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The effects of clonidine on cortical neurones: evidence for its action as a partial agonist at α_1-adrenoceptors

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Evidence obtained with receptor-labelling techniques indicates that both α_1- and α_2-adrenoceptors occur in brain tissue (Langer, 1980). Phenylephrine, a selective α_1-adrenoceptor agonist, is a potent excitant of cortical neurones (Bevan, Bradshaw & Szabadi, 1977). In the present experiments, we have compared the effects of phenylephrine with those of clonidine, a relatively selective stimulant of α_2-adrenoceptors (Langer, 1980).

Spontaneously active single neurones were studied in the somatosensory cortex of the halothane-anaesthetized rat; all the drugs were applied by microelectrophoresis (Bevan *et al.*, 1977).

The agonistic effects of clonidine and phenylephrine were compared on 34 cells which yielded consistent excitatory responses to phenylephrine; 15 of these cells were excited by clonidine, the remaining 19, however, did not respond to clonidine applied by ejecting currents of up to 150 nA. The relative potencies of the two drugs were compared by calculating the equicurrent magnitude ratio (Bradshaw, Pun, Slater & Szabadi, 1981b). The mean equicurrent magnitude ratio (clonidine/phenylephrine) was 0.139 ± 0.039 ($n = 29$).

The mobilities of the clonidine and phenylephrine ions were compared using the method of Bradshaw, Pun, Slater & Szabadi (1981a). Eight micropipettes were used. The transport number of noradrenaline, released from a 0.05 M [^{14}C]-noradrenaline bitartrate solution (1 mCi/mmol), was: 0.098 ± 0.007 (in the presence of 0.05 M clonidine hydrochloride) and 0.106 ± 0.006 (in the presence of phenylephrine hydrochloride 0.05 M). There was no statistically significant difference between the two values of transport number ($P > 0.1$; Student's t-test).

Since clonidine showed only a very weak agonistic activity, we examined whether it could antagonise excitatory neuronal responses to phenylephrine and noradrenaline. Acetylcholine was used as a control agonist. Successful drug-interaction studies were conducted on 15 cells. On each of these cells, in the presence of clonidine (clonidine hydrochloride 0.05 M, 5–10 nA), responses to phenylephrine and noradrenaline were antagonized, whereas the response to acetylcholine was not affected. The mean sizes of the responses to the agonists (expressed as percentage of control), in the presence of clonidine, were: 24.6 ± 3.3 (phenylephrine); 39.0 ± 7.4 (noradrenaline); 99.8 ± 4.3 (acetylcholine).

These results show, in agreement with previous findings (Anderson & Stone, 1974), that cortical neurones are sensitive to clonidine. Clonidine, however, appears to be a weak agonist: it was approximately ten times less potent than phenylephrine. This difference in potency is likely to reflect a genuine biological difference between clonidine and phenylephrine, since there was no significant difference between the mobilities of the two drugs. Finally, clonidine antagonized the response to phenylephrine, a selective α_1-adrenoceptor stimulant. Our results suggest that clonidine, similarly to its action in the rat aorta (Ruffolo, Waddell & Yaden, 1980), may act as a partial agonist at α_1-adrenoceptors on cortical neurones.

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Pharmacological evidence for the subclassification of central dopamine receptors in the rat

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The dopamine receptor agonist SK & F 38393 causes contralateral rotation in rats with nigrostriatal lesions but fails to cause stereotypy (Setler, Sarau, Zirkle & Saunders, 1978). The dopamine receptor antagonist clozapine fails to inhibit apomorphine-induced stereotypy in rats (Stille, Lauener & Eichenberger, 1971). To investigate whether these atypical actions are due to differences in dopamine receptors mediating the two responses, we have compared the actions of these drugs with other dopamine receptor agonists and antagonists in the two tests.

Rats with nigrostriatal lesions were prepared by infusing 6-hydroxydopamine (8 μg in 4 μl) into the substantia nigra on one side (fr. 2.2, h.–2.4, sag. 2.0, Konig & Klippel, 1963). In these rats agonists were injected intraperitoneally or directly into the striatum (fr. 7.5, h. 0.0, sag. 2.5) on the lesioned side via an indwelling cannula. Stereotypy (licking and chewing) was assessed in non-lesioned rats following the injection of agonists intravenously or, via indwelling cannulae, bilaterally into the olfactory tubercles (A 8.6, H–2.4, L 2.5, De Groot, 1959). The effects of antagonists (i.p.) on responses induced by agonists (i.p. or i.v.) were determined in both tests at the times of peak effects of both agonists and antagonists.

Dopamine and the 2-aminotetralins A-5,6 DTN and A-6,7 DTN (3–30 μg/rat intracerebrally) were non-selective and caused contralateral rotation and stereotypy. Apomorphine was also non-selective, causing contralateral rotation (0.1–1.0 mg/kg i.p.) and stereotypy (0.2–1.6 mg/kg i.v.). Unlike these agonists, SK & F 38393, 3–30 μg/rat intracerebrally, or 1–3 mg/kg peripherally, induced contralateral rotation but not stereotypy.

Contralateral rotation induced by SK & F 38393 (3 mg/kg i.p.) was inhibited by clozapine (ED_{50} 6.2 mg/kg i.p.) but not by haloperidol (0.8 mg/kg i.p.). Contralateral rotation induced by apomorphine (0.5 mg/kg i.p.) was inhibited by clozapine (ED_{50} 24 mg/kg i.p.) and haloperidol (ED_{50} 0.1 mg/kg i.p.). Stereotypy induced by apomorphine (1 mg/kg i.v.) was inhibited by haloperidol (ED_{50} 0.1 mg/kg i.p.) but not by clozapine (40–160 mg/kg i.p.). Like haloperidol, oxiperomide, pimozide and fluphenazine inhibited apomorphine rotation and stereotypy (ED_{50}'s 1.0, 1.1–4.0 and 0.05 mg/kg i.p. respectively) but failed to inhibit SK & F 38393-induced rotation. Cis-α-fluphenthixol, thioridazine and clothiapine (ED_{50}'s 0.2–0.4, 14.6–22 and 1–1.7 mg/kg i.p., respectively) were non-selective and inhibited rotation induced by SK & F 38393 or apomorphine and apomorphine stereotypy. Yohimbine (2 mg/kg), prazosin (2 mg/kg), propranolol (20 mg/kg), atropine (20 mg/kg), mepyramine (10 mg/kg) or methysergide (20 mg/kg) did not inhibit apomorphine rotation or stereotypy or SK & F 38393 rotation.

The present results provide evidence for a functionally related sub-classification of central dopamine receptors. It is proposed that contralateral rotation induced by dopamine agonists is mediated by two types of dopamine receptors. One of these receptors is classifiable in that it is selectively stimu-

lated by SK&F 38393 and is blocked by clozapine but not by haloperidol; the other receptor is blocked by both haloperidol and clozapine. A third receptor, mediating stereotypy, is *insensitive* to the agonist actions of SK&F 38393 and is blocked by haloperidol but not clozapine. In this classification the receptors sensitive to blockade by haloperidol are also sensitive to blockade by oxiperomide, pimozide and fluphenazine. Clothiapine, cis-α-flupenthixol and thioridazine do not discriminate between the three proposed receptor types.

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The effect of ADTN and some of its derivatives on dopamine receptor binding in rat striatum

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The potent dopamine receptor agonist ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) is more active than *iso*-ADTN (2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene) in several tests of dopamine receptor function. R-(+)-ADTN is more active than S-(−)-ADTN as a dopamine agonist and the dimethyoxy derivatives of ADTN or *iso*-ADTN are virtually inactive (Woodruff, Davis, Andrews & Poat, 1979). N,N-di-n-propyl-*iso*-ADTN, which structurally resembles apomorphine, is more active than *iso*-ADTN on the dopamine-sensitive adenylate cyclase (Cannon, Costall, Laduron, Leysen & Naylor, 1978).

We have compared the potencies of these ADTN derivatives, together with apomorphine and dopamine, in receptor binding assays, using [^{3}H]-(+)-sulpiride (26.2 Ci/mmol; 15 nM), [^{3}H]-(+)-ADTN (35 Ci/mmol; 5 nM) and [^{3}H]-cis-(Z)-flupenthixol (17.2 ci/mmol; 3 nM) as ligands. Binding assays were carried out in partially purified homogenates of rat striatum as described by (Freedman, Poat & Woodruff, 1981) except that [^{3}H]-cis-flupenthixol and [^{3}H]-ADTN binding assays were carried out in the absence of nialamide and ascorbate using GF/B filters. S-(−)-sulpiride (1 μM), cis-flupenthixol (1 μM) and (±)-ADTN (1 μM) were used to define specific binding in the sulpiride, flupenthixol and ADTN binding assays respectively.

The results are shown in Table 1, which shows that the rank order of potency of (+) and (−)-ADTN, *iso*-ADTN and N,N-dipropyl-*iso*-ADTN are similar whichever tritiated ligand is used. The potency ratios for (+) and (−)-ADTN in the ADTN binding assay are less than those obtained in a previous study which used lower specific activity ligand (Woodruff *et al.*,

Table 1

Drug	IC_{50} *agonist* [^{3}H]-sulpiride	[^{3}H]-cis-flupenthixol	[^{3}H]-ADTN
(+)-ADTN	16	244	1.7
N,N-dipropyl-*iso*-ADTN	16	251	0.4
(±)-ADTN	28	242	3.6
Apomorphine	32	69	7.1
(−)-ADTN	250	18,080	22.8
Dopamine	580	423	12.6
(±)-*iso*-ADTN	760	>> 10,000	30.4
Dimethoxy-*iso*-ADTN	>> 10,000	>> 10,000	268.0

Potencies of some drugs in dopamine receptor binding assays. IC_{50} values are concentrations (nM) causing 50% displacement of specific binding. Each value is the mean of at least 3 experiments carried out in triplicate.

1979). Our results do not provide evidence that the specific binding of tritiated sulpiride, cis-flupenthixol or ADTN in rat striatum represents binding to different populations of dopamine receptors.

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Effect of chronic antidepressant administration on rat frontal cortex α_2- and β-adrenoceptor binding

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Long-term administration of certain antidepressants is associated with adaptive changes in rat brain α_2-adrenoceptor sensitivity, as assessed by studying the effect of drug administration on the ability of a low dose of clonidine (CLON) to lower rat brain 3-methoxy-4-hydroxyphenylethyleneglycol sulphate (MHPG-SO_4) content. For example, chronic desipramine induces subsensitive α_2-adrenoceptors (McMillen, Warnack, German & Shore, 1980; Sugrue 1981a). In contrast, the sensitivity of the receptors is enhanced by chronic mianserin (Sugrue, 1980). Other chronically administered antidepressants such as iprindole, trazodone, nisoxetine and salbutamol are devoid of effect on rat brain α_2-adrenoceptor sensitivity (Sugrue, 1981b). In light of these observations, the effect of chronically administered desipramine, mianserin and iprindole on rat frontal cortex [^{3}H]-CLON binding was investigated since it could be speculated that changes in receptor sensitivity may be manifested by alterations in the characteristics of the radioligand recognition site. Drug effects on [^{3}H]-dihydroalprenolol (DHA) binding were also investigated since it has been suggested that the sensitivity of rat cortical α_2-adrenoceptors may be regulated by β-adrenoceptors (Maggi, U'Prichard & Enna, 1980). Electroconvulsive shock therapy (ECT) and the monoamine oxidase inhibitors pargyline and tranylcypromine were also studied.

Adult male Sprague-Dawley rats were used. Desipramine, mianserin and iprindole (all 10 mg/kg) were injected i.p. every 12 h for 14 days and rats were killed 12 h after the last injection. Pargyline (25 mg/kg) and tranylcypromine (5 mg/kg) were injected i.p. once daily for 14 days and rats were killed 24 h after the last dose. ECT (100 mA for 1 s) was applied by ear-clip electrodes once daily for 10 days and rats were killed 6.5 h after the last shock. Specific [^{3}H]-CLON and [^{3}H]-DHA binding was the difference in radioactivity bound in the presence and absence of phentolamine (10 μM) and ($\pm$)-propranolol (20 μM) respectively. Scatchard analysis indicated that both radioligands are bound to a single population of recognition sites.

Long-term administration of desipramine and iprindole, but not mianserin, reduced cortical [^{3}H]-DHA binding. Scatchard analysis indicated that both drugs decreased the number of binding sites (B_{max}) with no change in the dissociation constant (K_D). [^{3}H]-DHA binding was also decreased by chronic ECT. In contrast to these observations, none of the above procedures altered [^{3}H]-CLON binding. However, [^{3}H]-CLON binding was decreased by chronically administered pargyline and tranylcypromine. This was due to a decreased B_{max} with no change in K_D. The monoamine oxidase inhibitors also reduced cortical [^{3}H]-DHA binding.

In summary, the ability of chronically administered desipramine and mianserin to modify rat brain α_2-adrenoceptor sensitivity, as assessed by the CLON-induced reduction in MHPG-SO_4 content is not reflected in [^{3}H]-CLON binding studies thus indicating that the two phenomena are not interrelated. Moreover, the ability of chronic desipramine, iprindole and ECT to alter [^{3}H]-DHA, but not [^{3}H]-CLON binding suggests that an inverse reciprocal modulation of central adrenoceptors does not occur.

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Finally, rat cortical α_2-adrenoceptor recognition sites are down-regulated by the monoamine oxidase inhibitors pargyline and tranylcypromine.

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Involvement of dopamine in the analgesic response induced by footshock

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Analgesia induced by 30 s footshock is enhanced by inhibiting 5HT synthesis or by lesioning spinal 5HT neurons and is attenuated by 5HT releasers (Curzon, Hutson & Tricklebank, 1980, 1981). We now report a similar relationship with DA.

Latency to lick the paws on exposure to a hot plate at 56°C was determined three times in sequence on male Sprague-Dawley rats (weights 200–250 g) as follows:– Initially (L1); immediately after returning to the home cage for 30 s (L2); immediately after 30 s exposure to 1 mA inescapable footshock (L3). Results are expressed as percentage analgesia score (PAS) = (L3/L2) × 100. The ratio (L2/L1) × 100 provides a non-shocked control value.

In non-drug treated rats, PAS after footshock was greater than non-shocked control values (Table 1). Non-cataleptogenic doses of the DA antagonists haloperidol (0.2 mg/kg, i.p., 60 min before test) or pimozide (1 mg/kg, i.p., 60 min before test) further increased PAS after footshock. In agreement with these findings, intraventricular 6-hydroxydopamine (6-OHDA) (200 μg in 25 μl 0.9% NaCl containing ascorbic acid 1 mg/ml, 1 h after desipramine

Table 1 Effect of drugs on footshock induced analgesia

	Percentage Analgesia Score	
	No Shock	*Shock*
Vehicle	88.8 ± 27.1	203.5 ± 32.2**
Haloperidol	96.1 ± 20.1	274.7 ± 57.4**‡
Vehicle	110.3 ± 32.4	210.1 ± 33.7**
Pimozide	109.3 ± 30.3	265.7 ± 47.8**‡
Vehicle	109.2 ± 47.4	211.2 ± 14.3**
60HDA	91.2 ± 35.6	258.6 ± 63.9**†
Vehicle	82.9 ± 15.9	215.9 ± 52.4**
Amphetamine	81.9 ± 17.1	151.9 ± 23.2**†
Vehicle	105.5 ± 29.8	192.3 ± 31.4**
Apomorphine	95.7 ± 31.6	144.2 ± 34.7* ‡

Values are means ± s.d. $n = 7–10$ per group.
Significance of differences from non-shocked values: *, $P < 0.01$; **, $P < 0.001$.
Significance of differences from vehicle treated rats: †, $P < 0.05$; ‡, $P < 0.01$.

25 mg/kg, i.p. and 10 days before testing) increased PAS after footshock. Conversely, amphetamine sulphate (2 mg/kg, i.p., 90 min before test) or the DA agonist apomorphine (0.75 mg/kg, s.c. 30 min before test) decreased PAS after footshock. None of the above effects of drug treatments were manifest in non-shocked rats.

