THE DESCENDING CONTROL OF NOCICEPTION AND THE SITE OF ACTION OF MORPHINE IN THE ANAESTHETIZED CAT

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It has been concluded (Clark & Ryall, 1982a,b) that when the highly potent analgesic substance, etorphine, is microinjected into the brainstem, the antinociceptive action on dorsal horn neurones could be explained by the efflux of the opiate from the brain into the circulation and thence into the spinal cord. The antagonistic effect of naloxone was due to a similar mechanism. It was therefore of interest to determine whether the same mechanism could account for the antinociceptive effect of microinjected morphine, which is much less lipid-soluble than either etorphine or naloxone.

The discharge of dorsal horn neurones in L7 to noxious radiant heat was monitored in 29 cats with an intact spinal cord and in 8 cats with the cord transected at L2. Most experiments were carried out in pentobarbitone-anaesthetized animals except for three experiments in which the cord was intact but in which the animals were decerebrated one day earlier and anaesthesia was subsequently discontinued.

After spinal transection, the microinjection of morphine sulphate (0.1 M) to the brainstem only inhibited the excitation of dorsal horn neurones when the total amount injected approached the amount required to cause inhibition with intravenous administration in cats with intact or transected spinal cords. This suggests that morphine can leave the brain in significant amounts, as was found for etorphine.

However, when the cord was intact, unlike etorphine, dorsal horn neurones were more sensitive to morphine microinjected into the periaqueductal grey, nucleus raphe magnus or nucleus reticularis magnocellularis than they were to intravenously administered opiate or to microinjected opiate in spinal cats. With the cord intact, effective amounts of morphine microinjected into the brainstem ranged from 28 to 285 µg of base (mean 120 µg) in different animals, except in one experiment in which 1140 µg was required. The inhibitory effects were often small in extent and variable when the microinjection was repeated in the same animal. The amount required to cause inhibition was about ten times less than that found necessary in spinal cats or when injected intravenously. The effects with microinjection when the cord was intact cannot be explained simply by the entry of morphine into the circulation but are presumed to reflect a local action in the brain.

Nevertheless, it is considered that the local concentrations probably achieved by the microinjection of relatively large amounts of morphine in highly concentrated solutions at and near the site of injection in this and other similar studies are likely to be in excess of 10^{-4} M within a radius of 2 mm from the injection site. The concentrations are consequently greatly in excess of those which could ever be achieved by systemic administration of analgesic doses. It is therefore doubtful whether the inhibitory effects obtained by microinjection in the brainstem are of significance in relation to the hypothesis that systemic opiates cause analgesia in part by activation of descending inhibitory systems.

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EVIDENCE FOR THE PRESENCE OF $GABA_B$ RECEPTORS ON CEREBELLAR PURKINJE DENDRITES

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Baclofen-sensitive, bicuculline-resistant $GABA_B$ receptors are distributed throughout the mammalian CNS although there are regional variations. A region of particularly high density is the cerebellum where, unlike classical $GABA_A$ sites, $GABA_B$ sites are confined to the molecular layer (Wilkin et al. 1981). Their cellular location within this layer is, however, unknown. Previous studies with peripheral nervous tissue have indicated that $GABA_B$ sites are present on nerve terminals and when activated result in diminished transmitter release (Bowery et al. 1981). In an attempt to discern their location within the cerebellum we have utilised mutant mice characterised by abnormal cerebellar development. Four mutant types have been studied:- (a) Weaver (wv/wv 20-23 days old: n=5): (b) Staggerer (sg/sg 18-20 days old: n=4): (c) Lurcher (Lc/+ 75-89 days old: n=4): (d) Stumbler (stu/stu 20 days: n=7).

In the Weaver and Staggerer there is an absence of granule cells with a secondary diminution in the Purkinje cell dendrites. The Lurcher is devoid of cerebellar Purkinje cells with about 20% of the normal level of granule cells. The Stumbler aberration is restricted to a stunting of Purkinje dendrites. All other cells in the cerebellum of this mutant appear to be normal (Caddy and Sidman, 1981).

The presence of $GABA_B$ binding sites was determined by incubation of 10 µm cerebellar sections in ^3H-GABA (50 nM for 15 min) followed by a dry-mounting autoradiographic technique or liquid scintillation spectrometry (Wilkin et al. 1981). Frozen sections were prepared from cerebella perfuse-fixed with 0.1% paraformaldehyde in 0.01M phosphate buffer (pH 7.4). In all cases comparison was made with sections prepared from normal littermates.

Sections from the agranular mutants (Weaver and Staggerer) exhibited a 49% and 33% decrease in $GABA_B$ sites respectively after taking into account the decrease in cerebellar size. Whereas <5% of the normal $GABA_B$ site population was present in slices of the Lurcher cerebellum. Taken together these observations indicate that $GABA_B$ sites are unlikely to be located primarily on granule cell terminals since binding still occurred in their absence and failed to occur when a proportion of the cells remained as in the Lurcher. The observations also suggest that the presence of Purkinje cells is important for binding to occur. However, autoradiographic studies in the normal cerebellum indicate that $GABA_B$ sites are not on Purkinje perikarya. In the Stumbler mutant, binding to $GABA_B$ sites was reduced by 60%. Since in this mutant only the Purkinje dendrites are affected, this supports the notion that $GABA_B$ sites are located on the dendritic processes of Purkinje cells. Thus $GABA_B$ sites would appear to be postsynaptic in the cerebellum in contrast to their presence on nerve terminals in the striatum (Kilkpatrick et al. 1983) and peripheral autonomic systems (Bowery et al. 1981). It is therefore possible that within the cerebellum these sites are innervated by Basket or Stellate cell axons.

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ARE α_2-ADRENOCEPTORS INVOLVED IN MEDIATING THE NEURONAL EXCITATION TO NORADRENALINE IN THE CEREBRAL CORTEX?

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There is evidence that the excitatory responses of cortical neurones to noradrenaline are mediated by α_1-adrenoceptors: methoxamine and phenylephrine, selective α_1-adrenoceptor agonists, evoke only excitatory responses, and these responses can be antagonized by the selective α_1-adrenoceptor antagonists prazosin and haloperidol (Bradshaw et al, 1981, 1982). In the present experiments we examined whether α_2-adrenoceptors, which also occur in the cerebral cortex (Langer, 1980), might play a role in mediating neuronal responses to noradrenaline. In Experiment I, we examined whether the α_2-adrenoceptor antagonists yohimbine and RX781094 (Chapleo et al, 1981) can discriminate between neuronal responses to phenylephrine, a selective α_1-adrenoceptor agonist, and noradrenaline, a mixed α_1- and α_2-adrenoceptor stimulant (Langer, 1980). In Experiment II, we investigated the effects of the selective α_2-adrenoceptor agonist UK14304 (Cambridge, 1981).

Spontaneously active single neurones were studied in the somatosensory cortex of the halothane-anaesthetized rat; all the drugs were applied by microelectrophoresis (Bradshaw et al, 1982).

The effect of yohimbine on responses to noradrenaline and phenylephrine was compared on 8 cells. Acetylcholine was used as a control agonist. Yohimbine had no selective effect on the responses: responses to the two amines and acetylcholine were equally reduced in size. The effect of RX781094 was studied on 10 cells. On all of these, RX781094 equally antagonized responses to noradrenaline and phenylephrine; on 3 cells the antagonism of the responses to the amines occured when the response to acetylcholine was not affected; on 7 cells the response to acetylcholine was also reduced in size.

The effects of UK14304 and phenylephrine were compared on 24 cells; 4 of these yielded weak excitations to UK14304, the remaining 20, however, did not respond to UK14304 applied by ejecting currents of up to 150 nA. The effect of UK14304 on responses to phenylephrine and acetylcholine was compared on 22 cells. On all of these, the response to phenylephrine was antagonized, while the response to acetylcholine was hardly affected [percentage change in the size of the response in the presence of UK14304, mean $\pm$ s.e.mean: -86.6 $\pm$ 2.4 (phenylephrine); -9.5 $\pm$ 4.6 (acetylcholine); the antagonism of the response to phenylephrine was statistically significant ($P < 0.001$; t-test)]. On 10 of these cells responses to noradrenaline were also studied: responses to noradrenaline were antagonized (-50.2 $\pm$ 10.2, $P < 0.001$), although to a somewhat smaller degree than were responses to phenylephrine ($P < 0.01$).

The lack of a specific effect of the α_2-adrenoceptor antagonists on the response to noradrenaline argues against an α_2-adrenoceptor mediated component in this response. Furthermore, our observations with UK14304 suggest that this drug, similarly to clonidine (Bradshaw et al, 1982), acts at α_1-, rather than at α_2-adrenoceptors on cortical neurones.

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RESPONSES OF SINGLE RESPIRATORY-RELATED NEURONES IN THE RAT BRAIN STEM TO MICROIONTOPHORETICALLY APPLIED FENTANYL AND ITS CONGENERS

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Fentanyl is an anilidopiperidine analgesic which is 100 times more potent than morphine (Janssen, et al, 1963); its congeners are sufentanil, lofentanil and alfentanil. Within this series there are considerable variations of potency and duration of action. There is also marked depression of respiration. We have studied the effects of these drugs on respiratory-related (RRN) and non-respiratory (nRRN) neurones in the brainstem of the rat, using the technique of microiontophoresis.

Male Wistar rats (200-400g) were anaesthetised with urethane (1.75 g/kg i.p.) and prepared for use with conventional microiontophoretic techniques as previously described (Bradley and Dray, 1974). RRN were identified by their characteristic firing pattern. Neuronal activity and the diaphragm electromyogram were displayed on a dual-beam oscilloscope and photographed. Action potentials were also counted electronically. Depending on which phase of the respiratory cycle peak firing occurred, RRN were further classified as "inspiratory" or "expiratory". Five-barrelled micropipettes were used in all experiments. The recording barrel was filled with 4M NaCl. One other barrel was filled with Pontamine Sky Blue (2.5% in Na acetate buffer, pH 5.6) for current balancing, current controls and routine marking of neurones (Boakes, et al., 1974). The other barrels contained a selection of the following: fentanyl citrate, sufentanil citrate, lofentanil oxalate and alfentanil hydrochloride (all 25 mM, pH 4-5), naloxone hydrochloride (20 mM, pH 4.5-5.5), γ-aminobutyric acid (GABA 0.5M, pH 4-5), glycine (0.2 M, pH 4-5), picrotoxin (5 mM, pH 6.5), strychnine nitrate (5 mM, pH 6.5), tri-sodium citrate (25 mM, pH 4 or 8) and hydrochloric acid (0.1 N, pH 4).

The effects of fentanyl and its congeners were examined on 324 spontaneously active neurones (166 RRN and 158 nRRN). The main response observed with all four drugs was a depression of the firing rate of both RRN and nRRN. Alfentanil produced shallow depressant responses which were slow in onset and similar to the responses produced by etorphine but of shorter duration. Depressant responses to fentanyl, sufentanil and lofentanil could be classified into "fast", "slow" and "combined" responses. "Fast" responses had a rapid onset (<5 sec) and were of relatively short duration (20 sec. - 2 min). "Slow" responses had a slower onset (normally 20 - 30 sec) and lasted for more than 5 min. "Combined" responses consisted of an initial "fast" response, followed by a "slow" response. Naloxone antagonised the "slow" responses produced by all four drugs, including the "slow" component of the "combined" responses. However, the "fast" responses were naloxone-resistant.

The "fast" response was not mimicked by either current or pH controls (0.1 N HCl, pH 4). Nor was it associated with any changes in spike height, eg. due to a local anaesthetic effect. Both fentanyl and sufentanil still evoked "fast" responses when citrate was applied concurrently from an adjacent barrel. As the "fast" response showed some similarity to responses produced by GABA or glycine, the effects of picrotoxin and strychnine were examined. However, although they blocked GABA or glycine, respectively, "fast" responses to fentanyl were unaffected.

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CARDIOVASCULAR RESPONSES TO MICROINJECTION OF CARBACHOL AND 5-HT INTO THE NUCLEUS TRACTUS SOLITARII IN ANAESTHETIZED RATS

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Afferent neurones from baroreceptors terminate in the nucleus tractus solitarii (NTS), which also contains short interneurones and receives inputs from other regions of the CNS (Palkovits & Záborszky, 1977). It is therefore likely that various neurotransmitters are employed at this site. We have made a preliminary examination of the actions of two drugs, carbachol and 5-HT, on this nucleus.

Male Wistar rats (250-380 g) were anaesthetised with urethane (1.6 g/kg, i.p.). The ventral tail artery was cannulated to record arterial BP and heart rate. Core temperature was maintained at 37°C with a heating lamp. The head was fixed in a stereotaxic frame, using the orientation of Pellegrino et al, (1979). The NTS was located with a 30-g cannula-electrode at either AP-6.4, LO.7 (intermediate NTS) or AP-7.4, LO.1 (commissural NTS). The cannula was lowered in 0.2 mm steps at 1 min intervals, until the maximum response of hypotension and bradycardia was evoked by electrical stimulation (4V; 1 ms; 8Hz; for 5s). Drugs were injected at these sites in 0.1 µl saline. At the end of each experiment 0.1 µl dye was injected to locate the injection site. The brains were perfuse-fixed with formal saline and serial 50 µ coronal sections cut on a freezing microtome.

Carbachol in a small dose (25 ng (0.14 nmol)) produced an immediate sharp fall in BP, followed by a rise, when injected into the intermediate NTS. The secondary rise in BP may have been due to diffusion into the commissural NTS, since the same dose evoked an elevation of BP when injected here (Figure 1). A much larger dose of 5-HT (1 µg (5.6 nmol)) was needed to evoke measurable responses: an initial fall followed by a prolonged rise in BP was obtained from both intermediate and commissural regions of the NTS (Figure 1). Changes in heart rate with either drug were small and parallel to the changes in BP. Injection of saline had little effect; injection of either drug into adjacent structures produced delayed responses.

Thus whereas the NTS is relatively insensitive to 5-HT, the regional heterogeneity of the NTS in its response to carbachol is interesting and warrants further study.

