RECEPTOR-DRUG DISSOCIATION RATE OF UNLABELLED NEUROLEPTICS: RELATION TO BINDING AFFINITY AND LIPOPHILICITY

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An original filter method was developed for estimating in vitro at room temperature the receptor-drug dissociation rate of unlabelled compounds. Membrane preparations are incubated (37° C) with a concentration of unlabelled drug producing maximal receptor occupation. Then, samples are filtered under suction on a series of glass fibre filters. The vacuum is ruptured and an aliquot of either warmed labelled ligand or buffer is applied on the filters. They are kept for 5 min at room temperature, followed by suction and thorough rinsing of the labelled filters with cold buffer or short rinsing of the unlabelled filters with warm buffer. Filters are from 0 to 4 times 5 min incubated with a warm buffer aliquot followed by a final incubation with labelled ligand. The advantages of this method are its speed and the omission of a cooling-step, which fixes certain drugs on the membrane, in a way that upon subsequent incubation at 37° C, dissociation is obstructed.

Table 1 Half-life of dissociation and equilibrium binding constants of drugs for receptor sites (mean values, n=3-5) and apparent lipophilicity at pH 7.5

n	dopamine D_2		serotonin S_2		α_1-adrenergic		log (P_{app}) pH 7.5
	K_i nM	$t_{1/2}$ min	K_i nM	$t_{1/2}$ min	K_i nM	$t_{1/2}$ min	
	A	B	C	D	E	F	G
pimozide	1.2	> 25	33	> 25	41	> 25	5.07
metitepine	3.9	> 25	1.8	> 25	0.4	> 25	4.64
fluphenazine	6.2	> 25	33	> 25	8.9	> 25	3.99
chlorpromazine	18	24±3	20	> 25	1.7	~25	3.37
droperidol	0.8	11±2	4.1	9±2	0.8	11±6	3.37
azaperone	44	5±1	7.2	5±1	1.3	7±1	2.99
haloperidol	1.2	11	48	8±2	8.1	15±2	2.87
spiperone	0.16	> 25	1.2	23	10	12±2	2.77
pipamperone	124	5±1	5	10	62	8±2	1.63
sulpiride	31	7±1	> 1000		> 1000		-1.06

Spearman rank correlation coefficients between:
G and A r=-0.317, $p > 0.05$; G and C r=-0.110, $p > 0.05$; G and E r=-0.517, $p > 0.05$
G and B r= 0.660, $p < 0.05$; G and D r= 0.642, $p < 0.05$; G and F r= 0.775, $p < .05$

Table 1 shows for various neuroleptics the overall half-lives of receptor-drug dissociation and K_i-values for the dopamine D_2, serotonin S_2 and α_1-adrenergic receptor sites and the apparent partition coefficient of the drugs at pH 7.5. The most lipophilic drugs (log $P_{app} \gtrsim 4$) dissociate very slowly from all three receptors. Significant correlations are found between the lipophilicity of the drugs and the receptor dissociation rates. However some exceptions are noted: droperidol dissociates more rapidly than chlorpromazine, although both drugs have the same apparent lipophilicity; spiperone has a relatively low lipophilicity yet dissociates slowly from the dopamine D_2-receptor site. Some relationship is apparent with in vivo time of onset and duration of action of the drugs. No relationship is found between lipophilicity and K_i-values of the drugs.

TRYPTAMINE-INDUCED CHANGES IN 5-HYDROXYTRYPTAMINE RELEASE FROM MOUSE BRAIN SLICES

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The behavioural effects of peripherally administered tryptamine resemble those produced by 5-hydroxytryptamine (5HT) agonists. They are dependent, in part at least, on the presence of 5HT (Marsden and Curzon, 1979) and may involve release of 5HT (Irons and Marsden, 1983). However, in vitro studies, using superfusion techniques, have suggested that tryptamine can inhibit 5HT release (Cox and Ennis, 1982). The present study compares the effects of tryptamine on 5HT release from mouse brain slices using both fixed volume and superfusion systems.

Mouse (male CS-1) brain hypothalamic slices were used in all experiments. Under fixed volume conditions slices were preincubated for 5 min in 10^{-6}M pargyline after which tryptamine (10^{-6}M) was added and incubation continued for 20 min. Endogenous 5HT released into the incubation medium was assayed by HPLC with electrochemical detection (carbon paste working electrode at +0.65 V). Separation was performed on a Spherisorb 5 ODS reverse phase column using 0.1 M acetate-citrate buffer pH 4.1 containing 3% methanol. The superfusion method was essentially that described by Ennis et al (1981) but using slices preloaded with [^{3}H]-5HT (specific activity 13 Ci/mmol, Amersham International PLC). Basically two 4 min K^+ (30 mM) pulses (S_1, S_2) were given 15 min apart and drugs were added to the superfusate after the first K^+ pulse. Results are expressed as the % change in the S_2/S_1 ratio.

