BINDING OF [^{3}H]-BRADYKININ TO RENAL CORTEX MEMBRANES PREPARED FROM RAT AND GUINEA-PIG

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Bradykinin has effects on renal electrolyte metabolism. It reportedly alters blood flow, affects renal vasculature and directly influences the transporting epithelia (Kauker, 1980). It has been reported that guinea pig tissues possess high affinity binding sites for bradykinin and the present study compares renal binding sites for bradykinin from rat and guinea pig by ligand binding and autoradiography.

Crude brush border basolateral membranes were prepared by the method of Heidrich et al (1972). Aliquots (50-100µg protein) were incubated in 25 mM TES buffer, pH 6.8, containing 1 mM 1,10 phenanthroline, 1 mM dithiothreitol, 0.1 mM bacitracin, 5 µM SQ 20881 and 0.2% BSA with [^{3}H]-bradykinin in a total volume of 500 µl for 1 hour at 4°C. Non-specific binding was determined by the inclusion of 10 µM bradykinin. The reaction was terminated by filtration through Whatman GF/B filters and bound radioactivity determined by liquid scintillation spectrometry. Autoradiography was carried out on 10 µm slices mounted onto gelatin-coated glass slides. As far as possible incubation conditions were kept similar to those determined for membrane binding, ligand concentrations used were 0.5,1,20 nM, non-specific binding was determined using 10 µM unlabelled bradykinin and the incubation time was 90 min at 4°C. The slides were apposed to LKB ultrafilm for 6-8 weeks and analysed on a Quantimet densitometer.

High affinity saturable binding sites were detected in guinea-pig cortex membranes, the Kd was 125±23 pM and the Bmax 2.6 ± 0.27 pmol/mg protein. The binding was displaced by bradykinin analogues with the following order of potency;- bradykinin IC_{50} 0.155 ± 0.04; [lys]-bradykinin 0.163 ± 0.012; [Met-lys]-bradykinin 0.573 ± 0.101; [Tyr^8]-bradykinin 0.95 ± 0.15; [Tyr^1]-bradykinin 1.306 ± 0.103 nM, SQ 20881 15 µM and [DesArg^9]-bradykinin 50 µM. These results suggest that this binding site resembles the characteristics of a bradykinin receptor. In contrast the binding site in rat renal cortex membranes had a lower affinity of 45.3 ± 9.1 nM and Bmax 3.95 pmol/mg. Analogues displaced with a similar order of potency but with lower IC_{50}s i.e. bradykinin 37.3 ± 6.7; [lys]-bradykinin 103 ± 11.4 [Tyr^8]-bradykinin 93.3 ± 4.3 nM with again the angiotensin converting enzyme inhibitor SQ 20881 (30µM) and [DesArg^9]-bradykinin (100 µM) being less active. Similar results were obtained autoradiographically, at low ligand concentrations binding sites were located in guinea pig renal cortex and medulla. At these low concentrations only discrete binding in rat medulla was observed, while at higher ligand concentrations sites were located in rat cortex.

The results show that guinea-pig kidney contains high affinity binding sites for [^{3}H]-bradykinin. This is in keeping with the multiplicity of roles of bradykinin in this tissue.

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2P

DOPAMINE D_2 RECEPTOR MODULATION BY BRAIN NON-HAEM IRON.

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In the brain iron is unevenly distributed with highest concentration being present in globus pallidus, substantia nigra, putament and caudate nucleus (Youdim, 1985). Iron-deficiency (ID) in rats causes a selective subsensitivity of dopamine D_2 receptor as indicated by the reduced ^{3}H-spiperone binding Bmax in the caudate nucleus and diminished apomorphine induced behaviours (Ashkenazi et al., 1982). Furthermore, iron chelators o-phenanthroline and desferrioxamine inhibit the binding of ^{3}H-spiperone (Ben-Shachar et al., 1985). In the present study we have systematically examined the effect of ID on serum iron, brain non-haem iron, ^{3}H-spiperone binding in caudate nucleus and behavioural response to apomorphine as well as the influence of iron-supplementation in iron-deficient rats.

Male Sprague-Dawley rats (21 days old) were fed an iron-deficient diet for 4 weeks (Ben-Shachar et al., 1985). Determination of the above parameters at weekly intervals showed that by the fourth week there were reductions in serum iron (78%, $P<0.001$), brain non-haem iron (37%, $P<0.01$), ^{3}H-spiperone binding (41%, $P<0.01$) and apomorphine (2mg/kg) behavioural response (52%, $P<0.001$). These could all be reversed by supplementation of the iron-deficient diet with ferrous sulphate (200 ppm) for up to 2 weeks. Since the effect of ID on dopamine D_2 receptor cannot be reversed in vitro by the addition of ferric or ferrous sulphate (10^{-3}M), the results indicated the possible involvement of iron in DA D_2 receptor protein synthesis. In vivo incorporation of ^{14}C-valine in caudate nucleus of control (299±22 cpm/mg protein/hr, n=5) and iron-deficient (260±15 cpm/mg protein/hr, n=5) rats showed a non-significant reduction of protein synthesis. However, isoelectric focusing and two dimensional sodium dodecyl sulphate (SDS)-gel electrophoresis (Heydorn et al., 1983) of caudate nucleus and n-accumbens protein from iron-deficient rats indicated a significant (27%, $P<0.005$) reduction of a protein with molecular weight of 94K, which was restored to normal values in iron-supplemented animals. This value is identical to the molecular weight of purified dopamine D_2 receptor subunit as separated by SDS-gel electrophoresis (Amlaiky and Caron, 1985). The lack of alteration in microviscosity (fluidity, $\bar{\eta}$) of caudate nucleus membranes, prepared from iron-deficient (2.48±0.21, n=6) as compared to control (2.53±6.17, n=6) animals, would suggest that ID is not simply changing the microenvironment of the membrane bound receptor.

The data, however, could point to the involvement of iron in either receptor synthesis or incorporation of newly synthesized receptor at the postsynaptic site. The latter possibility was negated since subcellular fractionation of dopamine D_2 receptor in caudate nucleus from iron-deficient and control rats showed the same pattern of distribution, indicating that ID does not cause a loss of membrane bound receptor to the soluble (100,000xg supernatant) fraction.

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KAPPA OPIOID RECEPTORS LABELLED WITH PEPTIDE AND NONPEPTIDE RADIOLIGANDS - QUANTITATIVE AUTORADIOGRAPHIC STUDIES

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Kappa receptors represent one of the sub-types of opioid receptors known to exist in the mammalian central nervous system. Radioligand binding studies conducted on homogenates of dissected tissues have been used previously to ascertain the general regional distribution of kappa receptors with autoradiographic studies supporting and extending these observations. Although autoradio-graphic studies have provided more detailed information on receptor distribution they have been qualitative in nature and have relied primarily on the use of the synthetic nonpeptide ligands [^{3}H]bremazocine and [^{3}H]EKC. Since dynorphins are thought to be the endogenous peptides interacting with kappa receptors we have compared the properties and the brain regional localization of kappa receptors using [^{125}I]dynorphin (1-8) ([^{125}I]DYN) and [^{3}H]etorphine using quantitative autoradiographic (QARG) procedures (see Clark and Hall, 1986; Sharif et al. 1986 for details).

Under conditions of mu and delta receptor blockade and in the presence of protease inhibitors (Gillan et al. 1985) [^{125}I]DYN bound with high nanomolar affinity to guinea pig cerebellar membranes. Bound [^{125}I]DYN was readily displaced by unlabelled dynorphin (1-8), U50488 and etorphine (K_is = 2nM) but not by the delta-selective ligand, D-Pen-D-Pen-enkephalin.

QARG supported the homogenate-based data since U50488, a kappaselective drug, competed well for [^{125}I]DYN binding to kappa receptors in guinea pig cerebral cortex (layers V-VI), hippocampus and s.nigra, regions known to be enriched in kappa receptors (Table 1). Digital subtraction autoradiography also revealed an uneven pattern of localization of kappa receptors labelled with 0.2 nM [^{125}I]DYN and significant species differences also became apparent (Table 1). It is evident that the density of kappa receptors in the guinea pig cortex (layers V-VI), striatum, n.accumbens, s.nigra and the molecular layer of cerebellum is approx. 2-6 times greater than in the rat, while the MPOA of the rat apparently has approx. 18-times the concentration of kappa receptors than the equivalent region in the guinea pig.

In conclusion, our data indicate that [^{125}I]dynorphin (1-8) is a useful probe for localizing kappa receptors and that high resolution QARG can be employed to quantify and visualize these receptors in discrete regions and nuclei in the brain.

Table 1 Kappa Receptor Binding (fmol x 10^{-3}/mm^2)

Brain Region	Guinea Pig	Rat
C. Cortex (Layers I-II)	1.4 ± 0.24	0.8 ± 0.05
C. Cortex (Layers V-VI)	4.6 ± 0.34	2.0 ± 0.11
Striatum	3.6 ± 0.15	0.9 ± 0.10
N. accumbens	4.2 ± 0.23	2.3 ± 0.26
Medial preoptic Area (MPOA)	0.2 ± 0.05	3.6 ± 0.25
S. Nigra	2.9 ± 0.25	1.5 ± 0.16
Cerebellum (Molecular Layer)	2.2 ± 0.22	0.37 ± 0.05
Cerebellum (Granular Layer)	1.0 ± 0.08	0.27 ± 0.03

(means ± SEM of 4-6 determinations from 2 animals of each species; 0.2nM [^{125}I]DYN)

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4P

QUANTITATIVE AUTORADIOGRAPHIC LOCALIZATION OF CHOLECYSTOKININ RECEPTORS IN DISCRETE NUCLEI OF THE RAT HYPOTHALAMUS.

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The peptide cholecystokinin (CCK) was originally isolated from extracts of gut, but has since been found in high concentrations in a number of brain regions, including the hypothalamus - a structure critically involved in several important homeostatic processes, such as the regulation of food intake and neuro-endocrine function. The present study was undertaken to investigate the localization of CCK receptors within discrete nuclei of the hypothalamus by autoradiographic techniques and subsequently to elucidate a functional role for these receptors.

Sections of frozen rat brain (10um thickness) were cut on a cryostat microtome (-20°C), thaw-mounted onto gelatinized slides and incubated for 2h in HEPES buffer (10mM; pH 7.2; 25°C) containing [125I]Bolton Hunter-labelled CCK8 ([125I]CCK8; 0.25nM). After washing and drying, sections were apposed to Ultrofilm for 7-14 days followed by development in Kodak D19 (3 min). Non-specific binding was defined as that which remained when adjacent sections were co-incubated with [125I]CCK8 in the presence of excess unlabelled CCK8 (1uM). The resulting autoradiographic film was used as a negative to produce black and white images showing receptor distribution or analysed using a computer-assisted image analysis system to quantify receptor density.

CCK receptor binding sites were discretely localized in several hypothalamic nuclei, with highest concentrations found in the ventromedial nucleus (VMN), followed by the compact zone of the dorsomedial nucleus (CDMN) > supra-optic nucleus (SON) > paraventricular nucleus (PVN). Detailed distribution studies throughout the VMN indicated a heterogeneous localization of CCK receptor binding sites, with [125I]CCK8 localized in a "halo" formation within the nucleus. The presence of CCK receptors in the VMN is entirely consistent with the proposed role of CCK within the VMN in the regulation of food intake.

In order to investigate the functional role of receptors located in the PVN and SON, CCK receptor density was measured in conditions where the hypothalamic-pituitary axis was stimulated. Thus CCK receptor binding site density was found to be greatly elevated in the PVN and SON of both salt-loaded (11 days) and Brattleboro rats compared to controls. Furthermore, this increase in receptor density appeared to be localized only to the magnocellular (and not parvocellular) divisions of the PVN, a finding consistent with stimulation of magnocellular neurones in both salt-loaded and Brattleboro rats (Marley et al., 1984). In conclusion, the high density of CCK receptors in discrete nuclei of the hypothalamus, which can be up-regulated under certain pharmacological conditions, strongly supports a functional role for CCK in homeostatic mechanisms.

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INHIBITION OF GLUTAMATE RELEASE IN CULTURED NEURONES BY ADENOSINE MAY NOT INVOLVE ENHANCEMENT OF PRESYNAPTIC POTASSIUM CURRENTS

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The inhibition of synaptic activity by adenosine is thought to involve activation of A_1 adenosine receptors (Reddington et al, 1982). The release of many transmitters is inhibited by adenosine and its analogues; this includes the release of glutamate from brain slices (Dolphin and Archer, 1983) and from cerebellar granule cell cultures (Dolphin and Prestwich, 1985). We have also shown that endogenous adenosine inhibits glutamate release from these cultures (Dolphin et al, 1986a). Adenosine analogues have been shown to inhibit voltage activated calcium currents in rat dorsal root ganglion neurones (Dolphin et al 1986b), and to activate K^+ currents in several neuronal cell types (eg Haas and Greene, 1984). It is therefore unclear whether the mechanism of inhibition of transmitter release by adenosine involves inhibition of calcium currents or activation of potassium currents in the presynaptic terminal, or possibly another intracellular mechanism.

We have investigated the inhibition of K^+-stimulated release of newly synthesised [^{3}H]-glutamate by 2-chloroadenosine (2-CA, 2μM) and by the adenosine uptake blocker dipyridamole (5 and 10μM). A comparison was made using two concentrations of K^+ (50 and 130mM) for depolarization.

Rat cerebellar neurones were grown on glass coverslips, prelabelled with [^{3}H] glutamine, and perfused with artificial c.s.f. as previously described (Dolphin and Prestwich, 1985). Two 2 min periods of stimulated release (S_1,S_2 were included, using the indicated concentrations of K^+. On each day a pair of experiments was performed, a control experiment in the absence of drugs to give a control S_2/S_1 ratio, and an experiment in which either 2-CA or dipyridamole was present during the second stimulation period S_2 to examine the effect of the drug. The % change in S_2/S_1 ratio was determined for each pair of experiments.

The ability of 2-CA and dipyridamole to inhibit glutamate release was similar at the two depolarizing concentrations of K^+ (Table 1). This result suggests that an increase in a presynaptic K^+ conductance is not responsible for presynaptic inhibition by 2-CA or endogenous adenosine.