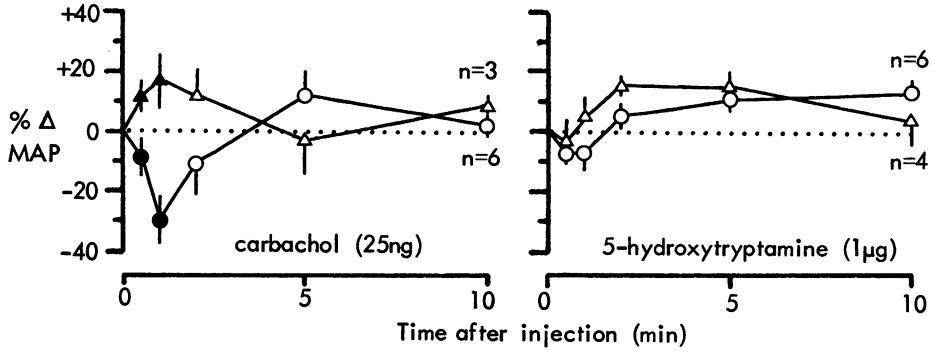

Figure 1. Drug-induced responses evoked from intermediate (O) and commissural (Δ) regions of NTS. Filled symbols indicate $P<0.05$ between ● and ▲ (Mann-Whitney test)

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BLOCKADE BY (-)3PPP OF THE DOPAMINE RECEPTORS THAT MODULATE THE RELEASE OF (^{3}H)-ACETYLCHOLINE IN RAT STRIATAL SLICES

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d-Amphetamine (Amph) inhibits the electrically-evoked release of ^{3}H-acetylcholine (^{3}H-Ach) from slices of rat corpus striatum through the activation of dopamine receptors by dopamine released from a reserpine-resistant pool (Langer et al., 1982). It was recently reported that the putative dopamine autoreceptor agonist, 3-(3-hydroxyphenyl) N-n-propylpiperidine, (+)3PPP, antagonizes d-Amph induced locomotor stimulation and stereotypy (Clark et al., 1982). We have studied the effects of (-)3PPP on the inhibition by d-Amph of the electrically-evoked release of ^{3}H-Ach in striatal slices from rats pretreated with reserpine.

Slices of corpus striatum from untreated or reserpine-treated rats (5 mg/kg, s.c. 24 hr) were labelled with ^{3}H-methyl-choline and superfused with Krebs' solution. Two periods of electrical stimulation (S_1 and S_2 ; 1 Hz, 2 msec, 2 min, 16 mA) were applied with a 44 min interval. (-)3PPP 1 µM was added 20 min before S_1 ; Amph and apomorphine (Apo) were added 20 min before S_2.

Table 1 : Antagonism by (-)3PPP of the inhibitory effects of Amph and Apo on electrically-evoked ^{3}H-Ach release from slices of rat striatum.

			n	S_1	S_2/S_1
(Reserpine)		Control	4	3.34 ± 0.18	0.85 ± 0.02
		Amph 1.0 µM	4	1.93 ± 0.33	0.39 ± 0.03*
(-)3PPP 0.1 µM)	+	Amph 1.0 µM	3	1.97 ± 0.60	0.50 ± 0.03*
(-)3PPP 1.0 µM	+	Amph 1.0 µM	3	2.73 ± 0.29	0.80 ± 0.01
(Untreated)		Control	8	2.36 ± 0.25	0.78 ± 0.02
		Apo 0.03 µM	8	2.07 ± 0.27	0.37 ± 0.02*
(-)3PPP 0.1 µM	+	Apo 0.03 µM	3	3.68 ± 0.37	0.49 ± 0.01*
(-)3PPP 1.0 µM	+	Apo 0.03 µM	3	1.63 ± 0.40	0.80 ± 0.04

*$p<0.001$, when compared with the corresponding control group.

(-)3PPP 1 µM added before S_2 did not modify the release of ^{3}H-Ach from untreated or reserpine treated rats. As shown in Table 1, the inhibition of electrically-evoked ^{3}H-Ach release by Amph 1 µM and Apo 0.03 µM was antagonized in a concentration-dependent manner by (-)3PPP. These inhibitory effects of Amph and Apo on ^{3}H-Ach release were also antagonized by 0.01 µM sulpiride (data not shown).

These results support the view that the (-) isomer of 3PPP is a dopamine receptor antagonist at dopamine receptors modulating ^{3}H-Ach release. Dopamine blocking properties of (+)3PPP have been recently reported (Watling et al., 1982). In addition, we have recently reported that (+) and (-)3PPP are devoid of agonist or antagonist activity at the dopamine autoreceptor that inhibits dopaminergic transmission in slices of the rabbit caudate (Langer et al., 1982). These results support the view that pharmacological differences exist between the dopamine autoreceptor and the dopamine receptor that modulates ^{3}H-Ach release in the striatum (Lehmann and Langer, 1982). In view of the effects obtained with (-)3PPP "in vitro", it is suggested that the antidopaminergic activity of (+)3PPP could be in part due to dopamine receptor blocking properties of the compound.

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ENDOGENOUSLY RELEASED DOPAMINE DISPLACES (^{3}H)-HALOPERIDOL FROM THE STRIATUM OF ANAESTHETIZED RATS

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A method has been described whereby ^{3}H-haloperidol labels specific sites in the striatum of anaesthetised rats (Forster & Ungerstedt, 1982) and utilises the brain dialysis technique developed by Ungerstedt et al (1982). Various neuroleptics and agonists displaced this ligand indicating that ^{3}H-haloperidol was labelling dopaminergic sites. However, in order to confirm that specific sites were being labelled, dopamine should be the most potent endogenous displacer. Therefore this present investigation was designed to determine if endogenously released dopamine could displace ^{3}H-haloperidol from the striatum of anaesthetised rats.

Dialysis fibres were implanted as described previously (Forster & Ungerstedt, 1982). An insulated, monopolar electrode (tip 0.3 mm) was lowered into the vicinity of the right nigro-neostriatal pathway, 5.2 mm posterior and 1.1 mm lateral of Bregma. Ringer solution was allowed to perfuse the dialysis fibre for 1h and perfusate samples were collected at 20 min intervals. Afterwhich the perfusing medium was changed to ^{3}H-haloperidol (14.2Ci. mmol.) and continued throughout the remainder of the experiment. Similarly, 20 min samples were collected and following the first hour the electrode was lowered into the nigro-striatal pathway (a total depth of 7.2 mm). Following this manipulation 2 further samples were collected so that any mechanical release of neurotransmitter returned to basal level before the onset of stimulation. The stimulation parameters were indentical to those used by Arbuthnott et al (1970) and the stimulation was made for 5 min at the start of a collection period (2 samples being collected between successive stimulations). Samples were analysed for dopamine and metabolites as described previously (Bennett et al, 1982) and radioactivity measured in 10 ul of each sample.

A significant increase in dopamine was observed with ^{3}H-haloperiodol prior to electrical stimulation. In each stimulation sample there was an increase in dopamine release of at least 100% which decreased to pre-stimulation levels in the 2 unstimulated samples. DOPAC showed similar changes to dopamine although the increase was not as great. HVA reflected the greatest change in metabolites, the increase being most pronounced in the sample collected following the stimulation, similarly the radioactivity was highest in these samples. The % increase in radioactivity being 10-30% in the stimulation samples and 14% in the post-stimulation sample.

Hence endogenous released dopamine displaces radioactivity from striatal sites, the displacement being of similar order to that seen when exogenous dopamine was perfused (Forster & Ungerstedt). Confirmation of dopamine release due to stimulation was demonstrated together with a concomitant increase in DOPAC and HVA.

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PERGOLIDE INHIBITS THE RISE IN NEURALLY RELEASED NORADRENALINE IN PITHED RATS SUBJECTED TO SPINAL CORD STIMULATION

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Pergolide is a dopamine receptor agonist. In conscious spontaneously hypertensive rats or in pentobarbital anaesthetized normotensive rats, this ergoline derivative decreases blood pressure and heart rate (Yen et al., 1979; Cavero, 1982). Furthermore, pergolide inhibits the pressor responses evoked by electrical stimulation of the spinal cord (Hahn, 1981; Cavero, 1982). This paper reports that the latter effect is associated with a decrease in the plasma content of noradrenaline.

Normotensive rats (Crl: SD^R CD, Charles River Laboratories, France) were adrenalectomized and given saline to drink for the week preceding the experimental procedure. A group of rats was anaesthetized with pentobarbitone and the effects of pergolide (30.0 µg/kg i.v.) studied on mean carotid artery pressure and heart rate. Other groups of rats were pithed, given i.v. methylatropine (1.0 mg/kg), d-tubocurarine (5.0 mg/kg) and lithium heparine (500 U/kg). A carotid artery was cannulated for measuring blood pressure and the other for blood withdrawal in ice cooled heparinized tubes. The plasma content of noradrenaline and adrenaline was measured using a double isotope technique (Brown and Jenner, 1981). A 1 ml sample of blood was taken 10 min before and 30 sec after beginning the stimulation of the spinal cord (0.5 Hz, 10 msec, 50 V for 60 sec). After the first sample, the animals were given 1.0 ml of freshly prepared rat plasma. The rats then received saline or pergolide (30.0 µg/kg, i.v.) as treatments.

In adrenalectomized pentobarbital anesthetized rats, pergolide produced a sustained decrease in arterial pressure which was 44.5 ± 4.2 mmHg (base-line value: 134.5 ± 1.3 mmHg) 15 min after its administration. In pithed rats, the plasma content of noradrenaline during spinal cord stimulation was not modified by saline, but it was significantly decreased by 49% in rats given pergolide. Similarly, this compound reduced by 60% the pressor response to this stimulation. In these rats, we did not detect adrenaline in the plasma.

These results indicate that in an in vivo preparation, a dopamine agonist, like pergolide, inhibits the elevation of plasma noradrenaline associated with the vascular response to stimulation of sympathetic nerve fibers.

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SELECTIVITY OF SOME ERGOT DERIVATIVES FOR α_1- AND α_2-ADRENOCEPTORS OF RAT CEREBRAL CORTEX

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A number of ergots, including bromocryptine, lergotrile and pergolide, possess actions as directly acting agonists at dopamine receptors of the D-2 subtype which make them useful in Parkinson's disease and hyperprolactinaemia (Schachter et al., 1980). Since many ergots have α-adrenergic actions, particularly on the peripheral nervous system, the aim of the present study was to examine the actions of new synthetic ergolines at central α_1- and α_2-adrenoceptors.

Washed cerebral cortical membranes from rat brain were incubated with ^{3}H-rauwolscine or ^{3}H-prazosin for 30 min at 25°C (McPherson & Summers, 1982). The incubations were terminated by rapid vacuum filtration through Whatman GF/B glass fibre filters and the retained radioactivity was counted by liquid scintillation spectrometry. Phentolamine (10 μM) was used to define non-specific binding. The binding of ^{3}H-spiperone to D-2 receptors present in membranes of the rat corpus striatum was determined as previously described (Beart & McDonald, 1982). Binding data were analysed by computer-assisted iterative curve fitting.

Table 1 Inhibition by ergot derivatives of specific ^{3}H-rauwolscine, ^{3}H-prazosin and ^{3}H-spiperone binding

	K_i (nM) ^{3}H-Rauwolscine	K_i (nM) ^{3}H-Prazosin	α_2/α_1 Selectivity	K_i (nM) ^{3}H-Spiperone	α_2/D-2 Selectivity
Lisuride	0.54	27	50	2.1	3.9
Lergotrile	15	4.8	0.32	9.0	0.58
Pergolide	52	560	11	3.5	0.07
LY-158A	36	330	9.3	8.8	0.25
LY-062	3.6	130	36	12	3.2
CM 29-712	94	510	5.4	34	0.36
CQ 32-084	35	>5 μM	>96	5.1	0.15
Bromocryptine	120	18	0.16	1.6	0.01
Dihydroergocryptine	12	12	1.1	19	1.6
LY-141865	>5 μM	>5 μM	-	680	-
Phentolamine	2.4	5.2	2.1	>5 μM	-

All ergot derivatives, except LY-141865 and CQ 32-084, were able to totally displace specific ^{3}H-rauwolscine and ^{3}H-prazosin binding (Table 1). Markedly different rank orders of potency were observed for the displacement of ^{3}H-rauwolscine and ^{3}H-prazosin binding by the ergots. A marked difference in the selectivity of the ergot derivatives for either the α_1- or α_2-adrenoceptor was thus obtained. The ergot derivatives also displayed high affinity for D-2 receptors (Table 1) and differed markedly in their selectivity for either D-2 receptors or α_2-adrenoceptors.

The data suggest that lergotrile, lisuride and LY-062 might be better considered as α-adrenergic rather than dopaminergic drugs. Noradrenaline has been implicated in rage, arousal and schizophrenia (Hornykiewicz, 1982), and ergolines such as pergolide and CQ 32-084 which show pronounced selectivities for D-2 receptors over adrenoceptors may well be preferable in clinical use.

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Wy 26157: A NOVEL SELECTIVE INHIBITOR OF 5-HT REUPTAKE

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There is considerable evidence that monoamine transport depends directly or indirectly, on the activity of sodium, potassium-activated, magnesium-dependent adenosinetriphosphatase (Na^+, K^+-ATPase). It has also been shown that imipramine inhibits 5-hydroxytryptamine (5-HT) uptake by an action at a site, associated with, but distinct from,the 5-HT re-uptake site (Briley et al, 1981). One possibility is that imipramine may interact with the Na^+, K^+-ATPase enzyme complex. Consistent with this hypothesis is the finding that the substrate for this enzyme (ATP) alters the inhibitory potency of imipramine on the 5-HT re-uptake process (Blake et al, 1981). To further examine this possibility, Wy 26157 (3,7-dihydro-7-(-3{ { 2-(5-hydroxy-1H-indol-3-yl)-ethyl}amino}propyl}-1,3-dimethyl -1H purine-2,6-dione, hydrochloride), a theophylline derivative of 5-HT, was synthesised as a potential selective inhibitor of 5-HT uptake.

Monoamine uptake was studied using synaptosomes prepared form rat cerebral cortex or rat striatum (Wood & Wyllie, 1981). In preliminary experiments, the drug sensitivity of uptake of ^{3}H-5-HT, ^{3}H-noradrenaline (NA) and ^{3}H-dopamine (DA) was analysed over a 4 min incubation period at 37°C in a medium of the following composition (mM): NaCl (136) ; KCl (5), $MgCl_2$ (1.2), $CaCl_2$(2-5), glucose (10) ascorbate (1), Tris (20), ^{3}H-amine (0.0001) which was adjusted to pH7.4 with HCl and gassed with pure oxygen. Energy independent accumulation was determined by replacement of NaCl with an equiosmolar concentration of LiCl. In more detailed kinetic analyses of the nature of 5-HT uptake inhibition by Wy 26157, uptake at varying concentrations (30-200nM) of ^{3}H-5-HT were employed.

Wy 26157 was a potent inhibitor of active 5-HT uptake with an IC_{50} of 166 ± 29nM (n=4). In contrast NA and DA uptake were not substantially affected at concentrations of Wy 26157 up to 3000nM. Detailed kinetic analysis revealed that Wy 26157 was a competitive inhibitor of the 5-HT uptake system with an apparent inhibitory affinity constant (K_I) of 84 nM.

