DOPAMINE RECEPTORS IN THE PARS INTERMEDIA OF THE PITUITARY GLAND

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Both the corticotroph cells of the anterior pituitary gland and melanotroph cells of the pars intermedia synthesise pro-opiocortin. Only the melanotroph cells however seem to be under tonic inhibitory control by dopaminergic neurones (Vermes, Mulder, Smelik & Tilders, 1980). We have studied dopamine receptor binding in the anterior pituitary, pars intermedia and pars nervosa.

Spiperone binding sites were localised at the light microscopic level using an in vitro autoradiographic approach (Young & Kuhar, 1979). Rats were killed by cervical dislocation and the pituitary glands rapidly removed and frozen. 13µm sections were cut on a cryostat microtome and thaw mounted onto cooled subbed slides. (^{3}H)-Spiperone binding sites were localised by incubation of the mounted sections in 0.4 nM (^{3}H)-spiperone (specific activity 39 Ci mmol^{-1}, Radiochemical Centre) in 0.17 M tris-HCl buffer pH 7.7, for 30 min at room temperature. After two 5 min washes in ice cold buffer and dipping in distilled water, the sections were dried in a stream of cold dry air. Parallel incubations were also conducted in which the rigid dopamine agonist 2-amin-6,7-dihydroxytetrahydronaphthalene (ADTN) 10^{-5}M was added to define specific dopamine receptors (Quik, Iversen, Larder & Mackay, 1978).

(^{3}H)-Spiperone binding was present throughout the pituitary gland. There was however a marked concentration of autoradiographic silver grains over the cells of the pars intermedia. These were of a similar density to the grains seen on control sections of corpus striatum. The (^{3}H)-spiperone binding in the pars intermedia was displaced by ADTN, suggesting that this binding was to specific dopamine receptors.

These results provide novel evidence that the inhibition of melanotroph cells by dopamine is mediated through specific dopamine receptors, and that the dopamine binding sites are considerably more concentrated in the pars intermedia than in the anterior or neural lobes where they have been previously described.

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THE BENZODIAZEPINE BINDING SITE: ONE RECEPTOR OR TWO?

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It has recently been shown by Braestrup and Nielsen (1980) that some β-carboline derivatives interact potently with benzodiazepine receptors but do not display mass action kinetics. In an elegant analysis based on these findings, they have suggested the presence of two independent subclasses of benzodiazepine receptors which have identical affinities for benzodiazepines, but differ tenfold in their affinities for β-carbolines. We have studied the on- and off- rates of (^{3}H)-flunitrazepam binding kinetics to further explore this postulated heterogeneity of binding sites. Although we find there to be at least two molecular species of binding-site/ligand complex, these species appear capable of interconversion depending upon the occupancy of the total binding-site population. Specific binding of (^{3}H)-flunitrazepam (0.35nM) to receptor sites in washed membrane preparations from rat brain was defined by addition of 2μM flunitrazepam; all experiments were performed at 4°C.

Association of (^{3}H)-flunitrazepam at a ligand concentration of 0.35nM appears to be into a single compartment with $t_{\frac{1}{2}}$ = 96s. Following 200-fold dilution of the radiolabel, (^{3}H)-flunitrazepam dissociates from two compartments, 67% from a slow compartment (A) ($k = 7.1 \times 10^{-4} s^{-1}$) and the remainder from a fast compartment (B) ($k = 4.3 \times 10^{-3} s^{-1}$). If dissociation was initiated by saturating the sites with 2μM diazepam, the relative sizes of the compartments changed to 39% (A). On the other hand, if 2μM ethyl-β-carboline-3-carboxylate (βCCE) was used to saturate the sites, compartment (B) was replaced by compartment (C) ($k = 6.7 \times 10^{-3} x^{-1}$) which comprised 68% of the total. For comparison, the kinetics of (^{3}H)-βCCE were also analysed: this ligand associated and dissociated (regardless of the method of initiation) monoexponentially. The rate constants were $k_{on} = 1.7\ s^{-1}M^7$ and $k_{off} = 6.5 \times 10^{-3} s^{-1}$.

We suggest that there is a uniform population of benzodiazepine binding-sites which can exist as more than one conformer. One conformer (B) is stabilised by agonists (e.g. benzodiazepines) whilst another (C) is stabilised by antagonists (e.g. β-carbolines).

A.D. is an MRC Student

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ETHYL β-CARBOLINE-3-CARBOXYLATE REVERSES THE EFFECT OF BENZODIAZEPINES IN A TEST FOR DETECTING ANXIOLYTIC ACTIVITY

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Ethyl β-carboline-3-carboxylate (βCCE) is a potent inhibitor of benzodiazepine binding to rat brain membrane fragments and has been identified in ethanolic hydrochloric acid extracts of human urine and human, porcine and rat brain (Braestrup et al, 1980). Pharmacologically, it has been shown to reverse the anti-convulsant, motor incoordinating and locomotor inhibitory actions of benzodiazepines (Oakley & Jones, 1980; Cowen et al 1981; Tenen & Hirsch, 1980). It was therefore of interest to determine whether βCCE can also reverse the actions of benzodiazepines which may be more indicative of anxiolytic properties.

The effect of benzodiazepines have been characterised in a simple procedure, based on that described by Soubrié et al (1976), in which the time water-deprived rats spend drinking in a novel environment is measured. Naive male Wistar rats, which have been water-deprived overnight, are placed singly in a clear perspex cylindrical surround through which a spout attached to a graduated water vessel projects. The apparatus is brightly lit from above to increase the aversive nature of the environment. The latency to onset of drinking, and the time spent drinking and the volume drunk in 5 and 10 min periods have been recorded. Average increases in the time spent drinking and volume drunk during 10 min for groups of 6-12 rats, have been adopted as the most reliable measures of potential anxiolytic activity.

Dose-dependent increases in the time spent drinking and the volume of water drunk have been observed for chlordiazepoxide (1.25-5mg/kg i.p.), nitrazepam (0.5-2mg/kg i.p.), diazepam (0.5-8mg/kg i.p.) and also phenobarbitone (15 and 30mg/kg i.p.). Amphetamine (1-4mg/kg i.p.) failed to increase either of the parameters measured. βCCE (0.5-5mg/kg i.v.) 5 min before testing had no significant effects on the pattern of drinking, but as shown in Table 1, 5 mg/kg i.v. βCCE significantly reversed the effects of 2 mg/kg i.p. diazepam and 5 mg/kg i.p. chlordiazepoxide.

Table 1 Reversal of diazepam and chlordiazepoxide-induced effects by βCCE

Treatment	Time spent drinking(s)	Volume drunk(ml)	Treatment	Time spent drinking(s)	Volume drunk(ml)
Control	50	1.1	Control	91	1.9
βCCE 5mg/kg i.v.	62*	1.3*	βCCE 5mg/kg i.v.	51*	1.2*
Diazepam 2mg/kg i.p.	122	2.4	Chlordiazepoxide 5mg/kg i.p.	155	3.3
Diazepam 2mg/kg i.p. + βCCE 5mg/kg i.v.	47†	0.8†	Chlordiazepoxide 5mg/kg i.p. + BCCE 5mg/kg i.v.	81†	1.7†

$p < 0.05$* vs control, † vs benzodiazepine alone. Mann Whitney U Test (n=12)

Thus the activity of these compounds in this test, which may reflect their anxiolytic properties, also is reversed by βCCE, indicating that βCCE does not discriminate between the diverse actions of benzodiazepines.

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REPEATED ELECTROCONVULSIVE SHOCK ENHANCES THE ANTICONVULSANT EFFECT OF BENZODIAZEPINES

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Repeated electroconvulsive shock (ECS) enhances certain monoamine-mediated behaviours in rats (Green, 1978). We have now investigated the effect of repeated ECS on a behavioural property of the benzodiazepines, namely their anticonvulsant activity.

Male Sprague-Dawley derived rats, weighing 100-125 g at the start of experiments, were given an ECS (125 v 1 s 50 Hz) via ear clip electrodes once daily for 10 days. Seizure thresholds were determined 24 h after the last treatment by infusing a 10 mg/ml solution of pentylenetetrazol (PTZ) into a tail vein (Nutt et al, 1980).

Following saline pretreatment there was no difference in seizure thresholds between ECS-treated rats and a handled control group. However, 20 min following administration of either diazepam (2 mg/kg i.p.) or the water-soluble flurazepam (10 mg/kg, i.p.) there was a significantly greater elevation of seizure threshold in the rats that had received ECS. In contrast, repeated ECS did not increase the sensitivity of animals to the anticonvulsant effect of either sodium valproate (400 mg/kg in saline) or progabide (200 mg/kg in Tween) (both administered i.p. 30 min before infusion).

Table 1 Changes in seizure threshold following repeated ECS

Pretreatment		Seizure threshold to PTZ (mg/kg)		P Value
		Handled	ECS x 10	
Saline		34±5(4)	37± 5(5)	N.S.
Flurazepam	10 mg/kg	43±5(6)	54± 7(8)	<0.005
Diazepam	2 mg/kg	43±7(4)	75±12(6)	<0.005
Valproate	400 mg/kg	49±9(5)	53± 9(8)	N.S.
Progabide	200 mg/kg	43±9(6)	45± 8(8)	N.S.
β-CCE	1 mg/kg	20±3(8)	30± 8(8)	<0.001

Numbers represent mean ± S.D. with number of animals in brackets. P value shows significance between ECS and handled group on Student's 't' test. None of the vehicles used altered seizure threshold compared with saline.

The selectivity of the ECS effect was further tested by administration of the proconvulsant benzodiazepine receptor ligand, β-carboline carboxylate (β-CCE) (Cowen et al, 1981). When β-CCE (1 mg/kg i.v.) was given 5 min before PTZ infusion the proconvulsant effect was significantly less in the ECS-treated group.

These findings suggest that repeated ECS may produce a specific change of BDZ receptor function. Since studies have failed to show alteration in BDZ binding following repeated seizures (Bowdler & Green, 1982), it seems that the enhanced BDZ response and reduced β-CCE effect may reflect an alteration in receptor state, perhaps a change to a more 'active' configuration.

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GABA AND DIAZEPAM-INDUCED REDUCTION OF CEREBRAL 5-HYDROXYTRYPTAMINE TURNOVER

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Recent assessment of benzodiazepine action has, in many instances, focussed on the γ-aminobutyric acid (GABA) system, usually at the expense of other central neurotransmitters (e.g. Costa, 1980). However, 5-hydroxytryptamine (5-HT) has also been implicated in the anxiolytic action of these drugs (Wise, Berger & Stein, 1972), although in many cases the doses of benzodiazepines used in these studies are far higher than those used clinically. This study has employed therapeutically comparable doses of diazepam to measure 5-HT turnover in rat cerebral cortex and to investigate the possible role of GABA in this effect.

Adult male Porton rats were treated chronically for 7 days with low doses of diazepam in 1% Tween (5 or 2.5 mg/kg/day, i.p.). Control animals received vehicle alone. The effect of this treatment, in conjunction with manipulating cerebral GABA mechanisms with either a single subconvulsive dose of picrotoxin (3 mg/kg, i.p., 1 hr before killing) or administration of GABA-transaminase inhibitors aminooxyacetic acid (AOAA; 25 mg/kg, i.p., 4 hr before killing) or ethanolamine-O-sulphate (EOS; 250 mg/kg/day, i.p., 7 days) was studied on the turnover of 5-HT in cerebral cortex. The turnover of 5-HT was estimated by calculating the ratio of the metabolite 5-hydroxyindoleacetic acid (5-HIAA): 5-HT in cortex.

Chronic treatment of rats with diazepam, at both 5 and 2.5 mg/kg doses, significantly reduced the ratio of 5-HIAA:5HT by 43 and 35% respectively ($p < 0.01$ and $P < 0.001$, 2-tailed t test and Mann-Whitney U test). Picrotoxin administered immediately after the last diazepam injection (5 mg/kg) reversed this effect; the 5-HIAA:5-HT ratio being now similar to that of controls. At this dose picrotoxin alone had no effect on 5-HT turnover. Using GABA-transaminase inhibitors to elevate cerebral GABA concentrations (Fonnum, 1981) both AOAA and EOS further enhanced the reduction in 5-HT turnover seen to chronic diazepam (2.5 mg/kg/day). Although AOAA alone did not significantly alter the 5-HIAA:5-HT ratio compared to control, vehicle-injected rats, EOS alone produced a significant reduction in this ratio (to 58% of control, $p < 0.001$).

Microinjection of picrotoxin into the dorsal raphé nucleus of control rats (0.1 μg in 0.5 μl, 30 min) enhanced 5-HT turnover in the cerebral cortex (39% increase from control, $2p < 0.05$). Raphé injections of picrotoxin in chronic diazepam pretreated animals (5 mg/kg/day, 7 days) reversed the fall in 5-HT turnover seen to the chronic benzodiazepine treatment, and the ratio of 5-HIAA:5-HT was now within control values.

Although it is already known that low doses of diazepam (< 10 mg/kg) decrease cerebral 5-HT turnover, these results show that this effect appears to involve a GABA mechanism as a drug which antagonises GABA receptors (picrotoxin) reverse this effect, while drugs which enhance GABAergic neurotransmission increase this action of diazepam.

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MORPHOLOGICAL CHARACTERIZATION OF (^{3}H)GABA ACCUMULATING NEURONS IN THE RAT NEOSTRIATUM BY GOLGI-STAINING AND ELECTRON MICROSCOPY

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The results of pharmacological, biochemical and electrophysiological experiments are in agreement that one of the transmitters of the striatonigral pathway is GABA and that it might also play a role in the neostriatum itself (Dray, 1979). The major class of striatonigral neuron has been identified as the medium-sized densely spinous type and if these neurons use GABA as a transmitter then their local axon collaterals may account for GABA-mediated effects within the neostriatum. However, it has been suggested that some local circuit neurons in the neostriatum might also use GABA (Iversen & Schon, 1973; McGeer & McGeer, 1975).