Results indicate that dopaminergic activity inhibits footshock induced analgesic response. Whether this indicates a true analgesia or an effect on the motor response to a noxious stimulus after footshock remains to be investigated.

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Dopamine receptors and dopamine dependent behaviours in iron-deficient rats

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Iron is the most abundant trace element in the body and is associated with enzymes and membrane structures (Jacob & Worwood, 1974). Its deficiency results in abnormal behaviour in man and rat (Pollitt & Leibel, 1976; Youdim & Green, 1977). Rats made nutritionally deficient have lower brain iron content and exhibit significantly lower drug (amphetamine, apomorphine and 5-methoxy-N,N-dimethyltryptamine) and non-drug induced behaviours (Youdim & Green, 1977; Youdim, Yehuda & Ben Uriah, 1981). These animals have normal brain levels of dopamine, noradrenaline and 5-hydroxytryptamine (Youdim & Green, 1977).

Youdim & Green (1977) suggested that the altered drug induced behaviours in nutritionally iron-deficient rats may be related to post-synaptic changes. Measurement of dopamine (D_1 receptor) dependent adenylcyclase in the caudate nucleus revealed no differences between iron-deficient and control adult rats. The examination of post-synaptic dopamine (D_2) (caudate nucleus), α- and β-adrenergic (cortex); muscarinic (hippocampus); 5-hydroxytryptamine (hippocampus) and GABA (cerebellum) receptors using radioactively labelled ligands have shown a selective and significant ($P < 0.01$) reduction (55%) in B max of [^{3}H]-spiperone binding sites in iron-deficient rats without a change in the receptor Kd. The reduction in dopamine (D_2) receptor B max can be achieved more readily in young (21 day old) as compared to adult (80 day old) rats fed iron-deficient diet. The production of haemolytic anaemia in rats by the use of phenylhydrazine had no effect on [^{3}H]-spiperone binding in the caudate.

The altered dopamine D_2 receptor B max can be correlated with studies on the hypothermic effect of amphetamine and the prolactin binding site number in the liver in rats. In control animals kept at an ambient temperature of 4°C, amphetamine (15 mg/kg) induces hypothermia and this hypothermia is blocked by haloperidol and pimozide (Yehuda & Wurtman, 1975). Iron-deficient rats (Hb 6.9 ± 1.1 g%) fail to develop extensive hypothermia following treatment with amphetamine (15 mg/kg). In this respect the iron-deficient rats behave similarly to neuroleptic treated animals (Youdim *et al.* 1981).

Prolactin induces its own receptor in the livers of rats after exogenous prolactin treatment for 7–14 days (Barkey, Shani, Lahav, Amit & Youdim, 1981). It is well established that blockade of dopamine receptor by chronic treatment with neuroleptics results in increased plasma prolactin (Costal & Naylor, 1980). Normal rats treated for 7 days with either haloperidol (10 mg/kg), chlorpromazine (5 mg/kg) or fluphenazine (5 mg/kg) showed increased liver prolactin binding sites of 60, 80 and 110% respectively over the control values. It is interesting to note that iron-deficient rats also show an increase of 60% in liver prolactin binding compared to their control group. The behavioural and biochemical changes produced by iron-deficiency can be restored to normal within 8 days of feeding rats the iron-deficient diet supplemented with iron. These results may go some way to explain the possible behavioural changes produced by iron-deficiency in man (Youdim, Yehuda, Ben-Shachar & Ashkenazi, 1981).

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Neuronal development in discrete areas of rat brain during neonatal thyroid deficiency

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Impaired choline acetyltransferase (ChAT) activity in whole brain of propylthiouracil (PTU)-treated neonatal rats (Ladinsky *et al.*, 1972) prompted this investigation of postnatal development in rat cerebrum. Female Wistar rats with 4 day-old litters were fed PTU (0.3% w/w) in 41B meal. ChAT, glutamate decarboxylase (GAD) and the atropine-sensitive binding of quinuclidinyl benzilate (QNB) were assayed in whole homogenates of neonatal brain tissue, choline (Ch) uptake was assayed in fine slices ($0.05 \times 0.05 \times 1$ mm; 3H-Ch, 0.5 μM; Na^+, 118 mM).

PTU-treatment caused consistently large deficits (30–40%) in ChAT activity in corpus striatum and occasional, small deficits (approx. 10%) in frontal cortex (Table 1). Small but significant deficits (approx. 12%) were also apparent in hippocampus. Ch-uptake was minimally affected in cortex, but was 25–35% impaired in striatum. There was no evidence of impaired GAD activity in these tissues or impaired QNB-binding in cortex and striatum. Striatal ChAT activity and Ch-uptake remained impaired after withdrawal of the PTU, effects on cortical ChAT were again uncertain (Table 1).

Postnatal neurogenesis in rat cerebrum is largely confined to microneurones (Altman, 1969). Thus late differentiation of cholinergic interneurones may explain the protracted development of Ch-uptake in

Table 1 Effect of PTU-diet on ChAT and GAD activities, choline uptake and QNB binding in cortex and striatum of developing rat brain

	Age (weeks)	*ChAT* (% control)	*Choline uptake* (% control)	*GAD* (% control)	*QNB binding* (% control)
CORTEX	3–4	91.0 ± 2.3 (2,7,73)	103.0 ± 4.9 (0,4,37)	87.3; 112.0 (0,2,24)	97.2 ± 3.2 (0,3,36)
	6–7	89.0 ± 3.3 (2,5,48)	101.9 ± 7.8 (0,3,26)	88.4 (0,1,12)	95.6 (0,1,12)
	12–13	89.6; 95.9 (0,2,20)	116.9; 128.7 (1,2,20)		
	W/D*	89.5 ± 1.1 (2,4,41)	99.9 ± 2.3 (0,4,41)		
STRIATUM	3–4	61.8 ± 2.4 (10,10,107)	80.5 ± 2.5 (3,4,37)	115.1 ± 4.5 (0,4,46)	89.2 ± 0.6 (0,3,35)
	6–7	66.7 ± 4.7 (7,7,69)	70.7 ± 5.3 (2,3,26)	109.8 ± 4.9 (1,3,34)	102.6 (0,1,12)
	12–13	56.3; 64.4 (2,2,20)	77.7; 72.2 (2,2,20)		
	W/D*	70.4 ± 3.7 (4,4,41)	77.8 ± 6.2 (3,4,41)		

Effects represented as means (mean per cent ± s.e.mean, except where results of individual experiments are given). Figures in brackets respectively: number of experiments in which the effect proved significant ($P < 0.05$), the total number of independent experiments, and the total number of animals (test and control, approx. equal numbers) used in all the experiments.

*PTU withdrawn for 3 or 6 weeks at 3 or 6 weeks age, pooled data.

the striatum (Sorimachi & Kataoka, 1975) and account for the differential effects of neonatal thyroid deficiency in rat cerebrum (Table 1). The persistent deficits in the striatum suggest fewer cholinergic nerve terminals, in agreement with morphological evidence of impaired axonal branching of neurones in rat caudate nucleus, in neonatal thyroid deficiency (Lu & Brown, 1978). The unimpaired GAD activities are consistent with the development of GAD before ChAT in rat brain (Coyle & Yamamura, 1976). Some degree of selective impairment of brain development with respect to neurone type and neurotransmitter may thus be achieved by timing the onset of experimental thyroid deficiency in relation to the onset of differentiation.

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Effect of adenosine on acetylcholine output from electrically stimulated brain slices

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Vizi & Knoll (1976) showed that adenosine decreases the release of acetylcholine (ACh) induced by electrical stimulation in the guinea-pig isolated ileum and by ouabain in brain slices. Their findings were confirmed by Hayashi, Mori, Yamada & Kumitomo (1978) in the guinea-pig ileum and by Harms, Wardeh & Mulder (1979) in potassium-depolarized striatal slices.

In the present study the effect of adenosine on ACh output was investigated in cortical slices stimulated at different frequencies and the dose-response relationship was also sought.

Rat superfused cortical slices were electrically stimulated at different frequencies according to Beani, Bianchi, Giacomelli & Tamberi (1978) and ACh in the perfusate was bioassayed.

Following the addition of adenosine (30 μM) to the superfusion fluid a 21% decrease in ACh release from the unstimulated slices was found. The decrease was 44% at 2, 57% at 5 and 37% at 10 Hz stimulation frequency. Table 1 shows the dose-dependent decrease in ACh output induced by adenosine at 5 Hz.

From these experiments it appears that ACh release from electrically stimulated slices is much more sensitive to the depressant effect of adenosine than from the slices at rest and a modulatory role of adenosine on cholinergic function can be envisaged.

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Table 1 Effect of adenosine on ACh output (ng/g/min ± s.e.mean) from electrically stimulated cortical slices

	At rest		*5 Hz*	
Adenosine (μM)	ACh output	% decrease	ACh output	% decrease
0	9.86 ± 0.5	—	119.00 ± 9.4	—
1	9.56 ± 0.8	3	60.03 ± 8.3**	50
30	7.80 ± 0.9*	21	50.72 ± 6.0**	57
100	6.28 ± 0.9*	36	36.11 ± 5.0	70

Each value is the mean ± s.e.mean of at least 4 experiments.
Statistically significant difference from no adenosine: *$P < 0.05$; **$P < 0.01$.

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Changes in locomotor activity in rats following acute and chronic treatments with nicotine

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Nicotine can stimulate or decrease the motor activity of rats and the type of effect may depend upon the dose, the interval before testing and previous exposure to the drug (Bovet *et al.*, 1967; Clarke & Kumar, 1981; Morrison & Stephenson, 1972; Stolerman *et al.*, 1973). Some of these major sources of variation were further analysed by continuously recording the motor activity of male hooded rats in photocell cages (30 cm × 30 cm × 30 cm). Movements between two parallel infra-red photobeams, spaced 23 cm apart, were counted every 20 min for 80 min after subcutaneous injection of nicotine or saline. Tests in non-tolerant rats ($n = 14$) were spaced 3 or 4 days apart and the order of doses was randomised. Nicotine (0, 100, 200, 400 μg/kg base) initially produced ataxia and reduced activity in the first 20 min, but 40–80 min after injection, activity was enhanced. Both effects were dose-related ($P < 0.002$). Activity did not change over days of testing.

Tolerance was studied by comparing rats ($n = 10$) given daily injections of nicotine (400 μg/kg) with control animals ($n = 10$) given saline instead. Both groups were tested twice weekly on consecutive days, once after nicotine and once after saline in a counterbalanced way. On saline test days, the rats given daily nicotine received the drug after their test. Thus the changing response to nicotine was monitored over 4 weeks in rats receiving weekly or daily nicotine. The initial depressant effect on activity changed to enhancement after a week's treatment with nicotine ($P < 0.001$), and tolerance to the depressant effect was also seen one week after a single dose in controls ($P < 0.002$). The motor stimulant effect appeared sooner and became more prominent as the experiment progressed ($P < 0.05$); these changes developed more rapidly in rats receiving daily rather than weekly doses of nicotine. On saline test days, the rats given daily nicotine became slightly less active over successive weeks than the controls ($P < 0.02$). After 5 weeks, nicotine (0, 100, 200, 400 μg/kg) stimulated activity throughout the session in a dose-related way ($P < 0.0001$). However, a larger dose of 800 μg/kg still caused some ataxia, and activity was stimulated less than with 400 μg/kg. Three weeks after chronic injections were terminated, the rats still displayed tolerance to the depressant effects of nicotine ($P < 0.0001$).

In non-tolerant rats ($n = 11$), the initial depression of activity by nicotine (400 μg/kg) was prevented by mecamylamine (0.5, 1.0 mg/kg s.c.) given 20 min before the nicotine; the antagonist alone did not alter activity. In tolerant rats ($n = 12$), stimulation of activity by nicotine was prevented by mecamylamine (0.1, 0.32, 1.0 mg/kg s.c.) in a dose-related way ($P < 0.001$), but in similar tests in other rats ($n = 8$), hexamethonium (0.2, 1.0, 5.0 mg/kg s.c.) was ineffective.

Interpretations of tests of activity using spaced doses of nicotine may therefore be complicated by a lack of knowledge about rates at which tolerance develops and is lost. A few treatments with nicotine can unmask stimulant actions of this drug, which are probably of central origin.

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Chronic L-dopa-benserazide treatment facilitates dopamine-dependent dyskinesias in the guinea-pig

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The ability of L-dopa to alleviate akinesia and induce dyskinesia has been associated with an action on striatal dopamine (DA) receptors, whose sensitivity may consequently change to thereby modify the action of L-dopa. Within the striatum of guinea-pig we have shown the DA receptors mediating hyperactivity and dyskinesia to have differing topography. In the present study we follow the behavioural changes consequent on L-dopa-benserazide treatment by injecting DA agonists both into the 'dyskinesia' site of the striatum and peripherally.

Male Dunkin-Hartley guinea-pigs (500–550 g) were stereotaxically implanted with guide cannulae to allow drug injection into a striatal area responsive to dyskinesia induction (Ant. 8.0, Lat. ±2.5, Vert. 8.2 mm below the skull surface; see Costall, De Souza & Naylor, 1980). Animals were initially divided into three groups which received vehicle, L-dopa (100 mg/kg i.p.) or L-dopa plus benserazide (50 mg/kg i.p.) for 14 days (the L-dopa alone failed to modify striatal DA content, but combined with benserazide it caused increases of 80%, 25% and 8% at 2, 4 and 24 h after treatment; serotonin levels were not changed). The experimental routine for 14 days was 09.00 h – observe for motor change/dyskinesia, 11.00 h – administer dopamine agonist peripherally or intrastriatally and observe response, 15.00 h – treat guinea-pigs with L-dopa or L-dopa-benserazide and observe for a further 3 hours. In addition to its failure to change striatal DA levels, L-dopa alone failed to cause behavioural change; subsequent studies therefore concentrated on the use of an L-dopa-benserazide treatment. Changes in motor behaviour to this treatment alone were observed on the first 4 days of treatment as sedation which developed within 20 min of injection and persisted for 70–80 minutes. From the 5th day of treatment the sedation was absent; in virtually all animals there developed a highly characteristic grimacing and munching response ('orobuccolingual movements') and, in approximately 30% of animals, involuntary forelimb movements. During the first 2–3 days of L-dopa-benserazide treatment the biting response to tetralin (2-(N,N-dipropyl)amino-5,6-dihydroxytetralin, 0.025 mg/kg s.c., 12.5 μg intrastriatal) was the same as that of control animals, but from the 4th day of treatment this increased sensitivity to the tetralin such that responses were obtained at 0.00625 mg/kg s.c. and 2.5 μg intrastriatal. Similarly, from the 4th day of treatment 50 μg intrastriatal DA could be used to elicit orobuccolingual dyskinesias (a dose ineffective in normal animals).

We conclude, firstly, that daily treatment with L-dopa and benserazide can induce dyskinesias in the guinea-pig. Secondly, that such an extended treatment regime can facilitate the induction of dyskinesias by dopamine and the dopamine agonist, 2-(N,N-dipropyl)-amino-5,6-dihydroxytetralin, at a striatal site of action.

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Neurotoxin lesions of the lateral septum and changes in social and aggressive behaviours

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Changes in social and aggressive behaviours have been reported following electrolytic lesions of the septum (Albert & Chew, 1980; Jonason & Enloe, 1971), but the roles of specific neurotransmitter pathways remain unknown.

Ascending 5-HT and NA pathways originating in the dorsal raphe and locus coeruleus, respectively, innervate the lateral septum (Azmitia & Segal, 1979; Ungerstedt, 1971). The present study compared the effects of 5,7-dihydroxytryptamine (5,7-DHT) and 6-hydroxydopamine (6-OHDA) induced septal le-

sions on social and aggressive behaviours.

