EFFECTS OF PUTATIVE ANION TRANSPORT INHIBITORS ON THE RELEASE OF 5-HYDROXYTRYPTAMINE INDUCED BY LOW CHLORIDE SUPERFUSION

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In recent experiments employing a superfused slice preparation (Boakes, Turner and Virmani, 1984) we have shown that the convulsants picrotoxin and strychnine significantly attenuate low chloride (low /$\bar{\text{Cl}}$/)-induced release of 5-HT from slices of rat cortex. These observations suggest that the release of 5-HT elicited in low /$\bar{\text{Cl}}$/ medium may be caused by movement of Cl ions through membrane Cl^- channels, some of which are receptor-linked. In further experiments we have now examined the effects on low /$\bar{\text{Cl}}$/ release of 5-HT of frusemide and 4-acetamido-4-isothiocyanostilbene 2, 2-disulphonic acid (SITS), agents which interfere with chloride transport mechanisms.

Eight slice fragments, obtained from rat parietal cortex were preloaded with tritiated 5-HT, and superfused at 0.5ml min^{-1} with Krebs bicarbonate medium containing 0.1mM pargyline at 37°C. After equilibration, the superfusate was collected as 2 min fractions and the fractional rate coefficient (FRC, Bowery et al, 1976) was calculated from the radioactivity. Low /$\bar{\text{Cl}}$/ values (3mM in all experiments) were obtained by substituting propionate for Cl in the superfusing medium. Drugs, dissolved in Krebs solution were introduced into the superfusing medium 2-30 min before a 10 min pulse of low /$\bar{\text{Cl}}$/ medium was delivered. In each experiment, 4 slice fragments were exposed to drug and low-/$\bar{\text{Cl}}$/ medium and 4 control fragments were exposed only to the low /$\bar{\text{Cl}}$/ medium pulse.

Frusemide antagonises the passive cotransport of Cl^- with Na^+ and K^+. In our experiments, frusemide (10mM and 5mM), in a dose-dependent manner, facilitated the rising phase of low /$\bar{\text{Cl}}$/ induced 5-HT release, depressed the peak FRC measured and delayed the return to basal release after the low /$\bar{\text{Cl}}$/ pulse. Both concentrations of frusemide also increased the basal release measured in normal /$\bar{\text{Cl}}$/ medium. These effects do not resemble the effects of picrotoxin and strychnine, which depress significantly both rising phase and peak FRC measured in low /$\bar{\text{Cl}}$/ medium but do not affect basal release. The proposed Cl^- channel blocker SITS affected low /$\bar{\text{Cl}}$/ induced release of 5-HT in a manner similar to the convulsants. For example, 1mM SITS delayed the appearance of label in the superfusate and the peak FRC measured in response to the low /$\bar{\text{Cl}}$/ pulse. Like picrotoxin and strychnine, this compound had no effect on basal 5-HT release.

In conclusion, the anion transport inhibitors frusemide and SITS have different effects on both basal and low /$\bar{\text{Cl}}$/-induced release of 5-HT. However, the mechanisms underlying these effects in this preparation are not fully understood.

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EFFECTS OF SUBSTITUTED TETRAHYDRO-β-CARBOLINES ON 5-HT UPTAKE AND RELEASE IN RAT HYPOTHALAMIC SLICES

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The selective inhibitor of 5HT uptake, citalopram, does not affect per se the electrically-evoked overflow of ^{3}H-5HT in hypothalamic slices from normal rats (Langer and Moret, 1982). However, in rats pretreated with para-chlorophenylalanine (PCPA) exposure to citalopram increased in a concentration-dependent manner the electrically-evoked overflow of ^{3}H-5HT (Galzin et al., 1983). Substituted tetrahydro-β-carbolines (THBC) are possible candidates as endogenous ligands of the 5HT uptake modulatory site labelled with ^{3}H-imipramine (Langer et al, 1984).The present experiments were carried out in order to determine the effects of substituted THBC's on ^{3}H-5HT uptake and release in hypothalamic slices from normal and PCPA treated rats.

Uptake experiments were performed in rat hypothalamic slices preincubated at 37°C (or 0°C for corresponding blanks) for 20 min in the presence of various concentrations of the drug. ^{3}H-5HT (50 nM final concentration) was added to the medium for 5 min and the uptake was terminated by transferring the slices in a large volume of cold Krebs' solution. The IC_{50} values on ^{3}H-5HT uptake were calculated by computer analysis. For transmitter release experiments rat hypothalamic slices were labelled with ^{3}H-5HT and superfused with Krebs' solution. Two periods (S_1 or S_2) of electrical stimulation were applied with an interval of 44min and drugs were added 20min before S_2. The parameters of stimulation were 3 Hz, 20 mA, 2msec for 2 min.

On the inhibition of ^{3}H-5HT uptake in normal rat hypothalamic slices, 6-OH-THBC (IC_{50}=70 nM) was more potent than 6-OCH_3-THBC (IC_{50}=600 nM) and THBC (IC_{50}=900 nM). Under the same experimental conditions, citalopram was a very potent inhibitor (IC_{50} =10 nM) while imipramine had an IC_{50} close to 150 nM. In normal rat hypothalamic slices, the electrically-evoked ^{3}H-5HT release was S_1 = 2.00 ± 0.08% (n=6). 6-OH-THBC (0.1 - 10 µM) did not affect per se either the stimulation-evoked overflow of ^{3}H-5HT, or the spontaneous outflow of radioactivity. The concentration-effect curve for THBC showed a clear biphasic effect since exposure to 1 µM THBC inhibited the electrically-evoked overflow of ^{3}H-5HT (S_2/S_1 = 0.51±0.04, n = 9 for THBC 1 µM, $p<0.01$ when compared to the control value S_2/S_1=1.20±0.12 n=6), while at higher concentrations THBC did not affect ^{3}H-5HT release. Moreover, THBC (0.1-10µM) significantly inhibited the spontaneous outflow of radioactivity. Under the same experimental conditions, 6-OCH_3-THBC (0.1-10µM) did not affect per se the electrically-evoked release of ^{3}H-5HT, but significantly decreased the spontaneous outflow of radioactivity. These results are probably related to the MAO inhibiting properties of THBC. Similar experiments were performed in PCPA-treated rats (300 mg/kg PCPA i.p. 48h before the experiment). It was previously reported that under these conditions, the endogenous levels of 5HT in the hypothalamus was decreased by 90% (Galzin et al, 1983). Exposure to substituted THBC's increased in a concentration dependent manner the stimulation-evoked release of ^{3}H-5HT with the same order of potency as shown for these compounds at inhibiting ^{3}H-5HT uptake (6-OH-THBC > 6-OCH_3-THBC > THBC).

These results suggest that PCPA pretreatment may induce changes in the sensitivity of the 5HT uptake system in the hypothalamus. We cannot exclude the possibility that PCPA pretreatment may affect the endogenous modulator of the 5HT transporter which appears to act through the ^{3}H-imipramine recognition site (Langer and Raisman, 1983 ; Barbaccia et al., 1983 ; Langer et al.,1984).

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EFFECTS OF FLUOXETINE ON IMIPRAMINE-BINDING IN VIVO AND IN VITRO

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In patients suffering from affective disorders both platelet uptake of 5-hydroxy-tryptamine (5-HT; Coppen et al, 1978) and imipramine-binding to platelet membranes (Briley et al, 1980) are low compared to controls. That these observations might reflect a central deficiency was suggested by the decreased imipramine-binding to cortical membranes from patients who suffered from depressive illness (Perry et al., 1983). Together, the data contribute to the notion of 5-HT mismanagement in these patients. Whether the platelet deficit is a state or trait phenomonen would, if resolved, assist in treatment or diagnosis.

Previously (Perry, 1983) we determined that circulating tricyclics did not interfere with the measurement of imipramine-binding. In this study, on the effects on patients suffering from depression, of fluoxetine, a non-tricyclic known to be a 5-HT uptake blocker, we measured platelet imipramine-binding at the beginning of the trial, after one week on placebo and at weekly intervals during daily treatment with the drug (60mg). The values obtained for imipramine-binding are:

Pretreatment 1	Pretreatment 2	Week 1	Week 2	Week 3
B_{max}	(% Pretreatment 1)			
(fmoles/mg Prot)				
427 ± 238(25)	90 ± 53(28)	42 ± 34(23)	38 ± 33(19)	18 ± 12(18)

Values are mean ± S.D. N = numbers in brackets.

Clearly there is a decrease in imipramine-binding with continued treatment. In previous studies on patients in whom platelet 5-HT uptake or imipramine-binding to platelet membranes has been measured, the values have either increased during treatment (Suranyi-Cadotte et al, 1982) or remained the same (Coppen et al, 1978). In none, have the values continued to decrease.

In order to investigate further the effects of fluoxetine we administered chronic (21 days) daily doses of fluoxetine (10mg/kg ip) to rats and found a greater than 50% loss in binding to cortical membranes. This is in contrast to the lack of effect observed after chronic administration of imipramine (Perry et al, 1983). Also, when samples of blood were preincubated with the IC_{50} for fluoxetine (30nM) and platelet membranes prepared, the imipramine-binding was decreased to less than 40% of the controls, independent of how many times the platelet membranes were washed. Given that the initial dose of fluoxetine should only have caused a loss of 50% binding, the actual effect of fluoxetine appears to be irreversible. It appears that fluoxetine has an effect which is not simply an interference with imipramine-binding.

The relationship between 5-HT uptake and changes in mood has, therefore, not been immediately possible to determine. However, these studies are continuing and imipramine-binding is being assessed in patients whose treatment with fluoxetine has been continued and in those whose treatment has been changed.

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[^{3}H]-IMIPRAMINE BINDING IN HUMAN BLOOD PLATELETS : CHANGES AFTER CHRONIC CHLORIMIPRAMINE TREATMENT

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The presence of high affinity binding sites for ^{3}H-imipramine (IMI) in brain and platelets is by now well established (Langer et al. 1981). This site is associated with the neuronal uptake for serotonin (5HT) and may be a biological marker in depression. In several studies, a significant decrease in Bmax was reported in platelets of untreated severely depressed patients when compared with control volunteers (Raisman et al. 1981, Paul et al. 1981, Asarch et al. 1981) while one group reported a small increase in Bmax in platelets of depressed manic melancholic patients (Mellerup et al.1982). This discrepancy may be due to differences in the time of washout from antidepressant medication. Therefore, it was thought of interest to study the changes in ^{3}H-IMI binding in platelets of normal volunteers during and after the administration of chlorimipramine(CMI). ^{3}H-5HT uptake was also measured in the same subjects as well as CMI concentrations in plasma.

Six volunteers (four male and two female,29 to 51 years old) in good health and giving informed consent, took CMI orally at 9.00 a.m. and 4.00 p.m. (total dose 50mg a day) for one week (days 1 to 7). Blood was taken at 9.00 a.m. on days 1 (baseline), 8, 15, 22, 29 and 36. The experimental procedures are described elsewhere (Poirier et al., 1984).

At D1, the parameters for ^{3}H-5HT uptake were the following : Km = 0.56 $\pm$ 0.09 μM (n=4) ; Vmax = 22.9 $\pm$ 5.7 pmoles/10^8 platelets/min (n=4). The dose of CMI administered was effective at inhibiting ^{3}H-5HT-uptake in blood platelets since at D8, the uptake of ^{3}H-5HT was completely blocked (Km not measurable ; Vmax < 2 pmoles/10^8 platelets/min). After one week washout, Vmax values of ^{3}H-5HT uptake were close to the controls and the kinetic parameters were fully recovered after two weeks of washout. Measurements of plasmatic concentrations of CMI and desmethyl-CMI were clearly correlated with the 5HT uptake inhibition observed in platelets .

In ^{3}H-IMI binding studies, the K_D values were found to be significantly higher at D8 (Kd = 0.61 $\pm$ 0.01 nM, n=5 at J1 ; Kd = 3.53 $\pm$ 0.99 nM, n=5 at D8 $p<0.05$), returning to control baseline values after one week of washout (Kd = 0.73 $\pm$ 0.08 nM, n=4 at J15). The Bmax of ^{3}H-IMI binding was decreased at J8, and remained significantly lower than control values after one week washout, while K_D values had already returned to baseline levels (Bmax = 1450.8 $\pm$ 171.3 fmoles/mg prot at D1, n=5, $p<0.05$ when compared with Bmax = 593.7 $\pm$ 154.9 fmoles/mg prot, n=5 at D8 and Bmax = 624.1 $\pm$ 209.7 fmoles/mg prot, n=4 at D15). The results clearly indicate that the Bmax values of ^{3}H-IMI binding are still affected by the CMI treatment after three weeks of washout.

It is concluded that previously reported variations of ^{3}H-IMI binding in depressed patients should be interpreted with caution because the duration of the washout period and the dose of antidepressant used in therapy could have influenced these determinations. It is likely that in some of the studies the differences observed between depressed and control populations may be due to a residual effect of previous antidepressant treatment because of a rather short washout period.

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INHIBITORY EFFECT OF DIHYDROERGOCRISTINE ON 5-HT RELEASE FROM RAT HYPOTHALAMIC SLICES

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Dihydroergocristine (DHEC) and other ergot derivatives have been shown to have multiple biochemical and pharmacological effects in the central nervous system and to be therapeutically active in brain dysfunction related to aging.

Three monoamine neurotransmitter systems appear to be implicated in the action of DHEC. Binding studies show that this drug interacts directly at the $\alpha 1$, $\alpha 2$, dopamine D_2 and $5HT_2$ receptors (Briley and Charveron, unpublished results). In addition, DHEC has been shown to increase acetylcholine release (Markstein, 1983). 5HT autoreceptors (Göthert, 1982), $\alpha 2$-adrenoceptors (Göthert and Huth, 1980) and cholinergic muscarinic receptors (Ennis and Cox, 1982) present on serotonergic nerve terminals modulate the release of serotonin from rat brain slices. The aim of the present study was to evaluate the effect of DHEC on 5HT release.

Experiments were carried out in 0.4 mm thick slices of the rat hypothalamus prelabelled with ^{3}H-5HT. Each slice was stimulated twice at 3 Hz for 2 min. The overflow of ^{3}H-5HT elicited electrically was calcium dependent. In the controls the percentage of total tissue radioactivity released was 2.28 $\pm$ 0.21, n = 11 (mean $\pm$ s.e.m.) during the first period of electrical stimulation (S1). The ratio between the two consecutive periods of electrical stimulation, S2/S1, was 1.07 $\pm$ 0.10, n = 11. Exposure to DHEC before S2 reduced significantly the stimulation-evoked overflow of the tritiated transmitter in a concentration-dependent manner (S2/S1 = 0.93 $\pm$ 0.14, n = 7, at 0.01 μM ; S2/S1 = 0.65 $\pm$ 0.08, at 0.1 μM, n = 6, $p < 0.05$; S2/S1 = 0.52 $\pm$ 0.07 at 1 μM, n = 6, $p < 0.005$). At these concentrations, the basal outflow of radioactivity was not modified. At 10 μM, the spontaneous release of ^{3}H-5HT was increased by more than 100%.

In the presence of phentolamine (0.1 or 1 μM) from the beginning of the superfusion, the effect of DHEC remained unchanged. The inhibitory effect of DHEC on electrically evoked release of ^{3}H-5HT was also unchanged in the presence of sulpiride 0.1 or 1 μM. The addition of atropine 1 μM or methiothepin 0.1 μM did not modify the DHEC-induced inhibition of ^{3}H-5HT overflow. At 1 μM, however, methiothepin antagonized the inhibition induced by 0.1 μM (S2/S1 = 1.01 $\pm$ 0.05, n = 5, $p < 0.005$ compared to DHEC alone), and by 1 μM DHEC (S2/S1 = 0.80 $\pm$ 0.07, n = 7, $p < 0.05$ compared to DHEC alone).

Thus $\alpha 2$-adrenoceptors, present on 5HT nerve terminals (Göthert and Huth, 1980), appear not to be implicated in the inhibitory effect of DHEC. In addition, the dopaminergic and cholinergic activities of DHEC do not seem to be responsible for its effect on the release of 5HT. Methiothepin (0.1 μM) is sufficient to shift the concentration-effect curve of LSD on the evoked release of ^{3}H-5HT to the right by a factor of 10 (Langer and Moret, 1982). However, 1 μM methiothepin was required to shift the DHEC concentration-effect curve to the same extent. At 1 μM, however, methiothepin provokes a 300% increase in ^{3}H-5HT release (Langer and Moret, 1982) suggesting that the apparent antagonism may be simply a summation of the positive and negative effects on release. Thus while a stimulation of the 5HT autoreceptor by DHEC cannot be excluded this may represent an oversimplification.

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EFFECTS OF DIAZEPAM ON THE CONCENTRATION AND BIOSYNTHESIS OF 5-HYDROXYTRYPTAMINE IN RAT BRAIN

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Previous studies in this and other laboratories have suggested that drugs which relieve the anxiogenic effects of aversive stimuli may exert their effects in part by inhibiting the secretion of 5-hydroxytryptamine (5-HT) in the hippocampus (Balfour 1982, Gray 1981). In this study the effects of diazepam (DZ) administration and its withdrawal on the concentration and the biosynthesis of 5-HT in rat hippocampus have been examined and compared with its effects in hypothalamus and cerebral cortex.

Male Sprague Dawley rats were treated intragastrically with DZ (5 or 25 mg/kg) or vehicle (40 percent (v/v) propylene glycol in water) either acutely or chronically for 40 days. One hour after the final injection the rats were killed by cervical dislocation, the brains rapidly removed and the hippocampus, hypothalamus and cerebral cortex dissected out (Glowinski & Iversen, 1966). The tissues were analysed for 5-HT or 5-hydroxyindole acetic acid (5-HIAA) using the method of Reinhard et al (1980) or were homogenised in ice-cold 0.32M sucrose and the homogenate used for the measurement of synaptosomal 5-HT biosynthesis (Benwell & Balfour 1982).

The acute administration of DZ (5 mg and 25 mg/kg) had no significant effects on the concentrations of 5-HT and 5-HIAA in any of the brain regions examined. However, 5-HT biosynthesis in hippocampal synaptosomes was significantly reduced ($p < 0.05$) from 0.313 ± 0.038 pmoles/mg protein/min ($n = 6$) to 0.194 ± 0.023 pmoles/mg protein/min ($n = 6$) following acute treatment with 25 mg/kg DZ. Chronic treatment with DZ (25 mg/kg) reduced the concentration of 5-HT ($p < 0.05$) from 0.172 ± 0.013 $\mu g/g$ ($n = 8$) to 0.133 ± 0.016 $\mu g/g$ ($n = 6$) in cerebral cortex. Hippocampal 5-HIAA was increased ($p < 0.05$) from 0.358 ± 0.034 $\mu g/g$ ($n = 8$) to 0.423 ± 0.027 $\mu g/g$ ($n = 8$) by chronic DZ (25 mg/kg). Hypothalamic 5-HT and 5-HIAA were not significantly altered by chronic DZ whereas withdrawal of DZ (25 mg/kg) for 24 hours following chronic treatment for 39 days reduced hypothalamic 5-HT ($p < 0.01$) from 0.493 ± 0.39 $\mu g/g$ ($n = 8$) to 0.324 ± 0.034 $\mu g/g$ ($n = 8$). No other significant changes were observed following DZ withdrawal. Neither chronic DZ (25 mg/kg) administration nor its withdrawal had significant effects on 5-HT biosynthesis in synaptosomes prepared from any of the brain regions studied although a decrease observed in synaptosomes from cerebral cortex of rats treated chronically with 25 mg/kg DZ approached statistical significance.

The investigation suggests that DZ can reduce 5-HT biosynthesis in the hippocampus but that this effect only occurs following acute administration whereas its effects on the concentration of 5-HT in cerebral cortex and hypothalamus were only observed following chronic treatment or withdrawal of the drug.

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SELECTIVE ACTIVATION OF THE MONOAMINE OXIDASE INHIBITING PRODRUG, MDL 72394 BY AADC OF CENTRAL MONOAMINE NEURONS

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MDL 72394, (E-β-fluoromethylene-m-tyrosine) is an inert bioprecursor which is decarboxylated by aromatic L-aminoacid decarboxylase (AADC) to yield the potent enzyme-activated irreversible inhibitor of monoamine oxidase (MAO), E-β-fluoromethylene-m-tyramine (Palfreyman et al. 1983). The predominant distribution of AADC in monoamine nerves of the brain suggested that MDL 72394 might inhibit selectively interneuronal MAO.

In the first experiment, rat brain synaptosomes and mitochondria (Gardner & Richards, 1981) were incubated with 5 µM MDL 72394 at 37°C and produced a 66±7 % inhibition of synaptosomal MAO (measured with [^{14}C] 5HT as substrate) compared with only 35±6 % inhibition of the mitochondrial fraction. In the presence of 10 µM monofluoromethyldopa (MFMD) to inhibit irreversibly AADC (Jung et al, 1979) the extent of MAO inhibition produced by MDL 72394 in the synaptosomes was reduced to 30±8 % ($p < 0.01$ versus MDL 72394 alone).