These results indicate that the theophylline derivative of 5-HT, Wy 26157, is a competitive inhibitor of the 5-HT re-uptake process in vitro with minimal effects on other monoamine transport systems. The site of action of Wy 26157 is therefore apparently dissimilar from the site of action of imipramine (Briley et al, 1981 Broadhurst et al, 1982). The competitive interaction may reflect either an action at the 5-HT recognition site or an interaction on the 5-HT carrier system.

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SUBCELLULAR FRACTIONATION OF (^{3}H)-IMIPRAMINE BINDING AFTER CHEMICAL OR ELECTROLYTIC LESION OF THE SEROTONINERGIC SYSTEM

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Studies carried out after electrolytic lesion of the dorsal raphe show that a large proportion of the ^{3}H-imipramine (^{3}H-IMI) binding sites are located on serotonin nerve terminals (Sette et al., 1981). After chemical lesion of the serotoninergic neurons with 5,7 dihydroxytriptamine (5-7 DHT), the endogenous levels of serotonin in the rat cortex decrease to a greater extent than the density of ^{3}H-IMI binding sites suggesting that ^{3}H-IMI labels sites on serotoninergic nerve terminals and also recognition sites which are insensitive to serotoninergic denervation (Brunello et al., 1982).

We examined the subcellular fractionation of ^{3}H-IMI binding sites in the rat cerebral cortex. We also measured ^{3}H-5HT uptake, endogenous levels of 5HT and some marker enzymes such as cytochrome oxydase as a mitochondrial marker and 5'Nucleotidase (5'ND) as a plasma membrane marker. In the nuclear (N) fraction the specific binding of ^{3}H-IMI was poor (3.3 % of the total) and ^{3}H-5HT uptake or endogenous serotonin were not detectable. In the heavy mitochondrial fraction (M) ^{3}H-IMI binding represented 40 % of the specific binding while nearly 90 % of the total ^{3}H-5HT uptake was present in this fraction. The (M) fraction had 60 % of the total endogenous 5HT. In the light mitochondrial fraction (L) we found only 16 % of the total ^{3}H-IMI binding, 9% of the ^{3}H-5HT uptake and 14 % of the endogenous 5HT levels. To our surprise, the microsomal fraction (P) contained 37 % of the ^{3}H-IMI binding sites although the uptake of 5HT was practically absent and the serotonin levels represented only 14 % of the total. Presented in the manner proposed by de Duve et al. (1955), histograms expressing the relative specific activity, reveal a similar distribution pattern for 5'ND and the ^{3}H-IMI binding in all fractions, whereas the cytochrome oxydase shows a specific enrichment only in the (M) fraction, reflecting the synaptosomal enrichment.

Serotoninergic denervation with 5,7 DHT or the electrolytic lesion of the raphe dorsalis modified the subcellular distribution of ^{3}H-IMI binding in the rat cortex in the following manner : the small amount of ^{3}H-IMI binding in the fraction (N) was not affected. In the fraction (M) ^{3}H-IMI binding was decreased by 30 %, the ^{3}H-5HT uptake was decreased by 70 % and the serotonin levels by 94 %. In the fraction (L), ^{3}H-IMI binding was decreased by 57 % and there was no detectable ^{3}H-5HT uptake or endogenous 5HT levels. Surprisingly, in the (P) fraction, the lesions produced an 81 % decrease in the specific ^{3}H-IMI binding and the small amount of endogenous serotonin was completely reduced after the lesion. It should be noted that, after the 5,7 DHT lesion, the Kd values of ^{3}H-IMI binding were significantly increased in fractions L and P.

It is concluded that ^{3}H-IMI binds poorly in the N fraction. The M fraction, which contains most of the ^{3}H-5HT uptake, and around 40 % of the total ^{3}H-IMI binding sites, was only marginally affected by denervation of the serotoninergic neurons. Additional studies are required to clarify the significance of the ^{3}H-IMI sites present in the P fraction and its pronounced decrease after chemical or electrolytic denervation.

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ONTOGENETIC DIFFERENCES IN STRIATAL ENKEPHALIN LEVELS IN RATS EXPOSED TO LOW LEVELS OF LEAD

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It has been suggested that some neuropsychological disorders of childhood may be related to modification of neurochemical function as a consequence of exposure to lead. Since the enkephalinergic system undergoes rapid development in the neonate we have begun studies to determine if the ontogeny of enkephalin is affected by low lead exposure.

Maternal rats were dosed with 300 and 1000 p.p.m. Pb in drinking water from conception to weaning as previously described (Carmichael et al, 1981). For each time point studied enkephalin was extracted and assayed as met-enkephalin equivalents essentially as described by Hughes et al (1977). Blood lead levels were determined by atomic absorption spectrophotometry.

Table 1 Effect of maternal lead dosing on striatal enkephalin levels of neonate rats

Age (days)	Dose (ppm) 0	300	1000
		Enkephalin (ng/g)	
10	386 ± 26 (12)	198 ± 18 (11)**	238 ± 39 (9)**
21	867 ± 150 (11)	660 ± 95 (8)	451 ± 62 (8)*
30	443 ± 42 (10)	573 ± 34 (10)*	448 ± 60 (7)
40	453 ± 64 (6)	323 ± 31 (9)	243 ± 20 (12)**

Each point represents mean ± s.e. mean. No. of observations in parentheses. Analysis of variance vs control * $p < 0.05$ ** $p < 0.01$.

Striatal enkephalin levels were as much as 50% lower than controls in lead exposed neonatal rats 10 and 21 days postpartum. Though there was an indication that this effect was reversible by 30 days (9 days after cessation of lead dosing) measures of enkephalin at a later time point indicated that the depressant effects of lead exposure were still being manifested. There was no correlation between enkephalin levels and individual blood lead levels.

These preliminary findings show that the ontogeny of striatal enkephalin is affected by low lead exposure, and indicate that the enkephalinergic system may play a part in the behavioural disorders observed in lead-exposed children.

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HISTAMINE STIMULATION OF INOSITOL 1-PHOSPHATE ACCUMULATION IN LITHIUM-TREATED SLICES FROM GUINEA-PIG BRAIN

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Histamine H_1-receptors are present in appreciable numbers in several regions of mammalian brain (Schwartz, Pollard & Quach, 1980). However, studies on the function of these receptors have been hampered by the limited number of biochemical responses which are known to be associated with H_1-receptor activation. Furthermore those responses which have been detected may be both species and tissue dependent. However, strong arguments have been made that all receptors involved in increasing intracellular calcium, which appears to be the general function of H_1-receptors, are associated with an increased hydrolysis of phospholipids (Michell, Kirk, Jones, Downes & Creba, 1981). A dramatic improvement in the sensitivity of the assay of receptor-stimulated phospholipid breakdown in brain slices has been achieved recently by the use of lithium, which inhibits the breakdown of the inositol 1-phosphate (Ins1P) released and consequently leads to the accumulation of Ins1P in the tissue (Berridge, Downes & Hanley, 1982). This appears to offer a simple and general approach to measuring a functional response closely linked to H_1-receptor activation in brain.

The accumulation of Ins1P in slices (300 x 300 μm) of regions of guinea-pig brain prelabelled with myo-[2-^{3}H]-inositol was measured in the presence of 10 mM LiCl essentially as described by Berridge et al (1982), using in general the batch elution technique to release bound Ins1P from the anion exchange resin. Stimulation of Ins1P accumulation was expressed as the ratio of Ins1P accumulated in the presence of histamine to that in its absence.

Histamine (100 μM) caused a stimulation of Ins1P accumulation in slices of guinea-pig cerebral cortex which after an initial lag period increased approximately linearly over 120 min. The mean stimulation after 60 min incubation was 2.0±0.1 fold (18 measurements). The stimulation was inhibited by 1 μM mepyramine, but not by 100 μM cimetidine. Dimaprit (1 mM) had no significant effect on the level of Ins1P. The EC_{50} for histamine was 13±2 μM. This is similar to the IC_{50} for histamine inhibition of [^{3}H]-mepyramine binding in homogenates of cerebral cortex (Daum, Hill & Young, 1982).

Mepyramine-sensitive stimulation of Ins1P accumulation by histamine was also observed in slices from cerebellum, 3.1±0.2 fold (8 experiments), hippocampus, 2.0±0.2 (3) and hypothalamus, 2.7±0.1 (3). Only a small response was obtained in striatal slices, 1.2 (1). The relative stimulations in the areas studied reflect, qualitatively, the relative densities of H_1-receptors (Hill, Emson & Young, 1978). The differences in the ratio of the stimulations given by carbachol and histamine (8.7 in striatum, 0.5 in cerebellum) indicates that the differences in stimulation by histamine are much more likely to reflect differences in receptor density in different areas rather than differences in the viability of slices.

These observations suggest that the stimulation of Ins1P accumulation in lithium-treated slice preparations should be of great utility in functional studies of H_1-receptors in brain.

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DL-AMINO-4-PHOSPHONOBUTYRATE BINDING SITES ON RAT BRAIN SYNAPTIC MEMBRANES

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DL-amino-4-phosphonobutyrate (APB) is an antagonist at excitatory amino acid receptors activated by glutamate, aspartate, kainate, quisqualate and N-methyl-D-aspartate (NMDA) (Davies & Watkins, 1982), and this activity appears to reside in the D-(-)-isomer. L-(+)APB however, has a potent synaptic depressant action, possibly by inhibiting transmitter release, or by preventing the postsynaptic effects of an as yet unidentified transmitter. It also possesses extremely weak NMDA-like activity.

With the recent availability of labelled DL-APB (Monaghan et al.,1982), we have investigated its binding to whole rat brain synaptic membranes. These were prepared as described previously (Sharif & Roberts, 1980) and the assays performed in 50 mM Hepes-KOH buffer (pH 7.4). Incubations were performed at 37^{0} with inclusion of 2.5 mM $CaCl_2$ in the medium. DL-^{3}H-APB (26.6 Ci/mmol; NEN) was usually present at a final concentration of 30 nM. Binding assays were terminated with a microfuge, and the non-specific binding determined by inclusion of 1 mM L-glutamate.

DL-^{3}H-APB binding was optimal at physiological pH and temperature, and strongly dependent on Ca^{2+} and Cl^{-} as has been reported for APB-sensitive binding of L-glutamate (Fagg et al., 1982). Analysis of untransformed data revealed the presence of a single population of binding sites of K_d = 1.30 $\pm$ 0.18 micromolar and B_{max} = 12.08 $\pm$ 0.5 pmol/mg protein (quadruplicate determinations).

Studies of the structural specificity of ^{3}H-APB binding revealed that L-(+)APB was at least 20 times more potent than the D-isomer, in inhibiting binding. L-isomers of agonists were also more active than the D-forms. The binding site was not sensitive to kainate, and APB binding was only very weakly inhibited by NMDA and its congener quinolinic acid. However, several NMDA-receptor-preferring antagonists, such as α-aminosuberate and α-aminoadipate were potent inhibitors.The most active substance was quisqualate, which has been proposed to interact with a GDEE-sensitive population of glutamate receptors.However, GDEE was only very weakly active here, although the quisqualate-like agonist, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA), was moderately active. Interestingly, γ-D-glutamyl glycine, which antagonises both kainate and NMDA-induced excitations, but which is inactive against quisqualate or glutamate, did not influence the binding of APB. A number of other transmitter substances and drugs, such as GABA, glycine, noradrenaline, carbachol, phencyclidine, and phenobarbitone, were inactive.

In summary, we have demonstrated the presence of specific binding sites for ^{3}H-APB on rat brain synaptic membranes, and which appear to be largely associated with the L-(+)-isomer. Experiments are now being directed to a detailed characterisation of these sites, and determination of their cellular localisation.

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MECHANISMS OF ACTION OF N-METHYL-D-ASPARTIC ACID AND L-GLUTAMIC ACID ON RAT HIPPOCAMPAL PYRAMIDAL NEURONES

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N-methyl-D-aspartic acid (NMDA) and L-glutamic acid (GLU) have powerful excitatory effects on hippocampal neurones. Recently it has been suggested that the changes in membrane conductance activated by NMDA are different from those activated by GLU (Hablitz, 1982). Such different mechanisms of action could be a result of the responses being mediated by different excitatory amino acid receptor types (Watkins, 1981). The present experiments sought to clarify further the mechanisms of action of NMDA and GLU on hippocampal neurones.

Intracellular recordings were made from neurones in the CA1 pyramidal cell region of the rat hippocampus in an in vitro brain slice preparation. Slices were superfused and maintained at 37°C as described by Henderson et al (1982). Recording electrodes were filled with 3M KCl and had resistances of 40-70 MΩ . Drugs were applied to the neurones under study either in known concentrations in the superfusate or by pressure ejection from a micropipette positioned above the surface of the slice in close proximity to the recording site.

NMDA (3-30μM) and GLU (1-10 mM) applied in the superfusate for 3-5min produced dose-dependant depolarisations which gave rise to the firing of action potentials when threshold was exceeded. Similar excitatory responses, but with a faster time course, were elicited following pressure ejection of NMDA (100 μM) and GLU (10 mM) for 1-5sec at 1-10lbf/in^2. The conductance changes underlying the responses to NMDA and GLU were determined while the membrane potential was clamped manually at resting potential. In the case of depolarisations of 25 mV or less, NMDA caused a decrease of up to 38% in membrane conductance in all cells studied. The effect of GLU on membrane conductance was variable between cells, producing no change, a small (<10%) increase or a small decrease in input resistance. However at high concentrations when larger depolarisations were elicited, both NMDA and GLU frequently produced an increase in membrane conductance.

Repeated pressure application of NMDA or GLU induced transient membrane depolarisations which were reproducible for periods of up to 2 h. Addition of DL-2-amino-5-phosphonovaleric acid (2APV; 1-100 μM), a specific NMDA antagonist (Watkins, 1981), to the superfusate resulted in a concentration-dependant reduction in the responses to NMDA. A 50% reduction of the response to NMDA was achieved with approximately 4 μM 2APV. The effects of 2APV were rapid in onset and offset, being maximal after 4 min of application with recovery of the response being complete within 7 min of washout. Responses to GLU were unaffected by concentrations of 2APV below 30 μM. The reduction of responses to GLU by concentrations of 2APV above 30 μM may represent a nonselective action of 2APV at higher concentrations.

The results provide further evidence that NMDA and GLU produce their excitatory effects by acting at separate receptor sites. Different receptor-activated ionic mechanisms appear to underly the responses to NMDA and GLU.