Male hooded rats, anaesthetized with halothane, received bilateral injections each of 5,7-DHT ($3 \times 0.4\,\mu g$ in $0.05\,\mu l$), 6-OHDA ($3 \times 0.4\,\mu g$ in $0.05\,\mu l$) or vehicle into the lateral septum (co-ordinates w.r.t. ear bar: A 7.4, L 1, V 3.8, 4.2 and 4.6). Two weeks post-operative recovery time was allowed. Lesions were verified biochemically by determination of septal 5-HT and NA.

Rats were isolated for five days prior to testing, and randomly assigned to be tested under high or low light. On the first test day, pairs of rats were placed in the unfamiliar test arena in the assigned light level, and the time spent in social interaction scored for 7.5 minutes. The social behaviours were largely investigatory such as sniffing or following. Locomotor activity was automatically recorded. After the first test, each rat was given 10 min in the test box, and the following day, all pairs were re-tested in the now familiar arena.

Aggression was assessed by placing the experimental rats into a long-established colony of 10 unoperated rats for 5 min, and scoring the frequency of dominance and subordination behaviours.

Overall, there was less social interaction when the test arena was unfamiliar ($F(1,21) = 37.7$, $P < 0.001$); but the 5,7-DHT group had significantly higher scores in the unfamiliar test conditions than did controls (lesion × familiarity ($F(1,21) = 13.8$, $P < 0.02$). In the colony aggression test, the incidence of dominance behaviours, and specifically of approaches to the residents, were reduced by the lesion ($U = 33$ and 34 respectively, $P < 0.05$).

In contrast, the 6-OHDA group did not differ from controls in the social interaction test, although increasing familiarity again increased responding ($F(1, 24) = 144.5$, $P < 0.001$). However, this group of rats was more aggressive: the incidence of dominance behaviours was increased ($U = 18$, $P < 0.01$), whilst the incidence of subordination behaviours was decreased ($U = 25.5$, $P < 0.02$). In particular, the frequencies of submissions and squeaks were reduced ($U = 10$, $P < 0.001$ and $U = 23$, $P < 0.02$, respectively).

The pattern of results shown by the 5,7-DHT group resembles that seen after 5,7-DHT lesions in the dorsal raphe nucleus (File *et al.*, 1979b), and after the chronic administration of benzodiazepines (File, 1980). The contrasting results from the 6-OHDA group resemble those seen after 6-OHDA lesions of the locus coerulus (Crow, *et al.*, 1978; File, *et al.*, 1979a).

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Apomorphine antagonizes the stereotyped behaviour produced by metoclopramide in the guinea-pig

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Although catalepsy induced by drugs in guinea-pigs is difficult to determine because of the 'freezing' behaviour which these animals show when handled, it was evident that metoclopramide (78 mg/kg, subcutaneously) did not produce catalepsy within the first 45 to 60 min of injection. On the contrary, metoclopramide consistently induced a state of hyperactivity which was followed by a period, lasting 15 to 45 min, of stereotyped circular movements of

the head and sniffing, which was usually accompanied by pressing of the nose against the walls or the floor of the cage. This behaviour has been reported as 'snout'- or 'nose-rubbing' in the pig and the guinea-pig respectively (Ely, Rumble & Sharman, 1978). 'Snout-rubbing' is induced by metoclopramide and other neuroleptic drugs in the pig (Fry & Sharman, 1976).

The stereotyped behaviour produced by metoclopramide in guinea-pigs is not blocked by haloperidol (Sharman, 1979). Furthermore, guinea-pigs which clearly showed signs of catalepsy following a dose of haloperidol (5 mg/kg), showed hyperactivity and stereotyped behaviour after an injection of metoclopramide (78 mg/kg s.c.) 15 min after this dose of haloperidol. Metoclopramide, like other neuroleptic drugs, blocks the stereotyped chewing-biting behaviour induced by apomorphine in the guinea-pig. However, in most cases, when both metoclopramide (78 mg/kg) and apomorphine (8 mg/kg) were administered simultaneously, neither the apomorphine-induced chewing-biting response nor the 'nose-rubbing' behaviour was observed. In the few cases in which circular movements of the head were observed, their frequency and duration were greatly reduced compared with those seen after metoclopramide alone. When smaller doses of apomorphine (2–6 mg/kg) were administered, blockade of the characteristic abnormal behaviour induced by metoclopramide was less evident or absent.

Atropine which can reverse the catalepsy induced by metoclopramide in rats (Ahtee, 1975) and enhance the stereotyped behaviour induced by amphetamine (Arnfred & Randrup, 1968), when administered at a dose of 5 mg/kg, blocked the abnormal behaviour induced by metoclopramide in guinea-pigs.

The response to metoclopramide in the guinea-pig shows many similarities to the dyskinetic behaviour induced by intrastriatal dopamine reported by Costall & Naylor (1975), in particular the fact that both responses are inhibited by apomorphine only at a dose of 8 mg/kg. Recently Fry, Sharman & Stephens (1981) and Sharman, Mann, Fry, Banns & Stephens (in preparation) have reported that in early weaned piglets showing stereotyped 'snout-rubbing' there is a reduced metabolism of dopamine in some parts of the brain. Thus it is possible that the production of some stereotyped behaviour may be mediated through dopaminergic mechanisms different from those involved in the production of stereotypies by apomorphine.

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Triazolam and related 1,4-benzodiazepines: spatial delayed alternation behaviour in the monkey *(Macaca mulatta)*

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Activity of two triazolo-1,4-benzodiazepines (Figure 1a & b) was compared with three other 1,4-benzodiazepines (Figure 1c, d & e) with different heterocyclic ring structures across the 1,2-position. Five male rhesus monkeys were tested on a spatial delayed alternation task 1 h after intraperitoneal injection of drug or drug vehicle (placebo control). The order of administration of drugs and placebo was randomised, and one week separated injections.

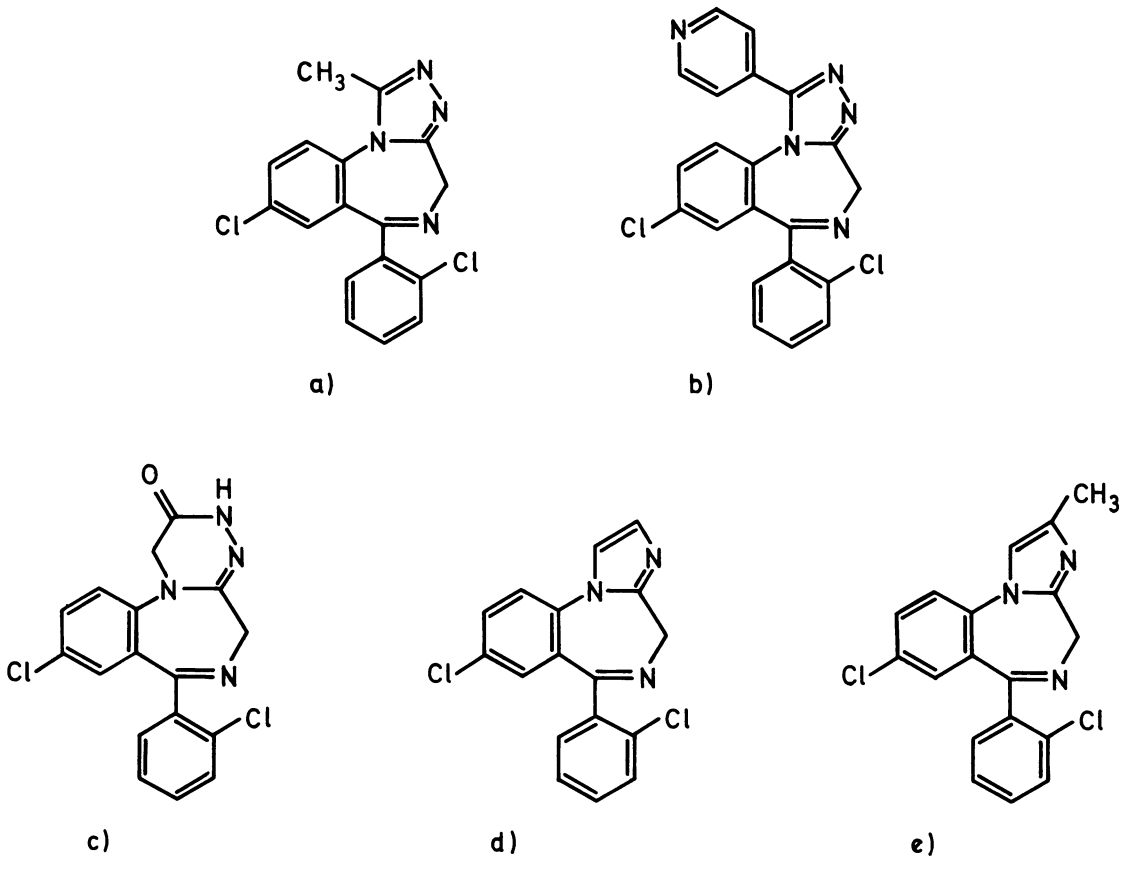

Figure 1 Structural formulae of compounds: (a) 8-chloro-6-(o-chlorophenyl)-1-methyl-4H-s-triazolo [4,3-a][1,4] benzodiazepine. (b) 8-chloro-6-(o-chlorophenyl)-1-(4-pyridyl)-4H-s-triazolo [4,3-a][1,4] benzodiazepine. (c) 9-chloro-7-(o-chlorophenyl)-3,5-dihydro-as-triazino [4,3-a][1,4] benzodiazepine-2-(1H)-one. (d) 8-chloro-6-(o-chlorophenyl)-4H-imidazo [1,2-a][1,4] benzodiazepine. (e) 8-chloro-6-(o-chlorophenyl)-2-methyl-4H-imidazo [1,2-a][1,4] benzodiazepine.

In the initial experiments the doses were 0.25, 0.5 and 0.75 mg/kg. Each dose of triazolam (a) and pyridyltriazolam (b) decreased the number of correct responses ($P < 0.001$) and increased total response time ($P < 0.01$ & $P < 0.05$ respectively). The highest dose (0.75 mg/kg) of the triazino compound (c) also reduced the number of correct responses ($P < 0.01$), but without an increase in total response time. There was no consistent effect with the imidazo (d) and methylimidazo (e) compounds.

Experiments with a lower dose range (0.005, 0.01 & 0.05 mg/kg) showed that triazolam (0.05 mg/kg) still reduced the number of correct responses. A higher dose range (1.0, 3.0 & 7.5 mg/kg) of the imidazo compound was without effect.

It would appear that the triazolo ring across the 1,2-position of a 1,4-benzodiazepine leads to marked behavioural activity which is not altered by a pyridyl radical. The triazino compound is associated with behavioural activity similar to that seen with diazepam and nordiazepam (Curry, Nicholson, Whelpton & Wright, 1977), whereas the imidazo compounds appear to have even less activity.

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Ciclazindol increases resting metabolic rate in rats in the absence of CNS stimulation

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The increase in resting metabolic rate (RMR) produced by ciclazindol has been attributed to an inhibition of noradrenaline (NA) uptake in brown adipose tissue with subsequent stimulation of Na^+, K^+-ATPase (Latham, Rothwell, Stock, White & Wyllie, 1981; Rothwell, Stock & Wyllie, 1981). Ciclazindol also inhibits brain NA (Sugden, 1974) and dopamine (DA) uptake, raising the possibility that the thermogenic response to this drug may contain a component of CNS stimulation. We describe here the effects of ciclazindol (compared with mazindol and (+)-amphetamine) on RMR and behavioural arousal.

Animals were male Sprague-Dawley rats (250–350 g) and drugs were administered i.p. Resting oxygen consumption (VO_2) was measured in a closed circuit respirometer (Stock, 1975) and behaviour was assessed using an arbitrary scoring system: 0 = sleep; 1 = motionless, drowsy; 2 = inactive but alert; 3 = normal activity e.g. exploration, grooming; 4 = overt stimulation e.g. hyperactivity, stereotypy. Scores were assigned every 10 min; the final behavioural score being the sum of 12 scores accumulated over the 2 h period immediately following dosing.

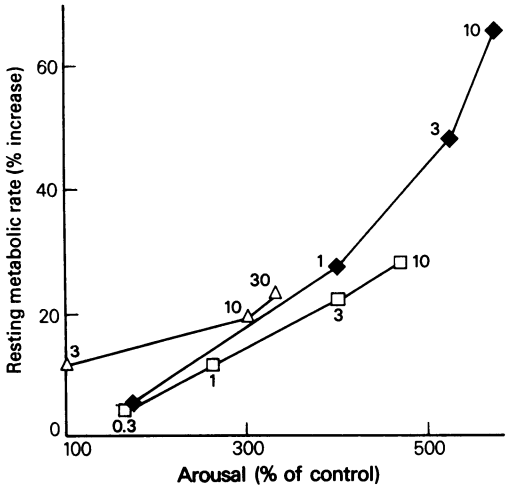

Figure 1 Correlation of metabolic and behavioural arousal effects of ciclazindol (△), mazindol (□) and d-amphetamine (◆). Numbers on the graph are drug doses in mg/kg i.p.

The behavioural stimulation produced by ciclazindol differed markedly from that produced by mazindol and (+)-amphetamine. The latter two drugs induced an increase in exploratory behaviour at low effective doses and hyperactivity and stereotypy at high doses. Ciclazindol did not induce stereotypy or hyperactivity but increased sleep latency at 10 and 30 mg/kg. Doses required to produce 40% of maximum arousal score were: ciclazindol (10 mg/kg), mazindol (1.8 mg/kg), (+)-amphetamine (0.42 mg/kg). The different behavioural effects of ciclazindol and mazindol may reflect different actions on brain DA. Mazindol (10 mg/kg) caused a 15% decrease in brain DA levels after 1 h; ciclazindol had no effect. Also, mazindol was a more potent *in vitro* inhibitor of DA uptake than ciclazindol.

The correlation of RMR with arousal (Figure 1) suggests that the metabolic and central stimulant properties of mazindol and amphetamine cannot be resolved whereas ciclazindol significantly increased RMR at doses (1, 3 mg/kg) which did not increase arousal.

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Pharmacokinetics and metabolism of Δ^1- tetrahydrocannabinol in rabbits and mice

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Plasma levels of Δ^1-tetrahydrocannabinol (Δ^1-THC), the major psychoactive component of marihuana, are typically in the ng/ml range during intoxication by the drug and rapidly fall to the pg/g range as the result of metabolism and uptake by tissues. Few accurate pharmacokinetic data are available as these levels are below the detection limits of existing assay techniques; these are in the range 0.5–1 ng/ml. Information on metabolites, particularly regarding their concentrations in tissues and their contribution to the drug's activity are also lacking.

We have developed a sensitive GC/MS assay method for Δ^1-THC in plasma based on metastable ion monitoring which is capable of measuring Δ^1-THC to 0.005 ng/ml (Harvey, Leuschner & Paton, 1980) and have used this to study Δ^1-THC pharmacokinetics in rabbits and mice. Female New Zealand white rabbits (2.5–3 kg) were treated intravenously with Δ^1-THC (1.0 or 0.1 mg/kg) in Tween 80 and isotonic saline either once or daily for 8 or 22 days. Serial blood samples were taken immediately after the last dose. Tissues were collected from animals killed at intervals to 30 days after dosing. Mice (male Charles River CD-1, 20–25 g) were treated intraperitoneally at doses of 30 and 10 mg/kg. Δ^1-THC was extracted from plasma samples with hexane, the extracts were methylated (CH_2N_2) and trimethylsilylated and the THC was measured using the metastable ion from the M^+. (m/z 386)$\rightarrow$[M-CH_3]$^+$. (m/z 371) transition with [$^2H_4$] CBN as the internal standard.

