinic receptors (mAChR), which mediate negative inotropic and chronotropic responses via opening of potassium channels and inhibition of adenylyl cyclase, comprise only the M_2 subtype (see Hulme *et al.*, 1990, for references). High muscarinic agonist concentrations (>1μM) additionally lead to polyphosphoinositide hydrolysis (Mizushima *et al.*,1989). As evidence indicates differential G-protein coupling for these two biochemical responses (Kohl *et al.*,1990), we have examined mAChR-mediated inositol phosphates (IPs) formation in guinea-pig cardiac tissue. Slices (0·35 x 0·35mm) of atria (GPA) and ventricles (GPV) were incubated with [2-^{3}H]-*myo*-inositol (0·67μM; 60min; with 5mM LiCl) followed by 60min stimulation with agonist. Total [^{3}H]-IPs were isolated by anion exchange chromatography.

The mAChR agonists (+)-cis-dioxolane and L-660,863 (3-(3-amino-1,2,4-oxadiazol-5-yl)-quinuclidine) concentration-dependently stimulated IPs formation to a maximum of 1.8-fold above basal levels (GPA: basal 157±12, max. 457±25; GPV: 152±10, 409±33; dpm/vial, mean ± s.e.mean, n=4), with the following -logEC_{50}s (±s.e.mean): atria, 5·7±0·1 and 6·3±0·3; ventricles, 5·8±0·2 and 6·0±0·2, compared with -logK_A values for M_2 mAChR mediating negative inotropy in the left atria of 6·0±0·2 and 7·6±0·1, respectively. Inhibition curves for mAChR antagonists (see Hulme *et al.*, 1990, for full chemical names), added 30min prior to agonist, were constructed against 10μM (+)-cis-dioxolane (approx. EC_{80}) and apparent -log K_i values are shown in table 1.

Table 1. Antagonist affinities (-logK_i) for mAChR-mediated IPs formation in guinea-pig cardiac tissue.

	atropine (9·3)	pirenzepine (6·5)	methoctramine (7·9)	AFDX-116 (7·2)	HHSiD (6·5)	p-F-HHSiD (nd)
GPA:	9.4±0.3	7·4±0·1	7·7±0·1	6·8±0·1	7·8±0·1	6·8±0·2
GPV:	9.3±0.2	7·4±0·1	†7·0±0·1	6·7±0·2	†6·9±0·2	6·7±0·1

†P<0.01, GPA versus GPV. All values are mean ± s.e.mean, n=4-6; -logK_i=-log{IC_{50}/(1+(EC_{80}/EC_{50}))}. Values in parentheses give -log K_B values for M_2-mAChR (Hulme *et al.*, 1990); nd=no data.

It is apparent that the antagonist affinity profile at mAChRs mediating IPs formation in guinea-pig cardiac tissues deviates from that expected for an M_2-mediated response, and that methoctramine and HHSiD can distinguish between the responses in ventricular and atrial tissues. These data, in addition to the high EC_{50} value for L-660,863 as compared to the -logK_A value for negative inotropy, may implicate a diversity among M_2 mAChR in mammalian cardiac tissue or a diversity of extra-receptor binding sites.

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353P INHIBITION OF PEPTIDOLEUKOTRIENE RELEASE FROM GUINEA-PIG HEART TISSUE BY ADENOSINE

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We have investigated the action of adenosine on the release of peptidoleukotrienes from guinea pig heart tissue stimulated by antigen challenge.

Male Dunkin-Hartley guinea pigs (200-250g) were sensitized with ovalbumin (Sigma, Grade II) in saline (100mg i.p. and 100mg s.c.). After 3-4 weeks the animals were killed by cervical dislocation, 15 min after receiving heparin (3000u kg^{-1} i.p.). The hearts were removed and the coronary circulation perfused free of blood. The cardiac tissue was then chopped into $1mm^3$ cubes with a McIlwain tissue chopper and divided into 800mg aliquots. Tissue aliquots were incubated in 6ml Tyrode's solution at 37°C in a water bath. Adenosine was added to a concentration in the range of 0.01μM to 100μM from 15min prior, to 10min after antigen challenge with 100μg ml^{-1} ovalbumin. In separate experiments incubations were carried out in the presence of 3μM indomethacin, and in addition the adenosine A_2 receptor antagonist 8-phenyltheophylline (8PT; 10μM) or the adenosine uptake blocker S-(p-nitrobenzyl)-6-thioinosine (NBTI; 1μM) were added to separate incubations, 10min before addition of adenosine. 15min after addition of antigen, the samples were put on ice and peptidoleukotrienes extracted from the supernatants by elution from C_{18} Sep-paks. Eluates were dried down under vacuum and peptidoleukotrienes were assayed on superfused guinea pig ileum in the presence of hyoscine and mepyramine, against an LTD_4 standard.

The effect of adenosine on the release of peptidoleukotriene was a 'bell-shaped' dose-response, exhibiting a maximal inhibition of 46.55±11.53% at a concentration of 1μM. At lower or higher concentrations the inhibitory effect of adenosine was reduced. The maximal effect of adenosine was achieved when the nucleoside was added 2min prior to or simultaneously with the addition of antigen (46.55±11.53 and 60.04±16.17%, at t = 2 and 0min respectively with 1μM adenosine). When adenosine was added for a shorter or longer time the inhibitory effect was reduced. If endogenous prostaglandins were inhibiting pLT release, incubation with indomethacin might have been expected to potentiate pLT release and perhaps unmask a greater inhibitory effect of adenosine. This was not the case; adenosine-mediated inhibition of pLT release in the presence of indomethacin was 34.1±18.4%, not significantly different from the result obtained with adenosine alone. On the other hand 8PT abolished the response to adenosine, whilst NBTI had no effect.

The results demonstrate that adenosine inhibits pLT release from antigen-challenged guinea pig heart tissue in a concentration- and time-dependent manner and suggest that the effect is mediated by increase of intracellular cyclic AMP levels. Adenosine release might represent a physiological control mechanism for the biosynthesis of peptidoleukotrienes.

We thank Pfizer UK for funding.

354P THE EFFECTS OF SHORT-TERM DILTIAZEM ADMINISTRATION ON LEFT VENTRICLE MASS AND COLLAGEN CONTENT IN DOCA-SALT HYPERTENSIVE RATS

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Left ventricular hypertrophy is associated with myocardial proliferation of fibrous tissue. Some antihypertensive drugs can reduce left ventricle (LV) mass but collagen regression may not occur, leading to further increased collagen concentration (Sen et al., 1977). The present study examined the effects of 7 weeks of diltiazem treatment on left ventricular hypertrophy in the rat.

Stable hypertension was induced in male Sprague-Dawley rats by 12 week treatment with desoxycorticosterone acetate (DOCA) and saline drinking fluid. They were then divided into two groups. One group (n=6) received water thereafter as drinking fluid (untreated hypertensives) and the other (n=5) received diltiazem hydrochloride solution (treated hypertensives). Diltiazem concentration was increased over 2 weeks from 0.3 to 0.7 mg/ml and kept at 0.7 mg/ml for a further 5 weeks. A third age-matched normotensive control group (n=6) received tap water. Systolic arterial pressure (SAP) was measured in all groups by tail-cuff plethysmography. Rats were killed and hearts excised. Dry mass of LV free wall with interventricular septum was determined. Total collagen content was estimated by hydroxyproline (HP) assay of the hydrolysed samples (Woessner, 1961).

After 7 weeks, SAP (mm Hg) in the treated hypertensives was 164 ± 5 (mean ± s.e. mean) compared with 200 ± 5 in the untreated group and 129 ± 2 in the normotensive group. Relative LV mass (mg/100 g body weight) in the treated group was 53.3 ± 2.2 compared with 69.3 ± 2.7 in the untreated hypertensives and 42.9 ± 0.9 in the normotensives. Ventricular HP concentration (mg/g) in the normotensive group was 2.38 ± 0.06 whilst that in the treated and untreated groups was 2.59 ± 0.14 and 2.61 ± 0.06 respectively. However, in the treated group, total HP content was 18.8% lower than in the untreated group and absolute LV mass was 18.5% lower. These figures parallel the difference in SAP between the two groups (18.0%).

These data show that blood pressure reduction with diltiazem can induce hypertrophy regression, in agreement with recent reports (e.g Grellet et al., 1988). Reduction of LV mass was accompanied by collagen regression and HP concentration was not elevated in the treated group. The decrease in SAP, absolute LV mass and total collagen content suggest that in the early stage of pressure reduction with diltiazem, the degree of hypertrophy regression is related to the fall in arterial pressure. However, chronic dosing studies to explore these relationships further are indicated.

The author is supported by an SERC award.

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Cl^- replacement by NO_3^- is antiarrhythmic in ischaemia and reperfusion in vitro (Ridley et al., 1989). The mechanism may involve intracellular pH (pH_i) changes. Phosphorus NMR spectroscopy can be used to measure pH_i, but only if extracellular PO_4^{3-} ($PO_4^{3-}{}_e$) is absent (Garlick et al., 1979). To permit comparison between arrhythmia data (derived using PO_4^{3-}-containing solution) with planned NMR studies, we have examined whether $PO_4^{3-}{}_e$ removal affects arrhythmias and their modulation by Cl^- replacement by NO_3^-.

Left regional ischaemia was induced in rat hearts (n=12/group) perfused with solution containing (in mM) NaCl 118.5, $NaHCO_3$ 25.0, KCl 4, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4 and glucose 11.1, pH 7.4, 37°C or similar solution modified by removal of PO_4^{3-} and/or by substitution of Cl^- by NO_3^- (presence and absence designated + and - respectively in Table 1). The incidences (%) of ventricular premature beats *(VPB)*, bigeminy *(BG)*, salvos *(S)*, ventricular tachycardia *(VT)* and fibrillation *(VF)* were recorded during 30 min regional ischaemia and 5 min reperfusion (*p<0.05 for Cl^- vs NO_3^-, **p<0.05 for PO_4^{3-} vs no PO_4^{3-}).

Table 1.

			Ischaemia-Induced Arrhythmias					Reperfusion-Induced Arrhythmias				
PO_4^{3-}	Cl^-	NO_3^-	*VPB*	*BG*	*S*	*VT*	*VF*	*VPB*	*BG*	*S*	*VT*	*VF*
+	+	-	100	100	100	100	67	100	33	100	100	78
-	+	-	100	92	100	100	83	100	63	100	100	88
+	-	+	*50	*17	*25	*0	*0	88	42	*25	*17	*0
-	-	+	83	*25	*25	*8	*8	67	25	*25	*8	*0

Table 1 shows that replacement of Cl^- by NO_3^- inhibited arrhythmias (in agreement with previous data; Ridley et al., 1989) and that $PO_4^{3-}{}_e$ removal had no influence on arrhythmias or their modification by replacement of Cl^- by NO_3^-. Heart rate and coronary flow, too, were not affected by $PO_4^{3-}{}_e$ removal (not shown). In conclusion, PO_4^{3-}-free solution can legitimately be used in studies concerning the antiarrhythmic effects of anion manipulation thus permitting arrhythmia data to be integrated with data derived from planned NMR studies.

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356P IN THE PITHED RAT, Ca^{2+}-INFLUX DEPENDENT AND INDEPENDENT α_1 ADRENOCEPTOR-MEDIATED PRESSOR RESPONSES ARE NOT MEDIATED BY α_{1A}- AND α_{1B}-ADRENOCEPTORS, RESPECTIVELY

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In pithed normotensive rats α_1-adrenoceptor agonists can increase diastolic pressure by triggering a Ca^{2+}-influx and by releasing Ca^{2+} from intracellular stores. Indanidine predominantly activates Ca^{2+}-influx, whereas cirazoline activates both Ca^{2+}-influx and Ca^{2+}-release from intracellular stores (Timmermans et al., 1985). We investigated whether the Ca^{2+}-influx dependent component of the pressor response is mediated by α_{1A}-adrenoceptors and the Ca^{2+}-influx independent proces is mediated by α_{1B}-adrenoceptors.

The experiments were performed in male normotensive rats (250-300g), anesthetized with hexobarbitone (150 mg/kg, i.p.) which were subsequently pithed and artificially ventilated with room air. The jugular vein and the carotid artery were canulated for the administration of drugs and the measurements of blood pressure, respectively. Nifedipine was injected into a carotid artery. Chlorethylclonidine was administered in conscious rats via a caudal vein.

The log dose pressor response curve of cirazoline was only slightly shifted to the right by pretreatment (-60 min, i.v.) with the irreversibly selective α_{1B}-adrenoceptor antagonist chlorethylclonidine, 12.5 mg/kg and 25 mg/kg being equieffective. Additional pretreatment with nifedipine (1 and 3 mg/kg, i.a.; -15 min) did not enhance the antagonism by chlorethylclonidine, nor enhanced chlorethylclonidine the attenuation of the pressor response of cirazoline by nifedipine. The log pressor response curve of indanidine was also hardly affected by chlorethylclonidine, nor enhanced chlorethylclonidine the attenuation of the pressor response by nifedipine (0.1 mg/kg). The log dose pressor response curve of indanidine underwent a dose-dependent rightward shift after pretreatment (-15 min, i.v.) with the selective α_{1A}-adrenoceptor antagonist 5-methyl-urapidil (0.05 mg/kg: 5-fold;0.15 mg/kg: 8-fold). 5-methyl-urapidil also caused a dose dependent rightward shift of the dose response curve of cirazoline (0.05 mg/kg: 5-fold;0.15 mg/kg: 13-fold). After pretreatment with nifedipine (1mg/kg) 5-methyl-urapidil was slightly more effective (0.05 mg/kg: 12-fold;0.15 mg/kg: 41-fold).

In conclusion, by studying the interaction between Ca^{2+}-influx dependent and independent α_1-adrenoceptor-mediated pressor responses with the selective α_{1A}-adrenoceptor antagonist 5-methyl-urapidil (Hanft & Gross, 1989) and selective irreversible α_{1B}-adrenoceptor antagonist chlorethylclonidine (Han et al., 1987) no indications were found that these contractile mechanisms are mediated by different α_1-adrenoceptor subtypes.

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357P EFFECTS OF AMINOGLYCOSIDE ANTIBIOTICS ON STEADY STATE AND POST-REST POTENTIATED FORCE OF CONTRACTION OF GUINEA-PIG ISOLATED ATRIA

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The negative inotropic effect of aminoglycoside-antibiotics is well known. This action is due to the displacement of calcium-ions from the outer surface of the cell membrane (Lüllmann & Schwarz, 1985). We investigated the effect of various aminoglycosides on the steady state (CF_{ss}) and the post-rest potentiated force of contraction (CF_{pr}) of mammalian cardiac muscle.
Guinea-pig isolated left atria were paced at a frequency of 3 Hz with interspersed rests of 4 sec duration every 15 minutes. This protocol was applied for at least 6 hours.
Aminoglycoside antibiotics in a millimolar concentration range acutely impaired both parameters. Significant differences were found concerning the negative inotropic potency of the various agents. The following EC_{50} values for the depression of CF_{ss} were detected: gentamicin 1.11 ± 0.09 mM, tobramycin 1.06 ± 0.19 mM, sisomicin 0.28 ± 0.03 mM, netilmicin 0.23 ± 0.01 and amikacin 0.15 ± 0.004 mM. The negative inotropic action of these compounds is lowered at high calcium concentrations in the medium ($[Ca]_o$). The relationship between log $[Ca]_o$ and the negative inotropic potency of gentamicin was found to be nearly linear (slope -1.12, pA_2 3.01).
Under control conditions both CF_{ss} and CF_{pr} decreased slowly within several hours. In the presence of aminoglycoside antibiotics CF_{pr} increased slowly, while CF_{ss} remained depressed during the course of the experiment. Compared to other positive or negative inotropic drugs this effect was found to be unique. The efficacy of inducing this effect was different for the aminoglycosides in the following rank order: gentamicin > sisomicin > tobramycin > netilmicin > amikacin. In contrast to the negative inotropic action of these drugs, the slow raise of CF_{pr} was found to be enhanced under conditions of elevated $[Ca]_o$.
After 6 hours the aminoglycosides were washed out. Both CF_{ss} and CF_{pr} increased markedly and remained stable at this level.
These results may indicate a slow accumulation of intracellular calcium. We therefore investigated interventions which are known to inhibit the calcium-extrusion out of the cell. For this purpose we used 0.5 mM amiloride (Floreani & Luciani 1984) and a low sodium medium (70 mM)(Langer et al. 1976). In contrast to the aminoglycosides, the later mediate a positive inotropic effect in heart muscle. Under the experimental conditions described above CF_{ss} and CF_{pr} developed in the course of the long-lasting experiments exactly the same as they have done in the presence of aminoglycosides.
We therefore conclude that aminoglycoside antibiotics may not only mediate negative inotropy by displacing calcium from the outer surface of the plasma-membrane but inhibit thereby concurrently the calcium-extrusion out of the cells. This effect becomes only visable during long-lasting experiments.