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THE ELECTROPHYSIOLOGICAL EFFECTS OF PRIFUROLINE AND LIGNOCAINE ON DOG VENTRICULAR MUSCLE AND PURKINJE FIBRES

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The new anti-arrhythmic drug prifuroline [3-(benzofuran-2-yl) 3,4-dihydro N,N-dimethyl 2H-pyrrol-5 amine] fumarate has been shown to possess some similarities with amiodarone concerning the effects on sinus node automaticity, atrionodal conduction and atrial and ventricular refractoriness, but quinidine-like effects on the His-Purkinje system after intravenous injection in anesthetized dog (Jaillon et al., 1982). We have studied the electrophysiological effects of prifuroline in comparison with those of lignocaïne on dog (Beagle) ventricular muscle and Purkinje fibres in vitro. The experimental arrangement of the preparation used was similar to that described by Bigger and Mandel (1970). The experiments were performed at 37°C on preparations placed in a chamber perfused at 6ml/min with a physiological salt solution (NaCl 130mM; KCl 5.4mM; $CaCl_2$ 1.9mM; $MgCl_2$ 1.1mM; $NaHCO_3$ 15.5mM; NaH_2PO_4 0.6mM ; glucose 11mM ; mannitol 11mM ; Na Pyruvate 1.8mM) equilibrated with O_2 95%:CO_2 5% at a pH of 7.35.

At $3x10^{-6}$M lignocaïne decreased $\dot{V}max$ of the rising phase of Purkinje action potential (AP) by 5.6% and increased conduction time between Purkinje fibers and ventricular muscle (CT P-V) by 4.3% ; at this dose the increase in AP duration (+5%) (measured at 90% repolarisation) of ventricular muscle was the only parameter modified. At 10^{-5}M lignocaïne decreased Purkinje AP duration by 13%, plateau amplitude of AP by 6.4%, the effective refractory period (ERP) by 11% and CT P-V was increased by 7%. At this dose $\dot{V}max$ of ventricular AP decreased by 8.8% and AP duration increased by 2.5%. Compared to lignocaïne, prifuroline was much more effective on the $\dot{V}max$ of Purkinje fibers and CT P-V : at 10^{-5}M prifuroline, $\dot{V}max$ decreased by 14% and CT P-V increased by 26%, these effects being doubled at $3x10^{-5}$M.AP amplitude of Purkinje fibers was also decreased by 4% and 13% by 10^{-5}M and $3x10^{-5}$M prifuroline respectively. $\dot{V}$ max of ventricular AP was also decreased by 13.7% at 10^{-5}M and 31% by $3x10^{-5}$M and AP duration was increased by 5% at 10^{-5}M prifuroline only.

Thus, prifuroline is much more active than lignocaïne on phase 0 of AP and conduction time, between Purkinje fibers and ventricular muscle. According to these results, prifuroline appears to inhibit the fast sodium channel. The present in vitro findings are in accordance with the in vivo effects of the compound on the dog Purkinje system and ventricular muscle.

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ALTERATION OF THE PROPERTIES OF SODIUM CHANNELS BY COMPONENTS FROM RED CELL MEMBRANES

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Electrogenic sodium transport across mammalian epithelia differs from that of amphibia in being insensitive to manipulations which increase tissue cAMP. Studies on the epithelium lining the rat descending colon have been made in an attempt to discover the reason for the deficiency. We find sensitivity to cAMP can be conferred by exposing the apical surface of the epithelium to vesicles from amphibian red cells. Rat colon was chosen for this study as it normally exhibits no electrogenic sodium transport and the short circuit current (SCC) is insensitive to amiloride. A single intraperitoneal injection of dexamethasone (6 mg Kg^{-1}) given 16-22 hours previously confers a high level of amiloride sensitive sodium transport (129.5±21.5 μA cm^{-2}, n=11) as shown by Will et al (1980). When normal colonic epithelia are exposed to forskolin (10 μM), an activator of adenylate cyclase, an increase in SCC due to chloride secretion is obtained (Cuthbert & Spayne, 1982). We show here that forskolin also increases SCC in dexamethasone treated tissues which is accompanied by an increase in tissue cAMP, an increase in net chloride secretion but with no effect on sodium absorption. As chloride secretion and sodium absorption are additive in terms of the electrical (SCC) responses it can be predicted that the amiloride sensitive short circuit current (ASCC) will not increase even after the SCC is elevated with forskolin. In eight separate experiments the value for SCC (for 0.6 cm^2) was 62.4±13.0 μA and for ASCC 69.4±14.9 μA. After the amiloride was washed away and the SCC had returned to the original value SCC was increased by forskolin to 139.0±11.9 μA but the ASCC remained unchanged at 57.4±14.8 μA. This protocol was made the basis for testing manipulations which might confer sensitivity to cAMP on the sodium transporting mechanism.

Dexamethasone treated colonic epithelia were exposed, on their apical surfaces, to sonicated suspensions of nucleated erythrocytes for 30 min after which the tissues were thoroughly washed. This procedure had no effect on SCC. ASCC was measured before and after SCC was increased with either forskolin or dibutyryl cAMP. In 10 experiments the ASCC (0.6 cm^2) was 60.3±7.9 μA before and 103.7±15.9 μA after SCC was increased, indicating that sensitivity to cAMP had been conferred. Vesicles prepared from purified red cell membranes had the same effect suggesting a membrane component or closely adhering substance is responsible. It appears that the mammalian epithelial sodium channel may have lost, during evolution, a component which confers sensitivity to the nucleotide and that this component is present in erythrocytes from lower phyla. Apparently the component can be added back into the membrane in a functional way.

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EFFECTS OF VERAPAMIL AND CINNARIZINE ON CANINE HINDLIMB PRESSOR RESPONSES TO α-ADRENOCEPTOR AGONISTS AND SYMPATHETIC STIMULATION

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In agreement with published results in the pithed rat (Van Meel et al, 1982), we have recently demonstrated in the dog hindlimb that pressor responses to the relatively selective α_2-adrenoceptor agonist B-HT 920 are preferentially antagonized by calcium entry blocking drugs (Llenas & Massingham, 1983). Cinnarizine, however, another drug claimed to possess calcium entry blocking properties (See Godfraind, 1981) produced quantitatively similar inhibitions of hindlimb responses to both preferential α_1 and α_2-adrenoceptor agonists suggesting that it may have a different site of action to verapamil in this preparation. This study extends these observations and examines the effects of verapamil and cinnarizine upon hindlimb responses to noradrenaline and lumbar sympathetic stimulation.

Dogs were anaesthetized with pentobarbitone, intubated and artificially respired. After heparinization the abdominal aorta was cannulated and, using a roller pump, the left hindlimb was autoperfused via the femoral artery. Two series of experiments were performed, the first using close arterial injections of noradrenaline (NA: 1-3µg), phenylephrine (PE: 6-12µg) and B-HT 920 (B-HT: 1-3µg) to the hindlimb of chlorisondamine (1mg/kg i.v.), atropine (1mg/kg i.v.) and propranolol (0.5mg/kg i.v. + 0.25mg/kg/hr i.v.) pretreated animals. The second group of dogs was not treated with chlorisondamine and low frequency lumbar sympathetic stimulation was carried out (LSS: 1 Hz, 1m sec, 1 min, supramaximal voltage) in addition to close arterial injections of NA and PE.

Following stable responses, either verapamil or cinnarizine was infused close arterially and 20-30 min later the agonists were re-examined. Hindlimb responses to B-HT were inhibited by 55 ± 5%, 72 ± 3% and 78 ± 5% (n=4), 20-30 min following the infusion of verapamil at 1, 3 and 10µg/kg/min i.a. respectively whilst PE responses were reduced by 15 ± 4%, 17 ± 4% and 38 ± 5% (n=8) during similar infusion rates of this drug. The LSS response was markedly reduced by verapamil, the inhibition-response curve not being significantly different from that to B-HT. The inhibition curve to NA occupied a position between B-HT and PE, the responses being inhibited by 27 ± 3%, 38 ± 4% and 49 ± 5% (n=8) respectively for the 3 verapamil infusion rates. Cinnarizine over the infusion range selected (10-100µg/kg/min i.a.) antagonized LSS, B-HT, NA and PE responses approximately equally, the inhibitions were between 30-45% for a rate of 10µg/kg/min i.a. and between 75-93% for 100µg/kg/min i.a. cinnarizine.

Since selective inhibition of α_2-adrenoceptor mediated vasoconstriction is a property of several structurally distinct calcium entry blockers these results question the use of cinnarizine as a selective calcium entry blocking drug and suggest it may have other or additional cellular sites of action. Finally if noradrenaline release upon sympathetic nerve stimulation is as resistant to verapamil in vivo as has been reported in vitro (Haeusler, 1972), then the marked inhibition of LSS by low doses of this drug is surprising since it has been suggested that the α_1-adrenoceptor subtype is predominantly innervated in this preparation (See Langer et al, 1981). Studies enabling a simultaneous, direct estimation of transmitter release and end organ response are required to resolve this observation.

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PHOTODYNAMIC EFFECTS OF ERYTHROSINE B ON THE ELECTRICAL AND MECHANICAL ACTIVITY OF GUINEA-PIG TAENIA COLI

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Erythrosine B, a widely used food colouring agent, has been found to affect several membrane transport systems including that for calcium (Colombini & Wu, 1981). We report here that erythrosine B has photodynamic effects on spontaneous and drug-induced electrical and mechanical activity of the isolated guinea-pig taenia coli (caecum), a smooth muscle preparation which exhibits Ca^{2+}-dependent action potentials. Recordings were obtained using either the sucrose-gap (Lowe, Matthews & Richardson, 1981) or muscle superfusion techniques (Brading & Sneddon, 1980) at 37°C. The preparation could be illuminated with a quartz-halogen light source (Schott KL 150) equipped with a heat filter and fibre optic probe. From sucrose-gap recordings it was found that erythrosine B, 10 μM, caused, but only in the presence of illumination, a marked stimulation in both electrical and mechanical activity of the taenia; action potential frequency increased and muscle contractions summated, with the eventual obliteration of phasic periods of relaxation. Increasing $[Ca]_o$ threefold, from 2.56 mM to 7.68 mM, enhanced the photodynamic effects of erythrosine B, whereas decreasing $[Ca]_o$ to 0.64 mM inhibited them.

Carbachol-induced contractions of the superfused taenia preparation were markedly inhibited by erythrosine B in the presence of illumination, but not in its absence. The inhibitory effect was dependent on light intensity since an approximately tenfold decrease in light intensity reduced the inhibitory effect of illumination (in the presence of erythrosine B, 10 μM) on the carbachol dose-response curve from $> 90\%$ to $\leqslant 20\%$; light alone had no such effects. Furthermore, in comparative experiments it was found that both carbachol-induced muscle contractions and those evoked by increasing $[K]_o$ tenfold to 47 mM were inhibited by erythrosine B, 10 μM, although for similar light intensities the carbachol-induced responses were more sensitive to inhibition than contractions elicited by potassium depolarization. It is therefore possible that receptor operated channels are more susceptible to the photochemical action of erythrosine B than are voltage operated channels.

Control experiments demonstrated that irradiating erythrosine B before tissue contact was ineffective in modifying muscle responses. The requirement for both photon energy and erythrosine B together in order to elicit the biological effects described here indicates the likely involvement of an erythrosine B transient or free radical species. Erythrosine B may therefore be a useful photochemical agent for probing the molecular organization and function of membrane ionic permeability mechanisms and calcium transport in smooth muscle and other cells.

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EFFECTS OF SULPHONYLUREAS AND INCREASED $(K)_o$ ON (^{86}Rb) EFFLUX FROM RAT PANCREATIC ISLETS

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The hypoglycaemic sulphonylurea tolbutamide has a dual effect on the potassium permeability of rat pancreatic islets in vitro as monitored by changes in ^{86}Rb efflux (Matthews & Shotton, 1982). Tolbutamide, 0.7 mM, caused first a decrease in efflux (the 'on' response) followed on drug removal by a large phasic, calcium-dependent, increase in ^{86}Rb efflux (the 'off' response). Subsequent work has revealed that the 'on' and 'off' responses display a different dose-dependency. The 'on' response is half-maximal at tolbutamide concentrations of 30 μM, maximal at 200 μM and decreased by concentrations $>$ 200 μM. In contrast, the 'off' response is half-maximal at 70 μM, maximal at 700 μM and further increases in tolbutamide (up to 5 mM) produce no further change in its magnitude. Glibenclamide produced a rapid decrease in ^{86}Rb efflux similar to that for tolbutamide but at a much lower concentration (10 μM) and with no 'off' response apparent over a wide range of concentration (1 to 100 μM). The decrease in ^{86}Rb efflux was sustained and returned to control values only slowly following removal of glibenclamide from the perifusion solution.

Both tolbutamide and glibenclamide depolarize β-cells and elicit continuous electrical spiking activity (Dean & Matthews, 1968; Meissner & Atwater, 1976). To explore further the relationship between ^{86}Rb efflux, potassium permeability and islet cell transmembrane potential, efflux was measured during depolarization of the islet cells by increasing the extracellular potassium concentration $[K]_o$. Increasing $[K]_o$ to 10, 20, and 47 mM produced corresponding increases in ^{86}Rb efflux from pancreatic islets of 35%, 128% and 185%, respectively. The magnitude of these effects correlates remarkably well with the islet β-cell transmembrane potentials measured at the same $[K]_o$ by Henquin & Meissner (1978). Substituting $[Co]_o$ for $[Ca]_o$ 2.56 mM prevents the gated entry of calcium through voltage dependent channels and this reduced by 51% the peak increase in ^{86}Rb efflux induced by $[K]_o$ 47 mM, whereas quinine, 10^{-5} M, used to prevent the calcium-dependent activation of potassium channels, decreased the response to $[K]_o$ 47 mM, in normal $[Ca]_o$, by 47%, although in both types of experiment a significant increase ($P < 0.001$) in ^{86}Rb efflux still occurred upon exposure to $[K]_o$ 47 mM.

It is concluded that both tolbutamide and glibenclamide produce an alteration in islet cell rubidium efflux by mechanisms that are not simply a consequence of depolarization caused by these agents; rather the rapid initial decrease in potassium permeability, as evidenced by the decrease in ^{86}Rb efflux, may itself serve to depolarize the β-cells, activate the entry of calcium through voltage-dependent channels and initiate insulin release. However, the two sulphonylureas differ in the potency and duration of their effects on islet cell ionic permeability, the sustained pharmacological action of glibenclamide being explained by the long-lasting decrease in potassium permeability that it produces. We conclude also that the islet-cell depolarization elicited by a raised $[K]_o$ increases membrane potassium permeability and efflux by at least two mechanisms; (i) an increase in $[Ca]_i$ which activates calcium-dependent potassium efflux and (ii) the activation of voltage-sensitive potassium channels giving an efflux which persists when the calcium-dependent component is inhibited.