Table 1

Concentration of K^+	% of control S_2/S_1 ratio in the presence of:	
	2-CA (2μM)	dipyridamole (10μM)
50mM	62.2±10.8 (7)	75.5± 4.0 (8)
130mM	62.9± 8.8 (5)	69.8±10.1 (7)

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6P

EFFECTS OF ANTICONVULSANTS ON NMDA RECEPTOR-MEDIATED EPILEPTIFORM ACTIVITY IN RAT CORTICAL SLICES SUPERFUSED WITH Mg^{2+}-FREE MEDIUM

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In several animal models of epilepsy, synaptic excitation mediated via N-methylaspartate (NMA) receptors appears to play a key role in the genesis of epileptiform activity (Schwarcz & Meldrum, 1985). For example, convulsant activity induced in a cortical slice preparation by various ionic and pharmacological manipulations can be blocked by competitive, eg. 2-amino-5-phosphonovalerate (2-AP5), and non-competitive, eg. ketamine, antagonists of NMA (Aram & Lodge, 1986). Removal from the superfusing medium of magnesium ions, which relieves a voltage-dependent block of the ion channel linked to the NMA receptor, produces spontaneous and evoked bursts of potentials. This preparation allows us to examine the action of clinically relevant anticonvulsants on epileptiform activity due to excessive NMA receptor-mediated depolarisation.

500uM slices of rat cerebral cortex were maintained at the interface of an artificial C.S.F. and humidified 5% CO_2 in O_2 (Thomson et al. 1985). A tungsten bipolar stimulating electrode was positioned in the corpus callosum. Extracellular glass microelectrodes were placed in layer II/III of the cingulate cortex to record spontaneous and evoked field potentials. Following perfusion with a magnesium-free medium, large negative-going potentials appeared spontaneously with up to 20 afterpotentials superimposed on the decay phase. Callosal stimulation evoked almost indistinguishable burst potentials. 2-AP5 (5-20μM), ketamine (5-20μM) and magnesium (100-400μM) reversibly blocked such epileptiform activity, reducing both the frequency of spontaneous bursts and the number of after potentials per burst.

Sodium valproate (0.5-4mM) and baclofen (1-50μM) reduced both the frequency of the bursts and the number of afterpotentials, whereas phenytoin (50-100 μM) and carbamazepine (10-50μM) reduced the number of afterpotentials with no reduction of the frequency of bursts. Similar concentrations of these four drugs also reduced epileptiform activity induced by 4-aminopyridine (50μM). Of the important antiepileptics, the concentrations of valproate and phenytoin are clearly higher than therapeutic levels but those of carbamazepine are in the clinical range.

Because the profile of action of carbamazepine was quite different to that of 2-AP5, ketamine and magnesium, it seems unlikely that this potent anticonvulsant shares the same mechanism of action. To test this view, we have used the cortical wedge preparation (Harrison & Simmonds, 1984) to examine the effects of carbamazepine on depolarisations evoked by agonists selective for the three glutamate receptor subtypes. In six slices superfused with carbamazepine 50μM, there was no significant reduction of depolarisation produced by NMA, quisqualate or kainate. This indicates that postsynaptic effects at glutamatergic synapses are unlikely to explain the anticonvulsant action of carbamazepine but other effects at such synapses, eg. a reduction in the release of transmitter, cannot be excluded. In conclusion, this cortical slice model can be used to compare different types of anticonvulsant. With further studies it should be possible to correlate the profile of a drug's effect on epileptiform activity with its effect on particular receptors or other membrane properties.

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MK-801 PREVENTS DEGENERATION OF STRIATAL NEURONES CAUSED BY INTRASTRIATAL INJECTION OF QUINOLINIC ACID

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Quinolinate (QUIN; pyridine-2,3-dicarboxylate) is an endogenous excitotoxin which causes neuronal degeneration by activating the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors (Schwarcz et al, 1984). Recently, Beal et al (1986) have suggested that injection of QUIN into the rat striatum produces a pattern of neuronal degeneration which closely mimics that observed in the basal ganglia of Huntington's disease victims. Results from this laboratory have shown that MK-801 is a potent, selective and non-competitive antagonist of NMDA receptors (Kemp et al, 1986), and when systemically-applied prevents NMDA-induced neurotoxicity in the rat hippocampus and striatum (Foster et al, 1986). In this study, we have evaluated the ability of systemically-administered MK-801 to protect against QUIN-induced neurotoxicity in the rat striatum.

Male Sprague-Dawley rats were given a sterotaxic injection of QUIN (in 1µl, pH7.4) into the right striatum under equithesin anaesthesia. Animals receiving MK-801 were injected i.p. 1 hour prior to QUIN injection. For histological analysis, animals were perfused transcardially with fixative 7 days later, and microtome sections stained with cresyl violet. For enzyme measurements, animals were killed at 7 days, their striata dissected and assayed for choline acetyltransferase (CAT) or glutamate decarboxylase (GAD) activity.

Unilateral intrastriatal injection of 120nmol QUIN in control animals (n=4) resulted in the degeneration of striatal neurons for several mm around the injection site; at 300nmol QUIN (n=4) the area of degeneration was more extensive. Pretreatment of rats with 10mg/kg MK-801 ip, 1 hour prior to QUIN injection, completely prevented striatal neuronal loss at both doses of QUIN (n=4 in each group). As shown in Table 1, pretreatment with MK-801 at a dose of 10mg/kg (i.p.) caused almost total protection of the QUIN-induced reductions of striatal CAT and GAD activity.

	120nmol QUIN				300nmol QUIN			
	Control	(n)	10mg/kg MK-801	(n)	Control	(n)	10mg/kg MK-801	(n)
GAD	54.6 ± 4.4	(4)	0.9 ± 12.5**	(4)	91.5 ± 1.2	(4)	4.0 ± 10.2**	(4)
CAT	18.1 ± 3.7	(5)	3.7 ± 2.6*	(5)	87.3 ± 7.3	(5)	4.9 ± 2.8**	(5)

Values are % reduction of enzyme activity in the QUIN-injected striatum compared to the uninjected contralateral side and are means ± SEM of n animals
* $P < 0.05$; ** $P < 0.01$

These data indicate that MK-801 can protect against neuronal degeneration in this animal model of Huntington's disease.

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8P

SODIUM AND CALCIUM DEPENDENCY OF N-METHYL-D-ASPARTATE INDUCED INCREASES IN CYCLIC GMP LEVELS IN IMMATURE RAT CEREBELLAR SLICES

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N-methyl-D-aspartate (NMDA) in the presence of magnesium produces a voltage dependent conductance increase to which both sodium and calcium ions contribute (Flatman et al, 1986; MacDermott et al, 1986), but whether calcium entry is a direct consequence of NMDA receptor activation or secondary to the depolarisation produced by NMDA is not clear. In immature rat cerebellar slices NMDA induces an increase in cyclic GMP (cGMP) levels (Garthwaite, 1982). As the effect is calcium dependent, this model provides a convenient neurochemical means of assessing the relevance of calcium to the actions of NMDA.

Cerebellar slices (400x400µm) from 8 day old rats (IFFA Credo, France) were preincubated for two hours at 37°C in oxygenated (95% O_2, 5% CO_2) Krebs buffer (mM; NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25; KH_2PO_4, 1.18; $MgSO_4$, 1.19; Glucose, 11) and aliquots (≃200µg protein) then incubated at 37°C in 500µl of this buffer containing test compounds. Antagonists were added 5 min before NMDA and the reaction stopped 5 min later by heating. After homogenisation and centrifugation cGMP was measured in the supernatant by radioimmunoassay (Amersham).

NMDA (20-160µM) produced a dose dependent increase in cGMP levels. The effects of NMDA were enhanced by omission of Mg^{++}. The effects of 80µM NMDA were antagonised by (IC_{50}, µM) 2-amino-5-phosphonovalerate (12) and D-alpha-amino-adipate (100) but not by 640µM glutamic acid diethyl ester or γ-D-glutamylamino methyl sulphonic acid. The effects of NMDA were abolished by omission of calcium from the medium and proportional to the concentration of calcium from 0.2 to 20mM. NMDA responses were antagonised by inorganic calcium antagonists including (IC_{50}, mM) Ni^{++}, 0.025; Co^{++}, 1.5; Cd^{++}, 1.5; La^{+++}, 4.5; Mn^{++}, 7), but not by 10 mM Ba^{++} or Sr^{++}. Responses to NMDA were unchanged when Ba^{++} or Sr^{++} were substituted for Ca^{++} in the medium. The effects of NMDA were blocked by organic calcium antagonists (IC_{50}, µM), flunarizine, 64; pimozide, 100; diltiazem, 240; verapamil, 450. Nifedipine (10µM) partially antagonised the effects of 20-160µM NMDA reducing its maximal effects. Tetrodotoxin (1µM) had no effect, but omission of sodium totally abolished the response to NMDA, which was restored by intermediate sodium concentrations. Zn^{++}, a tetrodotoxin resistant sodium channel blocker (Frelin et al., 1986) also antagonised the effects of NMDA (IC_{50} 0.1 mM).

The results show the dependence of the NMDA response on extracellular calcium. The continued effect in the presence of Ba^{++} or Sr^{++}, which pass through voltage dependent calcium channels clearly shows that calcium channel opening is a consequence of NMDA receptor activation. The opening of calcium channels would appear to be secondary to sodium entry, as removal of sodium abolished the effects of NMDA. This suggests that calcium entry is a consequence of NMDA induced depolarisation, rather than direct coupling to the receptor. This depolarisation may involve sodium entry through tetrodotoxin-resistant sodium channels.

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SYSTEMIC ADMINSTRATION OF MK-801 PROTECTS AGAINST ISCHAEMIA-INDUCED HIPPOCAMPAL NEURODEGENERATION IN THE GERBIL

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There is evidence that N-methyl-D-aspartate (NMDA) receptors may mediate the neuronal degeneration which follows cerebral ischaemia (Simon et al, 1984). MK-801 has recently been shown to be a potent and selective antagonist of NMDA receptors (Kemp et al, 1986). We have examined the effectiveness of peripherally administered MK-801 to protect against neuronal degeneration produced by complete forebrain ischaemia in gerbils.

Female mongolian gerbils (60-70g) were anaesthetised with a mixture of 2% halothane, 70% N_2O, 30% O_2. Cerebral ischaemia was induced by occlusion of the common carotid arteries for 5 min. MK-801 was administered either 1 hour prior to the ischaemic insult (0.03-10mg/kg i.p.) or at a dose of 10mg/kg i.p. at various times after the ischaemic episode. Four days later the animals were perfused with fixative. Measurements of neuronal damage were made from both hippocampi in cresyl violet stained coronal sections (40μm) corresponding to 1.5, 1.7 and 1.9mm caudal to bregma. The area was summed for the three sections giving the total area of degeneration in 6 hippocampal planes.

Table: Effect of MK-801 on the area of ischaemia-induced hippocampal degeneration

	Dose of MK-801, 1 hour pre-ischaemia (mg/kg)						
	Control	0.03	0.1	0.3	1	3	10
Area (mm^2)	7.3	5.3	4.9**	4.9*	1.5*	0.9*	0.8*
Mean ± SEM	±0.32	±1.00	±0.89	±0.78	±0.58	±0.51	±0.45
(n)	(12)	(10)	(10)	(10)	(10)	(10)	(10)

	MK-801 (10mg/kg) given post-ischaemia at (h)			
	Control	0.5	15	24
Area (mm^2)	7.32	1.28*	4.20*	3.15*
Mean ± SEM	±0.34	±0.54	±0.82	±0.76
(n)	(12)	(10)	(9)	(10)

** $P < 0.05$; * $P < 0.01$ (compared to controls; Mann Whitney U-test)

In all untreated controls, degeneration of hippocampal CA1 and CA2 area pyramidal neurones was seen. Pretreatment with MK-801 produced a dose-dependent decrease in the area of neuronal degeneration (Kruskal-Wallis ANOVA H=38.10; $P < 0.0001$). At higher doses the majority of animals were completely protected. MK-801 also protected against ischaemic neuronal damage when given up to 24 hours following the insult (Kruskal-Wallis ANOVA H=27.81; $P < 0.0001$).

These results further indicate that NMDA receptors play an important role in ischaemia-induced neuronal degeneration. The ability of peripherally administered MK-801 to prevent neuronal damage in gerbils when given 24 hours after the ischaemic episode, suggests a potential use of this compound as a neuroprotective agent in the treatment of human ischaemic neuropathologies such as stroke.

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10P

STUDIES ON THE USE-DEPENDENCY OF PHENCYCLIDINE-LIKE COMPOUNDS AS N-METHYLASPARTATE ANTAGONISTS *IN VIVO* AND *IN VITRO*.

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It is clear from pharmacological studies *in vitro* that the antagonism of N-methylaspartate (NMA) by phencyclidine (PCP)- and sigma opiate- like compounds is not competitive (Harrison & Simmonds, 1984; Lodge & Johnston, 1985; Martin & Lodge, 1985; Johnson & Snell, 1985). In initial studies on spinal neurones *in vivo* we found no evidence to support the idea that ketamine only exerted its action when the receptor-ionophore complex was in the activated state. Recently Kemp et al. (1986) have shown that MK801, a dibenzocycloalkenimine which shares some properties with PCP, produces a use-dependent block of responses to NMA.

We have therefore examined the action of ketamine and MK801 on responses of spinal neurones in pentobarbitone-anaesthetised rats to the electrophoretic ejection of excitatory amino acids. The time course of NMA antagonism by ketamine (4 cells) and MK801 (6 cells) was found to be the same whether frequent (every 1 or 2 min) or infrequent (every 10 min) ejections of NMA were used. Because recovery time from MK801 considerably outlasts that of 2-amino-5-phosphonovalerate (2-AP5), it was possible by administering MK801 during a supramaximal dose of 2-AP5 to see if MK801 would block the inactive state of receptor-ionophore complex. On all 6 cells the degree of block at a time when antagonism by 2-AP5 had recovered was the same as during control administrations of MK801. Various other protocols of administration have been tried. All our results support the view that NMA antagonism by PCP-like drugs *in vivo* is not use-dependent.

Like Kemp et al. (1986) we find that MK801 produces a use-dependent antagonism of NMA *in vitro* on cortical wedges (Harrison & Simmonds, 1984) but can find no clear evidence of this with either phencyclidine or ketamine. In view of the possibility that magnesium (Nowak et al. 1985) and PCP-like drugs may act in or near the NMA-activated ion channel and that the above observations were obtained from preparations maintained in magnesium-free medium, we have made a preliminary investigation of the action of MK801 in medium containing 1mM magnesium ions. Despite the fact that magnesium might be expected to facilitate the action of MK801 (see Harrison & Simmonds, 1984; Martin & Lodge, 1985), use-dependency could still be demonstrated in the presence of magnesium.