To demonstrate neuronal selectivity *in vivo* male Sprague Dawley rats were unilaterally lesioned with 6-hydroxydopamine (8 µg in 2 µl 30 min after 25 mg/kg i.p. desipramine) in the median forebrain bundle (MFB) at the level of the substantia nigra. One month later they were treated orally with MDL 72394 (0.1 mg/kg) plus carbidopa (10 mg/kg) ; carbidopa alone ; clorgyline (5 mg/kg) or distilled water and 4 h later MAO and AADC activities determined in the striatum and overlying cerebral cortex. The lesion reduced striatal AADC activity by 83±3 % without altering MAO activity or the activity of either enzyme in the cerebral cortex. MDL 72394 pretreatment markedly inhibited MAO activity of the intact striatum (58±5 %) which was significantly ($p < 0.05$) greater than the 37±5 % inhibition seen in the lesioned striatum In contrast, clorgyline inhibited MAO to a similar extent in both striata (52±7 % and 64±6 % in intact and lesioned striata, respectively) whereas inhibition by both MAO inhibitors was similar in the cerebral cortex (60-65 %) and did not differ between the sides.

In a final experiment, MFMD was injected unilaterally into the MFB (10 µg in 0.5 µl) of rats. After a time (20 h) sufficient to allow inactivated AADC to diffuse by axonal flow to the striatum AADC activity was reduced by 83±7 % while MAO activity was unchanged. Following a 4 h oral pretreatment with MDL 72394 (0.1 mg/kg) plus carbidopa (10 mg/kg) striatal MAO was inhibited on the non-injected side by 61±4 % compared with only 22±4 % inhibition on the MFMD injected side ($p < 0.02$). In contrast, clorgyline (5 mg/kg p.o.) pretreatment inhibited MAO to a similar extent (60-65 %) in the two striata.

Taken together, these experiments clearly indicate that MDL 72394 is being decarboxylated selectively within monoamine neurons of the brain.

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CARBACHOL-STIMULATED PHOSPHATIDYLINOSITOL HYDROLYSIS IN THE CEREBRAL CORTEX AFTER FREEZING AND POST MORTEM DELAY

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A considerable number of putative neurotransmitters stimulate the hydrolysis of phosphatidylinositol (PI) eg. acetylcholine, noradrenaline, 5-hydroxy-tryptamine, in the rat brain (Downes, 1983). The receptor coupling of this PI response in the normal human brain and possible alterations in neurological and psychiatric disorders therefore requires investigation. The feasibility of using post mortem (p.m.) tissue to study the PI response and the possibility of preserving the response by freezing has been investigated by measuring the PI hydrolysis elicited by muscarinic receptor activation in the rat cerebral cortex using the sensitive assay procedure of Berridge et al (1982).

Tissue was either frozen intact in liquid nitrogen, slow frozen intact in 0.32M sucrose or cross-chopped (350x350μm) using a McIlwain chopper and the resulting miniprisms either dispersed in Krebs Ringer bicarbonate (KRB) for immediate assay, or dispersed in either 0.32M sucrose or KRB containing 10% dimethylsulphoxide (DMSO) before freezing. The miniprisms dispersed in sucrose were slow frozen in an insulated container at -70°C, while those dispersed in 10% DMSO were frozen using the procedure described by Haan and Bowen (1981). In all cases the frozen tissue was rapidly thawed at 37°C. The miniprisms were labelled with myo-(2-^{3}H) inositol and 50μl aliquots were then incubated for 1h in KRB containing 10mM LiCl with and without 10^{-4}M carbachol. PI hydrolysis was calculated as ratio of cpm in inositol phosphate fraction/cpm in phospholipid fraction x 100.

No measurable PI hydrolysis was found in cerebral cortex that had been frozen intact and the incorporation of ^{3}H-inositol into phospholipid was low. However, Table 1 shows that PI hydrolysis could still be elicited after slow freezing miniprisms in 0.32M sucrose or freezing in 10% DMSO and after a 4h p.m. delay (brain left in skull at room temperature).

Table 1 Carbachol stimulated phosphatidylinositol hydrolysis in the rat cerebral cortex

Miniprisms	Control	10^{-4}M Carbachol
Fresh	4.67 ± 0.50 (15)	8.48 ± 0.94 (15)**
Frozen in sucrose	4.28 ± 0.61 (15)	7.24 ± 0.53 (15)**
Frozen in DMSO	4.06 ± 0.35 (12)	6.21 ± 0.62 (12)**
4h post mortem delay	8.95 ± 0.60 (6)	12.81 ± 1.45 (6)*

* P > .05, ** P < .01 Student's t test

However, after the post mortem delay there was an increase in the basal hydrolysis. Atropine (10^{-5}M) abolished the response to carbachol. Preliminary results show that in the normal human cerebral cortex PI hydrolysis can still be elicited with carbachol even 10h p.m. and the response can be preserved by slow freezing miniprisms in 0.32M sucrose.

These results suggest that PI hydrolysis can be studied in post mortem tissue and that it is possible to "brain bank" appropriately prepared tissue for such studies.

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THE BENZODIAZEPINE ANTAGOINST, Ro 15-1788, DOES NOT ALLEVIATE THE ETHANOL WITHDRAWAL SYNDROME IN RATS

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The possible involvement of β-carboline compounds in the action of ethanol has been controversial ever since it was first suggested that the condensation of the metabolite acetaldehyde with central monoamines formed compounds of this type. Bhattacharya et al., (1982) found that alcoholics in withdrawal had a greater urinary output (than control subjects) of a monoamine oxidase inhibitor, which was suggested to be a β-carboline. This activity coincided with an increase in a benzodiazepine receptor binding substance in the urine (Sandler, 1982). Benzodiazepine receptor ligands have now been described with effects opposite to those of the benzodiazepines ("contragonists"), being convulsant and anxiogenic (Cowen et al., 1981; Dorow et al., 1983). Such substances, occurring endogenously, could contribute to ethanol withdrawal. Their effects are prevented by the benzodiazepine antagonist Ro 15-1788 (Nutt et al., 1982), so we tested the effects of this antagonist in ethanol withdrawal.

Male Sprague Dawley rats (140-160g) were fed for 48 days on a liquid diet (Complan) containing ethanol, in concentrations starting at 7% and rising to 15%. Control animals received liquid diet only. On the day of withdrawal, 6-7h after removal of ethanol, animals were injected intraperitoneally with either Ro 15-1788 20 $mgkg^{-1}$ or its vehicle (Tween 80, 1 drop in 10 ml distilled water) 15 min later they were subjected to an audiogenic stimulus (electric bell, 1 min) and the numbers of animals which convulsed were recorded. The rating was carried out "blind". The convulsion thresholds of the control animals to pentylenetetrazol (PTZ) were measured by the method of Nutt et al., (1980). 15 min after i.p. injection of 10 or 20 $mgkg^{-1}$ Ro 15-1788 or Tween vehicle, to confirm the lack of effect of Ro 15-1788 in non-drug-treated animals.

Ethanol treated rats - numbers convulsing.

Tween 80	Ro 15-1788, 10 $mgkg^{-1}$	Ro 15-1788, 20 $mgkg^{-1}$
6/7	4/8	6/8

Control treated rats, PTZ convulsion thresholds (n=6) mean ± s.e.m.

Tween 80	Ro 15-1788, 10 $mgkg^{-1}$	Ro 15-1788, 20 $mgkg^{-1}$
23.6 ± 1.5	26.3 ± 1.5	27.3 ± 0.8

The number of animals having full convulsions was not significantly altered by Ro 15-1788 10 or 20 $mgkg^{-1}$ ($P > 0.05$, Fisher's exact test). This provides evidence that β-carboline compounds of the benzodiazepine contragonist type do not make a major contribution to the convulsive component of the ethanol withdrawal syndrome.

We thank the Wellcome Trust for funding and Roche for Ro 15-1788.

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SPECIFICITY OF PROSTAGLANDIN ACTION IN THE STRIATUM TO ANTAGONISE DYSKINESIAS

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The instrastriatal injection of prostaglandin E_1 and E_2 can antagonise the perioral dyskinesias induced by the peripheral treatment with the dopamine agonist 2-di-n-propylamino-5,6-dihyroxytetralin (Costall et al, 1984). The present study investigates whether this ability to antagonise striatal dopamine function can be extended to other behavioural situations and to the limbic system.

Rats were subject to standard stereotaxic surgery for the implantation of chronically indwelling guide cannulae to allow the bilateral injection of prostaglandin or neuroleptic agent into the nucleus accumbens (Ant. 9.0, Vert. 0.0, Lat. ± 1.6; De Groot, 1959) or caudate-putamen (Ant. 7.4, Vert. +1.O, Lat. ± 3.0). Hyperactivity was induced from the nucleus accumbens by acute administration of (+) amphetamine (10µg bilateral) or repeated administration of dopamine (25µg bilateral for 5 days) and the modification of established responses by intra-accumbens prostaglandin (PGE_1, PGE_2, PGD_2, $PGF_{2\alpha}$] or neuroleptic agent (fluphenazine, (-) sulpiride) determined (measurements in individual cages each fitted with one photocell unit set off-centre; interruptions of the light beams recorded electromechanically and the nature of the behaviour confirmed visually). For assessment of ability to modify striatal dopamine function the intrastriatal administrations of prostaglandin or the neuroleptic agents were followed by catalepsy assessments (timing of ability to maintain an abnormal imposed position), challenge with apomorphine (2.0mg/kg s.c.) and assessment of stereotyped behaviour (scored 0-4 according to the frequency/intensity of repetitive sniffing and biting), and challenge with 2-di-n-propylamino-5,6-dihyroxytetralin and assessment of perioral dyskinesias (scored 0-3 according to the frequency/intensity of biting and oral movements).

The intra-accumbens injection of prostaglandin E_1 dose-dependently reduced amphetamine hyperactivity (9-98% reductions at 0.01-1µg, $P<0.05$-$P<0.001$), prostaglandin E_2 and $F_{2\alpha}$(0.1-1µg) failed to modify the amphetamine response whilst prostaglandin D_2 enhanced the amphetamine response (20-44% increases at 0.1-1µg, $P<0.01$-$P<0.001$). The hyperactivity caused by repeated administrations of dopamine into the nucleus accumbens was reduced by both prostaglandin E_1 (90% reduction at 1µg, $P<0.001$) and E_2(80% reduction at 1µg, $P<0.001$), was unmodified by prostaglandin $F_{2\alpha}$,but was enhanced by prostaglandin D_2 (66% increase at 1µg, $P<0.001$). (-)sulpiride (5-40ng) and fluphenazine (0.5-1ng) dose-dependently reduced (20-86%, $P<0.05$-$P<0.001$) the hyperactivity caused by amphetamine.

When injected bilaterally into the striatum prostaglandin E_1, E_2, D_2 and $F_{2\alpha}$ all failed to induce catalepsy and to antagonise stereotyped behaviour caused by apomorphine. However, prostaglandin E_1 and E_2 were both potent to antagonise the dyskinesias induced by the tetralin compound (ED_{50} values 0.7 and 0.02ng intrastriatal respectively).

Thus, the present results show that whilst a hyperactivity induced from the nucleus accumbens of rat can be antagonised by prostaglandin E_1/E_2, the large (microgram) doses required contrast markedly with the high potency of these agents (effective in picogram doses) to antagonise dopamine agonist (tetralin) action within the striatum. The dopamine antagonist action in the striatum is shown to be directed at a specific behavioural response and does not appear to reflect a generalised ability to inhibit cerebral dopamine function.

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$GABA_B$ RECEPTOR STIMULATION INCREASES VASOACTIVE INTESTINAL PEPTIDE ACTIVATED CYCLIC AMP ACCUMULATION IN SLICES OF RAT CEREBRAL CORTEX

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$GABA_B$ receptors are characterised by their insensitivity to the $GABA_A$ antagonist bicuculline and their selective activation by baclofen (Hill & Bowery, 1981). The ability of guanyl nucleotides to decrease the saturable binding of ^3H -baclofen to $GABA_B$ receptors led to the suggestion that these receptors may be associated with the modulation of adenylate cyclase activity (Hill et al., 1984). Support for this notion has come from recent studies in which baclofen, while having no effects on basal adenylate cyclase activity, has been shown to enhance the effects of noradrenaline in stimulating cyclic AMP accumulation in slices of rat cerebral cortex (Hill, 1984). Vasoactive intestinal peptide (VIP) is also know to stimulate adenylate cyclase activity in the central nervous system (Quik et al., 1978). The present series of experiments was undertaken to investigate the effects of baclofen on VIP-stimulated adenylate cyclase activity in rat brain.

Slices of cerebral cortex were cross chopped at 260 um x 260 um intervals using a McIlwain tissue chopper and preincubated in oxygenated Krebs/bicarbonate Ringer's containing 0.005% ascorbate for 90 min at 37°C. Fifty microlitre aliquots of tissue slices (containing approximately 1 mg protein) were placed in tubes containing fresh Ringer's (final volume 500 ul) and incubated for a further 15 min at 37°C prior to the addition of drugs. Slices were then incubated for a further 10 min at 37°C followed by boiling for 3 min. Duplicate 50 ul samples of the extract were assayed for cyclic AMP content by the method of Brown et al., (1972). The protein contents of 50 ul aliquots of tissue slices were determined by the method of Lowry et al., (1951) and data calculated as pmol cyclic AMP/mg protein.

In the absence of any drug, basal cyclic AMP accumulation was 0.84 $\pm$ 0.12 pmol cyclic AMP/mg protein (n=12). In keeping with the results of Hill (1984), 100 uM (-)-baclofen did not significantly increase basal cyclic AMP accumulation. However, in the presence of 1 uM VIP, cyclic AMP accumulation increased to 19.52 $\pm$ 1.77 pmol cyclic AMP/mg protein (n=4), rising to 45.69 $\pm$ 3.10 pmol cyclic AMP/mg protein (n=4) in the presence of 10 uM VIP. In the presence of 100 uM (-)-baclofen, the responses to 1 uM VIP and 10 uM VIP were approximately doubled, to 39.75 $\pm$ 2.9 pmol cyclic AMP/mg protein (n=4) and 84.73 $\pm$ 4.1 pmol cyclic AMP/mg protein (n=4) respectively. The ability of (-)-baclofen to augment VIP-stimulated adenylate cyclase activity was mimicked by 100uM GABA (in the presence of 1 mM nipecotic acid), but was not antagonised by the GABA antagonist bicuculline methobromide (100 uM). In contrast to the (-)-isomer, 100 uM (+)-baclofen did not enhance VIP-stimulated cyclic AMP production.

These data suggest that the stimulation of $GABA_B$ receptors in rat cerebral cortex can augment VIP-stimulated cyclic AMP production.

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SELECTIVE POTENTIATION OF THE EFFECTS OF A BENZODIAZEPINE CONTRAGONIST AFTER CHRONIC FLURAZEPAM TREATMENT IN MICE

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We have shown previously that chronic treatment with the benzodiazepine contragonist FG 7142 caused sensitisation to its effects (Little and Nutt, 1984). We have now shown that chronic treatment with the benzodiazepine flurazepam also increased the effects of FG 7142 but did not alter the effects of other drugs acting at the GABA benzodiazepine receptor complex.

Flurazepam, 40 mg/kg i.p. was given once daily for 7 days to male CD1 mice, 30-35 g. Each animal was used once only and the results below are for groups of eight mice. 24 h after the last injection of flurazepam the effects of the following drugs on convulsion threshold, body temperature and locomotor activity was investigated. Convulsion threshold was measured by intravenous infusion of the convulsant drug (Nutt et al., 1980) and body temperature by rectal probe (ambient temperature 22°C). Locomotor activity was measured on pairs of mice for 1 h by Doppler probe.

Convulsion thresholds	Chronic treatment : S = saline, FZ = flurazepam		
FG 7142, 40 mg/kg, i.p.	S : no convulsions	FZ : 7/8 convulsed	
PTZ threshold, i.v. (mean ± s.e.m)	S : 37 ± 1.6	FZ : 33 ± 1.6,	mg/kg
PTZ threshold after flurazepam, 10 mg/kg, i.p.	S : 107 ± 9	FZ : 75 ± 5*	mg/kg
Bicuculline threshold, i.v.	S : 0.41 ± 0.02	FZ : 0.44 ± 0.03	mg/kg
PTZ threshold after muscimol 3.5 mg/kg	S : 51 ± 3.5	FZ : 51 ± 3.3	mg/kg
Locomotor activity, mean ± s.e.m.			
Flurazepam, 40 mg/kg, i.p.	S : 385 ± 100	FZ : 1505 ± 202*	(Controls: 1706 ± 191)
Temperature measurements just before and 30 min after injection, mean ± s.e.m.			
Flurazepam, 40 mg/kg, i.p.	S : 37.5 ± 0.1 → 32.9 ± 0.2,	FZ : 37.3 ± 0.1 → 35.8 ± 0.2*	
FG 7142, 20 mg/kg, i.p.	S : 37.9 ± 0.1 → 37.3 ± 0.2,	FZ : 37.7 ± 0.1 → 35.5 ± 0.2*	
PTZ, 20 mg/kg, i.p.	S : 38.1 ± 0.1 → 36.5 ± 0.3,	FZ : 37.9 ± 0.1 → 36.5 ± 0.2	
Muscimol, 1 mg/kg, i.p.	S : 38.3 ± 0.1 → 35.0 ± 0.8,	FZ : 38.2 ± 0.1 → 35.8 ± 0.6	

The expected tolerance to the effects of flurazepam was seen. FG 7142, normally only proconvulsant, caused convulsions and its hypothermic effects were increased. (*P<0.05, Mann-Witney 'U' test). The actions of pentylenetetrazol (PTZ), bicuculline, and muscimol, in the middle of the effect dose ranges, were unaltered.

The benzodiazepine antagonist Ro 15-1788, 10 mg/kg i.p. caused no changes in behaviour when given 24 h after the last dose of flurazepam, suggesting that the changes seen were not due to displacement of residual flurazepam from receptor sites by FG 7142, thus causing withdrawal.

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STRUCTURE-AFFINITY AND STRUCTURE-EFFICACY RELATIONSHIPS AT β-ADRENOCEPTOR SITES

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Recent studies (McPherson *et al.*, 1984) have shown that the selective β_1-receptor agonistic actions of RO363 (Iakovidis *et al.*, 1980) reflect both selective affinity and efficacy at β_1- as opposed to β_2-adrenoceptor sites. Since, on a structural basis, RO363 differs from isoprenaline through the presence of an oxymethylene bridge in the side-chain and an homoveratryl amine substituent, it was of interest to determine the contributions made by each of these groups to the overall activity of RO363. This was achieved by determining the affinity and relative efficacies of the oxymethylene derivative (OM-ISO) and the homoveratryl amine derivative of isoprenaline (Compound 16) at β_1- and β_2-adrenoceptor sites.

The dissociation constants (K_D; 1/affinity) of the molecules were determined from their ability to displace [^{125}I]cyanopindolol from guinea-pig left atrial and uterine membrane homogenates. The relative efficacies of the two drugs were calculated according to the method described by Furchgott & Bursztyn (1967) using the K_D values and equiactive responses interpolated from organ bath studies. For the latter, cumulative concentration-effect curves for positive inotropic responses in guinea-pig isolated driven left atria (2.5 Hz, 1 ms, 2 x threshold voltage) and relaxant responses in K^+-depolarized uteri from guinea-pigs, were first established for (-)-isoprenaline and thereafter to one of the test drugs. Comparison of dose-response curves indicated that Compound 16 and OM-ISO showed little selectivity for agonistic actions at β_1-/β_2-receptor sites.

Table 1 shows the mean negative log of the dissociation constants (pK_D) for the two drugs and for (-)-isoprenaline and RO363, and their mean relative efficacies ($\varepsilon_{ISO}/\varepsilon_{DRUG}$) determined from 3 - 6 experiments.

Table 1 Mean neg. log K_D values (pK_D) and relative efficacies of OM-ISO, Compound 16, (-)-Isoprenaline and RO363

	Atria		Uterus	
	pK_D	Relative Efficacy	pK_D	Relative Efficacy
(-)-Isoprenaline*	6.4 ± 0.1	1	6.0 ± 0.1	1
OM-ISO	7.4 ± 0.1	312 ± 104	6.8 ± 0.1	24 ± 3
Compound 16	4.2 ± 0.3	34 ± 6	4.3 ± 0.1	148 ± 40
RO363*	7.8 ± 0.1	25 ± 1	6.0 ± 0.1	2633 ± 1000

Values are means ± s.e.mean from 3 - 6 experiments.
*Data from McPherson *et al.* (1984)

The data indicates that the selective β_1-adrenoceptor agonistic actions of RO363 can be explained by the combined effects of both the oxymethylene and the homoveratryl groups which, in combination, have produced an increase in affinity at β_1-adrenoceptor and a reduction in efficacy at β_2-adrenoceptor sites.

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NEURO-EFFECTOR TRANSMISSION THROUGH POSTSYNAPTIC α_2-ADRENOCEPTORS IN HUMAN SAPHENOUS VEIN

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Alpha-adrenoceptor agonists produce contractions of human isolated blood vessels which can best be explained in terms of a mixed population of $alpha_1$- and $alpha_2$-adrenoceptors on the vascular smooth muscle (Stevens & Moulds, 1981). There is no clear evidence that endogenous noradrenaline neurotransmitter can act on these postsynaptic $alpha_2$-receptors, even in animal tissues (see Wilffert et al., 1982). We are now able to provide such evidence in human saphenous vein.