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358P DIFFERENTIAL RESPONSE OF ISOLATED HEARTS OF SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS TO ADRENERGIC AND CHOLINERGIC AGONISTS

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The prolonged existence of hypertension, both in humans and in experimental animals is known to be associated with important changes in cardiac morphology and function, predominantly characterized by an increase in left ventricular myocardial mass. Cardiac hypertrophy is not necessarily explained by increased mechanical stress alone. The autonomic nervous system, as well as certain growth factors, may also play a role. Little is known concerning the response of the heart of hypertensive humans and animals to inotropic drugs. We therefore investigated the functional response to various inotropic agents in isolated hearts from spontaneously hypertensive rats (SHR) and age matched Wistar Kyoto rats (WKY, controls). Accordingly, we studied the functional response to various ß-adrenoceptor agonists: isoprenaline ($ß_1+ß_2$), dobutamine ($ß_1>ß_2$), salbutamol ($ß_2>ß_1$) terbutaline ($ß_2>ß_1$) and to α-stimulants: methoxamine ($α_1$), cirazoline ($α_1$), phenylephrine ($α_1>ß_1$). In addition, the response to the muscarinic agonists metacholine and oxotremorine was established. In male SHR of 18 weeks old, mean arterial blood pressure and heart rate (determined i.a. in a carotid artery, under anaesthesia with pentobarbitone 75mg/kg), amounted to 184 mmHg and 448 beats/min. respectively. Male WKY of the same age had a mean blood pressure of 98 mmHg and a mean heart rate of 357 beats/min. The animals were sacrificed and the hearts were excised out and set on as Langendorff preparations, perfused with Tyrode at 37°C and paced at 5 Hz. The degree of cardiac hypertrophy was characterised by the ratio body weight/heart weight. Changes in contractile force and coronary flow were quantified by means of E_{max} values, obtained from concentration-response curves for each drug (table 1). Initial values of contractile force and coronary flow were the same for both SHR and WKY.

	WKY	SHR		WKY	SHR
Isoprenaline	152.10 ± 18.75	172.46 ± 41.57	Cirazoline	53.65 ± 11.72	15.99 ± 6.18
Dobutamine	113.63 ± 11.93	144.02 ± 15.10	Methoxamine	45.68 ± 11.27	28.78 ± 8.64
Salbutamol	67.55 ± 5.73	52.77 ± 7.01	Phenylephrine	50.18 ± 4.09	43.21 ± 13.82
Terbutaline	73.75 ± 8.51	78.43 ± 21.05			
			Metacholine	-58.05 ± 4.47	-28.39 ± 5.17
			Oxotremorine	-37.08 ± 5.19	-23.55 ± 2.49

table 1 E_{max}-values in % of the initial contractile force

There was no significant difference in increase of LVP induced by the ß-adrenoceptor agonists in SHR and WKY. However, the inotropic response to the $α_1$-adrenoceptor agonists cirazoline and methoxamine and to the muscarinic agonists oxotremorine and metacholine proved to be significantly weaker in hearts from SHR than in those from WKY ($p<0.05$).
These results show that long existing hypertension leads to a different response to $α_1$ and muscarinic stimulation. Whether these changes in inotropic response in SHR are caused by a decrease in receptor density or a uncoupling of second messenger systems remains to be elucidated.

359P MUSCARINIC RECEPTORS IN THE NORMOXIC AND ISCHAEMIC RAT HEART

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The effects of ischaemia on cardiac muscarinic receptors have not been investigated in detail. We therefore studied the influence of global ischaemia on muscarinic receptors, by means of both functional and radioligand binding experiments.

In functional studies the muscarinic receptor agonist oxotremorine reduced contractile force of the rat Langendorff heart (LH) by maximally 9.0 ± 1.5 % after 30 min ischaemia and by 27.6 ± 1.5 % in normoxic control preparations (means ± s.e.m., n=6; $p<0.05$). The effect of oxotremorine on cardiac frequency was not affected by ischaemia.

In radioligand binding studies the M_2-cholinoceptor density and affinity were assessed in left ventricular homogenates of rat LH under normoxic circumstances, after 30 min of ischaemia and after 30 min of ischaemia plus reperfusion, respectively. Neither ischaemia nor post-ischaemic reperfusion caused any change in receptor density or affinity.

In displacement experiments in ischaemic and reperfused left ventricular rat heart preparations oxotremorine potentiated ($p<0.05$) [^{3}H]NMS binding in concentrations ranging from 2×10^{-10} - 10^{-7} M. For maximally potentiated values we measured 122.0 ± 8.0 % (ischaemic) and 126.8 ± 14.1 % (reperfused). This was followed by a displacement at higher concentrations of oxotremorine. This phenomenon was abolished in the presence of 10^{-4} M Gpp(NH)p which is known to shift receptors to the low-affinity state. In normoxic ventricular preparations oxotremorine was unable to potentiate [^{3}H]NMS binding. The potentiation after ischaemia could be an allosteric effect mediated by ischaemia.

In conclusion, global ischaemia decreased the negative inotropic but not the chronotropic response to the muscarinic agonist oxotremorine. Although global ischaemia did not change the density and affinity of cardiac muscarinic receptors for [^{3}H]NMS, low doses of oxotremorine enhanced the binding of [^{3}H]NMS to ventricular homogenates.

360P EFFECT OF THE SPIROLACTAM NK-1 RECEPTOR ANTAGONIST, GR82334, ON NEUROKININ- AND ELECTRICAL STIMULATION-INDUCED OEDEMA IN THE RAT

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Application of noxious stimuli to the skin initiates an inflammatory response characterised by local oedema. This is thought to be a consequence of the release of peptides, particularly calcitonin-gene related peptide (CGRP) and neurokinins (NKs) from sensory nerves, which act synergistically, causing vasodilatation and plasma protein extravasation (PEV) respectively (see Holzer, 1988). On the basis of the activities of receptor selective NK agonists, it has been suggested that NK-1 receptors mediate NK-induced PEV in rat skin (Andrews et al, 1989). We now report the effects of an NK-1 antagonist, GR82334 (Hagan et al, 1990) on (i) NK-1 agonist-induced PEV in rat skin, (ii) the PEV response in rat paw to antidromic stimulation of the fibularis nerve and (iii) the increase in paw volume caused by co-injection into the paw of an NK-1 agonist with CGRP.

Pentobarbitone-anaesthetised male rats were treated with Evans Blue dye (30mg/kg iv) and then vehicle or GR82334 (0.01-2.5μmol/kg iv, 15min pretreatment). The NK-1 selective agonist, GR73632 (Hagan et al, 1989) was injected into dorsal skin (1-1000pmol) or the fibularis nerve was stimulated for 3min (10mA, 5Hz, 2ms). After 10min, PEV was quantified by measuring levels of Evans Blue dye in the skin. In some experiments, a range of doses of GR73632 (0.03-30nmol) were injected intraplantar in combination with CGRP (0.1nmol) and the effect of GR82334 on the resulting oedema was measured by plethysmometry.

GR82334 (0.5μmol/kg) was able to abolish the PEV response in skin induced by the NK-1 agonist, GR73632. GR82334 also attenuated, in a dose-related manner, the PEV response (μg Evans Blue/paw) to electrical stimulation of the fibularis nerve :- vehicle pretreatment (n=14): stimulated paw (S) 47.5 ± 5.9, unstimulated paw (US) 4.1 ± 0.4; GR82334 (0.3μmol/kg, n=9): (S) 9.0 ± 1.6, (US) 3.9 ± 0.9. The paw oedema response to GR73632/CGRP was maximal at 30min post-injection and was maintained for >4h. GR82334 (2.2μmol/kg sc, 30min pretreatment) antagonised this response, causing an 893-fold rightward shift in the dose-response curve to GR73632.

These results confirm the involvement of NK-1 receptors in NK-induced PEV in rat skin. Furthermore, the ability of GR82334 to abolish sensory nerve stimulation-induced PEV suggests that this response is predominantly mediated by NKs (substance P and NKA) acting at NK-1 receptors. GR82334 was able to markedly antagonise the synergistic effects of CGRP and an NK-1 agonist on paw oedema, suggesting that an NK-1 antagonist may be useful for the treatment of inflammatory conditions of neurogenic origin.

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361P ACTIONS OF CORTICOTROPHIN RELEASING FACTOR-41 (CRF-41) ON THE VASCULAR AND EXTRAVASCULAR CAPSULAR SMOOTH MUSCLE OF THE ISOLATED PERFUSED PIG SPLEEN

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CRF-41 has been located immunohistochemically within the human gut (Nieuwenhuijzen-Kruseman *et al.*, 1982) and high affinity CRF-41 binding sites, pharmacologically similar to those in the anterior pituitary gland, have been demonstrated in the mouse spleen (Webster & DeSouza, 1988). Although CRF-41 has been shown to possess some peripheral vascular actions (Gardiner *et al.*, 1988) neither its effects on the splenic smooth muscle nor its molar potency relative to other vasoactive substances has been elucidated.

In the present experiments 9 female large white pigs (mean weight 23.8 ± 0.5 kg), were anaesthetised with fluothane (ICI) . The spleen (mean weight 60.5 ± 3.2g) was removed, enclosed in a perspex plethysmograph, and perfused at constant flow with Krebs solution (37°C, equilibrated with 95% O_2/5% CO_2). Mean perfusion pressure was recorded continuously and changes in both splenic inflow resistance and spleen volume were assessed in response to the intra-arterial bolus administration of rat CRF-41 (Sigma), lysine-vasopressin (LVP), adrenaline (Ad) and noradrenaline (NA). It was clear that Ad could evoke substantial reductions (mean maximum 27.4 ± 5.6 ml) in volume of the pig spleen. Quantitatively the results confirmed in the pig spleen, previous observations (Davies & Withrington, 1975) in the dog spleen, that on a molar basis the vasoconstrictor potency ranking order on the splenic vascular smooth muscle is LVP > NA > Ad whilst, in contrast, on the capsular smooth muscle the order is Ad > NA > LVP.

Injection of CRF-41 (0.1 - 50 μg) caused variable changes in splenic inflow resistance although the predominant effect was an increase. The mean maximum increase in inflow resistance to CRF, irrespective of dose, was 51.6 ± 15.5% of control thus, although the response to CRF-41 was significant ($P < 0.05$), it was considerably smaller ($P < 0.001$) than the maximum responses to either NA or LVP. A small increase in spleen volume (maximum 6.2 ± 1.8 ml) was evident with the highest doses of CRF-41. Adrenaline, over the same molar dose range as CRF-41, caused a reduction in inflow resistance indicating vasodilatation, with a maximum fall of 27 ± 5.3% of control.

The experiments indicate that CRF-41 can cause a small, but significant splenic vasoconstriction. It may, therefore, be a factor in altering the splenic microcirculation in a manner appropriate to those circumstances leading to its release.

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362P EICOSANOID RELEASE FROM THE ANTRAL AND CORPUS REGIONS OF THE RAT STOMACH

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Non-steriodal anti-inflammatory drugs (NSAIDS) can cause antral ulceration in man (Stock and Bach, 1988). These drugs may cause damage by increasing leukotriene release via redirection of arachidonic acid metabolism. We have therefore investigated the effects of indomethacin and Merck (MK)886 (3-[3-(4-chlorobensyl)-3-t-butyl-thio-5-isopropylindol-2-yl]2,2-dimethylpropanoic acid) an inhibitor of leukotriene synthesis on eicosanoid release from the antral and corpus regions of the rat stomach *in vitro*. The corpus and antral regions of the stomach were isolated from female random hooded rats (100-125g) and incubated in Krebs solution for 30 mins at 37°C (basal release). Subsequently the bathing medium was removed and replaced with fresh solution containing (1) the calcium ionophore A23187 (10^{-5}M), (2) A23187 and indomethacin ($3x10^{-6}$M) or (3) indomethacin, A23187 and MK886 (10^{-4}M). Following a further 30 mins incubation period, the tissue was removed, blotted and weighed. Eicosanoids released into the medium were measured by radioimmunoassay. The results are presented in Table 1 where values quoted are mean ± S.E.M.

Table 1 **Eicosanoid release from the corpus and antral region of the rat stomach.**

Treatment	Antrum (ng/gram wet weight)			Corpus (ng/gram wet weight)		
	6-keto $PGF_{1\alpha}$	LTB_4	$LTC_4/D_4/E_4$	6-keto $PGF_{1\alpha}$	LTB_4	$LTC_4/D_4/E_4$
Basal	23.69±3.88	*0.17±0.14	6.53±0.71	23.95±2.33	*0.56±0.12	4.43±0.46
A23187	23.65±4.06	1.33±0.20	6.67±0.87	29.80±3.65	1.21±0.22	4.69±0.43
A23187 + Indo	*4.64±0.93	*2.73±0.03	*11.67±1.60	*7.11±1.76	1.72±0.23	4.53±0.63
A23187+Indo+MK886	+5.44±1.65	+1.02±0.93	+4.77±0.53	+4.15±0.67	+0.55±0.34	5.42±0.93

* $p<0.05$ = significant difference from A23187, or + = A23187/indo unpaired t-test (group no. 8-16)

The antral and corpus regions released more immunoreactive 6-keto $PGF_{1\alpha}$ than leukotriene (LT) under basal conditions. A23187 further increased LTB_4, but not $LTC_4/D_4/E_4$ release from the antrum and corpus. Indomethacin in the presence of A23187 significantly inhibited release of 6-keto $PGF_{1\alpha}$ from both regions but enhanced LT release only from the antrum. MK 886 significantly inhibited LT release from the antral region and LTB_4 from the corpus. Thus indomethacin can enhance the release of leukotrienes from the antrum *in vitro*, a mechanism which may be significant for ulcerogenesis *in vivo*.

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363P STIMULATION OF U937 HUMAN MONOCYTIC CELLS BY THROMBIN RESULTS IN INOSITOL PHOSPHATE FORMATION AND Ca^{2+} MOBILISATION BUT NOT THROMBOXANE B_2 SYNTHESIS

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The serine protease thrombin is a potent stimulator of a range of cells involved in haemostasis. In addition, thrombin has been shown to be chemotactic for mononuclear phagocytic imflammatory cells, although the effects of thrombin in these cells is not well understood. In the present study, we have used the human pro-monocytic cell line U937 as a model of monocyte-macrophage cells to examine the effect(s) of thrombin. The effects of thrombin on U937 cells were compared to its effects on human platelets.

U937 cells were differentiated into macrophage-like cells by treating them with 20% conditioned medium for 48 hours [Storch et al. 1990]. In some experiments, cells were labelled with [^{3}H]-myo-inositol, and [^{3}H]-inositol phosphates (IP) were subsequently resolved by Dowex anion exchange chromatography. Washed platelets were prepared as described by Joseph et al. [1990]. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) mobilisation and thromboxane (Tx)B_2 synthesis were measured in Fura-2-loaded cells and by radioimmunoassay respectively.

In differentiated U937 cells, thrombin (1-50U/ml) caused a transient 1.5-2.5 fold increase in $[Ca^{2+}]_i$ above resting levels (200nM). This effect of thrombin was inhibited by the specific thrombin antagonist, hirudin. Consistent with its ability to mobilise $[Ca^{2+}]_i$, thrombin accelerated the formation of [^{3}H]-IP_3 e.g. 60 sec after 50U/ml thrombin stimulation, IP_3 levels increased 1.3-fold above resting levels. Despite these stimulatory effects, thrombin failed to increase TxB_2 synthesis. In contrast, in differentiated U937 cells the chemotactic factor, FMLP (1μM), caused a 3-fold increase in TxB_2 synthesis under conditions where it evoked increases in IP_3 formation and $[Ca^{2+}]_i$ comparable to those triggered by thrombin. In platelets, thrombin caused a significant increase in the levels of both $[Ca^{2+}]_i$ and TxB_2, the threshold thrombin concentrations being 0.05U/ml and 1U/ml, respectively.

These results suggest that U937 cells express receptors for thrombin, which mediate the formation of IP_3 and mobilisation of $[Ca^{2+}]_i$. However the failure of thrombin to cause TxB_2 synthesis in U937 cells suggest that the thrombin receptor and/or the thrombin receptor-coupling mechanism in these cells is functionally distinct from the thrombin receptor/receptor-coupling mechanism present in other cells such as platelets.

This work was supported by a grant from the National Asthma Campaign

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364P 5-LIPOXYGENASE INHIBITION BY METHOXYALKYL THIAZOLES: COMPARISON WITH OTHER NON-REDOX INHIBITORS

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We have recently described a novel series of 5-lipoxygenase inhibitors, methoxyalkyl thiazoles, which are neither redox agents nor iron chelators (McMillan et al, 1990). The objective of this study was to compare a representative methoxyalkyl thiazole, ICI211965, with 2 other lipoxygenase inhibitors that are free from redox and iron chelation properties, REV5901 and WY50,295.