The differences in the use-dependency of MK801 between *in vivo* and *in vitro* preparations do not appear to be explained by differences in magnesium concentration nor by suggesting that MK801 acts *in vitro* at a site other than the PCP/sigma receptor :combination studies of ketamine and MK801 produce an additive effect on NMA dose ratios (Martin, unpublished observations). Other explanations such as those due to differences between cortical and spinal neurones, or to the low temperature (22^{0}C) of the cortical slices, or to the presence of endogenous PCP/sigma receptor ligands *in vivo* cannot presently be discounted.

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ADENOSINE INHIBITS NMA RECEPTOR MEDIATED EPILEPTIFORM ACTIVITY IN RAT CORTICAL SLICES

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It has been suggested that endogenous chemical substances such as adenosine, released during a seizure attack, may act as anticonvulsants in vivo. We have investigated the ability of purinoceptor agonists, and the adenosine antagonist, theophylline, to influence the appearance of spontaneous paroxysmal events in slices of rat cerebral cortex on removal of magnesium from the superfusion medium.

Grease seal techniques were used for recording responses of rat cortical slices (Harrison & Simmonds, 1984). Wedges (500 μm) comprising cerebral cortex and corpus callosum were prepared and perfused with magnesium-free oxygenated Krebs solution until stable responses were obtained. Under these conditions, large negative going potentials appeared spontaneously with up to 20 afterpotentials superimposed on the decay phase.

Bath application of adenosine receptor agonists for 20 min reduced both the frequency of spontaneous bursts and the number of afterpotentials recorded in a 5 min period. ED50s estimated from data normalised as % control activity were: adenosine 25 μM, 2-chloroadenosine 4.5 μM, L-phenylisopropyladenosine 0.22 μM.

316μM theophylline applied for 15 min increased both the frequency of bursts and number of afterpotentials recorded by 20 - 30 %. Effects of exogenously applied adenosine (100 μM) were also antagonised by this concentration of theophylline.

These results are consistent with the idea that activation of adenosine receptors suppresses epileptiform activity in the rat cortex. Ault & Wang (1986) have reported similar findings in hippocampal slices. The relative potencies of the agonists tested and the antagonism of adenosine by theophylline would suggest that such effects are mediated through the A1 adenosine receptor (Londos et al., 1980).

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12P

LOSS OF ADENOSINE UPTAKE RECOGNITION SITES: A CONSEQUENCE OF CEREBRAL ISCHAEMIA

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Adenosine has multiple effects on nervous tissue. It decreases or increases the level of cyclic AMP through A-1 or A-2 receptors coupled to adenylate cyclase (Williams, 1984) and inhibits the release of many different neurotransmitters (Harms et al, 1979). A high affinity uptake system for adenosine exists in brain (Bender et al, 1980) which presumably regulates the extracellular level of adenosine. Because the inhibition of synaptic neurotransmission following ischaemia may be due to increased release of adenosine (Lipton & Robacker, 1982) we have examined the effect of cerebral ischaemia on the adenosine uptake recognition site in the hippocampus of the mongolian gerbil, Meriones unguiculatus.

Twenty seven male gerbils (60-80g) were anaesthetised with 6mg pentobarbital: the left and right common carotid arteries were exposed in the paratracheal region and similtaneously occluded with microvascular clamps. Immediately following a 10 min occlusion, or 72h after, animals were decapitated and the hippocampus dissected and stored under liquid nitrogen. Binding of [^{3}H]-nitrobenzylthioinosine ([^{3}H]-NBI) to hippocampus membranes from three pooled samples was carried out as described by Marangos(1984). Non-specific binding was defined using 10µM dipyridamole. Each value represents the mean ± s.e. mean of three separate determinations.

The binding of [^{3}H]-NBI to gerbil hippocampus was saturable and characteristic of the adenosine uptake recognition site, having a high affinity for the selective adenosine uptake antagonists, NBI (Ki 0.56nmol.litre^{-1}), nitrobenzylthioguanosine (ki 2nmol.litre^{-1}) modest affinity for dipyridamole (ki 323nmol.litre^{-1}) and low affinity for 2-chloroadenosine (ki <5.00), caffeine (ki <5.00) and theophylline (Ki <5.00). Iterative non-linear analysis of binding isotherms from saturation studies demonstrated a single class of high affinity sites with a kd of 88 ± 20pmol.litre^{-1} and Bmax of 160 ± 31fmol.mg protein^{-1}. Global ischaemia for 10 min in the absence of reperfusion had no effect upon the number (135 ± 41 fmol.mg protein^{-1}) or affinity (85 ± 8 pmol.litre^{-1}) of adenosine uptake recognition sites. In contrast, 72h after global ischaemia a significant decrease ($P<0.05$) in the number of adenosine uptake recognition sites to 95 ± 15 fmol.mg protein^{-1} was observed in the hippocampus with no apparent change in affinity (71 ± 20.pmol.litre^{-1}).

Loss of adenosine uptake recognition sites coupled to possible reduction in the maximum reuptake capacity (Vmax)of the adenosine symport may through alterations in the level of adenosine be responsible in part for the pronounced effects that ischaemia has on synaptic neurotransmission. Alternatively, the decrease in adenosine uptake recognition sites, may simply reflect the neuronal cell death that is occurring in the hippocampus.

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MEDIAL THALAMIC NEURONES DO NOT APPEAR TO USE ASPARTATE OR GLUTAMATE FOR NEUROTRANSMISSION

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The anatomical connections of most thalamic nuclei are relatively well-defined yet little is known concerning their transmitter chemistry. We have been attempting to clarify this issue for those medial thalamic nuclei which separately innervate the caudate-putamen and deep parietal cortex (from parafascicular (PF) and centro-lateral thalamus, CL), the medial prefrontal cortex (from mediodorsal thalamus, MD) and the nucleus accumbens (from periventricular thalamus, PV). Although these neurones appear to be excitatory (e.g. Wilson et al, 1983), our previous studies suggest that they are unlikely to be cholinergic (Barrington-Ward et al, 1984; Kilpatrick & Phillipson, 1986). Our preliminary data also argue against S(+)-aspartate (ASP) or S(+)-glutamate (GLU) as transmitters in thalamo-caudate neurones (Kilpatrick & Stancliffe, 1985). The present study confirms and extends these findings to the above thalamic neurones.

Under halothane anaesthesia (1.5% in O_2) and full asepsis, male Porton rats (180-220g) received bilateral stereotaxic infusions of either the excitotoxin, sodium quinolinate (10 nmol in 0.2 µl) or vehicle into PF and CL (6 sites), MD (4 sites) or PV (4 sites). Four days later, animals were killed by cervical fracture and thalamic terminal regions microdissected. Tissues were individually assayed for both their ASP and GLU content by a reversed phase HPLC method and the high-affinity uptake of [^{3}H]R(-)-ASP (5×10^{-8}M) into P_2 synaptosomal preparations. An assay control was provided by a bilateral lesion of frontal and parietal cortices (CTX) with later analyses of caudate-putamen. Each lesion/injection site was examined histologically.

Table 1 indicates that whilst a CTX lesion could evoke a large decrease in R(-)-ASP uptake in caudate-putamen, none of the histologically-verified thalamic lesions caused a significant change in this parameter in their respective target areas. Further, CTX lesions reduced caudate-putamen ASP and GLU contents by -40% and -12%, respectively, yet these amino acids were unchanged in areas deprived of their thalamic input (not shown).

Table 1. [^{3}H]R(-)-ASP uptake after thalamic lesions (nmol/10 min/mg protein)

	CTX		PF/CL		MD		PV	
BRAIN AREA	Sham	Lesion	Sham	Lesion	Sham	Lesion	Sham	Lesion
Caudate	6.1±0.2	(-45%,*)	6.8±0.6	(+11%)	-		-	
Prefrontal cortex	-		-		13.8±1.6	(-12%)	-	
Nucleus accumbens	-		-		-		4.9±0.5	(-8%)
Parietal cortex	-		6.4±0.4	(+7%)	-		-	

Each result is the mean ± S.E.M. of 4-6 experiments. * $p < 0.01$.

Taken together, the data imply that these groups of medial thalamic neurones do not use either ASP or GLU as neurotransmitters and agrees with the relative lack of GLU-like immunoreactivity displayed by thalamic perikarya (Ottersen & Storm-Mathisen, 1984). The search continues.

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14P

THE ACTION OF BAYK8644 ON CALCIUM CHANNEL CURRENTS MODIFIED BY GTP-γ-S

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The possibility that Ca^{2+} channels are associated with nucleotide binding proteins has been studied; we have recently shown that intracellular application of guanosine 5'-O-3-thiotriphosphate (GTP-γ-S) alters the activation, inactivation and amplitude of voltage-dependent inward calcium currents (I_{Ca}) recorded from cultured rat dorsal root ganglion neurones (Scott and Dolphin, 1986). In the present study we have investigated the action of the dihydropyridine Ca^{2+} channel agonist BayK8644, to determine whether GTP-γ-S modifies the ability of BayK8644 to enhance whole cell calcium currents.

In the presence of TTX (2.5μM), TEA (25mM), $BaCl_2$ (2.5mM) and no $CaCl_2$ in the recording medium and using patch solution containing CsCl (140mM), control DRG neurones had an input resistance of 154±53 MΩ (n=7) compared with 151±31 MΩ (n=5) when GTP-γ-S (500μM) was included in the patch solution. Under dark conditions BayK8644 (5μM) was applied by low pressure ejection and induced an increase in conductance of 7.6±3nS (n=7) and 2.7±2.3nS (n=5) for control and GTP-γ-S containing cells respectively.

From a holding potential (V_H) of -80mV, high threshold I_{Ca} was activated at around +10mV and low threshold I_{Ca} at -30mV. Table 1 shows the mean±S.E. for inward calcium currents before and during application of BayK8644.

Table 1

	Control (n=10)		GTP-γ-S (n=10)	
	High threshold Current nA	Low threshold Current nA	High threshold Current nA	Low threshold Current nA
Before BayK8644 application	3.75±0.38	0.17±0.07	1.20±0.21	0.11±0.05
During BayK8644 application	**4.67±0.44	*0.54±0.20	*2.24±0.50	**0.46±0.11

*p<0.05, **p<0.01

The amplitude of both high and low threshold currents was increased by BayK8644 in control cells and those containing GTP-γ-S.

The action of BayK8644 was reversed by light, after 3 minutes illumination in the presence of BayK8644, both high and low threshold currents were reduced by between 20 and 50%.

These data suggest that in this system, BayK8644 can interact with Ca^{2+} channels underlying high and low threshold currents and that this is not prevented by the presence of intracellular GTP-γ-S.

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EXCITATORY EFFECT OF BRADYKININ ON SPINAL NEURONES

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The probable role of kinins in the genesis of slow pain has provided a stimulus for development of bradykinin antagonists (Vavrek & Stewart, 1985). However there is to our knowledge no convenient assay system for testing the effects of such antagonists on nervous tissue. Therefore we have examined the effect of bradykinin on the in vitro hemisected spinal cord of the immature (1 to 5 day old) rat. Two minute applications of bradykinin (1μM) produced depolarization (2.7 mV ± 0.8 s.e. of mean, n=4) of motoneurones recorded from ventral roots (L5). Measurement of the dose-dependence of this effect was made difficult due to desensitization. This conditioning effect of bradykinin persisted for up to 150 min following an initial application of 1μM bradykinin. The depolarizing action of bradykinin was abolished in the presence of tetrodotoxin, at a concentration sufficient to block regenerative activity, indicating that it was transmitted indirectly to motoneurones.

In the presence of the excitatory amino acid antagonists 2-amino-5-phosphono-pentanoate (100μM; 2 preparations) and kynurenate (2mM; 3 preparations) bradykinin-induced depolarizations were attenuated to less than 50% of control responses obtained after the washout of antagonists. Such attenuation of the effect of bradykinin did not occur in a preparation treated with a mixture of cholinergic antagonists (hexamethonium 250μM; atropine 1μM). This suggests that the bradykinin-induced depolarization of motoneurones was mediated, partially at least, by the release of endogenous amino acids. This indirect activation of motoneurones may have been due to transmitter released through bradykinin-induced depolarization of primary afferent terminals since isolated dorsal roots were found to be depolarized (0.32mV ± 0.12 s.e. of mean, n=5) by bradykinin (1μM). Similar size depolarizations were induced by bradykinin (1μM) in dorsal roots attached to two spinal cord preparations in the presence of tetrodotoxin. The depolarizing action of bradykinin on dorsal root fibres was subject to desensitization with a similar time-course to that observed in ventral roots.

It was of interest to compare the action of bradykinin on the superior cervical ganglion of the mature rat since the peptide has been reported to have a depolarizing action on this preparation (Watson, 1970). Ganglia were less sensitive to bradykinin than spinal cord preparations. Bradykinin (10μM) produced a mean depolarization of 0.28mV ± 0.06 s.e. of mean (n=3). The peptide (1-10μM) depolarized the postganglionic and not the preganglionic nerve. Unlike the spinal cord preparation doses of bradykinin could be applied to ganglia at 30 min intervals without producing significant desensitization.

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EFFECTS OF AMINE OXIDASE INHIBITORS UPON TRYPTAMINE METABOLISM AND TRYPTAMINE-INDUCED CONTRACTIONS OF RAT AORTA

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The enhanced potency of tryptamine (TR) in contracting the rabbit aorta after pretreating the tissue with iproniazid (IPN), may be due to inhibition of monoamine oxidase (MAO) (Stollak & Furchgott, 1983). Smooth muscle of the rat aorta contains a semicarbazide-sensitive amine oxidase (SSAO) in addition to MAO (Lyles & Singh 1985), and both enzymes are inhibited by IPN and certain other hydrazines (Lyles 1984). Here we have studied the effects of MAO and SSAO inhibitors upon TR metabolism and TR-induced contraction in rat aorta.

Deamination of [^{3}H]-TR was studied in homogenates of aortae from male rats (300-500g). Metabolism of TR (20μM-1mM) by SSAO was estimated in homogenates preincubated with 10^{-3}M clorgyline to inhibit MAO activities completely. Resulting apparent kinetic constants, (mean ± s.e. of 4 rats) for SSAO were 67 ± 3μM (Km), 20.0 ± 2.7 nmol/h/mg protein (Vmax). The clorgyline (10^{-3}M)-sensitive portion of total TR metabolism yielded kinetic constants (for MAO) of 21 ± 3μM (Km), 35.5 ± 12.5 nmol/h/mg protein. Inhibition curves for TR metabolism in homogenates preincubated with a range of clorgyline concentrations (10^{-10} to 10^{-3}M) revealed that approximate % contributions of MAO-A: MAO-B: SSAO to 5μM or 1mM TR metabolism were 80:10:10 and 55:20:25 respectively.