Human saphenous veins were obtained as leftovers from coronary artery bypass grafts of male patients (aged 38-64 years). Tissues were cut spirally and superfused at 37°C in Krebs-Henseleit solution.

Field stimulation for 3 min at a frequency of 5 Hz produced an isometric contraction of 1.00 ± 0.19 g (n=14). The $alpha_2$-receptor antagonist yohimbine produced a significant inhibition of this contraction over the concentration range 0.01-1 uM, whereas the $alpha_1$-antagonist prazosin produced a significant inhibition only at concentrations of 0.1 uM and above. The IC_{30} of yohimbine (concentration producing 30 % inhibition of stimulation-evoked contractions) was 13.2 nM (95 % confidence limits 6.6 - 26.3 nM). For prazosin, an IC_{30} could not be obtained in 2 of 6 experiments in which the inhibition by prazosin up to 1 uM was small, so that the mean IC_{30} of prazosin could only be expressed as not less than 250 nM. The calcium entry blocker nifedipine (10 uM) reduced stimulation-evoked contractions to 59.6 ± 5.9 % of control (n=3).

The inhibition of the stimulation-evoked contraction was postsynaptically mediated since the antagonists in these concentrations did not reduce stimulation-evoked ^{3}H-noradrenaline overflow: indeed yohimbine increased ^{3}H-noradrenaline overflow significantly in the concentration range 0.01-0.1 uM. The $alpha_2$-agonist xylazine produced concentration dependent isometric contractions and concentration-dependent inhibition of ^{3}H-noradrenaline overflow.

The postsynaptic potency of yohimbine obtained in this study agrees well with its potency at presynaptic $alpha_2$-receptors, whereas the potency of prazosin obtained is much less than its potency at postsynaptic $alpha_1$-receptors in other tissues (see Starke, 1981). The data suggest that neuro-effector transmission in human saphenous vein is mediated predominantly by postsynaptic $alpha_2$-adrenoceptors, and involves calcium entry through nifedipine-sensitive channels.

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RELEASE OF NORADRENALINE AND ADRENALINE FROM PERFUSED CULTURED BOVINE ADRENAL CHROMAFFIN CELLS

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Studies on the release of catecholamines from adrenal medulla have been central to the development of models of stimulus secretion coupling. In recent years several laboratories have used primary cultures of bovine adrenal chromaffin cells maintained in monolayer on tissue culture dishes. In order to further investigate the control of release of endogenous catecholamines and other mediators from chromaffin cells, we have, for the first time, developed a system where continuous perfusion of cells is possible.

Bovine chromaffin cells were dispersed and purified on Percoll gradients essentially as described by others (Wilson and Viveros, 1981) and were cultured on microcarrier beads (Cytodex 1). After several days in culture, aliquots of the beads (with cells attached) were introduced into small (200 µl) perfusion chambers. One min. fractions of the perfusate (0.5 ml/min. of Earls balanced salt solution) were collected and assayed for noradrenaline (NA) and adrenaline (AD) by high performance liquid chromatography with electrochemical detection.

The procedure enables the monitoring of basal release of NA and AD. Perfusion of cells with nicotine (10^{-5} M) resulted in an almost immediate increase in catecholamine response which decayed rapidly despite continued exposure to this drug (Figure 1A). This nicotine response was suppressed (80%) by mecamylamine (10^{-5} M) introduced into the perfusate 5 min. before the nicotine. Release induced by veratrine (5 x 10^{-5} M) was somewhat delayed in onset and displayed a slower rate of increase to a plateau which was sustained throughout the period of the experiment (Figure 1B). It can be seen that in both cases there was a preferential release of NA (the cells contained equal amounts of AD and NA) which was considerably greater than the preferential release from cells in the absence of perfusion. This difference may reflect the removal of released substances from the cell surface in the perfusion experiments. The different time course of veratrine-stimulated compared to nicotine-stimulated release may reflect fluctuations in the free calcium concentration inside the cells.

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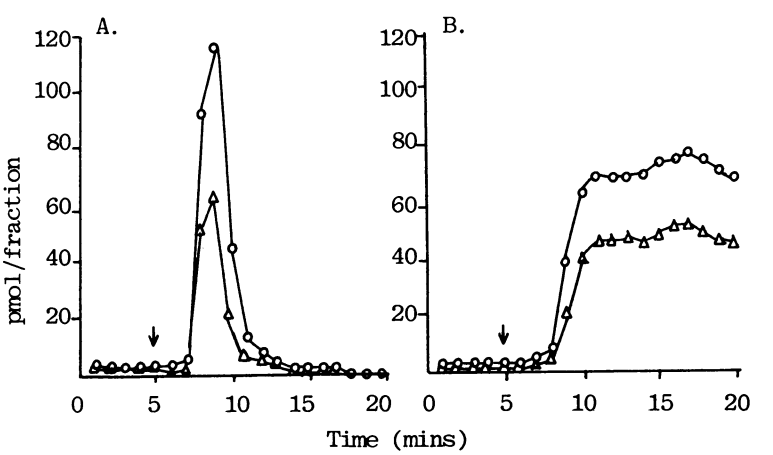

Figure 1

Release of NA (o) and AD (Δ) from perfused cells.

In A., nicotine (10^{-5} M) was added at 5 min. and in B. veratrine (5 x 10^{-5} M) was added at 5 min.

α_1 AND α_2 ADRENOCEPTOR RESPONSES IN HUMAN ISOLATED ARTERIES

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It has recently been reported that human cerebral arteries fail to constrict in response to the selective α_2-agonist clonidine (Toda, 1983). This observation constrasts with those obtained from other species (e.g. dog) in which clonidine mediated vasoconstriction has been interpreted as evidence for postsynaptic or extrajunctional α_2 adrenoceptors. The present study describes the responses of various isolated human arteries (renal, mesenteric, pulmonary, splenic, gastric and brachial) to a number of adrenergic agonists and antagonists.

Human arteries were obtained from organs or tissues resected at surgery and used immediately for investigation. Arteries of between 2mm and 6mm internal diameter were cut into rings of up to 7mm length, taking care to avoid any trauma. These rings were set up in the tissue bath according to the method described by Towart (1982). The tension applied was that which generated the maximum response to a 1 μM noradrenaline stimulus. This generally varied between 1g and 4g. The number of arterial segments obtained from each anatomical location varied from 3-8. The integrity of the endothelium was examined histologically.

No consistent responses were obtained to the highly selective α_2 adrenoceptor agonist, UK 14304, at a dose of 1nM to 10μM. All tissues contracted to adrenaline, noradrenaline and phenylephrine in a dose dependent manner from 3-300 nM. To ascertain whether these contractile responses were mediated partly through post-junctional α_2 adrenoceptors, the selective α_2 antagonist, RX 781094 (100-300nM), was incubated with the tissue for 15 min and the dose response curves repeated. In no tissue was the dose response curve antagonised by the presence of RX 781094. Doxazosin (100nM), however, caused a marked reduction in the constrictor response. In some instances the response to adrenaline (100nM) was augmented by RX 781094 (100nM) by up to 500%. This potentiation of the constrictor response in the presence of α_2 antagonists has also been reported in other species by Cocks and Angus (1983).

These observations support the hypothesis that α_2 antagonists inhibit the release of the endothelium derived relaxing ('Furchgott') factor (E.D.R.F.). The presence of the E.D.R.F. in these human blood vessels was demonstrated by the ability of 1μM Acetylcholine to relax noradrenaline preconstricted vessels - a response which was lost if the endothelium was deliberately removed by rubbing with a wooden stick. Further evidence for an α_2 receptor link to the E.D.R.F. was provided by the observation in the $PGF_{2\alpha}$ preconstricted pulmonary artery that noradrenaline 3nM-3μM produced a 30% relaxation in the presence of 1μM doxazosin. This response was abolished both by 1μM RX 781094 and by deliberate removal of the endothelium. In the arteries studied we found no evidence for a postsynaptic α_2 receptor mediating vasoconstriction. In contrast the endothelial α_2 receptor mediates vasodilatation. Our observations emphasise the importance of possible inter-species differences in vascular responses to adrenergic agents. Our data do not rule out the possibility that α_2 adrenoceptors mediating vasoconstriction may exist at other sites, e.g. smaller calibre arteries and arterioles or veins.

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MEASUREMENT OF CALCIUM ANTAGONIST POTENCY USING CARDIAC CELL CULTURES

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Ventricular tissue from embryonic chick can be maintained in primary culture under conditions which allow the formation of a monolayer or spherical aggregates and both display automaticity. The beating of these cultures is TTX (Na^+ channel) sensitive and calcium entry and contractility can also be inhibited by calcium antagonists (Marsh et al, 1983). The quantitative measurement of this contractility is an elaborate procedure in both types of culture. However, contractility can be measured qualitatively (on an all or none basis) when the cells are encouraged to form aggregates. A method has been developed from which calcium antagonist potency of compounds can be determined using inhibition of aggregate contractility as a marker. Calcium antagonist potencies have been compared with those obtained from the measurement of inhibition of slow action potential-induced contractions of depolarised guinea pig papillary muscles.

Hearts from 11-12 day old Leghorn J chicken embryos were aseptically dissected and cells dissociated with trypsin utilising the method described by Clusin (1981). Cell suspensions were cultured for a period of 3-4 days. Stock concentrations of standard calcium antagonists were added to the culture plates and inhibition of beating determined after 30 min incubation. Measurements of calcium dependent contractures of 20 mM K^+ depolarised guinea pig papillary muscles were made as described by Patmore & Whiting (1982). The effects of each calcium antagonist at a range of concentrations was determined following superfusion for 15 min.

Results from both experiments were expressed as % of control contractility. Inhibition-concentration relationships were fitted using a least squares iterative programme. Data are compared in Table 1.

Table 1 Comparison of calcium antagonist potencies

COMPOUND	Cardiac Cell Culture	Papillary Muscle
NICARDIPINE	6.94 (6.99-6.90)	8.08 (8.17-8.00)
NIFEDIPINE	6.71 (6.80-6.63)	7.43 (7.51-7.36)
VERAPAMIL	6.36 (6.40-6.32)	7.82 (7.96-7.71)
GALLOPAMIL	6.77 (6.78-6.76)	7.68 (7.76-7.62)
DILTIAZEM	5.83 (5.86-5.80)	6.92 (6.95-6.88)
LIDOFLAZINE	5.76 (5.87-5.68)	5.44 (5.54-5.36)
RYANODINE	5.28 (5.37-5.20)	<4.00

Values shown are pIC_{50} with SD range (n = 4-6)

There is a good correlation between the potencies determined using the two methods (r = 0.85). This cell culture method is also sensitive to the intracellular calcium antagonist, ryanodine. Cell culture estimates of pIC_{50} are lower than those from papillary muscle. This is due to the all or none end point of the former assay compared with the graded inhibition of contraction measured from the ventricular fibres. The cell culture assay is, thus, an effective and inexpensive method of evaluating calcium antagonist potencies.

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MEASUREMENT OF NATRIURETIC HORMONE LEVELS IN RODENTS AND MAN

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There is considerable evidence to suggest that hypertension is associated with an increase in levels of a natriuretic hormone, which controls sodium excretion and Na^+, K^+-ATPase (inter alia McGregor et al., 1981). In general, only an indirect assessment of hormone levels, determined from monitoring the associated changes in tissue Na^+, K^+-ATPase has been attempted. This may result in artifactual results due to removal of natriuretic hormone during the isolation procedure (Cross & Wyllie, 1984). In this report, we describe in vitro methodology suitable for the direct assessment of natriuretic hormone in extracts of biological material including serum.

Homogenates of rat brain (10% w/v in 0.32M sucrose) were prepared using an Ultraturrax homogeniser (speed setting 7, 30 sec). In a separate series of experiments, 10ml of blood was withdrawn from treated or untreated hypertensive patients and normotensive volunteers, into glass centrifuge tubes (no anti-coagulant). These were allowed to stand until full clot retraction occurred (30 min.) and centrifuged for 10 min. at $500g_{AV}$. The serum was decanted and assayed for natriuretic hormone activity. All assays were carried out blind on coded samples.

Aliquots (200µl) of brain homogenate or serum were added to 300mg activated Dowex cation exchange resin (30W - X8 (H^+), 200-400 mesh) in Eppendorf tubes. These were inverted and allowed to stand for 10 min. before centrifugation. Natriuretic hormone activity was assessed from the ability of the samples to inhibit Na^+, K^+-ATPase or displace ^{3}H-ouabain (Cross & Wyllie, 1984).

Unless samples were treated with Dowex, the concentrations of K^+ ions normally found in serum (3.5-4.5mM) resulted in substantial displacement of ^{3}H-ouabain. After treatment with Dowex the concentration of K^+ ions in brain samples or serum was reduced to < 0.2mM (n=25), a concentration which did not interfere with the assay. There was a good correlation ($r=0.95$, n=24) between data calculated from either assay (^{3}H-ouabain binding displacement or inhibition of Na^+, K^+-ATPase.

Elevated levels (143 ± 7%, n=12) of natriuretic hormone were found in samples prepared from brains of spontaneously hypertensive rats, when compared to aged-matched controls.

Mean arterial pressure in man showed a significant correlation with serum natriuretic hormone levels ($r=0.69$, $p < 0.001$, n=54). As in the case of experimental hypertension (Cross & Wyllie, 1984), natriuretic hormone levels were susceptible to pharmacological intervention. Baratol (25mg b.d., 4 weeks) produced parallel changes in mean arterial pressure, and natriuretic hormone levels (Table 1).

Table 1. The effects of baratol on mean arterial pressure and natriuretic hormone levels in man

Mean arterial pressure mmHg		Natriuretic Hormone level Ouabain-like units, nM	
Pre-Baratol	Post-Baratol	Pre-Baratol	Post-Baratol
127 ± 3	117 ± 5	16.3 ± 1.2	14.1 ± 0.9

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PARTIAL AGONISM AT CENTRAL AND PERIPHERAL α_2-ADRENOCEPTORS IN THE RAT

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Clough and Hatton (1981) reported that ICI 101,187 and ICI 106,270, like clonidine, produced centrally-mediated falls in blood pressure and heart rate but were less sedative than clonidine. It was found that the ICI compounds possessed agonist activity at both α1- and α2-adrenoceptors. α2-Adrenoceptor agonists induce mydriasis in the rat by a central mechanism (Berridge et al, 1983) involving α2-adrenoceptors that are similar pharmacologically to those in the vas deferens (Doxey et al, 1983a). We have determined the profiles of ICI 101,187 and ICI 106,270 at central α2-adrenoceptors mediating mydriasis and at prejunctional α2-adrenoceptors in the rat vas deferens.

ICI 101,187 and ICI 106,270 inhibited, dose-dependently, the stimulation-evoked twitch response of the vas deferens in pithed rats (prepared according to Doxey et al., 1983b). The maximal inhibitory responses were 83 ± 3% and 77 ± 4%, respectively and their respective cumulative ED50 values were 3.1 ± 0.7 and 9.6 ± 1.9 μg/kg,i.v. These profiles are similar to those obtained for these compounds in mouse isolated vas deferens (Clough and Hatton, 1981). In contrast, clonidine and UK-14,304 completely inhibited (100 %) the twitch response (ED50: 5.5 ± 0.5 and 2.9 ± 0.4 μg/kg,i.v. respectively). All four agonists were antagonised competitively by idazoxan (0.1 mg/kg,i.v.), a selective α2-adrenoceptor antagonist.

Pupil diameter was measured in pentobarbitone-anaesthetized rats as described by Berridge et al. (1983). Both ICI compounds partially dilated the pupil in a dose-related manner over the range 3-1000 μg/kg,i.v.) . ICI 101,187 showed a greater degree of agonism than did ICI 106,270; the maximal pupil dilatations were 2.7 ± 0.2 mm and 2.1 ± 0.1 mm, respectively. These responses were significantly less than the maximal responses of 3.9 ± 0.2 mm and 4.2 ± 0.1 mm seen after the agonists clonidine (1-100 μg/kg) and guanoxabenz (3-300 μg/kg,i.v.). The order of agonist potencies was clonidine > guanoxabenz > ICI 101,187 > ICI 106,270. The mydriatic effect of each agonist was antagonised competitively with idazoxan (0.5 mg/kg,i.v.).

The ICI compounds also exhibited a central α2-adrenoceptor antagonist component as judged by their ability to cause a dose-related (10-1000 μg/kg,i.v.) partial reversal (about 60%) of the full dilatation (>4mm) produced by guanoxabenz (0.1 mg/kg,i.v.). Furthermore pretreatment (-15min) with ICI 101,187 (0.1 mg/kg,i.v.) caused a 6-fold parallel rightward shift of the guanoxabenz dose-mydriatic response curve.

Since clonidine is a full α2-agonist in the tests employed in this study, it is tempting to speculate that the partial agonist activity of the ICI compounds at α2-adrenoceptors may account for their reduced sedative activity in rats. We suggest further that pupil measurement offers a simple and reliable approach to evaluate the activity of agonists and partial agonists at central α2-adrenoceptors.

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THE EFFECTS OF TEPROTIDE ON PRESSOR RESPONSES TO α-AGONISTS AND SYMPATHETIC NERVE STIMULATION

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Previous studies have shown that angiotensin converting enzyme inhibitors (ACE-inhibitors) antagonise the pressor responses to NA and to sympathetic nerve stimulation in the pithed rat (Hatton & Clough, 1982). Timmermans et al (1982) further claim that ACE-inhibitors selectively inhibit pressor responses via α_2-adrenoceptors. Our aims in this study were, firstly, to investigate whether ACE-inhibitors are selective for α_2 and, secondly, to study whether these effects occur via an AII-dependent mechanism.

Male Wistar rats (250g) were pithed and ventilated at 2.5ml/st (60st/min) with 40% O_2/60% N_2 to produce normal blood gases. The sympathetic vasopressor outflow (T6-T8) was stimulated at 40V, 0.05ms, 10 pulses, 0.5-10Hz.

Teprotide (1mg/kg) reduced the pressor response to AI (0.5ug/kg) by 77% thus indicating a substantial inhibition of ACE. Teprotide (1mg/kg) significantly reduced pressor responses to sympathetic nerve stimulation by approx. 50% at all frequencies studied (0.5-10 Hz). Infusion of AII (200ng/kg/min) failed to reverse the effects of teprotide. Peak responses to tyramine (0.2mg/kg) and NA (1ug/kg) were unaffected by teprotide (1mg/kg) but the late phases of the responses were reduced. Teprotide (1mg/kg) had little effect on the peak response to the α_1-agonist phenylephrine (3ug/kg) but the late phase of the response was significantly reduced. In contrast, teprotide (1mg/kg) significantly reduced both the peak response and the prolonged phase of the response to the α_1 agonist Indanidine (0.5mg/kg). Peak responses to the selective α_2 agonists xylazine (0.5mg/kg) and azepexole (0.25mg/kg) were unaffected by teprotide (1mg/kg). However the late, nifedipine-sensitive part of the responses to these agonists were significantly reduced. In all cases except for Indanidine, AII (50ng/kg/min) potentiated the peak responses to the agonists but failed to reverse the effects of teprotide on the late components of the responses.

In conclusion, the inhibitory effect of teprotide does not depend on the adrenoceptor subtype but depends on the duration of the response. Teprotide may not be acting via a circulating AII-dependent mechanism since AII infusion failed to reverse its inhibitory effects. Alternatively, as suggested by Bull & Drew (1984) teprotide may block a locally produced source of AII in vascular smooth muscle which cannot be replaced by AII infusion.

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CHARACTERISATION OF TWO HIGH AFFINITY BINDING SITES FOR [^{3}H] SOMATOSTATIN IN RAT BRAIN

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Somatostatin is a cyclic tetradecapeptide present in the mammalian central nervous system with a putative neurotransmitter role. The radioiodinated Tyr-1 and Tyr-11 analogues of somatostatin have previously been used to demonstrate specific binding sites for these ligands in brain (Reubi et al., 1981; Srikant & Patel, 1981; Epelbaum et al., 1982). However, these ligands present problems that have produced variable and conflicting results. Therefore we have used synthetic [4-^{3}H-Phe-6]-somatostatin (Allen et al., 1981), which is chemically identical to the natural ligand, and have unambiguously demonstrated two reversible and saturable binding sites for this ligand on brain membranes.