Release of LTB_4 from leukocytes and whole blood was measured as described previously (Foster et al, 1990; Proudman and McMillan, 1990). WY50,295 was a generous gift from Wyeth-Ayerst Research.

ICI211965 (1-[3-(Naphth-2-ylmethoxy)phenyl)]-1-(thiazol-2-yl)propyl methyl ether) selectively inhibited synthesis of the 5-lipoxygenase product, leukotriene B_4 (LTB_4), in rat blood with an IC_{50} value of 0.4μM. No significant inhibition of synthesis of the cyclo-oxygenase product, thromboxane B_2 (TXB_2), was observed at concentrations up to 100UM. ICI211965 had similar inhibitory potency against LTB_4 synthesis by human blood (IC_{50}= 0.45μM) and had no significant effect on TXB_2 synthesis up to 100μM. REV5901 and WY50,295 were also selective inhibitors of LTB_4 synthesis in rat blood but were less potent than ICI211965 with IC_{50} values of 5μ and 30μM respectively. However, REV5901 and WY50,295 were inactive as inhibitors of LTB_4 synthesis in human blood at concentrations up to 40μM and 100μM respectively. In plasma-free suspensions of rat peritoneal leukocytes, REV5901 was a potent inhibitor of LTB_4 synthesis (IC_{50}= 0.05μM) but the compound was approximately 80 times less potent as an inhibitor of LTB_4 synthesis by human leukocytes (IC_{50}= 4μM). Similar results in leukocytes were obtained with WY50,295.

These data demonstrate that ICI211965 is a more potent and more selective 5-lipoxygenase inhibitor than previously described inhibitors that are free from redox and iron chelation mechanisms, REV5901 and WY50,295. Both REV5901 and WY50,295, but not ICI211965, are more potent in rat than human systems and further studies are required to determine whether this is a result of a species differences in enzyme sensitivity or cellular uptake. We conclude that methoxyalkyl thiazoles, such as ICI211965, represent improved pharmacological probes for studying the pathophysiological roles of leukotrienes, particularly in human.

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365P INTRACELLULAR Ca^{2+} ($[Ca^{2+}]_I$) AND MORPHOLOGIC CHANGES IN HUMAN BASOPHILS

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During a series of studies designed to measure the changes in $[Ca^{2+}]_i$ in individual basophils we made the empirical observation that the increase in $[Ca^{2+}]_i$ was followed by morphologic changes which appeared to be temporally related to degranulation. To provide a first appoximation of these morphologic changes we measured the increase in the perimeter of the cells and related this to the increase in $[Ca^{2+}]_i$ and the subsequent release of histamine.

Human basophils(n=7, average purity = 80±3%) were challenged with anti-IgE(0.25μg/ml) and the changes in $[Ca^{2+}]_i$ and perimeter of each cell monitored for 15 minutes. $[Ca^{2+}]_i$ reached a peak value of 86±11nM Ca^{2+} within 1 minute and then declined to a new baseline 50-60nM above the pre-challenge value. In contrast, the changes in perimeter were slower, being unchanged after 1 minute and reaching a peak, 215±26% of control 5 minutes after the addition of the stimulus. The increase in perimeter was maintained for up to 15 minutes. Histamine release averaged 26±11%. F-met peptide(1μM) led to a rapid increase in $[Ca^{2+}]_i$, with the net peak of 183±31nM $[Ca^{2+}]_i$ attained within 30 seconds(n=6, average purity =83±2%). The increase in perimeter also occured more rapidly in basophils challenged with f-met peptide, reaching 203±14% of the control value within 2 minutes. The increase in perimeter was maintained for 5-15 minutes Histamine release was 56±14%. In contrast, in two experiments the phorbol ester, PMA, caused only a minimal net increase in $[Ca^{2+}]_i$ (20-30nM Ca^{2+}), but still initiated morphologic changes. The perimeter of the cells increased to 204±9% of the control value over 15-20 minutes and the histamine release was 42±3%.

These results suggest that though the increase in $[Ca^{2+}]_i$ precedes the increase in perimeter length when anti-IgE and f-met peptide initiate basophil responses there is no absolute requirement for an increase in $[Ca^{2+}]_i$ to occur prior to the morphologic changes.

366P EFFECT OF RECOMBINANT HUMAN INTERLEUKIN 5 (IL-5) ON PURIFIED GUINEA-PIG EOSINOPHILS: DIRECT CHEMOTACTIC ACTIVITY AND SYNERGISM WITH PAF-ACETHER

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It is recognized that blood and airways eosinophilia characterizes bronchial asthma and that eosinophils contribute to its pathogenesis. The factors responsible for eosinophil recruitment *in vivo* are poorly defined, although PAF-acether, LTB4 and cytokines appear to be involved. PAF-acether and LTB4 are potent chemotactic factors for normodense guinea-pig eosinophils as compared to FMLP, which is ineffective (E. Coëffier et al., Int. J. Immunopharmacol., in press). Recombinant human (rh) IL-5 is a powerful and selective stimulator of human eosinophil function and maintains eosinophil viability. IL-5 is a cytokine whose elaboration *in vivo* can explain the selective eosinophilia and eosinophil activation of disease. Our purpose was to study the mechanisms of the chemotactic responses of guinea-pig eosinophils, to better understand and modulate eosinophil recruitment to the site of allergic reactions. We compared the ability of IL-5, PAF-acether and LTB4 to promote eosinophil migration.

Eosinophils were purified from peritoneal lavages of polymyxin B-treated guinea-pigs (1 mg/ml i.p.; weekly during 6 weeks). Two populations were separated upon a discontinuous metrizamide gradient: one of low density (between 20 and 22% of metrizamide, purity: 63±3 %, n=27) and another of normal density (between 22 and 24% of metrizamide, purity: 87±2 %, n=26). Chemotactic activity of purified hypodense and normodense eosinophils was evaluated using a micro-Boyden chamber. Results are expressed as the number of migrating eosinophils (mean ± SEM) at 40 μm through a cellulose nitrate filter (3 μm pore size) in the presence of the agonist or of the solvent alone.

Rh IL-5 markedly stimulated eosinophil chemotaxis as compared to PAF-acether and LTB4. Its chemotactic activity increased dose-dependently between 50 and 1000 ng/ml and reached a peak at 500 ng/ml (96±34 migrating eosinophils, n=5, $P<0.05$, normodense eosinophils). In the same conditions, recombinant murine IL-5 was inactive on guinea-pig eosinophil migration. Guinea-pig eosinophils did not migrate in the presence of recombinant human IL-1ß and TNFα. Like PAF-acether and LTB4, and in contrast to its efficacy on normal density eosinophils, rh IL-5 stimulated marginally only low density eosinophils.

When eosinophils were preincubated during 5 min with rh IL-5 (50, 100 and 500 ng/ml), their migration by PAF-acether (100 and 10 nM) was significantly enhanced (173±23 migrating eosinophils with PAF-acether 10 nM after preincubation with rh IL-5 500 ng/ml *versus* 69±10 after preincubation with buffer alone, $P<0.01$). This priming was dependent of the dose of rh IL-5. By contrast, rh IL-5 failed to enhance migration by LTB4 (10 and 1 nM). We also demonstrated that FMLP (1 μM) does not induce eosinophil chemotaxis even in the presence of rh IL-5. Eosinophil migration by PAF-acether was significantly and concentration-dependently reduced when the cells were preincubated with the antagonists BN 52021 and WEB 2086. Both antagonists also reduced eosinophil migration by rh IL-5; inhibition was concentration-dependent but plateaued at 60%. Since this might indicate an involvement of PAF-acether with chemo-attraction by rh IL-5, cross-desensitization studies were performed. In both instances, the eosinophils were auto-desensitized to PAF-acether or to LTB4, but were not cross-desensitized to each other. Eosinophils desensitized to and by PAF-acether failed to migrate to rh IL-5, whereas eosinophil desensitization to and by LTB4 did not affect rh IL-5-induced migration. Eosinophils were not auto-desensitized to rh IL-5.

In conclusion, the *in vivo* eosinophil migration in guinea-pig allergy is not accounted for a direct effect of IL1 and TNF, but may involve IL-5, particularly associated to PAF-acether.

367P INVOLVEMENT OF INTERLEUKIN-1 IN THE RESPONSE TO TOPICALLY APPLIED CHEMICAL IRRITANTS

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Interleukin (IL)-1 has been shown to be present in the epidermis and stratum corneum (SC) of normal porcine skin using immunohistochemical and bioassay techniques (Ceriani et al., 1989; Moore & Cunningham, 1990). Since IL-1 induces proinflammatory effects in the skin, release of this mediator from the epidermis may contribute to the increased cutaneous blood flow and erythema seen after topical application of irritant chemicals to mini-pig skin.

In the present study, biologically active IL-1 was measured in homogenates of 0.1mm epidermal slices after topical application of benzalkonium chloride (B.Cl) 10% in dimethylsulphoxide (DMSO), hexyl nicotinate (HN) 10% in propylene glycol:isopropanol; 60:40 (PG:IPA) or the appropriate vehicle. Biopsies were taken when the increase in cutaneous blood flow, provoked by the irritants, was maximal and assayed as described previously (Moore & Cunningham, 1990). Skin to which irritants were applied immediately prior to biopsy served as controls.

The results shown in Table 1 indicate that whilst the amount of IL-1 in the epidermis of irritant-treated skin was decreased relative to control, the difference was not statistically significant. Furthermore, the amounts measured were no different to those in vehicle-treated sites. Assay of 3 sites from each of 4 pigs revealed an intersite variation in IL-1 levels of 15.2 ± 2.9%, the mean being 404.5 ± 58.7 pg/mg wet weight. Assay of one sample from each of 3 pigs on 4 separate occasions showed the interassay variation to be 8.1 ± 2.3%. Interestingly, measurement of IL-1 in untreated skin of varying depth revealed no significant difference in the amounts per mg wet weight of tissue extracted from samples comprised of epidermis and SC (0.1mm) when compared to those which also contained dermal tissue (Table 2.).

Table 1

Irritant	IL-1*	Vehicle	IL-1	control	IL-1
B.Cl	360.5 ± 89	DMSO	358.6 ± 104	B.Cl	606.7 ± 111
HN	412.5 ± 31	PG:IPA	579.0 ± 139	HN	749.0 ± 177

n=4. * expressed as mean pg IL-1 equivalents per mg wet weight of tissue ± s.e. mean.

Table 2

n	depth	IL-1*
3	0.1	478.6 ± 135.4
3	0.3	438.4 ± 72.7
3	0.5	458.9 ± 142.3

Thus, topical application of the irritants B.Cl and HN does not appear to cause mobilization of demonstrable amounts of IL-1 from the epidermis. Use of an IL-1 inhibitor may help to clarify whether this cytokine contributes to the inflammatory response to chemical irritants.

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368P MEDIATORS OF RAT PAW OEDEMA INDUCED BY PHOSPHOLIPASE A2

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Local administration of PLA2 to animals induces inflammatory reactions. Previous pharmacological studies have implicated pro-inflammatory lipids and amines in these responses, but these studies have been hampered by the use of relatively non-selective inhibitors (Marshall et al.,1986). We have used selective inhibitors of 5-lipoxygenase (ICI 207968 and Abbott A64077) and cyclo-oxygenase (Indomethacin) and an antagonist of PAF (WEB2170) to determine the role of lipid mediators in PLA2 induced paw oedema in the rat. In addition, the role of pro-inflammatory amines was investigated using a range of antagonists of 5HT and histamine.

Female Alderley Park rats (150-190g) were injected subplantar with either 10 units Naja Naja PLA2 or 0.5μgs PAF in 100μls of physiological saline. Oedema was assessed by plethysmography or by killing the animal and weighing the injected and non-injected feet. WEB 2170 was injected subplantar in 100μls of saline immediately prior to the injection of inflammogen. Other drugs were administered 1 hour prior to induction of inflammation, either orally, in HPMC, or subcutaneously in physiological saline. Effectiveness of cyclo-oxygenase and lipoxygenase inhibition was assessed in ex-vivo rat blood (Foster et al.,1990).

Subplantar injections of PLA2 (0.3-100 units/paw) and PAF (0.005-2.0μg/paw) produced dose-dependent oedema formation. Submaximal doses of PLA2 (10 units/paw) and PAF (0.5μg/paw) were selected for pharmacological studies. PLA2 induced oedema was significantly inhibited by dexamethasone (1mg/Kg p.o) but not by doses of A64077 (100mg/kg p.o.) or ICI 207968 (100mg/Kg p.o.) which reduced ex vivo LTB4 synthesis in blood to control levels. Indomethacin (10mg/Kg p.o.) completely suppressed ex-vivo synthesis of TXB2 but did not significantly inhibit PLA2 induced oedema when dosed alone or in combination with either A64077 or ICI 207968. WEB 2170 (20-200 μg/paw) virtually abolished PAF-induced oedema, but did not significantly inhibit oedema induced by PLA2. In accord with previous studies, PLA2-induced oedema was significantly inhibited by the non-selective amine antagonists cyproheptadine (10mg/Kg s.c.) and methysergide (10mg/Kg s.c.) Oedema was also inhibited by the selective 5HT2 antagonist ketanserin (10mg/Kg s.c.), but the H1 antagonist mepyramine (10mg/Kg p.o.) and the selective 5HT3 antagonist BRL34694A (5mg/Kg s.c.) were ineffective.

In conclusion, these data indicate that neither leukotrienes, prostaglandins nor PAF significantly contribute to rat paw oedema induced by Naja Naja PLA2. The response appears to be largely a 5HT2 dependent process presumably a consequence of mast cell degranulation. Future studies will investigate the mediators of inflammatory responses to a more relevant PLA2 that has been implicated in the pathobiology of arthritis

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369P COMPLEMENT C5a AND C3a INDUCED MEDIATOR RELEASE FROM DISSOCIATED HUMAN SKIN MAST CELLS

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The complement peptides C5a and C3a have both been shown to release histamine from human basophils but not human lung mast cells. As skin mast cells differ from those of the lung in both immunocytochemical and functional properties, we examined the ability of these anaphylatoxins to release preformed and newly generated mediators from human dispersed skin mast cells.

Mast cells were dispersed from human infant foreskin by enzymatic dispersion (Benyon et al., 1987) and complement peptides purified from human yeast activated serum (Hugli et al., 1981).

In concentration-response studies, both C5a and C3a released histamine in a concentration related manner with C5a being 50-100 times more potent. However, the extent of histamine release, 15-20%, was considerably less than that released from basophils. This was not due to catabolism of the peptides by mast cell proteases as C5a and C3a containing supernates from mast cell experiments were effective basophil histamine releasers. Removal of the C-terminal arginine from C5a and C3a abolished their activity on skin mast cells. In time course studies, histamine release induced by C5a and C3a was complete within 15 sec. With anti-IgE histamine release occurs within 5-7 min. Complement-induced histamine release is a non-cytotoxic process as evidenced by its complete inhibition by 2-deoxy-D-glucose and antimycin A, inhibitors of glycolysis and oxidative phosphorylation respectively. In contrast to IgE-dependent stimulation, anaphylatoxin-induced histamine release from human skin mast cells is independent of extracellular calcium. Both C5a and C3a at concentrations which induced 10-16% net histamine release, caused a negligible release of the newly generated mediator, PGD_2.

These results suggest that C5a and C3a stimulate human skin mast cells in a manner similar to substance P and related basic secretagogues. However, the activation site for C5a and C3a appears to be different to that for substance P as the substance P antagonist SPA $[(D\text{-}Pro^{4}\text{-}D\text{-}Trp^{7,9,10})]SP_{4\text{-}11}$ inhibited histamine release stimulated by substance P but not that induced by C5a or C3a.

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370P ET-1 RESPONSES IN THE GRANULATION TISSUE OF WOUND HEALING. POSSIBLE ROLE IN WOUND CONTRACTION?

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The cell responsible for wound closure is the contractile fibroblast like cell or "myofibroblast", which is derived from fibroblasts at the wound margins (Bouissou *et al.*, 1988). These cells are also present in other pathological conditions such as fibrotic lung and the nodules of Dupuytren's contracture, and have been shown to respond to numerous vasoactive substances including AGII, 5-HT and PG's (Majno *et al.*, 1971; Hurst *et al.*, 1986). In the present study we investigated the effects of the potent pressor peptide ET-1, on granulation tissue contraction *in vitro*, and the inhibitory action of the calcium antagonists flunarizine, verapamil and nitrendipine on this response.

Male Wistar rats (250-300g) were subcutaneously injected with 30ml of air and 1% croton oil, as previously defined by Selye, 1953. The granuloma pouch was then allowed to develop and the animals were killed at 7,14,21 and 28 days (n=12). Granulation tissue strips (5x20mm) were prepared from the pouch lining and suspended under 1g resting tension in Krebs-Henseleit solution aerated with 95% O_2/5% CO_2, thermostatically maintained at 37°C. The tissues were allowed to equilibrate for a period of 2hrs, cumulative dose response curves to ET-1 were then constructed.