Contractile effects of TR were studied with thoracic aorta rings, mounted at 37°C under 1g tension in Krebs buffer gassed with 5% CO_2 in O_2. A control cumulative dose response curve (DRC) to TR was obtained in each tissue, followed after washout, by 20 min exposure to added irreversible amine oxidase inhibitors. A repeat DRC was then obtained after extensive inhibitor washout. In preliminary control studies without inhibitors, sensitivity to TR did not change significantly when comparing first (EC_{50} 6.1 ± 1.3μM) with second DRCs (EC_{50} 6.2 ± 1.2μM) (n=30). Thus, the data below compares pre-inhibitor with post-inhibitor DRCs obtained on each tissue. At the doses used, the inhibitors did not depress maximal contractile effects of TR.

INHIBITOR	n	TRYPTAMINE EC_{50} (μM) PRE-INHIBITOR	POST-INHIBITOR	P
Iproniazid (10^{-3}M)	17	7.2 ± 1.2	2.1 ± 0.3	<0.001
Phenelzine (10^{-5}M)	9	13.3 ± 2.4	2.9 ± 0.4	<0.005
Semicarbazide (10^{-3}M)	8	8.7 ± 3.9	5.0 ± 2.6	>0.05
Clorgyline (10^{-6}M)	6	8.4 ± 2.2	6.1 ± 1.3	>0.05
Pargyline (10^{-4}M)	5	19.6 ± 3.1	6.3 ± 2.5	<0.05

At these concentrations, IPN and phenelzine are irreversible inhibitors of MAO and SSAO in aorta homogenates, and here produced significant potentiation of TR-induced contraction, whereas semicarbazide (SSAO inhibitor) and clorgyline (MAO-A selective at 10^{-6}M) did not. Pargyline (MAO-A + B inhibitor at 10^{-4}M) also potentiated TR significantly. Overall, these results suggest that inhibition of both MAO-A and B may be required to show this effect. Whether the additional ability of IPN and phenelzine to inhibit SSAO is important is not yet clear and this is under further investigation.

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INCREASE OF CONTRACTILE FORCE BY ADRENALINE THROUGH β_2-ADRENOCEPTORS IN VENTRICULAR MYOCARDIUM OF MAN

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Noradrenaline and adrenaline enhance with similar potency contractile force of human ventricle when tissue capture of catecholamines is prevented and α-adrenoceptors are blocked by phenoxybenzamine (Kaumann & al.,1982; Gille & al.,1985). These experiments suggest but do not prove that both catecholamines interact with β1-adrenoceptors. Binding assays have revealed that human ventricle contains a mixture of β1- and β2-adrenoceptors(Stiles & al.,1983). Do ventricular β2-adrenoceptors participate in the positive inotropic effects of adrenaline and noradrenaline? To answer this question we used the β1-specific CGP 20712 A (Kaumann,1986) and β2-selective ICI 118,551 (Bilski & al.,1983) as antagonists of the effects of the catecholamines. Left ventricular tissues were excised from patients with mitral lesion(n=3) or hypertrophic obstructive cardiomyopathy(n=6) dissected, set up at 37°C (5 µM phenoxybenzamine 2 h) and paced at 0.2 Hz as described (Kaumann & al.,1982). We also compared the fractions (f) of β1- and β2-adrenoceptors labelled with ^{3}H(-)bupranolol and the stimulation of the adenylate cyclase in membrane particles with the help of an equation for 2 non-interacting sites (Gille & al.,1985). An affinity estimate of the catecholamines for β1- and β2-adrenoceptors was made in membranes (0.1 mM GTP) by inhibition of binding of ^{3}H(-)bupranolol. Selective blockade of β2-adrenoceptors attenuated the increase in contractile force caused by adrenaline but not by noradrenaline. Specific blockade of β1-adrenoceptors shows that β2-adrenoceptors can mediate half of the maximum increase of contractile force caused by low concentrations of adrenaline and also contribute to the increase of contractile force elicited by high concentrations of noradrenaline.

Table 1

	Δ Contractile force % max		Δ Adenylate cyclase % max		β-Adrenoceptors			
	β1	β2	β1	β2	$f_{\beta 1}$	$f_{\beta 2}$	pK_D β1	pK_D β2
(-)Noradrenaline	100	?	36	64	0.71	0.29	5.7	4.4
(-)Adrenaline	100	50	24	76			5.5	5.5

Conclusions: 1) The β2-adrenoceptor-mediated increase of contractile force caused by low (-)adrenaline concentrations but only by high (-)noradrenaline concentrations is consistent with the higher affinity of (-)adrenaline compared to (-)noradrenaline. 2) The discrepancies between biochemical data obtained in membrane particles from human ventricle and inotropic data preclude further quantitative predictions of one from the other.

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PIG AORTIC ENDOTHELIAL CELLS CONTAIN BOTH SOLUBLE AND PARTICULATE GUANYLATE CYCLASE ISOENZYMES

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Endothelium-derived relaxing factor (EDRF) induces vasodilation by elevating the level of cyclic GMP in smooth muscle cells, but it is not known whether this cyclic nucleotide has any regulatory function in the vascular endothelial cell. The enzyme which catalyzes the formation of cyclic GMP, guanylate cyclase, exists as two isoenzymes: a soluble form that is activated by EDRF and by nitrovasodilators (Rapoport et al, 1983; Busse et al, 1985) and a particulate form that is activated by atrial natriuretic peptides (ANPs) (Winquist et al, 1984). We investigated whether pig aortic endothelial cells contained soluble and particulate forms of guanylate cyclase by examining the ability of a nitrovasodilator, glyceryl trinitrate (GTN), and an ANP, atriopeptin II (AP II), to elevate cyclic GMP content.

Endothelial cells were isolated from pig aorta using collagenase as previously described (Gordon and Martin, 1983). Cells were seeded into 9.6cm^2 multiwell plates and grown to confluence over the next 3-7 days. Cyclic GMP and cyclic AMP levels in the endothelial cells were determined by radioimmunoassay.

Resting levels of cyclic GMP and cyclic AMP in primary cultures of pig aortic endothelial cells were 0.035±0.004 pmol/ug DNA (n=18) and 19.78±1.92 pmol/ug DNA (n=18), respectively. GTN (0.1uM, 3 min) and AP II (0.01uM, 3 min) induced 3.2-fold and 17.4-fold increases in cyclic GMP, respectively. Methylene blue (20uM, 10 min), a selective inhibitor of soluble guanylate cyclase, blocked the elevation of cyclic GMP induced by GTN but not by AP II. The selective cyclic GMP phosphodiesterase inhibitor, M&B 22,948 (100uM, 3 min),induced a 7.0-fold increase in cyclic GMP content that was abolished by pretreatment with methylene blue (20uM, 10 min). Cyclic AMP levels were not changed by any of the above treatments.

These findings show that both soluble and particulate forms of guanylate cyclase are present in pig aortic endothelial cells. Elevation of cyclic GMP content by M&B 22,948 and its inhibition by methylene blue suggest that soluble guanylate cyclase is stimulated in the resting state, possibly by spontaneously released EDRF. Cyclic GMP, formed in resonse to locally produced EDRF or to circulating ANPs, may therefore have a regulatory role in the vascular endothelial cell.

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ADRENERGIC SEROTONERGIC AND EDRF-MEDIATED RESPONSES IN THE ATHEROSCLEROTIC THORACIC AORTA OF THE RABBIT.

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In aortic tissues of rabbits fed a cholesterol-rich diet, the contractile responses to serotonergic agonists are augmented (Henry and Yokoyama, 1980; Verbeuren et al. 1986) while those to adrenergic agonists are either unaltered (Henry and Yokoyama, 1980) or reduced (Verbeuren et al. 1986). The relaxations mediated by EDRF (endothelium-derived relaxing factor) are inhibited in atherosclerotic arteries (Habib et al., 1986; Verbeuren et al., 1986); in the rabbit abdominal aorta, which is only moderately affected by the cholesterol-induced atherosclerosis, the release of EDRF was not significantly altered at a time when the endothelium-dependent relaxations were reduced (Verbeuren et al., 1986).

The present study was designed to test whether in atherosclerotic blood vessels (1) only contractions mediated by $alpha_2$-receptors are reduced whereas those mediated by $alpha_1$-receptors are unaltered (Verbeuren et al., 1986); (2) alpha-receptor mediated effects of serotonin mask the enhanced response to the indoleamine (Verbeuren et al., 1986) and (3) the release of EDRF is altered in more heavily damaged tissues (e.g. the thoracic aorta).

Male New Zealand rabbits were fed a control or a cholesterol-rich (0.3%) diet for 16 weeks. Segments of the thoracic aortas of these rabbits were mounted in organ chambers for isometric tension recording (Verbeuren et al., 1986). In the atherosclerotic aortas the dose-response curves to noradrenaline, clonidine and phenylephrine were shifted to the right as compared to the control responses (Table 1). The responses to serotonin were not significantly altered by the atherosclerosis (Table 1). Phentolamine reduced the contractions to serotonin; in the presence of the alpha-blocker the contractile responses to the indoleamine were augmented in the atherosclerotic vessels (Table 1).

Table 1 : Adrenergic and serotonergic contractions in rabbit thoracic aortas.

	(% of maximal response)	
	Control	Atherosclerosis
Noradrenaline (3×10^{-7} M)	77.1 ± 3.0	53.2 ± 7.1*
Clonidine (10^{-6} M)	75.7 ± 4.8	28.3 ± 11.8*
Phenylephrine (3×10^{-7} M)	74.9 ± 2.6	49.3 ± 6.5*
Serotonin (10^{-6} M)	60.9 ± 5.3	71.5 ± 6.5
Serotonin + Phentolamine (3×10^{-6} M)	12.8 ± 2.9	37.1 ± 6.0*

In segments of thoracic aortas, mounted for the bio-assay of EDRF (Verbeuren et al., 1986) increasing concentrations of acetylcholine caused increasing amounts of EDRF in the perfusate. Significantly less EDRF was detected in the perfusate of the atherosclerotic thoracic aortas.

We conclude that in the atherosclerotic rabbit thoracic aorta (1) both responses to alpha-adrenoceptor agonists are reduced; (2) alpha-receptor blockade unmasks the enhanced response to serotonin and (3) the release of EDRF is impaired.

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20P

FUNCTIONAL RESPONSE TO DOBUTAMINE IN THE PITHED RAT 48 HOURS AND 7 DAYS AFTER MYOCARDIAL INFARCTION

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Loss of functional myocardial tissue after acute myocardial infarction causes a decrease in maximal contractile force of the heart, leading eventually to congestive heart failure. Contractility could be enhanced by sympathicomimetic drugs. However, a decrease in the number of beta adrenoceptors (desensitisation) limits the stimulatory effect of beta adrenoceptor agonists. Dobutamine, however, also has a alpha adrenoceptor agonistic effect.

We investigated the differences in maximal contractile force of the heart of pithed rats with sham operations, myocardial infarct operations, and without operations (control). Myocardial infarction was induced experimentally under aether anaesthesia by ligating the left coronary artery after left thoracotomy. Male Wistar rats weighing 200 to 250 g were used. 48 hours or 7 days later the rats were pithed. Systemic arterial blood pressure, heart rate (HR) and left ventricular pressure (LVP) were recorded. Left ventricular dP/dt was used to measure contractility. Dobutamine was administered intravenously in doses from 0.1 to 100 microg/Kg.

Under basal conditions, the heart rate after 48 hours, and systolic blood pressure (SBP) and LVP were lower in infarcted rats compared with sham operated rats. After stimulation with dobutamine in the 48 hour group, maximal dP/dt was 65 percent for infarcted and 80 percent for sham operated rats compared with control animals. After 7 days this was 54 and 96 percent respectively. In comparing sham with control animals, the observed decrease in SBP and LVP and maximal dP/dt after 48 hours had disappeared 7 days after the operation.

We conclude that the operation in itself after 48 hours still has a cardiodepressive effect, which is not detectable after 7 days and that after myocardial infarction dobutamine stimulation causes only a limited increase in maximal contractile force of the heart.

PRELIMINARY STUDIES ON ISCHAEMIA AND REPERFUSION-INDUCED DYSRHYTHMIAS IN ANAESTHETISED RABBITS.

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Many experiments on ischaemia-induced dysrhythmias are currently performed in either rats or dogs. Both species have disadvantages, e.g. rats have an unusual cardiac action potential, experiments in dogs are very expensive. This study assesses the feasibility of using anaesthetised rabbits as an alternative model.

Male New Zealand White rabbits (1.8 to 3.5 kg) were anaesthetised with diazepam 2.5mg kg^{-1} i.m. followed by Hypnorm (fentanyl and fluanisone) 0.4 ml kg^{-1} i.m. A Lead II ECG was recorded. The right femoral artery and vein were cannulated to allow blood pressure measurement and anaesthetic administration, respectively. A catheter was placed in the lumen of the left ventricle via the left carotid artery. The trachea was cannulated and sodium pentobarbitone was administered i.v. (18 to 48 mg kg^{-1}) to maintain anaesthesia. A left thoracotomy was performed at the 4th intercostal space and the rabbits were ventilated with room air at 38 strokes min^{-1}, 16 to 24 ml $stroke^{-1}$, with a positive end expiratory pressure of 1 to 2 cm H_2O. Arterial blood gases were monitored and the stroke volume adjusted to maintain PCO_2 within normal limits. A fine silk ligature was then placed around a coronary artery.

The left coronary artery was occluded at 4 different sites; anterior descending branch (LAD), circumflex branch within 2 to 3 mm from its origin (CX, high), circumflex branch within 1 to 2 mm from where it emerges from under the left atrial appendage (CX, lower) and the LAD and CX branches together close to their origin. Table 1 compares the incidence of ST-segment elevation, ventricular ectopic beats (VEBs), ventricular tachycardia (VT), ventricular fibrillation (VF) and AV block induced by coronary artery occlusion at these sites.

Table 1. Ischaemia-induced dysrhythmias in anaesthetised rabbits.