Δ^1-THC could be monitored in the rabbit for 7 days after a single 1 mg/kg dose and for 26 days after a 22-day treatment. Terminal elimination half lives obtained using non-linear regression analysis (NONLIN) ranged from 34.16 to 60.24 h (mean 60.49 h, $n = 5$) following a single dose and 77.94 h to 120.02 h (mean 94.36 h, $n = 5$) following multiple doses. This is consistent with data obtained from the dog (Garrett & Hunt, 1977) and rat (Kreuz & Axelrod, 1973) and confirms considerable accumulation and slow release of Δ^1-THC by tissues.

Similar results were obtained from mice. In addition metabolites were identified in liver and measured by GC/MS using conventional selected ion monitoring techniques in both liver and brain. Metabolism was rapid; up to 50 metabolites were detected in liver. Brain levels of Δ^1-THC were 0.6 μg/g at 20 min after the 10 mg/kg dose and then declined slowly. 7-Hydroxy-Δ^1-THC levels were maximal after 10 min, 6α-OH and 6α, 7-di-OH THC reached maximum concentrations at 20 minutes. These differential rates of uptake and release probably account for effects such as the differences in the time-course of the perceived 'high' produced by the drug and the observed plasma levels of Δ^1-THC in humans (Ohlsson, Lindgren, Wahlen, Agurell, Hollister & Gillespie, 1980).

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The inhibition of the first pass metabolism of propanolol in the pithed rat – correlation of *in vitro* and *in vivo* pharmacokinetic parameters

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The first pass elimination of propanolol can be described in terms of a perfusion-limited model. The activity of the drug metabolising enzymes *in vivo* is expressed as the intrinsic clearance of free drug (Cl'_{int}) (Rowland *et al.*, 1973; Wilkinson & Shand, 1975). This is derived from the intrinsic clearance of total drug (Cl_{int}) after correction for protein binding ($Cl_{int} = \alpha Cl'_{int}$ where α is the free fraction of drug in the blood). Cl_{int} is equivalent to $D/AUC_{(0)total}$ where D is the dose administered orally and $AUC_{(o)total}$ is the area under the total blood concentration versus time curve. *In vitro*, under first order conditions, Cl'_{int} is equivalent to V_{max}/Km.

This study has compared values for Cl'_{int} of propranolol obtained from *in vitro* studies with values for Cl_{int} obtained *in vivo* in the absence and presence of chlorpromazine (CPZ) or imipramine (IMIP).

The disappearance of propranolol *in vitro* was determined in a rat liver microsomal preparation after incubation at 37°C for 5 min. The incubates contained propranolol (1–20 μM), 0.5 ± 0.03 mg protein ml^{-1} and a NADPH generating system in excess. In some experiments CPZ (10 μM) or IMIP (10 μM) was added to the incubate. Km and V_{max} were calculated by Lineweaver-Burke analysis.

Rats were pithed under halothane anaesthesia and ventilated with 100% oxygen. [^{3}H]-Propranolol was injected over 30 s via a cannulated side branch of the hepatic portal vein (HPV). CPZ (4.0 mg/kg; 12.5 μmol/kg), IMIP (8.0 mg/kg; 28.5 μmol/kg) or 0.9% saline was infused via the HPV for 15 min before injection of propranolol. Blood samples were obtained at various times and [^{3}H]-propranolol was separated from its metabolites by liquid chromatography (Pritchard, Schneck & Hayes, 1979) and quantified by liquid scintillation counting. The results are shown in the Table.

There was no significant difference between Cl'_{int} and Cl_{int} in the control group. This supports the suggestion that propranolol, in the absence of inhibitors, is available for elimination by the liver whether bound or unbound within blood. In contrast, after CPZ or IMIP pretreatment, the difference observed between Cl'_{int} and Cl_{int} suggests that binding may become an important factor in the elimination of propranolol.

Table 1 The values for propranolol of the intrinsic clearance of free drug (Cl'_{int}) and the intrinsic clearance of total drug (Cl_{int})

Treatment	Cl'_{int} (ml min^{-1} kg^{-1})	Cl_{int} (ml min^{-1} kg^{-1})
Control	596 ± 54 (4)	803 ± 145 (5)
CPZ	183 ± 14 (4)*	96 ± 15 (5)*
Imipramine	268 ± 52 (4)†	93 ± 20 (4)†

Results are mean ± s.e.mean.
*, †, $P < 0.01$; non-paired t test.

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Deficient glucuronidation of 4-hydroxybiphenyl in hepatocytes isolated from diabetic rats

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The gross disturbances of carbohydrate metabolism that characterize diabetes mellitus might be expected to lead to an impaired ability of the liver to conjugate drugs and other foreign compounds with glucuronic acid, a derivative of glucose. This possibility appears to have been little explored and we have therefore studied the effects of streptozotocin (STZ)-induced diabetes on the glucuronidation and sulphation of a model substrate, 4-hydroxybiphenyl (4-OHBP), in rat isolated hepatocytes.

Female Wistar albino rats were rendered diabetic by a single injection of STZ (55 mg/kg i.p.) and all the animals so treated displayed polydipsia, polyuria, glycosuria and initial weight loss and were judged on these criteria to be diabetic. Two weeks after injection hepatocytes were isolated by collagenase digestion of liver slices as previously described (Fry, 1981). The viabilities and cell yields were similar in preparations isolated from control or diabetic rats. The isolated hepatocytes were incubated with either 100 μM 4-OHBP or with 100 μM 4-methoxybiphenyl (4-MBP), which is metabolized to 4-OHBP by Phase 1 metabolism, and the metabolites were assayed by fluorimetry (Wiebkin, Fry, Jones, Lowing & Bridges, 1978).

4-OHBP was conjugated with glucuronic acid to the extent of 28.2 ± 5.3 nmoles/2×10^6 cells/h in 'control' hepatocytes (mean $\pm$ s.d.). In hepatocytes isolated from diabetic rats this value was decreased to 14.9 ± 6.6 nmoles/2×10^6 cells/h, this difference being statistically significant ($P < 0.01$, $n = 14$). Activity of the alternative minor pathway of conjugation, i.e. sulphation, was not affected by the diabetes.

This defect in hepatic 4-OHBP glucuronidation found in STZ-induced diabetes was also apparent if 4-OHBP was generated *in situ* via demethylation of 4-MBP, although in this case there was a compensatory increase in 4-OHBP sulphation, probably because the concentration of 4-OHBP so generated was insufficient to produce saturation of the sulphation enzymes.

The finding of a defect in 4-OHBP glucuronidation in hepatocytes of diabetic rats is in agreement with earlier liver slice studies which demonstrated defective glucuronidation of testosterone (Schriefers, Ghraf & Pohl, 1966) and of 2-aminophenol (Muller-Oerlinghausen, Hasselblatt & Jahns, 1967) in alloxan-diabetic rats and, together, these findings point to there being a defect of hepatic glucuronidation in diabetes. Interestingly, activity of the hepatic mixed function oxidase system was not altered in the hepatocytes isolated from diabetic rats.

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A comparison of drug conjugation in male and female rat liver cubes and isolated microsomes

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The metabolism of drugs is achieved in two stages; phase I 'functionalisation' of the molecule, and phase II, conjugation of the functional group to an endogenous substrate. Many sex differences exist in phase I metabolism but little has been done on conjugation.

Fresh liver was sliced and cubed (0.5 mm) using a Mickle tissue chopper and suspended in Hank's balanced salt solution. The conjugation of bilirubin, testosterone, α-naphthol, *o*-aminophenol and *p*-nitrophenol was measured by the methods of Heirwegh *et al.* (1972), Rao *et al.* (1976), Otani *et al.* (1975), Arias (1961) and Isselbacher (1956) respectively. Linearity to time, tissue weight, and substrate concentration was established for all assays. The results obtained are given in Table 1.

Liver cubes from male animals conjugated α-naphthol faster, and p-nitrophenol slower than those from female animals. No sex difference was seen for the conjugation of testosterone.

Microsomes (where the enzymes responsible for glucuronidation are localized) were prepared by Ca^{2+} precipitation (Cinti *et al.*, 1972) activated by treatment with digitonin (2,4 mg/mg protein) or Triton X-100 (0,2 mg/mg protein), and glucuronidation measured using the above methods. The results obtained are shown in Table 1.

Microsomes from male animals metabolized p-nitrophenol about twice as fast as those from female microsomes but no other sex differences were seen.

Thus sex differences exist in the conjugation of α-naphthol and *p*-nitrophenol in the rat liver but

Table 1

Substrate	Microsomes male	Microsomes female	Liver cubes male	Liver cubes female
Bilirubin	5,00 ± 0,48	4,77 ± 0,22	n.d.	n.d.
α-Naphthol	493 ± 34	388 ± 46	16,9 ± 2,0	4,8 ± 0,4***
Testosterone	16,14 ± 1,78	12,13 ± 3,06	0,90 ± 0,10	0,78 ± 0,15
o-Aminophenol	4,31 ± 0,49	3,96 ± 0,11	1,89 ± 0,10	1,10 ± 0.09***
p-nitrophenol	15,9 ± 1,3	8,3 ± 0,6**	0,26 ± 0,05	0,32 ± 0,05*

Each figure represents the mean (± s.e.mean) of between four and nine observations, and results are expressed as nmoles product formed min^{-1} gm $liver^{-1}$.
* = $P < 0,02$; ** = $P < 0,01$; *** = $P < 0,001$. n.d. = not detectable.

these differences are not due to changes in the level of UDP-glucuronyltransferase (UDPGT) in the microsomes. These effects may be due to differences in the activation of the UDPGT (assuming all the conjugation in the cubes is glucuronidation, only about 3% of the latent UDPGT activity is seen in this preparation) or to sex differences in the activity of other conjugating enzymes.

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Metabolic conjugation of some carboxylic acids in the horse

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Non-steroidal anti-inflammatory drugs of the carboxylic acid type are widely used in equine medicine and occasionally encountered as doping agents. There is little metabolic information available on such compounds in the horse since the ability of the horse to carry out any of the common conjugations is unknown. It was thus of interest to study, in the horse, the metabolism of three aromatic carboxylic acids, whose fate in other species involves extensive conjugation.

[^{14}C]-labelled benzoic, salicylic and 2-naphthylacetic acids (see Table for doses) were administered by stomach tube on separate occasions to two gelded horses (weight 296–560 kg) and their urine collected for up to 7 days. The elimination of [^{14}C] was followed by scintillation counting, and urinary metabolites separated by solvent extraction, XAD-2 resin columns, thin layer and high pressure liquid chromatography and quantified by scintillation counting. Confirmation of the identity of the metabolites was achieved by gas chromatography-mass spectrometry. In order to permit the identification of benzoic acid metabolites in the presence of endogenous hippuric acid, (ring-d_5)-benzoic acid was co-administered with the [^{14}C]-benzoic acid.

Details of the fate of these three acids in the horse are given in Table 1, which shows that there is

Table 1 Urinary metabolites of some xenobiotic acids in the horse

Drug administration, urine collection and analysis as described in the text

Acid	Dose mg/kg	Dose [^{14}C]	% [^{14}C] dose in 0–24 h urine	% 0.24 h urinary [^{14}C] excreted as: Unchanged acid	Glycine Conjugate	Glucuronide	Taurine Conjugate	Other
Benzoic	5.35	150 μCi	100	1	96	2	n.d.	3-hydroxy* 3-phenylpropionic acid 2*
Salicylic	35	100 μCi	100	95	0.5	2	n.d.	Gentisic Acid 2
2-Naphthyl-acetic	2.24	110 μCi	20	19	61	10	10	n.d.

*Found in one animal only, n.d. = not detected.

substantial variation in the rate of elimination and route of conjugation according to the structure of the acid. Benzoic acid is rapidly and completely eliminated in the urine principally as its glycine conjugate, hippuric acid, with small amounts of the ester glucuronide, 3-hydroxy-3-phenylpropionic acid and unchanged benzoic acid also present. In contrast, although salicylic acid was also rapidly and completely eliminated in the urine, it was largely excreted unchanged, accompanied by very small amounts of the glycine and glucuronic acid conjugates and the oxidation product, gentisic acid. 2-Naphthylacetic acid was excreted slowly with only 20% excreted in the urine in 24 h and 65% in 6 days. The principal metabolite was 2-naphthylacetylglycine, with the taurine and glucuronic acid conjugates and the free acid also present.

This study has shown for the first time the ability of the horse to utilize three metabolic options for carboxylic acids, involving conjugation with glycine, taurine and glucuronic acid, and that the extent of these depends upon the structure of the acid. The finding of a novel metabolite of benzoic acid, 3-hydroxy-3-phenylpropionic acid, arising from the addition of a two carbon fragment to the carboxyl group of benzoic acid, suggests that there may be important links between the metabolism of xenobiotic acids and the intermediary metabolism of fatty acids.

Mary Varwell Marsh is an S.R.C. – CASE student.

The effect of passive immunisation with digoxin-specific antibodies on digoxin handling and response

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Several animal studies have suggested that digoxin-specific antibodies may be used to prevent or to reverse digoxin toxicity (Hess, Scholtysik & Riesen, 1978; Lloyd & Smith, 1978; Ochs & Vatner, 1978). Digoxin-specific Fab (Fragment antigen binding) fragments have also been used to reverse a case of digoxin overdose in man (Smith *et al.*, 1976).

The mechanism by which these antibodies exert their protective effect is not fully understood but one possible explanation is that these effects may stem from altered tissue distribution or elimination of digoxin. In an attempt to provide some information on this we have carried out two studies on the effect of passive immunisation with digoxin-specific antibodies, firstly on digoxin pharmacokinetics and tissue distribution in mice and secondly, on digoxin lethality in anaesthetized guinea-pigs.

In the first study, three groups of mice were pretreated with antidigoxin IgC, or Fab fragments (65 μg/kg, i.p.) or saline. At intervals up to 14 days thereafter the pharmacokinetics and tissue distribution of single doses of [^{3}H] digoxin (7.5 μg/kg, 20 μCi/kg i.v.) were determined. On all occasions plasma levels of digoxin were increased in both the

treated groups in comparison with controls and plasma clearance of digoxin was delayed (e.g. on day 1 – digoxin clearance in control group 0.21, IgC group 0.008, Fab group 0.10 $1\,kg^{-1}\,h^{-1}$). In general, tissue levels tended to be reduced in the treated animals.

In the second study, two groups of 5 guinea-pigs were anaesthetized with urethane. After 30 min the control group received saline and the other group Fab fragments (2 mg/kg i.p.). After a further 30 min, digoxin infusion (250 μg/ml, 3.8 ml/h i.v.) was commenced and ECG and heart rate monitored until death. The time to death and lethal digoxin dose required were significantly increased in the treated group (time to death, control group 31 min, Fab group 49.5 min; lethal dose, control 0.61 mg/kg, Fab 1.01 mg/kg). Serum and urinary digoxin levels were significantly increased in the Fab treated animals as compared to controls (e.g. serum-control group 0.46 μg/ml, Fab group 1.44 μg/ml). In general, as in the study in mice, tissue levels in the treated group were lower, significantly so in the case of the heart.

The second study indicates that pretreatment with digoxin-specific Fab fragments produces a significant protective effect against digoxin lethality. These effects may be related to alterations in digoxin disposition. The lower concentrations of digoxin in the heart at death in the Fab treated animals however are surprising.

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Some studies in the rat into the possible mechanism of action of the hypocholesterolaemic drug probucol

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Probucol [4,4′-(isopropylidenedithio) bis (2,6-di-tert-butylphenol)] is an effective hypocholesterolaemic agent in man (Heel, Brogden, Speight & Avery, 1978) and certain laboratory animals, but its mode of action remains unclear (Kritchevsky, 1980). The purpose of the present study was to see if we could find any evidence in the rat to support the suggestion (Kritchevsky, 1980) that probucol either (a) inhibited cholesterol synthesis, or (b) stimulated bile acid production.

For seven weeks night/day adapted male Wistar rats received either a normal 418 diet or one supplemented with probucol (0.25%). Serum cholesterol levels were not depressed, neither did probucol affect the activities of the three key hepatic microsomal enzymes which are rate limiting for cholesterogenesis, bile salt synthesis, or cholesterol ester formation (see Table 1). Probucol (0.5%) in the diet for two weeks did slightly ($P<0.05$) reduce serum cholesterol, but again the liver's capacity to synthesize either cholesterol or bile acids was not altered (see Table 1).