In the fixed volume experiments addition of tryptamine significantly increased the release of endogenous 5HT into the incubation medium (+108%, n=6) whereas in the superfusion system tryptamine (10^{-6}M) produced a significant decrease (-91%, n=8) in the K^+ stimulated [^{3}H]-5HT release compared with the control situation (two K^+ pulses but no tryptamine added). However, in the presence of the 5HT uptake inhibitor, paroxetine (10^{-5}M) tryptamine (10^{-6}M) significantly decreased 5HT release in both the fixed volume (-44%, n=18) and superfusion (-88%, n=8) systems. This decrease produced by tryptamine in the presence of paroxetine was reversed by the 5HT receptor antagonist, metergoline (10^{-6}M) and 5HT release increased by 44% (n=6) under fixed volume and by 197% (n=8) under superfusion conditions when compared with tryptamine plus paroxetine values.

These results suggest that the 5HT releasing action of tryptamine, seen in the fixed volume studies, involves tryptamine accumulation into the 5HT pre-synaptic nerve endings and subsequent displacement of 5HT. However, when accumulation is reduced, either by a 5HT uptake inhibitor or use of a superfusion system which prevents re-uptake, a receptor mediated inhibition of 5HT release is observed. In view of the low levels of tryptamine in brain the latter effect would seem to be of greater physiological importance.

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Cox, B and Ennis, C (1982) Psychopharmacology 78, 85-88
Ennis, C, Kemp, J D and Cox, B (1981) J.Neurochem. 36, 1500-1505
Irons, J and Marsden, C A (1983) Br.J.Pharmacol. 78, 13P
Marsden, C A and Curzon, G (1979) Neuropharmacol. 18, 159-164

IN VITRO RADIOLIGAND DISPLACEMENT STUDIES WITH BUCINDOLOL

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Bucindolol, 2-{2-hydroxy-3((2-(1H-indol-3-yl)-1, 1-dimethyl ethyl) amino)propoxy} benzonitrile hydrochloride is a new compound under investigation for the treatment of hypertension. In animal studies it has been shown to cause direct vasodilation, to have β adrenoceptor blocking properties and intrinsic sympathomimetic activity.

We have examined the relative affinity of this compound and one of its principal metabolites 5 hydroxy 2-{2 hydroxy-3-{{2-(H-indol-3-yl)-1, 1-dimethyl ethyl}amino} propoxy} benzonitrile for α_1, α_2 and β adrenoceptor binding sites using radioligand displacement techniques (Hannah et al, in press). [^{3}H]prazosin (3nM), [^{3}H] clonidine (11nM) and [^{3}H]dihydroalprenolol (DHA) (0.7 nM) were used as the specific ligands for α_1, α_2 and β adrenoceptors respectively. The ability of bucindolol and its 5 hydroxy metabolite to displace the ligands from binding sites on rabbit brain membranes was compared to that of established α and β adrenoceptor antagonists. The concentrations of drug required to displace 50% of bound radiolabelled ligand (IC_{50} values) are shown in the Table.

Table IC_{50} values for displacement of ^{3}H prazosin, ^{3}H clonidine and ^{3}H dihydroalprenolol from binding sites on rabbit brain membranes by α and β adrenoceptor antagonists.

	[^{3}H]Prazosin	[^{3}H]Clonidine	[^{3}H]Dihydroalprenolol
Bucindolol	1×10^{-8}	2×10^{-5}	8×10^{-6}
5 hydroxybucindolol	2×10^{-6}	2×10^{-5}	-
Propranolol	-	-	1×10^{-6}
ICI 118551	-	-	2×10^{-6}
Prazosin	8×10^{-8}	1×10^{-4}	-
Phentolamine	8×10^{-7}	3×10^{-7}	-
RX781094	4×10^{-3}	7×10^{-7}	-
Yohimbine	3×10^{-4}	2×10^{-5}	-

Bucindolol was more potent than atenolol but less potent than ICI 118551 and propranolol in displacing DHA from its binding site. Although the IC_{50} for bucindolol vs prazosin was very low suggesting a high affinity for the $\alpha_1$adrenoceptor binding site, the slope of the dose response curve was shallow and gave a Hill coefficient of 0.33 ± 0.21. At present we have no explanation for this finding. Hill coefficients for bucindolol vs DHA and bucindolol vs clonidine were 0.85 ± 0.18 and 0.9 ± 0.2 respectively. 5 hydroxy-bucindolol was more potent than the parent compound in displacing DHA from its binding site; however, the displacement curve was biphasic. This may indicate widely differing affinities for β_1 and β_2 adrenoceptors. 5 hydroxybucindolol showed a similar affinity for clonidine binding sites but a lower affinity for prazosin binding sites compared to the parent drug. In neither of these systems did the Hill coefficient differ significantly from one.

The potency of some drugs in our syste are somewhat different from those published elsewhere, while the low Hill coefficient for the discplacement of [^{3}H] prazosin by bucindolol could suggest abnormalities in bucindolol binding in brain.

The relationship of these findings to bucindolol's activity at α and β adrenoceptors in vivo needs further evaluation.

Hannah, J.A.M. et al Naunyn Schmiedeberg's Arch. Pharmacol. in press.