ET-1 caused concentration dependent contraction of granulation tissue strips *in vitro*. The 21 day granuloma pouch was found to be most sensitive, threshold contraction occurring at $1x10^{-10}$M and maximum contraction at $1x10^{-7}$M with an average 458.9 ± 55mg increase in tension. In subsequent antagonist studies the 21 day granuloma pouch was therefore used.

After construction of the first dose response curve the tissue was washed several times over a period of at least 1hr and then incubated for ½hr with test substance, a second dose response curve to ET-1 was then constructed. The calcium antagonists nitrendipine, flunarizine and verapamil significantly ($P<0.05$) dose dependently inhibited the ET-1 induced response. Verapamil (10^{-6}-10^{-4}M) was the most potent inhibitor with a 43% reduction in the maximum contraction with nitrendipine (10^{-6}-10^{-4}M) 39%, and flunarizine (10^{-6} & 10^{-5}M) 25% respectively. As it is known that agents which are important in wound healing such as TGFß, can stimulate endothelial cells to produce ET-1, it is possible that ET-1 could be involved in wound contraction, and that this could be inhibited by use of calcium antagonists.

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371P THE EFFECT OF CETIRIZINE ON ANTIGEN-INDUCED PLATELET DEPENDENT LEUCOPENIA IN THE GUINEA-PIG

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It has previously been demonstrated that antigen provocation of naive guinea pigs that 24h prior to challenge received platelets from sensitised animals will induce a leucopenia (Pretolani et al., 1990). In the present series of experiments, we have investigated the effect of the anti-allergic agent cetirizine and compared its effect to related H1 antagonists.

Male Hartley guinea pigs were sensitised by a s.c. injection of ovalbumin (OA) dispersed in 1 mg $Al(OH)_3$ on day 0 and 14 and the animals subsequently used on day 21-35. Sensitised animals were anaesthetised with sodium pentobarbitone and the carotid artery cannulated to allow blood collection. Blood was taken into 1/10 volume of 3.8 % sodium citrate in the presence of 1μM PGI2 . The blood was centrifuged and PRP prepared. The platelets were then washed twice with calcium free Tris buffer. The platelet concentration was adjusted to give a final concentration of 3.5×10^9 /ml and 1 ml of this suspension injected i.v. to naive guinea pigs. The following day, animals were anaesthetised, and the carotid artery and jugular vein cannulated to allow collection of blood samples, and i.v. drug administration respectively. 1 mg/kg of OA was injected i.v. and the number of leucocytes ennumerated every 15 min for the following 90 min. Cetirizine (10-30 mg/kg, i.v.), mepyramine (2 mg/kg, i.v.,) or terfenadine (50- 250 mg/kg, p.o.) were administered 1 h prior to OA challenge. Antigen challenge of guinea pigs that had received platelets from sensitised animals induced a 34.3 ± 8 % (n = 6) fall in the number of circulating leucocytes. Cetirizine induced a dose related inhibition of antigen-induced leucopenia. Mepyramine and terfenadine, at concentrations which inhibited the bronchoconstrictor response to histamine were inactive in this respect (% leucopenia 47.3 ± 4 and 32.0 ± 12, respectively). BWA4C was ineffective in modifying the leucopenia in contrast to that reported previously with BW755C (Pretolani et al., 1990). Heparin (100-500 U/kg, i.v.) however, inhibited the leucopenia.

Our data demonstrates that cetirizine is effective in inhibiting the platelet dependent leucopenia by an action independent of its ability to antagonise H1 receptors. The nature of the platelet -derived mediator(s) are unknown, but is unlikely to be a product of the lipoxygenase pathway. The efficacy of heparin suggests that charge interactions between the leucocyte and vascular endothelium maybe important in inducing the leucopenia. Further investigation into the interaction between antigen activated platelets, leucocytes and the vascular endothelium may be a rewarding area for future research.

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372P THE SITE OF INVERSION OF R(-)-IBUPROFEN: STUDIES USING *IN SITU* ISOLATED PERFUSED RAT INTESTINE/LIVER PREPARATIONS

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Stereospecific metabolic inversion of ibuprofen from the R(-)-enantiomer to the pharmacologically active S(+)-enantiomer occurs *in vivo*, and is believed to be mediated by formation of a thio-ester with co-enzyme A. The site of this inversion has been a subject of debate since Mehvar and Jamali (1988) claimed, on the basis of a pharmacokinetic model, that it occurs predominantly and pre-systemically in the gut. To obtain direct experimental data elucidating this issue, the fate of the isomers of ibuprofen was investigated using an array of *in situ* perfused rat organ preparations allowing vascular perfusion (55-60 min) of the separate or combined intestine and liver (Castle *et al.*, 1985; Pang *et al.*, 1985; Lennard *et al.*, 1983).

Ibuprofen was measured in vascular perfusate plasma and lumen perfusate using the stereoselective method of Averignos and Hutt (1987).

After addition of R(-)-ibuprofen (20 mg kg^{-1} body weight) to the closed (static) lumen of isolated 25 cm lengths of duodenum, jejunum or ileum and single-pass vascular perfusion (n=5 in each case) both isomers were measured in the lumen and in vascular perfusate plasma (mean plasma AUC values (± SD, μg ml^{-1} min) R(-)-ibuprofen: 1669 ± 115 (duodenum), 1687 ± 203 (jejunum), 2061 ± 188 (ileum); S(+)-ibuprofen: 23 ± 6 (duodenum), 14 ± 5 (jejunum), 26 ± 1 (ileum)). Addition of the same dose of S(+)-ibuprofen to the jejunum (n=5) resulted in AUC values of 1864 ± 238 for S(+)-ibuprofen and 6 ± 3 for R(-)-ibuprofen. After addition of R(-)-ibuprofen (30 μg ml^{-1}) to the recirculating vascular perfusate (100 ml) of the entire small intestine (n=6) AUC values were 1647 ± 34 for R(-) and 13 ± 3 for S(+). The same dose of R(-)-ibuprofen to combined intestine/liver (n=6) and liver only preparations (n=6) gave AUC values of 1011 ± 25 and 1021 ± 49 for R(-) and 220 ± 28 and 238 ± 22 for S(+), respectively. In all experiments, except those involving perfusion of the combined intestine/liver and the liver, the concentrations of the isomer opposite to that administered could be accounted for solely by the level of enantiomeric impurity (1.3% for R(-)-ibuprofen and 0.6% for S(+)-ibuprofen).

We conclude that inversion of R(-)-ibuprofen to the S(+) antipode occurs in the liver but does not occur on either mucosal or serosal sides of the small intestine of the rat.

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373P FLUNIXIN ABSORPTION FOLLOWING ORAL ADMINISTRATION IN FED AND FASTED PONIES

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Flunixin is a non-steroidal anti-inflammatory drug (NSAID) available in oral (paste and granule) formulations for use in the horse. The influence of feeding regimens on absorption of this drug in the horse have not been reported but this is of practical veterinary interest since the absorption of another NSAID, phenylbutazone, is delayed considerably when administered to horses with free access to hay. Maximum plasma concentrations may not be achieved for 18 hours and double or triple peaks in plasma concentration have been reported (Maitho et al, 1986, Lees et al, 1986). In vitro studies have demonstrated that both phenylbutazone and flunixin bind to hay and this is one potential cause of delayed absorption (Lees et al, 1988).

In this study six normal horses were used in a cross-over study to investigate the plasma concentration -time relationships of flunixin following oral administration as a paste (Finadyne Paste, Kirby Warwick) (a) when food was withheld for 4 hours before and 5 hours after dosing (-feed) and (b) when horses had access to hay for several hours both before and after dosing (+feed). An interval of 7 days between the two parts of the cross-over study was allowed.

Table 1	Ration	Tmax(h)	Cmax(µg/ml)	AUC_{0-30}(µg/ml.h)
	+ feed	7.66±1.74*	1.30±0.23*	11.11±1.56
	- feed	0.76±0.17	2.84±0.28	12.18±1.50

Results are expressed as mean ± s.e.mean (n=6); $p<0.05$ +feed vs. -feed ; paired t test.

The initial phase of absorption and AUC_{0-30} values were not reduced when the horses had access to feed, and onset and duration of action may therefore be unaffected by the feeding regimen. However, Cmax was reduced and Tmax was prolonged with the +feed regimen. The manner of and the extent to which the differing plasma concentration-time profiles influence the time-course of the anti-inflammatory actions of flunixin in the horse can be established only from further studies linking flunixin pharmacokinetics to its anti-inflammatory actions.

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374P R(-)IBUPROFEN-S-COENZYME A SYNTHETASE ACTIVITY: SUBCELLULAR LOCALISATION IN RAT LIVER AND INHIBITION BY CARBOXYLIC ACIDS

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A number of R(-)2-arylpropionic acid, non-steroidal anti-inflammatory drugs, including R(-)ibuprofen, have been shown to undergo metabolic chiral inversion *in vivo* in several species including man. This inversion is thought to proceed via the enzymatically-mediated, stereospecific formation of the acyl coenzyme A (CoA) intermediate, followed by spontaneous or enzymatic racemisation and hydrolysis to yield the racemic 2-arylpropionic acid. The enzymology of this process has yet to be fully characterised. In this study, the subcellular distribution of ibuprofen-S-CoA synthetase activity in rat liver and the inhibitory effects of a number of prototype carboxylic acids were investigated.

RS-Ibuprofen (2 mM) was incubated with microsomal, mitochondrial and cytosolic fraction from the livers of male Wistar rats (200-300 g), CoA (1 mM) and ATP (10 mM) for 10 min at 37°C. Carboxylic acids tested for inhibition included palmitate (1 mM), butyrate (10 mM), salicylate (10 mM), benzoate (10 mM) and R(-)naproxen (10 mM), all added as the respective sodium salt. The concentration of R(-) and S(+)ibuprofen in the samples was determined by an enantiospecific high performance liquid chromatography assay. Enzyme activity was expressed as the rate of substrate disappearance, in nmol/h/mg protein.

The microsomal fraction actively catalysed the disappearance of R(-)ibuprofen (264 ± 33 nmol/h/mg, mean ± SEM, n=5) (Figure 1). Synthetase activity had an absolute requirement for both ATP and CoA. Levels of S(+)ibuprofen did not change during the incubation, demonstrating that the reaction was enantiospecific, and that subsequent hydrolysis required a factor not present in the microsomal fraction. Neither the mitochondrial nor the cytosolic fraction possessed any detectable R(-)ibuprofen-S-CoA synthetase activity (< 25 nmol/h/mg).

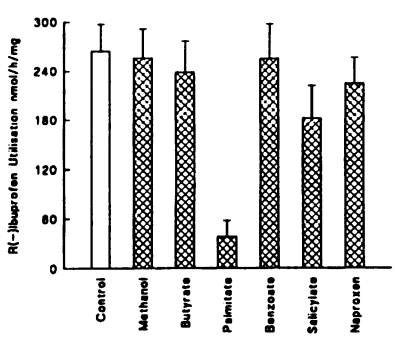


Figure 1. Effect of carboxylic acids on R(-)ibuprofen disappearance in rat liver microsomes (mean±SEM, n=5)

Hepatic synthetase activity was not significantly affected by the addition of butyrate, salicylate, benzoate or R(-)naproxen. However, in the presence of palmitate, activity was reduced to only 37 ± 20 nmol/h/mg, inhibition of 86% (Figure 1). This was not due to saturation of cofactor supply, as increasing the concentration of CoA and ATP did not prevent inhibition.

These studies demonstrate that, in rat liver, R(-)ibuprofen-S-CoA synthetase activity specific for the R(-)enantiomer is located in the microsomal fraction and this is inhibitable by palmitate. These observations suggest that the enzyme responsible is the long chain fatty acid: CoA ligase (E.C. 6.2.1.3), which is also localised in the microsomal fraction of rat liver, and for which palmitate is an excellent substrate.

(MTM was supported by a SERC CASE award studentship.)

375P TRANSDUCTION OF CELLS WITH P450IA2 CONFERS SUSCEPTIBILITY TO PARACETAMOL TOXICITY

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The cell line RLER52H-16 (R52), derived from rat liver epithelium (RLE), was transduced with the gene for murine P450IA2. A second cell line, RLERC100 (RC100), containing all of the DNA elements except the P450IA2 gene, served as a negative control (Battula 1989). Cells were grown to confluence in DMEM + 10% FCS, supplemented with antibiotics. For incubation with paracetamol, cells were switched to a serum free medium, consisting of EBSS with supplements.

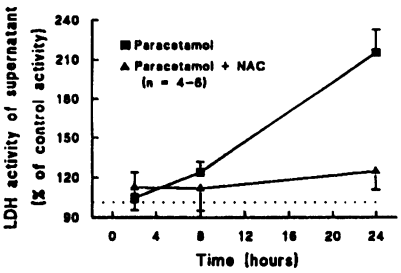


Figure 1. Effect of NAC on LDH leakage in R52 cells caused by paracetamol

The P450IA2 gene was shown to be functioning in the R52 cells by immunospecific Western blotting of P450IA2 (R.J. Edwards, Dept. Clin. Pharm. RPMS) and by the presence of 7-ethoxycoumarin *O*-deethylase activity. The RC100 cells were negative in both assays.

Following incubation of cells with 4 mM paracetamol for 4 h, then medium alone for a further 20 h, the activity of lactate dehydrogenase (LDH) in the supernatant increased to 1.17 µmol NADH oxidised/min/10^6 cells compared with 0.27 µmol NADH/min/10^6 cells for cells incubated in medium alone. The comparable activities for RC100 cells were 0.44 with paracetamol and 0.26 without paracetamol. R52 cells were incubated with 10 mM paracetamol for 2 hours and then in either fresh medium, alone or with 1.25 mM N-acetyl-L-cysteine (NAC). Cytotoxicity was apparent only after a lag period of 6 h (Figure 1). This contrasts with the much shorter interval required when hepatocyte suspensions were subjected to a similar protocol (Tee *et al.*, 1986). This loss of viability in R52 cells was associated with a rapid and profound depletion of cellular GSH (Figure 2). The addition of NAC largely prevented this loss of viability. GSH levels in these cells were approximately 200 % of control levels, at 24 hours.

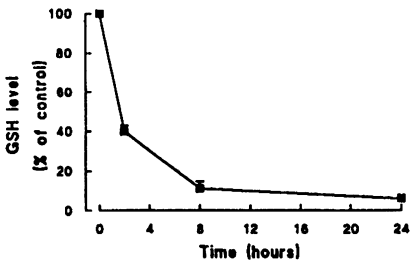


Figure 2. Effect of paracetamol on GSH content of R52 cells

The two phase model of paracetamol toxicity, characterised by a metabolic phase followed by a phase of progressive cell deterioration, has been shown to be valid in R52 cells. These cells expressing the P450IA2 gene become susceptible to paracetamol toxicity, demonstrating the critical role that this enzyme plays in such toxicity. The R52 cell line should prove extremely useful in studies of mechanisms of toxic cell injury.

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JWW is an MRC research student.

376P IRON CHELATORS AND CALCIUM ANTAGONISTS AS INHIBITORS OF IRON INDUCED NEURONAL LIPID PEROXIDATION

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The selective increase of iron and ferritin in the pars compacta of substantia nigra from Parkinsonian brains has led us to implicate a cytotoxic role for iron, resulting in oxidative stress This may result from the generation of oxygen free radicals via the interaction of iron in its transitional state with monoamine oxidase generated H_2O_2 and melanin (Sofic et al , 1990) The increase of lipid peroxidation (Dexter etal , 1989) and decrease of mitochondrial complex I (Mizuno et al , 1989) reported in substantia nigra of Parkinson's disease supports this notion

Both Fe^{2+} and Fe^{3+} (100µM) induced a time dependent increase of lipid peroxidation which paralleled the net increase of $^{45}Ca^{2+}$ uptake in rat brain cortical synaptosomal preparations The maximum effect ()400%) was observed at 4-5min The iron chelator Lazaroid (U47500A, 100µM) and desferrioxamine (100µM) completely abolished Fe^{3+} (100µM) initiated increase of $^{45}Ca^{2+}$ uptake However, dihydropyridine calcium antagonists nifedipine (20µM) and nisuldipine (20µM) were able to partially antagonize (50-60%) the net $^{45}Ca^{2+}$ uptake The anti-Parkinson selective monoamine oxidase (MAO) inhibitor l-deprenyl (10µM) was without an effect Nifedipine, nisoldipine and felodopine inhibited Fe^{3+} (100µM) induced lipid peroxidation with IC_{50} values 50±7, 110±8 and 65±7 µM respectively U474500A is also a selective inhibitor of MAO-A with Ki values of 95 and 750 µM for MAO-A and MAO-B respectively

This study indicates that iron initiated lipid peroxidation may be dependent on calcium influx through dihydropyridine sensitive L-calcium channels since calcium antagonists inhibit $^{45}Ca^{2+}$ uptake and lipid peroxidation similar to iron chelators. The possible use of such compounds as neuroprotective drugs may be considered valid

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377P RS-15385-197, A SELECTIVE α_2-ADRENOCEPTOR ANTAGONIST, HAS LOW AFFINITY FOR IMIDAZOLINE BINDING SITES ON HAMSTER ADIPOCYTES

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[^{3}H]-Idazoxan binds to 2 sites on hamster adipocytes: an α_2-adrenoceptor and a non-adrenoceptor site (MacKinnon et al., 1989). The lack of a selective radioligand for the imidazoline site necessitates the use of certain existing α_2-adrenoceptor radioligands coupled with blockade of the α_2-adrenoceptor component in order to study the imidazoline site in isolation. In this study we have compared the affinity of RS-15385-197 (Clark et al., 1989) at the α_2-adrenoceptor and the imidazoline site on hamster adipocytes.