Myant & Lewis (1966) estimated the rate of conversion of cholesterol to bile acid in man by measurement of $[^{14}C]O_2$ excretion in the breath following intravenous (26–$[^{14}C]$) cholesterol. We performed essentially similar studies in four groups of six normal light cycle rats which had eaten for one week either (i) a normal 41B diet, (ii) 41B + 5% cholestyramine, (iii) 41B + 1% cholic acid or (iv) 41B + 0.25% probucol. Cholestyramine significantly ($P<0.001$) increased, cholic acid significantly ($P<0.01$) reduced, while probucol did not affect the percentage of the injected $[^{14}C]$ cholesterol excreted as $[^{14}C]O_2$ in the rats' breath ($P[^{14}C]O_2$). After giving probucol

Table 1 Effect in the rat of either 0.25% probucol for 7 weeks or 0.5% probucol for 2 weeks on serum cholesterol and the hepatic microsomal enzymes which are rate limiting for cholesterogenesis (HMGCoA reductase) cholesterol esterification (ACAT) and conversion of cholesterol to bile acid (7α cholesterol hydroxylase)

	Duration of experiment	*Body weight* (g)	*Serum Cholesterol* mmol/1	*HMGCoA reductase* $nmol mg^{-1} min^{-1}$	*ACAT* $pmol mg^{-1} min^{-1}$	*7α Cholesterol hydroxylase* $pmol mg^{-1} min^{-1}$
41B alone	7 weeks	256±4.5 (12)	2.13±0.05 (12)	1.00±0.10 (6)	122± 7 (6)	8.1±1.5 (6)
41B + 0.25% probucol	7 weeks	262±5.0 (12)	2.27±0.10 (12)	0.99±0.11 (6)	133±11 (6)	6.8±0.9 (6)
41B alone	2 weeks	217±4.0 (12)	1.96±0.04 (12)	1.16±0.16 (6)	109± 8 (6)	11.9±2.0 (6)
41B + 0.5% probucol	2 weeks	220±3.5 (12)	1.72±0.09* (12)	1.54±0.14 (6)	109±11 (6)	8.9±0.4 (6)

*$P < 0.05$.

(0.5%) for two weeks to night/day reversed rats P[^{14}C]O_2 was slightly though not significantly higher than in an appropriate control group.

We conclude that these data do not support the hypothesis that probucol's cholesterol lowering action is due to either depression of hepatic cholesterogenesis or enhanced bile acid synthesis.

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Danazol – aromatase inhibitory properties

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The effectiveness of danazol (pregna-2,4-dien-20-yno[2,3-d]isoxazol-17-ol) in the treatment of benign breast disorders in humans (Nezhat *et al.*, 1980) and against rat mammary tumours (Peters *et al.*, 1977) prompted a study of its effects in patients with histologically proven, metastatic breast carcinoma. The preliminary results (Coombes, *et al.*, 1980) indicate that the drug may be effective for some patients and may provide a suitable alternative to oophorectomy in pre-menopausal patients (Coombes & Powles, 1980).

The mechanism of the anti-tumour action of danazol is not clear but the drug, *inter alia*, inhibits the release of LH and FSH (Potts *et al.*, 1980) and adrenal 21- and 11β-hydroxylation (Barbieri *et al.*, 1980), decreases progesterone production (Asch *et al.*, 1980), and binds to oestrogen receptor (Chamness *et al.*, 1980). Danazol is extensively metabolized in humans (Rosi *et al.*, 1977) but the role of the metabolites in the expression of the biological activity of the drug *in vivo* is not known.

Post-menopausal patients with breast cancer were given danazol (100 mg, thrice daily) either alone or in combination with aminoglutethimide (250 mg, thrice daily), tamoxifen (10 mg, twice daily), and hydrocortisone (20 mg, twice daily). Hormones were meas-

ured by radioimmunoassays before and after therapy for 2 months and the expected fall in gonadotrophins was revealed but also, *inter alia*, elevated plasma testosterone levels with danazol alone (7 patients; $1.8 \pm 1.2 \rightarrow 2.76 \pm 1.17$ nmol/l) or in combination (9 patients; $0.9 \pm 0.45 \rightarrow 1.58 \pm 0.37$ nmol/l). Neither aminoglutethimide nor tamoxifen caused elevation of testosterone levels. On the basis of these preliminary findings the effect of danazol and some of its metabolites (Rosi *et al.*, 1977) on the release of $[^3H_2]O$ from [1β, 2β-^{3}H]-testosterone by the aromatase in the microsomal fraction of human placental tissue (Graves & Salhanick, 1979) was investigated. An apparent K_m of 0.13 μM (30°, pH 7.4) was observed for testosterone in this system (no product inhibition or non-hyperbolic kinetics). With the substrate at the optimal concentration of 1.5 μM, danazol behaved as a competitive inhibitor of modest activity (K_i 44 μM), the major metabolite (17-hydroxy-2α-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one) and one of the minor metabolites (17-hydroxy-17α-pregn-4-en-20-yn-3-one, ethisterone), were weakly inhibitory but the other minor metabolites [17-hydroxy- and 6β, 17-dihydroxy-2-(hydroxymethyl)-17α-pregna-1,4-dien-20-yn-3-one] were inactive; *cf.* K_I values of 0.6 μM for aminoglutethimide [which inhibits both aromatase and desmolase (Santen *et al.*, 1979)] and 0.165 μM for 4-hydroxyandrost-4-en-3,17-dione [a potent experimental aromatase inhibitor (Brodie *et al.*, 1977)]. The inhibition of aromatase is probably a minor contributor to the anti-tumour activity of danazol.

Since 5/7 patients receiving danazol alone also showed reduced plasma levels of dehydroepiandrosterone sulphate ($2.23 \pm 1.45 \rightarrow 1.4 \pm 1.0$ μmol/l) desmolase inhibition was also considered as a contributory mechanism. In the combination, such an effect could be masked by the much stronger suppression ($1.06 \pm 0.2 \rightarrow 0.12 \pm 0.2$ μmol/l) caused by aminoglutethimide. However, danazol had very weak inhibitory activity against desmolase *in vitro* in the standard assay (Hochberg *et al.*, 1974).

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Time course of action of phenobarbitone on liver blood flow in the rat

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Phenobarbitone not only induces hepatic microsomal drug metabolizing enzymes but also increases liver blood flow in the rat (Ohnhaus, Thorgeirsson, Davies & Breckenridge, 1971; Nies, Wilkinson, Rush, Strother & McDevitt, 1976; Yates, Hiley, Roberts, Back & Crawford, 1978). In an attempt to determine whether liver blood flow, liver weight and microsomal enzyme levels are affected independently by phenobarbitone, we studied its onset of action and offset following treatment.

Groups of male Wistar rats (185–300 g) were injected intraperitoneally with phenobarbitone (80 mg/kg/day) for 1, 3 or 5 days and two groups were dosed for 5 days and left a further 2 or 4 days. As described previously, liver blood flow was determined in some rats using 15 μm diameter radioactive microspheres while liver microsomal cytochrome P450 and cytochrome c reductase levels were determined in other animals in the group (Yates *et al.*, 1978). Liver volume was measured by saline displacement to an accuracy of 0.05 ml and dry liver weight after freeze drying to constant weight.

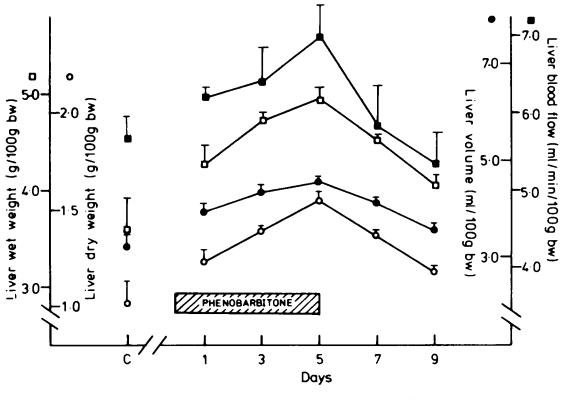

Figure 1 Effect of phenobarbitone (80 mg/kg/day given for up to 5 days as shown by the hatched area) on liver wet weight (□), dry weight (○), volume (●) and blood flow (■) in the rat. The ordinate indicates days after the commencement of treatment. C indicates the values obtained in animals treated for 1 day with saline (2 ml/kg). For each time point $n = 6$ but for the control group $n = 5$. The error bars represent 1 s.e.mean.

Figure 1 shows statistically significant increases ($P < 0.05$; Student's unpaired t-test) in liver volume, dry and wet weight after 1 day of phenobarbitone treatment when compared to 1 day saline-treated controls. After 5 days treatment these parameters and liver blood flow reached peak values and blood flow was significantly greater than control values.

Cytochrome P450 and cytochrome c reductase activity increased; the former peaked after 5 days of treatment, the latter after 3 days. The greatest values were 267% and 313% respectively above controls. After treatment stopped all parameters decreased concomitantly and were not significantly different from control values 4 days after treatment ceased. There was no continuation of elevated values of either liver blood flow or wet weight after ending treatment and it was not possible to differentiate the effect of phenobarbitone on liver blood flow, weight or microsomal enzyme activity with respect to its time course of action.

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Effects of distal diuretics, amiloride and hydrochlorothiazide, on renal magnesium clearance in rats

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The role of the distal tubule of the nephron in the renal handling of magnesium remains unclear. Brunette, Vigneault & Carriere (1974) have suggested a negligible role for the distal tubule, whereas others have suggested that under certain circumstances, a magnesium secretory mechanism exists at this site (Wen, Evanson & Dirks, 1970). We have previously reported that the administration of the distal diuretics, amiloride and triamterene, to conscious saline-loaded rats resulted in a reduced urinary excretion of magnesium (Devane & Ryan, 1981). We also carried out clearance experiments in anaesthetized rats and amiloride resulted in a small but significant fall in the fractional excretion of magnesium under these conditions (Devane & Ryan, 1980). We have now extended our studies to the effects of the two distally-acting diuretics amiloride and hydrochlorothiazide, on renal clearance of magnesium during a frusemide-induced diuresis.

Male Wistar rats were anaesthetized by intraperitoneal injection of thiobutobarbitone sodium (100 mg/kg, 'Inactin'). The left femoral artery was catheterized for obtaining blood samples. Donor rat plasma was infused into the right jugular vein (2.4 ml/h for the first hour; thereafter 0.4 ml/h). A bolus injection of 6 μCi [^{3}H]-inulin in 0.6 ml of 0.9% NaCl was given into the left jugular vein, followed by infusion of a modified Ringer containing 0.6 μCi [^{3}H]-Inulin/ml, at a rate of 60 ml/kg h. The trachea was cannulated. The left ureter was cannulated for urine collection and results refer to the left kidney. After 100 min equilibration, frusemide (1 mg kg^{-1} h^{-1}) was added to the infusion solution and, after 40 min, three control urine collections were made. Amiloride hydrochloride or sodium hydrochlorothiazide was then added to the infusion solution and, following 15 min equilibration, three further 15 min urine collections were made.

The effects of amiloride were investigated at four dose levels 0.02, 0.08, 0.20 and 2.00 mg kg^{-1} h^{-1}. Infusion of amiloride at all four doses resulted in a significant reduction in the fractional excretion of potassium (FE_K). A significant reduction in FE_{Mg} and FE_{Ca} occurred at the three higher doses (0.08, 0.20 and 2.00 mg kg^{-1} h^{-1}). A significant natriuresis only occurred at the two highest doses (0.20 and 2.00 mg kg^{-1} h^{-1}). The effects of amiloride on K and Mg excretion showed a significant dose-dependence when assessed by a one-way analysis of variance and an F-test, whereas the effects on Na and Ca did not show a significant dose-dependence. Infusion of hydrochlorothiazide (2.0 mg kg^{-1} h^{-1}) resulted in a significant natriuresis and a small increase in FE_K. The FE_{Mg} and FE_{Ca} remained unchanged.

These results confirm our previous findings that the K-sparing diuretic amiloride reduces the urinary excretion of magnesium. This magnesium sparing action is dose-related and does not appear to be consistently associated with a natriuresis. Another diuretic, hydrochlorothiazide, which acts in the distal tubule did not affect the urinary excretion of magnesium. Further studies are required to elucidate the mechanism(s) by which amiloride alters the renal handling of magnesium.

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Addressing receptors – a model study

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Drug receptors and other membrane proteins are often located in specialized membrane domains, for example synapses and Ranvier nodes. There is considerable interest at the moment in the mechanisms underlying specific localization. A membrane protein

is synthesized on ribosomes attached to the rough endoplasmic reticulum (RER) where it usually acquires a characteristic high-mannose core oligosaccharide. Migration to its final destination then proceeds via the Golgi apparatus, where the core oligosaccharide is trimmed and terminal sugars are added (Rothman & Fine, 1980). At some point in this pathway proteins must be sorted for delivery to specific destinations. The location and mechanism of this sorting facility are, however, unclear.

Transporting epithelia show marked polarization of their plasma membranes into apical and basolateral domains, in which targets for drug action such as ion channels and pumps and hormone receptors are uniquely located. Furthermore, several enveloped viruses have been shown to bud exclusively from one or other domain (Rodriguez Boulan & Sabatini, 1978). For example, vesicular stomatitis virus (VSV) buds from the basolateral membrane of MDCK monolayers, whereas influenza virus buds from the apical membrane. These viruses code for only a small number of proteins and host cell mechanisms are involved in their transport and assembly. Consequently, the fortunes of viral proteins may provide important information with regard to the sorting of proteins in epithelial cells.

In this study we have examined the interaction between VSV and avian influenza (fowl plague) virus (FPV) at the RER insertion step. Viral mRNA was translated in a rabbit reticulocyte lysate cell-free system containing [^{35}S]-methionine and supplemented with dog pancreas microsomes, a RER preparation from an epithelial tissue. Core glycosylation and resistance to proteolysis were used to screen for membrane insertion of VSV glycoprotein (G) and FPV haemagglutinin (HA). By varying the relative concentrations of RNA messages, while keeping the microsome concentration submaximal, we have demonstrated that nascent VSV G_o competes with nascent HA_o for membrane insertion. Lack of competition would have indicated that the two proteins had already been sorted and were being processed separately. In the event the result indicates that proteins destined for different cellular locations may have a common early pathway and that sorting occurs after insertion into the RER.

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Fluidity of membrane proteins and lipids: a structure/activity study of the effects of S-alkylthiouroniums

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General depressant compounds may act on biological membranes in one of two ways (which may not be mutually exclusive). They may interact directly with hydrophobic sites on excitable functional proteins or they may dissolve in the lipid bilayer of the membrane and perturb the lipid environment of adjacent proteins. The modification of membrane protein or lipid induced by such depressant drugs may be investigated with paramagnetic probes, compounds which are inserted into the membrane at fairly specific points and report on the properties of their immediate environment. These are recorded as changes in the electron spin resonance (ESR) spectra generated by the probes. We have used three such probes to examine the actions of some members of a homologous series of S-alkylthiouroniums on fluidity changes within protein and lipid regions of erythrocyte membranes. This series of compounds, notably the S-heptyl-, S-octyl-, S-nonyl- and S-decyl- derivatives has previously been shown to penetrate and stabilize erythrocyte membranes, their potency increasing with increasing chain length (Beresford & Fastier, 1980). Other membrane stabilizing drugs have been shown to alter the fluidity of membrane compounds (Chin & Goldstein, 1977; Lenaz *et al.*, 1979). We wished to determine whether the alkylthiouroniums behaved in a similar manner.