We have carried out experiments to classify morphologically and study the synapses of neurons in the neostriatum that accumulate (^{3}H)GABA by a combination of both autoradiography and Golgi-staining (Somogyi et al., 1981). Anaesthetized rats were injected into the neostriatum with 0.2 µl of (^{3}H)GABA (2-10 µCi, 60 Ci/mmol). After a survival time of 20-40 min the brains were fixed and 80 µm Golgi-stained and gold-toned sections were prepared; autoradiography was carried out on 1-2 µm sections.

In the light microscope it was found that only a small proportion of medium-sized neurons around the injection site were radio-labelled. Examination in the electron microscope of 17 labelled neurons from 3 animals showed that all the neurons were of the same type and characteristically had an indented nucleus with a moderate amount of cytoplasm and numerous cytoplasmic organelles. The sparse synaptic input to the perikarya consisted of boutons forming both symmetrical and asymmetrical membrane contacts. Golgi-stained and gold-toned neurons that were also selectively labelled with the (^{3}H)GABA had round perikarya with several dendrites that branched often. The dendrites had a varicose appearance, had only occasional spines and sometimes followed a recurving course. Electron microscopic examination showed them to be of the same type as the neurons that were (^{3}H)GABA labelled but not Golgi-impregnated.

The morphology of the GABA-accumulating neurons was distinct from other characterized neurons in the neostriatum, i.e. the medium-sized densely spinous and the large long-dendrite neuron, that project to the substantia nigra (Somogyi et al., 1981; Bolam et al., 1981); the neurons that stain immunoreactively for substance P or somatostatin (unpublished); and the giant neurons of the neostriatum. These results demonstrate that there is a specific morphological class of neostriatal neurons that accumulate locally administered (^{3}H)GABA and since we have observed neurons of this Golgi type with extensive axonal aborizations we suggest that they are the GABAergic interneurons of the neostriatum.

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GABA RECEPTOR BINDING IN PHYSIOLOGICAL SALT SOLUTION

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Saturable binding sites for GABA on brain synaptic membranes can be readily demonstrated in Na^+-free organic buffer solutions using radiolabelling techniques (eg Zukin et al. 1974). Binding in such solutions is taken to represent attachment to post-synaptic receptors since unlike binding to GABA transport sites it does not require sodium ions (eg Lester et al. 1981). Tris-citrate buffer solution (TCB) is frequently employed although other buffer systems can be used (Tunnicliff & Smith, 1981). Whilst most GABA agonists displace specifically bound ^{3}H-GABA from membranes incubated in TCB we believe that this buffer may interfere with receptor binding sufficient to prevent the detection of some potential GABA-mimetics. For example, we have recently observed that ethylenediamine (EDA) is a GABA-mimetic when applied iontophoretically to rat brain neurones in vivo and in vitro when applied to rat isolated superior cervical ganglia (Perkins et al. 1981). However, EDA does not displace ^{3}H-GABA from rat brain membranes when incubated in TCB. We have therefore attempted to measure GABA receptor binding in the same physiological salt solution, Krebs'-Henseleit (KHS), used in our ganglion assay system with the addition of nipecotic acid to prevent binding to transport sites.

Rat synaptic membranes were prepared as described by Zukin et al (1974) and stored at -15°C. For the assay membranes were thawed and washed four times in KHS before incubation in 1 ml KHS containing ^{3}H-GABA (5 nM), or ^{3}H-muscimol (5 nM) to label $GABA_A$ sites. 100 μM (±)nipecotic acid and (±)baclofen (the latter to saturate $GABA_B$ sites, Hill & Bowery, 1981) were also included with or without a potential unlabelled displacer. Binding of (±)^{3}H-baclofen (20 nM) to $GABA_B$ sites was assayed in KHS alone. After incubation for 10 min at 20°C the mixture was centrifuged (7000 g for 10 min). The tritium content of each pellet was determined by liquid scintillation spectrometry. The specific portion of ^{3}H-GABA (49.3±1.05%,n=23) and ^{3}H-muscimol binding (79.6±0.34%,n=16) was determined with isoguvacine (100 μM) and ^{3}H-baclofen binding (24.8±1.1%, n=12) with (±)baclofen (100 μM). Under these conditions only compounds shown to be active at GABA receptors displaced the bound ligands. The inhibitor of GABA transport cis-3-aminocyclohexane carboxylic acid (ACHC) was inactive at concentrations up to 1 mM. Muscimol and GABA displaced ^{3}H-GABA and ^{3}H-muscimol from $GABA_A$ sites with the same potencies as in TCB. Isoguvacine was marginally less active in displacing ^{3}H-GABA in KHS (IC_{50}=300 nM cf GABA IC_{50}=103 nM) than in TCB (isoguvacine≈GABA). Bicuculline methobromide was significantly more active in displacing ^{3}H-GABA from $GABA_A$ sites in KHS (IC_{50} values = 4.8±0.18 μM in KHS, 27.6±3.67 μM in TCB, n=6 in each case). However, the most important observation was that EDA which failed to displace any of the radioligands in tris-citrate or tris-HCl buffer now displaced both ^{3}H-GABA and ^{3}H-muscimol from $GABA_A$ and ^{3}H-baclofen from $GABA_B$ sites in a dose-dependent manner. IC_{50} values were 5.08±0.67 μM (^{3}H-GABA - $GABA_A$ site), 2.80±0.20 μM (^{3}H-muscimol - $GABA_A$ site) and 3.0±0.15 μM (^{3}H-baclofen - $GABA_B$ site) (mean±s.e.mean, n=3 with each ligand). In all cases EDA produced the same maximal displacement as GABA.

We conclude that post-synaptic GABA binding sites can be reliably demonstrated in physiological salt solutions containing nipecotic acid. This may be preferable to tris-buffer solutions for the screening of potential GABA-mimetics.

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THE EFFECTS OF AMNESIA-PRODUCING DRUGS ON HIPPOCAMPAL POTENTIATION IN THE RAT

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The administration of scopolamine or of certain benzodiazepine drugs (notably lorazepam) produces temporary amnesia in humans. The central noradrenergic system has also been hypothesised to be involved in learning. An intact hippocampal region is necessary for normal human memory and it is possible that processes necessary for normal memory underlie the dramatic potentiation of responses evoked by electrical stimulation of hippocampal afferents. The effects of lorazepam, scopolamine and propranolol on potentiation produced by electrical stimulation of hippocampal commissural fibres have been investigated in the rat.

Results are reported from 24 rats anaesthetised with urethane (1.5 g/kg i.p.). Recording electrodes were positioned in areas CA1 and CA3 of dorsal hippocampus. A stimulating electrode was located homotopically in the contralateral CA3 region. Electrode positions were determined electrophysiologically and later confirmed histologically. Stimuli (typically 1 mA for 100 μs) were delivered at 1 Hz or as tetani (10 Hz for 10-20 s). Tetani were given every 15 or 20 min. For each rat stimulus parameters were initially adjusted to produce potentiation of the evoked responses during the tetanus (frequency potentiation) and were then held constant. Drugs were administered in 300 μl saline via a cannula in the femoral vein and were washed in with another 300 μl saline. A potentiation latency was measured for each tetanus as the time from the start of the tetanus to the start of potentiation of the population spike (mean 5.5 s). For each rat a mean percentage change in this latency from one tetanus to the next was calculated in the absence of drug administration. This mean was subtracted from the percentage change for tetani following administration of a drug. This relative latency change was averaged across rats.

For tetani delivered 5 to 20 min after injection, lorazepam (250 μg/kg) produced a significant increase in the relative latency (CA1: mean = 21.8 ± 3.3% s.e.mean, 10 rats, $P<0.001$; CA3: 26.4 ± 7.9%, 6 rats, $P<0.05$). In CA1 scopolamine (10 mg/kg) produced a smaller (less than lorazepam: $P<0.01$) but reliable ($P<0.05$) increase in relative latency (6.7 ± 2.2%, 7 rats) for tetani delivered 5 to 20 min after administration, but the effect in CA3 (7.0 ± 6.2%, 4 rats) was not significant. Propranolol (3 mg/kg) produced no significant effect (CA1: 3.6 ± 3.9%, 8 rats; CA3: 9.5 ± 4.6%, 6 rats). Injections of lower doses of drug or of saline alone did not produce significant changes in relative latency.

Thus a delay in the onset of potentiation was only found in the tetanus immediately following injection of the amnesia-producing drugs lorazepam and scopolamine.

No significant effects of the drugs were found on the amplitudes or wave-forms of the potentials evoked by 1 Hz stimulation, or on the peak amplitude of frequency potentiation, or on the amplitude or duration of post-tetanic potentiation. Long-lasting (>5 min) potentiation was not observed in these experiments. After certain tetani, after-discharges were observed. These were significantly reduced ($P<0.01$) in intensity or duration for tetani delivered 5 to 20 min after lorazepam ($\geq$250 μg/kg) or propranolol (3 mg/kg) compared to control periods.

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α_2-AGONIST-INDUCED MYDRIASIS IN THE RAT: AN EFFECT MEDIATED BY α_2-ADRENOCEPTORS IN THE CNS?

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It is well known that clonidine induces a pronounced mydriasis in a number of animal species. In the cat, this effect is thought to be central in origin and is produced by a marked inhibition of the parasympathetic innervation of the iris (Koss & San, 1976; Koss, 1979). An α_2-adrenoceptor mechanism appears to be involved in this effect since it is antagonised by yohimbine. In the present study we have measured the mydriasis produced by α_2-adrenoceptor agonists in the rat and, in addition, have investigated the sensitivity of this effect to the selective α_2-adrenoceptor antagonist RX 781094 [2-(2-(1,4-benzodioxanyl)) - 2-imidazoline HCl](Chapleo et al., 1981) administered by several routes. Yohimbine was also included in some experiments for comparison.

Rats (Sprague-Dawley, 300-350g) were anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.) and prepared for drug administration by cannulation of a femoral vein. Pupil diameter was measured under constant illumination by means of a Beck Luminex pupillometer. Dose-response curves were constructed by administering increasing i.v. agonist doses at 5 min intervals following pretreatment with either drug vehicle or the antagonists RX 781094 and yohimbine. The cumulative i.v. doses of the antagonists causing a 50% reversal of the maximal mydriasis produced by the selective α_2-agonist guanoxabenz (300 µg/kg) were used to assess antagonist potency. Reversal of guanoxabenz mydriasis was also examined after i.c.v. administration of RX 781094 or instillation of RX 781094 solution (25 µl; 0.1 and 0.3% w/v) onto the cornea of one eye.

The α_2-agonists clonidine (1-100 µg/kg),UK 14304-18 (1-300 µg/kg)(Cambridge, 1981)[2] and guanoxabenz (1-300 µg/kg) all produced a long-lasting (1h), dose-related mydriasis. Pretreatment with RX 781094 (0.5 mg/kg) shifted the agonist dose-response curves to the right (approximately 30 fold) in a parallel fashion. Yohimbine was less potent; 1.5 mg/kg i.v. producing a 10 fold shift. Both RX 781094 and yohimbine reversed guanoxabenz mydriasis in a dose-related manner;their respective ED_{50} values being 42 and 900 µg/kg,i.v.

Topical application of RX 781094 solution to one eye produced a slow (5-10 min) and similar reversal of guanoxabenz mydriasis in both eyes simultaneously. This suggested that RX 781094 produces reversal not by a local action in the eye but rather by being absorbed systemically and antagonising guanoxabenz at a central site. This was supported by the finding that i.c.v. administration of RX 781094 (1.5 - 15 µg/10 µl) caused a fairly rapid (2min) and dose-related antagonism of guanoxabenz mydriasis.

In conclusion, α_2-adrenoceptor agonists induce mydriasis in the rat by an action in the CNS. In view of the antagonism of this mydriasis by the highly selective and specific α_2-adrenoceptor antagonist RX 781094 it would appear that an α_2-adrenoceptor mechanism is involved.

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EFFECTS OF ADRENOCEPTOR MODULATION ON DRINKING CONFLICT IN RATS

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Central noradrenergic pathways originating in the locus coeruleus have been implicated in anxiety and α and β receptor antagonists and clonidine modulate the potentiated startle model of anxiety (Davis et al, 1979). However, there have been few such studies in conflict models, particularly drinking conflict. Conflict was established between thirst (24h water deprivation) and drinking contingent footshock (230 μA, 300 msec every 100 licks) in male Wistar rats (180-230g) (Gardner et al, 1981). Drugs were administered intraperitoneally, 30 min prior to testing. Diazepam (1-8 mg/kg) evoked a marked increase in punished drinking (PD). The selective α_1 antagonist, prazosin (5-40 mg/kg), evoked an increase of similar magnitude (Fig. 1) and smaller increases were evoked by phentolamine (5-40 mg/kg) and piperoxan (20-40 mg/kg). The selective α_2 antagonist, yohimbine (1-8 mg/kg), also increases PD. Clonidine (12.5-200 μg/kg) and propranolol (3-25 mg/kg) were without significant effect.

The possibility that these adrenergic drugs may act by increasing thirst (Meyer and Hertting, 1980) has been investigated. Hypertonic saline (8-32%, 4 ml/kg) increased drinking in 24h water deprived unpunished rats (UPD). The same doses had a relatively small effect on PD (Fig. 1). Doses of diazepam which markedly increased PD caused only a small increase in UPD similar to that of hypertonic saline. Thus, only a small component of the increased PD with diazepam may be due to increased thirst. However, increases in PD and UPD with yohimbine were similar (Fig. 1) and increased thirst may be a more major component in its anti-punishment effect. The sedative properties of the other α blockers decreased UPD, making interpretation difficult.

The relative contributions of antipunishment and thirst induction in the actions of these agents requires further investigation, but when testing new compounds in this model of anxiety the potential "false positive" of thirst induction should be considered.