CHRONIC ETHANOL ADMINISTRATION TO RATS INCREASES (^{14}C)-SERINE INCORPORATION INTO SYNAPTOSOMAL PHOSPHOLIPIDS

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The development of ethanol tolerance is associated with alterations in synaptosomal membrane lipids (Littleton & John, 1977) and in the activity of some enzymes of membrane lipid metabolism, eg, Ca^{2+}-dependent phospholipase A_2 (Littleton & Nhamburo, 1983). Ca^{2+}-mediated base exchange is the principal route of phosphatidyl serine synthesis in neuronal tissue (Goracci et al, 1973) and involves exchange of serine with phosphatidylethanolamine. Paton and Wing (1981) have reported its inhibition by the general anaesthetic halothane, but there have been no previous reports of alteration in activity by ethanol.

Male Sprague Dawley rats (200-250g) received intoxicating concentrations of ethanol by inhalation for 1-10 days. Crude synaptosomal fractions of brain (Cotman, 1974) in 0.32M sucrose, containing 1mM dithiothreitol, were used to assess base-exchange activity (Natsuki et al, 1978). Aliquots (500µg protein) were incubated with ^{14}C-serine (specific activity 53mCi/m.mol; Amersham) for a period of 40 min at 37°C. The reaction mixture also contained HEPES buffer 40mM, pH 8.0 and 50µM unlabelled serine. The reaction was stopped by the addition of ice-cold chloroform : methanol : HCl (2:1:0.02 v/v/v). Lipid extracts were prepared and aliquots taken for liquid scintillation counting of ^{14}C. When phospholipid classes were separated by thin-layer chromatography, approximately 90% of radiolabel was recovered in the phosphatidylserine fraction.

Base exchange activity was calculated as p.moles radiolabel incorporated per mg protein. Incorporation was not measurable in the presence of 1mM E.G.T.A. but increasing the external concentration of Ca^{2+} ($CaCl_2$ added to incubation mixture) caused a progressive increase in activity so that, in the presence of 2mM $CaCl_2$, the incorporation was approximately 3 times that when no $CaCl_2$ (or EGTA) was added. Ethanol (50mM) *in vitro* consistently reduced the base exchange activity (10-15%) only when the external Ca^{2+} concentration was high (2mM).

Synaptosomal fractions from brains of rats exposed to ethanol for 1,6 and 10 days showed a progressive and significant increase in enzyme activity. This was still observed in fractions taken from rats killed at the peak of the physical withdrawal syndrome when ethanol concentrations *in vivo* were very low. These increases relative to controls were obtained regardless of the added external Ca^{2+} concentration and presence of ethanol (50mM) *in vitro* still produced a consistent inhibition of base exchange activity.

The chronic administration of ethanol to rats therefore increases the activity of the enzymes involves in the Ca^{2+}-dependent serine-ethanolamine base exchange reaction. This probably does not alter the relative proportions of phospholipids in synaptosomal membranes (unpublished) but may reflect a general increase in Ca^{2+}-dependent synaptic processes associated with ethanol tolerance, since similar changes occur in phospholipase A_2 activity (Littleton & Nhamburo, 1983) and the stimulated release of neurotransmitters (Lynch & Littleton, 1983).

Cotman, C.W. (1974) Methods of Enzymology, vol.31, p.445. Academic Press, New York
Goracci, G. et al (1973) J. Neurochem. 20, 1167
Littleton, J.M. & John, G.R. (1977) J.Pharm.Pharmacol. 29, 579
Littleton, J.M. & Nhamburo, P.T. (1983) Br.J.Pharmacol. (in press)
Lynch, M.A. & Littleton, J.M. (1983) This meeting
Natsuki, R. et al (1978) Mol.Pharmacol. 14, 448
Paton, W.D.M. & Wing, D.R. (1981) Br.J.Pharmacol. 72, 393

A23187-INDUCED DOPAMINE RELEASE IN VITRO IS ENHANCED IN PREPARATIONS FROM RATS MADE PHYSICALLY DEPENDENT ON ETHANOL IN VIVO

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The presence of ethanol is generally inhibitory to the stimulated release of neurotransmitters (Carmichael & Israel, 1975) and this has been ascribed to an inhibitory effect of ethanol on voltage-dependent Na^+ or Ca^{2+} channels (Harris & Hood, 1981). When preparations are taken from brains of ethanol-tolerant animals the inhibitory effect of ethanol in vitro on electrically stimulated release of acetylcholine is reduced. Moreover, in the absence of ethanol the electrical stimulation of these preparations released a greater fraction of acetylcholine than in controls (Clark, Kalant & Carmichael, 1977). The mechanisms of such changes are of interest because they may represent the neuronal basis of ethanol tolerance and dependence.