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INHIBITION OF UTERINE AND VASCULAR MUSCLE BY CALCIUM ANTAGONISTS, HYDRALAZINE AND SODIUM NITROPRUSSIDE

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The potencies and selectivities of calcium antagonists as inhibitors of mechanical activity vary between cardiac muscle and various smooth muscles. These drugs inhibit contractility of both rat and human uterine muscle and may have a place in the treatment of premature labour. In the present study the potencies and selectivities of several calcium antagonists have been compared with those of hydralazine and sodium nitroprusside using isolated uterine and hepatic portal vein preparations from the rat.

Uterine strips or hepatic portal veins were removed from Sprague-Dawley rats on day 22 of pregnancy before 10.00 h. Contractions were recorded isometrically and quantified using integrators. Control experiments showed, that after an initial 30 min during which time the tissues were washed frequently, the integral of spontaneous contractility of both tissues did not change. Tissues were exposed to approximate IC50s of the drugs in Table 1 to determine the time to equilibration of inhibitory responses; these times varied between 20 and 50 min. To measure potencies the drugs were added cumulatively at times greater than for equilibration of response. The integrals of contractility were measured in the 10 min (portal vein) or 15 min (uterine strip) periods before subsequent drug additions and expressed as a % of the control integral enabling calculation of an IC50 on each tissue. In other experiments oxytocin (0.1 mu/ml) was added to the uterine strips for 10 min every 40 min and potencies of the drugs against uterine contractility due to oxytocin determined.

The calcium antagonists were potent inhibitors of the three preparations (Table 1); hydralazine and sodium nitroprusside were much less potent.

Table 1 Inhibitory potencies* of some calcium antagonists, hydralazine and sodium nitroprusside

Compound	Uterus		Portal Vein
	Spontaneous	Oxytocin-induced	Spontaneous
Nifedipine	9.36±0.36	8.17±1.50	8.22±0.12
Methoxyverapamil	8.22±0.46	7.94±0.36	7.95±0.26
Diltiazem	8.18±0.56	6.65±1.20	6.66±0.32
Cinnarizine	6.87±0.54	6.10±0.48	5.54±0.36
Hydralazine	4.48±0.51	<4	3.47±0.31
Sodium nitroprusside	<3	N.D.	4.99±0.39

* Potencies as mean -log molar IC50 with 95% confidence intervals. n=4-6. N.D.= not determined.

The results show that the rank order of potencies of the calcium antagonists and hydralazine in the 3 experimental situations did not differ. The calcium antagonists exhibited some selectivity for inhibition of spontaneous contractility of the uterus relative to the hepatic portal vein but there was no selective inhibition of oxytocin-induced contractions of the uterus.

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EFFECTS OF FOUR CALCIUM ANTAGONISTS ON S-T SEGMENT ELEVATION AND CORONARY VENOUS EFFLUX OF LACTATE AND K^+

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S-T segment elevation caused by temporary occlusion of a coronary artery is a good indicator of myocardial ischaemia (Maroko et al., 1971). According to Marshall & Parratt (1979) there may be correlation between S-T segment elevation and potassium efflux. Higuchi (1981) on the other hand suggests a correlation between S-T and lactate levels. However studies have shown that some drugs do not uniformly modify these parameters. It seemed of interest therefore, to investigate several calcium antagonists on S-T segment elevation and on the efflux of lactate and K^+.

The method used was derived from that of Maroko et al. (1971). Thirty-five mongrel dogs of both sexes were randomly assigned to five treatment groups: placebo (physiological saline). bepridil (1 mg/kg), diltiazem (0.1 mg/kg), nifedipine (0.01 mg/kg) or verapamil (0.1 mg/kg). The doses were selected on the basis of their ability to induce a maximum increase (100%) in coronary venous PO_2.

Two 10-min occlusions of a branch of the left anterior descending coronary artery were made at 1-h intervals. The first was made to record changes before treatment; the second was made 5 min after an i.v. injection of placebo or active compound. S-T segment elevation was determined before, and at three times during, occlusion. In order to determine lactate and K^+ levels, blood samples were collected from a vein draining the ischaemic area as well as from an artery, following the method of Thomas et al. (1970).

Table 1. Effects of calcium antagonists on S-T segment elevation and on the efflux of lactate and potassium caused by temporary ischaemia. Each treatment group contained seven animals. Values are % change from control (first occlusion) *P<0.05.

	Placebo (vehicle)	Bepridil 1 mg/kg	Diltiazem 0.1 mg/kg	Nifedipine 0.01 mg/kg	Verapamil 0.1 mg/kg
S-T elevation	- 4	- 53*	- 28*	- 32*	- 45*
Lactate efflux	- 13	- 61*	- 39*	- 37*	- 55*
K^+ efflux	+ 19	- 27	- 22	0	- 44*
Blood pressure	- 1	- 13*	- 7*	- 17*	- 9*
Heart rate	0	- 9*	- 7*	+ 3	- 2

Table 1 shows that all four calcium antagonists significantly reduced S-T segment elevation. This effect was not accompanied by bradycardia in animals treated with nifedipine or verapamil. At doses causing a similar degree of bradycardia, bepridil was about twice as active as diltiazem in reducing S-T segment elevation. All four compounds significantly lowered lactate production. Changes in the coronary venous efflux of potassium from the ischaemic zone varied; bepridil and diltiazem slightly reduced K^+ release; verapamil greatly inhibited release and nifedipine had no effect.

The results indicate that all four calcium antagonists reduce S-T segment elevation and lactate release. Verapamil appears to be distinguished from bepridil, diltiazem and nifedipine by its marked inhibitory effect on K^+ release.

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MD 260355: A NEW CALCIUM ANTAGONIST

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MD260355, [chloro-4 dimethoxy-2, 5(piperidino-2)ethoxy-6]phenyl-1 (hydroxy-4)phenyl -3 propanol-1 was tested for antagonism of potassium-induced contraction of dog coronary artery and calcium-induced contraction of rat thoracic aorta.

MD260355 inhibited in a concentration dependent manner the slow component of contraction induced by potassium depolarization in strips of the isolated dog coronary artery [IC50 : 1.3 10^{-8} M (table 1)]. In rat thoracic aorta, MD260355 potently antagonized the calcium-induced contractions. The concentration-response curves were shifted to the right and the antagonism was competitive. The apparent pA_2 was 9.8 (9.1-10.7). The slope of the Schild regression was 0.92. The apparent pA_2 for piprofurol, a compound chemically related to MD260355 was 9.5 (8.5-10.9) (Pourrias et al, 1982) and it was 8.3 (7.9-8.9) for verapamil.

In order to establish whether the antagonism of vascular smooth muscle contraction was specific, the inotropic effects of MD260355 were investigated in the isolated papillary muscle of the guinea pig heart. The muscles were suspended in a Krebs-Henseleit solution at 37°C and their electrically evoked contractions recorded by conventional methods. The concentration inducing a 50 % depression of the amplitude of the papillary muscle contraction (DC50) was 4.9 10^{-6} M. In the same conditions, DC50 of piprofurol was 4.6 10^{-6} M.

These results suggest that the MD260355 is a potent Ca^{++} antagonist with potentialy less cardiac depressor activity and better selectivity for arterial smooth muscle than piprofurol or verapamil.

Table 1 : Calcium antagonistic effects of MD260355, piprofurol and verapamil in isolated, potassium depolarized dog coronary artery and guinea-pig papillary muscle

	ACTIVITIES	
	Dog coronary artery IC50 M	Guinea-pig papillary muscle DC50 M
MD260355	1.3 (0.5 - 3.5)10^{-8}	4.9 (0.3 - 71) 10^{-6}
PIPROFUROL	2. (1.6 - 2.6)10^{-8}	4.6 (0.7 - 26) 10^{-6}
VERAPAMIL	2.8 (1.3 - 5.8)10^{-7}	1.7 (0.14 - 23)10^{-6}

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PRE- AND POSTSYNAPTIC ACTIONS OF METHOHEXITONE AT THE VERTEBRATE NEUROMUSCULAR JUNCTION

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It has been shown that methohexitone, in low concentrations, has a differential action on the contractions produced by repetitive nerve stimulation as compared with the depolarization and contracture responses produced by exogenous acetylcholine (ACh) in the isolated chick biventer cervicis (BVC) nerve-muscle preparation (Bell & Wali, 1981; Elliott & Wali, 1983). In addition, Wali (1982), using a sucrose-gap apparatus, has shown that methohexitone produced a hyperpolarization in the membrane potential of the chick BVC muscle.

The present experiments are an extension of those previously reported by Elliott & Wali (1983) and are designed to further investigate the possibility that methohexitone may act both pre- and postsynaptically at the chick neuromuscular junction.

The effect of methohexitone (88uM) on the presynaptic release of ACh was investigated by using radio-labelled choline (tritiated choline, ^{3}H-methyl choline, specific activity of 15 Ci.mmol^{-1}). The preparation was loaded with labelled choline (bath concentration of 2uCi.ml^{-1}) for 2 h and methohexitone was added during repetitive nerve stimulation, at 0.2 Hz with 5V and 0.5 ms pulse duration, and in the absence of such stimulation. Aliquots and samples from the digested preparations (by hyamine hydroxide, 1M) were taken for measurement of radioactivity in a liquid scintillation counter.

In the absence of repetitive nerve stimulation, methohexitone (88 uM) produced a significant increase (at 0.1% level) in the amount of radioactivity in the control muscles, suggesting that it may increase the spontaneous release of ACh from the nerve terminals. The mean ($\pm$SEM) number of counts min^{-1}(CPM) of radioactivity in the control muscles and in the muscles treated with methohexitone were 4,608$\pm$142 and 11,394$\pm$173 CPM, n=10, respectively.

Using intracellular recording technique, the mean resting membrane potential of the chick BVC muscle was -72$\pm$1.6 mV (in 20 cells, 6 experiments). Methohexitone produced a hyperpolarization in the chick BVC muscle (to -85$\pm$1.8 mV, n=6, $P < 0.001$). Methohexitone reduced the amplitudes of the depolarizations produced by 0.5 mM ACh, by about 79% $\pm$2.1 (control value, 15$\pm$0.05 mV, n=6).

In other preparations, e.g. rat neuromuscular junction, it has been shown that methohexitone increased the frequency of the miniature endplate potentials (mepps), whereas it, simultaneously, reduced the amplitudes of the mepps (Westmoreland, Ward & John, 1971).

Although the ionic basis of methohexitone-induced hyperpolarization is not yet entirely clear, it has been shown that barbiturates can reduce the sensitivity of the postsynaptic membrane to applied ACh (Thesleff, 1956) and that they can also block the endplate ion channels responsible for membrane depolarization (Adams, 1976). In conclusion, methohexitone seems to act at both pre- and postsynaptic membranes of the vertebrate neuromuscular junction.

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DOSE-DEPENDENT DISPOSITION OF TRANS-ANETHOLE IN THE RAT

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trans-Anethole (*trans*-*p*-methoxypropenyl benzene) occurs naturally in the oils fennel and star anise. It is widely used in foods, medicines and alcoholic beverages. Various studies have found *trans*-anethole to be mildly hepatotoxic at high (0.5-1g/kg) doses to rodents. We have previously reported that the related compounds estragole and *p*-propylanisole exhibit dose-dependent metabolism in rodents. We now report on the disposition of *trans*-anethole in the rat over a wide dose range.

Female Wistar albino rats (200 g b.w.) received [*methoxy*-^{14}C]-*trans*-anethole p.o. at doses ranging from 0.05-1500mg/kg. They were housed in cages to permit the separate collection of urine, faeces and $^{14}CO_2$ in the expired air. Table 1 gives details of the excretion of ^{14}C by various routes in relation to dose size. Faecal excretion of ^{14}C was < 0.01% of dose.

Table 1. Excretion of ^{14}C by rats given [*methoxy*-^{14}C]-*trans*-anethole p.o. at various doses

	Dose (mg/kg)			
% ^{14}C dose excreted in 72h in :	0.05	5	50	1500
Urine	27.6	32.8	41.2	56.6
$^{14}CO_2$ in expired air	56.3	53.9	41.5	31.5
Total	83.9	86.7	83.0	88.3

Figures are means of at least 4 animals at each dose.

Urinary metabolites were analysed by solvent extraction, TLC, HPLC, MS and GC-MS. Anethole was completely metabolized by rats at all doses. The major ^{14}C-metabolites are two stereo isomeric diols (4-(1', 2'-dihydroxypropyl)-anisoles) *p*-methoxy-acetophenone and *p*-methoxy-benzoic and cinnamic acids, excreted free and conjugated with glycine and glucuronic acid. Additionally, the O-demethylation product *p*-hydroxy propenylbenzene is excreted as its glucuronide.

The data demonstrate that the fate of anethole is influenced markedly by dose size. At low doses, O-demethylation leading to $^{14}CO_2$ excretion is favoured, and as the dose increases, this pathway becomes less important throwing emphasis upon oxidation of the side chain. With increasing dose, there are increases in the relative proportion of urinary ^{14}C as the diols and *p*-methoxybenzoyl-glycine.

There is a great discrepancy between human exposure to anethole from foods and beverages (ca. 60µg/day) and the doses used in animal toxicity tests. The use of very high doses of anethole in animal toxicity tests causes "metabolic overload" which distorts the pharmacokinetics of the test compound. As in the cases of estragole and *p*-propylanisole, these data show the importance of considering dose-dependent metabolism when attempting to assess the significance to man of animal data obtained at doses vastly in excess of human intake.

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INHIBITION BY THROMBOXANE A_2 ANALOGUES OF THE PULMONARY METABOLISM OF THROMBOXANE B_2

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One possible mechanism of the formation of 13,14-dihydro-15-keto-thromboxane B_2 (TXDK) in guinea-pig lung involves the uptake and metabolism of thromboxane B_2, TXB_2 (Robinson et al, 1982). We now present evidence which further defines the processes involved in this conversion.

Lungs from male Dunkin-Hartley guinea-pigs (450-550 g) were perfused at 10 ml/min for 5 min with Krebs' solution containing 1 μCi of ($^3H_8$)-TXB_2 (10 ng/ml) and 250 nCi of inulin-(^{14}C)-carboxylic acid to which various concentrations of thromboxane A_2analogues were added. The disposition of TXB_2 within the lung was studied by measurement of (a) the tritium tissue to medium ratio (T/M) achieved at the end of the experiment and (b) the metabolism of TXB_2 to TXDK as quantified by radio-TLC of perfusate extracts (Robinson et al, 1982).