The tritiated ligand is sufficiently stable in the presence of 0.01% bacitracin to allow incubation at 30^{o}C for 30mins for equilibrium binding to be reached. Under these conditions there was no detectable degradation of the label as determined by HPLC of the incubation media. Displacement of ^{3}H-somatostatin by increasing concentrations of unlabelled somatostatin revealed, on Scatchard analysis, two high affinity binding sites on whole rat brain membranes (Kd_1 0.4 ± 0.1nM, $Bmax_1$ 81.6 ± 0.9fmoles/mg protein, Kd_2 22.9 ± 6.2nM, $Bmax_2$ 309.5 ± 29.8fmoles/mg protein; n=6). The association rate constant (kon) was found to be 1.03 x 10^6 M^{-1} s^{-1}, and the dissociation rate constant (koff) measured at 4.49 x 10^{-4} M^{-1} s^{-1}. The corresponding dissociation constant (koff/kon) was calculated to be 0.44nM, in good agreement with the higher affinity binding sites found from the equilibrium binding studies. The regional distribution of ^{3}H-somatostatin specific binding (at 1nM label concentration) was highest in the cortex (76.3 ± 2.8fmol/mg protein) and the hippocampus (74.9 ± 10.2fmoles/mg protein), lowest in the pons/medulla (5.3 ± 1.9fmoles/mg protein) and the cerebellum (4.9 ± 1.9fmoles/mg protein) and intermediate in the striatum (23.1 ± 2.7fmoles/mg protein), midbrain (20.7 ± 2.5fmoles/mg protein) and hypothalamus (14.8 ± 1.2 fmoles/mg protein).

We conclude that somatostatin binding sites are enriched in the cortical regions of rat brain and that two distinct high affinity binding sites are present. The physiological significance of these two binding sites is unclear, but these two receptor subtypes could correspond to binding sites for the somatostatin-14 and somatostatin-28 known to be present in mammalian brain.

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TRH IS ENDOGENOUS TO RAT OLFACTORY BULB - EVIDENCE FROM CHEMICAL AND SURGICAL LESIONS

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The tripeptide,thyrotropin-releasing hormone (TRH), is distributed heterogeneously throughout the mammalian central nervous system - the hypothalamus(HYP) , amygdala (AMG) and olfactory bulb (OB) being the most enriched regions. However, the anatomy and neuronal circuitary of TRH-utilising pathways is poorly understood . The present study sought to discern the origin and possible site(s) of termination of TRH-using projections in the rat OB by employing lesioning techniques coupled with biochemical and morphological determinations.

Unilateral,stereotaxic injections (AP = 9 mm, ML = 1.5 mm, DV = 3 mm) of 15 µg /3 µl kainic acid (KA) in rat OB was accompanied by a 43 ± 13% (n = 4) depletion of endogenous TRH(measured by radioimmunoassay relative to sham-operated controls) 2 weeks post lesion. TRH levels were unchanged in HYP , AM and hippocampus (HP) . TRH depletion in the OB was accompanied by a 61.7 ± 1% (n = 5; $P < 0.001$) reduction in bulbar [^{3}H]MeTRH receptor binding and Scatchard plots of competition experiments indicated a specific decrease in receptor number with little change in binding affinity (controls, K_d = 3.4 ± 0.6 nM, lesioned, K_d = 5.4 ± 1.1 nM).Similarly, TRH receptor density remained unaltered in 5 other brain areas and pituitary following KA lesions and 4 weeks after surgical bulbectomy. The latter treatment also did not change levels of TRH or its receptors in the OB 2 weeks post lesion.

The neuronal specificity of KA was demonstrated by unaltered activity of the presynaptically localised choline acetyl transferase in centrifugal cholinergic afferents. A concomitant 57 – 70% decrease of postsynaptically localised receptors for TRH, benzodiazepines and muscarinic drugs after KA lesions of the OB provided further evidence for the perikaryal site of action of this neurotoxin.

While destruction of the serotonergic fibres (> –90% 5HT) to the OB with 5,7-dihydroxytryptamine (Sharif et al.1983 a,b) did not effect levels of TRH or its receptors, 6-hydroxydopamine (6-OH DA; 8 µg) injections in the OB reduced the peptide receptors by about 35% .

Histological studies following kainate lesions of the OB revealed an apparent loss of granule cells with apparently minor changes in morphology of other neurones.

Table 1. Endogenous TRH levels (pg / mg wet weight)

Treatment	OB	HYP	AMG	HP
Controls	137 + 23	250 + 37	47 + 6	13 + 3
KA lesioned	64 + 13*	203 + 22	47 + 15	10 + 2

(n = 3–5 ; * $P < 0.02$)

In conclusion , this investigation has identified high affinity receptors for TRH in rat OB where high levels of the peptide are found (Table 1).The results of KA , 6-OH DA and surgical deafferentations suggest that bulbar TRH is intrinsically produced for local use and that the majority of TRH receptors here appear to be located on granule cells. A lower number of receptors also appear to be associated with terminals of dopaminergic and / or noradrenergic neurones .

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SK&F 83566 AND 83692: BENZAZEPINES WITH SELECTIVE AND STEREOSPECIFIC ACTIONS AT THE D_1 DOPAMINE RECEPTOR.

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The identification of the benzazepines SK&F 38393 and SCH 23390 as, respectively, selective agonists and antagonists at the brain D_1 dopamine receptor, has allowed the functional role of this site to be investigated; the activity of SK&F 38393 at the D_1 receptor is confined to the R-enantiomer, a configuration shared by SCH 23390. We have further investigated structural and stereochemical requirements for selective interactions with the D_1 receptor, using the new benzazepine derivatives R- and S- SK&F 83566 (7-bromo-SCH 23390), and SK&F 83692 (7-hydro-SCH 23390).

Relative potencies of drugs to displace the binding of 0.3nM ^{3}H-piflxol (^{3}H-PIF, D_1 receptors) and of 0.1nM ^{3}H-spiperone (^{3}H-SPIP, D_2 receptors) were assayed using rat striatal membranes (O'Boyle & Waddington, 1984). R- but not S-SK&F 83566 potently and stereoselectively displaced ^{3}H-PIF, while these drugs negligibly displaced ^{3}H-SPIP and with little stereoselectivity. SK&F 83692 displaced ^{3}H-PIF more potently than ^{3}H-SPIP (Table).

	IC_{50}(nM)		
	^{3}H-PIF	^{3}H-SPIP	$\frac{^3\text{H-PIF}(D_1)}{^3\text{H-SPIP}(D_2)}$
SCH 23390 (R)[a]	1.0±0.3	1,565±31	0.0006
SK&F 38393 (RS)[a]	2020±230	29,970±9810	0.068
SK&F 83566 (R)	1.9±0.6	2710±770	0.0007
SK&F 83566 (S)	561±215	11400±2200	0.049
SK&F 83692 (RS)	216±9	3440±840	0.063

Mean ± S.E mean (n=3). [a]From O'Boyle & Waddington (1984).

Substitution of Br for Cl at the 7 position in SCH 23390 (SK&F 83566) retained potent and specific activity at the D_1 receptor, and the R-Configuration was stereoselectively prefered. Substitution of H for Cl at the 7 position in SCH 23390 (SK&F 83692) retained a preference for the D_1 receptor but with a loss of affinity similar to that of SK&F 38393 (a7-OH compound). R-configuration benzazepines with 7-halogen-substituands appear to potently and stereoselectively recognise the D_1 but not the D_2 dopamine receptor.

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CLONIDINE REVERSES RESERPINE-INDUCED HYPOTHERMIA IN THE MOUSE VIA ACTIVATION OF CENTRAL POST-SYNAPTIC α_2-ADRENOCEPTORS ?

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The alpha$_2$-adrenoceptor agonist, clonidine, has been reported to inhibit reserpine-induced hypothermia in the mouse over the dose range 0.125-0.32mg.kg^{-1}p.o. (Gouret et al, 1977). Behavioural studies (Delini-Stula et al, 1979) indicate that clonidine is selective for alpha$_2$-adrenoceptors only at doses $\leq$0.1-0.2mg.kg^{-1}; higher doses also stimulate alpha$_1$-adrenoceptors. This report describes experiments designed to determine the location and the nature of the alpha-adrenoceptors involved in the anti-reserpine action of clonidine.

Groups of 8 female Tuck T/O strain mice (weighing 19-25g) per dose were used. Animals were injected with reserpine (2.5mg.kg^{-1} s.c.) and maintained at an ambient temperature of 18°C for 18 hours before receiving clonidine or vehicle (0.5% HPMC in distilled water, 10ml.kg^{-1}). Rectal temperatures were recorded prior to drug administration and at 2h intervals for up to 6h post-clonidine.

Clonidine produced a significant reversal ($p<0.05$, Students t-test) of reserpine-induced hypothermia over the dose range 0.032-1.25mg.kg^{-1} i.p. The dose-response relationship, however, was bell-shaped with the maximum effect (8-9°C increase in body temperature) evoked by a dose of 0.2mg.kg^{-1} of clonidine. When administered i.c.v. to reserpinized mice in sterile 0.9% saline, clonidine also evoked a thermogenic response, but was approximately twenty times more potent (dose per body weight) than when given i.p.

The selective alpha$_2$-adrenoceptor antagonists yohimbine (Starke et al, 1975), Wy 26392 (Lattimer et al, 1982) and idazoxan (Doxey et al, 1983) antagonized the hyperthermic action of clonidine (0.2mg.kg^{-1} i.p.) in reserpinized mice in a dose-related manner when given p.o. 30 min prior to clonidine. The doses of these agents calculated to inhibit the anti-reserpine effect of clonidine by 50% were 0.6, 1.2 and 0.25mg.kg^{-1} p.o. respectively (Linear regression analysis). Other experiments showed that the dose-response curve to clonidine in reserpinized mice was shifted to the right in a parallel manner by pretreatment with idazoxan, 0.3mg.kg^{-1} p.o.

Treatment of reserpinized animals with two doses of the catecholamine synthesis inhibitor, α-methyl-p-tyrosine (300mg.kg^{-1} i.p. 1h prior to-and 100mg.kg^{-1} 16h after reserpine) markedly attenuated (60-90%) the thermogenic action of the noradrenaline uptake inhibitor, desipramine (0.32-12.5mg.kg^{-1} i.p.). In contrast, the anti-reserpine effect of clonidine (0.032-0.2mg.kg^{-1} i.p.) was only slightly reduced (13-35%) by this type of pretreatment.

With regard to a) the sensitivity of the response to clonidine to challenge with selective alpha$_2$-antagonists, b) the relative lack of effect of inhibition of catecholamine synthesis, and c) the greater potency of clonidine i.c.v. than i.p.- it would appear that the thermogenic action of clonidine in reserpinized mice is mediated via activation of alpha$_2$-adrenoceptors, which are located postsynaptically within the central nervous system.

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THE EFFECTS OF KETOPROFEN, DEXAMETHASONE AND CORTICOTROPHIN ON THE HYPERTHERMIC EFFECT OF POLYINOSINIC-POLYCYTIDYLIC ACID

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Polyinosinic-polycytidylic acid (Poly I:C), an known interferon inducer, produces a hyperthermic response when given parenteally and which resembles endotoxin fever. (Lindsay et al, 1969). If this hyperthermia is a true fever then it should be inhibited by antipyretic agents. To prove this Poly I:C was administered both by intravenous injection and directly into the third cerebral ventricle of conscious rabbits in which rectal temperature was monitored continuously. The effect of the non-steroidal anti-inflammatory drug ketoprofen, the steroid dexamethasone and the hormone corticotrophin (ACTH) on this response was studied.

Poly I:C in a dose of 250 µg/kg i.v. was found to produce a characteristic biphasic hyperthermic response, this dose being sub-maximal. Ketoprofen (2 mg/kg s.c.) when administered 30 minutes before the Poly I:C completely suppressed the subsequent reponse. In addition ketoprofen in the same dose produced complete antipyresis when administered after the febrile response had been initiated. This was true whether the ketoprofen was given during the development of either the first or second phase or during the plateau phases. Poly I:C in a dose of 12.5 µg when injected into the third cerebral ventricle produced a long lasting monophasic hyperthermia. This also was completely suppressed by ketoprofen. In contrast, dexamethasone (2 mg/kg i.v.) given 1 hour before the Poly I:C (250 and 50 µg/kg i.v.) had no significant effect on the developing hyperthermia. Similarly when dexamethasone was administered during the plateau phase following i.c.v. injection of Poly I:C there again was no significant antipyresis. ACTH (1 unit/kg) produced a fall in temperature when given after the initiation of a febrile response by Poly I:C, however a similar hypothermia was produced in control animals receiving just ACTH. From these results it is concluded that Poly I:C is producing a true febrile response. The inability of either dexamethasone or ACTH to attenuate the febrile response is at the present time not understood.

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THE SEROTONIN AGONIST 8-OH-DPAT ELICITS FEEDING IN NON-DEPRIVED RATS

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There is considerable pharmacological evidence to indicate that serotonin may be involved in the neural regulation of food intake. Thus, drugs which stimulate central serotonin mechanisms (e.g. fenfluramine) reduce food intake and body weight in animals and man (Blundell and Latham, 1978). Recently, an ergot congener 8-hydroxy-2 (di-n-propylamino) tetralin (8-OH-DPAT) has been synthesized, which is claimed to possess potent and selective agonist activity at central serotonergic receptors (Ahlenius et al., 1981). Administration of 8-OH-DPAT to male rats produced a behavioural syndrome which is characteristic of activation of postsynaptic serotonin receptors (flat body posture, headweaving, forepaw treading, tremor) and, in addition, stimulated sexual behaviour (Ahlenius et al., 1981). The latter finding was somewhat surprising as sexual behaviour is well known to be enhanced after serotonin synthesis is inhibited. The present study shows that 8-OH-DPAT has a similarly paradoxical effect on food intake as this was enhanced in drug treated rats. The subjects (96, adult male Sprague-Dawley rats weighing 250-350 g) were housed in individual cages on a 12 h dark/light cycle (lights on 6 a.m.) with food and water freely available. The rats were placed in individual test cages for overnight habituation prior to a 2 hour test the next day. Drug injections were administered SC in a saline vehicle at doses of 0, 3.75, 7.5, 15, 30, 60, 125, 250, 500, 1000, 2000 and 4000 μg/kg (n = 8 rats per group). At the start of the test each animal was given a weighed amount of food (3 standard pellets) and 3 wood blocks; water was freely available. At the end of the 2 hour test the food was reweighed and intake calculated. In addition, the behaviour of the rats was continuously recorded on video tape for subsequent analysis using a microcomputer. Only 1/8 control animals exhibited a feeding response (intake 0.2 g). In contrast, 8-OH-DPAT treatment produced a striking increase in food intake. Kruskal Wallis ANOVA confirmed that 8-OH-DPAT had a significant effect on feeding ($H = 35.6$, 11df, $p < 0.001$). Individual comparisons by Mann Whitney U-test revealed that 15, 30, 60, 125, 250, 500, 1000, 2000 and 4000 μg/kg 8-OH-DPAT elicited significant increases in food intake. Analysis of video recordings indicated that the elicited response was specific to feeding since drug-treated rats did not gnaw wood blocks to a greater extent than controls. Furthermore, the feeding elicited at low doses (15-60 μg/kg) was independent of various motor effects which are only apparent at high doses of 8-OH-DPAT (125-4000 μg/kg). It has been proposed on the basis of receptor binding studies that 8-OH-DPAT may be a selective serotonin autoreceptor agonist (Gozlan et al., 1983). The present finding of 8-OH-DPAT-induced feeding appears to be consistent with this proposal.

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EFFECT OF pH ON THE BINDING OF [^{3}H]-PRAZOSIN TO RAT CEREBRAL CORTEX

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Prazosin, a selective α_1-adrenoceptor antagonist, has been used extensively in direct binding studies on the α_1-adrenoceptor (Bylund and U'Pritchard, 1983). In preliminary experiments, we found the affinity of this ligand to vary considerably when experiments were performed at different pH. It was of interest, therefore, to characterise further these effects of pH on the binding of [^{3}H]-prazosin.

In all experiments a Tris EDTA (50 mM Tris, 5 mM EDTA; pH of assay buffer) washed, rat cerebral cortex membrane preparation was used. Experiments were performed at pH 7.0, 7.8 or 8.6 in a Tris-EDTA buffer system (Tris 50 mM, EDTA 0.5 mM). In saturation binding experiments [^{3}H]-prazosin (1×10^{-11} to 8×10^{-9} mol.litre^{-1}) and the membrane preparation (0.5 mg.prot.ml^{-1}) were incubated in a total volume of 1 ml at 25°C for 45 min. For competition studies fixed [^{3}H]-prazosin concentrations of 0.1, 0.5 or 1 nM were used at pH 7, 7.8 and 8.6 respectively. After equilibration bound ligand was separated from free ligand by vacuum filtration. Non-specific binding was defined using 1×10^{-5} mol.litre^{-1} phentolamine. Data were analysed using the iterative non linear curve fitting programme LIGAND (Munson & Rodbard, 1980).

Under the three conditions of pH employed in these experiments [^{3}H]-prazosin labelled a homogenous population of sites. The Kd value for [^{3}H]-prazosin however, showed marked differences with a decrease in the affinity of the ligand as the pH of the assay medium increased [Kd (nM): pH 7.0 = 0.065; pH 7.8 = 0.12; pH 8.6 = 0.29]. Between pH 7.0 and 7.8 no difference in Bmax was detected (169 and 170 fmol.mg protein^{-1} at pH 7.0 and 7.8 respectively). At pH 8.6 the Bmax was significantly reduced (102 fmol.mg protein^{-1}).

Competition experiments were performed to determine if the change in affinity of [^{3}H]-prazosin was related to the prazosin molecule itself or the binding site. The affinity of phentolamine, imiloxan (RS-21361), idazoxan, clonidine, noradrenaline and amidephrine was significantly (two to three fold) greater at pH 7.8 than at pH 7.0. The affinity of the compounds at pH 8.6 although significantly greater than at pH 7.0 was not different to the affinity obtained at pH 7.8. For all the compounds studied, the effect of pH on the affinity of the competitors was opposite to that observed for [^{3}H]-prazosin, indicating that the effect of pH on the binding of [^{3}H]-prazosin was related more to changes in the prazosin molecule than to an effect on the binding site.

These results demonstrate that the affinity of both [^{3}H]-prazosin and competing ligands for the α_1-adrenoceptor may be markedly influenced by the assay pH.

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THE EFFECTS OF NICOTINE ON THE BEHAVIOUR OF TWO STRAINS OF RATS IN A COMBINED MAZE-OPEN FIELD

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There is indirect evidence to suggest that nicotine may alleviate the effects of stress in rats although there appear to be no reports of experiments designed to test this hypothesis directly (Balfour, 1982). In this study the effects of nicotine were investigated in two inbred strains of rats (Roman High Avoidance (RHA/Verh) and Roman Low Avoidance (RLA/Verh), reported to differ in their levels of emotionality and response to nicotine (Driscoll and Battig, 1982), using a combined maze-open field apparatus in which a central open field was illuminated to provide an aversive stimulus (Martin et al, 1982).

Groups of rats of each strain (n = 9 or 10/gp) were given daily subcutaneous injections of saline (1ml/kg) or nicotine (0.4mg/kg) and, after each injection, placed in the maze for 20 minutes. Their activity in the maze was monitored remotely and stored on a PDP11 computer for subsequent analysis by multivariate analysis of variance (Battig, 1983).

The RLA/Verh rats were less active than the RHA/Verh rats throughout the experiment ($F(1,33) = 27.3$; $p < 0.001$). Nicotine initially depressed the total activity of both strains of rats (day 1) whereas, from day 4, a stimulatory effect was observed. The percent of the total activity occurring in the outer part of the maze, away from the illuminated area, decreased with the number of days of treatment ($F(9,29) = 14.2$; $p < 0.001$) whereas both the number of open field crossings, expressed as a percent of total activity ($F(9,297) = 21.6$; $p < 0.001$) and the mean time spent in the open field ($F(9,297) = 11.7$; $p < 0.001$) increased. The rats also approached the open field more often ($F(9,297) = 9.6$; $p < 0.001$). In the RHA/Verh rats nicotine had no significant effect on the relative number of open field crossings or approaches to the open field or on the time spent in the open field. In the RLA/Verh rats nicotine reduced the number of rats which entered the open field on each day of the experiment. In this strain nicotine increased the proportion of the total activity occurring in the outer part of the maze ($F(1,11) = 11.2$; $p < 0.001$) and decreased the relative number of approaches to the open field ($F(1,17) = 12.0$; $p < 0.001$). Interestingly on day 1, a higher proportion (7/9) of the saline-treated RLA/Verh rats entered the open field than the saline-treated RHA/Verh rats (5,9) and, of the nicotine-treated groups, 5/9 of the RHA/Verh rats entered the central field whereas only 1/10 of the RLA/Verh rats entered.

The study failed to support the hypothesis that nicotine can alleviate the effects of an aversive stimulus and indeed suggested that, in some circumstances, it may enhance the effects of such stimuli.

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We have previously reported that AH 19437 is a specific thromboxane receptor blocking drug (Coleman et al, 1981 a,b), and this compound proved a valuable tool in the development of the hypothesis that distinct receptors exist for each naturally-occurring prostanoid (Kennedy et al, 1982). More potent analogues of AH 19437 have since been identified and we now describe the thromboxane receptor blocking actions of one of these, AH 23848 ([1α(Z),2β,5α]-(±)-7-[5-[[(1,1 -biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid).

Guinea-pig lung strip (GPL) and dog saphenous vein (DSV) containing TxA_2-sensitive (TP-) receptors, guinea-pig fundus (GPF), cat trachea (CT) and chick ileum (ChI), all containing PGE_2-sensitive (EP-) receptors, and dog iris (DI) containing $PGF_{2\alpha}$-sensitive (FP-) receptors, were prepared as described by Kennedy et al, 1982. Preparations were suspended in organ baths, except for experiments in which TxA_2 was studied, in which case cascade superfusion was employed (Coleman et al, 1981c). Antagonist activity was determined as described by Kennedy et al (1982).