In the experiments reported here, the release of ^3H from rat brain slices of corpus striatum preincubated with [^3H] dopamine has been studied in a superfusion system. Slices were prepared from control and ethanol-treated rats. Release of [^3H] dopamine was stimulated for 2 seven-minute periods during the experiment (S_1 and S_2) by including 12μM A23187 in the superfusing Krebs solution. The Ca^{2+} ionophore, A23187 causes release of neurotransmitter by allowing Ca^{2+} entry into the nerve terminal in a manner which by-passes the voltage-dependent channels (Akerman & Nicholls, 1981). Thus any changes in A23187-induced transmitter release from ethanol-treated slices compared with controls are unlikely to be a consequence of alterations in depolarisation or voltage-dependent ion flux.

Preparations of corpus striatum from control rats and rats made physically dependent on ethanol took up ^3H to a similiar extent. When superfused with A23187 however, the preparations from ethanol-dependent rats released a significantly greater fraction of ^3H than did controls. This was true of both the S_1 period of stimulation, where in preparations from ethanol-dependent rats, A23187 released a 4-fold greater fraction of [^3H] dopamine, and in the S_2 period, where the difference was 2.3-fold. If ethanol-treated rats were sacrificed at the peak of the physical syndrome of withdrawal, when blood ethanol concentrations *in vivo* were very low, the fraction of [^3H] dopamine released was increased 3-fold above control.

It is concluded that the increased fraction of neurotransmitter released from slices prepared from rats which have received ethanol *in vivo* may reflect an increased sensitivity of the release process to Ca^{2+} entry caused by the ionophore. Alternatively, the terminals from ethanol-treated rats may contain a higher Ca^{2+} concentration than normal and thus be closer to the threshold concentration of Ca^{2+} required for neurotransmitter release. Either mechanism could overcome the inhibitory effect of the presence of ethanol on transmitter release and could lead to a greater release of transmitter on withdrawal of the drug. The change may therefore play a role in the process leading to tolerance and physical dependence.

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Akerman, K.E.O. & Nicholls, D.J. (1981) Eur. J. Biochem. 115, 67-73
Carmichael, F.J. & Israel, Y.J. (1975) J. Pharmacol. Exp. Ther. 193, 824-834
Clark, J.W. et al., (1977) Can. J. Physiol. 55, 758-768
Harris, R.A. & Hood, W.F. (1980) J. Pharmacol. Exp. Ther. 213, 562-568

CHANGES IN TRH IN SPECIFIC RAT BRAIN REGIONS AND LUMBAR SPINAL CORD FOLLOWING METERGOLINE, AMITRIPTYLINE AND CHLORIMIPRAMINE

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Thyrotrophin releasing hormone (TRH) is widely distributed in the CNS and evidence suggests TRH may function as a neurotransmitter or neuromodulator (see Yarbrough, 1979). TRH has been shown to coexist with 5-hydroxytryptamine (5HT) in ventral spinal cord neurones (Gilbert et al, 1982). In view of the reported potentiating action of TRH on the changes in 5HT metabolism induced by imipramine (Rastogi, 1981) and reports that TRH improves depression (see Yarbrough, 1979) we have examined changes in TRH levels after chronic administration of two antidepressants, chlorimipramine and amitriptyline and the 5HT antagonist metergoline.

Male Wistar rats were given either metergoline (2 mg/kg), chlorimipramine (15 mg/kg) or amitriptyline (15 mg/kg) twice daily i.p. for 14 days. Control animals received vehicle alone. Four hours following the final injection, animals were killed and TRH measured by radioimmunoassay in various brain regions and the lumbar spinal cord.

Table. Effect of Amitriptyline, chlorimipramine and metergoline on TRH in specific CNS regions.

Treatment		Suprachiasmatic Nucleus	Nucleus Accumbens	Lumbar Spinal Cord
Amitriptyline	Control (6)	3.58±0.23	0.52±0.22	0.37±0.03
	Drug (6)	9.81±1.14**	2.99±0.21**	1.58±0.40**
Chlorimipramine	Control (12)	2.56±0.39	0.38±0.06	0.50±0.04
	Drug (12)	1.84±0.27	0.27±0.03	0.69±0.05*
Metergoline	Control (18)	2.87±0.36	0.36±0.09	0.56±0.06
	Drug (18)	2.73±0.42	0.25±0.06	0.77±0.04*

Results given as pg/μg protein±SEM. *P<0.01 **P<0.001 (Students t-test).

Chronic administration of amitriptyline, chlorimipramine or metergoline markedly increased the TRH content of the lumbar spinal cord. Amitriptyline also significantly increased TRH in the suprachiasmatic nucleus and the nucleus accumbens (Table). None of the drugs altered TRH levels in the septum or median eminence. Previous studies have shown changes in spinal cord and accumbens TRH following lesions of 5HT neurones, changes in 5HT synthesis (Lighton et al, 1983) and repeated ECS (Bennett et al, 1983). The present results indicate that antidepressants also alter TRH levels. It remains to be determined if these changes are related to 5HT neuronal activity though it is interesting that amitriptyline, which blocks 5HT reuptake and is a 5HT receptor antagonist (Ögren et al, 1979) had the most marked effects on TRH.