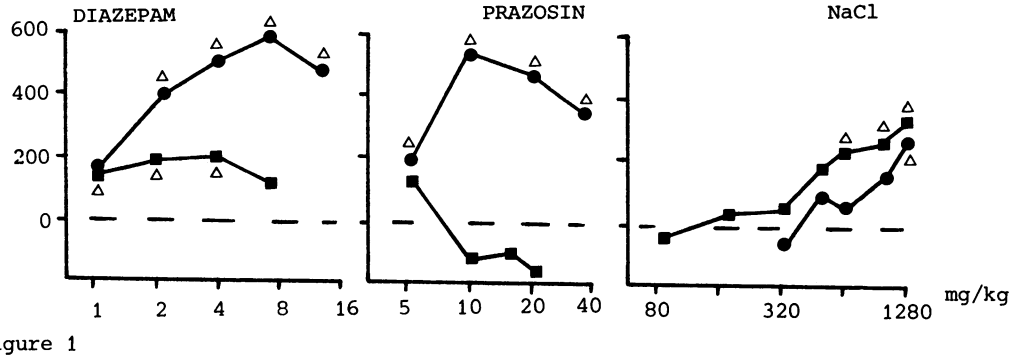

Figure 1

Dose response curves of the mean change from control in the total number of licks over a 3 minute period in the punished (●—●) and unpunished (■—■) conditions. All drugs were administered i.p. thirty minutes prior to testing with at least 8 animals per dose group. △ $p < .05$

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EVIDENCE FOR REGULATION OF AMINO ACID-MEDIATED TRANSMISSION IN RAT OLFACTORY CORTEX SLICES BY ENDOGENOUSLY RELEASED ADENOSINE

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When rat olfactory cortex slices are perfused in vitro at ambient temperature stimulation of the lateral olfactory tract evokes a characteristic series of field potentials comprising a monosynaptically-evoked e.p.s.p. (N-wave) and two longer latency massed postsynaptic potentials (late N- and I-waves; Pickles & Simmonds, 1976, 1978). Adenosine (1μM-1mM) depressed the amplitude of the field potentials in a concentration-dependent manner (see also Scholfield, 1978); IC_{50} values (μM); N-wave, 267 ± 58; late N-wave, 41 ± 7 and I-wave, 24 ± 6 (means ± s.e., n = 8) whereas the tract action potential was unaffected. Cyclic AMP, 2'-deoxyadenosine and adenosine N^1-oxide exhibited similar effects but were considerably less potent. These depressant actions of adenosine were reduced by the adenosine receptor antagonists theophylline (IC_{50} for depression of the N-wave by adenosine in the presence of 0.3mM theophylline was 758 ± 74μM; mean ± s.e., n = 8) and 3-isobutyl-1-methylxanthine (IBMX), (IC_{50} for depression of the N-wave by adenosine in the presence of 1mM IBMX was 810 ± 95μM, mean ± s.e., n = 4) and potentiated by dipyridamole, an inhibitor of adenosine uptake (IC_{50} for depression of the N-wave by adenosine in the presence of 10μM dipyridamole was 120 ± 16μM, mean ± s.e., n = 4).

In other experiments, the effects of drugs on tissue cyclic AMP levels were assessed (assay of Brown et al., 1971; all values quoted in pmol mg^{-1} protein). Application of adenosine (100μM) or of the excitatory neurotransmitter candidates L-aspartate and L-glutamate (5mM) to slices for 15 min significantly increased cyclic AMP levels (basal level, 20 ± 5; plus adenosine, 725 ± 27; plus L-aspartate, 609 ± 22; plus L-glutamate, 490 ± 98; means ± s.e., n = 6, $P < 0.001$). These effects were antagonized by 0.3mM theophylline (basal level with theophylline alone, 30 ± 6; plus adenosine, 405 ± 17; plus L-aspartate, 95 ± 7; plus L-glutamate, 82 ± 4; means ± s.e., n = 6, $P < 0.001$). Similarly, electrical stimulation of the lateral olfactory tract of slices (50V, 50μs duration, frequency of 5 min^{-1}) in the presence of dipyridamole (50μM) also increased cyclic AMP levels by a mechanism antagonized by 0.3mM theophylline (basal level with dipyridamole alone, 21 ± 3; with stimulation, 231 ± 17; plus theophylline, 19 ± 2; with stimulation plus theophylline, 37 ± 4; means ± s.e., n = 4). In other experiments, theophylline (1mM) antagonized the depressant actions of L-aspartate on the N-wave amplitude (IC_{50} value in mM for aspartate alone was 7.2 ± 0.9; plus theophylline, 13.2 ± 1.7; mean ± s.e., n = 5, $P < 0.01$).

It is concluded that synaptic activation of olfactory cortex slices releases adenosine, a process possibly depending on the release of the neurotransmitter candidates aspartate and glutamate. The adenosine so released interacts with extracellular receptors thereby regulating neurotransmission and inducing cyclic AMP synthesis although there is no experimental evidence that the latter two effects are directly related.

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KETAMINE ANTAGONISES N-METHYL-ASPARTATE AND SYNAPTIC EXCITATION OF SPINAL NEURONES

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Many anaesthetics enhance long latency spinal inhibitions (Eccles et al. 1963). Our preliminary studies have shown that ketamine i.v. did not consistently affect either long latency or short latency inhibitions. Polysynaptic, but not monosynaptic, reflexes were usually reduced.

We have therefore investigated the action of ketamine in pentobarbitone-anaesthetised cats and rats, and in decerebrate cats (initially anaesthetised with CT 1341), on the responses of spinal neurones to electrophoretically administered excitatory amino acids.

(±) ketamine HCl (4-25 nA, 100 mM in 100 mM NaCl, pH 4) reduced the responses of all dorsal horn neurones tested (cat 35 : rat 19) to N-methyl-aspartate (NMA) more than to quisqualate or kainate. Responses to L-glutamate and L-aspartate were less affected than those to NMA, ketamine showing little selectivity between the two natural transmitter candidates. Similarly intravenous ketamine (2.5-10 mg/kg) selectively reduced responses to the local ejection of NMA of all 9 neurones tested.

Synaptic excitations of 9 cells following afferent nerve stimulation were reduced by both intravenous and electrophoretic ketamine in parallel with reduction of responses to NMA. Although such studies are in their initial stages it appears that long latency excitation from high threshold afferents are more sensitive to ketamine than early excitations (<15 ms latency) from low threshold afferents.

Responses of 9 neurones to GABA and glycine in decerebrate cats were not affected by ejection of ketamine with currents up to 3 times greater than those for NMA antagonism.

These findings demonstrate the importance of NMA - receptors in normal synaptic functions of the mammalian C.N.S. and raise again the question of identity of the natural excitatory transmitter(s). The results also suggest a site of action for the anaesthetic-analgesic properties of ketamine at the NMA receptor.

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A COMPARISON OF THE EFFECTS OF TWO ISOMERIC Gn-RF ANALOGUES ON DMBA TUMOURS IN RATS

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The anti-tumour effects of D-pyroGlu^{1}-D-Ser(Bu^{t})^{6}Azgly^{10}-Gn-RF (ICI 152910) have been compared with those of its isomer, D-Ser(Bu^{t})^{6}Azgly^{10}-Gn-RF (ICI 118630) which Nicholson & Maynard (1979) have shown previously to cause regression of DMBA tumours in rats similar to that observed with tamoxifen (100 µg daily) when 0.5 µg was administered i.m. twice daily for 20 days.

Tumours were induced in rats (Sprague-Dawley, 150 g-170 g) by the administration of DMBA (20 mg in 1 ml arachis oil, p.o.). Tumours of the required size (≮1.5 cm mean diameter) were biopsied under ether anaesthesia prior to treatment with the Gn-RF analogues and tumour volumes were determined at weekly intervals during the treatment period (20 days) and for a further 5 weeks. All biopsy specimens and, on sacrifice of the animals at the end of week eight, all solid tumours of sufficient size (≮300 mg) were blotted free of excess fluid, frozen in liquid N_2 and stored at -70° until analysed for oestrogen- and progesterone-receptor contents using the dextran coated charcoal technique.

Drugs (either 0.05 or 0.5 µg of 118630, or 5 or 50 µg of 152910) were administered s.c. in citrate buffer (0.1 ml) twice daily at 9 a.m. and 4 p.m. for 20 consecutive days.

In two experiments using 118630 (0.5 µg) 10 out of 14 and 10 out of 11 tumours respectively regressed to <10% of their original size during the treatment period compared with 5 or 6 out of 12 using a dose of 0.05 µg. With 152910, 6 out of 10 (50 µg dose) and 5 out of 10 (5 µg dose) regressed to the same level. Amongst control animals only one tumour out of twenty spontaneously regressed to the 10% level.

The incidence of new tumours arising during the treatment period was 3 and 1 respectively for experiments using the 0.5 µg dose of 118630, and 0 for the 50 µg dose of 152910 compared with 14 and 8 respectively in the control groups. Following withdrawal of treatment the incidence of new tumours was not greatly different in treatment and control groups except for animals treated with ICI 152910 in which 14 and 15 new tumours were observed in the 5 and 50 µg treatment groups respectively.

With one exception regression of tumours to <10% of their original size and rapid regrowth following withdrawal of 118630 occurred if oestrogen or progesterone receptor contents of the tumours were high. No such correlation was observed using 152910.

On sacrifice the average progesterone receptor content (per mg protein) of tumours increased in the order: control < 5 µg 152910 < 50 µg 152910 < 0.05 µg 118630 < 0.5 µg 118630, the difference from control with 0.5 µg of 118630 being significant at the 0.01 level, and the ratio of the mean DNA content to mean protein content was significantly lower than control in all treatment groups other than with the low dose of 118630. We conclude that the incorporation of D-pyroglutamate in place of its L-isomer produces a significant reduction in anti-tumour activity. This lower activity would appear not to be due to more rapid inactivation and elimination.

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INTRAVENTRICULAR TETRAHYDROPAPAVEROLINE INCREASES ALCOHOL CONSUMPTION IN RATS

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The finding that intraventricular (IVT) administration of low doses of tetrahydroisoquinolines (TIQ's) increases alcohol consumption in rats (Myers & Melchior,1977; Melchior,1979) has been confirmed by one group (Duncan & Deitrich,1980) but not by another (Smith et al..1980). We present here independent verification of the original finding that IVT administration of the TIQ, tetrahydropapaveroline (THP), to rats can increase alcohol intake.

Male Sprague Dawley rats were individually housed under standard conditions of lighting and temperature. Each was implanted with a guide cannula over the right lateral ventricle. Following recovery, these animals were tested for alcohol preference according to a 12-day test sequence described by Melchior (1977): a choice between alcohol, which increased in concentration from 3-30% on consecutive days, and tap water was offered in bottles, the position of which altered daily. Body weight, alcohol and water intake were monitored daily so that the animals could be divided into three groups of approximately equal alcohol preference.

During the following 12 days animals were presented with the alcohol preference schedule as before but also received daily IVT injections of 5 µl saline (control group; C: n = 5), 0.1 µg THP in 5 µl saline (low dose THP; LTHP: n = 6) or 1.0 µg THP in 5 µl saline (high dose THP; HTHP: n = 6). The results are shown in Table 1, the lower dose of THP markedly increased alcohol intake, paradoxically the effect was less marked in HTHP-treated rats.

Table 1 Average alcohol consumption during the 12 day schedule before and during intraventricular administration of saline or THP

Group	Before IVT administration		After IVT administration	
	Av.g/kg alcohol/ rat/day (± S.E.M)	Proportion of alcohol to water consumed/rat/day	Av.g/kg alcohol/ rat/day	Proportion of alcohol to water consumed/rat/day
C	2.3 ± 0.2	0.30 ± 0.05	1.9 ± 0.4	0.20 ± 0.03
LTHP	2.1 ± 0.2	0.23 ± 0.03	3.2 ± 0.4**	0.38 ± 0.03***
HTHP	3.2 ± 0.2	0.24 ± 0.04	2.6 ± 0.8	0.27 ± 0.03*

*$p < 0.05$; **$p < 0.002$; ***$p < 0.001$ (comparisons with control animals by analyses of variance on differences between scores before and after administration of THP)

The effect was greatest in the LTHP- and the HTHP-treated animals at alcohol concentrations in the range 7-20%. Whether TIQ administration can increase alcohol intake in rats to a sustained level where withdrawal effects typically occur is still not known.

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THE EFFECT OF HEPARIN ON PLATELET AGGREGATION IN VIVO

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A convenient and reproducible method for studying platelet aggregation and disaggregation in vivo has recently been described (Davies, Morley, Page & Paul; 1981). In this method, III-Indium oxine labelled platelets are monitored in the thoracic (C1) and abdominal (C2) regions of an anaesthetized guinea-pig (40 mg/kg pentobarbitone) using collimated 1" crystal scintillation probes. Count rates are collected by a dual channel gamma spectrometer (Nuclear Enterprises) incorporating a microcomputer which retains up to 99 successive counts for each channel, for determination of the ratio (C1/C2). The intravenous administration of aggregating agents such as ADP (0.01-10 mg/kg), collagen (100-500 ug/kg) and platelet activating factor (PAF)(30-100 ng/kg) produces an increase in counts in C1 and a concomitant decrease in counts in C2 producing an increase in ratio C1/C2.

Although heparin is used extensivly as an anti-coagulant its effects on platelet aggregation are not clearly established (Mustard and Packham, 1975). Mandatory use of anti-coagulants in other in vivo techniques precludes analysis of the effect of heparin on platelet aggregation and disaggregation in vivo. This technique permits analysis of the effects of heparin on platelet aggregation and disaggregation in vivo since systemic anti-coagulants are not necessitated.

Heparin (500 units/kg) produces minimal effects on the primary aggregation response to ADP (10 mg/kg). However, the more sustained component of platelet retention is diminished by heparin (500 units/kg), in contrast to the potentiation of the ADP respose reported elsewhere (Eika, 1972). The sustained component may correspond to the secondary phase of aggregation induced by ADP in vitro (Constantine, 1966), since the secondary wave of ADP induced aggregation often does not occur in heparinized platelet-rich plasma (Mills and Roberts, 1967). In contrast to previous reports (Klein & Bell, 1974; Bell, Anderson, & Anderson; 1977), heparin itself did not produce platelet aggregation in these animals. Intravenous collagen (300 ug/kg) caused a more sustained response than ADP and this is also diminished in the pres ence of heparin (500 units/kg). These results indicate that heparin is able to affect both platelet/ platelet or platelet/fibre interactions in vivo.

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FATE OF (^{14}C)-CAPTOPRIL COVALENTLY BOUND TO PLASMA PROTEINS IN THE RAT

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Captopril (CP) is a novel angiotensin I converting enzyme inhibitor used in the treatment of hypertension. The nature and time course of the adverse reactions to captopril are consistent with there being an underlying immunological mechanism; and it was therefore suggested that CP, or one of its metabolites, may act as a hapten and induce antibody formation (Hoorntje, et al., 1980). We have investigated the relationship between the metabolism of CP and the formation of CP plasma protein conjugates in vivo.