Krebs' perfused control lungs converted 30.3 ± 2.4% TXB_2 to TXDK (n= 18) and retained substantial amounts of tritium (T/M ratio 3.79 ± 0.11) but not of the ^{14}C extracellular space marked (ratio 0.17 ± 0.01). Co-perfusion with the 9,11- and 11, 9-epoxymethano analogues of PGH_2 (U44069 and U46619), which possess thromboxane A_2-like activity in a variety of preparations (Coleman et al, 1981; Jones & Wilson, 1980) produced concentration-dependent inhibition of tritium retention and TXB_2 metabolism (IC_{50} v. metabolism: 0.67 and 0.53 μM). In both cases inhibition of tritium retention and metabolism was linearly related to analogue concentration.

In contrast pinane thromboxane A_2 (PTA_2) and the 9,11-etheno-16-p-fluorophenoxy PGH_2 analogue EPO11 (Dong & Jones, 1982) exhibited different behaviour. Both were potent inhibitors of TXB_2 metabolism (IC_{50} values 0.68 and 2.57 μM respectively) and this effect was also linearly dependent on analogue concentration. However, their effects on tritium retention, although inhibitory, showed no linear concentration dependency.

Specificity of U46619 was demonstrable: at 0.6 μM U46619 the inactivation of 10 ng/ml PGF_{2a} was 85.8 ± 0.5% with a tissue to medium ratio of 1.64 ± 0.03 which was not significantly different from control values of 87.2 ± 3.9% and 1.59 ± 0.06 (n = 4). Even at 1.2 μM U46619 (enough to totally abolish TXB_2 metabolism), the effect was only slight: metabolism of PGF_{2a} was reduced by 18.7% and tritium retention was down by 15% ($p < 0.01$, n = 3). Prostaglandin F_{2a} itself failed to inhibit the accumulation and metabolism of 10 ng/ml TXB_2 at concentrations (0.6 and 0.9 μM) at which U46619 exhibited potent activity (Hoult and Robinson, 1983).

These results show that (i) the four TXA_2 analogues are potent inhibitors of TXB_2 metabolism in perfused guinea-pig lung; and (ii) their different effects on tritium retention and TXB_2 metabolism suggest that at least two consecutive but closely-coupled processes (amenable to differential intervention) are probably involved in the pulmonary handling of TXB_2. Moreover, they imply that thromboxane A_2 itself may be subject to similar proce sses in lung tissue.

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THROMBOXANE A_2-INDUCED PLATELET ACTIVATION: RELATIONSHIP BETWEEN RECEPTOR OCCUPANCY AND PHOSPHATIDYLINOSITOL METABOLISM

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Platelet activation, a process dependent upon a rise in the cytoplasmic free calcium concentration ([Ca-f]) (Rink et al, 1981), can be induced by several agonists including PGG_2, PGH_2, TxA_2 and stable mimetics that combine with distinct receptor(s) in/on platelets (MacIntyre, 1981). It has been proposed that agonist-induced degradation of phosphatidylinositol (PI) and resultant formation of phosphatidic acid (PA) are direct consequences of receptor activation which precede, and may evoke, increased membrane permeability to calcium (Michell, 1979). We have used the stable TxA_2-mimetic, U44069, and the specific TxA_2 receptor antagonist, EP 045 (Jones et al, 1982) to examine the relationship between TxA_2 receptor activation and PI turnover in human platelets. Suspensions of platelets in calcium-free, phosphate-free buffer were prepared from citrated platelet-rich plasma. For PI turnover studies, platelets were incubated with [^{32}P]-PO_4 (25-30 μCi/ml) and resuspended in fresh buffer prior to addition of U44069 (3.3 nM-3.3 μM). [^{32}P]-Phospholipids were extracted, separated by two dimensional thin-layer chromatography, identified by exposure to iodine vapour and quantified by liquid scintillation spectrometry (MacIntyre & Pollock, 1982). For radioligand binding studies, platelets were incubated with [^{3}H]-U44069 (3.3 nM-1 μM) in the presence and absence of excess unlabelled U44069. Free and bound ligand were separated by centrifugation and assayed by liquid scintillation counting (Armstrong et al, 1983).

Three components of [^{3}H]-U44069 binding could be distinguished, only one of which was stereospecific, saturable and displayed a high affinity (K_D = 70 ± 13 nM; mean ± S.E., n = 4) for the radioligand. U44069 induced a concentration dependent stimulation of PI turnover, as indicated by formation of [^{32}P]-PA. Half maximal stimulation of [^{32}P]-PA formation was observed at U44069 = 51 ± 9 nM (mean ± S.E., n = 4), a concentration at which approximately 50% of the high affinity binding sites for U44069 are occupied. EP 045 (0.1 - 10 μM) inhibited, in a concentration dependent manner, U44069-induced [^{32}P]-PA formation and binding of [^{3}H]-U44069 to the high affinity binding site.

These results indicate that the degree of [^{32}P]-PA formation induced by U44069 is approximately proportional to the extent of receptor occupancy and that U44069-induced PI turnover is suppressed by a specific TxA_2 receptor antagonist (EP 045). This supports the concept that PI degradation is a direct consequence of TxA_2-receptor interaction. A cause and effect relationship between TxA_2-induced PI turnover and increased [Ca-f] remains to be established.

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THE EFFECT OF EP 045, A THROMBOXANE A_2 ANTAGONIST ON LEUKOTRIENE-INDUCED CONTRACTIONS OF PARENCHYMAL STRIPS AND HUMAN BRONCHUS

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Contractions of superfused guinea-pig parenchymal strips(GPP) induced by leukotrienes (LT) B_4, C_4, D_4 and E_4 are inhibited by indomethacin (cyclo-oxygenase inhibitor) and carboxyheptylimidazole (thromboxane A_2 (TxA_2))synthetase inhibitor (Piper & Samhoun, 1981). This suggests that a major part of the constrictor effect of these LTs is mediated by a myotropic cyclo-oxygenase product(s), probably TxA_2. In order to further elucidate the mechanism of action of LTs in the lung, we have investigated the effect of EP 045 ($\pm$5-endo-{6-carboxyhex-2-Z-enyl-6-exo N-(phenyl carbamoyl) bicyclo heptane hydrazonomethyl bicyclo [2,2,1] heptane}) which directly antagonises the action of TxA_2 (Jones, Peesapati & Wilson, 1982) on LT-induced contractions.

Lungs from rat, rabbit and guinea pig were perfused free from blood, parenchymal strips (30 x 3 x 3 mm) were cut and superfused with Tyrode. Spirally-cut strips of human bronchus were prepared from specimens obtained from patients undergoing pneumonectomy. Dose-related contractions were obtained for LTs B_4, C_4, D_4 and E_4 before and during infusion of EP 045 (250 ng/ml). U-44069 ((15S)-hydroxy-9α,11α-(epoxymethano)-prosta 5Z,13E-dienoic acid) (0.25 - 0.5 μg) a TxA_2 mimic and acetylcholine (Ach) (0.5 - 2 μg) were given as control agonists. EP 045 significantly reduced contractions of GPP produced by 30 pmol of LTB_4, C_4 and D_4 producing reductions of 92, 67 and 84% respectively. The duration of contractions was greatly prolonged despite the reduction in height. This may be due to the antagonism of relaxant prostaglandins E_2 and I_2. When higher doses of LTs B_4, C_4, D_4 and E_4 (100 - 150 pmol) were given, contractions of LTB_4 remained antagonised (90% reduction) whilst those due to LTC_4, D_4 and E_4 partially increased in height (35 - 40% reduction) and their duration remained increased. The remaining contractions to LTC_4, D_4 and E_4 were antagonised by FPL-55712 and were probably due to a direct long-lasting action of LTs. Contractions of human bronchus by LTs C_4 and D_4 (3 - 100 pmol) were not significantly reduced by indomethacin but were antagonised by EP 045 and by FPL-55712. LTB_4 was active only at higher doses (3 - 100 x 10^{-11} mol) and tachyphylaxis rapidly developed. Contractions of rat or rabbit parenchymal strips were not significantly reduced by EP 045. EP 045 completely abolished contractions produced by U-44069 in all preparations but did not affect those produced by Ach.

These results show that while contractions of superfused GPP caused by low doses of LTs C_4, D_4 and E_4 are significantly inhibited by a TxA_2 antagonist higher doses are not inhibited to such a great extent. However, LTB_4-induced contractions are inhibited equally at the lower and higher dose range. These results also show that contractions of GPP induced by Ach and LTs involve different mechanisms since Ach-induced contractions are not antagonised by EP 045. In rat and rabbit it appears that LTs C_4, D_4 and E_4 have a direct contractile action as they are unaffected by EP 045 or indomethacin. LT-induced contractions of human bronchus produce an anomaly in that they are not significantly inhibited by indomethacin, but are significantly inhibited by EP 045. These results suggest that the TxA_2 antagonist EP 045 may be active in antagonising a non-cyclo-oxygenase product.

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SYNTHESIS OF PROSTACYCLIN RECEPTORS FOLLOWING DESENSITISATION

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Prostacyclin (PGI_2) binds to membrane receptors (Blair & MacDermot,1981) and activates adenylate cyclase (Blair et al,1980) in NCB-20 neuronal somatic hybrid cells. Culture of these cells with carbacyclin, a stable prostacyclin analogue, results in a decrease in the maximum PGI_2-stimulated adenylate cyclase activity (Vmax) and an increase in the concentration of ligand necessary for half maximum enzyme activation (Kact) (Blair et al, 1982). These changes are accompanied by a reduction in the number of prostacyclin receptors (Bmax = 542.5 fmoles ^{3}H-PGI_2 bound/mg protein in desensitised cells compared to 1076 fmol/mg in control cells). The Kd of binding to desensitised cell membranes (50.0nM) is greater than that of binding to control membranes (18.1nM) (Blair et al,1982).

To investigate whether these changes are reversible, non-dividing cells (cultured in cytosine arabinofuranoside) were incubated for 16h with carbacyclin, and then washed three times with Dulbecco's modified Eagle medium. The cells were then cultured for variable lengths of time before harvesting. The stimulation of adenylate cyclase by 4 μM PGI_2 was measured. In desensitised cells, PGI_2-stimulated adenylate cyclase activity was decreased to 41% of control values. After a 48h recovery period, this increased to 82% of control values with half-time of approximately 18h. After desensitisation the Vmax for PGI_2 stimulated adenylate cyclase activity decreased from 90.1 to 44.0 pmoles cyclic AMP/min/mg protein and the Kact increased from 41.7nM to 142.9nM. After resensitisation the Vmax was restored to 60.9 pmoles cyclic AMP/min/mg protein and the Kact returned almost to that of control cells.

The inhibition of RNA or protein synthesis by actinomycin D or cycloheximide arrested resensitisation of carbacyclin treated cells. It follows that protein synthesis is required for either the synthesis of new receptors or the recycling of "used" receptors during resensitisation. In addition, there is an insufficient RNA pool to allow the protein synthesis necessary for resensitisation.

There was a decrease in total protein in cells treated with cycloheximide for 16h due to cessation of protein synthesis. However, PGI_2-stimulated adenylate cyclase activity decreased more rapidly than total cell protein and so a reduction in Vmax (pmol cyclic AMP/mg protein) was observed. This decrease was similar in magnitude to that seen in cells treated with carbacyclin for 16h. However, the increased Kact of desensitised cells was not observed in cycloheximide treated cells. Cells treated concurrently with carbacyclin and cycloheximide showed a Vmax only slighly less than that of cells treated with either agent alone, and a Kact similar to that of carbacyclin treated cells. In conclusion, cycloheximide reduces the maximum PGI_2-stimulated adenylate cyclase activity to the level seen in desensitised cells without causing the increase in Kact. These results indicate that desensitisation of PGI_2 receptors is complex and involves at least two processes, namely a loss of receptors and an affinity change. Furthermore, since cycloheximide has little additional affect on PGI_2 responsiveness during desensitisation by carbacyclin, it follows that synthesis of the PGI_2-receptor protein may be switched off during prolonged treatment with carbacyclin.

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EFFECT OF LEUKOTRIENES (LTs) ON PULMONARY FUNCTION AND LUNG IRRITANT RECEPTORS IN CATS AND DOGS

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Inhalation of an aerosol of LTD_4 or LTC_4 in man causes bronchoconstriction and may predispose subjects to cough during subsequent forced expiratory manoeuvres (Holroyde et al, 1981). It is possible that the lowering of the cough threshold and also part of the bronchoconstriction result from an action of LTs on sensory nerves in the airways, such as the rapidly adapting "irritant" receptors (Fillenz & Widdicombe, 1972).

Chloralose-anaesthetised dogs (n=4) and cats (n=4) were bilaterally vagotomised and prepared for measuring total lung resistance (R_L), dynamic lung compliance (Cdyn) and irritant receptor discharge (IRD) as previously described (Dixon et al 1979). Histamine (dogs) or 5HT (cats) was given i.v. (10 µg/kg) every 10 min and peak responses recorded. Leukotriene aerosols were then administered (dogs: LTC_4 1 µg/ml for 5 mins; cats LTD_4 20 µg/ml for 10 breaths) and dosing with histamine or 5HT continued. This procedure was also carried out in 6 cats with intact vagi (IRD was not recorded).

In vagotomised dogs, histamine consistently increased R_L and IRD, and reduced Cdyn. LTC_4 slightly but significantly raised resting R_L (from 2.0 $\pm$ 0.1 to 2.5 $\pm$ 0.1 $cmH_2O/l/s$; mean $\pm$ s.e. mean) but had no effect on resting Cdyn or IRD. LTC_4 did not affect responses to histamine. In vagotomised cats, 5HT increased R_L and IRD, and reduced Cdyn. LTD_4 slightly but significantly elevated resting R_L (from 24.2 $\pm$ 1.0 to 28.3 $\pm$ 1.6 $cmH_2O/l/s$; mean $\pm$ s.e. mean) but had no effect on Cdyn or IRD and did not affect responses to 5HT. Vagally intact cats showed similar responses but in addition, 5HT-induced increases in R_L were substantially potentiated by LTD_4 (5HT-induced R_L change increased from 17.8 $\pm$ 2.5 to 52.0 $\pm$ 14.3 $cmH_2O/l/s$; mean $\pm$ s.e. mean).

These studies show (1) LTC_4 and LTD_4 at the concentrations used do not affect lung irritant receptors in cats and dogs, (2) these LTs have very weak bronchoconstrictor activity in these species, and (3) in cats, LTD_4 can induce airway hyperreactivity by a vagally-dependent mechanism. This mechanism remains to be investigated, but may involve enhancement of afferent C fibre activity (Paintal, 1977), or enhancement of responsiveness to efferent nerve stimulation (Sheller et al, 1982).