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Bennett, G W et al (1983) Br.J.Pharmacol. (in press)
Gilbert, R F et al (1982) Neuroscience 7, 69-87
Lighton, C et al (1983) Neuropharmacol. (submitted)
Ögren, S-O et al (1979) J.Neurol.Trans. 46, 85-103
Rastogi, R B et al (1981) Brain Res.Bull. 7, 449-451
Yarbrough, G G (1979) Progress in Neurobiology 12, 291-312

EFFECTS OF BUPROPION ON BODY WEIGHT AND METABOLISM IN DIETARY OBESE RATS

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Bupropion is a novel antidepressant which, although structurally related to amphetamine, appears to lack the pharmacological profile of an amphetamine-like stimulant in the CNS (Hamilton et al 1983). In view of the ability of conventional antidepressants to affect appetite, food-intake and body weight, the action of bupropion on body weight and glucose metabolism has been investigated.

Groups of 6 female Wistar rats (University of Bath strain), initial weight 200±2.1g, were allowed continous access to Oxoid 41B pellet either alone or with chocolate, meat, biscuit and "Rice Krispies" (Kirby et al 1978). All rats were weighed daily at 10.00h before oral dosing with either bupropion (2.5 or 7.5mg/kg) in water or water alone. After 28 days the rats were killed, and blood and liver samples were taken for assay of glucose and glycogen respectively.

Body weight of pellet-fed rats was unaffected by bupropion , but the dietary-obese animals showed a significantly reduced ($p < 0.05$) gain in weight (Table 1). Conversely bupropion significantly reduced ($p < 0.001$) blood glucose concentrations in the pellet-fed animals, but failed to induce any further decrease in the already-lowered blood glucose levels of the dietary-obese rats. No changes in liver glycogen were observed.

The ability of bupropion to lower blood glucose and to reduce the rate of weight gain in 'cafeteria-fed' animals was reminiscent of the action of fenfluramine, to which bupropion bears a structural relationship; it was therefore of interest to investigate whether bupropion also shared the ability of fenfluramine to increase glucose uptake into skeletal muscle (Kirby & Turner 1975). Hemidiaphragms were prepared from male Wistar rats (120±0.7g) previously fasted overnight, and glucose uptake was measured in the presence of 100μU/ml insulin (Kirby & Turner 1975). Bupropion 100ng/ml did not affect glucose uptake, but at a concentration of 250 ng/ml glucose uptake was increased by 0.62±0.26mg glucose/g wet weight/90min (mean±s.e.m., n=6) while a concentration of 500ng/ml bupropion increased glucose uptake by 0.88±0.22mg glucose/g wet weight/90 min (mean±s.e.m., n=6 $p < 0.02$).

Thus bupropion appears to have weak anorectic properties and to behave like fenfluramine in terms of the parameters of glucose metabolism measured.

Table 1. Effect of bupropion 7.5mg/kg on weight gain, blood glucose & liver glycogen.

	Pellet-fed		Dietary-obese	
	control	bupropion	control	bupropion
weight gain (g)	27.1±3. 0	26.9±2.10	84.6±6. 2	63.1±7. 2
blood glucose (mg%)	69.7±0.53	62.0±1.76	59.7±0.83	58.3±1.36
liver glycogen (mg/g wet wt)	49.2±0.97	50.0±1.18	48.9±1.16	49.9±0.99

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Hamilton, M.J. et al (1983) Br. J. Clin. Pharmac. 15, 367-377
Kirby, M.J. & Turner P. (1975) Postgrad. Med. J. 51 (Suppl. 1) 73-76
Kirby, M.J. et al (1978) Br. J. Pharmac. 64 442P.

USE OF AN IN VITRO MAMMALIAN SPINAL CORD PREPARATION TO EXAMINE THE EFFECTS OF MONOAMINES ON EVOKED RESPONSES

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There is increasing evidence that the monoamines noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) have a role in the regulation of synaptic transmission in the spinal cord (Willis, 1982). Investigation of this in vivo is difficult because of the limitations on direct quantification imposed by the techniques of iontophoresis, and pressure ejection from microelectrodes, and the uncertain effects of the blood-brain barrier on substances administered systemically. The in-vitro hemisected cord offers the advantages of allowing direct application of known quantities of test compounds to the tissue under controlled conditions without the intervention of the blood-barrier. Isolated preparations of amphibian and neonatal mammalian spinal cord have demonstrated the usefulness of this approach in investigating the effects of drugs upon spinal responses (Tebecis & Phillis 1967, Evans 1980), but it has so far proved difficult to apply the same technique to mature mammalian tissue. We have developed a viable hemisected spinal cord preparation from golden hamsters which has allowed us to investigate the effects of monoamine upon evoked reflex responses.