Adipocytes were isolated from fat pads of male Syrian hamsters (2-3 months old 100-150g) by collagenase digestion as described by MacKinnon et al., (1989). [^{3}H]-Idazoxan binding to adipocyte membranes was carried out by the method of MacKinnon et al., (1989).

[^{3}H]-Idazoxan bound to a single population of high affinity sites (Kd = 2.26 ± 0.02 nM, Bmax = 373 ± 25 fmol./mg protein) in hamster adipocytes. Saturation experiments repeated in the presence of 100 nM RS-15385-197 reduced the density of [^{3}H]-idazoxan binding sites (Bmax = 260 ± 24 fmol./mg protein) without affecting the affinity (Kd = 2.20 ± 0.21 nM) of the radioligand indicating that [^{3}H]-idazoxan labelled two sites of similar affinity.

Table 1 Displacement of 1.5 nM [^{3}H]-idazoxan from hamster adipocytes

	pKi		
	site 1	site 2	selectivity
idazoxan	8.34 ± 0.06		-
yohimbine	7.28 ± 0.02	4.48 ± 0.16	631
phentolamine	7.92 ± 0.14	5.21 ± 0.02	513
l-noradrenaline	6.21 ± 0.10	<4.00	>162
l-adrenaline	6.92 ± 0.12	<4.0	>832
RS 15385-197	8.38 ± 0.01	<4.00	>23988

In competition experiments, yohimbine and phentolamine but not RS-15385-197, l-adrenaline or l-noradrenaline, displaced non-specific [^{3}H]-idazoxan binding when defined by 10 μM phentolamine. When defined by 1 mM phentolamine, specific [^{3}H]-idazoxan binding was inhibited by yohimbine and phentolamine with low Hill slopes which could be resolved into 2 components (Table 1). RS-15385-197 inhibited approximately 25% specific binding with an affinity consistent with binding to α_2-adrenoceptors. Idazoxan exhibited a monophasic displacement curve consistent with idazoxan having equal affinity for both sites. The lack of affinity of RS-15385-197 towards imidazoline binding sites makes it ideal for precluding α_2-adrenoceptors in tissues containing an heterogenous population of sites.

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378P EVIDENCE AGAINST A ROLE FOR BROWN FAT IN THE ANTI-OBESITY EFFECTS OF EPHEDRINE IN RATS

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The use of sympathomimetics, such as ephedrine (EPH), to reduce obesity in rodents has been assumed to depend on activation of brown adipose tissue (BAT) thermogenesis (Dulloo and Miller, 1987), but changes in BAT activity following chronic treatment with EPH have not been assessed. In the present study, changes in thermogenic responses, energy balance, body composition and BAT activity were determined in Sprague-Dawley (S-D) rats treated for 21 days with a dietary admixture containing EPH and caffeine (1g/kg diet and 1.4g/kg diet, respectively) or EPH (1g/kg) alone.

Compared to controls, rats in both treatment groups displayed a marked reduction (60-90%) in the acute thermogenic responses to ED_{50} doses (5mg/kg, s.c.) of EPH and ED_{100} doses (400μg/kg, s.c.) of noradrenaline. The thermogenic responses to these agonists could be restored to control levels within 5 days following withdrawal of the EPH treatment. Despite these reductions in thermogenic responses, the biochemical parameters of BAT activity (GDP binding to isolated BAT mitochondria, tissue protein content) were unaltered.

The metabolizable energy (ME) intake of the treated rats was significantly reduced (13-26%), which in turn resulted in a reduction in energy expenditure (9-21%). However, expenditure expressed as a percentage of ME intake was elevated, resulting in significant reductions in the efficiency of energy utilization (32-44%) and body fat (29-37%); body water and protein content was unaltered. The changes in energy balance and body fat of rats treated with EPH were more modest than those in rats treated with a combination of EPH and caffeine.

The anti-obesity effects of EPH observed in this study appear to be the result of an increase in energy expenditure as well as a reduction in energy intake. The increase in energy expenditure does not appear to involve BAT, a conclusion consistent with human studies on EPH (Astrup et al, 1984).

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379P MODIFICATION OF THE ANTI-OBESITY EFFECTS OF BRL35135A IN LEAN AND OBESE RATS

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The dependence on the level of endogenous sympathetic (SNS) activity of the anti-obesity effects of a β_3-adrenoceptor agonist, BRL35135A (BRL), were investigated in sympathectomized Sprague-Dawley (S-D) and adrenalectomized obese Zucker (fa/fa) rats; bilateral adrenalectomy (ADX) restores SNS and brown adipose tissue (BAT) activity, and arrests the developement of obesity in fa/fa rats (York et al, 1985). Chronic chemical sympathectomy (SNX) of S-D rats using guanethidine sulphate (2.5g/kg diet) was verified by measurement of oxygen consumption, interscapular BAT temperature and heart rate responses to directly and indirectly-acting sympathomimetics. SNX rats exhibited decreases in BAT thermogenic activity (mitochondrial GDP-binding capacity) and protein content, and a 16% increase in total body fat content; energy intake was unaltered. By contrast, ADX fa/fa rats showed increases in BAT thermogenic activity and protein, and a 15% decrease in body fat; energy intake was decreased by 40%.

Chronic BRL-treatment (5mg/kg diet) of control S-D and sham-operated fa/fa rats produced significant increases in BAT activity and protein, but only in the fa/fa rats did this result in a significant decrease (33%) in body fat content. BRL-treatment of SNX S-D and ADX fa/fa rats produced similiar increases in BAT activity and protein as those seen in intact animals. However, the anti-obesity effects of BRL were only apparent in SNX S-D rats (16% decrease in total body fat content compared to controls, or a 28% decrease compared to untreated SNX rats). Energy intake in all groups was not affected by treatment with BRL.

These results could explain why the anti-obesity effects of selective β_3-adrenergic agonists, such as BRL, are seen in genetically obese rodents, but not in lean rodents. The changes in energy balance and body composition induced by BRL were seen only in rats where endogenous SNS activity was depressed (e.g intact fa/fa, or SNX S-D). When SNS activity was normal (control S-D), or normalised (ADX fa/fa), BRL treatment had no effect.

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380P IMPORTANCE OF ENDOGENOUS TACHYKININS IN THE MECHANISM OF SUSTAINED HYPERALGESIA TO PGE_2 IN THE RAT

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It has been shown that repeated injections of prostaglandin E_2 (PGE_2) for 15 days into the rat paw caused chronic swelling and hyperalgesia (Willis and Cornelsen, 1973). This model has been used to study the peripheral analgesic effect of dipyrone (Ferreira *et al.*, 1987). Recently, it has been shown that a substance P antagonist inhibited the development of sustained hyperalgesia induced by multiple injections of PGE_2 (Nakamura-Craig and Smith, 1989). The present study further describes the modification of the long-term treatment to induce sustained hyperalgesia in the rat paw, in which PGE_2 is injected twice daily for 4 days. Furthermore the importance of endogenous tachykinins in this phenomenon has been investigated.

Male Wistar rats (150-170g, n=5 per group) were used. Hyperalgesia in the rat paw was measured by a modification of the Randall-Selitto test (Ferreira *et al.*, 1987). The intensity of hyperalgesia (mean ± s.e.mean) was quantified as the variation in reaction time (Δ reaction time, s) obtained by subtracting the value measured after administration of the hyperalgesic substance from the control reaction time. PGE_2 (100 ng) was injected into the rat paw twice daily to study the development of sustained hyperalgesia.

Daily subplantar injections of PGE_2 (100 ng, twice daily) over 4 days led to the development on the fifth day of an intense hyperalgesia (Δ reaction time, 21.3±0.1s) that was still present on the 15th day (8.3±0.17s). Nineteen days after the begining of the dosing with PGE_2, when the sustained hyperalgesic effect had subsided, challenge with a sub-threshold dose of PGE_2 (0.1 ng), PGI_2 (0.5 ng) or dopamine (0.5 ng) into the paw caused significant ($P<0.001$) hyperalgesia that lasted for at least 5 hours. Thus, the effect of a single sub-threshold dose of the above compounds and their subsequent effect in sensitized paws at 3 h, respectively were as follows: PGE_2 (0.47±0.5s; 16.6±1.0s); PGI_2 (-0.1±0.4s; 18.9±0.6s); dopamine (0.06±0.5s; 15.7±0.5s). This sensitization of nociceptors to challenge with hyperalgesic substances lasted for at least 25 days after the start of the multiple injection regimen. Pretreatment of adult rats with capsaicin to deplete sensory neuropeptides, 2 weeks prior to the study (Esplugues *et al.*, 1989) inhibited by 80.5±5% ($n=5$; $P<0.001$) the development of sustained hyperalgesia induced by multiple injections of PGE_2 observed on the fifth day of administration.

The present findings suggest that endogenous tachykinins participate in the mechanisms of sensitization of nociceptors by PGE_2. It is suggested that the sustained hyperalgesia induced by multiple injections of PGE_2 could be associated with chronic pain and therefore could be used as a new chronic experimental model.

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381P DIMINISHED BINDING OF [^{3}H]DTG TO CEREBELLAR SIGMA RECOGNITION SITES IN POST-MORTEM SAMPLES FROM HALOPERIDOL-TREATED PATIENTS WITH SCHIZOPHRENIA

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The proposed involvement of sigma receptors in psychosis and antipsychotic drug action prompted an investigation of these sites in post-mortem brain tissue from patients with schizophrenia. Previous preliminary reports have indicated decreased density of sigma recognition sites in such tissue (e.g.Simpson et al., 1990); our initial aim was to test the hypothesis that such changes related to a down-regulation of receptors by prior treatment with neuroleptic drugs. Specific binding of 5nM [3H]ditolylguanidine (DTG) to membranes prepared from the cerebellar cortex, a region of the brain not particularly implicated in the neuropathology or pathophysiology of schizophrenia, was determined in a series of cases previously assessed as meeting research criteria for schizophrenia and a group of age-matched controls. Specific binding was defined by displacement with 2μM haloperidol and incubations were carried out at room temperature for 90 min.

Table 1 Binding of 5nM [3H]DTG to human cerebellar cortex membranes in schizophrenia

Controls (n=17)	127 ± 25	
Schizophrenics (n=16)	99 ± 28	p<0.02
Treatment with:		
Haloperidol (n=5)	75 ± 20	p<0.002
No haloperidol (n=11)	111 ± 24	

Values are mean ± s.d in fmol/mg protein.
Significant differences vs. controls calculated by Mann-Witney U test.

There is a clear deficit of sigma recognition sites in the schizophrenic group. Although saturation binding analysis was not undertaken, it was considered that the results obtained closely reflected receptor density; in a separate experiment the ratio of binding at two concentrations (1 and 4nM) was equal (0.30) in the two groups indicating that the KD values were equivalent and thus competition from residual neuroleptic in the brain tissue was not apparently influencing ligand binding.

Although a retrospective assessment of medication is not always reliable, it proved possible approximately to subdivide the schizophrenic cases in terms of the history of neuroleptic drug treatment that they received. This indicated that prior treatment with haloperidol is particularly associated with low DTG binding. The group described as "no haloperidol" above had all received phenothiazines except for one patient, unmedicated at death, who previously had thioridazine. Several patients had received more than one neuroleptic, but of the patient groups who had chlorpromazine or fluphenazine alone, no significant deficit in these sites was observed. One interpretation of these data is that haloperidol, but not phenothiazines to the same extent, can induce a down-regulation of sigma receptors. This has been suggested from some animal experiments (e.g. Lee and Picarelli, 1989).

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382P SIGMA SITE LIGANDS AND CIRCLING BEHAVIOUR IN RATS

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The central distribution of sites labelled by di-o-tolylguanidine (DTG), a compound which has specific affinity for σ receptors, suggests a functional role for these receptors in the regulation of movement (Weber et al, 1986). Recent studies by Walker et al. (1988) showing that intracerebral injections of DTG elicit postural movements, support this hypothesis. The aim of the present study was to evaluate the specificity of the circling behaviour induced by DTG. The method of Walker et al (1988) was used to investigate the effects of compounds with high affinity for σ sites. These included (+)-SKF 10.047, (-)-SKF 10.047 and di-o-methyl-tolylguanidine (DMTG), compounds which also bind to the PCP sites associated with the NMDA receptor complex. MK-801 was also tested because of its ability to bind to PCP receptors without having any significant affinity for σ sites.

Experiments were carried out in male Sprague-Dawley rats (Charles River, France) weighing 150-200 g. Intranigral injections (0.5 μl/rat) were performed through indwelling cannulae implanted in the left substantia nigra. Complete contralateral turning was recorded for 20 min.

As previously described, DTG produced dose-dependent contralateral rotations at doses from 0.6 μg with a maximal effect of 6 turns/min at 2.5 μg. DMTG provoked very similar turning at doses of 1 to 10 μg. (+)-SKF 10,047 and (-)-SKF 10.047 induced similar dose-dependent effects (from 1 to 10 μg). In contrast, MK-801 did not produce any significant turning up to 1 μg. The turning behaviour induced by DTG or (+)-SKF 10,047 was not antagonised by naloxone (1 mg/kg, sc). These results suggest that the circling observed after intranigral injection of selective or non-selective σ ligands is related to the interaction of these compounds with the σ sites present in this area.

The effects of haloperidol, BMY 14802 and rimcazole on the rotational behaviour induced by DTG (2.5 μg) were also investigated. Haloperidol inhibited DTG-induced rotations at 0.1 mg/kg ip and BMY 14802 produced a dose-dependent inhibition of DTG turning (3-30 mg/kg ip).At the highest dose tested, 20 mg/kg ip, rimcazole produced a decrease in DTG-induced rotations which was not, however, statistically significant. These results suggest that rotational behaviour produced by DTG may be a useful model for studying the interaction of new drugs with σ sites and might also be used to clarify the potential involvement of σ sites in the regulation of the activity of the nigrostriatal dopaminergic pathway.

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Walker, J.M., Matsumoto, R.R., Bowen, W.D., Gans, D.L., Jones, K.D. and Walker, F.O. (1988) Neurology 38, 961-965

383P COMPARISON OF κ-OPIOID BINDING IN HOMOGENATES OF GUINEA-PIG AND RABBIT BRAIN

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Although κ-opioid binding sites can be labelled with the highly selective ligand [^{3}H]-U-69593, there is increasing evidence that this tritiated ligand does not label all the binding sites previously characterised as κ-binding sites (Lahti et al., 1985; Hunter et al., 1989). In the present experiments, the binding of several κ-ligands has been compared in homogenates of guinea-pig and rabbit brain.

In homogenates of guinea-pig brain, the binding capacity found with [^{3}H]-U-69593 was 3.17 ± 0.24 pmol g^{-1} (n=4). After suppresion of μ- and δ-binding, the binding capacities of [^{3}H]-bremazocine and [^{3}H]-ethylketazocine were 6.70 ± 0.78 pmol g^{-1} (n=5) and 6.93 ± 0.63 pmol g^{-1} (n=4), respectively, whereas that of [^{3}H]-diprenorphine was only 3.42 ± 0.56 pmol g^{-1}(n=3). Similar results were obtained in homogenates of rabbit brain (Paterson, 1990).

In displacement assays in homogenates of guinea-pig and rabbit brain, κ-opioids inhibited the binding of [^{3}H]-U-69593 with Hill slopes of unity. In contrast, the selective κ-ligands, U-69593, PD 117302 and ICI 197067 displaced the binding of [^{3}H]-bremazocine, [^{3}H]-ethylketazocine and [^{3}H]-diprenorphine in the presence of μ- and δ-blockers with low Hill slopes (<0.77), whereas the benzomorphan κ-opioids, bremazocine, ethylketazocine and Mr 2266 had Hill slopes of unity.

These findings are further evidence that κ-opioid binding sites, as originally defined by Kosterlitz et al.(1981), are not homogeneous.