Occlusion site	n	ST elevation	VEBs	VT	VF	AV block
LAD	8	25%	12%	0%	0%	0%
LAD + CX	9	100%	89%	22%	44%	33%
CX, high	21	100%	76%	14%	19%	10%
CX, lower	11	100%	73%	9%	45%	9%

CX occlusion at the lower site is probably best since this only causes a minor fall in arterial pressure 1 min after occlusion (75 ± 4 / 49 ± 3 to 68 ± 4 / 44 ± 3 mmHg). A marked reduction in arterial pressure was observed when the CX artery was occluded near its origin (77 ± 2 / 55 ± 3 to 57 ± 2 / 42 ± 2 mmHg, $P < 0.001$).

In 18 of the rabbits which survived CX occlusion (either site) the ligature was released after 15 or 20 min of ischaemia. In 2 animals there was no evidence of reperfusion but in all the others rapid reversal of the ischaemia-induced ST-segment elevation was evident. Reperfusion-induced VEBs, VT and VF occurred in 81%, 44% and 25% of the rabbits, respectively.

These results suggest that occlusion of the left CX coronary artery in anaesthetised rabbits may provide a reasonable alternative or additional model to study ischaemia and reperfusion-induced dysrhythmias.

FAILURE OF ALLOPURINOL AND OXYPURINOL TO REDUCE THE INCIDENCE OF REPERFUSION VENTRICULAR FIBRILLATION IN ANAESTHETISED BEAGLES

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The myocardial xanthine oxidase system has been implicated as a source of oxygen free-radicals generated during reperfusion of the ischaemic myocardium (McCord, 1985). Allopurinol, an inhibitor of xanthine oxidase has been reported to reduce both myocardial infarct size in anaesthetised dogs and arrhythmogenesis in anaesthetised rats induced by coronary artery occlusion/reperfusion (Chambers et al, 1985, Manning et al, 1984). Conversely, allopurinol has been shown to be ineffective at reducing both infarct size and the incidence of ventricular fibrillation (VF) in two models of coronary artery occlusion/reperfusion in anaesthetised dogs. (Reimer and Jennings, 1985, Wainwright and Parratt, 1986).

The purpose of this study was to determine whether allopurinol or oxypurinol could reduce the incidence of VF in a canine occlusion/reperfusion model.

Anaesthetised beagle dogs of either sex (n=30, wt. 8.9-15.4kg) instrumented for drug infusion, blood pressure, blood gas monitoring, and lead II electrocardiogram were used. A left thoracotomy was performed and the heart supported in a pericardial cradle. The left anterior descending coronary artery was dissected free distal to the bifurcation of the circumflex and septal arteries. A suture was placed under the artery proximal to all major diagonal branches and a polythene snare positioned to allow for a 20 minute occlusion of the artery with subsequent reperfusion.

4 Experimental groups were used. In group 1, animals served as a control group and received no treatment. In group 2, animals received intravenous allopurinol ($20mgkg^{-1}$) 1h before coronary occlusion. In group 3, animals received allopurinol, administered orally ($20mgkg^{-1}$) once daily for 7 days and then a further dose was administered intravenously ($20mgkg^{-1}$) 1h before coronary occlusion. In group 4, animals received intravenous oxypurinol ($10mgkg^{-1}$) 1h before coronary occlusion.

The table below summarises the incidence of occlusion/reperfusion VF in all groups.

Group	n	VF occlusion	VF reperfusion	Survivors
1	8	1/8	4/8	3/8
2	6	1/6	4/6	1/6*
3	9	3/9	4/9	2/9*
4	7	0/7	4/7	3/7*

*No statistical difference in survival rate between groups by chi-square analysis at $p<0.05$.

The haemodynamic status (blood pressure and heart rate) and blood gases of both control and treated animals were similar before and after drug infusion,

In conclusion, the results of this study demonstrate that allopurinol and oxypurinol at the doses used both failed to reduce the incidence of VF thereby questioning the role of xanthine oxidase in the pathophysiology of ischaemia-induced arrhythmogenesis in the anaesthetised dog. Although in our studies, myocardial xanthine oxidase inhibition was not determined, in view of the known potency of these inhibitors (Elion, 1978), it is highly unlikely that lack of enzyme inhibition could account for our negative results.

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EFFECTS OF TOCAINIDE ENANTIOMERS ON EXPERIMENTAL ARRHYTHMIAS PRODUCED BY PROGRAMMED ELECTRICAL STIMULATION

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Tocainide is a class Ib antiarrhythmic agent, structurally related to lignocaine and well absorbed after oral administration (Zipes & Troup, 1978,). The racemic mixture comprises 2 enantiomers but it has been shown in animal studies that the R enantiomer is up to 3 times as potent as the S enantiomer (Byrnes, 1979). We have developed a chronic canine model where re-entrant arrhythmias, similar to those responsible for sudden death in man, maybe initiated by programmed electrical stimulation (PES). Using this model, we have studied the anti-arrhythmic and electrophysiological effects of the racemic mixture and enantiomers, and compared the results with a control group receiving no drug. After anaesthesia with sodium methohexitone, 10 mg/kg, artificial ventilation with 1.5% halothane, and thoracotomy, coronary artery ligation was performed in adult greyhounds in a 2-stage procedure (Harris, 1950). Myocardial pacing wires were placed within and adjacent to the infarcted area. 7-30 days later the conscious dogs underwent PES. Using stimuli of 4 msec duration at twice diastolic threshold, ventricular pacing was carried out with a basic cycle length of 350 msec. A single extrastimulus was introduced at 350 msec and the delay reduced at 20 msec intervals until refractory. Second and third extrastimuli were similarly introduced until an arrhythmia was produced or the protocol was exhausted.

Groups of 6 dogs with reproducible ventricular tachycardia (sustained ventricular tachycardia or 4 or more ventricular ectopic beats) were randomly allocated to receive increasing intravenous doses of SR tocainide, S tocainide, R tocainide or placebo. Results were ranked for abolition of arrhythmias, no change or death, and compared with placebo using the non-parametric Mann-Whitney U test. PR and corrected QT intervals, QRS duration and effective and functional refractory periods were measured before and after each treatment.

In the placebo group the arrhythmia in 4/6 dogs remained unchanged and 2 died. SR tocainide (21.3 $\pm$ 5.3 mg/kg) prevented a ventricular tachycardia in 3/6 dogs; 2 dogs died and in 1 no change was seen. S tocainide (7.1 $\pm$ 3.3 mg/kg) prevented ventricular tachycardia in 4/6 dogs; 1 dog died and 1 showed no change. R tocainide (9.0 $\pm$ 5.9 mg/kg) prevented ventricular tachycardia in 5/6 dogs, with no change in one. PR and corrected QT intervals and QRS duration were unchanged in all groups and refractory periods were unaltered.

In conclusion, R tocainide was significantly better than placebo ($p<0.01$) in preventing ventricular tachycardia and death in conscious dogs after programmed electrical stimulation; S tocainide was also better ($p<0.05$) but the effect of SR tocainide was not significant compared with placebo. Both enantiomers exhibited antiarrhythmic effects at doses lower than those required for the racemic mixture. This study indicates that the enantiomers of tocainide are more effective than the racemic mixture; no additive anti-arrhythmic effect occurs with the racemic mixture and adverse effects may be potentiated.

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ARE 5-HT RECEPTORS INVOLVED IN THE ANTIHYPERTENSIVE EFFECTS OF URAPIDIL

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Urapidil is a novel antihypertensive agent which reduces blood pressure with little or no associated tachycardia (Van Zwieten et al., (1985). In addition to a peripheral vasodilator effect due to post-synaptic α_1-adrenoceptor blockade, a centrally mediated reduction in sympathetic tone and an increase in vagal activity are suggested to be contributory factors to its antihypertensive effects (Sanders & Jurna, 1985; Ramage, 1986). However, the mechanism of the centrally mediated effects is not known. In this communication we provide evidence that urapidil has appreciable affinity for the 5-HT_{1A} receptor which may be relevant to the central component of its antihypertensive activity.

The affinity of urapidil for a number of central neurotransmitter recognition sites was measured as previously described in detail (Fozard et al, 1986). Agonist/antagonist activity at 5-HT_{1A} receptors was investigated using the electrically stimulated guinea-pig ileum (Fozard & Kilbinger, 1985). Functional α_1-adrenoceptor antagonist potency was determined on several isolated tissues using phenylephrine as the agonist.

Table 1. Affinity of Urapidil for central neurotransmitter recognition sites.

	5-HT_{1A}	5-HT_{1B}	5-HT_2	α_1	α_2	D_2
pIC_{50} mean±SEM(n)	7.1 ± 0.1(4)	4.5 ± 0.2(3)	4.7 ± 0.3(4)	6.2 ± 0.1(3)	5.0 ± 0.1(3)	5.3 ± 0.2(3)

Table 2. α_1- Adrenoceptor antagonist potency of urapidil in isolated tissues.

	Rat aorta	Rabbit aorta	Guinea pig aorta	Rat portal vein	Rabbit fundus
pA_2 mean±SEM(n)	7.3 ± 0.4(3)	7.1 ± 0.2(4)	7.3 ± 0.3(3)	7.4 ± 0.1(4)	6.9 ± 0.2(4)
Slope Schild plot	-1.0 ± 0.2	-1.0 ± 0.1	-0.8 ± 0.1	-1.0 ± 0.1	-0.9 ± 0.1

Urapidil clearly discriminates between central 5-HT receptor subtypes and is highly selective for the 5-HT_{1A} recognition site; moreover the binding data show urapidil to be some 10-fold less active at α_1-adrenoceptors and about 100-fold less active at α_2- and dopamine D_2 receptors (Table 1). The affinity of urapidil for 5-HT_{1A} recognition sites correlates well with its potency to antagonize the neuronal inhibitory effects of 8-OH-DPAT in the electrically stimulated guinea-pig ileum (pA_2 = 7.3). In addition it has potent agonist effects in this test system (pIC_{15} = 7.5) which are blocked completely by metergoline 5×10^{-7}. The potency of urapidil as an α_1-adrenoceptor antagonist in several isolated tissues (table 2) is similar to its potency as a mixed antagonist/agonist at 5-HT_{1A} receptors. A reduction of central sympathetic tone and/or an increase in vagal activity are effects characteristic of the mode of action of compounds like 8-OH-DPAT which have selectivity for central 5-HT_{1A} receptors (See Mir & Fozard, 1986). The activity of urapidil at these sites may thus be relevant to the centrally mediated component of its antihypertensive response.

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POTENTIATION OF THE EFFECTS OF DOPAMINE IN THE RABBIT SPLENIC ARTERY IN VITRO BY IBMX OR FORSKOLIN

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Vascular dopamine receptors in the rabbit splenic artery have been characterised previously (Hilditch and Drew, 1981). However some preparations do not respond to dopamine. Dopamine increases cAMP levels in other arterial preparations (Collier et al., 1983; 1984) and thus its effects in the splenic artery may involve this mechanism. Consequently we have attempted to increase the responsiveness of the preparation by inhibiting the breakdown of cAMP with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) or by enhancing the effect of dopamine with the adenylate cyclase subunit stimulant forskolin.

Splenic artery rings pretreated as described by Hilditch and Drew (1981) were contracted with the thromboxane A_2-mimetic U-46619. Dopamine (10^{-8} - 10^{-4} M) was administered until reproducible responses were obtained. Preparations were classified as insensitive or sensitive depending on whether dopamine produced less than or greater than 50% relaxation of the spasmogen-induced tone. Both types were incubated with IBMX (10^{-5} M) for 30 min before re-contracting the preparations. Previously insensitive preparations relaxed to dopamine whereas in sensitive preparations the effect of dopamine was potentiated 10-20 fold by IBMX (Fig. 1). Forskolin (10^{-7} M) produced similar effects to IBMX.

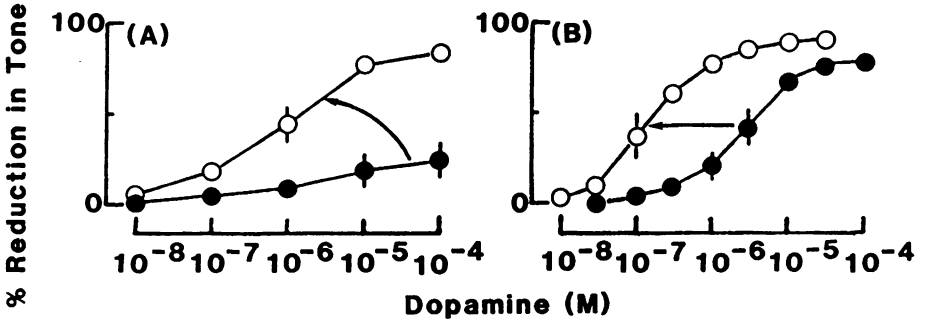

Fig. 1: The effect of dopamine in (A) dopamine-insensitive (B) dopamine-sensitive preparations before (●) and after (○) IBMX (10^{-5} M) (n=4).

In IBMX treated preparations epinine and 6,7-ADTN, like dopamine, produced concentration-related relaxation of U-46619-induced tone. SK&F 38393 and N,N-di-n-propyldopamine (DPDA) had little effect. The agonist equipotent concentration ratios in IBMX treated preparations were: epinine (0.2) >6,7-ADTN (1) = dopamine (1) >SK&F 38393 (> 10) >DPDA (> 45) which was similar to that obtained in untreated preparations (Hilditch and Drew, 1981). SCH 23390 behaved as a competitive antagonist versus dopamine in untreated (pA_2 = 10.65 ± 0.45) (Hilditch and Drew, 1985) and IBMX treated preparations (pA_2 = 10.47 ± 0.55).

Agonist and antagonist data suggest that the effect of dopamine in both untreated and IBMX treated preparations is mediated via the same receptor. Both IBMX and forskolin increase the responsiveness of the preparations which suggests that the relaxant effect of dopamine is mediated via cAMP.

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FENOLDOPAM INDUCES VASODILATION IN THE RAT HINDQUARTERS, POSSIBLY VIA GANGLIONIC DOPAMINE RECEPTORS

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Fenoldopam was reported to be a selective DA_1 -receptor agonist (see e.g. Hahn et al., 1982). In the rat, it induces vasodilation in the renal(Lokhandwala & Steenberg,1984) and superior mesenteric (Dupont et al.,1985) vascular beds through stimulation of postsynaptic DA_1-receptors. In the present study, we investigated the effect of local administration of fenoldopam in the autoperfused hindquarters of the rat.

The experiments were done in normotensive Wistar rats weighing 300-410g, anesthetized with pentobarbital. Autoperfusion of the rat hindquarters was performed at constant flow, using an extracorporeal circuit as described previously, except that the lumbar sympathetic chains were not ligated (Dupont et al.,1985).