Male Wistar rats (250-300g) were anaesthetised with urethane and the trachea, the carotid artery and the jugular vein cannulated. ^{14}C-CP (5μCi; 4 mg/kg) was administered intravenously and arterial blood samples collected after 5, 30, 60, 120 and 180 minutes when the animals were killed and the radioactive content of the lungs, kidney, spleen and liver determined after dissolving in tissue solubiliser.

After extraction from plasma with methanol, ^{14}C-CP and its metabolites were separated and quantitated by radiochromatography as described by Wong et al., (1979). The amount of ^{14}C-CP covalently bound to the methanol precipitated plasma proteins was determined by equilibrium dialysis after dissolution in aqueous sodium dodecyl sulphate (Sun & Dent, 1980).

Free ^{14}C-CP was cleared relatively quickly from plasma, but we were unable to determine any pharmacokinetic parameters because the plasma concentration-time profile appeared curvilinear. There was extensive covalent binding of ^{14}C-CP to plasma proteins at 5 minutes and after 180 minutes 35 ± 5% of total radioactivity in plasma was covalently bound. Other metabolites identified in plasma were captopril disulphide and ^{14}C-CP cysteine mixed disulphide.

Captopril readily forms covalent disulphide linkages with plasma proteins in vitro (Wong et al., 1979) and we were therefore able to prepare rat plasma in which ^{14}C-CP (68 μg/ml) was at least 94% covalently bound. Free metabolites were removed by repeated washing of the plasma proteins with 0.9% saline in an Amicon B15 protein concentrator. After infusion of the plasma protein conjugate (1 ml) into rats, as described above, the plasma concentration of ^{14}C-CP covalently bound to plasma protein declined mono-exponentially with a half-life of 71.1 ± 2.2 min and an apparent volume of distribution of 16.5 ± 1.6 ml. After 180 minutes there was a significantly greater accumulation of radioactivity in the liver, spleen and the lung than in corresponding experiments in which free ^{14}C-CP was given. However, the greatest accumulation was in urine where there was no evidence for protein bound ^{14}C-CP; the major metabolites were ^{14}C-CP cysteine mixed disulphide and free ^{14}C-CP. Thus, although captopril readily forms covalent bonds with plasma proteins, the resulting drug protein conjugate(s) dissociate in vivo. If captopril does act as a hapten, the relative rates of these processes may partly determine the toxicity of the drug.

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EFFECT OF THE ANTIMALARIALS CHLOROQUINE AND PRIMAQUINE ON DRUG METABOLISM IN THE RAT

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Chloroquine (CQ, a 4-aminoquinolone) is the most widely used drug in the treatment of the asexual erythrocytic form of the plasmodial life cycle, whereas primaquine (PQ, an 8-aminoquinolone) eliminates the liver form. CQ has been reported to inhibit the activity of imidazole-N-methyltransferase, alcohol dehydrogenase and succinic dehydrogenase (Cohn, 1965; Fiddick & Heath, 1967; Sanabria et al., 1959) and PQ to inhibit the metabolism of CQ (Gaudette & Coatney, 1961). We have therefore investigated in detail the effects of CQ and PQ on drug metabolism in the rat in vivo and in vitro.

Groups of 5 male Wistar rats were given CQ, PQ or saline, i.p., 30 min before determination of a) pentobarbitone (40 mg/kg) sleeping time (CQ and PQ, 10, 20 and 50 mg/kg), b) zoxazolamine (60 mg/kg) paralysis time (CQ and PQ, 50 mg/kg) and c) ^{14}C-antipyrine (5 μCi/kg; 15 mg/kg)kinetics (CQ and PQ, 50 mg/kg). The demethylation of aminopyrine (2.5 mM) was studied in vitro in the presence of CQ and PQ (0.001, 0.01, 0.1, 1 and 10 mM) and the deethylation of ethoxyresorufin (ERR) 250 nM, in the presence of CQ and PQ (250 nM). Finally, the effect of two fixed concentrations of CQ (3.0 and 10 mM) and PQ (0.01 and 0.1 mM) on the kinetics of aminopyrine n-demethylation (aminopyrine, 0.25, 0.75, 1.5 and 2.5 mM) was investigated.

PQ administration increased pentobarbitone sleeping time in a dose related manner (control, 94.0 ± 9.4 min; 10 mg/kg, 137.0 ± 2.4, $P < 0.01$; 20 mg/kg, 197.0 ± 7.5. $P < 0.001$; 50 mg/kg, 269.0 ± 2.9, $P < 0.001$; mean ± s.e.mean), prolonged paralysis time (control, 140.0 ± 10.0; 50 mg/kg, 341.5 ± 25.6 min, $P < 0.01$) and decreased antipyrine blood clearance from 2.17 ± 0.19 to 0.86 ± 0.12 ml/min, $P < 0.001$). CQ showed no effect on pentobarbitone sleeping time or zoxazolamine paralysis time, but decreased antipyrine clearance from 2.17 ± 0.19 to 1.11 ± 0.18 ml/min ($P < 0.01$). Both drugs inhibited aminopyrine demethylation. The concentration of inhibitor causing 50 per cent inhibition (IC50), for CQ was 10 mM and for PQ approximately 0.1 mM. Lineweaver-Burk plots showed CQ to inhibit competitively whereas PQ inhibition was non-competitive at 0.01 mM and uncompetitive at 0.1 mM. ERR deethylase activity decreased by 14.3% and 19.1% in the presence of CQ and PQ (250 nM; i.e. equimolar with substrate) respectively.

Since antipyrine, ERR and zoxazolamine are regarded as substrates for cytochrome P-448 and aminopyrine, antipyrine and pentobarbitone substrates for P-450 enzymes, the present study has shown that PQ and CQ inhibit both enzyme systems although CQ appears to be more selective.

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RENAL EXCRETION OF DIGITOXIN IN A RAT ISOLATED PERFUSED KIDNEY PREPARATION: THE INFLUENCE OF PROTEIN BINDING

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The effect of altered plasma protein binding on glomerular filtration is well understood; only the unbound drug is filtered. The influence of such changes on renal tubular secretion and reabsorption is less clear, in particular little quantitative information is available (Rowland and Tozer, 1980). The isolated perfused kidney preparation, which retains such excretory functions, offers an ideal opportunity to study the effect of protein binding changes without complicating systemic processes. In this study the effect of changes in the fraction unbound (fu) on the renal clearance (CL_R) of digitoxin, a drug which is highly bound to albumin and undergoes extensive tubular reabsorption in man (Lukas, 1971) was evaluated.

An isolated perfused rat kidney preparation based on that of Bowman (1975), was developed using both bovine serum albumin (B.S.A.) and dextran in the perfusion media. Kidney function was evaluated by recording urine flow, sodium reabsorption, glucose reabsorption and glomerular filtration rate (G.F.R.), the latter being measured as the clearance of (^{14}C)-inulin. Using (^{3}H)-digitoxin to determine the clearance, three perfusions were carried out at fu values of 0.03, 0.12, 0.40, 0.51, and 0.99. Each perfusion involved seven ten minute clearance periods. Fractions unbound were determined using ultracentrifugation.

Renal function was similar to that reported previously using B.S.A. in the perfusate (Bowman and Maack, 1972), the mean values (± standard deviation) were; urine flow 81.39 (±30.21)$\mu\ell$ min^{-1}, percent sodium reabsorption 91.75 (±3.39), percent glucose reabsorption 96.75 (±0.80) and G.F.R. 802.2 (±271.0)$\mu\ell$ min^{-1}. The use of dextran (5%^{w}/v) in place of B.S.A. (6% ^{w}/v) in the perfusate produced no significant changes in renal function. Similarly digitoxin (20ng ml^{-1}) did not significantly alter the functional characteristics of the preparation. Thin layer chromatographic analysis of urine samples indicated that digitoxin was not transformed or degraded during the course of the perfusions.

In all cases the fraction of filtered digitoxin which was excreted was low, (mean = 0.31), indicating extensive tubular reabsorption. The renal clearance of digitoxin was not correlated with urinary pH (r = 0.02) but did show a weak correlation with urine volume (r = 0.58).

Over the 33-fold change in fu seen in this study, there appeared to be a linear relationship between digitoxin clearance (corrected for G.F.R.) and the fraction unbound in the perfusate ($y = 0.2368x + 0.0134$, $r = 0.9952$).

These findings agree with suggestions that the rate of tubular reabsorption is proportional to the concentration gradient of diffusible drug across the renal tubular boundary (Levy, 1980). Furthermore, support is given to the previously held theory that the renal clearance of drugs which have a low renal clearance, owing to extensive tubular reabsorption, will be sensitive to changes in their binding to plasma proteins (Rowland and Tozer, 1980).

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EFFECT OF VARIOUS INHIBITORS ON ANTIPYRINE METABOLITE KINETICS IN RAT IN VIVO

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Antipyrine (AN) is a widely used test compound for assessing hepatic mixed function oxidase activity in vivo. Recently there has been interest in the measurement of the oxidative metabolites of AN in urine. Evidence has been presented (Danhof et al, 1979) which indicates that the formation of these metabolites is controlled by different forms of cytochrome P-450. We have investigated the effect of three commonly used inhibitors of oxidative drug metabolism (Netter, 1980) on the metabolite kinetics of AN.

Twenty four male Sprague-Dawley rats were divided into 4 groups and each received (N-$^{14}CH_3$)-AN (50 mg/kg; 2 μCi/kg; i.p.) on 2 occasions 1 week apart. On the second occasion the control group received no other drug while the other rats were administered i.p. either metyrapone (M; 25 mg/kg), SKF 525A (S; 25 mg/kg) or α-naphthoflavone (N; 100 mg/kg) 1 h prior to AN dosing. During the studies the rats were housed in all-glass metabolism cages modified for continuous $^{14}CO_2$ collection; $^{14}CO_2$ being the end product of the single carbon species removed from AN by demethylation. Urine was collected over 30 h and assayed for 3-hydroxymethylantipyrine (3HM), 4-hydroxyantipyrine (4H), norantipyrine (NOR) and parent drug by reverse-phase h.p.l.c. following conjugate hydrolysis and solvent extraction. The metabolite parameters obtained from each animal under control and inhibited states are reported as means ± s.d. and are compared by paired t test.

M, S and N administration resulted in marked changes in the $^{14}CO_2$ exhalation rate (CER)-time profiles which were consistent with inhibition of AN metabolism. The maximum CER attained was decreased from 0.085 ± 0.013 to 0.040 ± 0.011 % dose/min ($P<0.001$) in the 3 inhibitor groups. M and S also increased the time taken to achieve maximum CER from 75 ± 15 to 240 ± 110 min ($P<0.05$). CER-time plots allowed the time course for the inhibition process to be monitored. Under control conditions, semi-logarithmic plots of CER against mid-point time gave terminal linear phases with half-lives of 139 ± 17 min over the time period 100 to 500 min. Similar terminal half-lives were observed after M and N administration but in the former case the linear terminal phase was not reached until 400 ± 100 min. The effect of S was more prolonged resulting in a terminal half-life increase from 142 ± 16 to 520 ± 300 min over the time period 200 to 600 min. Urinary excretion of AN was increased from 2.0 ± 1.0 to 4.3 ± 3.0 ($P<0.005$) following administration of inhibitor. 3HM formation was inhibited from 20.0 ± 1.6 to 10.1 ± 5.7 % dose ($P<0.01$) by S resulting in an increase in the parallel pathway 4H (26.0 ± 4.4 to 38.8 ± 5.0 % dose, $P<0.01$). The effects of M were less specific and no significant change in either urinary metabolite was observed. Urinary recovery of 4H and NOR was not significantly altered by N although 3HM increased from 16.9 ± 3.9 to 22.4 ± 3.7 % dose ($P<0.01$). In contrast the control group of rats showed minimal variations in urinary excretion products and CER parameters between the two AN tests.

In conclusion AN metabolite formation is altered quantitatively and qualitatively by the inhibitors studied. The data presented are not consistent with a single enzyme catalysing all 3 oxidations. Thus it provides further evidence for the involvement of multiple forms of cytochrome P-450 in AN metabolism in vivo.

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METABOLIC INTERMEDIATES OF PARACETAMOL WHICH MAY EXPLAIN ITS MECHANISM OF TOXICITY

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Liver failure in cases of paracetamol poisoning is believed to be the result of irreversible binding of an unstable metabolite to hepatic macromolecules to cause cell death. The nature of the toxic species is not yet known but the most popular candidate appears to be N-acetylbenzoquinonimine (Hinson et al, 1980). We present data that suggests that 4-hydroxy-2,3-dihydroacetanilide 2,3-oxide may play an important role in the toxic action of the drug.

Human urine collected following theraputic and toxic doses of paracetamol was analysed for metabolites using HPLC. On the basis of drug related chromatographic peaks having retention times identical with those of reference samples, either before or after enzymic hydrolysis, two of the minor metabolites were identified as 2- & 3- hydroxyparacetamol. Thus it appears that paracetamol is oxidised in such a way that the ultimate products are metabolites substituted with either sulphur at the 3- position (Potter et al, 1974) or with oxygen which we have shown may be at the 2- or 3- position. It has been found that for various substituted benzenes e.g. napthalene, reaction of the epoxide, generated by hepatic oxidation, with glutathione gives rise to acid-labile derivatives which were considered to be 1-hydroxy-2-S-(N-acetylcysteinyl) 1,2-dihydroaromatics or 'premercapturates' (Knight and Young, 1958). We therefore incubated aliquots of urine from volunteers following a 1.5g oral dose of paracetamol with HCl at pH 0.3 and 37°C for 20 hours after enzymic hydrolysis (sulphatase & β-glucuronidase, Sigma) and reassayed the samples for O- substituted (2- & 3- hydroxyparacetamol) and S- substituted*** (3-S-cysteinyl & 3-S-(N-acetylcysteinyl) paracetamol) metabolites. In order to assess possible breakdown of these final metabolites in acid, standard samples were incubated under identical conditions. Breakdown is indeed substantial and was found to be 50% for the O- substituted compounds, 85% for 3-S-cysteinyl paracetamol, and 76% for 3-S-(N-acetylcysteinyl)paracetamol after 20 hours. We therefore corrected for this decomposition in assessing the concentrations of these compounds in urine before such treatment. Greatly elevated levels of S-substituted metabolites and 3-hydroxyparacetamol were found after pH 0.3 treatment.