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THE ACTIONS OF LEUKOTRIENES AND FPL55712 ON GUINEA-PIG ISOLATED HEART: LACK OF SPECIFICITY OF FPL55712

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Leukotrienes (LTs) have been shown to reduce both contractility and coronary flow in the guinea-pig isolated heart (Letts & Piper, 1982). In the present study we have compared the actions of LTC_4, LTD_4, LTE_4 and LTF_4 in this preparation. We have also examined the effects of the SRS-A antagonist FPL55712 (Augstein et al., 1973) on responses to LTC_4, vasopressin and the TxA_2-mimetic U-46619 (Coleman et al., 1981).

Guinea-pig isolated hearts were perfused via retrograde cannulation of the aorta at a rate of 7 ml/min with modified Krebs-Hensleit solution (Reinhardt et al., 1976) gassed with 5% CO_2 in oxygen and maintained at 37°C. Perfusion pressure was measured from a side arm of the aortic cannula and the force of ventricular contraction by a strain gauge attached by thread to the apex of the ventricle. Agonists were infused at a rate of 200 µl/min until a peak response was obtained (1-2 min). Dose-effect curves were constructed cumulatively.

LTC_4 (10^{-10} - 3 x 10^{-8} mol/l, n=5) caused concentration-related increases in perfusion pressure of up to 26 ± 13 mmHg (mean ± s.e. mean) and decreases in contractility of up to 40 ± 4%. LTD_4 (10^{-9} - 10^{-5} mol/l, n=5) also increased perfusion pressure by up to 25 ± 5 mmHg, although the concentration-effect curve was shallower than that for LTC_4. Low concentrations (up to 10^{-8} mol/l) LTD_4 caused small increases in contractility (maximum effect 6 ± 1% at 3 x 10^{-9} mol/l) whilst higher concentrations caused small decreases of up to 10 ± 3%. LTE_4 (10^{-8} - 3 x 10^{-5} mol/l, n=5) caused concentration-related increases in perfusion pressure of up to ± 10 5 mmHg, but had no consistent effect on contractility. LTF_4 (10^{-9} - 10^{-6} mol/l, n=5) increased perfusion by up to 9 ± 2 mmHg and decreased contractility by up to 14 ± 2%. Because of differences in slopes of concentration-effect curves, quantitative comparisons of potency were difficult, but the rank order for increasing perfusion pressure and reducing contractility was LTC_4 > LTD_4 > LTF_4 > LTE_4.

FPL55712 (3.8 x 10^{-6} and 10^{-5} mol/l for 30 min) reduced resting perfusion pressure by up to 20 mmHg and contractility by up to 40%. The lower concentration of FPL55712 had little or no effect on responses to LTC_4, whilst the higher concentrations caused a 10-15 fold shift to the right of the LTC_4 concentration effect curve. However, this effect was not specific since the same concentrations of FPL55712 antagonised increases in perfusion pressure and reductions in contractility caused by U-46619 (2.9 x 10^{-10} - 2.9 x 10^{-6} mol/l) and vasopressin (10^{-4} - 10^{-2} IU/ml) to a similar degree. FPL55712 therefore appears to be of limited value as a tool for elucidating the role of endogenous leukotrienes in the heart.

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EFFECTS OF GENDER AND THE OESTROUS CYCLE ON THE DISTRIBUTION OF EXOGENOUS (^{14}C)-PALMITATE IN LIPIDS OF RAT ISOLATED LUNG

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We have studied the uptake and metabolism of ^{14}C-palmitic acid (PAL) in rat isolated lung in order to compare its fate with that of another long-chain fatty acid, arachidonic acid (AA) already investigated by us (Al-Ubaidi & Bakhle, 1980; Bakhle & Zakrzewski, 1982). Although much is known about the pulmonary metabolism of PAL (van Golde, 1976), our particular experimental conditions have not been used. Lungs from male or female rats were isolated and perfused with Krebs solution at 8 ml/min via the pulmonary circulation as described previously (Al-Ubaidi & Bakhle, 1980). After equilibration each lung received a 3 min infusion of ^{14}C-PAL (4μCi per infusion; 40μM final concentration) and 7 min later perfusion was stopped. The lung was extracted, the extracts analysed by chromatography and the radioactivity in each fraction measured (Bakhle & Zakrzewski, 1982).

The distribution of ^{14}C retained in the lung at 10 min between free fatty acid (FFA) neutral lipid (NL) and phospholipid (PL) fractions was much the same in males and females: FFA, 75 ± 5 vs. 78 ± 1; NL, 4 ± 0.8 vs. 2 ± 0.1; PL, 22 ± 5 vs 20 ± 1 (males, n=6 vs females, n=24) all values as % total lung radioactivity. These values may be compared with those obtained by Bassett, Hamosh, Hamosh & Rabinowitz (1981) of 68% incorporation of ^{14}C-PAL into PL after 2 h perfusion of isolated lung. Further analysis of the PL fraction into phosphatidyl-choline (PC), phosphatidyl ethanolamine (PE) and a fraction containing phosphatidyl-inositol (PI) disclosed significantly ($P < 0.05$) more labelling in the PE fraction in females 7.5 ± 0.7 (n=24) vs 4.1 ± 1.0 in males (n=6). These values for PAL differ in several respects from those already obtained for AA. In particular there was a higher labelling of the NL fraction with AA (20-30% label) and in the PE and PI fractions label from AA was 2 - 3-fold higher (Al-Ubaidi & Bakhle, 1980; Bakhle & Zakrzewski, 1982).

The distribution of label from ^{14}C-PAL in lung of virgin female rats was also studied at the different stages of the 4-day oestrous cycle; the stages were monitored by vaginal smears (Bakhle & Zakrzewski, 1982). Differences were most obvious between pro-oestrus and the other stages. Thus, during pro-oestrus, the label in FFA was lowest (74 ± 3%, n=6) but that in PE was highest (9.4 ± 1.4%,n=6) of all the stages. Label in PC remained constant over the oestrous cycle at about 10% of total lung radioactivity. The cycle-related changes for PAL were much less than those for AA where FFA and total PL as well as the PC, PE and PI fractions show marked variations (Bakhle & Zakrzewski, 1982) From these results we would conclude that the metabolism of exogenous PAL under our conditions differs from that of exogenous AA quantitatively and qualitatively although it still shows some susceptibility to sex hormonal influences.

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ANTI-INFLAMMATORY DRUGS AND BACTERIAL INFECTION

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A major problem encountered in the clinical use of anti-inflammatory corticosteroids is that of increased susceptibility to bacterial infection. This effect is not shared by non-steroid anti-inflammatory drugs, which are selective inhibitors of cyclo-oxygenase and are commonly used to reduce the fever and pain associated with infection (Robinson et al., 1974). It has been proposed that BW755C, which is a dual inhibitor of cyclo-oxygenase and lipoxygenase, has a steroid-like profile of anti-inflammatory actions (Higgs et al., 1979). The inhibition of lipoxygenase prevents the production of leukotriene B_4 which is thought to be an important mediator of leukocyte movement and activation (Ford-Hutchinson et al., 1980). Because phagocytic leukocytes are an integral part of defence against bacterial infection, any drug which impairs leukocyte function may increase host susceptibility to infection. We have now compared the effects of BW755C, dexamethasone, hydrocortisone and indomethacin on experimental infection in mice and rabbits.

A chronic Salmonella typhimurium infection was established in groups of 22-24 CBA/Ca mice following inoculation of 10^4 virulent organisms. 30 days after infection approximately 60% of the mice had salmonella in the faeces and all animals killed at this time had positive liver and spleen cultures. Drugs were administered orally to chronically infected mice by addition to the powdered diet. Hydrocortisone (10-25 mg/kg/day) caused a dose-dependent reduction in resistance to infection resulting in death 7-23 days after treatment commenced. Post-mortems indicated that salmonella counts in liver and spleen cultures from these animals were considerably elevated. In a separate experiment administration of hydrocortisone (25 mg/kg/day) for 28 days to uninfected mice did not result in death. No deaths were recorded in groups of animals receiving BW755C (10-50 mg/kg/day) or indomethacin (1.0-2.5 mg/kg/day) and after 28 days treatment salmonella counts in organs and faeces of these animals did not differ significantly from those in control groups.

Groups of four male Dutch rabbits (1.8-2.6 kg) were injected intra-dermally with virulent cultures of Staphylococcus aureus ($1.25\text{-}5.0 \times 10^7$ organisms in 100 µl). Drugs were administered intra-muscularly once a day for 4 days prior to infection. 24 h after inoculation of S. aureus, a skin lesion developed which consisted of a raised white central area of pus surrounded by a larger area of erythema. Dexamethasone (1 mg/kg/day) caused a significant increase in the size of skin lesions but indomethacin (20 mg/kg/day) and BW755C (50 mg/kg/day) did not significantly alter the response.

These experiments confirm the observations that corticosteroids, unlike non-steroid anti-inflammatory drugs, lower resistance in experimental infection (Robinson et al. 1974). In common with other non-steroid anti-inflammatory drugs, BW755C does not exacerbate infection. It is possible, therefore, that BW755C could selectively suppress inflammatory reactions mediated by endogenous arachidonate metabolites, without impairing the response of these cells to bacterial chemotactic factors. This would be a significant clinical advantage over steroids, which appear to have a more fundamental effect on defence mechanisms.

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INHIBITION OF PLATELET ARACHIDONIC ACID METABOLISM IN VIVO IN THE RAT: ITS EFFECT ON INTRAVASCULAR PLATELET AGGREGATION

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Smith and Duncan (1981) showed that collagen (40μg/kg i.v.) caused a fall in the circulating platelet count of anaesthetised animals. We now examine the relationship between arachidonic acid metabolism and collagen-induced intravascular platelet aggregation (CIPA) in the rat.

Rats were anaesthetised and the platelet count measured as described by Smith and Duncan (1981). Blood samples were taken by cannulating a carotid artery (Mallarkey and Smith, unpublished). Plasma levels of thromboxane B_2 (TXB_2) and 6-keto prostaglandin $F_1\alpha$ (6-keto $PGF_1\alpha$) were measured by radioimmunoassay.

TXB_2 and 6-keto $PGF_1\alpha$ were undetectable before injection of collagen (sensitivity 0.3 and 0.2 ng/ml respectively). After two control responses to collagen, the non-steroidal anti-inflammatory drugs (NSAIDs) indomethacin and piroxicam and BW755C, an inhibitor of both cyclo-oxygenase and lipoxygenase enzymes (Higgs et al, 1979), were given i.v. 5 min before the next injection of collagen.

DRUG	DOSE mg/kg i.v.	% INHIBITION of CIPA	Plasma TXB_2 ng/ml	Plasma 6-keto $PGF_1\alpha$ ng/ml
Control	-	-	4.2 ± 1.0 (5)	N.D. (5)
Indomethacin	8	52.5 ± 1.6 (5)	N.D. (5)	N.D. (5)
Indomethacin	16	34.5 ± 2.0 (5)	N.D. (5)	1.4 ± 0.2 (5)
Piroxicam	0.8	30.1 ± 3.2 (11)	N.D. (4)	N.D. (5)
Piroxicam	1.6	25.0 ± 4.2 (4)	N.D. (5)	0.7 ± 0.1 (5)
BW755C	2	19.8 ± 7.4*(9)	13.0 ± 1.4[1](5)	N.D. (5)
BW755C	16	31.4 ± 6.5 (4)	5.1 ± 1.0 (5)	1.5 ± 0.1 (5)

N.D. = Not detected * = Potentiation [1] = SIG.P<0.005
Figures in brackets indicate number of observations

These results confirm previous observations that NSAIDs inhibit cyclo-oxygenase. It has also been reported that high doses inhibit both cyclo-oxygenase and lipoxygenase activity (Siegel et al, 1979). This would prevent production of 12-hydroperoxyarachidonic acid (12-HPAA). Its positional isomer, 15-HPAA inhibits prostacyclin synthesis in vitro (Gryglewski et al, 1976). If 12-HPAA exerts similar activity in vivo then inhibition of lipoxygenase by high doses of NSAIDs would explain our observations. The reduced activity of the NSAIDs at higher doses could be due to RBC lysis as plasma samples were slightly pink. The resultant release of ADP would add to the CIPA.

We could not demonstrate inhibition of CIPA by low doses of BW755C consistent with cyclo-oxygenase inhibition, but the potentiation observed may have been due to lipoxygenase inhibition when more arachidonic acid would then be available for conversion to TXA_2. The inhibition of CIPA found at higher doses of BW755C could be due to both enzymes being inhibited. If BW755C is only a lipoxygenase inhibitor at 2 mg/kg, the absence of detectable levels of 6-keto $PGF_1\alpha$ remains to be explained.

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EFFECTS OF ANTI-INFLAMMATORY DRUGS ON SKIN ALLOGRAFT SURVIVAL IN THE RAT

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Previously we reported changes in the local levels of cyclo-oxygenase products (COP) in rat skin grafts during the normal course of graft establishment and rejection. Furthermore it was shown that the immunosuppressive agent,cyclosporin A (CSA) was able to suppress the COP levels in allografts and prolong allograft survival (Fan & Lewis, 1982a). It was questioned whether an interference with arachidonic acid (AA) metabolism by non-steroid anti-inflammatory drugs can modify the survival time of allografts.

In the present study, groups of WAG rats ($RT1^u$) bearing two allografts from DA rats ($RT1^a$) were treated with different drugs, starting on the first post-transplant day until the grafts were rejected or up to a maximum of 14 days in the case of CSA. Grafting technique and blood flow measurements were as described earlier (Fan & Lewis, 1982b). The results are summarised below:

Group	Daily dose	Route	Mean allograft survival time days $\pm$ s.e.m.(n)	P value
No treatment			9.4 $\pm$ 0.1 (30)	-
Cyclosporin A	1 x 20 mg/kg	i.m.	34.7 $\pm$ 0.4 (10)	< 0.001
Indomethacin	2 x 1.5 mg/kg	p.o.	7.1 $\pm$ 0.6 (6)	< 0.001
Benoxaprofen	2 x 15 mg/kg	p.o.	6.9 $\pm$ 1.1 (8)	< 0.001

Radioimmunoassays for PGE_2, $PGF_{2\alpha}$, 6-oxo-$PGF_{1\alpha}$ and TxB_2 contents in skin graft extracts revealed that indomethacin caused a 40 - 50% inhibition of the total COP while benoxaprofen produced no significant effect on the total COP, although it significantly reduced 6-oxo-$PGF_{1\alpha}$. It is not known if all these drugs modified the levels of lipoxygenase products but it appears that the ability of CSA to interfere with the formation/release of AA metabolites in the allograft could be a component of its immunosuppressive activities. However, mere modification of either the cyclo-oxygenase or the lipoxygenase pathway of AA metabolism by anti-inflammatory drugs cannot prevent rejection. On the contrary, both indomethacin and benoxaprofen accelerated rejection which indicates that an AA metabolite, possibly PGI_2, might normally exert an immunosuppressive activity representing a 'fine tuning' of an immune response.