In the first group of rats, fenoldopam was infused (30 µg/kg/min i.a.). It had no effect on systemic blood pressure or baseline perfusion pressure, but it reduced the pressure responses induced by preganglionic lumbar sympathetic nerve stimulation (supramaximal voltage, 1 ms, 4 Hz) to 70.1 $\pm$ 1.4 % (mean $\pm$ s.e. mean, n = 6) of control responses. In contrast, increases of perfusion pressure induced by local administration of noradrenaline and phenylephrine (n = 6) were not modified by the fenoldopam infusion.

In all further experiments, the hindquarters vascular tone was maintained at 165-180 mm Hg by Vasopressin (0.01-0.05 U/kg/min i.a.). Bolus injections of fenoldopam were given i.a. at 5 min intervals. In control rats (n=6), fenoldopam 30,100 and 300 µg/kg produced reductions in perfusion pressure of 7.4 $\pm$ 1.0, 9.1 $\pm$ 1.0 and 10.9 $\pm$ 1.6 % ($p < 0.05$), while bolus injections of saline had no influence. When rats were treated with hexamethonium (20 mg/kg i.v., n = 6), baseline perfusion pressure was reduced ; fenoldopam, administered after baseline perfusion pressure was restored by increasing the dose of vasopressin, had no influence. Likewise in rats where the lumbar sympathetic nerves were sectioned (n=6), bolus injections of fenoldopam failed to reduce baseline perfusion pressure.

Administration of phentolamine (100 µg/kg i.a., n=6) or prazosin (100 µg/kg i.a., n=6) reduced baseline perfusion pressure ; fenoldopam given after restoration of the perfusion pressure had no influence. Treatment of rats with the DA_2-receptor antagonist domperidone (10 µg/kg i.a., n=6) did not influence the vasodilator effect of fenoldopam ; however, treatment with the DA_1-receptor antagonist SCH 23390 (R)-(+)8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol (50 µg/kg i.a., n=6 ; 200 µg/kg i.a., n=6) or the non-selective dopamine receptor antagonist RS-sulpiride (1 mg/kg i.a., n=6) antagonized the vasodilator effect of fenoldopam.

The results of this study show that the vasodilator effect of fenoldopam in the rat hindquarters in due to inhibition of sympathetic tone. As presynaptic dopamine receptors on the sympathetic nerve endings belong to the DA_2-type, our findings suggest that fenoldopam induces vasodilation in the rat hindquarters by activation of ganglionic dopamine receptors.

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MOXONIDINE (BE 5895) IS A FULL AGONIST AT PRESYNAPTIC (α_2-) AND POSTSYNAPTIC (α_1-) ADRENOCEPTOR SITES

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The centrally acting agent clonidine reduces arterial blood pressure by activation of central alpha-2-adrenoceptors. Like clonidine, most imidazoline alpha-agonists possess high affinity for the receptor, but exert only a partial agonist action (Ruffolo et al. 1979). Moxonidine (BE 5895), 4-chloro-5-(2-imidazoline-2-ylamino)-6-methoxy-2-methyl-pyrimidine, is a new imidazoline alpha-agonist with established antihypertensive action in man (Plänitz, 1984). The major site of action is central, as direct injection into the vertebral artery of cats or cisterna cerebello-medularis of rabbits results in a greater blood pressure reduction than i.v. injection of same doses (Armah & Stenzel, 1982).
Recently moxonidine has been reported to be a full agonist at alpha-2-receptors of human platelet membranes and to inhibit adenylate cyclase stimulated by PGE_1 in a yohimbine sensitive manner. Similar to adrenaline (K_{act} = 1,4 ± 0,2 µM), moxonidine was also found to induce platelet aggregation (K_{act} = 4,8 ± 0,6 µM), in contrast to clonidine which does not induce platelet aggregation (Bergerhausen, 1985). This paper describes further studies to characterise the actions of moxonidine on alpha-receptors.
In the isolated ^{3}H-noradrenaline preincubated rabbit pulmonary artery, moxonidine inhibits ^{3}H-overflow induced by intramural electrical stimulation at 1 Hz. Maximal inhibitory action was 90 % for moxonidine but 65 % for the partial agonist clonidine. IC_{50} = 7,9 x 10^{-8}M moxonidine vs 2,3 x 10^{-7}M clonidine.
In the isolated thoracic aorta of the rabbit, which is endowed with a high population of alpha-1-receptors (Docherty et al., 1981) moxonidine exerted vasoconstriction (IC_{50} = 3,2 x 10^{-6}M) with an intrinsic activity 100 % that of phenylephrine; in contrast to 40 % for clonidine. In the isolated rabbit vena cava (endowed with alpha-2-receptors) moxonidine (IC_{50} = 6,3 x 10^{-6}M) had an intrinsic action again comparable with phenylephrine, whilst clonidine, guanabenz, guanfacine and UK 14304 were all partial agonists. Antagonism of moxonidine at postsynaptic sites in rabbit aorta and V. cava gave pA_2-values of 8,75 for prazosin but 6,2 for yohimbine. Thus moxonidine was specificially stimulating alpha-1-receptors at postsynaptic sites with an intrinsic activity of 1. In receptor binding assays with ^{3}H-rauwolscine (87 Ci/mmol) and ^{3}H-prazosin (33 Ci/mmol) bound to rat cortex membranes, moxonidine competed with ^{3}H-rauwolscine with an overall K_i-value of 460 nmol/l and with ^{3}H-prazosin at K_i = 36,5 µmol/l. Moxonidine binding inhibition of ^{3}H-rauwolscine was potentiated by Mg^{++}, but shifted to lower potency by Mg^{++}/GppNHp and 150 mmol/l NaCl (in a manner similar to (-)noradrenaline. Thus moxonidine behaves as an agonist at ^{3}H-rauwolscine binding sites.
In conclusion moxonidine exerts full agonist action at alpha-2- and alpha-1-receptor sites, similar to the neurotransmitter noradrenaline, except that moxonidine shows higher pre-/postsynaptic selectivity than noradrenaline.

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DIFFERENTIAL EFFECTS OF α_1- AND α_2-ADRENOCEPTOR BLOCKADE ON REGIONAL MYOCARDIAL BLOOD FLOW IN ACUTELY ISCHAEMIC MYOCARDIUM

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Protective effects of alpha-adrenoceptor blockade have been demonstrated against the early phase of ventricular arrhythmias occurring within the first 10 minutes of acute myocardial ischaemia. It is uncertain however whether at least part of this action might result from redistribution of coronary blood flow and inhibition of alpha-adrenoceptor mediated vasoconstriction within ischaemic areas. Such vasoconstriction has been suggested to be mediated by alpha2-rather than alpha1-adrenoceptors and to be more evident in regions of poor residual flow (Heusch et al, 1985).

Studies have been performed therefore in 23 open chest pentabarbitone anaesthetised dogs to assess effects of alpha1- and alpha2-adrenoceptor blockade on regional myocardial blood flows during successive 10 minute periods of left anterior descending coronary occlusion before and after intracoronary admin. of 10μg/Kg doxazosin (n=8), an alpha1-adrenoceptor blocking agent, 10μg/Kg rauwolscine (n=7), an alpha2-adrenoceptor blocking agent, and placebo (n=8). Efficacy of rauwolscine at this dose was confirmed by greater increases in left ventricular dp/dt after left sympathetic stimulation (30sec,8Hz,8V) prior to final coronary occlusion. Regional flows in each study were determined from 80 endocardial and 80 epicardialbiopsy sites within a 4 x 5cm area of anterior left ventricle after tracer microsphere injection (25 million spheres) before and after left sympathetic stimulation (30sec, 8Hz,8V) at 4 and 4.5 minutes respectively of onset of these 2 periods of coronary occlusion.

Mean ischaemic area flows were comparable during pre-treatment occlusions between groups and similar increases in flow followed sympathetic stimulation. In the absence of nerve stimulation, no significant differences in ischaemic area flows were found following either doxazosin, rauwolscine or placebo treatment.

Following left sympathetic stimulation ischaemic area flows were increased in the placebo group, but unchanged following doxazosin, and significantly reduced following rauwolscine (see Table).

CHANGES IN EPICARDIAL BLOOD FLOW (ml/g/min) WITH SYMPATHETIC STIMULATION.

Pretreatment Ischaemic area flow range	Placebo	Doxazosin (Alpha1)	Rauwolscine (Alpha2)
0 to 0.20	+0.04±0.03	+0.11±0.09	-0.01±0.02
0.20 to 0.40	+0.16±0.07	+0.02±0.06	-0.07±0.07*
0.40 to 0.60	+0.36±0.16	+0.04±0.08	-0.15±0.07*

*$p<0.05$ vs placebo

Findings therefore would not support the concept that redistribution of flow constitutes a major mechanism of antiarrhythmic action of alpha-adrenoceptor blockade during early ischaemia and would be consistent with induction of adverse haemodynamic effects of coronary steal following alpha2- but not alpha1-adrenoceptor blockade.

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ANTI-ISCHAEMIC PROPERTIES OF R 58735 IN ISOLATED HEART PREPARATIONS.

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R 58735 (4-[(2-benzothiazolyl)methylamino]-α-[(4-fluorophenoxy)methyl]-1-piperidine ethanol) is a compound that displays strong anti-hypoxic activity. In the present study the anti-ischaemic activity as well as the effect on inotropy of R 58735 were investigated and compared with nifedipine in isolated guinea-pig hearts. Paced hearts (frequency 5 Hz) were perfused at 37°C with Tyrode solution containing 1.8 mM calcium. LVP was measured by placing a latex balloon in the left ventricle. After equilibration for 30 min, and where appropriate a 20 min pretreatment with R 58735 or nifedipine, the hearts were used as normoxic controls. A 90 min period of global ischaemia at 37°C was induced by stopping the perfusion. After a 30 min reperfusion period the hearts were frozen in liquid nitrogen and freeze-dried and ATP and CrP were assayed. Functional recovery was calculated as the percentage of the pre-ischaemic LVP value of the control hearts. During the reperfusion period the drug-pretreated hearts were perfused with the same drug concentration as before ischaemia.

Under normoxic conditions neither R 58735 ($3x10^{-7}$ and 10^{-6}M), nor nifedipine (3×10^{-8} and $6x10^{-8}$ M) induced changes in the endocardial CrP and ATP content compared with the control value (32.45 ± 1.8, n=6 and 19.30 ± 0.6, n=6, μmol/g dry weight, respectively). At the end of the reperfusion period, a functional recovery of 12 ± 0.8% (n=6) was observed while CrP and ATP returned to 14.3 ± 1.6 and 8.8 ± 0.9% of the normoxic value, respectively. Both R 58735 and nifedipine gave a clear protective effect towards ischaemia. R 58735 displayed a maximal functional recovery of 52.6 ± 1.9% (n=6) while CrP and ATP returned to 36.9 ± 2.8 and 28.4 ± 1.4% of the initial value, respectively.

Nifedipine displayed a maximal protective effect at a concentration of $6x10^{-8}$ M. The functional recovery was 55.4 ± 3.4% (n=6), CrP and ATP recovered up to 34.33 ± 2.6 and 14.4 ± 1.6% of the initial values, respectively. At concentrations of R 58735 that displayed protective effects (i.e. at $3x10^{-7}$ and 10^{-6} M) the negative inotropic effect amounted to 28.3 ± 2.1 (n=6) and 42.6 ± 2.8% (n=6) reduction of LVP, respectively, whereas for nifedipine at protective concentrations (i.e. $3x10^{-8}$ and $6x10^{-8}$ M) a negative inotropic effect of 54.2 ± 3.1 (n=6) and 64.1 ± 4.8% (n=6) was observed. Obviously, for nifedipine the cardiodepressant and cardioprotective effects occur within the same dose range. However, R 58735 displays a protective effect at concentrations that cause less cardiodepressant activity. In conclusion, R 58735 appears to be a compound with a potent anti-ischaemic activity and little cardiodepressant potency.

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MULTIPLE LATE PHASE REACTIONS AND CELLULAR CORRELATES IN AN IN VIVO GUINEA-PIG MODEL OF BRONCHIAL ASTHMA

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Allergen-induced late phase asthmatic responses occur several hours after immediate reactions with further cyclic decrements in lung function sometimes being recorded for several days afterwards (Newman Taylor et al, 1979). Late phase responses have been considered to be a more relevant clinical model than the early response for studying pathogenetic mechanisms in bronchial asthma and development of bronchial hyperreactivity. The association of asthma with inflammation has received much attention, but evidence for this has been largely indirect. We report on a guinea-pig model showing immediate and multiple late phase responses following allergen challenge. Cytological and histopathological evidence of local inflammation during the late responses is presented.

Male guinea-pigs (500-600g), sensitized by ovalbumen (1%, inhaled for 3 min weekly for 2 weeks), were challenged by aerosolized ovalbumen (2% for 5 min, test) or vehicle (control) under cover of mepyramine (10 mg/kg i.p.). Specific airways conductance (sGaw) was assessed by whole body plethysmography in conscious animals. Bronchoalveolar lavage (BAL) was performed by introduction of 2 x 5 ml saline into the lungs of freshly sacrificed animals. BAL fluid was centrifuged and the cell pellet resuspended in Eagles minimum essential medium and a total cell count performed. Cell identification and differential cell counts were performed on cytocentrifuged preparations using a May-Grunwald-Giemsa stain. Lungs were removed for fixation and histological examination.

In 53 guinea-pigs, ovalbumen challenge produced a 24.0±3.5% reduction in sGaw within 5 min. After recovery, further falls in sGaw of 57.3±4.9% and 38.7±4.0% at 17 and 72 h respectively were observed. BAL, performed at 6, 17 and 72 h, revealed no significant changes in macrophage, lymphocyte and mast cell numbers. Neutrophil and eosinophil numbers were significantly ($p<0.05$) elevated (Table 1). Neutrophils were maximally elevated at 6 h and 17 h, while eosinophil infiltration increased up to 72 h when these cells constituted 54% of the total nucleated cells. Histological examination of lung sections confirmed the infiltration of neutrophils and eosinophils into the bronchial lumen but not the alveoli.