Fully mobile, young hamsters weighing 20-40gm were decapitated under light halothane anaesthesia and the spinal cords dissected out under cold mammalian artificial cerebrospinal fluid (c.s.f.). Cords were hemisected and mounted in a bath perfused with c.s.f. at 21^{o}C. After a recovery period of approximately 1 hour reflex discharges up to 4mV in amplitude could be recorded from the lumbar ventral roots on stimulation of the corresponding dorsal roots. These evoked responses showed clear mono- and polysynaptic components and remained stable for up to 8 hours, allowing detailed pharmacological investigations. Replacement of the calcium in the c.s.f. by 2mM manganese abolished the responses, showing them to be of synaptic origin and not caused by direct activation of the motoneurones by stimulus spread.

Dose response curves have been plotted for the effects of bath applied adrenaline, nor-adrenaline, dopamine and 5-HT upon the amplitude of the evoked ventral root reflex. Dopamine and 5-HT were both found to depress the response whereas adrenaline and noradrenaline produced potentiation. The optimum concentration for potentiation of the response by adrenaline was 1 µM which caused an increase in the amplitude of the monosynaptic response by 18.0% (SE$\pm$ 1.7, n=5). Noradrenaline was found to be less potent, causing a maximum potentiation of 23.3% (SE+ 5.9, n=4) at a concentration of 50µM. At the same concentration 50µM, 5HT and dopamine depressed the response to 70.0% and 80.2% of control values respectively. A reduction in the size of the response was also observed at concentration of noradrenaline above 100µM. Addition of the adrenergic antagonist phentolamine (1µM) to the perfusion medium abolished the potentiation of the response produced by both adrenaline and noradrenaline.

Investigations are in progress to study the effects of these amines on the properties of individual units within the cord. We feel that this preparation will prove a useful tool in the study of the pharmacology of the spinal cord.

Evans, R.H. (1978) Br.J.Pharmac. 62, 171
Tebecis, A.K. & Phillis, J.W. (1967). Comp.Biochem.Physiol. 23, 553
Willis, W.D. (1982) Progress in Sensory Physiology. 3, 40

PSEUDO NON-COMPETITIVE INTERACTION OF ENDOGENOUS AND EXOGENOUS NORADRENALINE WITH CEREBRAL α_2-ADRENOCEPTORS

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There is now substantial evidence indicating the alpha$_2$-adrenoceptors are negatively coupled to adenylate cyclase and that the affinity of agonists, but not antagonists, is characteristically reduced by Na^+ and guanine nucleotides (Limbird, 1981). However, a recent report suggests that the binding of the antagonist ^{3}H-yohimbine to rat cerebral cortical alpha$_2$-adrenoceptors is markedly increased by these modulators (Woodcock & Murley, 1982). In the present experiments, we provide evidence that this phenomenon relates to retained endogenous noradrenaline in certain membrane preparations.

Rat cerebral cortical membranes were prepared and washed in hypertonic sucrose buffer (A) (Woodcock & Murley, 1982) favouring the formation of synaptosomes or hypotonic Tris-HCl buffer (B) and assays performed as previously described (Cheung et al, 1982) using the alpha$_2$ specific radioligand ^{3}H-yohimbine. Saturation binding curves were performed in parallel experiments in the presence or absence of NaCl (200 mM) and Gpp(NH)p (10 µM). Endogenous noradrenaline retained in the membranes was measured by HPLC with electrochemical detection.

Binding capacities (B_{max}) and dissociation constants (K_D) were calculated from Scatchard plots. B_{max} in preparation A (63.6 ± 6.7 fmol/mg protein ; mean ± SEM; n = 3) was considerably lower than preparation B (182.5 ± 37.8) ($P < 0.05$). NaCl and Gpp(NH)p significantly increased binding capacity in preparation A (156.3 ± 22.9) but had no significant effect on B (220.9 ± 36.4). The apparent affinity for ^{3}H-yohimbine was identical in all preparations. The noradrenaline content of membranes from preparation A (1.98 ± 0.24 pmol/mg protein) was at least 10 fold greater than those from B (< 0.2 pmol/mg protein), and would, if assumed to be entirely free, be equivalent to an approximate concentration of 5 nM per tube in the binding assays.

In separate experiments, the effect of exogenously added noradrenaline on ^{3}H-yohimbine binding in membranes prepared in hypotonic buffer (B) was examined. The apparent B_{max} was reduced by 40-50% in the presence of 10 nM noradrenaline, and again this effect was readily reversed when incubations were performed in the presence of NaCl and Gpp(NH)p.

These results suggest that noradrenaline interacts with antagonist binding to the alpha$_2$-adrenoceptor in a 'pseudo non-competitive' manner as recently described for agonists at the dopamine D_2 receptor (Sibley & Creese, 1980). Thus, endogenous noradrenaline retained in the synaptosomal membranes can preferentially occupy a proportion of receptor sites in the high affinity state and lead to an apparently low binding capacity which is reversed by Na^+ and guanine nucleotides. These findings may have wider implications since they could complicate the interpretation of certain 'down regulation' receptor studies in which the influence of membrane preparation and retained agonists have not been fully assessed.