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STIMULATION OF PROSTACYCLIN RELEASE FROM THE PIGLET LUNG THROUGH PURINERGIC RECEPTORS

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Prostacyclin (PGI_2), a potent vasodilator and inhibitor of platelet aggregation, is generated by vascular tissue. Although its plasma level is normally too low to have significant effects in vivo (Blair et al., 1982), production is stimulated by other vasoactive agents (Hong, 1980) which supports the concept that PGI_2 is important in local control of haemostasis and vascular tone. Adenosine triphosphate (ATP), another potent vasoactive agent, transiently attains high extracellular concentrations in response to injurious stimuli (Green & Stoner, 1950), is released into the circulation by exercising muscle (Forrester, 1981) and stimulates prostaglandin release from several perfused organs (Needleman et al., 1974). We have now investigated the effects of ATP and other adenosine derivatives on PGI_2 generation by the piglet lung.

Isolated lungs were perfused (10 ml/min) via the pulmonary artery with Krebs solution containing 4.5% (w/v) Ficoll 70 (Hellewell & Pearson, 1982). Agonists were administered as a 100 µl bolus injection, venous effluent was collected every 18 sec for 3 min, and PGI_2 was determined in each sample by radioimmunoassay of its stable metabolite, 6-keto-$PGF_{1\alpha}$. Basal release of PGI_2 was 1.0-1.5 ng/ml. Bolus injections of ATP elicited a dose-dependent stimulation of PGI_2 release with a threshold response to 0.1 µmoles ATP (this amount was diluted at least X50 in the pulmonary vasculature) and a maximum level of 14 ng/ml, achieved with 3 µmoles ATP. The response was transient and returned to baseline level within 3 min. ADP was approximately equipotent with ATP, while AMP and adenosine were almost inactive. These results are consistent with the stimulation of PGI_2 release occurring through a P_2 purinergic receptor mechanism (Burnstock, 1979).

Since ATP is rapidly metabolised by discrete ectonucleotidases in the pulmonary circulation (Ryan & Ryan, 1977) we tested the non-metabolisable ATP analogue, adenosine-5'-O-(3-thiotriphosphate) (ATP-γ-s) as a stimulus of PGI_2 release. This compound also stimulated PGI_2 generation in a dose-dependent manner and was more effective than ATP. A 3 min infusion of ATP or of ATP-γ-s (final concentration $\sim$ 50 µM) resulted in a single peak of PGI_2 of similar duration to that obtained following a bolus injection.

The results suggest that the pulmonary circulation possesses purinergic receptors through which ATP at physiological concentrations can induce large transient increases in PGI_2 release. This phenomenon may play a significant role in the control of pulmonary haemostasis.

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(^{3}H)-NORADRENALINE RELEASE FROM THE MOUSE VAS DEFERENS BY ELECTROTONIC DEPOLARIZATION OF NERVE TERMINALS

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Electrophysiological evidence indicates that electrotonic depolarization of nerve terminals releases acetylcholine even under conditions of complete blockade of the inward sodium current by tetrodotoxin (Katz & Miledi, 1965; Illes & Thesleff, 1978). We have now studied biochemically whether a similar mode of transmitter release can be elicited in postganglionic sympathetic nerves of the mouse vas deferens.

Vasa deferentia of mice were preincubated with ^{3}H-noradrenaline and subsequently superfused with Krebs solution containing cocaine 10 µM and phentolamine 30 µM (see Illes et al, 1982). The intramural nerves were stimulated at a frequency of 0.5 Hz for periods of 2 min. Either impulses of a current strength of 50 mA and a pulse duration of 1 ms ('50 mA impulses') or impulses of a current strength of 200 mA and a pulse duration of 2 ms ('200 mA impulses') were used. The outflow of total tritium and in addition of ^{3}H-noradrenaline and ^{3}H-3,4-dihydroxyphenylglycol (^{3}H-DOPEG) was measured by liquid scintillation spectrometry. ^{3}H-noradrenaline was separated from its metabolites by the method of Graefe et al (1973).

In contrast to 50 mA impulses, 200 mA impulses evoked tritium overflow even in the presence of 0.5 µM tetrodotoxin. A substitution of the total Na^+ in the medium by $Tris^+$ abolished responses to 50 mA impulses, but massively increased responses to 200 mA impulses. The basal outflow of tritium consisted mainly of ^{3}H-DOPEG with only a small percentage of ^{3}H-noradrenaline. Stimulation at both 50 and 200 mA led to a selective increase in the outflow of ^{3}H-noradrenaline, which was dependent on the external Ca^{2+}-concentration (0-10 mM). Omission of Ca^{2+} from the medium practically abolished the overflow of ^{3}H-noradrenaline evoked by 200 mA impulses, whereas the overflow of total tritium was only reduced. In contrast to the overflow caused by stimulation at 50 mA, that caused by stimulation at 200 mA was not reduced by the inhibitors of Ca^{2+} currents, Mg^{2+} (20 mM) and Co^{2+} (5 mM), and was not enhanced by the inhibitors of K^+ currents, tetraethylammonium (5 mM) or 4-aminopyridine (1 mM). Similarly, even in concentrations larger than those needed to reduce tritium overflow at 50 mA, normorphine (40 and 100 µM) and fentanyl (4 µM) failed to reduce tritium overflow at 200 mA.

It is concluded that high intensity electrical field stimulation elicits a tetrodotoxin-resistant, calcium-dependent release of noradrenaline, probably by direct depolarization of nerve terminals. Electrophysiological studies have shown that noradrenaline can be released both in a synchronous and non-synchronous manner. The former is represented by nerve stimulation-induced excitatory junction potentials (e.j.ps) and the latter by spontaneous e.j.ps. The release evoked by high intensity stimulation may be mainly non-synchronous, as opposed to the synchronous release triggered by action potentials. In addition, high intensity stimulation seems to increase the outflow of ^{3}H-metabolites in a calcium-independent manner.

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A STUDY OF PRE- AND POSTSYNAPTIC α_2-ADRENOCEPTORS IN THE PITHED RAT

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Alpha adrenoceptors have been subclassified into two groups, α_1 and α_2 based on the affinity of agonists and antagonists for the two sites. (Langer, 1981). The α_2 receptors have been further divided, anatomically, into pre and postsynaptic subgroups. There is some debate as to whether the pre and postsynaptic α_2 adrenoceptors are identical. (De Jonge et al, 1981; Hicks 1981).

Using a series of α_2 adrenoceptor antagonists we have attempted to differentiate in vivo, between the pre and postsynaptic α_2 adrenoceptors, and have compared them with the classical postsynaptic α_1 adrenoceptor. Experiments were performed in pithed rats (female Sprague-Dawley, 230-270g). Pressor dose response curves (postsynaptic response) were obtained by cumulative i.v. administration of B-HT 933 (α_2) or methoxamine (α_1). Presynaptic α_2 adrenoceptor stimulation was measured as an inhibition by B-HT 933 of electrically stimulated tachycardia (Gillespie et al., 1970). Shifts, measured at 50% of the maximal response, in each of the dose response curves were used to assess the potency of antagonists at the receptor sites. The selectivity of an antagonist for a given receptor is indicated by the ratio of the shift in the dose response curves obtained at one site divided by that obtained at another. (see Table).

Drug	Dose (mg/kg)	$\frac{\alpha_2 Pre}{\alpha_1 Post}$	$\frac{\alpha_2 Pre}{\alpha_1 Post}$	Drug	Dose (mg/kg)	$\frac{\alpha_2 Pre}{\alpha_2 Post}$	$\frac{\alpha_2 Pre}{\alpha_1 Post}$
Wy 26703	0.3	0.8	3.4	RS21361	1.0	1.3	1.0
	3.0	2.1	23.4		10.0	0.9	5.3
Wy 25309D	0.3	2.0	7.3	Yohimbine	0.3	1.1	5.6
	3.0	2.5	56.4		1.0	1.2	10.6
RX 781094	0.3	2.0	2.8	Phentolamine	1.0	1.2	0.6
	3.0	2.1	27.7				

The ratio α_2Pre/α_2Post did not differ markedly from unity for RS 21361 yohimbine or phentolamine. Ratios of 0.8-2.5 were obtained for Wy 25309D, 26703, (Lattimer, 1982) and RX 781094. These ratios, unlike those obtained for the α_2/α_1 comparison were not large enough to suggest a significant difference between the pre and post synaptic α_2 adrenoceptors.

With the exception of phentolamine, the compounds showed a selectivity for the presynaptic α_2, compared with the α_1 adrenoceptor, which increased with dose. This result suggests that the selectivity of an α_2 adrenoceptor antagonist should be qualified by a consideration of its potency and the dose administered.

In conclusion, in a study using α adrenoceptor antagonists, no difference could be found between the pre and postsynaptic α_2 adrenoceptors. The selectivity of α_2 adrenoceptor antagonists increased with dose over the range employed.

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HYPOXIA BLOCKS THE NON-ADRENERGIC NON-CHOLINERGIC INHIBITORY NERVES IN THE BOVINE RETRACTOR PENIS MUSCLE

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During a study of the effects of varying the O_2 tension on the responsiveness of isolated smooth muscles to drugs and to nerve stimulation (Bowman & McGrath, 1982), it was noticed that the relaxations evoked by stimulating the non-adrenergic, non-cholinergic (nanc) inhibitory nerves in the isolated rat anococcygeus and bovine retractor penis muscles became progressively smaller as the oxygen content of the gassing mixture was reduced, while contractions in response to noradrenergic stimulation were, if anything, increased in size.

In this communication, we describe further observations on the effects of hypoxia on the inhibitory responses of the bovine retractor penis muscle to field stimulation, and to some agonists that cause relaxation. Hypoxia was induced by changing the gas mixture with which the bathing fluid (Krebs' solution) was bubbled from 5% CO_2 in O_2 to 5% CO_2 in N_2.

In the isolated bovine retractor penis muscle, complete abolition of the inhibitory response evoked by field stimulation (1 - 2 Hz for 10 s) occurred within 2 min of removal of O_2 from the gassing mixture. When the relaxations evoked by field stimulation were abolished, relaxations in response to sodium nitroprusside, prostaglandin E_1, VIP, or the inhibitory factor extracted from the bovine retractor penis muscle, remained unaffected. With more prolonged hypoxia (10 - 20 min), the relaxations produced by these agonists became slower in onset, but undiminished in size.

Hypoxia of 20 min duration did not affect the inhibitory responses of the isolated taenia of the guinea-pig caecum, nor the motor responses of the isolated guinea-pig bladder, evoked by field stimulation of their intrinsic nanc nerves.

The site of the block of the neurally-evoked responses of the bovine retractor penis caused by hypoxia is unlikely to be nerve conduction, or stimulus-release coupling, since transmission in other autonomic nerves was not affected; nor is the cause of the block likely to be inability of the muscle to relax, since agonists still produced relaxation. The results are compatible with the possibility that a novel transmission mechanism exists in which an oxygen-requiring enzyme may convert an inactive precursor of the transmitter into its functional form. These results also add support to the conclusion, based on the blocking actions of apamin and oxyhaemoglobin (Bowman & Gillespie, 1981), that the nanc inhibitory mechanism in the taenia of the guinea-pig caecum differs from that in the bovine retractor penis muscle.

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Ca^{++}-FREE AND Ca^{++}-REQUIRING α_1 RESPONSES IN RAT ANOCOCCYGEUS HAVE DIFFERING AGONIST POTENCY SERIES

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In rat anococcygeus, agonists which are α_2 at pre-junctional sites, contract smooth muscle with greater potency than is expected from their α_1 effects in other tissues, yet are blocked by "selective" α_1-antagonists (Docherty & Starke, 1981; McGrath, 1982). In rat aorta, which shows parallel features (Ruffolo et al., 1982), "α_1-agonists", but not "α_2-agonists", initiate contraction in the absence of extracellular Ca^{++} (Godfraind et al., 1982). This communication analyses the Ca^{++} dependence of contractions to α-agonists in rat anococcygeus.

Isometric tension was recorded in vitro (Gillespie, 1972). Responses to a range of concentrations of noradrenaline (NA), amidephrine (AMID) and xylazine (XYL), and to depolarisation with KCl (100 mM) were obtained in the absence of Ca^{++}. At each concentration of agonist, Ca^{++} was then added in steps to the Krebs' saline in order to calculate pD_2 values for Ca^{++}, i.e. -log ([Ca^{++}] producing 50% of maximum tension attained with [Ca^{++}] $\leqslant$ 5 mM). EDTA (20 μM) was present throughout, cocaine (3 μM) for NA experiments.

(a) Without Ca^{++}: NA (0.03 - 30 μM) produced concentration-related contractions which reached a peak within 1 min then declined over 10 min. This contraction, as a proportion of the "Ca^{++} Present Response" (CPR), increased with NA concentration to 21 ± 1% (n=8) at 3 μM. After nifedipine (10 μM), the response was transient, a rapid peak (20 s) declining within 1 min. AMID (0.05 - 50 μM) produced concentration-related contractions which were slower but higher than for NA (42 ± 3% of CPR, n=6, at 0.5 μM). XYL produced virtually no contraction even at concentrations which were "maximal" with Ca^{++} present (30 μM). Nifedipine (10 μM) reduced contractions to AMID.

(b) Ca^{++} concentration/response relationship: The pD_2 for Ca^{++} increased with increasing concentration of each agonist to an optimum then decreased, e.g.

drug	[optimal]	pD_2	mean CPR	mean "Ca^{++} free"
NA	0.3 μM	3.91	6.3 g	0.69 g
AMID	0.5 μM	3.88	6.5 g	2.70 g
XYL	30 μM	3.72	5.7 g	0.05 g
KCl	100 mM	3.35	6.8 g	0.60 g

With nifedipine (10 μM), the Ca^{++} pD_2's for any concentration of the three agonists fell to 3.4 - 3.6 and for KCl to 2.8.

Thus two aspects of α_1 activation have been identified which have different potency series for agonists: (i) a Ca^{++} free response for which xylazine is ineffective, (ii) a Ca^{++} requiring response activated by all three agonists. In the involvement of Ca^{++}, neither corresponds precisely to KCl-induced depolarisation. An explanation of this may require either sub-division of the α_1 category into two further receptor sub-types or the concept of two modes of activation of one receptor.

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