Supported by a grant from St Thomas's Research Endowments Committee.

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384P EFFECT OF NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL ON HIPPOCAMPAL NORADRENALINE RELEASE AS MEASURED BY *IN VIVO* MICRODIALYSIS

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It is currently believed that noradrenergic neurones in the locus coeruleus have a central role in the mechanism of opiate dependence and withdrawal (Redmond & Krystal, 1984; Rasmussen *et al.*, 1990). The opiate-withdrawal syndrome in rats is associated with an increased firing of noradrenergic neurones and an increase in noradrenaline (NA) turnover in rat forebrain (Aghajanian, 1978; Bongianni *et al.*, 1986; Valentino & Wehby, 1989). However, it is important to demonstrate that these effects are accompanied by an increased release of noradrenaline. Here we examine the effect of naloxone-precipitated morphine withdrawal on NA release, measured in rat hippocampus in vivo, using microdialysis.

Microdialysis was carried out, as previously described (Done & Sharp, this meeting), on rats which had received an 8 day course (single daily injections) of either saline or increasing doses of morphine (2.5-100 mg/kg s.c.). Following a baseline period of 2-3 h morphine withdrawal was precipitated by an injection of naloxone (1 mg/kg i.p.) in rats 3 h, 24 h and 3 weeks after the last morphine injection.

Basal levels of NA in hippocampal dialysates were about 50% of normal 3 h after the last injection of morphine, but were not different from controls at 24 h. Naloxone induced an immediate and striking increase in NA levels when administered both 3 h and 24 h after the last morphine injection, these effects being maximal (+325 ± 70% and +150 ± 10% respectively, n=4 rats) 40-60 min post-naloxone and declining over the following 2 h. In contrast, naloxone injection did not alter NA output in rats 3 h after the last injection of saline or 3 weeks after the last injection of morphine; confirming that spontaneous morphine withdrawal had occurred in the latter group of rats.

In summary, this study shows that naloxone-precipitated morphine withdrawal results in an increase in hippocampal NA release in vivo. The microdialysis technique may prove to be a useful method for investigating the mechanisms underlying morphine withdrawal-induced activation of noradrenergic neurotransmission in vivo.

C.D. is an MRC student funded by SmithKline Beecham. P.S. is an Oxford RHA Research Fellow in Psychopharmacology.

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385P THE DISTRIBUTION OF TETRAHYDROAMINOACRIDINE IN RAT BRAIN FOLLOWING INTRAPERITONEAL ADMINISTRATION

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Tetrahydroaminoacridine (THA) is a cholinesterase inhibitor which may have therapeutic effects in some patients with Alzheimer-type dementia (Summers et al, 1986). A number of other pharmacological properties of THA have been identified, for example, release of monoamines from cortical and striatal slices of rat brain (Robinson et al, 1989). However such effects have generally been observed at concentrations of THA greater than those required to inhibit acetylcholinesterase, so their importance to the clinical effect is unknown. In the present study we have determined brain extracellular THA concentrations using intracerebral dialysis.

Male Lister hooded rats (200-300g) were used throughout. Dialysis probes were stereotoxically implanted into the striatum using guide cannulae 24h before THA administration (5mg kg^{-1} i.p.), and perfused at a rate of 1µl min^{-1} with artificial c.s.f.

THA concentrations in 20 or 40µl samples of perfusate were determined by h.p.l.c. (Forsyth et al 1988). Tissue samples were homogenised in 0.4M perchloric acid and the 10,000g supernatant taken for THA analysis.

In the striatum, peak tissue concentrations of THA occurred 1h post-administration and were 40 times greater than free extracellular concentrations (Figure 1). Tissue concentrations in cortex, cerebellum and hippocampus were similar to that found in the striatum.

These data indicate that striatal tissue markedly concentrates THA. They further show that while THA does not apparently release dopamine from the striatum *in vivo* (De Souza et al, 1990), the tissue concentration of the drug nevertheless rises to levels which have been shown to release dopamine from tissue slices *in vitro* (Robinson et al, 1989).

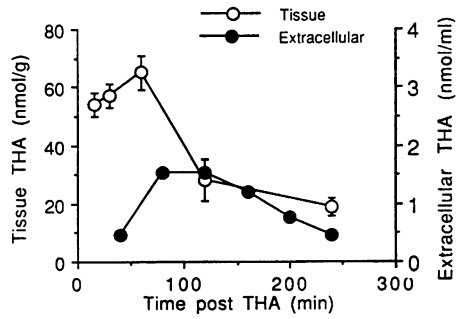


Figure 1. Tissue and extracellular concentrations of THA in rat striatum following THA (5mgkg^{-1}i.p.)

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386P SYSTEMIC AND INTRAHIPPOCAMPAL INJECTION OF PARAQUAT PRODUCES MOTOR SEIZURES AND BRAIN DAMAGE IN RATS

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Paraquat dichloride (1,1'-dimethyl 4,4'-biperidinium dichloride) is a herbicide known to be toxic in man. It is also a potent reversible inhibitor of acetylcholinesterase activities (Seto and Shinohara, 1988) and is able to cross the blood brain barrier in mammals (Corasaniti et al., 1990). Inhibition of acetyl-cholinesterase in the brain increases central acetylcholine concentrations which may lead to neuronal depolarization and cell death (Olney et al., 1983). This study examined the neuropathological effects of paraquat given either systemically or directly into the hippocampus of rats.

Stainless steel guide cannulae were chronically implanted into a dorsal hippocampus of adult male Wistar rats (250-280 g) under chloral hydrate (400 mg kg^{-1}) anaesthesia (co-ordinates: AP = 4.0 mm; L = 2.0 mm; V = 2.4 mm). Unilateral hippocampal injection of paraquat (0.1 - 1.0 µmol in 1 µl) produced motor limbic seizures within a few minutes followed at 24 h, by neuronal damage of the CA1 and CA3 pyramidal cell layers, pyriform cortex, dentate gyrus and hilus fascia dentata (n = 9 rats). Given systemically, paraquat (20 - 100 mg kg^{-1} s.c.; n = 15 rats) produced clonus of the fore limbs and rearing followed at 24 h (10/15 rats) by neuronal degeneration in the pyriform cortex. Smaller doses (10 nmol i.h. or 5 mg kg^{-1} s.c.) were ineffective.

Atropine (50 µmol) co-administered with paraquat (1 µmol) into the hippocampus prevented motor seizures and neuronal degeneration (6 rats). Atropine (150 mg kg^{-1} i.p.) also prevented the effects of paraquat (100 mg kg^{-1} i.p.) administered 1 h later; neuro-degeneration was observed in 2 out of 6 rats tested. In conclusion our results show that systemic or intrahippocampal injection of paraquat produced motor seizures and brain damage which were prevented by muscarinic receptor blockade with atropine.

Partial support from Minstero dell'Università e Ricerca Scientifica (Rome) is acknowledged.

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387P FUNCTIONAL INTERACTION BETWEEN MUSCARINIC ANTAGONISTS AND THE ATYPICAL NEUROLEPTIC, CLOZAPINE, ON ACCUMBENS AND STRIATAL DOPAMINE METABOLISM *IN VIVO*

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Chronic treatment with most neuroleptics is associated with the emergence of extrapyramidal side-effects (Marsden and Jenner, 1980). However, some neuroleptics are distinguished by having a lower incidence of these side-effects and have been named "atypical" (eg clozapine) as opposed to the "typical" neuroleptics (eg haloperidol) (Herman and Pleasure, 1963). This property of clozapine has been related to it ability to block muscarinic receptors in the striatum (Miller and Hiley, 1974). While the anticholinergic properties of clozapine are well recognise, it is still not clear if these properties have functional consequence within the brain. In the present study, we have examined whether the muscarinic antagonists (atropine and scopolamine) alter the in vivo increase in striatal and accumbens dopamine metabolism induced by both haloperidol and clozapine.

Male Wistar rats (250-280 g) were anaesthetised with chloral hydrate (400 mg/kg i.p.) and stereotaxically implanted with one electrically-pretreated 12 µm carbon fibre electrode in the striatum and one in the nucleus accumbens for the subsequent measurement of extracellular DOPAC using in vivo voltammetry (Marsden et al., 1988). DOPAC was recorded every five minutes during a stabilisation period of 45 min before drug administration. Saline (1ml/kg s.c.), scopolamine (1 mg/kg i.p.) or atropine (20 µg i.c.v.) were administered and 15 min later animals received saline (1 ml/kg s.c.), haloperidol (1 mg/kg s.c. or 10 µg i.c.v.) or clozapine (30 mg/kg i.p.). Extracellular DOPAC was recorded for 60 min after drug administration and the results expressed as percentage differences (± s.e.m.) compared to the baseline value (n=6 or more per groups). Data were assessed by two way analysis of variance for repeated measures.

Scopolamine or atropine alone (n=6) did not alter extracellular DOPAC in the nucleus accumbens or the striatum. Clozapine (n=6) increased extracellular accumbens and striatal DOPAC by 62% ±5.4 and 65% ±6.8 respectively 60 min after administration. Pretreatment with scopolamine partially blocked the increase in DOPAC induced by clozapine in the nucleus accumbens (31% ±3.4, above basal level) and in the striatum (20% ±3.6) 60 min after administration (n=10). Atropine (i.c.v.) blocked the increase in striatal extracellular DOPAC induced by clozapine (3% ±6.5 compare to pre-injection baseline level) and partially attenuated the increase of DOPAC in the nucleus accumbens (15% ±5.0). In contrast, neither scopolamine nor atropine altered the increase in DOPAC induced by haloperidol. In conclusion, the present results demonstrate a functional interaction between central cholinergic system and the atypical neuroleptic clozapine on both in vivo striatal and accumbens dopamine metabolism but to a greater extend in the striatum.

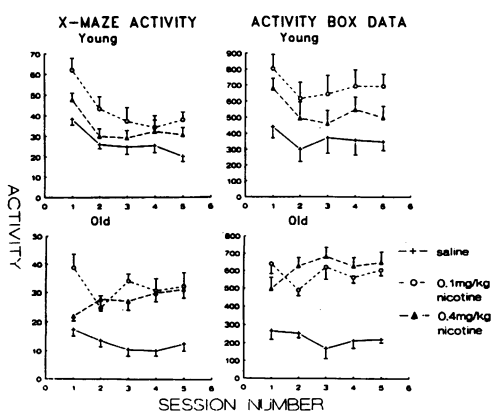
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388P AGE AS A FACTOR IN THE PSYCHOSTIMULANT RESPONSE TO NICOTINE

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Previous studies have demonstrated that nicotine invokes an increase in the locomotor activity of nicotine-tolerant rats and that this is mediated by increased dopamine release from the mesolimbic system of the brain (Clarke et al 1988). The process of ageing is associated with deterioration of this system (Moretti et al 1987) and the present studies were designed to examine the effect of ageing on the locomotor response to nicotine.



Two groups of male Sprague-Dawley rats, one young (10-11wks) and one old (21-23 months) were pretreated with 5 subcutaneous injections of saline or 0.1 mg/kg or 0.4 mg/kg nicotine (n=6/group). The animals were given 10 daily injections and 5 min later placed alternately in an activity box or an X-maze. Activity was recorded automatically for 20 min. (measured in the activity box using an Animex meter; in the X-maze as the number of runway entries) using a counterbalanced design.

The activity of the old rats (Figure 1.) was lower than that in the young animals (significant in maze $p<0.001$, but not on the activity box $p=0.057$). Both doses of nicotine increased the activity of the animals in the maze and the box ($p<0.001$). Analysis of covariance indicated that the effect of 0.4 but not 0.1 mg/kg, on activity in the maze was independent of its effect in the activity box. Further examination of the data demonstrated that the effect was greater in old animals ($p<0.001$) than in young ($p<0.01$). The data suggest that the effect of nicotine (0.4 mg/kg) on exploratory activity is enhanced in old rats.

Figure 1: The effects of nicotine on the activity of young and old animals. Data are expressed as mean ± SEM.

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389P NEOSURUGATOXIN IS AN ANTAGONIST AT THE SOMATIC MUSCLE CELL NICOTINIC RECEPTOR IN THE PARASITIC NEMATODE *ASCARIS SUUM*

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Neosurugatoxin (NSTX), a toxin isolated from the Japanese ivory mollusc Babylonia japonica (Kosuge et al, 1981), has a more potent blocking action at neuronal nicotinic receptors than at nicotinic neuromuscular junction receptors (Hayashi et al, 1984; Luetje et al, 1990). We have used this toxin to further characterise a nicotinic receptor in the parasitic nematode Ascaris suum. The potency of α-bungarotoxin is too low to be compatible with this receptor being a neuromuscular junction type (pA_2 5.85; Colquhoun et al, 1990). Acetylcholine elicits a dose-dependent depolarization of Ascaris muscle cells and an increase in input conductance. NSTX was applied to the preparation for 3 min. prior to and during the application of acetylcholine. NSTX blocked the response to 5 μM ACh with an IC_{50} of 117 ± 29 nM (n=4). NSTX (100 nM) shifted the dose-response curve for acetylcholine to the right in a manner which might indicate competitive antagonism (Figure 1). Further studies will be required to determine the pA_2 value for NSTX at this receptor and the slope of the Gaddum-Schild plot. The block was reversed to 69 ± 8% (n=7) of the control value after a 30 to 60 min. wash.

Thus NSTX is about 10 times more potent than α-bungarotoxin supporting the view that the Ascaris muscle receptor is most like a neuronal nicotinic receptor.

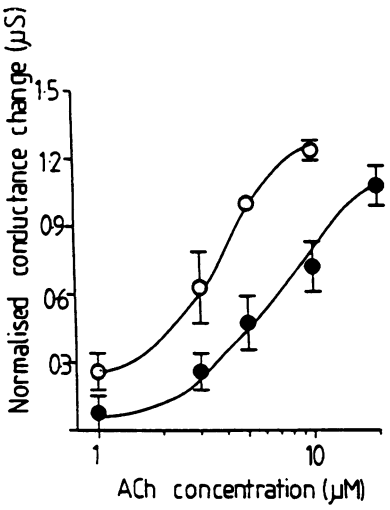


Figure 1. NSTX blocks the response to acetylcholine. Responses are normalized with respect to the response to 5 μM acetylcholine. O control, ● with 100 nM NSTX. (n=3 ± s.e. mean)

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390P TETRAETHYLAMMONIUM (TEA) ANTAGONIZES MUSCARINIC INHIBITION OF VOLTAGE-GATED CALCIUM CURRENT IN NEUROBLASTOMA x GLIOMA HYBRID (NG 108-15) CELLS

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The voltage-gated Ca current in NG 108-15 cells is inhibited by ACh (Higashida *et al.*, 1990). We have studied this effect using a range of agonists and conditions.

The whole cell patch-clamp technique was used to record Ca currents in chemically-differentiated cells (Higashida *et al.*, 1990). Dishes of cells were perfused with medium (22°C; pH 7.4, 95% O_2/5% CO_2 bubbled) containing (mM): NaCl 120; KCl 3; $CaCl_2$ 2.5; $MgCl_2$ 1.2; $NaHCO_3$ 23; HEPES 5; glucose 11, tetrodotoxin 0.5μM. Patch electrode solution contained (mM): CsCl 110; TEA 25; $MgCl_2$ 3; EGTA 3; HEPES 40, pH 7.4. Ca currents were evoked with 100ms steps to 0mV from -80mV at 0.1Hz.

Maximal Ca current inhibition by ACh was 37% ± 2% (n=44) in cells having "T", "N" - and "L"-like current components; inhibition was <5% in 88 cells having only "T"-like currents (cf. Docherty, 1988). Initially, medium contained TEA (120mM), instead of NaCl, to suppress K^+ currents (Docherty, 1988), as low concentrations of other neurotransmitter receptor agonists inhibit the Ca current under these conditions (Docherty & McFadzean, 1989). ACh was weakly active in high TEA medium and muscarine was virtually inactive, but when TEA was reduced to 10mM or omitted, ACh and muscarine became more potent. TEA had similar effects on responses to oxotremorine-M (Table 1).

Table 1 — Agonist EC_{50} values (μM; s.e. mean range in brackets)

	Control	n	TEA 10mM	n	TEA 120mM	n
ACh	0.32 (0.25-0.41)	4	4 (3.8-5.2)	4	7.9 (7.7-8.3)	3
(±) Muscarine	0.54 (0.38-0.76)	4	21	2	» 300	3
Oxotremorine-M	0.14 (0.09-0.21)	5	2.8(2.4-3.3)	5		

It was also noted that TEA (10mM) reduced the apparent potency of atropine (10nM-10μM) by 10-15 fold (n=5), possibly reflecting complex receptor interactions of a weak and a strong antagonist. The dose-ratio for TEA 120mM is less than that predicted from the dose ratio for TEA 10mM if antagonism is competitive. This may be because TEA at 120mM additionally inhibits cholinesterase; the EC_{50} for ACh plus neostigmine (1μM) is 0.11μM (0.09-0.14, n=12).