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Cheung, Y.-D. et al (1982) Eur. J. Pharmacol., 84, 79
Limbird, L. (1981) Biochem. J., 195, 1
Sibley, D.R. & Creese, I. (1980) Eur. J. Pharmacol., 65, 131
Woodcock, E. & Murley, B. (1982) B. B. Res. Comm., 105, 252

IONIC REGULATION OF DL-(^{3}H)-2-AMINO-4-PHOSPHONOBUTYRATE BINDING TO L-GLUTAMATE-SENSITIVE SITES ON RAT BRAIN SYNAPTIC MEMBRANES

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The application of binding studies to the characterisation of excitatory amino acid receptors has been impeded by the lack of suitable radiolabelled ligands. With the recent availability of DL-^{3}H-2-amino-4-phosphonobutyrate (APB), (22.6 Ci/mmol; NEN) we have reported that APB interacts with a population of Cl^- and Ca^{2+} -dependent sites (Butcher et al., 1983), which appears to correspond with a specific APB-sensitive, L-^{3}H-glutamate binding component on rat brain synaptic membranes (Fagg et al.,1982). In this study we have carried out a detailed analysis of the ionic requirements for glutamate-sensitive DL-^{3}H-APB binding.

Whole brain synaptic membranes were prepared as described previously (Sharif & Roberts,1980) and assays were performed in 50mM HEPES-KOH buffer (pH 7.4) with the addition of specific ions. Incubations were at 37°C with a final DL-^{3}H-APB concentration of 30nM. After 10 min, binding assays were terminated by centrifugation in a Beckman microfuge, and non-specific binding was determined by inclusion of 1mM L-glutamate.

Specific DL-^{3}H-APB binding was almost undetectable in HEPES-KOH buffer alone. The inclusion of Cl^- (2.5mM) resulted in a large increase in binding, to 123 ± 24 fmol/mg protein. This effect was highly specific for Cl^-, since no other anion apart from Br^-, could even partially reproduce this effect. Ca^{2+} (2.5mM) further increased binding to 246 ± 23 fmol/mg protein, but only in the presence of Cl^-. Other divalent cations partially mimicked the effect of calcium, while the monovalent cation, sodium, decreased the binding stimulated by Cl^-/Ca^{2+}. EC_{50}'s for the effects of each ion were: chloride, 631uM; calcium, 631uM, and sodium, 6.13mM. Saturation analysis data are shown in Table 1.

Table 1 Ionic dependence of DL-^{3}H-APB binding

Assay medium	K_D (uM)	B_{max} (pmol/mg protein)
(HEPES-KOH buffer +)		
1mM NH_4Cl	1.3	4.6
2.5 mM NH_4Cl	1.48	7.6
2.5mM $CaCl_2$	1.36	12.06
1mM $(CH_3COO)_2Ca$ + 2.5mM NH_4Cl	1.44	10.17
2.5mM $CaCl_2$ + 100mM CH_3COONa	4.69	14.58
Krebs medium	4.65	13.58

Ca^{2+} and Cl^- appear to act by increasing receptor density, while Na^+ decreases receptor affinity. This raises the possibility that binding may be regulated by two ionic mechanisms.

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Butcher, S.P., Roberts, P.J. & Collins, J.F. (1983) IRCS Medical Science 11, 42-43

Fagg, G.E., Foster, A.C., Mena, E.E. & Cotman, C.W. (1982) J. Neuroscience 2, 958-966.

EFFECTS OF NEUROLEPTICS ON THE INTRACELLULAR AND EXTRACELLULAR ACCUMULATION OF CYCLIC AMP IN DOPAMINE-STIMULATED BRAIN SLICES

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Dopamine (DA) receptors have been classified as D_1 receptors which mediate stimulation of adenylate cyclase and c'AMP accumulation but which have no other clearly established CNS function, and D_2 receptors which do not activate cyclase but which are probably responsible for the therapeutic actions of neuroleptics. Recently, Stoof and Kebabian (1981) have reported that activation of D_2 receptors inhibits the release of c'AMP from superfused striatal slices stimulated with D_1 agonists. We have used a non-superfusion brain-slice procedure to examine the effects of various neuroleptics/D_2 antagonists on the DA-stimulated accumulation of c'AMP in both the supernatant and tissue.

Rat striatal slices were incubated in Krebs buffer containing 1 mM IBMX at 37° for 90 min. with agitation and were then transferred to test tubes containing antagonists. After gentle vortexing for 10 min., DA was added (final concentration 50 μM in 370 μl) and the tubes were vortexed for a further 20 min. 100 μl of supernatant was removed for c'AMP radioimmunoassay and 20 μl concentrated HCl was added to each tube, which served both to stop the reaction and to elute tissue c'AMP, which was measured with a protein binding method.