Erythrocyte ghost membranes were prepared from human blood suspended in phosphate buffer (5 mM pH 7.4). Changes in protein fluidity were monitored with the protein-reactive spin-labelled probe

molecule, 4-maleimido-2,2,6,6-tetramethylpiperidinooxyl (MAL-6), this being incubated overnight with the ghost membranes. Changes in lipid fluidity were monitored with two spin-labelled stearic acid probes, N-oxyl-4,4,-dimethyloxazolidine derivatives of 5-ketostearic acid (5 NS) and 16-ketostearic acid (16 NS). These were incubated for 3 h with the erythrocyte ghost membranes. Samples of the labelled erythrocytes were incubated for 30 min with different concentrations (0.02, 0.2, 2, 20 mM) of the alkylthiouroniums before their ESR spectra were recorded at room temperature in a Varian E4 spectrometer. The results obtained from four separate experiments, each consisting of four replicates, were subjected to an analysis of variance.

Each of the four thiouroniums produced a dose-related change in both protein and lipid fluidity. The results obtained with S-decyl- and S-nonyl-thiouronium are shown in Figure 1. Protein fluidity was reduced significantly ($P < 0.01$) by all four drugs at concentrations of 2 and 20 mM. Lipid fluidity was measured in two regions of the membrane. (The paramagnetic group in 5 NS reports on conditions close to the surface of the membrane while that in 16 NS reports on conditions existing more deeply within the hydrophobic core.) The results obtained with 16 NS indicated that all the thiouroniums increased the fluidity of the lipid core. Significant ($P < 0.01$) increases were produced with 2 and 20 mM of each compound. The lipid order close to the surface was less affected, as shown using 5 NS. Only S-decyl- and S-nonyl- thiouronium produced a significant ($P < 0.01$) increase in lipid fluidity, and then only at 20 mM.

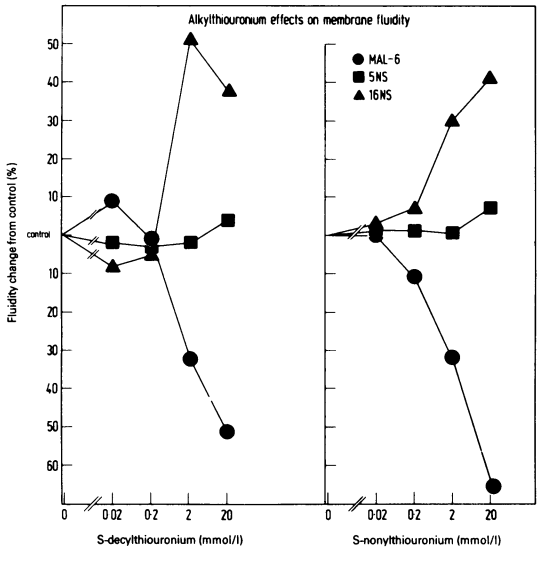

Figure 1 Changes in membrane fluidity produced by S-decylthiouronium and S-nonylthiouronium. Changes in protein fluidity are monitored with the paramagnetic probe, MAL-6; changes in lipid fluidity with the probes 5 NS (surface fluidity) and 16 NS (core fluidity).

The results as a whole indicate that the S-alkylthiouroniums affect the protein and lipid fluidity in an inverse manner, protein fluidity being reduced while lipid fluidity is increased. No other single study of depressant drugs has demonstrated this inverse relationship. Whether the protein and lipid effects are independent or inter-related remains to be determined.

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Adenosine-receptor antagonism and bronchodilator activity by methyl-substituted xanthines

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The widely used bronchodilator theophylline is a potent adenosine-receptor antagonist. This mechanism may explain e.g. its diuretic and CNS-stimulant effect (Rall, 1980). The present report examines structural requirements of xanthines for adenosine-receptor antagonism versus bronchodilation and suggests a possibility to produce antiasthmatic xanthines which do not antagonize adenosine.

Effects on isometric tension were examined in isolated tracheal rings with intrinsic tone and in electrically stimulated (0.1 Hz, 1 ms, supramaximal voltage) guinea-pig myenteric-plexus longitudinal muscle preparations, mounted in 50 ml organ baths containing normal Krebs solution (37°C; 95% O_2, 5% CO_2). Dipyridamole (1 μg/ml) was used to potentiate adenosine effects only on tracheal smooth muscle.

The tracheal preparations were relaxed by all xanthines (Table 1). Tracheal relaxant effects of adenosine were studied in the presence of concentrations, less than EC_{50}, of the xanthines. In the myenteric-plexus preparations a slightly enhanced amplitude of twitch responses was produced by each tested xanthine; adenosine inhibited the twitches. Cumulative concentration-response lines to adenosine alone and in the presence of different concentrations of xanthines (Fluka, A.G.; G. Kjellin, AB Draco) were obtained. All 1-methylated xanthines shifted to the right, in a concentration-dependent way, the concentration-response lines to adenosine both in tracheal and in myenteric-plexus preparations (Table 1).

In conclusion, xanthines with methyl-substitution in the 1-position antagonized adenosine at both neural and smooth muscle receptors. The stimulant effect by xanthines on the myenteric-plexus preparations was unrelated to adenosine-antagonism and may not be interpreted as due to the presence of an inhibitory adenosine tone. Bronchodilation and adenosine-antagonism were also unrelated, suggesting that antiasthmatic xanthines lacking adenosine-antagonistic properties may be produced.

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Table 1 Adenosine-receptor antagonism

$\left(\text{dose ratios, DR } \frac{EC_{50} \text{ of adenosine in the presence of xanthines}}{EC_{50} \text{ of adenosine in the absence of xanthines}} \pm \text{s.e.mean}\right)$ and tracheal relaxant potency

$\left(\frac{EC_{50} \text{ of theophylline}}{EC_{50} \text{ of other xanthines}} \pm \text{s.e.mean}\right)$ by methylxanthines

Xanthines methyl-position(s) indicated	*Adenosine-antagonism*						*Tracheal relaxant potency*	
	myenteric-plexus			*trachea*				
	DR	c†	*n*††	DR	c	*n*		*n*
1-	5.5±2.2	(0.10)	3	1.5±0.1	(0.015)	3	0.18±0.01	4
	13.7±6.4	(1.0)	4	5.6±1.1	(0.150)	4		
1,3-†††	4.1±0.8	(0.10)	10	3.0±0.5	(0.010)	2	1	4
	11.0±2.4	(0.30)	4	13.4±0.8	(0.060)	3		
1,7-	4.9±0.2	(0.15)	2	1.9±0.4	(0.015)	4	0.46±0.08	4
	10.8±1.8	(1.50)	6	12.4±2.2	(0.150)	4		
1,3,7-	2.8±0.3	(0.10)	4	2.0±0.2	(0.030)	8	0.76±0.06	4
	13.1±2.5	(0.90)	4	10.2±1.8	(0.300)	4		
1,3,8-	4.8±0.4	(0.08)	4	1.8±0.1	(0.008)	5	1.10±0.12	4
	11.5±3.8	(0.70)	3	6.6±0.9	(0.069)	4		
3-	1.3±0.2	(0.75)	4	0.7±0.1	(0.100)	4	0.26±0.02	4
7-	1.1±0.3	(3.0)	3	1.0±0.2	(0.300)	4	0.16±0.02	4
8-	1.2±0.6	(3.0)	3	1.5±0.2	(0.300)	6	0.09±0.01	4
3,7-	2.3±0.8	(0.30)	5	0.9±0.1	(0.030)	4	0.39±0.03	4
3,8-	2.5±1.1	(0.60)	3	0.8±0.1	(0.060)	4	0.55±0.05	4

†Concentration of xanthines in mmol/l; ††number of animals; †††theophylline.

The selective muscarinic antagonist actions of pancuronium and alcuronium

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There is now considerable evidence that there are subtypes of the muscarinic acetylcholine receptor distinguishable by agents such as pirenzepine and stercuronium (Hammer, Berrie, Birdsall, Burgen & Hulme, 1980; Brown, Forward & Marsh 1981). Studies *in vitro* have demonstrated that pirenzepine has greater affinity as an antagonist for muscarinic receptors in sympathetic ganglia than in atria (Barlow, Caulfield, Kitchen, Roberts & Stubley 1981). In contrast, the tachycardia produced by the neuromuscular blocking drugs stercuronium, gallamine and pancuronium is thought to be due mainly to a selective antimuscarinic action at the cardiac vagus neuroeffector junction (Saxena & Bonta 1970; Li & Mitchelson 1980). The present studies were carried out using both *in vivo* and *in vitro* preparations to determine the selectivity of pancuronium and alcuronium as antagonists for the muscarinic receptor in atrial and sympathetic ganglion preparations.

Rats (male, CD, 300–400 g) were anaesthetized with phenobarbitone (50 mg/kg i.p.), adrenalectomized bilaterally and then pithed. In these preparations muscarine (2.5–10 μg/kg i.v.) caused dose-dependent bradycardias (20–130 beats/min). Following dosing with pancuronium (0.1–1.0 mg/kg i.v.) or alcuronium (1.0–5.0 mg/kg i.v.) the bradycardia produced by muscarine was blocked. Furthermore, in the presence of these neuromuscular blocking drugs the same doses of muscarine which had earlier produced bradycardia now produced tachycardias which could subsequently be antagonized by atropine (10 μg/kg i.v.) or pirenzepine (10–30 μg/kg i.v.). In further studies, pretreatment of the animals with reserpine (5 mg/kg i.p.; 24 h prior to study), or propranolol (1 mg/kg i.v.) also abolished these muscarine-induced tachycardias, indicating a sympathetically-mediated effect.

The antagonist effects of pancuronium against muscarine-induced responses in the isolated spontaneously beating atria and superior cervical ganglion preparations of the rat were also determined. The effects measured were decreases in rate in atria and depolarizations in ganglia. Dose ratios were obtained using the 'non-sequential' method described by Barlow *et al.* (1981). Mean pA_2 values were calculated from the relationship

$$pA_2 = -\log\left(\frac{\text{antagonist concentration}}{\text{dose-ratio} - 1}\right)$$

In these preparations, as expected from the *in vivo* data, pancuronium was considerably more potent as an antagonist of the effects of muscarine in atria ($pA_2 = 7.0$, $n = 6$) than in ganglia ($pA_2 = 5.2$, $n = 6$).

It can be concluded that the tachycardia produced by muscarine in the presence of pancuronium and alcuronium probably results from activation of muscarinic receptors in sympathetic ganglia. The *in vitro* studies indicate that the neuromuscular blocking agents may selectively antagonize cardiac inhibitory muscarinic responses preferentially to excitatory muscarinic responses in sympathetic ganglia.

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Can endogenous prostaglandins exert a negative feedback control of lipolysis?

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There has been considerable confusion about the relationship between lipolysis and endogenous prostaglandins (PGs) (see Fain, 1973; Fredholm, 1978). It has been suggested that PGs form a negative feedback mechanism upon lipolysis (Illiano & Cuatrecasas, 1971).

We have studied the PG biosynthesis and the effect of cyclo-oxygenase inhibitors during lipolysis in human adipocytes stimulated by isoprenaline and theophylline *in vitro*. Human subcutaneous adipocytes were incubated in Medium 199 supplemented with 4% w/v free fatty acid deficient BSA for 48 hours. Isoprenaline (10^{-6}M) and theophylline (3×10^{-4}M) added at zero time induced lipolysis (measured in terms of glycerol released) as is well documented (Fain, 1973). In the resting state there was also a small production of PGs which was increased after stimulation by both lipolytic agents. Samples were subjected to radioimmunoassay for PGE_2, $PGF_{2\alpha}$, 6-oxo-$F_{1\alpha}$ and TxB_2 after extraction of medium into diethyl ether and removal of free fatty acids by silicic acid chromatography. The major product was found to be PGE_2 which increased almost two-fold from basal levels of 4 ng.6×10^4 cells.48 h to 7 ± 0.3 ng.6×10^4 cells.48 h with isoprenaline and 7.33 ± 2.1 ng.6×10^4 cells.48h with theophylline. Time-course studies showed that the rate of formation of PGE_2 corresponded to that of the lipolytic products. Indomethacin (2.8×10^{-6}M) and meclofenamate (3.37×10^{-6}M) inhibited PG biosynthesis by 80% in all samples. However, isoprenaline-induced lipolysis was unaffected by the cyclo-oxygenase inhibitors, whilst theophylline-induced lipolysis was found to be significantly potentiated (from $65 \pm 8\,\mu$g to $95 \pm 14\,\mu$g glycerol.6×10^4 cells.48 h $P < 0.001$ by indomethacin and to $84 \pm 10\,\mu$g glycerol.6×10^4 cells.48 h by meclofenamate $P < 0.02$).

Recently, Schimmel, McMahon & Serio (1981) showed that the sensitivity of adipocytes to antilipolytic agents is reduced by adenosine, which is produced by fat cells *in vitro* (Schwabe, Ebert & Erbler, 1973). Our experiments show that removal of PGs by indomethacin or meclofenamate caused potentiation of lipolysis only in the presence of theophylline which is known to antagonize the action of adenosine on fat cells (Londos, Cooper, Schlogel & Rodbell, 1978).

It is suggested, therefore, that PGE_2 formed in fat tissue during lipolysis can exert a negative feedback effect on lipolysis but the effect is only evident when the action of adenosine is abrogated.

These findings help to clarify the conflicting results obtained from earlier studies on the role of PGs in adipose tissue.

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The effects of anisomycin on the febrile responses to intracerebroventricular bacterial pyrogen and prostaglandin E_2 in cats

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Anisomycin, a potent and reversible inhibitor of protein synthesis in eukaryotic cells (Grollman, 1967) was used to investigate the mechanism underlying the hyperthermic responses of cats to intracerebroventricular (i.c.v.) bacterial pyrogen (*Shigella dysenteriae*), and prostaglandin E_2. Ruwe & Myers (1979) reported that anisomycin significantly delays or prevents the fever produced by intravenous pyrogen in cats.

Experiments were carried out in unrestrained female cats exposed to ambient temperatures of 5°, 20° and 38°C. Each animal received either anisomycin (15 mg/kg s.c.) or saline (2 ml s.c.) 30 min before administration of the pyrogenic agent. Rectal temperature was monitored continuously throughout each experiment. The above dose and time interval for anisomycin administration greatly diminished the responses to intravenous pyrogen (*Shigella dysenteriae* 2 μg/kg) at all three ambient temperatures.

The hyperthermic responses to PGE_2 (1 μg i.c.v.) were significantly attenuated at all three ambient temperatures, as recorded by thermal response indices (TRI) (Milton & Wendlandt, 1971) measured over a period of 2 h. The responses to PGE_2 over a longer period of time were complicated by an anisomycin hyperthermia of long latency. Anisomycin pretreatment was found not to have any significant effect on the febrile response to centrally administered bacterial pyrogen (100 ng). This was true whether the TRI over 5 h or the absolute magnitude of the fever at 5 h was used as a measure of the degree of fever. Further investigation into this lack of effect has indicated that the time course of action of anisomycin is a very important factor. In experiments carried out at room temperature, with the same doses of anisomycin and bacterial pyrogen, we found that when anisomycin was administered 90 min after the pyrogen i.e. just before the onset of the pyrogen hyperthermia, it caused a very marked inhibition of the febrile response over the following 3 h period.

The behavioural effects of the cats were also altered by anisomycin, which caused a marked reduction in activity at all ambient temperatures studied. Anisomycin also produced vigorous shivering during the rising phase of body temperature following its administration at 5° and 20°C. At 38°C shivering was observed only occasionally.

The attenuation of PGE_2 fever by anisomycin injected subcutaneously contrasts its lack of effect on such a fever when administered centrally as was reported by Ruwe & Myers (1979). Anisomycin, given subcutaneously to mice, in doses similar to those used in our experiments, has been shown to cause approximately 80–90% inhibition of brain protein synthesis during the first 2 h following its administration (Flood, Rosenzweig, Bennet & Orme, 1974). These results indicate that an intact protein synthesis mechanism is required for the development of normal hyperthemic responses to various pyrogenic agents.