Table 1 Neutrophils and eosinophils in BAL

	Neutrophils ($\times 10^6$)		Eosinophils ($\times 10^6$)	
Time	Control (n=6)	Test (n=6)	Control (n=6)	Test (n=6)
6	0.07 ± 0.05	0.52 ± 0.09*	0.60 ± 0.08	1.18 ± 0.33
17	0.04 ± 0.01	0.69 ± 0.17*	0.33 ± 0.11	1.94 ± 0.53*
72	0.09 ± 0.03	0.20 ± 0.06	0.82 ± 0.37	3.46 ± 0.74*

* $p < 0.05$ (test vs. control)

The similarities between these observations and late reactions in man suggest that this may be an appropriate animal model of bronchial asthma.

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AIRWAY HYPER-ACTIVITY AND EOSINOPHILS IN RATS TREATED WITH SEPHADEX PARTICLES

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Asthma is characterised by an airways hyper-reactivity and a blood eosinophilia. The effect of producing a blood eosinophilia in the rat on airways reactivity was studied.

The intravenous injection of Sephadex particles (G200) 0.125-0.5 mg/rat produced a specific, dose-dependant increase in the number of blood eosinophils from a mean value of $0.17 \pm 0.07 \times 10^6$ cells/ml to a mean maximum of $0.32 \pm 0.03 \times 10^6$ cells/ml 7 days later. A second injection given on day 14 when the eosinophil numbers had fallen to control levels produced a further dose-dependant increase in blood eosinophilia which peaked 5 days later. At this time there was also an increase in the number of eosinophils in the lung lavage fluid and in the lung tissue (table 1) but with no change in numbers of other leucocytes in the blood or lung lavage fluids. The respiratory sensitivity to 5-hydroxytryptamine (5HT) was determined on day 19 using the method of Konzett and Rossler. Urethane (1.5 gm/kg i.p.) was used as the anaesthetic. There was a significant decrease in the dose of 5HT required to increase the overflow volume to 50% of the maximum in the groups of rats treated with the higher doses of Sephadex (Table 1).

Number of eosinophils (Mean ± s.e.m. n = 5 or more)	Dose of Sephadex; mg/rat			
	0	0.125	0.25	0.5
Blood, millions/ml	0.14 ± 0.02	0.36*** ± 0.04	0.46*** ± 0.04	0.67*** ± 0.06
Lung lavage fluid millions/ml	0.009 ± 0.002	0.12* ± 0.04	0.16** ± 0.04	0.39** ± 0.11
Lung tissue, cells/mm^2	6.4 ± 2.3	48.1 ± 22.6	43.9*** ± 7.5	90.2*** ± 17.2
Dose (µg/kg) 5HT to increase overflow volume to 50% max	44.8	41.2	34.1$^+$	25.1$^+$
95% confidence limits,given i.v.	41-50	37-47	31-38	22-29

P = 0.05 to 0.01*; 0.01 to 0.001**; <0.001***; <0.05$^+$

It remains to show whether the eosinophilia is causal or merely coincidental with the hyper-reactivity to 5HT.

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COMPARISON OF TIMOLOL AND ITS ISOMER, L-714,465 ON THE AIRWAYS OF ASTHMATIC PATIENTS

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Timolol eye drops are widely used for the treatment of glaucoma. Inadvertent administration to patients with asthma continues to cause severe and even fatal episodes of asthma (1). The R-entantiomer of timolol, L-714,465 (1%) when given as eye drops to normal individuals caused less bronchial beta-adrenoceptor blockade than timolol 1% (dose ratio 1:13) (2), whereas it is only four times less potent in reducing intraocular pressure. The present study was designed to look at the airway response to increasing concentrations of L-714,465 and timolol or placebo when given as eye drops to asthmatic subjects.

Ten asthmatic subjects aged 18-51 years previously shown to bronchocontrict to timolol eye drops participated in the study. The subjects attended on 3 days at least one week apart. They abstained from all drugs for 8 hours prior to the study. After resting for 10 mins baseline measurements of specific airways conductance (sGaw), FEV_1 and heart rate (HR) were made. Subjects then received increasing concentrations (0.1-4%) of timolol or L-714,465, or methyl cellulose placebo, of 1 drop in each eye in random order in a double blind manner. Measurements of sGaw followed by FEV_1 were made at intervals up to 30 mins after each dose. If the FEV_1 had not decreased by 20% at 30 mins the next concentration of drug was given up to a maximum of 6 doses. At the end of the study bronchoconstriction was reversed with ipratropium bromide and salbutamol.

There was no difference in mean baseline values of FEV_1, sGaw or HR on the 3 test days. Geometric mean values of $PC_{15}FEV_1$ were 0.28% for timolol and 0.88% for L-714,465 ($p<0.05$). Geometric mean values for PC_{20}sGaw were 0.14% for timolol and 0.59% for L-714,465 (NS). Mean dose ratios for the two beta blocking drugs for FEV_1 and sGaw were 3.89 and 3.93 respectively.

Thus in the present study L-714,465 was 4 times less potent than timolol in causing bronchoconstriction in asthmatic subjects, compared with a 13 times lesser potency in normal subjects in our previous study (2). Since it is 4 times less potent in reducing intraocular pressure it would appear to offer no advantage over timolol in clinical use.

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PURINERGIC INVOLVEMENT IN THE NANC BRONCHODILATORY RESPONSE IN THE ANAESTHETISED CAT

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When resting bronchial smooth muscle tone is high, vagal stimulation causes non-cholinergic non-adrenergic bronchodilation (Diamond & O'Donnell,1980; Irvin et al 1980).We have investigated the transmission of this response using the P_2 and P_1 purine receptor antagonists α,β-methylene ATP (mATP) (Kasakov & Burnstock, 1983) and 8-phenyltheophylline (8PT) (Griffith et al, 1981) respectively.

Anaesthesia was induced by halothane in female cats, weight 2.7 - 3.1 kg and maintained by injection of α-chloralose (100mg/kg) via a cannulated left femoral vein. Cats were artificially ventilated on room air and respiratory measurements of airflow and transpulmonary pressure were made. Dynamic lung compliance and airways resistance were computed according to the method of Amdur and Mead (1958). Drugs were administered via a cannula inserted in the right jugular vein and advanced to the entrance of the right atrium to ensure their rapid distribution to the pulmonary circulation. Arterial pressure and heart rate were monitored continuously via a cannula into the right femoral artery. 5HT (20 - 50ug/kg/min) was continuously infused into the cannulated left femoral vein to increase bronchial tone. Stimulation of the cut vagus nerve (25v, 0.5ms pulse width, 15Hz) for 30 seconds evoked bronchodilatory responses that were maximal 5-30 seconds following cessation of electrical stimulation. The bronchodilatory responses were unaffected by sequential intravenous administration of atropine, propranolol, prazosin, idazoxan (or phentolamine) each drug given at dose level of 1mg/kg.

Both 8PT (10mg/kg) and mATP (2.5mg/kg as 5 boluses of 0.5mg/kg over 7 minutes) caused initial evanescent bronchoconstriction. The P_2 antagonist mATP did not significantly antagonise the nerve induced bronchodilation either in the presence or in the absence of atropine and the alpha and beta-adrenoceptor antagonists.The P_1 antagonist 8PT caused a small reduction in the neurally evoked bronchodilatory response in the presence of muscarinic and alpha and beta-adreneroceptor blockade. However, following administration of the adenosine uptake inhibitor dipyridamole (10mg/kg), it was found that 8PT markedly attenuated the bronchodilator response.

In conclusion, the results of the present study indicate that the non-adrenergic non-cholinergic nervous system present in the airways of the cat is at least partly purinergic mediated, and P_1 rather than P_2 receptors appear to be involved in the mediation of the bronchodilator response.

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34P

BRONCHIAL ANAPHYLAXIS IN ASCARIS SENSITIVE DOGS

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Bronchial asthma is a disease of complex aetiology attributed to the release of potent inflammatory mediators from sensitised cells. Whilst there is evidence suggesting a role for bronchial mast cells in initiating the responses characteristic of asthma, contributory roles for eosinophils, macrophages and platelets have also been suggested and the importance of each cell type is of some debate. Furthermore although there is little doubt of a patho-physiological role for some of the mediators released from these cells their relative importance remains obscure. We have therefore investigated the mechanisms of bronchial anaphylaxis in an animal model using bronchoalveolar lavage to assess mediator release and cellular infiltration.

Experiments were carried out in four chloralose anaesthetised (80 mg/kg) Beagle dogs (10.5-15 kg) sensitive to ascaris suum. Total airways resistance (R_L) and dynamic compliance (C_{dyn}) were measured and airways response to histamine (5 or 10 µg/kg i.v) determined. 5 ml saline was instilled into one side of the lung, using a fibre optic bronchoscope wedged in a 4th generation bronchi and lavaged 90 sec later with 50 ml saline; the other side of the lung was challenged with antigen (10-50 µg) before lavage. Lung response to histamine was measured 2 and 24 hrs post-challenge and the lavage repeated. The recovered lavage fluid collected on ice, was spun and 2.79 µM indomethacin, 50 µM NDGA and 10mM cysteine added to the supernatant. The pellet was resuspended and examined for major cell types.

Antigen challenge elicited an increase in R_L when compared to control (4±2.3 and 0.5 ± 0.5 cm H_2O/l/sec respectively), and a fall in C_{dyn}. This was associated with increased responses to histamine 2 hrs post-challenge (R_L 4.38 ± 1.2 compared to 1.5 ± 0.37 cm H_2O/l/sec prior to challenge at 5 µg/kg histamine). Changes in C_{dyn} in response to histamine were unaltered.

Histamine (106 ± 25.3 ng/ml) and PGD_2 (1.4 ± 0.56 ng/ml) concentrations were greater in the lavage fluid recovered from the challenged lobe than from the control (45.5 ± 12.1 and 0.22 ± 0.03 ng/ml respectively). There were no changes in TXB_2, $PGF_{2\alpha}$, PGE_2 or 6-oxo-$PGF_{1\alpha}$ concentrations. Histamine concentrations were still elevated compared to the control lobe 2 hrs post-challenge (48 ± 22.5 and 154.8 ± 35.4 ng/ml respectively) whereas PGD_2 and 6-oxo$PGF_{1\alpha}$ concentrations were elevated in both. Both lung function, histamine and prostanoid concentrations had returned to control levels by 24 hrs post-challenge. N-acetylglucosaminidase activity was greater in the lavage fluid from the challenged lobe 24 hrs post-challenge (5.27 ± 0.26 compared to 3.12 ± 0.26 µU/ml) as were protein concentrations and eosinophil number (3.9 ± 1.3 compared to 0.27 ± 0.13 x 10^4 cells/ml). Neutrophilia was seen in both lobes 2 and 24 hrs post-challenge.

We conclude that changes in R_L and C_{dyn} seen following antigen challenge, in this model, and the airways hyperreactivity seen 2 hrs post-challenge may be due to mast cell activation. Furthermore the anaphylactic bronchospasm is associated with eosinophil infiltration 24 hrs post-challenge. The neutrophilia, however, may be more a function of the lavage rather than the challenge.

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SENSITIZATION OF GUINEA-PIGS BY AEROSOL: EFFECT OF PARAQUAT

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Sensitized guinea-pigs have been used extensively as laboratory models of asthma, both in vivo and in vitro, usually following initial sensitization by injecting the antigen (generally ovalbumin) intra-peritoneally (i.p.) and sub-cutaneously (s.c.). Anaphylactic bronchospasm can be provoked 2-3 weeks later, by challenge via either of these routes, or by aerosol (Payne & Nucci 1987). There is evidence in man that atopic asthma is triggered by inhalation of the allergen, and it is likely that the initial sensitization also occurs via this route. In this paper we report a method for sensitizing guinea-pigs by an aerosol of ovalbumin (OA). In addition we have also compared the respective cardiopulmonary effects of subsequent challenge with OA given either intravenously (i.v.) or by aerosol.

Male guinea-pigs (250-300g) were placed in a plexiglass chamber ventilated with air, exposed to an aerosol of OA (0.2%) for 30 minutes for 3 alternate days, and used 2 to 3 weeks after the first sensitization. A control group of animals was sensitized by injection of 50mg i.p. and 50mg s.c. of OA. Each sensitized animal was anaesthetised with sodium pentobarbitone (40 mg/kg i.p.), and both vagi nerves cut at the level of the neck. The trachea was cannulated and the lungs ventilated mechanically (54 strokes/min of 1 ml air/100g body wt). Pulmonary inflation pressure was measured from a lateral port in the afferent limb of the ventilator circuit with a pressure transducer. Blood pressure/heart rate were measured from a catheter placed in the left carotid artery. Aerosols of OA (1%, 5 sec.) were generated by ultrasonic nebuliser (Lees & Payne 1986).

In animals sensitized i.p. and s.c., aerosol challenge provoked almost total bronchoconstriction, accompanied by a rise in mean arterial blood pressure (MABP) from 53.8±3 mmHg to 88±4.6 mmHg, n=4, $p<0.005$. In animals sensitized by OA aerosol, aerosol challenge again provoked almost total bronchoconstriction, but in contrast there was no significant change in MABP (62±4 mmHg; 66±5 mmHg; pre- and postchallenge, respectively n=6) However i.v. challenge with OA (1 mg/kg) in aerosol-sensitized guinea-pigs provoked almost total bronchoconstriction, and a significant increase in MABP from 50.2±2 mmHg to 116.6±1 mmHg, n=5, $p<0.005$). These results suggest that sensitization by aerosol promotes a more localised (bronchial) anaphylactic reaction on subsequent challenge with aerosolized antigen, than in animals sensitized i.p. and s.c.

Damage to the lung epithelium, mainly the type 1 pneumocyte (Smith & Heath, 1973) is a characteristic feature of the toxicity of paraquat. When aerosol-sensitized guinea-pigs were pretreated with paraquat (35 mg/kg, i.p. 12h earlier) aerosolised OA induced almost total bronchoconstriction, accompanied by a significant rise in MABP from 54±4 mmHg to 97±13 mmHg, n=7, $p<0.005$. Whether this cardiovascular effect is due to the destruction of the lung epithelium, therefore allowing the antigen to reach the circulation, or to cell migration into the lungs which could amplify the anaphylactic response, remains to be established.

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BRONCHODILATOR ACTION OF INHALED IPRATROPIUM AND FENOTEROL IN NORMAL SUBJECTS: A TEACHING EXERCISE FOR MEDICAL STUDENTS.

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A pharmacology practical class for pre-clinical medical students was devised, which illustrated the basic principles involved in carrying out a double blind trial, had direct clinical relevance, and brought together several disciplines within the medical school.