AH 23848 (10^{-9} - 10^{-6} mol/l) inhibited contractile responses of GPL and DSV to the stable TxA_2-mimetic, U-46619 (Coleman et al., 1981c), causing concentration-related parallel shifts to the right of concentration-effect curves. Analysis of these results (Arunlakshana & Schild, 1959) gave pA_2 values of 9.5 (9.0-10.3, n=7) on GPL and 8.3 (7.4-9.1, n=4) on DSV. In neither case did the slope of the Schild plot differ significantly from unity ($p>0.05$). The effects of AH 23848 on these preparations were specific, in that at a concentration of 10^{-4} mol/l, the compound had no effect on contractile responses of GPL to histamine or of DSV to 5-hydroxytryptamine. When tested in cascade superfusion at 10^{-7} mol/l, AH 23848 antagonised responses of GPL and DSV to U-46619 (dose ratios 84, range 40-150, n=3, and 13, range 5-30, n=3 respectively) and TxA_2 (dose-ratios 22, range 9-57, n=3, and 17, range 8-88, n=3 respectively). These effects were specific in that responses of GPL to acetylcholine and DSV to 5-hydroxytryptamine were unaffected. AH23848 had little or no antagonist activity at other prostanoid receptor types. Thus AH 23848 (10^{-5} mol/l) had no effect on contractile responses of DI to either $PGF_{2\alpha}$ or U-46619, of ChI to PGE_2 or U-46619, or of GPF to PGE_2, although it did weakly antagonize contractile responses of GPF to U-46619 (concentration ratio = 10.4 (6.0-18.0, n = 12). Furthermore, AH23848 (10^{-5} mol/l) had no effect on relaxant responses of CT to PGE_2.

In conclusion, AH 23848 qualitatively resembles AH 19437 in that it selectively blocks TP-receptors, but quantitatively AH 23848 is approximately 100 times more potent on DSV and 1000 times more potent on GPL. The difference in potency of AH 23848 on DSV and GPL warrants further investigation, since it supports the proposition that subtypes of TP-receptor exist (see Coleman et al., 1984).

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THE EFFECTS OF AH23848, A NOVEL THROMBOXANE RECEPTOR BLOCKING DRUG, ON PLATELETS AND VASCULAR SMOOTH MUSCLE

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The pharmacology of the potent thromboxane receptor blocking drug, AH23848 (Brittain et al., 1984), has been examined further on vascular smooth muscle and on platelets in vitro and in vivo.

The methods used for the in vitro studies have been described in detail elsewhere (Lumley & Humphrey, 1981; Kennedy et al., 1982). When TxA_2 was used it was prepared by incubation of PGH_2 with human platelet microsomes. The experiments in anaesthetised dogs were carried out under barbitone anaesthesia and mesenteric blood flow was measured using an electromagnetic flow probe as previously described (Lumley et al., 1982).

AH23848 competitively and specifically antagonised the contractions of strips of human isolated pulmonary vessels and rat isolated thoracic aorta produced by U-46619 with mean pA_2 values and slopes of Schild regressions (95% confidence limits) of 7.82 (7.51-8.13) and 0.96 (0.75-1.17), n = 8, and 7.90 (7.52-8.28) and 1.05 (0.76-1.34), n = 4, respectively. AH23848 (1.0×10^{-8} - 1.0×10^{-7} mol/l) also antagonised TxA_2- and PGH_2-induced contractions of rat aortic strips but had no effect on 5-HT or potassium chloride induced contractions even at 1.0×10^{-5} mol/l.

On human platelets in whole blood AH23848 specifically antagonised U-46619-induced platelet aggregation with a mean pA_2 value and slope of Schild regression of 7.83 (7.58-8.08) and 1.28 (0.95-1.61),n = 4. AH23848 also antagonised TxA_2-, PGH_2- and collagen-induced human platelet aggregation (approximate pA_2 against TxA_2 of 8.15) but had no effect on ADP-induced aggregation even at 1.0×10^{-5} mol/l.

In the anaesthetised dog AH23848 (10 - 300 µg/kg i.v.) specifically antagonised U-46619-induced vasoconstriction in the mesenteric arterial bed and inhibited collagen-induced platelet aggregation ex vivo giving DR_{10} values (intravenous dose to produce a ten-fold rightward shift of the agonist dose-response curve) of 125 (43 - 358), n = 5, and 99 (38 - 254), n = 4, µg/kg respectively. When administered orally to conscious dogs AH23848, 0.1 and 1.0 mg/kg, specifically antagonised collagen-induced platelet aggregation ex vivo resulting in peak collagen concentration-ratios (collagen EC_{50} after drug divided by collagen EC_{50} before drug) of 7 (5- 11), n = 10, and 23 (12 - 46), n = 5 respectively. The peak effect occurred within one hour of dosing and a significant effect remained even after 11 hours at the higher dose. Repeated oral dosing with AH23848 (1 mg/kg at 12 hourly intervals for 7 days) in the conscious dog resulted within 24 hours in a sustained antagonism of platelet aggregation with a minimum collagen concentration-ratio ex vivo of at least 15 over the remaining period of dosing.

Thus AH23848 is a potent, specific, orally active, long lasting thromboxane receptor blocking drug which is active in various animal models of occlusive vascular disease (e.g. see Coker & Parratt, 1984).

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EFFECTS OF A NEW THROMBOXANE RECEPTOR ANTAGONIST, AH23848, ON THE ACUTE CARDIOPULMONARY RESPONSES TO E.COLI ENDOTOXIN IN CATS

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The intravenous administration of E. coli endotoxin to anaesthetised cats results in a fall in arterial blood pressure (MABP), increases in pulmonary arterial pressure (PAP) and in airways resistance, and a reduced lung compliance (Parratt, 1973; Parratt et al., 1982). Thromboxane synthetase inhibition with dazoxiben attenuates the pulmonary hypertension. However, it also augments plasma concentrations of both $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$, the major metabolite of the vasodilator PGI_2 (Ball & Parratt, 1983) and this complicates the interpretation of results. The aim of the present study was to investigate the role of thromboxane in the early stages of endotoxaemia using the new thromboxane receptor antagonist AH23848 (Brittain et al., this meeting).

Cats of either sex were anaesthetised with sodium pentobarbitone and prepared for cardiopulmonary measurements (Parratt, 1973). AH23848 (1 mg kg^{-1} i.v.) or vehicle (10% ETOH in 2 ml saline) was administered i.v. 30 min prior to E. coli endotoxin (2 mg kg^{-1} over 20s). AH23848 alone caused marked but transient effects on PAP and MABP and intratracheal pressure (ITP). For example, MABP increased within 10-20s from 104±10 mmHg to a peak of 125±9 mmHg ($P<0.001$) but returned to 101±10 mmHg after 5 min. The results of endotoxin administration were:-

Pretreatment		Pre-endo.	2	7	30 (min) post-endo.
Vehicle	MPAP	16±1	34±4***	38±2***	19±2
AH23848	(mmHg)	17±1	26±2†	20±2†††	16±1
Vehicle	MABP	104±6	59±9*	82±10	82±9
AH23838	(mmHg)	105±6	104±8††	89±8	90±6
Vehicle	ITP	6.5±0.3	10.9±1.6*	11.2±1.4*	9.3±1.0*
AH23838	(mmHg)	6.2±0.4	7.2±0.5†	7.1±0.5†	6.2±0.4†

*$P<0.05$, **$P<0.01$, ***$P<0.001$; comparison with pre-endo. value; Wilcoxon test.
$^{\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.001$; comparison between group values; Mann Whitney U-test.

Pretreatment with AH23848 thus prevented the pulmonary hypertensive effect of endotoxin and markedly attenuated its effect on ITP. After endotoxin arterial PO_2 fell from 77±5 mmHg to 46±6 mmHg ($P<0.01$, at 30 min); AH23848 attenuated this (81±4 mmHg to 69±7 mmHg ($P<0.05$). These results support the hypothesis that thromboxane is largely responsible for the early cardiopulmonary effects of endotoxin.

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ANTIARRHYTHMIC ACTIVITY OF THE THROMBOXANE ANTAGONIST AH23848 DURING CANINE MYOCARDIAL ISCHAEMIA AND REPERFUSION

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We have demonstrated previously that thromboxane A_2 is released into blood draining from the acutely ischaemic canine myocardium and proposed that it may be an arrhythmogenic factor during ischaemia (Coker et al, 1981) and following reperfusion (Coker et al, 1982). The present study was designed to investigate this hypothesis further, by examining the effect of the thromboxane antagonist AH23848 (Brittain et al, 1984) on arrhythmias induced by coronary artery occlusion and reperfusion.

Chloralose anaesthetised, open-chest greyhounds were prepared for occlusion of the left anterior descending coronary artery (LAD). Catheters were placed in the coronary sinus draining the essentially normal myocardium, and in a local coronary vein draining the area rendered ischaemic by occlusion of the LAD. The plasma concentrations of thromboxane B_2 (the stable breakdown product of thromboxane A_2) and 6-keto $PGF_{1\alpha}$ (a metabolite of prostacyclin) were measured by radioimmunoassay.

The administration of AH23848, 1 mg/kg i.v. 30 min prior to LAD occlusion, caused slight transient increases in arterial blood pressure, left ventricular pressure (LVP) and LVdP/dt max. Plasma prostanoid concentrations in the aorta, the coronary sinus and the local coronary vein were not altered by AH23848. During coronary artery occlusion the release of thromboxane and prostacyclin into blood draining from the ischaemic myocardium was similar in both control and drug-treated dogs. For example, by 7 min post-occlusion local coronary venous thromboxane B_2 concentrations had increased from 51 ± 7 to 142 ± 31pg/ml in controls, and from 97 ± 25 to 186 ± 55 pg/ml in dogs which received AH23848 (values are mean ± s.e. mean; n = 9).

Pretreatment with AH23848 reduced the number of ectopic beats that occurred during the first 30 min of myocardial ischaemia to 339 ± 111 compared with 736 ± 153 in controls ($P = 0.05$, Mann-Whitney U-test). One dog in each group died in ventricular fibrillation during coronary artery occlusion. Release of the ligature around the LAD after 40 min of ischaemic caused ventricular fibrillation in 7 out of 8 control dogs, usually within 2 min of reperfusion. The incidence of reperfusion-induced ventricular fibrillation was reduced to 2 out of 8 in the dogs which received AH23848 ($P<0.05$, Chi-squared test). The occluded zone or 'area at risk' was similar in both groups; 35 ± 1% of the free left ventricular wall in controls and 33 ± 2% in the AH23848 group. The magnitude of the ST-segment depression which had developed after 30 min of ischaemia was also similar; 0.38 ± 0.07 mV and 0.33 ± 0.10 mV respectively.

These results indicate that the thromboxane antagonist AH23848 reduced ischaemia-induced arrhythmias by approximately 50% and markedly suppressed ventricular fibrillation following reperfusion. Thus, this study provides further evidence that thromboxane A_2 may be arrhythmogenic during myocardial ischaemia and may be a particularly important factor in the genesis of reperfusion-induced ventricular fibrillation.

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IS THE URINARY EXCRETION OF PGE FROM THE RAT INFLUENCED BY CHANGES IN URINE FLOW?

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Urine pH has recently been demonstrated to be a major determinant of renal PGE excretion when studied at constant urine flow in the conscious rat (Haylor et al 1984 a,b). In the present investigation we have performed a parallel experiment to determine whether the urine flow is a determinant of urinary PGE excretion from the rat, when urine pH is maintained at a constant level. The importance of studying the influence of urine flow on urinary PGE excretion relates to the use of this measurement as an index of renal PGE synthesis (Frolich et al 1975). Drugs which have been proposed, from urinary measurements, either to increase (e.g. frusemide, clonidine) or to decrease (e.g. indomethacin) renal PGE synthesis, also produce parallel changes in urine flow.

Female Wistar rats (Sheffield University 200-250g body weight) received an oral load of ammonium chloride (NH_4Cl)1%, 2ml/100g body weight 1-hour prior to anaesthesia (sodium thiobutabarbitone 150mg/kg i.p). Each rat received a continuous intravenous infusion of NH_4Cl 1% delivered at 175 ul/min into the right jugular vein. Urine samples were collected from a catheter placed in the bladder via a small abdominal incision, at 15 minute intervals over a 2-hour period. All urine sample were analysed for pH, osmolality and electrolytes, while the remainder of each sample was immediately adjusted to pH 5 with HCl and stored at $-20^{o}C$ awaiting assay. The PGE content of urine was measured by a radio-immunoassay procedure following prior extraction into acid media and column chromatography (Haylor et al 1984b). Each assayed sample was adjusted for recovery.

Time period (min)	45 - 60	60 - 75	75 - 90	90 - 105	105 - 120
Urine flow (ul/min)	42 ± 8	84 ± 13	134 ± 13	177 ± 14	221 ± 10
Urine pH	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
Urine PGE excretion rate (pmol/hr)	61 ± 7.1	52 ± 6.4	59 ± 2.5	71 ± 3.3	87 ± 6.4
Urine osmolality (mosm/kg.H_2O)	755 ± 81	544 ± 46	422 ± 15	401 ± 7	382 ± 10

A positive linear correlation was obtained ($r = 0.89$ $P<0.002$) between urine flow and urinary PGE excretion when only samples with a urine osmolality below 500 mosm/kg.H_2O were included.

The results indicate that (a) the rat does not exhibit a species difference in the relationship between urine flow and urinary PGE excretion as proposed by Fejes-Toth et al (1983), (b) the urinary excretion rate of PGE may not be able to be used as an index of renal PGE synthesis unless changes in urine flow are taken into account.

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ON THE MECHANISM OF DEXTRAN-INDUCED HISTAMINE SECRETION FROM RAT PERITONEAL MAST CELLS

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The polysaccharide dextran is an effective releaser of histamine from isolated rat peritoneal mast cells. However, the detailed mechanism involved in this response is not known. In particular, it is unclear whether the compound interacts with specific glucoreceptors on the mast cell membrane (Moodley et al, 1982) or rather combines with and cross links cell-fixed IgE antibody (Hanahoe, 1984). The present study is aimed at distinguishing between these possibilities.

Rat peritoneal mast cells were obtained by direct lavage and histamine secretion was determined as previously reported (White & Pearce, 1982). Dextran (Fisons, mol. wt. 110000) and anti-rat IgE (Miles) were used as secretory stimuli and lysophosphatidylserine (Sigma, 1 μM) was used to enhance the response. In some experiments, animals were first sensitized to the nematode *Nippostrongylus brasiliensis* and cells subsequently challenged with specific antigen (White & Pearce, 1982).

A number of experimental approaches were then adopted. (1) Histamine release induced by anti-IgE was inhibited in dose-dependent manner by purified, soluble myeloma rat IgE. However, this protein had only a weak and variable effect on secretion evoked by dextran. (2) Glucose and polyglucoses of low molecular weight (1030) blocked histamine release produced by dextran but not that produced by anti-IgE or antigen. (3) Mast cells derived from specific pathogen-free rats, which might be expected to possess negligible amounts of natural cell-fixed antibody, accordingly showed a very weak response to challenge with anti-IgE but responded normally to dextran. (4) Antigen and dextran showed different patterns of desensitization. Maximal desensitization to antigen was observed by challenge in a medium free of divalent cations followed by subsequent addition of lysophosphatidylserine and calcium together, whereas maximal desensitization to dextran was observed by challenge in the presence of calcium followed by subsequent addition of the lipid alone. In cross-desensitization experiments, primary challenge with antigen desensitized the cells to subsequent stimulation with both antigen and dextran but initial challenge with dextran produced desensitization to this stimulus alone. (5) In keeping with previous reports (Holgate et al, 1980), stimulation with anti-IgE or antigen induced a transient elevation in the intracellular concentration of cyclic AMP. This change accompanied or preceded histamine release and has been suggested to be an integral part of the activation sequence in the mast cell for IgE-directed ligands. In sharp contrast, dextran induced release of histamine with no concomitant elevation of cyclic AMP.

In total, the present study shows that there are fundamental biochemical differences between histamine secretion induced by dextran and IgE-directed ligands and that the former compound can activate the mast cell independently of cell-fixed IgE antibody. As such, our data are consistent with an interaction of the polysaccharide with specific glucoreceptors on the mast cell membrane.

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EFFECTS OF α_2-ANTAGONISTS AND $\alpha\beta$-METHYLENEATP ON RESPONSES OF RAT VAS DEFERENS TO TWIN PULSE FIELD STIMULATION

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Desheathed rat vása suspended in Krebs solution gassed with 5% CO_2 in O_2 were maintained at 37°C and equilibrated at 0.5 g resting tension. Tension responses to twin pulse field stimulation (TPFS - 0.8 ms width, 33V every 5 min) were digitised at a sampling frequency of 100 Hz and stored on floppy disc under the control of a BBC microcomputer (developed from Marshall & Sparks, 1981). The interval separating the second stimulus from the first of each TPFS was varied between 3 and 120 s. The minimum interval of 3 s ensured that baseline tension was re-established before the second stimulus was applied. The response to the first stimulus had the characteristic two components, a first peak at 300 ms with the second phase prominent at 600 msec. At 3 s separation the second response differed from the first in having a greater first peak height and a depressed second phase component. The latter result is compatible with NA released by the first pulse impairing the NA release evoked by the second pulse. These differences progressively diminished as the separation-interval was increased, the second response approaching the shape and size of the first response which remained constant throughout. Thus any auto-feedback inhibition becomes ineffective within 2 to 5 min.

In the following experiments a separation-interval of 3 s was used for TPFS. α_2-adrenoceptor blockade might be expected to restore the inhibited second phase (600 ms) in the second response, but yohimbine (10^{-9} to 10^{-6} M) failed to do so. At 10^{-9} M yohimbine) elicited a small overall potentiation of both responses to TPFS: at 10^{-8} and 10^{-7} M there was a larger overall potentiation of the second response; this exceeded the potentiation of the first response which was confined to the first peak (300 ms): at 10^{-6} M both responses showed a potentiated first peak but the second phase of the first response was considerably inhibited. Another selective (Michel et al., 1981) α_2-antagonist RS 21361 (10^{-9} to 10^{-6} M) produced similar effects with potentiation of the first peaks predominating in both responses; at 10^{-6} M a marked inhibition of the second phase of the first response only was evident. These results do not readily conform with the hypothesis of an α_2 mediated inhibition of the noradrenergic component of the 2nd response.

$\alpha\beta$-methyleneadenosinetriphosphate (MATP, 10^{-5} M) a P_2 purinoceptor-desensitizer (Meldrum & Burnstock, 1983) contracted the vas on the first but not the subsequent application. Both responses to TPFS were depressed overall in the presence of MATP. However, after washout the second phases of both responses recovered more quickly than did the first peaks.

It is evident from these results that factors other than α_2-feedback inhibition may be important in the interaction between responses of the vas to successive stimuli. The dramatic effects of MATP in this system suggest the possibility of purinergic involvement affecting all aspects of the responses.

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UPTAKE AND RELEASE OF 5-HYDROXYTRYPTAMINE BY SYMPATHETIC NERVE TERMINALS IN RAT ATRIA

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There is a high content of 5-hydroxytryptamine (5-HT) in rat atria, much of it being associated with granules within atrial muscle cells (Theron et al, 1978). However, there have been reports that 5-HT can be accumulated in noradrenergic sympathetic nerves in blood vessels (Verbeuren et al, 1983) and vas deferens (Thoa et al, 1969). The possibility that noradrenergic sympathetic nerves in rat atria could also accumulate 5-HT was explored in the present study. To this end, the uptake of ^{3}H-5-HT and the subsequent release of radioactivity by field stimulation of intramural nerves was investigated in isolated atria from control rats and rats pretreated with 6-hydroxydopamine (6-OHDA, 50 mg/mk., i.v., 72 and 48 h before use), and similar experiments were also carried out with ^{3}H-noradrenaline (^{3}H-NA).

Rat isolated atria suspended in Krebs-Henseleit solution bubbled with 95% O_2/5% CO_2 and maintained at 37°C were incubated with 0.5 μM of ^{3}H-5-HT or 0.1 μM of ^{3}H-NA for 10 min. It had been previously established that these concentrations were optimum for uptake of the respective amines. After a 15 min period of repeated washing with drug-free solution, the content of radioactivity in the atria was determined. The tissue/medium ratios for radioactivity retained in the atria were 2.08 (s.e.mean = 0.07, n = 3) after incubation with ^{3}H-5-HT and 6.36 (s.e.mean = 0.26, n = 4) after ^{3}H-NA. In atria from 6-OHDA-pretreated rats, the corresponding ratios were 1.58 (s.e.mean = 0.06, n = 5) and 1.01 (s.e.mean = 0.09, n = 5). These findings indicate that destruction of sympathetic nerves within the atria results in a decrease in 5-HT uptake.