We thank Pfizer for providing anisomycin.

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Evidence that the complement fragment C5a mediates increased vascular permeability in response to zymosan in the peritoneal cavity of the rabbit

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Our previous results have suggested that inflammatory oedema in response to intradermally-injected zymosan (yeast cell walls) results from synergism between two chemical mediators – a vasodilator prostaglandin (PGE_2 or PGI_2) and a vascular permeability-increasing substance, C5a (Williams & Jose, 1981). These deductions were based on experiments using combinations of pharmacological agents in the skin *in vivo* and the generation of permeability-increasing activity in blood plasma *in vitro*. The skin provides an opportunity for quantitative measurements of microvascular changes but direct identification of mediators is difficult. For this reason we have investigated responses in the peritoneal cavity.

Zymosan (10 mg/ml, 50 ml volume) was injected into the peritoneal cavities of rabbits (male NZW) and samples of peritoneal exudate collected on ice into heparin (10 units/ml) at intervals up to 24 h. Plasma protein leakage into the cavity was monitored using intravenously-injected Evans blue dye. Leakage was first detectable at 15–30 min; dye concentration increased most rapidly over the first 2–3 h and was still high at 24 h. Polymorphonuclear (PMN) leucocytes, predominantly neutrophils, accumulated progressively over the 24 h period; the largest rate of increase was from 2 h (approximately 10^5/ml) to 12 h (5×10^7/ml).

After centrifugation exudate supernatants were assayed for permeability-increasing activity in rabbit dorsal skin using the 30 min accumulation of intravenously injected [^{125}I]-albumin (Williams, 1979). A permeability-increasing substance (peritoneal inflammation product, PIP) was detected in peritoneal fluid in samples taken as early as 10 min. PIP had many characteristics in common with C5a purified from zymosan-activated blood plasma. In 2 h samples activity equivalent to 7.3 ± 2.3 μg of C5a per ml of exudate was measured by $2 + 2$ assay against a purified plasma C5a standard ($n = 5$ experiments). The common characteristics were: (a) a vasodilator prostaglandin had to be added to exudate samples in order to induce oedema in skin; (b) mepyramine and aprotinin had no effect on responses to PIP; (c) the activity eluted as a single peak, corresponding to M.W. 18,000, on Sephadex G-100 and was retained by the cation exchange resin CM Sephadex C-25 at pH 6; (d) responses to PIP were of long duration in the skin; and (e) responses could not be elicited in rabbits depleted of circulating PMN leucocytes (Wedmore & Williams, 1981).

That the addition of exogenous prostaglandin to peritoneal exudate samples was necessary in order to induce oedema in the skin suggests little active vasodilator substance was present. Samples obtained at 2 h contained only 3.4 ± 0.9 ng/ml of PGE_2 whereas the same samples contained 67.5 ± 13.4 ng/ml of 6-oxo-$PGF_{1\alpha}$, a stable product of PGI_2 (radioimmunoassay of 4 samples). This supports our original contention that PGI_2 may have a role in inflammation because of its vasodilator and exudation-potentiating activity (Peck & Williams, 1978).

These results suggest that increased vascular permeability in response to zymosan in the peritoneal cavity is mediated by C5a which may synergize with PGI_2 to induce plasma exudation.

M.J.F. is an M.R.C. Scholar.

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In vivo properties of leukotriene B_4

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Leukotriene B_4 isomer III (LTB_4), a product of the lipoxygenase pathway of arachidonic acid metabolism, stimulates the movement and aggregation of leucocytes *in vitro* (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980; Smith, Ford-Hutchinson & Bray, 1980). It has been shown that this compound also induces leucocyte accumulation *in vivo* when injected into the guinea-pig peritoneal cavity (Smith *et al.*, 1980), the rabbit skin (Carr, Higgs, Salmon & Spayne, 1981) and the rabbit eye (Bhattacherjee, Eakins & Hammond, 1981). LTB_4 causes vascular permeability changes in rabbit, rat and guinea-pig skin in the presence of vasodilators, including PGE_2 (Bray, Cunningham, Ford-Hutchinson & Smith, 1981). In the present communication we describe further effects of LTB_4 *in vivo*.

The intravenous administration of LTB_4 (1 μg) into the rabbit produced a transient but profound neutropenia, the maximal effect occurring 1 min after administration but returning to normal within 3 min. No changes in peripheral monocyte or platelet counts were observed. Infusion of a 5 ng/ml solution of LTB_4 over a hamster cheek pouch preparation (Atherton & Born, 1972) resulted in an increase in the number of leucocytes adhering to and passing through the vascular endothelium. The effect was reflected by the decreased numbers of rolling granulocytes counted in the post-capillary venules 2–3 min after commencing the perfusions. The peak response occurred after 8–10 min and was partially reversed during the 20 min after the treatment was ended. Histological examination of rabbit skin sites 30 min after intradermal injection of 1, 10 and 100 ng of LTB_4 showed a significant accumulation of polymorphonuclear leucocytes (PMNs). The injection of PGE_2 (100 ng) alone had no effect but when injected with LTB_4, a significant enhancement of PMN accumulation occurred with the highest dose of LTB_4. PMNs accumulate in skin chambers, containing LTB_4 (200 ng) in 2 ml of Hanks balanced salt solution (HBSS), when these are applied for 5 h over skin abrasions on the human forearm. No PMNs were detected in the presence of HBSS alone whereas in the chambers containing LTB_4 the mean number of PMNs was 894×10^3 (range $12-4400 \times 10^3$, $n = 9$).

These results show first, that extravascular LTB_4 causes the chemoattraction of leucocytes and the adherence to vascular endothelium *in vivo* and secondly, provide support for the view that LTB_4 should be considered as a natural mediator of inflammatory responses as are histamine, bradykinin, prostaglandins and the complement derived peptide, C5a.

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Actions of LTC, LTD in guinea-pig isolated hearts and the effect of indomethacin and FPL 55712

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The leukotrienes (LT) C_4 and D_4 have previously been shown to be potent constrictors of the guinea-pig (GP) coronary vasculature (Letts & Piper, 1981). In various GP isolated heart preparations they had no direct actions on spontaneously beating atria or electrically-driven right ventricular strips. In perfused working hearts (Langendorff preparation) they produced decreases in coronary flow followed by smaller, variable decreases in the force of contraction and the spontaneous rate. LTC_4 caused greater reduction in coronary flow than LTD_4. These actions of the leukotrienes have been further investigated in the presence of the cyclo-oxygenase inhibitor indomethacin and the SRS-A antagonist FPL 55712. GP hearts were perfused with Ringer-Locke solution at 37°C and changes in coronary flow, force of contraction and rate were recorded as previously described (Letts & Piper, 1981). After a 45 min equilibration period each heart received a single 5 min infusion of LT. When required, indomethacin and FPL 55712 were dissolved in the Ringer-Locke solution.

On a comparative basis, the results indicate LTC_4 (1.6×10^{-8}M) has a greater efficacy in reducing coronary flow (71 ± 6 vs. $48 \pm 2\%$; $n = 4$, 4 respectively) whereas LTD_4 (2×10^{-8}M) has a greater efficacy in reducing the force of contraction of the working hearts (37 ± 11 vs. $24 \pm 7\%$; $n = 4$, 4 respectively).

Indomethacin ($0.3 - 1.4 \times 10^{-5}$M) significantly decreased the peak reduction in coronary flow to 1.6×10^{-9}M LTC_4 (46 ± 3 to $21 \pm 3\%$; $n = 4$, 5 respectively), but not 2×10^{-8}M LTD_4 (48 ± 2 to $43 \pm 3\%$; $n = 4$, 5 respectively). However, the initial fall in flow during the first min of LTD_4 infusion was significantly decreased (45 ± 2 to $31 \pm 5\%$; $n = 4$, 5 respectively).

Indomethacin inhibited the decreases in contractility of the working hearts to both LTC_4 and LTD_4 but only the LTD_4 reduction was significant (37 ± 11 to $9 \pm 7\%$; $n = 4$, 5 respectively).

FPL 55712 (3.8×10^{-6}M) significantly blocked the peak LTC_4-induced decrease in flow (46 ± 3 to $7 \pm 3\%$; $n = 4$, 3 respectively) and also the initial phase of LTD_4-induced reduction in flow (48 ± 2 to $27 \pm 6\%$; $n = 4$, 4 respectively). There was no significant difference in the LTD_4-induced reduction after a 5 min infusion. Both the LTC_4- and LTD_4-induced decreases in contractility were completely antagonized. Neither LTC_4 nor LTD_4 induced arrythmias or caused significant differences in heart rate.

The results show there were qualitative and quantitative differences between the actions of the LTs on GP isolated working hearts. LTD_4 caused a more rapid reduction in flow and a greater decrease in contractility. The flow reduction was partially inhibited by FPL 55712 and indomethacin. In contrast, reduction in flow to LTC_4 was slower in onset; this and the decrease in force were significantly blocked by indomethacin and FPL 55712. This suggests that cyclo-oxygenase products may play a variable role in the actions of LTC_4 and LTD_4.

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Inhibition of cyclooxygenase activity by prednisolone

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Corticosteroids inhibit phospholipase A_2 activity by inducing synthesis of an inhibitory peptide, macrocortin (Blackwell, Carnuccio, Di Rosa *et al.*, 1980). The possibility that they may have an analogous effect on cyclooxygenase activity has not been widely explored. The present study was designed to investigate the effect of prednisolone on the cyclooxygenase activity of human rectal mucosa in organ culture.

Rectal biopsies from colitics not receiving corticosteroids were cultured (Hawkey & Truelove, 1981)

with or without prednisolone for periods of up to 40 h. Two concentrations of prednisolone were used, 8.33×10^{-7} M and 5.66×10^{-4} M, corresponding to concentrations achieved in the plasma with oral therapy and in the rectum with topical therapy. Basal synthesis of prostaglandin (PG) E_2 was calculated from the amount in the medium at the end of culture measured by radioimmunoassay or by laminar flow bioassay after extraction and thin layer chromatography. At the end of culture the biopsies were homogenized and incubated for 30 min at 37°C with arachidonic acid (6.1×10^{-5} M), adrenaline (3.0×10^{-3} M) and reduced glutathione (1.3×10^{-3} M). These incubation conditions lead to selective enhancement of PGE2 synthesis and the amount synthesized was operationally designated as the cyclooxygenase activity.

Prednisolone had no effect when added directly to homogenates without prior organ culture. No significant metabolism of authentic PGE2 (10–30 pmol) could be shown under the incubation conditions used to measure cyclooxygenase activity (mean recovery after incubation 95.0% ± 4.9%, s.e.mean, $n = 10$). The basal synthesis of PGE2 (mean and 95.0% confidence limits derived from logarithmically transformed data) over 40 h was reduced to 37.2% (27.1–50.9%) of control values by prednisolone (8.33×10^{-7} M, $P < 0.001$, $n = 26$, measured by bioassay). The cyclooxygenase activity after culture with this dose of prednisolone was 29.8% (22.2–41.0%) of control values ($P < 0.001$). The time course of these effects was investigated using radioimmunoassay.

The inhibition of basal synthesis developed within 2 h of culture and was time and concentration dependent. Inhibition of cyclooxygenase activity did not develop until 6 h and thereafter was time and concentration dependent. At 6 h prednisolone 5.66×10^{-4} M reduced synthesis of PGE2 to 69.8% (53.0–91.9%) of control values ($P < 0.05$, $n = 7$). Cycloheximide (2×10^{-5} M) reduced cyclooxygenase activity to 67.1% (44.6–100.9%) of control values ($0.1 > P > 0.05$). In the presence of cycloheximide, prednisolone had no additional inhibitory effect (101.6%, 81.2–127.2% of values after cycloheximide alone. With 2 h cultures treatment with cycloheximide enhanced cyclooxygenase activity (138.5%, 127.3–157.7%, of control values, $P < 0.05$, $n = 11$).

These data suggest that prednisolone can affect cyclooxygenase activity in human rectal mucosa. The possible mechanisms include induction of an inhibitory peptide, inhibition of synthesis of fresh cyclooxygenase enzyme or diversion of arachidonic acid metabolism to a product other than PGE_2.

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Effect of the thromboxane antagonist EP 045 on arachidonic acid responses in the rabbit, guinea-pig and sheep

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Intravenous injection of arachidonic acid (AA) produces multiple effects on the cardiovascular and bronchial systems as a result of prostaglandin and thromboxane biosynthesis (see Rose & Kot, 1977). We have investigated the effect of the thromboxane receptor antagonist EP 045 (Jones & Wilson, 1981) on AA responses in the rabbit, guinea-pig and sheep.

In the urethane-anaesthetized rabbit AA (0.3 mg/kg) caused severe respiratory distress and a precipitous fall in arterial blood pressure characterized by a reduction in pulse pressure; death occurred within 2 min in 40% of the animals. With AA (1 mg/kg) similar effects were seen and the mortality rate rose to 75%. EP 045 (0.25 mg/kg) injected 2 min prior to the AA (1 mg/kg) challenge afforded no protection, whereas at 1–5 mg/kg no deaths occurred. Falls in blood pressure were still seen, but they were similar to those obtained following intravenous injection of either PGE_2 or PGI_2 (no diminution in pulse pressure). Indomethacin (0.125 mg/kg i.p.)

completely protected against AA death, and abolished the cardiovascular response.

In the guinea-pig (Dial-urethane anaesthesia) intravenous AA (0.1–0.3 mg/kg) produced bronchospasm, as indicated by a rise in tracheal insufflation pressure, and a pressor response of small magnitude (5–10 mm Hg). EP 045 (2 mg/kg) blocked the bronchoconstriction and reversed the pressor response to a prolonged depressor effect. Indomethacin (0.5 mg/kg i.v.) inhibited all responses to AA. The bronchoconstrictor and pressor actions of 11,9-epoxymethano PGH_2 were blocked by EP 045 but not by indomethacin.

11,9-Epoxymethano PGH_2 (0.2–0.5 μg/kg) and AA (0.1–0.2 mg/kg) injected intravenously into the sheep (pentobarbitone anaesthesia) produced an immediate and short-lived fall in blood pressure probably due to pulmonary vasoconstriction. PGD_2 (0.025–0.5 μg/kg) on the other hand produced a rise in blood pressure after a delay of 5–8 s, due to peripheral arteriolar constriction (Jones, 1978). EP 045 (2–5 mg/kg) blocked the response to AA and 11,9-epoxymethano PGH_2 but had no effect on the PGD_2 response. This confirms our earlier proposal that the PGD-sensitive pressor system is quite distinct from thromboxane-sensitive systems found in vascular smooth muscle.

Our results are consistent with conversion of AA *in vivo* to prostaglandin endoperoxides and/or thromboxane A_2 resulting in bronchoconstriction and vasoconstriction and to other products of prostaglandin synthetase, perhaps PGE_2 or PGI_2, which are responsible for the vasodilator effect. EP 045 apparently acts at the thromboxane receptor site since it has no inhibitory action on thromboxane biosynthesis, at least in human platelets.