Mean Concentrations of O- & S- Substituted Metabolites in Human Urine

CONCENTRATION (μg/ml) IN:-		3-hyroxyparacetamol	S- metabolites***
Urine after enzymic hydrolysis (sulphatase and β-glucuronidase)	mean	7.57*	2.66*
	s.e.	0.94*	0.36*
Urine after enzymic hydrolysis and pH 0.3 incubation for 20h	mean	14.64	13.88
	s.e.	3.91	1.92
*corrected as described in text		(n=14) p<0.02	(n=8) p<0.001

These results suggest that pre-aromatic acid-labile metabolites of paracetamol are present in urine and constitute a significant proportion of the oxidised metabolites. This points to the fact that both O- and S- substituted metabolites arise from nucleophilic attack on an epoxide ring. We therefore propose that in the human, pathways of O- and S- substitution are indeed linked, and that 4-hydroxy-2,3-dihydroacetanilide 2,3-oxide is a likely intermediate in the hepatic metabolism of paracetamol and may also play a part in its toxicity.

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PROTECTION AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY BY ASCORBATE IN MICE

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The risk of hepatotoxicity in man following overdosage with N-acetyl-p-aminophenol (APAP, paracetamol) has caused considerable concern, since its onset is delayed and its outcome often fatal. The characteristic hepatic lesion, centrilobular necrosis, is similar in man and experimental animals (Jollow et al, 1974). L-ascorbic acid (LAA) has been shown to lower mortality due to APAP administration in mice (Raghuram et al, 1978) and appears to inhibit the covalent binding to protein of the reactive metabolites generated from APAP (Lake et al, 1981).

This study investigated the prophylactic efficacy of various dosage forms of ascorbate, given alone or in combinations against the hepatotoxicity produced by a challenge dose of 450mg APAP/kg p.o. in male MF1 mice. As indices of hepatotoxicity, the liver weight to body weight ratio (RLW) and an histological score of liver necrosis were measured 24h after dosing with APAP.

No significant protection was given by LAA at 50, 150 or 300mg/kg given at the same time as APAP, nor at 150 or 300mg/kg given 1h afterwards. Doses of 150mg LAA/kg given both at the same time as and 24h after APAP also failed to give protection. However, other forms of ascorbate did show a protective action. A significant reduction in the APAP-induced increase in RLW and liver necrosis score was evident with ascorbyl palmitate (ASC P) at 300, 600 and 900mg/kg (see Table). At doses of 600 and 900mg/kg these indices did not differ significantly from the vehicle controls. The level of protection given by a microencapsulated form of LAA (MEAA) appeared to be related not only to the dose (see Table) but to the fate of the microcapsules in the gastrointestinal tract. Significant positive correlations were found between the numbers of intact microcapsules in the gut 24h after APAP and both indices of hepatotoxicity. This suggests that disintegration of MEAA favours protection and that the pharmacokinetic profile of LAA relative to the time scale of the hepatotoxic processes may be critical. The results indicate that ascorbic acid in certain forms may have potential use in the development of an APAP formulation with improved safety.

Table Mortality, RLW and liver necrosis after APAP with ASC P and MEAA in mice

Treatment (mg/kg)			No. of	Deaths at	Mean	Frequency in	Mean necrosis
APAP	ASC P	MEAA	mice	24h (%)	RLW	survivors(%)	score+ in survivors
- (Vehicle control)			10	0	4.69**	0	0**
[a]450 (Treated control)			30	33	6.53	95	7.50
450	300	-	20	20	5.31**	81	4.75*
450	600	-	20	30	4.80**	43	1.42**
450	900	-	20	5	4.80**	21	1.52**
450	-	300	20	30	6.36	93	6.35
450	-	600	20	20	5.91	63	5.31

+maximum score 12. *Difference from (a) significant by Duncan's Multiple Range test, *P<0.05; **P<0.01.

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THE APPLICATION OF THE $^{14}CO_2$ BREATH ANALYSIS IN STUDIES OF THE DISPOSITION OF N-(^{14}C)METHYL LABELLED XENOBIOTICS IN MICE

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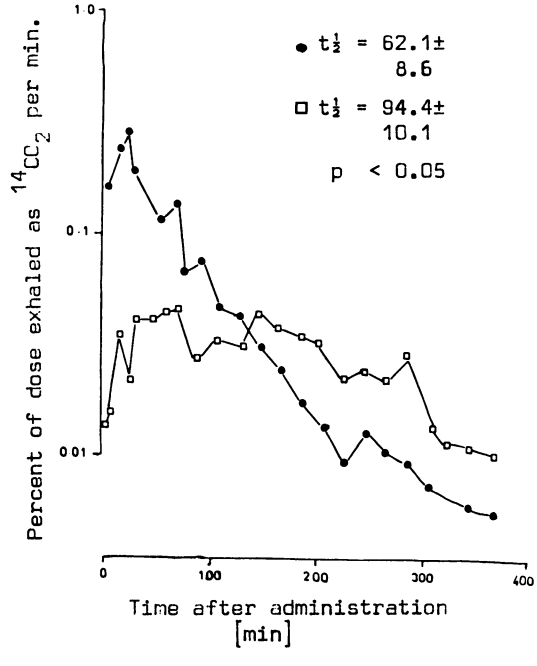
The exhalation with the breath of $^{14}CO_2$ as a metabolite of [^{14}C] aminopyrine has been used to study the disposition of aminopyrine, as the rate at which $^{14}CO_2$ appears with the breath after administration of [^{14}C]aminopyrine parallels the elimination of the drug from plasma (Lauterburg & Bircher, 1976). We have applied this method to other ^{14}C-labelled N-methyl xenobiotics (Gescher & Raymont, 1981) and studied the influence of a variety of factors on the $^{14}CO_2$ exhalation rate after administration of 1-[^{14}C]methyl labelled caffeine ([^{14}C]Caf) and [^{14}C]-methyl labelled hexamethylmelamine ([^{14}C]HMM). [^{14}C]Caf or [^{14}C]HMM were administered i.p. to male CBA Ca mice (20-25 g), the exhaled $^{14}CO_2$ was trapped continually and samples of exhaled radioactivity were counted. Whereas the administration of 1, 5, 40 and 80 mg/kg [^{14}C]HMM lead to identical $^{14}CO_2$ exhalation rate profiles, the $^{14}CO_2$ plot obtained with 2 mg/kg [^{14}C]Caf differs markedly from the profile obtained with 40 mg/kg (Figure 1), which is in accordance with the reported dose dependency of the plasma elimination kinetics of caffeine (Burg & Werner, 1972). The presence of either the PC6A plasma cell tumour in male BalbC mice 7 days after inoculation or the M5076 ovarian sarcoma in male BDF_1 mice 21 days after implantation did not affect the disposition of $^{14}CO_2$ after administration of [^{14}C]HMM (90 mg/kg, s.c.). When HMM was given subcutaneously the $^{14}CO_2$ exhalation profile differed significantly from the $^{14}CO_2$ plot observed after i.p. administration.



To measure exhaled $^{14}CO_2$ as a metabolite of N-[^{14}C]-methyl xenobiotics may constitute a sensitive and simple method to investigate factors which influence the metabolic disposition of these agents.

Figure 1: $^{14}CO_2$ exhalation rate plot after administration of [^{14}C]Caf 2 mg/kg (●) or 40 mg/kg (□). Points are means of at least six experiments.

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THE METABOLISM OF MONURON (N-(4-CHLOROPHENYL)-N',N'-DIMETHYLUREA) IN MICE

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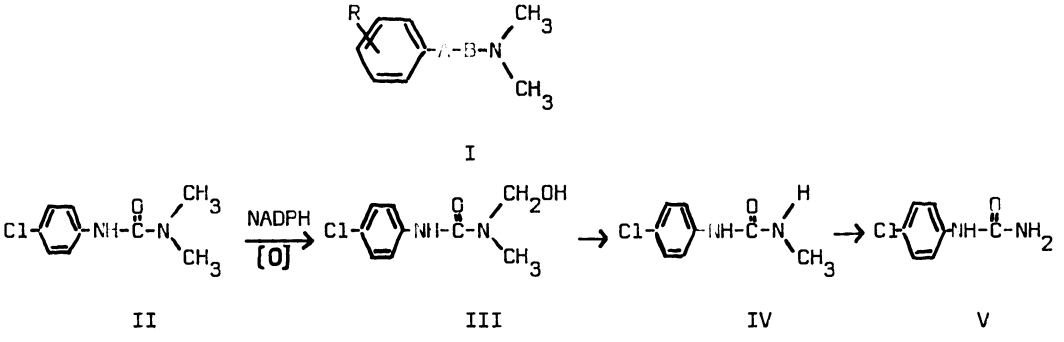
We are interested in the biological fate of N-methyl groups from xenobiotics which are substrates for oxidative metabolism by the liver and have made a study of compounds of the general formula I (Scheme 1).



Scheme 1: Metabolism of monuron

We report here that the herbicide monuron (II) is metabolised by mouse liver microsomes to a species which gives a positive test in a colourimetric assay for formaldehyde but which is not a substrate for enzymes which metabolise free formaldehyde (method: Gescher et al, 1979). Analysis of the metabolites of monuron by a HPLC method (Ultrasphere 5μODS, methanol/water 10% to 100% linear gradient) was performed on urine after administration of 200 mg/kg i.p. to male, 20g BalbC mice, and on products formed after incubation with various preparations of mouse liver in vitro. Samples were prepared for chromatography by the addition of methanol and centrifugation. The major urinary metabolite was 4-chlorophenylurea (V), a result similar to that seen in the rat (Ernst & Bohme, 1965). A conjugate in the urine was hydrolysed enzymatically to yield the monodemethylated product (IV). Incubation of 1mM monuron (II) with mouse hepatocytes, whole liver homogenate, 9000g supernatant and microsomes gave a product with a similar retention volume to p-chlorophenylurea (V). However on treatment with acid, alkali or on heating the sample this metabolite decomposed with a corresponding increase in the amount of N-(4-chlorophenyl)-N'-methylurea (IV). The chemical ionisation mass spectrum of this unstable metabolite was identical with a synthetic sample of IV. This suggests to us the possibility that the labile hydroxymethyl compound (III) had been formed. The glucoside conjugate of this compound (III) was reported as a product of the metabolism of monuron in cotton plants (Frear & Swanson 1972), but in animals and man such N-hydroxymethyl metabolites are rare. The ability of certain N-methyl compounds to form nascent formaldehyde may be of both toxicological and pharmacological significance.

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ENHANCED EXCRETION OF 2,4,5,2',4',5'-HEXACHLOROBIPHENYL BY RATS ON RESTRICTED FOOD INTAKE

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2,4,5,2',4',5'-Hexachlorobiphenyl (6-CB) is a neutral lipophilic compound which has no remaining adjacent pair of unsubstituted carbon atoms, and thus is no longer appreciably metabolisable (Hutzinger et al., 1972; Bickel & Muehlebach, 1980; Jondorf, 1981). This was demonstrated by Matthews & Anderson (1975) and more recently by our group (Muehlebach & Bickel, 1981) in experiments with rats on unrestricted food intake.

We found that after a single i.v. dose of 6-CB (0.6 mg/kg), only 17% of the dose was excreted in the faeces in infinite time, and urinary excretion was limited to 0,8%. Under ordinary physiological conditions, about 75% of the administered dose of 6-CB was irreversibly stored in the adipose tissue.

In the present series of experiments, male rats of the same strain (Sprague-Dawley derived) initially weighing ca. 250g and given single i.v. doses of 6-CB (or ^{14}C-labelled 6-CB) at 0.6 mg/kg were divided into 3 different groups with respect to dietary manipulation. The first group was on unrestricted food intake before injection, and was put on 25% of the ad libitum diet at 2 weeks after injection of 6-CB. The second group was put on 50% of the ad lib. diet for 2 weeks before injection, and immediately after injection, was restricted to 25% of the ad lib. food intake. The third group was restricted to 25% of the ad lib. food intake for 2 weeks before injection, and continued on this restricted food intake after injection of the 6-CB.

In the first group at 2 weeks post-injection, faecal and urinary excretion of 6-CB (measured by g.l.c. or ^{14}C methods) was 5.1% and 0.6% of the dose respectively. 47% of the dose was retained in adipose tissue and 29% in the skin. In the following 6 weeks on 25% diet, the weight of the rats became stabilised at ca. 160g. As the adipose depots diminished, their 6-CB content was redistributed. Faecal and urinary excretion rose to 36% and 1.5% respectively, and skin retention rose to 47% of the dose.

In the second group, the reduction to the equilibrium weight (150-160g) took less than 2 weeks post-injection. As the residual adipose reserves were used up, the rate of faecal and urinary excretion was accelerated. After 7 weeks a plateau had been reached. Faecal and urinary excretion totalled 54% and 3.3% of the dose respectively. Only 23% of the dose was retained in the skin.

In the third group which had almost reached the reduced equilibrium weight before the injection of 6-CB, there was no initial slower rate of excretion as in the second group above. A plateau had been reached at 7 weeks, at which time, faecal and urinary excretion totalled 48% and 1.3% respectively. Skin retention was 20% of the dose.

The concentration of 6-CB in brain, (the only organ with a high lipid content which is not affected by weight loss under the conditions here described) remained low in all three groups of animals, which implies that untoward neurological symptoms are unlikely to occur when a course of fasting reduces the body burden of 6-CB.