This study was supported by a grant from the Medical Research Council.

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391P ACETYLCHOLINE DOPAMINE INTERACTIONS: CHOLINERGIC TERMINALS MAKE SYNAPTIC CONTACT WITH DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA

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Acetylcholine (ACh) has been implicated as a neurotransmitter of terminals in the substantia nigra (SN). Indirect evidence suggests that these terminals (probably derived from neurons in the brainstem tegmental nuclei) make contact with the dopaminergic neurons of the SN. Thus 1) the pars compacta (SNC) contains a higher density of cholinergic terminals than the pars reticulata (SNR), 2) dopaminergic neurons contain high levels of acetylcholinesterase and 3) they respond electrophysiologically to ACh (see Gould,Woolfe & Butcher, 1989). Furthermore, stimulation of the region of the pedunculopontine nucleus causes an excitation of dopaminergic neurons that is sensitive to nicotinic receptor antagonists (Clarke, Hommer, Pert & Skirball, 1987) The object of the present experiment was to test directly, by morphological means, whether dopaminergic neurons in the SN receive synaptic input from cholinergic terminals.

Sections of the SN of perfuse-fixed brains of rats and ferrets were incubated by a double immunocytochemical procedure (Levey et al 1986) to reveal cholinergic axons using an antibody against choline acetyltransferase (ChAT) and then dopaminergic neurons using an antiserum against tyrosine hydroxylase (TH). The immunoreactive sites were revealed by peroxidase reactions but using different chromogens for each antigen which were distinguishable in both the light and electron microscopes. In the light microscope ChAT-immunoreactive axons and boutons were seen in both the SNC and SNR and were often closely apposed to TH-immunoreactive neurons. In the electron microscope the ChAT-immunoreactive terminals were found to form conventional asymmetrical synapses. On many occasions the ChAT-immunoreactive terminals were found to make direct synaptic contact with the TH-immunoreactive dendrites and perikarya. The dendrites of the dopaminergic neurons that descend into the SNR were found to receive multiple inputs from cholinergic fibres that formed 'climbing-fibre' like contacts.

These findings demonstrate that one of the major targets of cholinergic terminals in the SN are the dopaminergic neurons and provide an anatomical substrate for an interaction between ACh and dopamine in the SN. Furthermore, although the classical view that the balance between the levels of activity of ACh and dopamine for 'normal' basal ganglia function occurs within the striatum (Lehmann & Langer, 1983) the detection of cholinergic terminals in contact with dopaminergic neurons implies that at least part of this balance resides in the SN.

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392P ^{45}Ca EFFLUX FROM GUINEA-PIG ATRIA IS NOT DECREASED DURING DIGITALIS INTOXICATION

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Cardiac glycosides in toxic concentrations cause a massive Ca overload in heart muscle cells. From radioisotope experiments it was concluded that the Ca efflux from cardiac cells is decreased during digitalis intoxication. Experiments were performed to examine the influence of tissue Ca accumulation on the determination of ^{45}Ca efflux from guinea-pig left atria.

After labelling the exchangeable Ca pool of the atria with ^{45}Ca for 60 min, the washout of radioactivity into tracer-free Tyrode solution was studied for 300 min. After 90 min of washout, the ^{45}Ca efflux followed a single exponential course, corresponding to a constant fractional loss of ^{45}Ca per min from the muscle. In the presence of 5×10^{-6} M ouabain, the atria developed a contracture and the tissue Ca content increased from 1.65 to 10 mmol x kg^{-1} after 300 min. Only after addition at the beginning of the washout period, ouabain caused a decrease of the rate coefficient of ^{45}Ca efflux to 67 ± 4 % of the control value. Upon preincubation with ouabain for 30 and 90 min, the efflux rate reached 79 ± 6 % and 103 ± 7 % of the control value, respectively. In order to test whether the apparent decrease of the ^{45}Ca efflux rate was due to the massive Ca accumulation of the tissue after addition of ouabain at the beginning of the washout period, 3 mM EGTA was added to the washout medium to prevent the tissue Ca uptake. Under this condition, the ^{45}Ca efflux proceeded with a rate coefficient similar to the control value.

The results suggest that the Ca overload of myocardial cells during digitalis intoxication is not primarily caused by a reduction of Ca efflux from the cells but more likely by a massive increase of Ca influx.

393P THE USE OF HYPERMEDIA FOR STUDENT CENTRE LEARNING

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Hypermedia is the result of combining computer multimedia such as text, graphics, sound and animation with non-linear organisation of information. In non-linear (unstructured) systems information is linked by the strength and nature of it's association with other information, mimicking the way associative memory patterns are laid down in the brain. When combined with the storage and retrieval power of the computer Hypermedia becomes a powerful engine for managing and presenting information. Informal evaluations report improved pluralistic cognitive styles and increased student motivation when using Hypermedia (Landow, 1987). Here we demonstrate a computer program which uses Hypermedia to teach the functional anatomy of the rat. This highly interactive program uses HyperCard™ and the Apple® Macintosh® microcomputer to illustrate the flexibility and educational potential of Hypermedia for undergraduate teaching.

A network of high resolution photographic images and diagrams of a rat dissection is presented. As users move the mouse pointer across an image they reveal labels of the structures they are viewing. By clicking the mouse button on a number of structures or areas of the image users are able to progress to more detailed images/diagrams in a manner comparable to removing layers to reveal the layer beneath. They may move forward, backward or laterally within a network of information building up a picture of the relationships which exist. In 'Browse Mode' users are invited to adopt a browsing approach and work through the information at their own pace. They may retrieve associated text and by selecting keywords be led into a network of related textual information. This text may be altered by a user with access to a password, allowing teachers to tailor the program to specific groups of students. In 'Test Mode' labelling is suppressed while randomly generated questions invite users to identify structures on the image. A score of correctly answered questions is maintained. The third mode 'Browse & Test' combines the features of the modes mentioned above. A comprehensive map of the dissection allows users to instantly target areas of the dissection or establish where they have already been as the program keeps track of their movements. This complex and interactive medium is simple to use and encourages pursuit of a dynamic, non-linear approach to learning.

This work was supported by The Lord Dowding Fund (NAVS).

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394P A COMPUTER SIMULATION OF EXPERIMENTS PERFORMED ON FROG SKIN TO TEACH THE PRINCIPLES OF THE EPITHELIAL TRANSPORT OF IONS

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The in vitro frog skin preparation is used extensively to teach the principles of active and passive ion transport across tight epithelia. The output of the tissue, at least for teaching purposes, is relatively simple to model and computer aided learning (CAL) programs based on this preparation have been reported previously (Levin & Levin, 1986). Here we describe a highly interactive CAL program which is aimed at undergraduate students on biology/medical courses and is based largely on a number of experiments which may be performed on the frog skin preparation.

The program runs on any true IBM compatible microcomputer with graphics capability (Hercules, CGA, EGA, VGA). It is written in Turbo Pascal (Borland) and utilises an easy-to-use, windows-like menu system.

The principles underlying both passive and active transport, details of the methods used to measure these phenomena and the methods for calculating ion flux are explained using a combination of text, high-resolution colour graphics and animation. Experiments may be performed to investigate the effects of varying ion concentrations on both types of transport. Passive ion transport is determined from the open-circuit transepithelial p.d and data is displayed using a simulated analogue millivoltmeter. Students may change the concentrations of Na^+ and K^+ in both the mucosal and serosal bathing fluids and measure the p.d directly from the monitor. Active transport may be measured using either a short-circuit current technique or by a radioactive tracer method (two- or four-compartment bath). Ion concentrations may be varied as before and, depending on the choice of method, the monitor display shows either an analogue millivoltmeter and digital microammeter or the collection, sampling and counting of the radiolabel. The action of a number of drugs (amiloride, oxytocin, ouabain) on active ion transport may also be investigated by the short-circuit current method.

This work was supported by the Lord Dowding Fund (NAVS).

Levin, R. A. & Levin, R. J. (1986) Journal of Physiology, 376, P2.

395P THE LANGENDORFF HEART - A COMPUTER SIMULATION

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Computer simulations of experiments, which involve the use of animals or animal tissue, are increasingly being incorporated into undergraduate teaching programmes in pharmacology (Hughes, 1984; Brown et al, 1989; Dewhurst & Howells, 1990). Here we describe a computer aided learning (CAL) program based on a number of experiments which may be performed on the isolated perfused mammalian heart (Langendorff) preparation. The program, which is highly interactive, is aimed at undergraduate students of pharmacology and, in addition to being a useful teaching aid, offers a realistic alternative to performing the experiment for real.

The program is written in Turbo Pascal (Borland) and will run on any IBM compatible microcomputer using any of the standard graphics cards (e.g. VGA, EGA, CGA, Hercules). It is easy to use and makes use of an attractive windows-like menu display, from which the user may select experiments to demonstrate the action of:

a range of drugs (sympathomimetics, parasympathomimetics, cardiac glycosides, coronary vasodilators), with or without selected antagonists or potentiators;

a selection of ions (sodium, potassium, calcium);

changing ventricular pre-load on ventricular force (Starling's Law);

The simulated experimental results (ventricular force, heart rate and coronary blood flow) are displayed simultaneously in high resolution colour graphics in a form comparable to that of a chart recorder. A time scale and appropriate vertical scales are included on the trace and where, for example, the experiment involves the action of an agonist + antagonist/potentiator, it is possible, at the press of a key to 'flip' between that trace and that of the agonist alone, a facility which allows better visualisation of drug effects.

Background information on the preparation, and experimental method is presented as text, and HELP windows, the contents of which are under the control of the teacher, may also be used to offer further assistance to students using the program or perhaps to outline a number of tasks for the students to complete.

This work was supported by the Lord Dowding Fund (NAVS).

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396P COMPUTER SIMULATION OF ANIMAL EXPERIMENTS IN PHARMACOLOGY

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The main objective of pharmacology practical classes is to demonstrate drug actions taught in theory classes. The growing concern over animal rights and financial and ethical constraints are making it more difficult to use animals to achieve this objective. As a result a number of computer simulations of animal experiments have been developed recently. A software package containing seven programs simulating the following animal experiments will be demonstrated.

1 Effect of drugs on isolated heart of frog
2 Effect of drugs on blood pressure, heart rate and respiratory rate of dog
3 Effect of drugs on locomotor activity of rat
4 Effect of drugs on rabbit eye
5 Effect of drugs on ciliary motility of oesophagus of frog
6 Evaluation of analgesics using mice
7 The dose response curve and matching assay of histamine

These interactive simulation programs written for IBM PC compatibles are meant for undergraduates studying Pharmacology. They are aimed at giving a good understanding of drug actions and helping the user to remember them better. The programs are menu driven and each one has both tutorial as well as examination modes to be chosen by the user. In the examination mode the user is given a few unknown drugs to identify.

When performing any of the simulated experiments the user can decide the sequence of drug administration. In some experiments drug doses are fixed and in others it is open to the user to decide. Drug responses are subject to biological variations. Unknown drugs, provided by the program are randomly selected from the list of available drugs. The user can stop the experiment at any stage. Readings are expected to be recorded and tabulated during experiments. A model table is available for each experiment in the tutorial mode. At the end, the user is to draw conclusions and answer some questions which appear on the screen.

The program provides not only an inexpensive but also an easy way of demonstrating drug effects on different animal preparations. This would be useful in an environment where the use of animals is restricted due to various reasons.

397P CONTINUOUS 24 HOUR MONITORING OF BLOOD PRESSURE AND HEART RATE IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS

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Spontaneously hypertensive rats are conditioned (individually housed under same conditions as during testing) and sham dosed on 2 occasions during the 7 days pre operative. Rats are housed in specially designed cages and racks.

Under aseptic conditions, the left carotid artery is isolated and a heparinised-saline filled polythene catheter is implanted. The catheter is exteriorised at the back of the neck using a trochar and the ventral wound is sutured. A skin button (Instech) is inserted in place over the catheter and either implanted subcutaneously in the dorsal scapula region or fitted to a rat jacket (Alice King). On the day of surgery, analgesics (Temgesic 15μg b.i.d.) and antibiotics (Milimycin - Procaine penicillin, 40 mgs) are administered subcutaneously. The rat is then attached whilst recovering from anaesthetic (Halothane - 2%) via a stainless steel spring tether to a single channel fluid swivel (Instech). A 3-way port connects the tether system with a continuous, slow speed infusion pump (Instech Lambda pump - model 700) and a disposable pressure transducer (Spectramed).

Blood pressure signals are analysed on a beat to beat basis by an in house software package running on a PC 286 based system. Each computer is capable of monitoring 8 rats simultaneously and deriving systolic, diastolic, mean blood pressure and heart rate. Data is averaged to minute mean values and stored to disk and printer. Signal integrity is monitored using a commercial software package which converts a PC 286 into an 8 channel oscilloscope. Editing, graphing and statistics are performed by a separate in house software package.

On days 4 and 5 post operative, placebo data are recorded and if there are variations greater than 15% on an hour to hour comparison, then these rats are not used for drug treatment. Data from dosing day (day 6) is compared to placebo day (day 5), time point by time point, to eliminate circadian or cyclical artefacts, significance being analysed using Student's 2 day 't' test for group variates.

The advantages of the sytem are: rats are subjected to less stress bringing about a marked reduction in resting heart rate when compared to previously used, restrained rat screens - average resting heart rate reduced from approximately 500 to 350 beats per minute. Previous screening methods employed both a dosed and placebo group of animals. However, with this technique, each animal acts as its own control eliminating the need for a placebo group and thereby reducing the number of animals needed to test a compound by 50%. At the same time, we have seen a significant enhancement of data quality and continuous monitoring provides more detailed drug profiles, which ultimately may also lead to a reduction in group sizes.

Acknowledgements:
All in house software written by Mr M Posnett, Research and Engineering Laboratories, ICI Pharmaceuticals.

398P IDENTIFICATION OF THE PUTATIVE 5-HT$_4$ RECEPTORS IN GUINEA-PIG ASCENDING COLON

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397P CONTINUOUS 24 HOUR MONITORING OF BLOOD PRESSURE AND HEART RATE IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS

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399P THE ANTICONVULSANT ACTION OF CI-977 MAY INVOLVE THE GLYCINE/NMDA RECEPTOR COMPLEX

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ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work.

1P **Kay G & Elliott JM** Differential effect of lithium on β-adrenoceptor down-regulation in lymphoblast cell lines from patients with bipolar depression and healthy control subjects

2P **De Paermentier F, Crompton MR, Katona CLE & Horton RW** α_2-adrenoceptor binding sites in cortical samples from depressed suicide victims

3P **Lawrence KM, Falkowski J, Jacobson RR & Horton RW** A plasma inhibitor of platelet [^{3}H]imipramine binding is increased in depressive illness

4P **Hudson AL. Mallard NJ & Nutt DJ** Autoradiographic localization of non-adrenoceptor idazoxan binding sites (NAIBS) in rat brain

5P **Holman RB & Thomas DN** Regulation of endogenous noradrenaline release in rat hippocampus and frontal cortex: *in vivo* microdialysis

6P **Elliott JM, Payvandi N & Heal DJ** Characterisation of a high-affinity binding site for [^{3}H]UK 14304 in rat brain membranes which is insensitive to GTP

7P **Payvandi N, Elliott JM & Heal DJ** Guanine nucleotides - a novel method for defining specific binding of agonist radioligands to α_2 adrenoceptors

8P **Dexter DT, Wells FR, Javoy-Agid F, Agid Y, Daniels SE, Marsden CD & Jenner P** Ferritin levels in the brains of subjects with Lewy bodies at post-mortem who did not show clinical symptoms of Parkinson's disease in life

9P **Trout SJ, Patel J & Kruk ZL** Characteristics of autoreceptor control of dopamine release in olfactory tubercle in the absence of uptake inhibition

10P **Daly SA & Waddington JL** The psychopharmacology of three new, selective D-1 dopamine receptor antagonists in relation to two directions of D-1:D-2 interaction

11P **Strijbos P, Browning J, Ward M, Forder R, Carey F, Horan M & Rothwell N** Central and peripheral actions of recombinant lipocortin-1 and a fragment of lipocortin-1 on the responses to interleukin-1β

12P **Stock MJ & Carlisle HJ** Paradoxical thermoregulatory operant responses for heat in rats receiving isoprenaline

13P **Stock MJ & Carlisle HJ** Use of selective β-antagonists to potentiate thermal responses to a non-selective β-agonist

14P **Le Feuvre RA, Aisenthal L & Rothwell NJ** The involvement of corticotrophin releasing factor (CRF) in the thermogenic and anorexic actions of 5-HT

15P **Buckingham JC, Safieh B, Singh S & Kendall MD** Interactions of corticotrophin and glucocorticoids in the control of thymulin release in the rat

16P **MacLennan SJ, Turner MA, Prentice DJ & Martin GR** Evidence for activation of histamine-H_1 receptors by sumatriptan

17P **Spriggs TLB, Mallard NJ, Marshall RW & Sithers AJ** Functional discrimination of α_{1A} and α_{1B} adrenoceptors in rat vas deferens

18P **Przyborski SA, Levin RJ & Young A** The muscarinic M_1 agonist, McN-A-343, activates rat proximal and distal duodenal electrogenic secretion *in vitro* by a postganglionic neural pathway

19P **Whiting RL, Ford APDW, Baxter GS, Harris GC, Sharif NA & Eglen RM** Pharmacological and biochemical analysis of muscarinic receptors in guinea-pig ileum *in vitro*

20P **Eglen RM, Harris GC, Taylor M, Pfister J & Whiting RL** Characterization of muscarinic receptors mediating release of epithelial derived relaxant factor (EpDRF) *in vitro*

21P **Spina D & Page CP** Does hypoxia account for epithelial derived inhibitory factor (EpDIF) using a co-axial bioassay?