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l-benzylimidazole: a potent and selective inhibitor of 'thromboxane synthetase' *ex vivo*

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The balance of prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) is important for the maintenance of haemostasis since prostacyclin is a potent vasodilator and inhibitor of platelet aggregation whereas TXA_2 is a vasoconstrictor and induces platelet aggregation: Preferential synthesis of TXA_2 could thus lead to thromboembolic problems. Because both prostacyclin and TXA_2 are generated from prostaglandin endoperoxides, selective inhibition of the enzyme thromboxane synthetase is an attractive possibility in the treatment of disorders involving excessive platelet aggregation. Such inhibitors should not only reduce TXA_2 formation but also encourage prostacyclin generation which would exert additional inhibition of platelet aggregation. Imidazole and several of its l-substituted analogues are potent and selective inhibitors of thromboxane synthetase *in vitro* (Moncada, Bunting, Mullane, Thorogood, Vane, Raz & Needleman, 1977; Blackwell, Flower, Russell-Smith, Salmon, Thorogood & Vane, 1978; Tai and Yuan, 1978). We now report on the action of one such compound, l-benzylimidazole, as a thromboxane synthetase inhibitor.

l-Benzylimidazole was administered as its fumarate salt to male New Zealand white rabbits (2.5–3 kg) by intravenous and oral routes. Thromboxane B_2, the stable hydrolysis product of TXA_2, was measured by specific radioimmunoassay in diluted sera derived from 1 ml samples of whole blood allowed to clot at 37°C for 45 min in glass tubes (Patrono, Ciabattoni, Pinca, Pugliese, Castrucci, De Salvo, Satta & Peskar, 1980), and PGE_2 and 6-keto-$PGF_{1\alpha}$ (the decomposition product of prostacyclin) were likewise determined in the samples. The control, pre-drug, concentration of TXB_2 in sera was 398.4 ± 23.3 ng/ml ($n = 17$) whereas neither PGE_2 nor 6-keto-$PGF_{1\alpha}$ were detectable. 2 min after intravenous administration of 10 mg/kg l-benzylimidazole fumarate, thromboxane formation

was inhibited more than 80%; the inhibition decreasing to 20% 1 h after drug administration. Oral doses (10 and 20 mg/kg) produced dose related inhibition of thromboxane synthesis; maximum inhibition of approximately 40 and 70% respectively occurred after 30–45 min and an inhibitory effect was maintained for approximately 3 h. Inhibition of thromboxane synthesis was alwazs associated with increased formation of PGE_2 and 6-keto-$PGF_{1\alpha}$; approximately 50% of the prostaglandin endoperoxides presumed to be available due to reduced thromboxane synthesis was converted to PGE_2 and 2–5% was converted to 6-keto-$PGF_{1\alpha}$.

These data show that l-benzylimidazole is an effective inhibitor of thromboxane synthetase when given by intravenous or oral routes. The concomitant increase of PGE_2 and 6-keto-$PGF_{1\alpha}$ observed when thromboxane synthesis was suppressed demonstrates that the cyclo-oxygenase was unaffected by l-benzylimidazole confirming that the latter is a selective inhibitor of thromboxane synthetase. The experimental procedure described can be readily adapted to monitor pharmacokinetic parameters of l-benzylimidazole and other inhibitors of thromboxane synthetase.

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A cinematographic study of thromboxane A_2-induced ulceration of the canine gastric mucosa

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Prostaglandin $F_{2\alpha}$, thromboxane and the acute response to *E. coli* endotoxin in anaesthetized cats

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The acute response to intravenously administered *E. coli* endotoxin in anaesthetized cats consists of an increase in pulmonary artery pressure and airways resistance, a reduction in lung compliance and a transient systemic hypotension. It has been demonstrated previously (Parratt & Sturgess, 1977) that prostaglandin (PG) $F_{2\alpha}$ has actions appropriate for a mediator of this initial response. We now report the results of a study designed to establish whether $PGF_{2\alpha}$ is released from the lungs as a consequence of endotoxin administration, and to determine whether other prostanoids, e.g. thromboxane (Tx)A_2 are also involved.

Cats of either sex were anaesthetized with sodium pentobarbitone and prepared for the measurement of haemodynamics (Parratt, 1973) and respiratory function (Houston & Rodger, 1974). After

thoracotomy an 18G needle was inserted downstream into the pulmonary artery. Blood samples were obtained from the aorta and the pulmonary artery 5 min before and 2, 7 and 30 min after administration of *E. coli* endotoxin (2 mg/kg i.v.) and analysed for $PGF_{2\alpha}$ and TxB_2 (the stable breakdown product of TxA_2) by radio immunoassay.

The results are summarized in Table 1.

In the animals which exhibited an acute (pulmonary) response to endotoxin there was a marked early release of $PGF_{2\alpha}$ and $TxB_2\alpha$ from the lungs. The concentrations of $PGF_{2\alpha}$ measured in aortic blood correlate well with pulmonary artery pressures ($r = 0.70$), lung compliance ($r = 0.71$) and airways resistance ($r = 0.94$). Similar relationships exist between TxB_2 concentrations and the measured parameters. In contrast, there were no significant changes in the plasma concentrations of these prostanoids measured in animals which did not respond to endotoxin.

The results indicate that the severity of the acute pulmonary responses to endotoxin is related to the amount of $PGF_{2\alpha}$ and thromboxane released. This study does not, however, preclude the possibility that other mediators derived from arachidonic acid may be involved in the overall response.

Table 1 Effects of endotoxin on mean pulmonary arterial pressure and on plasma concentrations of $PGF_{2\alpha}$ and TxB_2. Values are means ± s.e.mean ($n = 4$ or 5)

	Before endotoxin	*Time after endotoxin (min)*		
Group A		*2*	*7*	*30*
Mean pulmonary artery pressure (mmHg)	14 ± 1	42 ± 4†**	29 ± 7	21 ± 3
Pulmonary arterial $PGF_{2\alpha}$ (pg/ml)	155 ± 29	487 ± 68*	431 ± 61*	314 ± 77
Aortic $PGF_{2\alpha}$ (pg/ml)	135 ± 29	724 ± 97**	296 ± 40*	176 ± 11
Group B				
Mean pulmonary artery pressure (mm Hg)	15 ± 2	35 ± 1†**	31 ± 4*	14 ± 1
Pulmonary arterial TxB_2 (pg/ml)	314 ± 65	651 ± 67*	457 ± 67	334 ± 22
Aortic TxB_2 (pg/ml)	190 ± 116	965 ± 176**	500 ± 90**	260 ± 29

† Change in pulmonary compliance and airways resistance at this time −57 ± 6% and + 251 ± 42% respectively;
†† Change in pulmonary compliance and airways resistance at this time − 43 ± 8% and + 149 ± 54% respectively.
*$P < 0.05$; **$P < 0.01$ dependent *t*-test.

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The influence of stimulant and relaxant drugs on prostacyclin production by the rat pregnant myometrium

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Prostacyclin (PGI_2) is the major arachidonic acid metabolite produced by myometrial tissue from late pregnant rats (El Tahir & Williams, 1980a). Myometrial PGI_2 output can be stimulated by some peptides but $PGF_{2\alpha}$ is ineffective (Williams & El Tahir, 1980). We have extended these studies to include other oxytocic drugs to which receptor antagonists are available and also some uterine relaxants.

Female Wistar rats were killed on days 18–21 of pregnancy. Myometrial tissue was separated as previously described (El Tahir & Williams, 1980). Samples were suspended in Krebs solution (25% w/v). Segments were preincubated at 37°C for 20 min. In test samples the relevant drug was added to the medium after 10 min and incubation continued for a further 10 min. Some tissue samples were preincubated with mepacrine (6.77 mM), atropine (4.8 μM) or propranolol (67.6 μM) for 10 min before drug addition. The following drugs were used carbachol (CCh; 55 μM); 5-hydroxytryptamine (5-HT, 21 μM); histamine (23 μM); salbutamol (21 μM) and porcine relaxin, 15 guinea-pig units (GPU)/ml (potency 500 GPU/mg). After preincubation arachidonic acid (AA) 329 μM was added to some samples. All myometrial preparations were then chopped and incubated for 15 min at 20°C. Incubation fluid was then aspirated and the PGI_2 content estimated by inhibition of ADP-induced aggregation of rabbit platelet-rich plasma. PGI_2 release by test samples (ng/mg over 15 min) was compared with a paired control sample and the level of significance of the drug-induced release was estimated by a paired 't' test. Output was then expressed as a % of the basal release.

Drug-stimulated changes in PGI_2 release were: CCh $120 \pm 10\%$ (mean ± s.e.mean; $P < 0.01$; $n = 7$); 5HT $85 \pm 10\%$ ($P < 0.05$; $n = 5$). Inhibition of PGI_2 output was seen with histamine $28 \pm 5\%$ ($P < 0.05$; $n = 6$); salbutamol $42 \pm 6.4\%$ ($P < 0.01$, $n = 7$) and relaxin $48 \pm 6\%$ ($P < 0.02$; $n = 6$). Atropine did not affect basal PGI_2 output but abolished CCh-stimulated release. Mepacrine abolished the increased PGI_2 output evoked by CCh and 5HT. Relaxin inhibited oxytocin-stimulated release of myometrial PGI_2 release, which is mediated via phospholipase A_2 activation (Williams & El Tahir, 1980) but did not affect AA-stimulated production.

It is concluded that the influence of oxytocic and relaxant drugs upon myometrial PGI_2 release are related to changes in phospholipase A_2 activity. These changes are probably a consequence of drug-receptor combination.

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TR4979 a selective 'ψ' prostanoid receptor agonist in the airways

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Of the three classes of prostanoid receptors identified in airways (Gardiner & Collier, 1980) it seems likely that two of these, the 'χ' contractant and the 'ψ' relaxant/inhibitory, act to partially mediate and modulate asthma respectively. However, the ability of the naturally occurring prostanoids to stimulate all three prostanoid receptors, albeit to varying degrees, decreases their therapeutic potential as modulators of asthma. Using test models for all three prostanoid receptors we report that TR4979, a 15-deoxy, 16-hydroxy, 17-cyclobutyl analogue of PGE_1, is a selective 'ψ' agonist in the airways.

TR4979 was evaluated as an agonist of 'ψ' receptors by determining its relative relaxant potency to PGE_1 on the guinea-pig isolated trachea (GPT) (Piper, 1969) and human isolated bronchial muscle (HBM) (Sweatman & Collier, 1968), under normal basal tone levels and on the cat isolated trachea (CT) (Lulich, Mitchell & Sparrow, 1976) at raised tonal levels induced by 5HT. The latter test system has been shown to contain predominantly 'ψ' receptors with no 'χ' receptors (Apperley, Coleman, Kennedy & Levy, 1979). TR4979 was 0.83 (0.5–1.5), 0.06 (0.03–0.13) and 0.02 (0.0004–0.4)× as potent as PGE_1 on the GPT, HBM and CT respectively. Propranolol (5×10^{-7} g/ml) produced no change in the dose response curves of PGE_1 and TR4979 on the GPT, strengthening the likelihood of their acting specifically on a prostanoid relaxant receptor(s).

Two further test models were used to evaluate the activity of TR4979 on 'χ' receptors in airways. In the presence of indomethacin (10^{-6} g/ml), the GPT loses its basal tone consequently it cannot relax to bronchodilator agents unless tone is artificially induced by a contractile agonist. Although this preparation at zero tone shows no response to β-agonist and methylxanthines it contracts to low concentrations of PGE_2 (10^{-9} g/ml) and PGE_1 (10^{-8} g/ml). TR4979, however, had no contractant activity over a wide concentration range (10^{-10}–10^{-4} g/ml). The cat isolated lung strip (Lulich, Mitchell & Sparrow, 1976) seems to contain a predominance of 'χ' receptors with little or no 'ψ' receptors, although under normal inherent tonal conditions it relaxes to isoprenaline. PGE_2 and PGE_1 both contract this preparation at 10^{-7} g/ml whereas TR4979 again had no effect (10^{-8}–10^{-5} g/ml). If we compare the concentrations of TR4979, PGE_1 and PGE_2 producing a 10% contractile or relaxant effect in the previous two GPT preparations we can determine their selectivity ratios for the 'χ' or 'ψ' receptors. A value >1 represents a predominantly 'ψ' effect and <1 a 'χ' effect. The following ratios were obtained using this method: PGE_2, 0.1; PGE_1, 10 and TR4979, >100; confirming the greater selectivity of TR4979 for 'ψ' receptors.

Finally we used the cat cough test (Gardiner, Copas, Elliott & Collier, 1978) to determine the 'ω' prostanoid irritant activity of TR4979. Conscious restrained cats were challenged with aerosols of PGE_1, PGE_2, TR4979 or placebo and the cough or irritant activity was measured. PGE_1 and PGE_2 acted as potent irritants in the cat whereas TR4979 produced little or no cough/irritant effects at any of the doses tested (10^{-7}–10^{-4} g/ml). Using this data and that for relaxation of the CT, a selectivity ratio for cough ('ω') against relaxation ('ψ') was calculated again using the concentration of agonists which produced a 10% threshold effect. The following ratios were obtained where a value >1 represents a predominantly 'ψ' effect and <1 a 'ω' effect: PGE_2 and PGE_1, 10; TR4979, 10,000.

Taken together these observations suggest that TR4979 is a selective 'ψ' prostanoid receptor agonist in the airways.

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Regulatory mechanisms in histamine-induced tone in swine isolated tracheal smooth muscle

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There is evidence that some contractile drugs cause the release of relaxant prostaglandins (PG; PGE_2) in isolated preparations of airways smooth muscle (Orehek, Douglas & Bouhuys, 1975; Gryglewski, Dembinska-Kiec, Grodzinska & Panczenko, 1976; and others). This observation could explain why indomethacin and aspirin enhance drug-induced contractions in guinea-pig isolated airways (Oreheck *et al.*, 1975). More recently, however, it has been suggested that immunological and possibly histamine-induced contractions are modified by the release of contractile products of the arachidonic acid lipoxygenase pathway (Adcock & Garland, 1980; Hitchcock & Kokolis, 1981). Results obtained in a preparation of swine isolated tracheal smooth muscle are now presented.

The preparations were suspended in organ baths containing Krebs solution at 37°C gassed with 95% O_2/5% CO_2 and tension was recorded isometrically. The effects of a number of drugs, with known effects on arachidonic acid metabolism, were examined on drug-induced contractions. Preparations were run in pairs – one tissue served as control to test for any sensitivity change to the contractile drugs acetylcholine (3–10 μM); histamine (100 μM); 2-pyridylethylamine (2-PEA 1.6 mM) whilst the other was used with the inhibitor drug. Control tissues were treated with vehicle (ethanol or Krebs). Contractions elicited in the test preparations in the presence of inhibitor were expressed as percentages of the pre-drug responses. The results are shown in Table 1.

Indomethacin and sodium salicylate (1 h) caused marked potentiation of histamine-induced contractions whereas they had small or no potentiating effect on contractions elicited to acetylcholine. Indomethacin also enhanced contractions to 2-PEA a H_1 receptor agonist. However, when 5, 8, 11, 14-eicosatetraynoic acid (ETYA) was administered along with indomethacin all potentiation was abolished. The phospholipase A_2 inhibitor mepacrine (15 min) caused pronounced inhibition of histamine-induced responses without affecting the responses to acetylcholine.

These results suggest a) that contractile responses are regulated by products of arachidonic acid other than PGs or metabolites of the cyclo-oxygenase pathway and b) this regulatory response is relatively selective for histamine H_1 receptor occupants.

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Table 1 Percentage changes in drug-induced contractile responses in the swine isolated trachea (mean ± s.e.mean)

	Acetylcholine 3 μM	*Acetylcholine* 10 μM	*Histamine* 100 μM	*2-PEA* 1.6 mM
Indomethacine (1 μM)	+33.5 ± 22.8 (6)	+42.6 ± 14.9* (6)	+213.3 ± 50.1** (6)	+90.2 ± 9.0*** (4)
Indomethacin (1 μM) + ETYA (10 μM)	+0.3 ± 11.7 (6)	—	−31.6 ± 14.1† (6)	—
Sodium salicylate (250 μM)	+0.9 ± 1.3 (4)	—	+126.9 ± 41.0* (6)	—
Mepacrine (100 μM)	−5.5 ± 4.6 (4)	—	−85.5 ± 9.7*** (4)	—

*$P < 0.05$; **$P < 0.02$; ***$P < 0.01$, compared to pre-drug responses (paired *t*-test); †$P < 0.001$ compared to indomethacin alone (unpaired *t*-test). Numbers in parenthesis refer to number of preparations. Acetylcholine responses at 3 μM and 10 μM were approximately 50% and 70% E_{max}. Histamine responses at 100 μM were approximately 50% E_{max}. Responses to 1.6 mM 2-PEA were similar to those with histamine.