In experiments on the release of radioactivity, atria were incubated for 20 min with 0.5 μM of ^{3}H-5-HT or 0.1 μM of ^{3}H-NA, then washed with drug-free solution for 60 min: midway through this time, a priming stimulus (1 ms pulses at 1 Hz for 30 s) was given to facilitate removal of non-specifically bound radioactive compounds. The effluxes of radioactivity induced by three periods of stimulation (1 ms pulses at 2 Hz for 30 s) at 30 min intervals were studied. In the first period, the stimulation-induced (S-I) releases were 3050 d/min (s.e.mean = 531, n = 8) and 9912 d/min (s.e.mean = 1305, n = 6) for atria incubated with ^{3}H-5-HT and ^{3}H-NA, respectively. The S-I releases of radioactivity were maintained at these levels for the second and third periods. When the second period of stimulation was carried out in calcium-free solution, the S-I effluxes of radioactivity were reduced to 10.6% (s.e.mean = 5.2, n = 5) and 16.7% (s.e.mean = 5.2, n = 3) of control values for ^{3}H-5-HT and ^{3}H-NA, respectively. When tetrodotoxin (0.25 μM) was present during the second period of stimulation, the S-I effluxes were zero (n = 4) for ^{3}H-5-HT and 2.75% (s.e.mean = 1.0, n = 4) of control for ^{3}H-NA. In atria from 6-OHDA-pretreated rats, the S-I effluxes were about 3% and 21% for ^{3}H-5-HT and ^{3}H-NA, respectively, of values for atria from untreated rats.

These findings indicate that 5-HT, like NA, that has been taken up into sympathetic nerve terminals can be subsequently released by nerve impulses.

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RELATIONSHIP OF METABOLISM OF ADENINE NUCLEOTIDES TO PRESYNAPTIC INHIBITION OF TRANSMISSION IN RAT VAS DEFERENS

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The 5'-adenine nucleotides (AMP, ADP and ATP) caused presynaptic inhibition of neurotransmission in rat vas deferens and this action was apparently dependent on their hydrolysis to adenosine (Clanachan et al, 1977; Willemot and Paton, 1981). A series of 2' and 3' substituted adenine nucleotides also produced presynaptic inhibition in vas. However, these nucleotides appeared to have direct actions as their activities were not reduced by addition of adenosine deaminase (Willemot and Paton, 1981). The aim of the present study was to investigate further the mechanism of action of adenine nucleotides in rat vas deferens by using HPLC to follow their metabolic fate.

Longitudinal contractions of the mid-portions of rat vasa deferentia were recorded isometrically at 37°C and elicited by continuous field stimulation at 0.2 Hz. Dose-responses curves were constructed by cumulative addition of nucleotide to the bath and the concentration producing 50% inhibition of contractions (IC_{50}) determined by linear regression. In separate studies, adenine nucleotides (30 μM) were added to the bath for 5min. Aliquots of the bathing medium were then analysed by HPLC using a Waters M440 dual channel detector at 254 and 280 nm. The minimal detectable amount of purine was approximately 2 pmoles per injection.

After 5min exposure to AMP, ADP, ATP and NAD, the bathing medium contained 2.1 ± 0.5, 1.3 ± 0.2, 1.2 ± 0.4 and 0.35 ± 0.08 μM adenosine respectively (mean ± s.e. mean). No evidence was obtained that the other nucleotides studied (2'-AMP; 3'-AMP; 2',3'-cyclic AMP; 2'5'-ADP; 3'5'-ADP; NADP; cyclicNADP) were hydrolysed to adenosine. Since no adenosine was detectable, the bathing medium contained < 0.03 μM adenosine.

All the nucleotides studied produced inhibition of responses to field stimulation, mean IC_{50} values ranging from 5-32 μM. All were antagonised by 30 μM theophylline, mean IC_{50} values being greater than 100 μM.

The nucleotides studied thus produced presynaptic inhibition of neurotransmission in rat vas deferens through activation of a P_1-purinoceptor. The actions of AMP, ADP and ATP at this receptor were to a considerable extent indirect since these nucleotides were partially hydrolysed to adenosine (Willemot and Paton, 1981; present study) while their actions were significantly reduced by addition of adenosine deaminase (Willemot and Paton, 1981). However, the other nucleotides studied all acted directly at this presynaptic receptor since they were not subject to hydrolysis to adenosine. It is possible that NAD acted partially indirectly since it was hydrolysed to adenosine to a small extent (present study) and its action was reduced slightly by exogenous adenosine deaminase (Willemot and Paton, 1981).

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COMPARISON OF ACTIVITIES OF MUSCARINIC AGONISTS ON RABBIT AORTA AND GUINEA PIG ILEUM

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Stimulation of the muscarinic receptor present on the endothelium produces vascular smooth muscle vasodilatation (Furchgott & Zwadski, 1980). The muscarinic receptor mediating this response, may be distinct from those muscarinic receptors present in the CNS, myocardium or G.I. tract (Eglen & Whiting, 1984). The purpose of the present study is to determine if there is differential activities of muscarinic agonists on gastrointestinal and vascular smooth muscle.

The agonist potencies (pD_2) and intrinsic activities for the two tissues, obtained as described previously (Clague et al, 1984; Eglen & Whiting, 1984), are shown in Table 1. Acetylcholine, carbachol, methacholine, bethanechol and muscarine exhibited little or no selectivity for either tissue. Acetyltropine methiodide and 3-acetoxy methyl piperidine methiodide (3-AMP) were significantly ($p < 0.01$) selective towards those receptors present on the aorta. In contrast, oxotremorine, pilocarpine and suberyldicholine exhibited highly significant ($p < 0.001$) selectivity towards the receptors present on the ileum.

Table 1 Muscarinic agonist potencies (pD_2) and intrinsic activities (α)

AGONIST	ILEUM		AORTA	
	pD_2	α	pD_2	α
Acetylcholine	6.15 ± 0.08	1.0	6.70 ± 0.10	1.0
Carbachol	6.45 ± 0.06	1.0	6.30 ± 0.08	1.0
Methacholine	6.22 ± 0.05	1.0	6.52 ± 0.06	0.5
Bethanechol	5.30 ± 0.06	1.0	5.00 ± 0.10	0.6
Oxtremorine	7.22 ± 0.10	1.0	6.05 ± 0.07	0.5
Muscarine	5.52 ± 0.06	1.0	6.10 ± 0.10	1.0
Acetyltropine	4.22 ± 0.03	1.0	5.52 ± 0.11	1.0
3-AMP	3.52 ± 0.04	1.0	4.40 ± 0.07	0.4
Suberyldicholine	5.52 ± 0.07	1.0	NO EFFECT	-
Pilocarpine	5.30 ± 0.06	1.0	NO EFFECT	-
McN-A-343	NO EFFECT	-	NO EFFECT	-

Values are mean ± SEM, n = 4 in each case.

The results obtained suggest that the receptors present on the aorta and ileum are different. Pilocarpine and suberyldicholine were found to exhibit full agonist activity in the ileum preparation but, conversely, no activity in the aortic preparation. The results, using these agents, provides the clearest evidence for different receptor subtypes on the two tissues. In addition, the lack of activity of McN-A-343 indicates a low number of M-1 receptor subtypes in these tissues. It should be noted, however, that differences in species and efficacy may also contribute to the differences in agonist potencies observed.

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CHOLINERGIC-MEDIATED CONTRACTIONS OF GUINEA PIG STOMACH, GALL BLADDER AND BLADDER; EFFECTS OF METOCLOPRAMIDE, BRL20627 AND SCH23390

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The ability of metoclopramide to enhance the contraction responses of stomach musculature caused by field stimulation (FS) of cholinergic nerves is well established (see review by Harrington, 1983). A similar action has been described for the benzamide derivative BRL20627 (McClelland et al, 1983) and the benzazepine compound SCH23390 (Costall et al, 1984). The present study investigates whether the actions of metoclopramide, BRL20627 and SCH23390 to enhance cholinergic mediated contractions in the stomach can also be detected in other tissues receiving a cholinergic innervation, the urinary bladder and gall bladder.

Male Dunkin-Hartley guinea pigs (500-600g) were killed by cervical trans-section. The stomach, gall bladder and urinary bladder were removed and strips (stomach longitudinal body, mucosal layer removed, 20mm; urinary bladder 30mm, gall bladder 10mm in length) taken and suspended in a 30 ml tissue bath, subjected to a resting tension of 1g and bathed at 37°C in oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution. Tissues were allowed to equilibriate for 45-60 min before electrical stimulation or the addition of drugs. Tension changes were detected by Grass tension transducers and displayed on a multichannel Grass recorder. Field stimulation (FS) of the muscle strips was achieved through platinum wire electrodes placed approximately 5mm apart (supramaximal voltage, 0.1ms pulse width). Tissues were stimulated for 30s at 5 min intervals.

FS of the muscle strips (stomach, 0.125-10Hz, urinary bladder 0.25-20Hz and gall bladder 0.5-20Hz) caused repeatable, frequency-related contraction responses on which were superimposed the spontaneous activity of the tissues. Atropine (10^{-9}-10^{-7}M) caused a concentration-related antagonism to abolish contractions of the stomach and gall bladder but only a partial antagonism (50%) of the contraction response of the urinary bladder. Metoclopramide, BRL20627 and SCH23390 (10^{-7}-10^{-5}M) enhanced the contraction responses of all 3 tissues, but to varying degrees (see example data obtained at 2.5Hz, Table 1). (A concentration-related enhancement of the spontaneous activity of the urinary bladder and development of spontaneous activity in the gall bladder preparations was also observed following treatment with metoclopramide and SCH23390). Atropine (10^{-8}-10^{-7}M) caused a concentration-related and complete antagonism of the potentiated contraction responses caused by metoclopramide, BRL20627 and SCH23390 (10^{-5}M) in all 3 tissues.

Table 1 Enhancement of FS-induced contractions of smooth muscle strips from stomach, gall bladder and urinary bladder

Tissue	Tension change (g) in control tissues at 2.5Hz	Metoclopramide 10^{-7}	Metoclopramide 10^{-6}	Metoclopramide 10^{-5}	SCH23390 10^{-7} (M)	SCH23390 10^{-6}	SCH23390 10^{-5}	BRL20627 10^{-7}	BRL20627 10^{-6}	BRL20627 10^{-5}
Stomach	1.28	333*	573	716	210	240	297	218	318	311
Gall bladder	0.096	295	454	727	306	513	1533	430	730	566
Urinary bladder	1.02	127	172	222	134	176	236	125	131	140

*Values indicate % increase above control contraction responses at 2.5Hz

It is concluded that the ability of metoclopramide, BRL20627, and SCH23390 to enhance cholinergic mediated contraction responses in the stomach can be extended to other tissues, the gall bladder and, although less effective, the urinary bladder.

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FUNCTIONAL INTERACTION BETWEEN CARBACHOL AND VARIOUS DRUGS WHICH RELAX OR CONTRACT THE ISOLATED TRACHEA OF THE GUINEA PIG

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Experiments have been carried out on guinea pig isolated trachea to estimate dose/state curves for carbachol in the presence of fixed concentrations of either isoprenaline, aminophylline or verapamil (acting as functional antagonists) or of histamine or the calcium ionophore calimycin (acting as synergists). Curves obtained in the presence and absence of each interactant have been shown to fit the general null equation $A2/A1 = \alpha + \beta A2 + \gamma/A1$ where A2 and A1 are concentrations of carbachol which produce equivalent states in the presence and absence of the interactant respectively. Using each interactant values of KF1 for carbachol have been calculated from the formula $KF1 = (\alpha - 1)/2\gamma$. (Mackay, 1981).

The values of KF1 obtained using any one interactant vary with time. However the rank order of the values of KF1 obtained under optimal conditions of tissue stability are verapamil=aminophylline >= isoprenaline > histamine >= calimycin.

The numerical values of KF1 obtained using these interactants range from approximately $4x10^6$ 1/mole (using verapamil) to $1x10^5$ 1/mole (using calimycin). According to the simplest quantitative model of functional interaction $KF1 = K(afR/b + 1)$ where K is the affinity constant of the agonist for the receptors, f is the intrinsic efficacy and a and b are chain constants. It can readily be shown that in this case the interactant whose site of action is nearest to the agonist/receptor interaction should produce the lowest value of KF1 for any single agonist (Mackay, 1982). Preliminary estimates have also been made of the affinity constant of carbachol for the muscarinic receptors of the guinea pig trachea using propylbenzilylcholine mustard (PrBCM) as an irreversible antagonist. The values obtained are close to $1x10^5$ 1/mole. The concentration/state curves used to estimate KF1 with calimycin covered a carbachol concentration range of approximately $1x10^{-7}$ to $1x10^{-5}$M whereas those used with PrBCM were over the range $1x10^{-6}$ to $2x10^{-4}$M.

These results suggest that the affinity constant of carbachol for the muscarinic receptors in guinea pig trachea is close to $1x10^5$ 1/mole and that these are functional at carbachol concentrations from $1x10^{-7}$ to $2x10^{-4}$M.

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β-FUNALTREXAMINE PRETREATMENT OF GUINEA-PIG MYENTERIC PLEXUS ALTERS μ-AGONIST SENSITIVITY WITHOUT AFFECTING OPIOID BINDING SITES

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Pretreatment of the guinea-pig myenteric plexus-longitudinal muscle with β-funaltrexamine (β-FNA), the fumaramate methyl ester derivative of naltrexone, causes a decrease in the sensitivity of the tissue to the agonist action of morphine but not of ethylketazocine (Takemori et al, 1981). In untreated preparations, β-FNA is an agonist and it was proposed that β-FNA is a reversible κ-agonist but an irreversible μ-antagonist (Ward et al, 1982).

In the myenteric plexus-longitudinal muscle the agonist potencies (IC_{50}) of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin, a selective μ-ligand, morphine, a relatively selective μ-ligand and U50,488-H, a selective κ-ligand, were determined. The tissues were then incubated with β-FNA (100 nM) for 30 min. Following washing with drug-free Krebs solution for 60 min to remove residual β-FNA, the agonist potencies of the three compounds were determined again. The IC_{50} values for [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin and morphine were increased to ten times their initial values whereas the IC_{50} value for U50,488-H was not altered. The antagonist potency (K_e, nM) of naloxone against the selective μ-ligand [D-Ala^2,MePhe^4, Gly-ol^5]enkephalin, was the same in β-FNA-treated and untreated tissues.

In vasa deferentia from mouse and hamster β-FNA had low agonist activity at δ-receptors. In the presence of 100 nM β-FNA the potencies of [D-Pen^2,D-Pen^5] enkephalin and [D-Ala^2,D-Leu^5]enkephalin respectively were reduced six-fold; after prolonged washing agonist potency was partly restored.

In the rabbit vas deferens, β-FNA was found to be a weak agonist with no antagonist activity (IC_{50} = 987±241; n = 11).

In homogenates of both guinea-pig brain and myenteric plexus-longitudinal muscle, β-FNA was a potent inhibitor of opioid binding using a 40 min incubation at 25°C. In brain homogenates, the K_I values were 0.40±0.03 nM (n = 4) at the μ-site, 17.9±2.5 nM (n = 3) at the δ-site and 2.75±0.46 nM (n = 3) at the κ-site. The corresponding values in the myenteric plexus were 0.83±0.16 nM (n = 3) at the μ-site and 12.1±3.0 nM (n = 3) at the κ-site.

Following incubation with β-FNA (100 nM or 1000 nM) for 30 min at 37°C, homogenates of either brain or myenteric plexus were washed twice using a 15 min incubation at 37°C in drug-free Tris buffer. In neither tissue did pretreatment with β-FNA affect the binding of the μ-ligand [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5] enkephalin or of the κ-ligand, [^{3}H]-bremazocine in the presence of unlabelled μ- and δ-ligands.

The mechanism by which β-FNA changes the sensitivity of the myenteric plexus to μ-agonists is unclear. From the binding studies, it appears that β-FNA does not bind irreversibly at any of the opioid binding sites. Thus β-FNA may interfere with the coupling between the binding site and the effector system.

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CHARACTERISATION OF α-ADRENOCEPTOR-MEDIATED RESPONSE IN RAT GASTRIC FUNDUS

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Inhibitory and excitatory responses to α-adrenoceptor agonists in gastrointestinal smooth muscle have previously been reported (Ogle & Wong, 1971; Wikberg, 1981; Sahyoun et al, 1982). In the present study we examined the effects of selective α-adrenoceptor agonists and antagonists on rat gastric fundus in an attempt to characterise the responses.

Strips of rat gastric fundus were prepared by the method of Vane (1957) and suspended in Krebs medium containing propranolol (2 μM). Atropine (2 μM) was also present except in experiments investigating cholinergic-nerve induced responses. Tone was induced by the addition of either $BaCl_2$ (0.1-1 mM) or 5-hydroxytryptamine (0.01-0.1 μM) to the bathing fluid. Electrical field stimulation (EFS) was carried out via silver/silver chloride ring and hook electrodes (1 ms pulse width; 0.2-20 Hz; supramaximal voltage).

In low tone preparations, none of the α-adrenoceptor agonists studied (noradrenaline (NA), the α_1-adrenoceptor agonists cirazoline and phenylephrine and the α_2-adrenoceptor agonists guanoxabenz and UK 14304) produced any contractile effects at the concentrations used ($\leq$ 3 μM). In conditions of raised tone, NA (0.01-1 μM), cirazoline (0.1-3 μM) and phenylephrine (0.1-3 μM) all produced dose-related relaxations which were abolished by the α_1-adrenoceptor antagonists prazosin (1μM) and corynanthine (1-2 μM). The α_2-adrenoceptor antagonist, idazoxan, had no effect on the relaxations at concentrations up to 0.1 μM. Neither guanoxabenz nor UK 14304 produced any relaxant effect in raised tone ($\leq$ 3 μM).

Contractile responses to EFS were obtained in low tone preparations in the absence of atropine. The contractions were potentiated by neostigmine (1 μM) and abolished by atropine (2 μM). UK 14304 (0.01-1 μM) produced a dose-related inhibition of these cholinergic nerve-induced contractions which was partially reversed by idazoxan (0.01-0.1 μM). Under conditions of raised tone in the presence of atropine (2 μM), EFS produced inhibitory responses which were only partially reduced by guanethidine (2-4 μM) and prazosin (1μM). These non-adrenergic non-cholinergic (NANC) inhibitory responses in the presence of guanethidine were reduced in a dose-related manner by UK 14304 and this reduction was antagonized by idazoxan (0.01-0.1 μM).

In conclusion, α-adrenoceptor-mediated responses in the rat gastric fundus consist of (1) post-junctional α_1-adrenoceptor-mediated relaxation; (2) prejunctional α_2-adrenoceptor-mediated inhibition of both cholinergic excitatory and NANC inhibitory nerve-induced responses. No evidence for excitatory postjunctional α_1-or α_2-adrenoceptor-mediated effects was found.

J. Kelly holds an SERC CASE award in collaboration with Reckitt & Colman plc

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THE EFFECTS OF 8-HYDROXY-2-(DI-N-PROPYLAMINO) TETRALIN ON THE CARDIOVASCULAR SYSTEM OF THE CAT: COMPARISON WITH CLONIDINE

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Both biochemical and behavioural evidence suggests that 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) is an agonist at central 5-hydroxytryptamine (5-HT) receptors (Hjorth et al. 1982) with selectivity for the 5-HT_{1A} subtype of the putative 5-HT_1 receptor (Middlemiss and Fozard, 1983 ; Tricklebank, 1984). In this report, we describe the cardiovascular response to 8-OH-DPAT in the cat and draw comparisons with the effects of clonidine, a centrally-acting hypotensive agent with selectivity for α_2-adrenoceptors.

Cats were anaesthetised with α-chloralose (70 mg/kg) and pentobarbitone sodium (12 mg). Simultaneous recordings of brachial arterial pressure (BP), heart rate (HR), femoral arterial conductance (FAC) and preganglionic sympathetic nerve activity (PSNA) were made as previously described (Ramage, 1984). Skeletal muscle paralysis was produced by decamethonium (0.25 mg/kg). 8-OH-DPAT or clonidine were injected intravenously (i.v.) in increasing doses at 4-7 min intervals; control animals received equivalent injections of saline.

8-OH-DPAT (0.5-128 μg/kg ; n=5) caused a significant, dose-dependent fall in BP which reached a maximum (max.) of -66 ± 11 mmHg (mean±s.e.). At the lowest doses (0.5-8 μg/kg), the decrease in BP was not associated with any change in PSNA ; at higher doses, PSNA declined to reach a maximum of -48 ± 9 %. There was a small decrease in HR at the lower doses ; at higher doses (32-128 μg/kg), HR fell markedly to a maximum of -113 ± 9 beats/min. The fall in BP was associated with a small but non significant increase in FAC. Clonidine (0.125-8 μg/kg ; n=5) also caused a dose-dependent fall in BP (max. -45 ± 6 mmHg) and HR (max. -38 ± 6 beats/min). Unlike 8-OH-DPAT, these effects were consistently associated with a fall in PSNA (max. -83 ± 3 %) and a decrease in FAC which was significant at 2 μg/kg. Idazoxan (0.5 mg/kg), given i.v. 40 min after the last injection of clonidine or 8-OH-DPAT, reversed the effects of clonidine on BP, HR and PSNA, but antagonized only the hypotensive response to 8-OH-DPAT and then only partially. The bradycardia caused by 8-OH-DPAT was reversed by atropine methylnitrate (0.1 mg/kg).