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EFFECT OF DEUTERATION ON THE PHARMACOKINETICS OF IMIPRAMINE

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Pharmacokinetic studies, in man and experimental animals, have shown that the antidepressant activity of imipramine is associated with the metabolite desmethylimipramine (DMI) (Dingell et al, 1964; Gram, 1974, 1977) formed by N-demethylation, and it has been suggested that imipramine itself may possess tranquillising properties (Maxwell, 1961; Dingell et al, 1964). The deuterium isotope effect has found application in pharmacology for the slowing of drug deactivation and elimination (Blake et al, 1975; Baillie, 1978), and for this reason deuterated-imipramine was prepared and its pharmacokinetics studied. It was thought that deuteration of imipramine might slow the metabolic N-demethylation and aromatic hydroxylation and thus give rise to higher plasma levels of imipramine relative to DMI and in this way initially sedate the patient and later exhibit its antidepressant effects.

Imipramine was specifically deuterated in the aromatic rings (4 deuterons) and in one N-methyl group of the side chain (3 deuterons), both individually (imipramine-d_4, imipramine-d_3) and combined (imipramine-d_7). The pharmacokinetic properties of these analogues were compared with unlabelled imipramine following intravenous and oral administrations. The rat was used, as this species shows a similar metabolism profile to the human (Dingell et al, 1964).

Half life of elimination of imipramine was extended from 160 min to 210 min, and total area under plasma concentration time curve was significantly increased following intravenous administration (10 mg/kg) of the d_7-analogue. Total systemic clearance was decreased from 85 to 59 ml/kg/min but the volume of distribution was unaffected. Oral administration of the d_3-analogue (50 mg/kg) also resulted in extension of the half-life to 210 min and an increase in the area under the curve. Peak plasma levels of imipramine were slightly increased as was the fraction of the oral dose absorbed (4.3 to 7.3%). No changes in the pharmacokinetic properties of imipramine were observed following oral administration (50 mg/kg) of the d_4-analogue.

A small but significant isotope effect (1.33) on the metabolic conversion of imipramine to DMI was thus observed, and this was consistent with the majority of isotope effects observed for N-demethylation reactions (magnitude <2) which have produced modified pharmacological properties (Elison et al, 1963; Abdel-Monem, 1975; Nelson et al, 1975). This isotopic effect extended the biological half-life of imipramine and decreased total systemic clearance, but appeared not to disturb the relative plasma levels of imipramine and DMI, indicating that no pharmacological change would be apparent. This has been confirmed by gastro-intestinal motility tests (charcoal meal test in rats) and on the isolated rabbit jejenum preparation.

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TIME-DEPENDENT KINETICS OF LIGNOCAINE IN THE ISOLATED PERFUSED RAT LIVER

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Lignocaine kinetics in man during continuous i.v. infusion appear to be time-dependent (Bax et al, 1980). In part, this is related to a progressive increase in plasma binding of the drug (Routledge et al, 1980) but studies in dogs also suggest a lowering of intrinsic clearance with time (Le Lorier et al, 1977; Vicuna et al, 1978). We have investigated this phenomenon using the isolated perfused rat liver preparation (IPRL). After a bolus injection of lignocaine (0.3 mg) into the reservoir (150 ml; re-circulating mode) elimination of the drug was a first-order process ($t_{\frac{1}{2}}$ = 5.25 ± 0.22 (s.d.) min; n = 4). At higher doses (7.5, 15.0 mg) non-linear kinetics were observed showing both sex- and time-dependence. The latter was not due to any deterioration in the preparation and there was no evidence of a hepatotoxic effect of the drug. The shapes of the drug concentration - time curves were consistent with saturable metabolism plus product inhibition. Prolongation of lignocaine $t_{\frac{1}{2}}$ observed (a) during multiple bolus injection (3 x 1.5 mg at 15 min intervals: $t_{\frac{1}{2}}$ (1st dose) = 6.11 ± 0.39 min; $t_{\frac{1}{2}}$ (3rd dose) = 8.30 ± 0.52 min ($p<0.05$)) and (b) following 120 min pre-treatment with high dose lignocaine ($t_{\frac{1}{2}}$ = 11.89 ± 0.59 min; $p<0.05$ vs control) provided additional evidence for this mechanism. After addition of known end-product metabolites (7.5 mg) of lignocaine to preparations already metabolising the drug, only monoethylglycinexylidide (MEGX) consistently impaired the elimination of lignocaine, prolonging $t_{\frac{1}{2}}$ from 6.88 ± 0.89 min to 10.31 ± 2.18 min. However, evidence was obtained that a direct effect of MEGX was not responsible for the observed time-dependent kinetics when this metabolite was produced endogenously. Shand et al (1973) accounted for the apparent time-dependent kinetics of propranolol in the IPRL by saturation of non-specific hepatic binding sites. Similar studies of the hepatic binding of lignocaine using the IPRL in the 'once-through' mode indicated the presence of high affinity - low capacity and low affinity - high capacity binding sites (K_D = 0.12 and 31 μM; capacity 0.03 and 0.43 $\mu M.g^{-1}$ liver, respectively). However, spectral studies of the interaction of lignocaine with liver microsomes by Von Bahr et al (1977) suggest that, rather than being non-specific, these sites could represent the enzymes responsible for aromatic hydroxylation and N-deethylation, respectively. Under conditions of impaired lignocaine elimination the Rosenthal binding curve for hepatic binding of the drug showed a marked shift to the left indicating that non-linear binding may not be the mechanism of the change in lignocaine kinetics with time. These results suggest that the time-dependent kinetics of lignocaine in the IPRL may be caused by an intermediate product related to the N-deethylation pathway which inhibits further metabolism of the drug.

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THE EFFECTS OF MEFENAMIC ACID ON TONE AND EVOKED RESPONSES IN HUMAN ISOLATED CEREBRAL ARTERIES

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The effectiveness of some non-steroidal anti-inflammatory drugs in the treatment of migraine suggests a possible role of the prostanoids (PGs) in the aetiology of this condition. Mefenamic acid is one of the clinically effective drugs and appears to act by inhibiting both synthesis and action of PGs (Bennett et al 1980). To try to clarify the role of PGs in migraine we have recorded the direct effects of mefenamate and its interaction with various PGs and vasoconstrictor amines on human intra-cranial arteries.

These vessels were obtained from post-mortems performed 24-72 h after death. The vessels were cut into spiral strips and suspended in Krebs' solution (5% CO_2 in O_2; 37°C; 0.5g load) to record isotonic contractions as described previously (Grimmer & Leathard, 1981). The contact time for each PG was 4 min and for each amine 1min. The mefenamic acid was applied as the sodium salt, prepared by neutralization with equimolar NaOH.

From preliminary dose-response curves, standard sub-maximal doses of the PGs and amines were selected for testing before and in the presence of mefenamate. PGs A_1, E_1, E_2, $F_{2\alpha}$ and 5-hydroxytryptamine(5-HT),and noradrenaline(NA)caused contraction whereas prostacyclin (PGI_2)caused relaxation. The mefenamate (50µg/ml) was allowed to equilibrate for 20 min, during which time the muscle relaxed (129±20% of maximum elicited with PGI_2,n=6) whereas simultaneous control experiments showed no relaxation (8.9±11.8% PGI_2,n=5) ($p<0.01$). Table 1 summarises the effects of mefenamate on evoked responses. Each PG was significantly antagonised($P<0.001$, t-test for unpaired data) although the degree of antagonism varied. Contractions to NA and 5-HT were also reduced.

Table 1 Sodium mefenamate on amine and PG-induced responses

Drug/Dose	Sodium mefenamate 50µg/ml			Control		P
	% initial response			% initial response		
	n	mean (±s.e.mean)		n	mean(±s.e.mean)	
5-HT 100 ng/ml	4	34.4	13.1	4	56	23.9	>0.1
5-HT 1 µg/ml	6	69.6	7.1	5	90	5.9	<0.05
NA 100 ng/ml	4	30.7	12.7	4	89.2	6.9	<0.01
NA 1µg/ml	6	53.0	5	5	88.7	3.5	<0.001
PG A_1 100 ng/ml	6	0	0	5	63.6	18.9	<0.001
PG E_1 10 ng/ml	6	30.9	9.3	5	99.2	7.6	<0.001
PG E_2 10 ng/ml	6	6.1	3.1	5	59	4.5	<0.001
PG $F_{2\alpha}$100ng/ml	6	0.6	0.66	5	76.7	7	<0.001
PGI_2 10 ng/ml	6	13.9	6	5	128.6	29.7	<0.001
PGI_2 100ng/ml	6	24.3	3.8	5	125.8	24.5	<0.001

The relaxation caused by mefenamate alone suggests that these arterial strips are continuously generating an excitatory PG which maintains vessel tone. The ability of mefenamate additionally to antagonise both inhibitory and excitatory responses of cerebral arteries to various PGs may contribute to its effectiveness in treating migraine.

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CHANGES IN BLOOD FLOW AND HISTAMINE CONTENT OF RAT SKIN GRAFTS

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Previously we have shown that the vasodilatation which occurred during the healing-in of rabbit skin autografts and allografts was associated with the concomitant increase of histamine content in the grafts. Furthermore, the onset of allograft rejection (as indicated by cessation of allograft blood flow) coincided with a peak histamine content which was more than three times that in autografts. In addition, mepyramine delayed the cessation of allograft blood flow thus suggesting that in rabbits the vasoconstriction was probably mediated by histamine (Lewis & Mangham, 1978).

In the present investigation we have examined this view further using the rat, a species in which histamine does not cause vasoconstriction. Male inbred rats of WAG(AgB2) and DA(AgB4) strains were used for allografts (DA WAG) and isograft controls (WAG WAG). Grafting technique and blood flow measurements were as described earlier (Fan & Lewis, 1981). Water-soluble mediators in each graft were extracted by homogenization in 7 ml ice-cold phosphate buffered saline and subsequent centrifugation (30,000g, 4°C, 20 min). The clear supernatant fluids thus obtained were assayed for pharmacological mediators using a cascade superfusion technique. Histamine content was further quantified fluorometrically.

Blood flow was first detected in the grafts on day 3 after grafting. A similar ^{133}Xe clearance pattern was observed in isografts and allografts up to day 6 after which time the allograft blood flow diminished within 3 to 4 days, whereas the isograft blood flow gradually returned to that of normal skin by day 14. The histamine content in the grafts was reduced by 40% during the 24 h after grafting and in allografts by 60% by day 3. The isograft histamine level, after the initial drop, gradually increased to the normal range by day 9, whereas the allograft histamine level remained low throughout the rejection process.

Therefore the changes in histamine content of skin grafts in rats was quite different from that in rabbits. The explanation is probably that rabbits possess many circulating basophils and platelets which contain histamine. These cells appear in the allograft during the rejection reaction and therefore increase the histamine content. On the other hand, in rats the histamine is largely located in tissue mast cells which are probably degranulated when the skin is removed from the donor, thus causing the initial reduction of histamine content in the grafts. Only in the surviving isograft, but not the allograft, does the histamine content return to normal.

The present finding is consistent with an earlier observation of Kahlson et al (1960) that in rat granulation tissue the histamine content was also lower than that in normal skin, although the histidine decarboxylase (HDC) activity was considerably higher. Furthermore, Moore & Schayer (1969) observed a similar increase in HDC in grafted tissue although they did not measure histamine content.

In view of the recent work on the immunoregulatory activities of histamine, it is possible that the reduction of histamine in allografts is related to the rejection process.

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THE INVOLVEMENT OF CENTRAL β-ADRENOCEPTORS IN BLOOD PRESSURE CONTROL IN THE RAT

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The presence of β-adrenoceptors in specific areas of the brain and their involvement in the control of blood pressure has been demonstrated in cats by Phillipu and Kittel (1977) and in rats by Zawoiski (1980). The areas of the brain studied were those which may be involved in the defence reaction (Abrahams et al., 1960). We report here studies in spontaneously hypertensive rats (SHR) demonstrating the involvement of β-adrenoceptors in the pressor responses to electrical stimulation of this brain area.

Male SHR of the Alderley Park strain (170-200g) were anaesthetised with an alphaxolone/alphadolone mixture ("Saffan", Glaxo; 18mg/kg i.v. for induction and 0.3-1.4mg/kg/min i.v. for maintenance) and b.p. and heart rate recorded from a cannulated carotid artery. Rats were stereotactically implanted with hollow stainless steel electrodes (uninsulated tip length 100μm) and electrically stimulated (200μa, 2ms pulse width, 5s duration) in the area immediately dorsal to the posterior hypothalamus (A 3.5, L 0.5, H +4.0 from ear bar zero, skull horizontal). This caused a marked frequency-dependent rise in heart rate and b.p. consistent with the cardiovascular changes which result from stimulation of the defence area in other species (Abrahams et al, 1960).

Electrical stimuli were delivered at various frequencies and a control frequency-response curve constructed. Drugs were subsequently injected via the hollow electrode in 1μl of artificial CSF and the electrical stimuli repeated. The effects of three cumulative doses of each drug were studies in each of 6 rats. For each frequency-response curve, that frequency causing a 50mm Hg rise in systolic pressure was calculated (F_{50}) and the dose of drug causing a two-fold increase in the control F_{50} determined by linear regression. Injection of D,L-propranolol resulted in a dose-related inhibition of the pressor responses (Table). This was not related to peripheral effects as the drug did not cause a bradycardia. The order of potency of the isomers was L>DL>D; the stereo-specificity suggesting that the activity observed is a consequence of β-adrenoceptor antagonism rather than membrane stabilisation. Atenolol, a β_1-selective antagonist, had no significant activity at doses up to 40μg.

Table Effects of propranolol and atenolol on pressor responses to electrical stimulation of the defence area in the SHR

Drug	Dose (μg) to cause a two-fold increase in the control F_{50} (s.e.mean)
L,-propranolol	2.8 (2.5 - 3.3)
D,L-propranolol	4.8 (3.3 - 6.9)
D,-propranolol	13.5 (13.3 - 13.8)
Atenolol	40

Thus, inhibition of the pressor responses to stimulation of the brain area described by microinjection of propranolol would suggest the involvement of central β-adrenoceptors in this phenomenon. Since the β_1-selective antagonist, atenolol was ineffective the receptors are possibly of the β_2-subtype.