The effects of two inhaled bronchodilator drugs, ipratropium bromide (I), and fenoterol hydrobromide (F) were compared with those of placebo (P). The students made observations on themselves and analysed the results later in their statistics course. This introduced in an interesting way the important principles of informed consent, the controlled clinical trial based on objective assessment, the correct use of metered dose inhalers, and the fact that inhaled drugs may have systemic effects.

FEV1 was measured with a Vitalograph spirometer, heart rate by counting the radial pulse, and tremor assessed by the time taken to thread needles. The 47 male and 32 female non-asthmatic student volunteers were randomised into three groups, so that 26 took ipratropium, 26 fenoterol and 27 placebo (propellant only) from identical metered dose inhalers. Under supervision, each student inhaled 4 puffs using the correct technique (Newman et al, 1981). Baseline measurements were made before inhalation, and 5, 15, 30, 45 and 60 min later.

	FEV1 (mls, BTPS)			Heart Rate (beats/min)		
	P	F	I	P	F	I
Baseline mean (SD)	3737(642)	3881(850)	3986(698)	80.2(12.0)	81.0(9.6)	77.4(10.9)
Change mean (SE) after						
5 min	-5 (39)	62 (27)	36 (36)	2.8 (1.2)	8.7(2.2)	0.0 (1.3)
15 min	-64 (31)	77 (26)	68 (33)	1.4 (1.3)	8.2(2.0)	0.0 (1.3)
30 min	-80 (29)	67 (30)	75 (41)	1.3 (1.6)	5.7(1.5)	-1.4 (1.3)
45 min	-57 (29)	61 (30)	68 (50)	-1.2 (1.4)	5.5(1.3)	-1.8 (1.3)
60 min	-42 (31)	64 (34)	103 (46)	-1.4 (1.2)	7.3(2.8)	-2.4 (1.2)

The table shows that both drugs increased FEV1 significantly; the maximum effect occurred 15 min after fenoterol and 60 min after ipratropium. Heart rate increased 5 min after fenoterol, and the effect lasted for 60 min. Needle threading time was prolonged after fenoterol, but the effect was variable (not shown).

These results show that inhaled ipratropium and fenoterol both cause bronchodilation in normal subjects. The effect of ipratropium suggests that resting airway calibre is partly governed by parasympathetic tone.

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MAINTAINED HYPOTENSIVE EFFECT OF HUMAN α-CGRP AFTER INFUSION FOR UP TO 4 HOURS IN CONSCIOUS RATS.

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Recent work on guinea-pig atrium showed that tachyphylaxis to the positive inotropic and chronotropic effect of rat α-CGRP occured within 15-20 min (Franco-Cereceda & Lundberg,1985). Human α-calcitonin gene-related peptide (hα-CGRP) evoked hypotension in normotensive and spontaneously hypertensive rats (Marshall et al,1986, Craig et al,1986). Therefore in the present experiments up to 4h infusions of hα-CGRP were given to see if tachyphylaxis developed to the hypotensive effect of the peptide in conscious rats.

Female normotensive Sprague Dawley rats (150-250g) or Wistar-Kyoto derived spontaneously hypertensive rats (SHR,170-200g) were anaesthetised with halothane and the left external jugular vein and left carotid artery were cannulated and exteriorised. After recovery, mean arterial pressure (MAP) and heart rate (HR) were recorded via the carotid artery and the jugular vein was connected to an infusion pump for drug administration. In some experiments, each dose of hα-CGRP (10^{-11}-10^{-9} mol kg^{-1} min^{-1}, given cumulatively) was infused for 10 min. In other experiments hα-CGRP, $3x10^{-10}$ mol kg^{-1} min^{-1} was infused for 1h or 4h. Control rats received appropriate infusions of saline. In addition, saline was infused for at least 10 min prior to hα-CGRP and for 20 min afterwards.

Human α-CGRP produced a dose-related hypotension in normotensive rats from a pre-drug MAP of 112±4 mm Hg (mean ± s.e.mean) to 89±7 mm Hg and 65±6 mm Hg with 10^{-10} mol kg^{-1} min^{-1} and 10^{-9} mol kg^{-1} min^{-1} respectively. Dose-dependent tachycardia accompanied the hypotension, with a maximum increase to 529±16 b min^{-1} from a pre-drug HR of 384±12 b min^{-1}. Saline administration did not affect either parameter. In SHR's, the pre-drug MAP was 178±4 mm Hg. Human α-CGRP 10^{-10} and 10^{-9} mol kg^{-1} min^{-1} produced falls in MAP to 160±6 mm Hg and 78±4 mm Hg respectively. The initial HR, 406±14 b min^{-1}, was increased by hα-CGRP to 503±7 b min^{-1}. In both normotensive rats and SHR's, when saline was infused subsequent to hα-CGRP, MAP and HR returned towards resting levels.

Normotensive rats infused with $3x10^{-10}$ mol kg^{-1} min^{-1} hα-CGRP for 1h had a MAP of 116±6 mm Hg (0 min) which fell to 93±5 mm Hg at 20 mins. This was maintained over the infusion time, e.g. 91±8 mm Hg at 55 mins. During the infusion the HR rose from 378±8 b min^{-1} at 0 min to 528±8 b min^{-1} at 20 mins and 510±10 b min^{-1} at 55 mins. Saline infusion over the same time did not affect either HR or MAP. The same dose ($3x10^{-10}$ mol kg^{-1} min^{-1}) of hα-CGRP when infused into normotensive rats for 4h, gave a well maintained hypotensive effect, with a fall from 121±3 mm Hg at 0 min to 81±6 mm Hg at 20 min, 85±4 mm Hg at 1h and 87±3 at 4h. The increase in HR was also sustained, being 375±6 b min^{-1} at 0 min, 518±9 at 20 min, 498±13 b min^{-1} at 1h and 503±7 b min^{-1} at 4h. Infusion of saline did not affect either MAP or HR. After both 1h and 4h infusions, MAP appeared to recover more slowly than HR.

In conclusion, infusing hα-CGRP into conscious normotensive rats and SHR's, produced dose-related falls in MAP and a rise in HR. The present results with 1h and 4h infusions of peptide demonstrate that tachyphylaxis does not develop to the hypotensive effect of hα-CGRP. These findings in vivo contrast with the recently reported work on guinea-pig atrium (Franco-Cereceda & Lundberg, 1985).

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EFFECTS OF HUMAN CALCITONIN GENE-RELATED PEPTIDE OF HUMAN ENDOTHELIAL CELLS

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The vasodilatation mediated by human calcitonin gene related peptide (hCGRP) is dependent on intact vascular endothelium in vitro (Hughes et al, 1985). We have previously reported to this Society that hCGRP activates adenylate cyclase but does not release prostacyclin from bovine aortic endothelial cells (Crossman et al, 1986). We have now examined the response of human endothelial cells to hCGRP, with measurement of prostacyclin release and activation of adenylate cyclase.

Human umbilical vein endothelial cells were prepared by a modification of a technique described previously (Jaffe et al, 1973). Endothelial cells were grown to confluence (24-72 h) and then sub-cultured on microbeads as described previously for superfusion studies (Gordon & Martin, 1983). Basal release of 6-oxo-$PGF_{1\alpha}$, the stable hydrolysis product of prostacyclin, was 200 pg/ml/10^6 cells, measured by radioimmunoassay of the superfusate. There was a dose-dependent increase in the release of 6-oxo-$PGF_{1\alpha}$ when the perfusate was changed to one containing selected concentrations of hCGRP. When hCGRP was 1 μM, release of 6-oxo-$PGF_{1\alpha}$ was 1230 ± 534 pg/ml/10^6 cells (n = 4). The rise was ten-fold less than maximal stimulation achieved with 1 μM bradykinin, which was included at the end of each experiment as a positive control.

Adenylate cyclase activity was measured in membrane preparations of endothelial cells by the method of Salomon et al (1974). hCGRP produced a dose-dependent increase in adenylate cyclase activity from a basal level of 4.7 pmol cyclic AMP/min/mg protein to a maximum of 20 pmol cyclic AMP /min/mg protein (n = 2). The Kact value was 190 nM (n = 2). A linear Eadie-Hofstee transformation suggested a simple bimolecular interaction between hCGRP and a single receptor population. In similar experiments there was no significant bradykinin-dependent enzyme activation at concentrations up to 1 μM.

The interaction of hCGRP with endothelium produces at least two distinct biological responses. First, there is a 4-fold increase in adenylate cyclase activity. Secondly, the vasodilator prostaglandin, prostacyclin, is released in a concentration-dependent manner. There is, however, no bradykinin dependent activation of adenylate cyclase, although prostacyclin is released in large amounts. This suggests that prostacyclin release and the activation of adenylate cyclase seen in response to hCGRP are not causally related.

hCGRP is a potent vasodilator in vivo and in vitro and the relaxation of vascular smooth muscle is dependent on the presence of an intact endothelium. The vasodilator properties of hCGRP may be mediated, at least in part, by vasodilator factors, including prostacyclin, which are released from vascular endothelium.

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THE EFFECTS OF CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P ON RESISTANCE AND CAPACITANCE VESSELS

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The vasoactive neuropeptides calcitonin gene-related peptide (CGRP) and substance P are co-localized in primary sensory neurones (Lundberg et al, 1985). We have compared the effects of brachial artery infusion of CGRP and substance P on resistance vessels, measuring forearm blood flow by venous occlusion plethysmography. We examined also the effect of the instillation of CGRP and substance P on venous tone of superficial veins, at rest, when preconstricted with noradrenaline, or when stimulated to constrict by the single deep breath reflex. Methods involved a lever device attached to a Harvard displacement transducer.

Brachial artery infusion of CGRP at doses of 1.25, 2.5, 3.75, 5.00 and 10 pmoles/min for 8 min caused a dose-dependent increase in forearm blood flow from basal 3.28 ± 0.56 ml/min/100 ml forearm volume to 9.08 ± 2.12 ml/min/100 ml at the highest dose ($n = 6$, $P < 0.01$, analysis of variance). The onset of vasodilatation was rapid, 90% of maximum dilatation, occurring within the first 4 min of infusion of any dose. The biological effective half-life was 20 min. Substance P was effective at lower doses. Infusions of 0.25, 0.5, 1, 1.5 pmoles/min caused a dose-dependent increase in forearm blood flow from 2.57 to 7.6 ml/min/100 ml forearm volume. The onset of vasodilatation was rapid but, in contrast to CGRP, the offset of vasodilatation after cessation of the substance P infusion was also rapid, returning to basal flow within 5 min.

Vasodilatation persisted during a prolonged infusion of CGRP 10 pmol/min for 30 min, but during an infusion of substance P 1.5 pmoles/min for 30 min there was a 16% decrease from maximum blood flow.

Infusion of CGRP at up to 10 pmoles/min into a superficial vein had no effect on resting venous tone, or that following 70% maximum constriction by noradrenaline. The vasoconstrictor response to a single deep breath was not inhibited by CGRP infusion. Substance P had no effect on resting venous tone, but relaxed promptly those veins constricted by noradrenaline, and also inhibited the venoconstrictor reflex. The lowest effective dose of substance P that reversed noradrenaline-mediated venoconstriction was 100 fmoles/min, but there was rapid development of tachyphylaxis and a dose response curve could not be constructed.

In summary, CGRP and substance P are potent dilators of resistance vessels. Substance P is also a venodilator, while CGRP appears to be a selective arterial dilator. Although CGRP and substance P are co-localized in some nerves, their different patterns of offset of actions and tachyphylaxis suggests that they have different modes of action on blood vessels. Both CGRP and substance P may be involved in the control of vascular smooth muscle tone.

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ß-ADRENOCEPTORS IN GUINEA-PIG GASTRIC FUNDUS - ARE THEY THE SAME AS THE 'ATYPICAL' ß-ADRENOCEPTORS IN RAT ADIPOCYTES?

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The ß-adrenoceptors mediating lipolysis in rat adipocytes are atypical, being resistant to ß-adrenoceptor antagonists (Stanton, 1972: Harms et al. 1980). Recently, a novel class of ß-adrenoceptor agonists has been discovered, which are highly potent and selective at this atypical adipocyte ß-adrenoceptor (Wilson et al., 1984). These compounds therefore provide evidence for a novel type of ß-adrenoceptor in rat adipocytes, and also serve as tools with which to investigate the presence of such receptors elsewhere.

The ß-adrenoceptors mediating gastrointestinal smooth muscle relaxation are also resistant to ß-adrenoceptor antagonists (Wikberg, 1977; Grivegnée et al., 1984; Bond et al., 1986; Dettmar et al., 1986). We have therefore tested a lipolytic ß-adrenoceptor agonist, BRL35135A (BRL, Wilson et al., 1984) on a preparation of gastrointestinal smooth muscle, guinea-pig stomach fundus (GPF). Strips of GPF were suspended in Krebs solution at 37°C containing atropine (0.4µM), phenoxybenzamine (1µM) and indomethacin (2.8µM), and were contracted by the addition of prostaglandin $F_{2\alpha}$ (2.9µM).

BRL relaxed GPF in a concentration-related fashion, with a mean EC_{50} (95% C.L.) of 63.3 (43.5-92.0, n=28)nM. Its potency was unaffected by the presence in the bathing solution of mepyramine (0.1µM), ranitidine (100µM), SCH 23990 (0.01µM), domperidone (1µM), methysergide (0.1µM), yohimbine (10µM) or phentolamine (1µM). Thus, BRL is unlikely to act at histamine, dopamine or 5-HT-receptors or α-adrenoceptors, and the presence of indomethacin rules out an action via endogenous prostanoid release. BRL exhibited marked tachyphlaxis with a mean rightward displacement of a second concentration-effect curve of about 16-fold. The ß-adrenoceptor agonist, isoprenaline (1SO), noradrenaline (NA) and salbutamol (SAL) did not exhibit tachyphylaxis. Concentration-effect curves to BRL were parallel to those to 1SO, NA and SAL and the rank order of agonist potency (equipotent concentration, 1SO=1) was 1SO (1) ≃ BRL (1.25) > NA (3.7) > SAL (37). Propranolol (10µM) caused only 33 (14-77, n=5), 23 (11-48, n=7), 4 (2-10, n=6) and 5 (0.5-44, n=5) fold rightward shifts (mean (95% confidence limits) of n values) of concentration-effect curves to 1SO, NA, SAL and BRL respectively.

The relatively high potency of BRL on GPF, together with the relatively weak antagonism of ß-adrenoceptor agonists by propranolol support the concept of the existence in GPF of 'atypical' ß-adrenoceptors similar to those in rat adipocytes. However, the reason for the observed tachyphylaxis to BRL is at present unclear.

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