A bolus dose of 8-OH-DPAT (32 μg/kg) in vagotomized animals caused a large fall in BP with no accompanying bradycardia. The fall in BP in both vagotomized and intact preparations was not antagonized by Wy 26392 (0.3 mg/kg), a potent and selective α_2-adrenoceptor antagonist (Paciorek et al. 1984).

Thus, 8-OH-DPAT, like clonidine, has prominent hypotensive and bradycardic activity in the anaesthetized cat at low doses injected intravenously. Unlike clonidine, however, the fall in BP is not accompanied by peripheral vasoconstriction, is independent of a reduction in PSNA, at least at the lowest doses used, and is resistant to blockade by selective α_2-adrenoceptor blocking agents.

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HYPOPHYSECTOMY ENHANCES THE ABILITY OF HALOPERIDOL TO INDUCE STRIATAL DOPAMINE RECEPTOR SUPERSENSITIVITY IN THE RAT

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Previously we found that hypophysectomy did not prevent striatal dopamine receptor supersensitivity in response to repeated high doses of haloperidol (Jenner et al 1981). However, Hruska and Pitman (1982) suggested that hypophysectomy increased the threshold dose of the neuroleptic required. We now examine the effect of hypophysectomy on the ability of a range of doses of haloperidol to induce striatal dopamine receptor supersensitivity in rats.

Male Wistar rats (initial weight 310 $\pm$ 2 g) were hypophysectomised or sham operated by the parapharyngeal technique. Two weeks postoperatively, animals received haloperidol (0.625 - 5.0 mg/kg ip) or 0.9% saline daily for 14 days. One hour after the final dose trunk blood and striatal samples were collected and assayed for haloperidol and prolactin levels. The remaining animals were allowed a 3 day drug washout period. (n = 6-7 in all cases).

Hypophysectomised animals did not gain weight over the period of dosing and showed adrenal and testicular regression. Hypophysectomy was confirmed histologically. Haloperidol treatment elevated plasma prolactin levels in sham-operated animals only. After 14 days administration striatal and plasma haloperidol levels in individual animals varied markedly in both groups. Analysis of variance across the haloperidol dose range showed plasma, but not striatal, haloperidol concentrations to be greater in hypophysectomized animals (Table 1). The number of specific striatal ^{3}H-spiperone (0.03 - 1.0 nM; defined using 10^{-5}M ($\pm$)-sulpiride) binding sites (Bmax) was increased by 2.5 & 5.0 mg/kg ip haloperidol treatment in sham-operated animals (Table 1). However, in hypophysectomised animals all doses of haloperidol elevated Bmax for specific ^{3}H-spiperone binding.

Table 1 Striatal ^{3}H-spiperone binding in animals after repeated haloperidol and plasma haloperidol levels

Dose	Sham operated		Hypophysectomised	
	Bmax (pmol/g)	Plasma haloperidol (ng/ml)	Bmax (pmol/g)	Plasma haloperidol (ng/ml)
Saline	16.7$\pm$0.8	0	14.2$\pm$0.9	0
0.625	16.0$\pm$2.1	9.4$\pm$2.7	19.7$\pm$0.4*	12.7$\pm$7.5
1.25	18.9$\pm$1.7	7.4$\pm$3.3	19.5$\pm$1.1*	87.2$\pm$75.0
2.5	21.6$\pm$2.0*	46.4$\pm$21.5	22.5$\pm$1.4*	127.8$\pm$69.3
5.0	21.0$\pm$1.6*	156.4$\pm$60.5	20.0$\pm$2.3*	283.5$\pm$76.0

* $p < 0.05$ compared to saline treated animals

Hypophysectomy does not prevent neuroleptic-induced increases in striatal dopamine receptor numbers. The pharmacokinetic changes produced by hypophysectomy may enhance the response to the neuroleptic, as suggested by acute experiments (Lloyd et al 1983).

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BEHAVIOURAL AND BIOCHEMICAL CONSEQUENCES OF BILATERAL INFUSION OF MPTP INTO RAT FOREBRAIN

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Whilst peripherally administered 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) can cause motor deficits in primates resembling those of Parkinson's disease, it has been difficult to reproduce these effects in the rat (Boyce et al, 1984; Chiueh et al, 1984). However, a continuous discrete infusion of MPTP directly into rat substantia nigra (SN) can cause a bradykinesia and hypokinesia (Bradbury et al, 1984). Here we assess the specificity of this action by determining whether similar motor deficits can result from MPTP infusion into two dopamine (DA)-containing forebrain regions, the nucleus accumbens (ACB) and caudate-putamen (CP).

Male Sprague-Dawley rats (n = 6-10) having stereotaxically implanted cannulae were subject to bilateral infusion of MPTP or its vehicle into the ACB or CP effected by Alzet osmotic minipumps (10-25μg/day, 0.48μl/h, 13 days). Individual rat locomotion was measured in individual photocell cages and grouped rat 'reactivity' on Automex activity meters. Ability to co-ordinate movement on a vertical wire grid was also assessed and videotape recordings allowed independent ratings. At critical stages of behavioural change (days 3 and 9 of infusion) animals were killed for a determination of levels of DA, noradrenaline, serotonin (5-HT), 3, 4-dihydroxyphenylacetic acid, homovanillic acid (HVA)and 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex, suprarhinal cortex, tuberculum olfactorium/ACB, CP and SN using HPLC with electrochemical detection.

The bilateral infusion of 10μg/day MPTP (a dose causing maximal behavioural change when infused into the SN) into the ACB failed to modify motor behaviour. This dose infused into the CP transiently (on day 2-3) reduced locomotion as measured in the photocell cages, but not on the Automex meters, and caused transient bradykinesia of the fore-limbs. These motor deficits were exaggerated when 25μg/day MPTP was infused into the CP, with 30-53% reduction in motor responding on days 2-4. However, these motor deficits rapidly diminished to 'normal' by day 6-8.

The major biochemical changes following the infusion of 25μg/day MPTP into the CP were recorded on day 9 when levels of striatal DA (normal content 7.4 ± 0.2 ng/mg), HVA (normal content 0.76 ± 0.04 ng/mg) and 5-HIAA (normal content 0.39 ± 0.04 ng/mg) were reduced by 36%, 46% and 33% respectively ($P < 0.01$ - $P < 0.001$), and limbic DA (normal content 5.2 ± 0.2 ng/mg) was reduced by 32% ($P < 0.01$). The biochemical changes recorded for the 3rd day of MPTP infusion into the CP were in the SN where DA levels were elevated by 46% ($P < 0.001$, normal levels 0.32 ± 0.036 ng/mg) and 5-HT levels were elevated by 35% ($P < 0.01$, normal content 0.56 ± 0.043 ng/mg) and in the CP where HVA levels were reduced by 43% ($P< 0.001$).

It is concluded that the marked motor deficits caused by the intra-SN infusion of MPTP in the rat cannot be replicated by infusions into the CP or ACB. Further, those motor deficits which do occur transiently when MPTP is infused into the CP are not associated with depletions of CP DA. Indeed, a delayed depletion of striatal DA occurs independently of any measureable motor deficit. The correlations between the behavioural and biochemical consequences of the action of MPTP in the forebrain remain to be established.

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IN ALBINO AND PIGMENTED MICE MPTP SELECTIVELY DEPLETES DOPAMINE FROM STRIATAL AND LIMBIC AREAS BUT NOT FROM HYPOTHALAMUS

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N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces Parkinsonism in man and primates, causing a 90% decrease in the dopamine (DA) content of monkey striatum (Burns et al, 1983; Langston et al, 1983). Chronic MPTP administration to albino rats, however, did not alter striatal DA function (Boyce et al, 1984). We have investigated whether this was due to an inadequate dose of MPTP or to the lack of melanin pigmentation in albino rodents. We now report the effects of chronic MPTP administration to both albino and pigmented mice on monoamine function in three brain regions innervated by different DA pathways.

Male albino CD1 mice (24-27g) or male pigmented C57 BL/10 mice (23-26g) were administered MPTP (25 or 10 mg/kg i.p.) or vehicle (0.3% v/v acetic acid i.p.) daily for 16 days. After 11 days withdrawal from drug the striatum, tuberculum olfactorium (TUO) and hypothalamus were removed prior to biochemical analysis by HPLC/ECD (Diggory & Buckett, 1984).

In albino and pigmented mice chronic MPTP (25 or 10mg/kg) administration induced a dose-dependent decrease in the levels of DA and its metabolites (DOPAC and HVA) in the TUO and particularly in the striatum, but did not alter dopamine function in the hypothalamus (Table 1). The effect was most marked in pigmented mice where MPTP (25 mg/kg) reduced striatal and TUO levels of DA by 50% and 33% respectively compared to decreases of 29% and 17% in albino mice. In both strains of mice the regional levels of DOPAC, the major intraneuronal DA metabolite, were more readily decreased than HVA (Table 1). The regional concentrations of noradrenaline, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were all unaltered by chronic MPTP (25 or 10mg/kg) treatment.

Table 1 The effect of chronic MPTP (25 or 10mg/kg x 16 days) administration on regional concentrations of dopamine and its metabolites in albino and pigmented mice.

Brain area	Albino mice			Pigmented mice		
Treatment	DA	DOPAC	HVA	DA	DOPAC	HVA
Striatum Vehicle	14443±484	944±42	1329±49	15949±558	694±44	1149±43
MPTP(25)	10229±637**	709±37**	1162±51*	8006±810**	398±36**	869±54**
MPTP(10)	13105±513	844±45	1383±66	12239±938**	556±45*	1067±62
Hypothalamus						
Vehicle	445±24	169±11	188±24	356±20	122±15	199±10
MPTP(25)	485±20	169±12	179±15	386±21	109± 5	179±10

*p<0.05; **p<0.001: Student's t-Test. Values = mean ± SEM (ng/g) n=18-20/treatment

Chronic MPTP administration therefore selectively depletes dopamine, but not other monoamines, from the striatum and TUO of albino and particularly pigmented mice without affecting the hypothalamus. However, no marked changes in spontaneous or DA-dependent behaviours were seen in these mice (Buckett & Luscombe, 1984) indicating that a greater degree of DA dysfunction is required to induce a Parkinson-like state in mice.

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PERSISTENT MOTOR CHANGE FOLLOWING THE UNILATERAL INFUSION OF MPTP INTO THE STRIATUM OF RAT BRAIN

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The unilateral infusion of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) into the rat substantia nigra (SN) can cause body asymmetries which persist throughout infusion and for many weeks after withdrawal of the MPTP (Bradbury et al, 1984). The present studies assess whether such chronic changes effected from the cell body region of the nigrostriatal system can also be effected from the terminal region, the striatum (CP).

Male Sprague-Dawley rats having stereotaxically implanted cannulae were subject to unilateral infusion of MPTP or vehicle into the SN or CP by Alzet osmotic minipumps (0.48µl/h for 13 days, 1.7-17ng/min, n = 6-12). Spontaneous and apomorphine-induced (0.25mg/kg s.c.) asymmetries and circling behaviour were assessed daily during infusion and for 95 days post-infusion. Groups of rats were killed at critical stages of behavioural change (days 3 to 4) for determination of levels of dopamine (DA), noradrenaline, serotonin, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid and 5-hydroxyindoleacetic acid in the frontal cortex, suprarhinal cortex, tuberculum olfactorium/nucleus accumbens, CP and SN using HPLC with electrochemical detection.

The unilateral infusion of MPTP into the CP caused an initial spontaneous ipsilateral asymmetry reversing to a contralateral response on day 2 of infusion which was maintained throughout the remaining infusion period and subsequently for 95 days post-infusion. Whilst apomorphine caused little change in the spontaneous asymmetry on day 2, ipsilateral asymmetry and circling responses were marked throughout the remaining infusion period. Within 1-2 days of ceasing infusion the apomorphine responses became contralateral in direction, and were maintained thus for the 95 day post-infusion period. Generally, these responses were more intense and better maintained beyond the 20th day post-infusion when a 10µg/day dose of MPTP had been employed.

The spontaneous asymmetries seen after MPTP infusion into the CP were similar to those observed for infusion into the SN, where ipsilateral asymmetry on the first 2 days of infusion converted to a contralateral response maintained throughout the remainder of the experiment (106 days). However, in contrast to the CP infusions, the SN infusions of MPTP lead to only a brief period of contralateral responding to apomorphine (day 2-4) which converted to an ipsilateral response persisting throughout the remaining infusion and post-infusion periods. Responses to the 10µg/day infusion of MPTP were again the more persistent.

The MPTP infusions into the CP specifically reduced the striatal content of DA (25-31%; $P < 0.01$) and DOPAC (38-42%; $P < 0.001$) whilst the MPTP infusion into the SN reduced the nigral content of DA (29-45%; $P < 0.001$) and DOPAC (38-60%; $P < 0.001$) with a small reduction in the level of striatal DA (24-29%; $P < 0.01$).

Thus MPTP infused unilaterally into both the CP and SN can effect spontaneous contralateral asymmetries which persist for at least 95 days following withdrawal of the MPTP. However, the functional state of the nigrostriatal system as revealed by apomorphine challenge would appear to be differentially affected by MPTP infused into the cell body or terminal region.

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THE ROLE OF CYTOCHROME P-450, NADPH-CYTOCHROME P-450 REDUCTASE AND LIPIDS IN SEX DIFFERENCES IN HEPATIC DRUG METABOLISM

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Sex differences exist in the hepatic microsomal metabolism of many drugs in the rat (Skett et al, 1980; Skett & Young, 1982) but the biochemical differences underlying this sexual dimorphism have been little investigated. Sex differences have been seen in cytochrome P-450 isoenzymes with male- (Kamataki et al, 1981) and female-specific forms of the enzyme (MacGeoch et al, 1983) being reported. Some correlation between the presence of these sex-specific forms and differences in drug metabolism have been noted. Sex differences in microsomal lipid composition have also been noted (Holtzman et al, 1970) and have been postulated to account for some sex differences in drug metabolism (Belina et al, 1975). This study investigates the roles of the components of the hepatic mixed-function oxidase in determining the sex differences in drug metabolism using the recently developed vesicle reconstitution method of Ingelman-Sundberg (1980). Cytochrome P-450 (P-450) and NADPH-cyt. P-450-reductase (reductase) were separated by n-octylamino-Sepharose 4B chromatography and, in the latter case, ADP-agarose chromatography (Guengerich et al, 1980). Microsomal lipids were isolated by chloroform/methanol extraction and used without further separation. Cross-over studies were performed using male and female P-450, reductase and lipids and assaying the drug-metabolising activity using lignocaine as substrate (Skett et al, 1980).

Table 1 Influence of P-450, Reductase and Lipid on Metabolism of Lignocaine by a Vesicular Reconstitution System

P-450	Reductase	Lipid	N-Deethylase*	3-Hydroxylase*
M	M	M	254±63	12±2
M	M	F	63±20	12±2
M	F	M	47±19	14±4
M	F	F	45±8	11±2
F	F	F	72±7	17±6
F	F	M	50±11	18±10
F	M	F	60±12	21±8
F	M	M	73±16	22±1

M = male, F = female, * - pmoles product min^{-1} nmole $P\text{-}450^{-1}$

Sex differences similar to those seen in microsomes were observed in the total male and female reconstitutions. Incorporation of female reductase and/or lipid into male P-450 containing vesicles, however, caused the disappearance of the sex difference in lignocaine N-deethylase (Table 1). The non-sex-differentiated enzyme, lignocaine 3-hydroxylase was unaffected. These results indicate that all three components of the mixed-function oxidase system are necessary for expression of the sex differences in drug metabolism seen in the rat.

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THE EFFECTS OF CHRONIC ETHANOL TREATMENT AND WITHDRAWAL ON IMMUNOREACTIVE SERUM INSULIN LEVELS IN LACG AND DIABETOGENIC C57 BL MICE

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C57 BL mice prefer ethanol solution to water. Furthermore they have high plasma glucose levels (pgl) which are lowered by compulsory ethanol consumption to levels similar to ethanol-avoiding LACG mice (Connelly et al, 1983). Treatment of C57 mice with insulin, glibenclamide and phenformin reduces their pgl and causes ethanol aversion. Insulin resistance has been proposed to account for the mild diabetes observed in C57 mice (Goas et al, 1979), but investigations into the effects of acute insulin administration on behaviour, and chronic insulin treatment on pgl and ethanol preference, have failed to confirm this (Connelly & Taberner, 1983). On withdrawal from chronic ethanol treatment C57 lose their preference for ethanol despite more profound hyperglycaemia (Connelly & Taberner, 1983). An investigation into the effects of chronic ethanol treatment and withdrawal on serum immunoreactive insulin (IRI) levels was therefore performed in LACG and C57 mice.

Adult LACG and C57 drug-naive, ethanol-tolerant (Unwin & Taberner, 1980), and 48 hour withdrawn mice, in both the fed and 24 hour fasted states, were bled by cardiac puncture under light ether anaesthesia. Radioimmunoassay for insulin was performed by the method of Herbert (1965), using commercially available guinea pig anti-insulin serum and (I^{125})-insulin.

Serum IRI levels (μIU/ml) were not significantly different between LACG and C57 mice in the fed state: LACG♂ 25.2 ± 5.6 (16), LACG♀ 13.7 ± 3.7 (8), C57♂ 20.0 ± 5.2 (9), C57♀ 14.7 ± 1.8 (12), or the fasted state: LACG♂ 7.1 ± 1.0 (9), LACG♀ 6.9 ± 2.3 (12), C57♂ 8.1 ± 1.2 (9), C57♀ 5.5 ± 1.8 (9), $p > 0.05$, t-test. Although chronic ethanol treatment significantly lowers the pgl of C57♂ and ♀ mice (Connelly et al, 1983) it had no significant effect on serum IRI in either sex of LACG or C57 mice compared to naive. Serum IRI was significantly increased in withdrawn mice compared to naive animals: C57♂ 62.4 ± 9.8 (11), $p < 0.01$, C57♀ 43.8 ± 4.9 (11) $p < 0.01$; LACG♀ 34.9 ± 9.4 (13), $p < 0.05$. On fasting, serum IRI in C57 mice, unlike LACG, remained elevated: C57♂ 18.7 ± 2.3 (11), $p < 0.001$, C57♀ 21.4 ± 2.6 (10), $p < 0.001$; LACG♂ 6.1 ± 0.4 (16), $p > 0.05$, LACG♀ 7.1 ± 2.1 (8), $p > 0.05$.

These results do not support the suggestion that the mild hyperglycaemia exhibited by drug-naive C57 mice is due to insulin resistance, which is typified by hyperinsulinaemia. However, on withdrawal from chronic ethanol treatment, C57 mice show increased hyperglycaemia, and hyperinsulinaemia. This indicates that mice previously tolerant to ethanol exhibit insulin resistance. This deterioration in the glucose homeostasis of C57 mice is now associated with a loss of ethanol preference.

D.M.C. is an M.R.C. Scholar.

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INTERRELATIONSHIP BETWEEN Na^+, K^+-ATPASE AND K^+-ATPASE IN GASTRIC MUCOSA

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Acid secretion in the gastric mucosa is dependent on adenosinetriphosphatase systems|ATPases, EC.3.6.1.3 (Forte, 1971)|. Circumstantial evidence indicates an intimate association between one particular ATPase and hydrogen ion secretion, ie. the potassium - stimulated, magnesium - dependent enzyme or K^+-ATPase. This enzyme however displays many of the biochemical characteristics of other members of the class including Mg^{++}-ATPase and Na^+, K^+-ATPase. The purpose of the present study was to examine the interrelationships of these three ATPases, using the putative selective inhibitor of K^+-ATPase, picoprazole (Wallmark et al, 1983), the selective inhibitor of Na^+, K^+-ATPase, ouabain, and the non-selective enzyme inhibitor oligomyocin.

ATPase activity was measured in microsomal material prepared from rabbit or hog stomachs. Fundic mucosa was homogenised in 10 volumes 0.25M sucrose, 50mM Tris-HCl buffer (pH 6.9 at 5°C). All isolation procedures were carried out at 0-5°C. The homogenate was centrifuged at 10,000 G_{av} for 20 min, the supernatant recentrifuged at 100,000 G_{av} for 2h (with one washing), and the final pellet resuspended by ultrasonication in 20 volumes 50mM Tris-HCl buffer (pH6.9 at 37°C for K^+-ATPase, pH 7.4 at 37°C for Na^+, K^+-ATPase). ATPase activity was measured in the presence of $MgCl_2$ (5mM) and either KCl (20mM) or KCl (20mM) and NaCl (100mM). The reaction was initiated by addition of ATP (2mM) and terminated after 20 min at 37°C, by addition of 0.8% dodecyl sulphate. The phosphate released was measured spectrophotometrically. Drugs were pre-incubated with the enzyme for 30 min.

The addition of either KCl or KCl + NaCl resulted in a significant elevation of enzyme activity (73±8% and 94±4%, respectively), when compared to enzyme activity in the presence of only $MgCl_2$. On this basis, it can be concluded that Na^+, K^+-ATPase activity and K^+-ATPase activity were of similar magnitude in microsomal material prepared from pig or rabbit.