22P **Norman P, Cuthbert NJ & Gardiner PJ** Differences between the thromboxane receptors of rat and guinea-pig lung. 1. Studies with agonists

23P **Cuthbert NJ, Norman P, McKenniff MC & Gardiner PJ** Differences between the thromboxane receptors of rat and guinea-pig lung. 2. Studies with thromboxane antagonists

24P **Nials AT, Coleman RA, Hartley D & Sheldrick RLG** AH13205 - a novel selective prostanoid EP_2 agonist

25P **Frossard N, Boichot E, Le Gall G, Lagente V, Mencia-Huerta JM, Lockhart A & Braquet P** Relation between *in vivo* and *in vitro* bronchoconstriction to substance P in aerosol-sensitized guinea-pigs

26P **Longmore J, Miller M & Weston AH** The relationship between the effects of lemakalim on tension and its effects on membrane potential and K permeability in bovine tracheal smooth muscle

27P **Herbert CA, Edwards D, Boot JR & Robinson C** Further studies of the eosinophil-dependent enhancement of the permeability of the bronchial mucosa to albumin

28P **Bertelsen H & Johansen T** Extracellular magnesium reduces the secretory response of rat peritoneal mast cells to compound 48/80 in a calcium-free medium

29P **Giembycz MA, Rabe K, Dent G & Barnes PJ** Identification and partial characterisation of cyclic nucleotide-dependent phosphodiesterase activities in guinea-pig peritoneal eosinophils

30P **Shahid M, Billington C & Nicholson CD** Evidence for two distinct Ca/calmodulin stimulated cyclic nucleotide phosphodiesterases (PDEs) in rabbit cardiac ventricle

31P **Allen MJ & Carpenter JR** Vanadium compounds, but not heparin, inhibit mechanical responses to IP_3 in saponin-treated guinea-pig ileum longitudinal smooth muscle

32P **Buchan KW & Martin W** Forskolin, but not 8 bromo cyclic GMP, modulates thrombin-induced elevations of cytosolic calcium in bovine aortic endothelial cells

33P **van Tits LJH, Michel MC & Brodde O-E** Lectin-stimulated proliferation and second messenger generation in human lymphocytes following isoprenaline infusions

34P **Donnelly LE, Boyd RS & MacDermot J** ADP-ribosyltransferase in NG108-15 cells and its role in the regulation of $G_{s\alpha}$ activity and abundance

35P **Bushfield M, Lavan BE, Milligan G & Houslay MD** The tumour-promoter okadaic acid induces the phosphorylation and inactivation of G_i-2

36P **Kendall DA & Firth JL** Modulations of cyclic AMP formation in human cerebrocortical slices

37P **Kendall DA & Wilson VG** A crude bovine brain extract containing clonidine displacing substance inhibits cyclic AMP formation in brain slices

38P **Souness JE, Turner NC, Diocee BK, Hassal GA & Wood LJ** Characterization of guinea-pig eosinophil cyclic AMP phosphodiesterase (PDE)

39P **Turner NC, Souness JE, Wood LJ, Diocee BK, Hassal GA & Scott L** Effects of phosphodiesterase inhibitors on granulocyte superoxide generation

40P **Quilley CP, Lin Y-S & McGiff JC** Renal actions of angiotensin II are modified by an inhibitor of cytochrome P450-dependent arachidonic acid metabolism

41P **Godfraind T, Dessy C, Tajimi M & Karaki H** Action of staurosporine on Ca signal and contraction evoked by phenylephrine in rat aorta

42P **O'Neill LAJ, Stylianou E, Edbrooke MR, Farndale R, Woo P & Saklatvala J** Understanding how interleukin 1 activates cells: induction of the transcription factor KFκB by IL1 in the murine thymoma line EL4 NOB-1

43P **Knott C & Reynolds F** Fetal to maternal antiepileptic drug transfer in the rabbit

44P **Kerwin RW, Murphy M, Robinson P & Stephenson J** Distribution of cholecystokinin receptors in post-mortem samples of medial temporal lobe from control subjects and schizophrenic patients

45P **Singh L, Field MJ, Hughes J, Vass CA & Woodruff GN** Central administration of a CCK-B receptor agonist induces anxiety

46P **Hayward NJ, Harding M, Lloyd SAC, McKnight AT, Woodruff GN & Hughes J** The effects of CCK-B/gastrin antagonists on stimulated gastric acid secretion in the anaesthetised rat

47P **Ebenezer IS, Parrott RF, Forsling ML & Baldwin BA** The effect of intravenous cholecystokinin on vasopressin and cortisol release in the conscious pig

48P **Raiteri M & Paudice P** 5-hydroxytryptamine and cholecystokinin interact in the brain via 5-HT_3 receptors

49P **Allen SP, Piper PJ, Chester AH & Yacoub MH** Leukotrienes C_4 and D_4 are potent vasoconstrictors of the human saphenous vein *in vitro*

50P **Stubbs CM, Waldron GJ, Connor HE & Feniuk W** Characterisation of neurokinin receptors mediating relaxation of canine isolated middle cerebral artery

51P **Shaw N, Foulkes R, Andrew DP & Hughes B** The effect of monoclonal antibodies to CGRP on CGRP-induced vasodilatation in pig coronary artery rings

52P **Schwietert HR, Wilhelm D, Wilffert B & Van Zwieten PA** The phasic and tonic responses to phenylephrine may reflect a divergence in the excitation-contraction coupling of α_1-adrenoceptors in rat portal vein

53P **Forster C & Armstrong PW** Abnormal vascular responsiveness following one week of rapid ventricular pacing in dogs

54P **Haddock PS, Woodward B, Hearse DJ & Dodds R** Modification of bovine and rat sodium/potassium ATPase by oxidised and reduced glutathione and other sulphydryl compounds

55P **Williams FM, Tanda K & Williams TJ** Investigation of the effect of cycloheximide on ^{111}In-neutrophil accumulation in ischaemic/reperfused rabbit myocardium

56P **Heijnis JB, Mathy M-J & Van Zwieten PA** Effects of nifedipine in isolated hearts from diabetic and control rats during global ischaemia and reperfusion

57P **Scheuffler E, Hugtenberg J, Belsner K, Mozes A, Pfeifer M, Wilffert B & Peters T** Effect of dipyridamole and nifedipine on cellular and interstitial adenine nucleotide catabolites in ischaemic guinea-pig hearts

58P **Hohlfeld Th, Strobach H & Schrör K** Further evidence that the cardioprotective action of defibrotide in acute myocardial ischaemia is prostacyclin-mediated

59P **Eschenhagen Th, Nose M, Schmitz W, Scholz H, Haverich A, Hirt S, Döring V & Kalmar P** Increased mRNA level of the α-subunit of inhibitory G-proteins in end-stage myocardial failure

60P **Dewar D, Chalmers DT, Graham DI & McCulloch J** Autoradiographic mapping of non-NMDA glutamate receptor subtypes in the human hippocampus: alterations in Alzheimer's disease

61P **Gill R & Lodge D** The neuroprotective action of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) in a rat focal ischaemia model

62P **Tacconi S, Ratti E, Marien MR, Gaviraghi G & Bowery NG** Regional inhibition of [^{3}H]MK801 binding in rat brain by CPP and 7-Cl-kynurenate: a receptor autoradiography study

63P **Neal MJ & Cunningham JR** MK801 inhibits ischaemia-induced release of glutamate, aspartate and GABA from the rat retina *in vitro*

64P **Foster AC, Donald AE, Grimwood S, Leeson PD & Williams BJ** Activities of 4-methyl derivatives of HA-966 at the glycine site of the N-methyl-D-aspartate receptor from rat brain

65P **Kemp JA, Priestley T, Marshall GR, Leeson PD & Williams BJ** Functional assessment of the actions of 4-methyl derivatives of HA-966 at the glycine site of the N-methyl-D-aspartate receptor

66P **Saywell K, Singh L, Oles RJ, Vass C, Leeson PD, Williams BJ & Tricklebank MD** The anticonvulsant properties in the mouse of the glycine/NMDA receptor antagonist, L-687,414

67P **Hutson PH, Thorn L, Bristow LJ & Tricklebank MD** R(+)-HA-966, a glycine /NMDA receptor antagonist, blocks increased mesolimbic dopamine metabolism induced by psychostimulant drugs

68P **Bristow LJ, Baucutt LJ, Hutson PH, Thorn L & Tricklebank MD** R(+)-HA-966, a glycine/NMDA receptor antagonist, attenuates PCP and amphetamine-induced hyperactivity in rodents

69P **O'Connor VM & Fry JP** Modification of arginine residues reveals [^{3}H]strychnine binding sites sensitive to use-dependent cation channel blockers in mouse spinal cord membranes

70P **Fletcher AE & Bowery NG** Autoradiographical determination of strychnine-insensitive glycine receptor modulation of [^{3}H]MK801 binding in rat CNS

71P **Wermelskirchen D, Urenjak J, Beile A, Khan S, Nebel U, Wirth A, Wilffert B, Tegtmeier F & Peters Th** Flunarizine and R 56865 suppress veratridine-induced increase in O_2 consumption and ^{45}Ca uptake in rat cortical synaptosomes

72P **Lambert PD, Barnes S, Hughes J, Woodruff GN & Hunter JC** The effect of CI-977 on potassium-stimulated glutamate release from rat cortical slices

73P **Elliott PJ, Turpin MP, Mason GS & Hagan RM** Behavioural activity of neurokinin agonists following intracerebroventricular injection in mice

74P **Vizcarra E, Esplugues JV, Ramos EG, Canet A, Barrachina MD & Esplugues J** Modulation by capsaicin-sensitive sensory neurones of gastric secretory responses induced by peptone

75P **Kuo H-P, Rohde JAL, Barnes PJ & Rogers DF** Cigarette smoke-induced goblet cell secretion in guinea-pig trachea *in vivo*: undiluted vapour phase activation of capsaicin-sensitive nerves

76P **Buckley TL, Jose PJ, Brain SD & Williams TJ** The release of calcitonin gene-related peptide (CGRP) by capsaicin in the rabbit skin *in vivo*

77P **Bevan S, Hothi S, Hughes GA, James IF, Rang HP, Shah K, Walpole CSJ & Yeats JC** Development of a competitive antagonist for the sensory neurone excitant, capsaicin

78P **Dray A, Campbell EA, Hughes GA, Patel IA, Perkins MN, Rang HP, Rueff A, Seno N, Urban L & Walpole CSJ** Antagonism of capsaicin-induced activation of C-fibres by a selective capsaicin antagonist, capsazepine

79P **Dickenson A, Dray A, Hughes GA & Walpole CSJ** The selective capsaicin antagonist capsazepine inhibits capsaicin-induced antinociception: electrophysiological studies in rodents

80P **Belvisi MG, Stretton D & Barnes PJ** Nitric oxide modulates cholinergic neurotransmission in guinea-pig tracheal smooth muscle

81P **Hobbs AJ, Brave AJ, Bhat S, Tucker JF & Gibson A** The nature of α-chymotrypsin-resistant NANC relaxations in guinea-pig trachea

82P **Finberg JPM & Vardi Y** Evidence for a role of EDRF in penile erection

83P **Martin W, Gillespie JS, Liu X & Gibson IF** Effects of N^G-substituted analogues of L-arginine on NANC relaxation of the anococcygeus, retractor penis and penile artery

84P **Boeckxstaens GE, Pelckmans PA, Bogers JJ, Bult H, De Man JG, Oosterbosch L, Herman AG & Van Maercke YM** Nitric oxide released upon stimulation of non-adrenergic non-cholinergic nerves in the rat gastric fundus

85P **Taylor SJ, Hamilton GF & Kilpatrick GJ** Inhibition of field stimulated non-cholinergic contractions of the guinea-pig ileum by histamine H_3 receptor activation

86P **Dunn PM** Ganglion blocking activity of dequalinium in frog and rat sympathetic ganglia *in vitro*

87P **Eigenhuis JJ, Leeuwin RS, Mantel P, van Wilgenburg H & Zeegers A** Benzodiazepines can inhibit motor activity by blocking impulse propagation

88P **Carrington AL, Ettlinger CB, Tomlinson DR & Calcutt NA** The effects of aldose reductase inhibition on nerve conduction abnormalities of diabetic rats

89P **Taneja DT & Clarke DE** Evidence for a noradrenergic innervation of "atypical" β-adrenoceptors in guinea-pig ileum

90P **Braun M, Ney P, Szymanski Ch, Bruch L & Schrör K** 13,14-dihydro-PGE_1, a potent inhibitor of human platelet and neutrophil activation

91P **Santana AC, de Nucci G & Bakhle YS** Effects of Ph CL28A in two models of acute inflammation induced by carrageenan in rats

92P **Armstrong RA & Jones RL** Cicaprost potentiates plasma exudation induced by EP_3-selective PGE analogues, in the absence of other inflammatory mediators

93P **Duncan GS, Peers SH, Carey F, Forder RA & Flower RJ** Antiserum to lipocortin 1 reverses the acute anti-inflammatory actions of dexamethasone in the rat

94P **Ahluwalia A, Peers SH & Flower RJ** Topical application of betamethasone-17-valerate cream inhibits oedema formation in rat skin

95P **Whelan CJ & Johnson M** Salmeterol, but not salbutamol, has anti-inflammatory activity in guinea-pig skin

96P **Von Uexkull C, Nourshaugh S & Williams TJ** Characterisation of inflammatory responses induced by live *E. coli* in rabbit skin

97P **Cadogan A & Humphrey PPA** Classification of the 5-HT receptor mediating cardio-excitation in the snail, *helix aspersa*

98P **Kaumann AJ, Brown AM & Raval P** Putative $5\text{-}HT_4$-like receptors in piglet left atrium

99P **Smits JF, Schoemaker RG, Daeman MJAP & Debets JJM** Haemodynamic consequences of interference with the renin-angiotensin system following myocardial infarction in rats

100P **Puttick RM & Terrar DA** Actions of propofol and enflurane on single calcium channel currents in guinea-pig ventricular myocytes

101P **de Silva R, Myers R, Manjil LG, Lewthwaite P, Bloomfield PM & Mann GE (introduced by AR Baydoun)** Investigation of rat myocardial peripheral type benzodiazepine receptors

102P **Brodde O-E, Khamssi M, Michel-Reher M & Michel MC** Chronic β_1-blocker treatment differentially regulates β-adrenoceptors and muscarinic receptors, but does not affect the amount of G_i-protein in the human right atrium

103P **Wang X-L & Ravens U** Reduction of cardiac outward currents by α-adrenoceptor stimulation: a subtype-specific effect?

104P **Ruck A, Kendall DA & Hill SH** β-adrenoceptor desensitization and agonist-induced astroglial differentiation

105P **Carroll MA, Falck JR & McGiff JC** Renal vascular effects of cytochrome P450-dependent arachidonate metabolites

106P **Quilley J, McGiff JC & Oyekan AB** Cytochrome P450 enzymes (P450) in the renal vasodilator response to arachidonic acid (AA) and the relase of vasoactive products

107P **Thiemermann Ch, Hecker M, Achim Mester P, Al-Damluji & Vane JR** L-arginyl-L-phenylalanine (Arg-Phe) is a potent pressor peptide in the anaesthetized rat *in vivo*

108P **Gardiner SM, Compton AM, Compton PA, Bennett T, Bose C, Foulkes R & Hughes B** Antagonism of the haemodynamic actions of rat islet amyloid polypeptide by human α-calcitonin gene-related peptide [8-37] in conscious rats

109P **Batin P, Gardiner SM, Compton AM & Bennett T** Haemodynamic effects of the non-peptide, angiotensin II antagonist, DuP753, in conscious Brattleboro rats

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British Journal of Pharmacology

Proceedings Supplement

Proceedings of the British Pharmacological Society Meeting

St George's Hospital Medical School

17–19 December, 1990

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