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CARDIOVASCULAR CONTROL IN ADRENALECTOMIZED RATS

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Seven days after operation, adrenalectomized rats maintained on 1% saline are hypotensive (Gardiner & Milmer, 1980). The hypotension could be due to a reduction in extracellular fluid volume (Moses, 1965) in which case reflex sympathetic hyperactivity and reduced vagal tone would be expected unless baroreflex mechanisms are reset to the low pressure. In the present study we have assessed the effect of autonomic antagonists on resting BP and heart rate (HR) and the influence of BP on pulse-interval in rats adrenalectomized 7 days previously. In addition we have studied the effects of the opiate antagonist, naloxone, on BP and HR in adrenalectomized rats since others (Guillemin et al, 1977) have shown that adrenalectomy raises the level of circulating β-endorphins; these are powerful depressor agents which might contribute to the hypotension.

Male Wistar rats were either bilaterally adrenalectomized (n=8) or sham-operated (n=7) and given 1% saline instead of tap-water to drink. Seven days after operation, rats were anaesthetized with sodium methohexitone (Brietal, Lilly; 60 mg/kg i.p.) and catheters implanted in the abdominal aorta for BP recording and in the right jugular vein for drug administrations; HR was derived from the BP recording. The animals were allowed at least 5 h to recover from anaesthesia. BP and HR were then measured for 30 min at rest and for a further 30 min after administration of naloxone (2 mg/kg). One h later, baroreflex sensitivity was assessed by regression analysis of the line relating systolic BP to the pulse-interval of the succeeding beat during an increase in pressure induced by methoxamine (0.4 mg/ml; 0.2 ml/min for 15 s). Atropine (1 mg/kg) propranolol (1 mg/kg) and phenoxybenzamine (0.2 mg/kg) were then given at 20 min intervals.

At rest, BP was significantly ($P<0.001$) lower in adrenalectomized rats (137 ± 5/82 ± 4 mmHg; systolic/diastolic; mean ±s.e. of mean) than in sham-operated rats (166 ± 3/105 ± 3 mmHg) while HR was significantly ($P<0.001$) higher (adrenalectomized = 433 ± 10 beats/min; sham-operated = 380 ± 5 beats/min).

Naloxone did not affect BP in either sham-operated or adrenalectomized rats. There was no significant difference between the baroreflex sensitivity in adrenalectomized rats ($y = 1.40x-66$) compared to sham-operated rats ($y = 1.38x-74$). Adrenalectomized rats showed a smaller HR response to atropine (+12 ± 1 beats/min) than sham-operated rats (+75 ± 4 beats/min) and a larger bradycardia with propranolol (adrenalectomized = -111 ± 7 beats/min; sham-operated = -80 ± 7 beats/min). There was a small reduction in BP after administration of propranolol to adrenalectomized but not sham-operated rats and phenoxybenzamine caused a more marked hypotension in adrenalectomized rats (-19 ± 3/-17 ± 2 mmHg) than sham-operated rats (-11 ± 2/-9 ± 3 mmHg).

The results show that circulating β-endorphins do not contribute to the hypotension seen in adrenalectomized rats 7 days after operation. The hypotension is accompanied by a tachycardia which is due to a combination of sympathetic hyperactivity and reduced vagal tone; this may be a reflex response to extracellular fluid volume depletion. Unlike the situation in hypertension, baroreflex mechanisms do not appear to reset after several days of hypotension.

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CHANGES IN CARDIOVASCULAR REACTIVITY DURING THE DEVELOPMENT OF ADRENAL REGENERATION HYPERTENSION IN RATS

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Adrenal regeneration hypertension (ARH) develops in saline-drinking rats following unilateral adrenal enucleation and contralateral adrenalectomy, with (Skelton, 1959) or without (Hall & Hall, 1965) unilateral nephrectomy. The development of high BP is associated with regeneration of the adrenal cortex and raised plasma steroid levels of both mineralocorticoids (e.g. Gallant & Brownie, 1979) and glucocorticoids (e.g. Buckingham & Hodges, 1975). Adrenocortical hypersecretion may cause an increased sensitivity of vascular smooth muscle to vasoconstrictor agents, in a manner analogous to that resulting from exogenous administration of corticosterone or deoxycorticosterone (Schömig et al, 1976; Berecek & Bohr, 1978). We have therefore investigated the possibility that cardiovascular hyperreactivity contributes to the development of ARH.

The systolic BP (tail cuff) of 2-kidney adrenal enucleated (AE) rats was significantly lower (117.2 $\pm$ 2.5mm Hg) than that of sham-operated controls (131.3 $\pm$ 2.4mm Hg) 6 days after surgery ($p<0.001$). After 10 days, systolic BP was not significantly different and by 21 days systolic BP of AE rats (147.5 $\pm$ 3.1mm Hg) was significantly higher ($p<0.001$) than controls (125.8 $\pm$ 2.2mm Hg). Pressor responsiveness to sympathetic nerve stimulation (50V, 1ms, 0.25 - 8.0Hz for 10s) and to i.v. noradrenaline (0.03 - 3.0 μg/kg) and angiotensin II (0.01 - 1.0 μg/kg) administration was studied in the pithed rat preparation (Gillespie & Muir, 1967) 10 days (pre-hypertensive) or 21 days (hypertensive) after surgery. During these investigations animals were artificially respired with oxygen-enriched air, and maintained at 37°C. Each experiment was concluded with blood withdrawal for blood gas and pH analysis. Despite their normal BP at 10 days, cardiovascular reactivity tests revealed a significant attenuation of pressor responsiveness to all frequencies of sympathetic nerve stimulation and to i.v. noradrenaline administration (0.1 - 3.0 μg/kg) in AE rats. Pressor responsiveness to angiotensin II was also reduced, but not significantly. After 21 days, however, cardiovascular responsiveness to all three pressor stimuli was found to be normal. Initial BP and final blood pCO_2, pO_2 and pH values did not differ significantly between pithed preparations from AE or control rats at either stage.

The rise in BP following adrenal enucleation was therefore associated with a recovery of cardiovascular pressor responsiveness from sub-normal to control levels. These findings do not support the hypothesis that increased cardiovascular reactivity contributes to the development of high BP in rats with regenerating adrenals.

K.E.M. is an S.R.C. CASE Research Student in association with ICI Pharmaceuticals Division.

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EXTRASYNAPTIC LOCATION OF VASCULAR α_2- AND β_2-ADRENOCEPTORS IN THE NORMOTENSIVE RAT

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The adrenoceptors involved in the pressor and tachycardic effects of catecholamines applied i.v. or released from sympathetic nerve endings were compared in pithed normotensive rats in order to obtain additional evidence for an extrajunctional location of vascular α_2- and β_2-adrenoceptors as suggested by Yamaguchi & Kopin (1980) and Langer et al (1980, 1981a, b).

Intravenous administration of (-)-noradrenaline and (-)-adrenaline to pithed normotensive rats (200-300 g) gave rise to a dose-dependent increase in diastolic pressure and heart rate. The characterization of the adrenoceptors involved in these effects was achieved with the selective α_1-, α_2-, β_1- and β_2-adrenoceptor antagonists prazosin (0.1 mg/kg), rauwolscine (1 mg/kg), atenolol (1 mg/kg) and ICI 118,551 (0.3 mg/kg), respectively. Accordingly, (-)-adrenaline behaved as a mixed α_1-, α_2-, β_1- and β_2-adrenoceptor agonist, whereas (-)-noradrenaline appeared to activate α_1-, α_2- and β_1-adrenoceptors.

The nicotinic agonist DMPP (1,1-dimethyl-4-phenylpiperazine iodide), which induces a release of catecholamines from the sympathetic neurones and the adrenal medulla, caused an increase in diastolic pressure and heart rate upon i.v. application to pithed rats. These effects were antagonized by reserpine and bilateral adrenalectomy. In the cardiovascular effects of DMPP, α_1- and β_1-adrenoceptors played a major role. At high doses of DMPP only, the participation of α_2-adrenoceptors could be identified. A vascular effect mediated by β_2-adrenoceptors was hardly demonstrable. After removal of both adrenal glands, the source for catecholamines are the sympathetic nerve endings only. Under this condition, DMPP could no longer activate α_2- and β_2-adrenoceptors.

(-)-Noradrenaline released neuronally, stimulated α_1- and β_1-adrenoceptors, but not α_2-adrenoceptors in contrast to the observations made for this catecholamine after i.v. administration (see above). Vascular β_2-adrenoceptors were not triggered by the catecholamines liberated from sympathetic nerve endings. After i.v. administration, (-)-adrenaline, however, activated vascular β_2-adrenoceptors.

The results are explained by the presence of predominantly α_1- and β_1-adrenoceptors in postganglionic sympathetic synapses and α_2- and β_2-adrenoceptors outside the synaptic cleft. The extrasynaptic adrenoceptors are possibly controlled by adrenaline from the adrenals.

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DOPAMINE STIMULATES POSTSYNAPTIC α_2-ADRENOCEPTORS IN THE MESENTERIC BED OF THE DOG

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Dopamine produces vasodilation of the dog mesenteric bed in vivo via postsynaptic dopamine receptors (Yeh et al., 1969). However this vasodilator effect of dopamine is only seen clearly if the preparation is pretreated with an α-adrenoceptor blocking agent, phenoxybenzamine being the most commonly used drug. This is due to the α-adrenoceptor stimulating properties of dopamine which produce a vasoconstrictor response. As we have previously reported the presence of postsynaptic α_2-adrenoceptors on vascular smooth in the anaesthetized dog (Langer et al., 1980), we have investigated the vasoconstrictor response elicited by dopamine to define the α-adrenoceptor subtype involved.

Dogs were anaesthetized with pentobarbitone (35 mg/kg i.v. + 6 mg/kg/h i.v.), and the femoral artery and brachial vein cannulated for the measurement of blood pressure and administration of drugs respectively. A laparotomy was performed, and the superior mesenteric artery located and cleaned. A flow probe (Carolina Medical) was placed around the artery and a needle (26G) placed in the artery to allow intra arterial administration of agonists. All antagonists were given intravenously. The dogs were ganglion and β-adrenoceptor blocked (chlorisondamine 1 mg/kg, atropine 1 mg/kg, propranolol 0.5 mg/kg + 0.25 mg/kg/h). Intra-arterial injections of phenylephrine (0.3-30 µg) and M-7 (a preferential α_2-adrenoceptor agonist, Drew, 1980) (1-100 µg) produced a dose related decrease in mesenteric blood flow. In contrast dopamine (1-300 µg), produced a biphasic response : an initial decrease followed by an increase in mesenteric blood flow at each dose, the magnitude of both responses being proportional to the dose injected. The increase in blood flow produced by dopamine was blocked by (+) butaclamol (300 µg/kg) a dose which had no significant effect on the decrease in blood flow. The decrease in mesenteric blood flow produced by phenylephrine was inhibited by prazosin (30-100 µg/kg), but that produced by dopamine or M-7 was not significantly reduced by 300 µg/kg of prazosin. On the other hand the decrease in blood flow produced by M-7 and dopamine was dose-dependently reduced by yohimbine (30-1000 µg/kg), whilst that produced by phenylephrine was not significantly affected by 100 µg/kg of yohimbine.

These results demonstrate that dopamine, like M-7 stimulates postsynaptic α_2-adrenoceptors in the mesenteric bed of the dog producing vasoconstriction. These receptors can be differentiated pharmacologically from α_1-adrenoceptors which are also present in the mesenteric bed. It is of interest that dopamine stimulates postsynaptic dopamine receptors and postsynaptic α_2-adrenoceptors in vascular smooth muscle over the same dose range. In contrast dopamine is considerably less potent as an agonist at presynaptic α_2-adrenoceptors than at presynaptic dopamine receptors on noradrenergic nerve endings (Langer and Dubocovich, 1979).

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CONTINUOUS INFUSION OF GUANFACINE AND CLONIDINE AND PRECIPITATION OF THEIR WITHDRAWAL SYNDROMES IN THE RAT

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Clonidine is known to evoke a withdrawal syndrome after cessation of prolonged treatment in man and in rats (for reviews see Weber, 1980 and Thoolen et al, 1981). Recently, the clonidine-like antihypertensive drug guanfacine has been shown to elicit considerably fewer and less severe withdrawal signs after cessation of treatment in hypertensive patients than does clonidine (Zamboulis & Reid, 1981).

In the present study, the effects on heart rate and blood pressure of a 12-day continuous subcutaneous infusion of guanfacine (10 mg/kg/day) and clonidine (500 µg/kg/day) and their withdrawal syndromes in the spontaneously hypertensive (SH) and normotensive rat were compared. Withdrawal was effected by either removal of the minipumps or i.p. injection of the α_2-adrenoceptor blocking agent yohimbine (Weitzell et al,1979), at the twelfth day of the infusion period. Blood pressure and heart rate were measured via permanently indwelling abdominal aortic catheters.

At the given infusion rates, clonidine and guanfacine reduced the mean arterial pressure and heart rate of the SH rats to the same extent throughout the infusion period. Following removal of the clonidine-charged minipumps, a marked overshoot of heart rate and a period of blood pressure lability, due to the appearance of transient blood pressure upswings, occurred, together with other withdrawal signs as body shivering, immobility, ptosis and piloerection. The mean arterial pressure returned to the control level within 5 h after cessation of treatment. The discontinuation syndrome lasted from about 3 to 50 h after withdrawal.

The cessation of guanfacine treatment in SH rats gave rise to a minor overshoot of heart rate. A much shorter period of blood pressure lability and less severe as compared to clonidine withdrawal was observed. The guanfacine withdrawal signs appeared considerably later (12-14 h) after pump removal than after suspension of clonidine treatment. Following discontinuation of the guanfacine infusion, the mean arterial pressure had not returned to control value until after 70-80 h.

In the normotensive rats, clonidine or guanfacine did not reduce the mean arterial pressure during the infusion. Cessation of treatment resulted in similar withdrawal patterns as observed in the SH rats.

At the twelfth day of the infusion period of clonidine and guanfacine in the SH rat, yohimbine (1,3 and 10 mg/kg, i.p.) dose-dependently precipitated withdrawal signs as mentioned above. The severity of the symptoms was similar in the guanfacine- and the clonidine-treated group. At the doses mentioned yohimbine did not elicit such signs in saline-treated control SH rats. The overshoot of heart rate and the appearance of behavioural changes as body shakes, shivering, jumping and wet dog shakes were more pronounced after the yohimbine challenge (10 mg/kg, i.p.) than after removal of the minipumps.