Picoprazole selectively inhibited rabbit K^+-ATPase (IC_{50} 7.0±1.3µM, n=7) in comparison with Mg^{++}-ATPase (IC_{50} >500µM) and Na^+, K^+-ATPase (IC_{50} >1000µM). Ouabain was a potent inhibitor of Na^+,K^+-ATPase (IC_{50}, 3.2±0.3µM, n=4). A component of K^+-ATPase was inhibited by ouabian over the same concentration range, although the maximum inhibition achieved was only ∿ 30%. Mg^{++}-ATPase activity was unaffected by ouabain. Oligomyocin in the concentration range 30-60µM inhibited all three enzymes by at least 50%.

It appears, therefore, that K^+-ATPase and Na^+, K^+-ATPase are closely associated, yet distinct. The finding that ouabain, the classical inhibitor of Na^+, K^+-ATPase, inhibits a portion of K^+-ATPase may reflect contamination from endogenous ions, in particular sodium ions. Equally, this may represent an interaction of ouabain at a common potassium-ion binding site on the two enzymes. Although picoprazole was found to be a selective inhibitor of K^+-ATPase, it was not a specific inhibitor. This may also be a reflection of the close association of the ATPases in the gastric mucosa.

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THE PHARMACOKINETICS AND TISSUE LOCALISATION OF PYRIMETHAMINE IN THE MOUSE: EFFECT OF DOSE SIZE

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Pyrimethamine-sulphonamide combination therapy is a first choice in areas of chloroquine resistant Plasmodium falciparum malaria. However, the pharmacokinetics of pyrimethamine remain poorly documented. Our objectives were firstly to investigate the disposition of pyrimethamine in the mouse, an important animal model in experimental malaria chemotherapy and secondly, to establish the effect of dose size on **a)** the pharmacokinetics of this compound, and **b)** the mass fate and tissue localisation of ^{14}C labelled pyrimethamine.

Eight groups of male T.F.W. white mice (mean wt 25g.) were provided with a Dixons 41B diet and drinking water ad libitum. The plasma disposition of pyrimethamine was determined in four of these groups A-D, (n = 5 per group) which were each dosed with pyrimethamine base, suspended in 5% v/v Tween '80 at 12.5, 25, 50, and 75 mg/kg i.p. respectively. The plasma concentration/time profiles for pyrimethamine over 30 hours were followed, drug levels being determined by a previously described H.P.L.C. technique (Coleman et al. 1984).

The urinary excretion of unchanged pyrimethamine, ^{14}C radioactivity excretion in faeces and urine, and also the mass fate of ^{14}C radioactivity were determined in groups E-H (n = 6 per group) in which, mice were dosed with ^{14}C pyrimethamine (0.5 µCi) as above. Faeces and urine were collected serially for 7 days, at which time the mice were sacrificed and the residual tissue localisation of ^{14}C radioactivity was determined in the various soft tissue organs, remaining carcasses and cage washings. Unchanged pyrimethamine in urine was determined by H.P.L.C. and the ^{14}C radioactivity, in each tissue and excreta, by liquid scintillation counting. Statistical evaluation was by one way analysis of variance, accepting $p < 0.05$ as significant.

Following i.p. administration of pyrimethamine, peak plasma levels were attained within 1-2 hrs, and declined monoexponentially with an elimination half-life of 4.6 to 5.6 hrs at all four doses. The mean values for $AUC_{(0\rightarrow\infty)}$ increased linearly with the dose of pyrimethamine ($r = 0.978$, $p < 0.001$), and the mean values for clearance and half-life were not significantly different between groups. This indicates the plasma pharmacokinetics of pyrimethamine to be independent of dose.

The percentage of the administered dose excreted as unchanged drug (1.3 - 3.3%) and ^{14}C radioactivity (21.7 - 29.1%) did not change with increasing dose. In contrast the cumulative excretion of ^{14}C radioactivity in faeces after the higher doses of 25, 50, 75 mg/kg, (16.7 - 22.8%) was significantly less than that seen with the lowest dose (50.3%). This suggests extensive biliary excretion of radioactivity, and that the capacity of this process may have been exceeded with the three highest doses. Seven days after administration of the highest doses (25, 50 and 75 mg/kg) a significantly greater percentage of ^{14}C pyrimethamine was localised in the soft tissue, i.e. heart, lung and kidney (7.8 - 13.8%) gut (5.4 - 9.4%) and particularly the liver (25 - 27.9%) when compared with the 12.5 mg/kg dose of the drug (1.2, 1.0, 0.5% respectively). Following each dose, between 85 and 97% of the administered radioactivity was accounted for. These studies indicate, that with higher doses of pyrimethamine, parent drug and/or its metabolites may accumulate in soft tissue, particularly the liver; but without appreciable effects on the plasma disposition and urinary excretion of the drug.

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THE USE OF LIVER CUBES IN THE ASSESSMENT OF PHASE I DRUG METABOLISM IN THE RAT

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Liver cubes have been shown to be a good model for in vivo conjugation of drugs (Graham & Skett, 1981). This model is closer to the in vivo situation than microsomes and even isolated hepatocytes as the intact cellular and tissue structure is maintained in the preparation. This study investigated the possible use of liver cubes as a model of in vivo phase I metabolism of drugs.

Liver cubes were prepared by a modification of the method of Pollard and Dutton (1982). Animals were killed by cervical dislocation and the liver quickly excised. The liver was cut by hand in 1 mm slices and then further cut in two dimensions by a McIlwain tissue chopper to yield 1 mm cubes of tissue. Drug metabolising capacity was assayed using [^{14}C]-lignocaine as substrate and the metabolites separated and quantitated as previously described (Skett et al, 1980). As this is an intact cell preparation, it was expected that conjugates would also be formed and, therefore, hydrolysis with arylsulfatase/β-glucuronidase was performed overnight to breakdown sulfate and glucuronide conjugates before analysis of the phase I metabolites. This hydrolysis/extraction procedure led to the extraction of 95% of the original radioactivity - the remaining 5% in the aqueous fraction was assumed to be other conjugates or hydrolysis products. Cell damage as estimated by release of lactate dehydrogenase into the medium and NADPH stimulation of lignocaine metabolism was 30%. This is not as good as the "snips" technique of Pollard and Dutton (1982) but the more rapid preparation and the use of the whole lobe (considering the uneven distribution of enzymes in the liver) in the "cube" method compensates for this loss of viability. Optimum conditions for assay were investigated and found to be 0.5 g liver, 30 min incubation at 37°C and 50 μM substrate. These conditions were used subsequently throughout the study.

Table 1 Metabolism of Lignocaine in cubes prepared from male, female and castrated male rats

	N-Deethylase*	3-Hydroxylase*
Male	1870±315	304±33
Female	494±51**	289±54
Castrated Male	602±186**	232±58

* - pmoles product min^{-1} g $liver^{-1}$ ** - $p < 0.05$ compared to male control

Liver cubes exhibited sex differences in the metabolism of lignocaine as seen in microsomes (Skett et al, 1980) (Table 1) with N-deethylation being higher in the male- than in the female-derived cubes but 3-hydroxylation being non-sex-differentiated. Castration of male animals one week prior to preparation of cubes leads to a more female pattern of metabolism in the cubes. This again is similar to that seen in microsomes.

Liver cubes thus provide a rapidly prepared, physiologically relevant model for the study of in vivo phase I metabolism and can be used to demonstrate physiological control of drug metabolism.

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INHIBITORY EFFECT OF PERMIXON ON TESTOSTERONE 5α-REDUCTASE ACTIVITY OF THE RAT VENTRAL PROSTATE

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Benign prostatic hyperplasia (BPH) is a disease associated with the presence of high levels of the active metabolite of testosterone, dihydrotestosterone (DHT) (Wilson, 1980). The accumulation of DHT is due, in part, to an increased activity of the enzyme testosterone-5 α reductase. Permixon, an extract of the plant Serenoa repens B. and a new treatment for BPH (Champaut et al., 1984), has been shown to be an antagonist of the cytosolic androgen receptor in rat prostate (Stenger et al., 1982 ; Carilla et al., 1984) and in human foreskin fibroblasts (Sultan et al., 1984). We report here the effect of Permixon, on the activity of testosterone-5 α reductase of the rat ventral prostate.

Rat ventral prostate was homogenised in a glass-glass Potter homogeniser in sucrose ($8.8.10^{-1}$M), $CaCl_2$ ($1.5.10^{-3}$M). An aliquot of the homogenate was incubated in a phosphate (4.10^{-2}M) buffer at pH 6.6 with NADPH (5.10^{-4}M) and ^{3}H-testosterone (25 nM, 100 Ci/mmol NEN) for 15 min. at 25°C. The reaction was stopped by adding 6 volumes of chloroform/methanol (2/1, V/V). The organic phase, which contained the ^{3}H-steroids, was separated by centrifugation (5 min. at 600 g) and evaporated under nitrogen. The residue was redissolved in methanol and applied (25 µl) to an aluminium silica-gel sheet, which was developed with dichloromethane/ether (4/1, V/V).

The labelled steroids were localised by the use of unlabelled markers which were revealed using acetaldehyde (15 min. at 110°C). The positions corresponding to the radioactive steroids were cut out and the radioactivity of each sections counted.

The metabolism of ^{3}H-testosterone was inhibited by progesterone, taken as reference, in a dose-dependent manner (IC_{50} = 59.7 ± 2.3 nM). Similarly, Permixon is a specific inhibitor of testosterone-5 α reductase (IC_{50} = 88.2 ± 2.6 µg/ml).

In order to verify that the physical nature of Permixon (an oil) was not responsible for its activity, we investigated several oils. Arachide, Onagre, Sunflower oils, and liquid paraffin, are all inactive at 800 µg/ml. Only Palm Oil and Tadenan (a plant extract commercialised for the treatment of BPH), have a very weak inhibitory effect, (about 50% at 800 µg/ml).

These results show that the liposterolic extract of Serenoa repens B., Permixon, is an inhibitor of testosterone-5 α reductase in the rat prostatic cell. Permixon has also been shown to be an androgen receptor antagonist in the rat prostatic cell (Carilla et al., 1984). Thus two complementary biochemical activities are available to explain the clinical activity of Permixon in BPH.

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THE DISPOSITION OF PHENYLBUTAZONE IN THE HORSE

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Phenylbutazone (PBZ) is the most widely used non-steroidal anti-inflammatory drug in equine medicine. Although the Rules of Racing prohibit its use in most countries including Great Britain, it is the only anti-inflammatory drug permitted in horses competing under Federation Equestre Internationale (FEI) rules. Despite its extensive use, uncertainty still exists regarding the disposition of PBZ in the horse.

[^{14}C] PBZ (7.1mg/kg containing 100μCi) was administered by stomach tube to two gelded horses. One of these horses received PBZ i.v. on a separate occasion.

Urine and faeces were collected as voided for up to 80 hours. Blood samples were obtained from an indwelling jugular cannula. The elimination of ^{14}C was monitored by scintillation counting, the faecal samples being combusted prior to analysis. Urinary metabolites were separated and characterised by TLC, HPLC, nad GC mass spectrometry.

Urinary excretion of ^{14}C is essentially complete after 72 hours, and accounts for 55% of the dose following both oral and i.v. administration. No faecal elimination of ^{14}C was observed up to 20 hours post dosing, but in the time period 20-70 hours some 21% (p.o.) and 29% (i.v.) of the dose were recovered. Examination of appropriate rate plots shows that faecal excretion was not complete within the collection period. This indicates that both renal and biliary excretion are major routes of elimination of the drug from the body. In addition, because of the similarity of excretion balance and plasma AUCs (total ^{14}C) for both routes of administration, we may conclude that PBZ is completely absorbed after oral administration.

The major urinary metabolites were unchanged PBZ (4%), p- and γ-hydroxyPBZ (20% and 14% respectively). In addition, a minor metabolite (1.5%) was present, and available analytical data suggests that it is p, γ-dihydroxyPBZ. Overall, these four compounds account for 40% of the dose (72% of urinary ^{14}C). In addition, three further metabolites have been separated by HPLC, but their identities are as yet unknown. Up to 10 hours after dosing γ-hydroxyPBZ is the major urinary metabolite, but thereafter p-hydroxyPBZ predominates, and preliminary data also demonstrate this change in the γ-hydroxyPBZ/p-hydroxyPBZ ratio occurring in the plasma. Inspection of the excretion rates appears to exclude the possibility that the formation of either metabolite is a zero order process. A possible explanation of this change in metabolite pattern with time involves enzyme inhibition either by PBZ or a metabolite.

METABOLISM OF LIGNOCAINE IN THE PERFUSED RAT LIVER IN SITU FOLLOWING FLOW VARIATIONS IN HEPATIC ARTERY AND PORTAL VEIN

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The portal vein (PV) and hepatic artery (HA) blood supply to the liver may be functionally different. Systemic availability of lignocaine (L) introduced into the HA exceeds that following PV perfusion (Ahmed *et al*, 1981). This difference may reflect acinar distribution or access of blood to the hepatic drug metabolising enzymes. We have approached this problem by analysing metabolites of L in effluent liver perfusate.

Male Wistar albino rats weighing between 350 and 500g were anaesthetised with pentobarbitone (70mg/kg, i.p.). The HA and PV were cannulated and the liver perfused with Krebs Medium containing human red blood cells at a haemoglobin concentration of 5-6% (wt/vol) Perfusate from the liver was collected via the vena cava. Livers were perfused with PV:HA flow contributions of 5:5 and 10:0 mls/min. L and four Phase I metabolites, 3-hydroxylignocaine (3-OH L), monoethylglycinexylidide (MEGX), 3-hydroxy MEGX (3-OH MEGX) and glycinexylidide (GX) were assayed in effluent perfusate after L perfusion of 4mg/l by HPLC. Enzymic hydrolysis of the perfusate prior to analysis with sulphatase and/or β-glucuronidase also allowed the conjugates of these metabolites to be measured. A summary of the results is given:-

PV:HA FLOW CONTRIBUTION →→	5:5	10:0 (mls/min)
Free metabolites		
3-OH MEGX	4.9 ± 0.3	5.0 ± 0.7
3-OH L	4.3 ± 0.2	5.0 ± 0.4
MEGX	ND	0.6 ± 0.2*
GX	16.6 ± 1.2	5.4 ± 0.4**
Total metabolites		
3-OH MEGX	47.2 ± 2.7	50.9 ± 1.2
3-OH L	29.4 ± 1.2	13.0 ± 0.5**
MEGX	ND	8.6 ± 0.4**
GX	23.6 ± 1.4	27.7 ± 1.4**

Figures represent mean±sem (n=20) of proportion of total metabolite recovery; ND = not detected; *p< 0.05, **p< 0.01.

The metabolites of L in the perfused rat liver vary with PV:HA ratio. As the PV flow contribution increased from 50 to 100% there was a significant increase in the proportion recovered as GX and MEGX, and a significant decrease in 3-OH L (as measured by total metabolites). The measureable appearance of MEGX in effluent perfusion fluid is dependent on a PV contribution greater than 5mls/min. Sulphate conjugation of all 4 metabolites and glucuronidation of all but GX significantly altered with PV flow.

The results indicate that the 2 hepatic blood supplies have some degree of mutually exclusive routes through the liver sinusoids and that the enzyme distribution of these sinusoids differ. This variation is reflected in the differences in the metabolism of L.

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FACTORS AFFECTING THE AMINOPYRINE BREATH TEST

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There is considerable interest in investigating the factors regulating hepatic drug metabolism in both man and animals. Recently the determination of ^{14}C-aminopyrine (^{14}C-AP) kinetics by monitoring the breath $^{14}CO_2$ exhalation-rate (^{14}CER) has been reported as a model for assessing hepatic drug metabolic function in the rat (Desmond et al, 1980; Houston et al, 1981). However, in most of these studies adult male rats have been used. We have extended these investigations by examining the effects of diurnal rhythm (i.e. late morning and late afternoon), sex, diet, cimetidine (Cm) and pregnancy on the ^{14}CER in rats. The ^{14}CER was determined in Wistar rats on 30 min samples taken over a 3-hour period following a single dose of ^{14}C-AP (2μ CI/kg) by i.p. route. The exhaled $^{14}CO_2$ peak occurred at 60 min in all cases, except in the pregnant females (90 mins), following injection of ^{14}C-AP after which there was a mono-exponential decline in the ^{14}CER during the sampling period.

TABLE 1

The effect of various treatment on the Aminopyrine Breath Test (Mean + S.D.)

Treatment Group (number)	Half Life $t_{\frac{1}{2}}$ (min)
I Males (n = 5) Morning	35.0 ± 3.1
II Males (n = 5) Afternoon	73.1 ± 26.3
III Males (n = 4) Fasted	60.2 ± 22.4
IV Males (n = 4) Cimetidine	58.6 ± 4.7
V Females (n = 6) Morning	46.5 ± 12.9
VI Females (n = 6) Cimetidine	61.2 ± 16.8
VII Pregnant (n = 3) Full term	95.4 ± 14.2

The results showed a significant difference ($P < 0.05$) in the ^{14}CER between the late morning (11 a.m. to 2 p.m.) and late afternoon (4 p.m. to 7 p.m.) studies - Group I and II. When the male rats were fasted for 72-hours prior to injection of ^{14}C-AP (Group III) the $t_{\frac{1}{2}}$ was significantly prolonged (compared with the Males non-fasted in Group I). There was no statistically significant difference between groups of male and female rats (Gp I and V). Cimetidine (100 mg/kg) given concurrently with ^{14}C-AP prolonged the ^{14}CER in male (Group IV) but not significantly in female (Group VI) rats. The ^{14}CER was markedly prolonged in the pregnant group (VII). These observations confirm the usefulness of the ^{14}CER and emphasize the need for care in the design of such studies.

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THE FATE OF DINITROPHENYL-ALBUMIN CONJUGATES IN THE RAT

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It is well established that the toxicity of some drugs and other chemicals is caused by chemically reactive metabolites (Trush et al, 1982) which bind covalently to endogenous macromolecules. The type of toxicity is determined by the location and function of the target macromolecule. However, little is known of the subsequent fate of conjugates formed from chemically reactive metabolites and endogenous macromolecules in vivo.

In this study we have examined the disposition and metabolic fate of protein conjugates of the mild arylating agent dinitrofluorobenzene (DNFB), which binds covalently to lysyl residues of proteins (Knight and Green, 1979).

Human serum albumin (HSA; Sigma, fraction V) or rat serum albumin (RSA; purified by affinity chromatography of whole rat serum) was conjugated with [^{3}H]DNFB and purified by dialysis to produce dinitrophenyl (DNP)-conjugates with varying DNP:albumin molar ratios (0.5:1 to 24:1). Urethane anaesthetized male Wistar rats (200-300g) received DNP-albumin conjugate via the jugular vein. Blood samples from the carotid artery and bile were collected over 3h. Plasma, bile, urine and the major organs were analysed for radioactivity. Bile was analysed by h.p.l.c. (Partisil ODS-2, 10 µm, 25 cm column) using a linear gradient of methanol (30 - 60% at 2%/min) in $NH_4H_2PO_4$ buffer (43 mM; pH 2.3) flowing at 2 ml/min.

Plasma disappearance of radioactivity was monoexponential. The volume of distribution (Vd) of all conjugates was consistent with intravascular distribution confined to plasma, whereas the apparent clearance (CL) of radioactivity differed widely with the degree of conjugation (Table 1).

TABLE 1 Mean (± s.e.) pharmacokinetic values for [^{3}H]DNP-albumin conjugates

	n	Vd(ml)	CL(ml/h)	$t_{\frac{1}{2}}$ (min)	% dose in liver
HSA-DNP$_{0.5}$	8	14 ± 1	1.4 ± 0.1	406 ± 55	5 ± 0.8
HSA-DNP$_{20}$	7	14 ± 1	11.4 ± 0.6**	53 ± 3**	31 ± 2**
RSA-DNP$_{0.6}$	4	12 ± 1	2.3 ± 0.2	212 ± 10	5 ± 0.1
RSA-DNP$_{24}$	5	17 ± 1*	7.2 ± 0.8**	102 ± 7**	38 ± 3**

* $P < 0.05$ **$P < 0.001$ (Student's t-test comparison for non-paired data)

Uptake of conjugates into the liver and excretion of radioactivity into bile were much greater (6- to 8-fold) after administration of highly conjugated, as compared with lightly conjugated, albumin. The major radiolabelled urinary and biliary metabolite of highly conjugated albumin was chromatographically identical to chemically synthesized N^2-acetyl-N^6-dinitrophenyl-lysine (ac-DNP-lys) and had the same UV absorption maximum (360 nm). Its identity was confirmed by enzymic and acid degradation and by comparison of its mass spectrum with that of authentic ac-DNP-lys. The degree of conjugation of both foreign (HSA) and 'self' (RSA) protein influences the rate of uptake into the liver and the subsequent excretion of metabolites into bile. Highly conjugated albumin appears to undergo rapid hydrolysis and to yield a simple amino acid conjugate which is acetylated and excreted into bile and urine. Such metabolites might provide markers for assessment of the binding of reactive metabolites to endogenous macromolecules in vivo.

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