The present findings suggest, that the longer half-life of guanfacine compared to clonidine is a major factor responsible for the difference in severity of the withdrawal symptoms after cessation of treatment with the two drugs in the rat.

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INTRINSIC SYMPATHOMIMETIC ACTIVITY (ISA) OF β-ADRENOCEPTOR BLOCKING AGENTS: A MYOFIBRILLAR PHENOMENON?

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Certain beta adrenoceptor blocking agents exert positive inotropic and chronotropic effects on the myocardium at concentrations lower than those which suppress contractility. The nature and mechanism of this cardiostimulant action, known as "intrinsic sympathomimetic activity" (ISA), are not yet completely understood. In some cases, ISA has been attributed to a partial agonism at beta adrenoceptors as it can be at least partly inhibited by (±) propranolol. Nevertheless, there remains a propranolol insensitive fraction of ISA which cannot be explained in terms of beta adrenoceptor stimulation. Furthermore, the ISA of sotalol and INPEA appears to be totally insensitive to (±) propranolol and is therefore assumed not to be mediated by beta adrenoceptors (cf. Kaumann & Blinks, 1980). Sympathetic excitation of the myocardial cell is widely accepted to be mediated by an increase in cyclic AMP and a concomitant enhancement of Ca^{++} influx during the plateau phase of the action potential. In view of this, we investigated whether ISA, in terms of a positive inotropic effect, requires a functioning sarcolemma including beta adrenoceptors or whether a direct stimulant effect on the intracellular contractile protein system is involved.

Thin (< 0.3 mm) strips were dissected from hog right ventricular trabeculae and then extracted with detergents and glycerol according to Herzig & Herzig (1974). By this "skinning" technique, the myocardial cells are deprived of the membranous systems of sarcolemma, sarcoplasmic reticulum and mitochondria, the myofibrils remaining perfectly contractile. Preliminary binding studies with the radioligand ^{125}iodocyanopindolol (^{125}ICYP) (cf. Engel, 1981) indicate that beta adrenoceptors are quantitatively extracted from our preparation. As diffusion barriers are eliminated, the functionally isolated contractile structures are activated in a buffer containing MgATP as energy source. With variation of the free Ca^{++}ion concentration in this "intracellular" medium (10^{-8} - 10^{-5} M Ca^{++}), graded isometric contractions were elicited, the EC_{50} being close to 10^{-6} M Ca^{++}. Application of DCI (dichloroisoproterenol), pindolol (Visken ^{R}) or sotalol at buffered Ca^{++} concentrations of 10^{-8} - 10^{-5} M induced an increase in isometric force development of up to 30 %. The EC_{50} values for Ca^{++} activation were reduced. Relaxation at 10^{-8} M Ca^{++} was not affected. Each of the drugs showed maximum activity at concentrations near 10^{-6} M, the effect being smaller at both lower and higher drug concentrations. This is very close to results obtained in intact myocardial preparations by Kaumann & Blinks (1980). Application of (±) propranolol in concentrations of up to 10^{-4} M did not inhibit the activation of the "skinned" preparations by the drugs mentioned above.

It is concluded from our data that 1) at least part of the ISA is independent of binding to beta adrenoceptors (ISA is also observed in the absence of a functioning sarcolemma and in presence of (±) propranolol) and may 2) be due to an increase in the Ca^{++} sensitivity of the contractile structures rather than an enhancement of Ca^{++} influx.

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EFFECTS OF HYDRALAZINE AND ITS HYDRAZONES ON THE RAT AORTIC STRIP

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Hydralazine (H) is a vasodilator with a short plasma half-life but prolonged action. Hydralazine hydrazones have been implicated in the hypotensive activity of H (Barron et al., 1977; Haegele et al., 1978). It is uncertain whether the hydrazones have intrinsic activity or act through reconversion to H. The relaxant action of H and of hydralazine acetone hydrazone (acetonide), of hydralazine pyruvate hydrazone and hydralazine α-ketoglutarate hydrazone on the rat aortic strip was investigated in this study.

Rat aortic spiral strips were suspended under 1g resting tension in Krebs-Henseleit solution (KHS) at 37°C, aerated with 5% CO_2 in O_2. After a 60 min equilibration period, sustained contracture was produced by replacing the KHS with a 30 mM K^+ modified KHS (Na^+ - K^+ substitution). Individual test compounds were then added cumulatively to the bath and the tension was measured isometrically. After each response, aliquots of the bath fluid were removed and analysed for each compound by HPLC.

H and acetonide caused dose-dependent relaxation of the aortic strip (Figure 1). The effect of acetonide could not be attributed to H as there was only 15% re-conversion to H. Unlike H, the effect of acetonide appeared to be only partially reversible. The pyruvate hydrazone produced only 8% relaxation at the highest concentration (5.9×10^{-4}M). The α-ketoglutarate hydrazone was inactive. Decomposition of the pyruvate and α-ketoglutarate hydrazones to H in KHS was less than 2% in 1h.

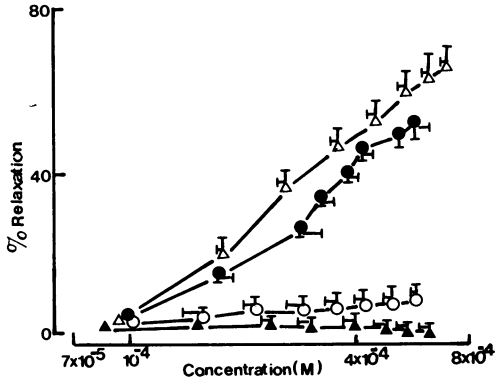

Figure 1. Dose-response curves to H (△), acetonide (●), pyruvate hydrazone (○) & α-ketoglutarate hydrazone (▲). (Mean ± s.e., n=4).

These results differ from those obtained with the rabbit aortic strip (Barron et al., 1977; Haegele et al., 1978) in which all three hydrazones were reported to be active. Since this may represent a species difference in the response to hydralazine hydrazones, comparison of their hypotensive activity in man will require the use of a human arterial preparation.

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COMPARISON OF THE ACTION OF HYDRALAZINE AND D600 ON THE MAMMALIAN HEART

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The results of recent experiments in blood vessels (McLean et al, 1978; Warburton & Weston, unpublished) suggest that some of the inhibitory actions of hydralazine in these tissues result from the blockade of calcium influx. In an attempt to clarify the effects of hydralazine in vascular tissue, its actions on the slow calcium channel of mammalian heart have been studied. The operation of this channel is relatively well understood and there seem to be similarities between the cardiac slow calcium channel and a calcium channel present in vascular muscle. D600 was used as a reference calcium antagonist.

Papillary muscles were dissected from the right ventricles of rats, guinea-pigs and rabbits and bathed at 37°C in a physiological salt solution (PSS) containing atenolol 10^{-5}M. During a 2h equilibration period the preparations were stimulated (0.5Hz, 10V, 2ms) and the resulting tension changes recorded isometrically. When the PSS K^+ concentration was increased from 6mM to 20 mM and the stimulus strength from 10V to 20V (Mascher, 1970), slow, calcium-dependent action potentials and accompanying mechanical changes were produced.

D600 (10^{-7} - 10^{-4}M) inhibited mechanical responses in the rat, guinea-pig and rabbit partially depolarised papillary muscles. There were no significant differences between the D600 log EC 50's in the three types of papillary muscle and the mean maximum inhibition observed was approximately 75%. In contrast the effects of hydralazine were species specific. In the guinea-pig hydralazine (10^{-5} - 10^{-3}M) produced an increase in tension development of 81±6% (mean±s.e.mean, n=6) which was almost abolished by cimetidine $3X10^{-5}$M. This finding supports the observations of Gershwin & Smith (1967) who showed that hydralazine was capable of releasing histamine from guinea-pig hearts. In the rabbit hydralazine (10^{-5} - $4X10^{-3}$M) reduced mechanical responses by 12±4% (mean±s.e.mean, n=6) whilst in the rat, hydralazine (10^{-5} - $4X10^{-3}$M) produced a reduction in tension development of 53±8% (mean±s.e.mean, n=6). The hydralazine:D600 log potency ratio in the rat was different from that in the rabbit ($P<0.001$). The inhibitory actions of hydralazine and of D600 in rat papillary muscle were reversed by increasing the PSS Ca^{2+} concentration to 12.5mM and enhanced by decreasing the Ca^{2+} concentration to 0.5mM.

The ability of hydralazine to inhibit the slow calcium channel in rat and rabbit papillary muscle is consistent with those observations in vascular muscle which show that hydralazine is capable of blocking calcium influx. However, the difference between the hydralazine:D600 log potency ratios in these tissues suggests that the two drugs do not share the same mechanism of action. Preliminary electro-physiological experiments have shown that there are marked differences in the shapes of rat and rabbit papillary muscle action potentials and the effects of hydralazine and of D600 on these is currently being studied.

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NORADRENALINE METABOLISM IN THE RABBIT PERFUSED HEART

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There have been many studies concerning the metabolism of noradrenaline released from the sympathetic transmitter stores of isolated tissues. Most of these studies have examined the metabolic fate of radiolabelled noradrenaline after its uptake into the transmitter stores (see review by Langer, 1974). The aim of the present study was to examine the efflux of endogenous noradrenaline (NOR) and its metabolites, 3,4-dihydroxyphenylglycol (DOPEG) and 3,4-dihydroxymandelic acid (DOMA), from the rabbit perfused isolated heart. The compounds were separated by alumina adsorbtion and HPLC and quantified by electrochemical detection.

The venous effluent from 6 hearts was collected over a 90 min period and contained 186 + 20 pmol/min per g tissue of DOPEG, whilst the efflux of DOMA and NOR were below the limits of detection (12 and 13 pmol/min per g respectively). The absence of substantial amounts of DOMA was surprising in view of its presence in the spontaneous efflux from rabbit hearts previously perfused with (^{3}H)-noradrenaline (Steppeler et al., 1980).

To examine the effects of noradrenaline perfusion on the efflux of DOMA, hearts were perfused with noradrenaline in concentrations of either 0.1, 1.0 or 10 μM for a period of 18 min and the spontaneous efflux of compounds was measured over the subsequent 90 min. As the concentration of noradrenaline in perfusion medium was increased so did the efflux of DOPEG, reaching 7255 + 743 pmol/min per g (n=4) for the 10 μM perfusion. DOMA was also present after the 1.0 and 10 μM noradrenaline perfusions, its spontaneous efflux being 1535 + 225 pmol/min per g (n=4) after the 10 μM perfusion. These results suggest that the efflux of DOMA is a consequence of exposure of the tissue to exogenous noradrenaline. The noradrenaline-evoked efflux of both DOPEG and DOMA were virtually abolished if cocaine (10 μM) was present during the perfusion with noradrenaline, indicating an intraneuronal site of formation for these metabolites.

Further experiments were carried out to determine whether DOMA may in some circumstances be formed from endogenous noradrenaline. The efflux evoked by tyramine (10 μM) consisted mainly of DOPEG with smaller amounts of NOR also being present. DOMA was not detected. Similarly, after the release of noradrenaline by nicotine (30 μM) or by nerve stimulation (5 Hz for 30 min) no DOMA was detected in the perfusion medium.

These results suggest that DOMA is at best only a minor metabolite of noradrenaline formed only when the intraneuronal cytoplasmic concentrations of noradrenaline are very high such as during exposure to exogenous noradrenaline.

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CAN ATP ACTIVATE P1 PURINE RECEPTORS IN THE GUINEA-PIG ATRIUM?

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Purine nucleosides and nucleotides exert negative inotropic and chronotropic effects on the mammalian heart (Burnstock,1980). In the guinea pig atrium, adenosine is a more potent negative inotrope than A.T.P. and theophylline is a competitive antagonist of the effects of both these agonists (Bilski,et.al., 1981, Collis et.al. 1981). The purine receptor mediating the negative inotropic response can therefore be classified as P1 (Burnstock, 1978). It has been proposed that in some tissues A.T.P. must be hydrolysed to adenosine in order to activate the P1 receptor (Christie and Satchell,1980). The purpose of the present study was to examine this possibility in the guinea pig atrium.

Guinea pig left atria were suspended in organ baths containing oxygenated Krebs solution (37°C). The atria were mounted on punctate electrodes and attached to isometric force transducers. They were electrically stimulated (4Hz, 3msec, threshold voltage, 0.5 - 3V), and continuously washed with Krebs solution. Electrical stimulation did not activate adrenergic or cholinergic nerve endings in the atria as atenolol (10^{-6}M, n=4) and atropine (10^{-6}M, n=6) had no effect on the amplitude of contraction.

Adenosine deaminase (1u/ml) abolished the negative inotropic response to adenosine (10^{-6}-10^{-3}M, n=6), however responses evoked by A.T.P. (10^{-6}-10^{-3}M, n=6) were not altered. An inhibitor of 5′ nucleotidase (α,β -methylene A.D.P. 10^{-5}M) also did not alter responses evoked by A.T.P. (n=4). Responses evoked by 5′ A.M.P. were abolished by adenosine deaminase (1u/ml) and 5′ nucleotidase (0.5u/ml), however this mixture of enzymes did not modify the response to A.T.P.(n=6).

The stable α, β-methylene isosteres of A.T.P. (n=6) and A.D.P. (n=4) were not active as negative inotropic agents on the atrium. However, β, γ-methylene A.T.P. (n=7) was slightly but significantly ($P<0.05$) more potent ($pD_2 = 4.7 \pm 0.22$) than A.T.P. ($pD_2 = 4.1 \pm 0.16$, n=11). Hydrolysis of β, γ-methylene A.T.P. to A.M.P. did not occur since adenosine deaminase (1u/ml) and 5′ nucleotidase (0.5u/ml) did not alter the response (n=4).

These results indicate that A.T.P. can act directly at the P1 receptor, without requiring hydrolysis to adenosine. The low potency of the α, β-methylene isosteres of A.T.P. and A.D.P. cannot be due to their resistance to hydrolysis as β, γ-methylene A.T.P. is active at the P1 receptor. Consequently, the structural conformation of the polyphosphate chain of A.T.P. and its methylene isosteres must be important in determining agonist activity at P1 purine receptors.

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