Table 1 Effect of DA antagonists on DA-stimulated c'AMP (expressed as % basal)

	Supernatant		Tissue	
Antagonist (μM)	50 μM DA	DA + Antagonist	50 μM DA	DA + Antagonist
Sulpiride (100)	244 ± 7	469 ± 43	178 ± 9	229 ± 25
Domperidone (10)	222 ± 13	474 ± 34	175 ± 7	202 ± 15
Haloperidol (10)	222 ± 13	328 ± 31	175 ± 7	132 ± 10
α-Flupenthixol (10)	183 ± 8	118 ± 8	141 ± 5	117 ± 5

Results are the mean and SEM of 9 observations obtained in 3 experiments. All antagonists produced significant differences from DA alone ($P < 0.05$ by ANOVA). Basal values (pmol c'AMP/mg protein, n = 7): supernatant 0.52 ± 0.11; tissue 12.2 ± 1.1. Propranolol, yohimbine and prazosin (all 10^{-5} M) were ineffective.

The results in Table 1 are consistent with the known D_1/D_2 selectivities of the antagonists. Domperidone and sulpiride are selective D_2 antagonists and enhanced DA-stimulated c'AMP in both supernatant and tissue to the same extent. Haloperidol (a less selective antagonist) was also stimulatory in the supernatant, though to a lesser extent, but inhibited DA-stimulated c'AMP in the tissue. α-Flupenthixol is non-selective and at all effective concentrations had only a inhibitory effect. Supernatant c'AMP levels provide a more sensitive measure of c'AMP stimulation than tissue levels, but the results with haloperidol indicate that the two are not always clearly related. The opposite effects of selective and non-selective neuroleptics leaves in doubt the significance of changes in DA-stimulated c'AMP production for the therapeutic actions of neuroleptics. The possibility remains that D_2-mediated inhibition of c'AMP stimulated by a different agonist-receptor system may be of therapeutic significance.

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Stoof, J.C. & Kebabian, J.W. (1981) Nature 294, 366-368.

A COMPARATIVE STUDY OF (^{3}H)-DOMPERIDONE BINDING IN THE RAT STRIATUM AND HYPOTHALAMUS

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It has recently been suggested that (^{3}H)-domperidone ((^{3}H)-DOM) is the most suitable radioligand for dopamine D-2 receptors in rat striatum (Lazareno & Nahorski, 1982). The high affinity, low non-specific binding, high specific activity and selectivity for D-2 receptors of this radioligand make it suitable for quantifying low densities of dopamine receptors, so we have compared the binding of (^{3}H)-DOM in rat striatum with that in hypothalamus, an area possessing a relatively low level of dopaminergic innervation which is important in many neuroendocrine functions.

Striata and hypothalami were dissected out from female Wistar rat brain and membranes prepared by homogenisation and centrifugation and stored at -70°C. (^{3}H)-DOM binding assays were performed by incubating radioligand with membranes (200 µg protein) in Tris/HCl buffer (50mM, pH 7.4 at 25°C) containing 0.002% bovine serum albumin and 120mM NaCl, for 45 mins at 25°C. Bound and free ligand were separated by rapid filtration over Whatman GF/C filters, followed by 3 x 5ml washes of the above buffer. Specific binding was defined as that displaced by 1µM d-butaclamol.

In preliminary studies it was found that specific binding increased linearly with protein concentration up to 300µg/ml in both striatum and hypothalamus. Specific binding reached equilibrium within 45 mins at 25°C, with half times of 8-10 mins for association and 6-9 mins for dissociation in both striatum and hypothalamus. Scatchard analysis of saturation curves for the striatal binding of (^{3}H)-DOM (0.025-2.5nM) indicated binding to a homogenous population of sites, with K_D = 0.35 ± 0.05nM and B_{max} = 210 ± 20 pmol/g protein. A similar analysis of hypothalamic (^{3}H)-DOM binding proved technically difficult due to the very low level of specific binding (<20% of total at 0.4nM (^{3}H)-DOM compared to 65% for striatal binding), but approximate values were K_D = 0.5nM and B_{max} = 35 pmol/g protein.

Pharmacological characterisation of (^{3}H)-DOM binding (0.4nM) to striatal membranes indicated a typical dopamine D-2 receptor profile; IC_{50} values (nM) for displacement of binding by various drugs were: d-butaclamol, 3; l-butaclamol, 10,000; domperidone, 1; spiperone, 0.2; sulpiride, 50; metoclopramide, 60; dopamine, 1000; ADTN, 200 ; apomorphine, 100; phentolamine, 6000; 5HT, >10,000. The low level of specific (^{3}H)-DOM binding in hypothalamus made it impossible to obtain accurate estimates of drug potencies, but approximate IC_{50} values (nM) were: d-butaclamol, 10; l-butaclamol, 1000; spiperone, 1; sulpiride, 50; dopamine, 3000; phentolamine, >1000; cinanserin, >>1000.

The binding characteristics of (^{3}H)-DOM in rat striatum were thus very similar to those reported by others (Lazareno & Nahorski, 1982; Huff & Molinoff, 1982), confirming the suitability of this radioligand for dopamine D-2 receptor studies. The characteristics of (^{3}H)-DOM binding in hypothalamus on the whole resembled those in striatum, with similar kinetics, K_D values, and relative potencies of displacing drugs. Binding site density was only around 15% of that in striatum, with a B_{max} value similar to that previously reported for hypothalamic D-2 sites (List & Seeman, 1981). It appears, therefore, that (^{3}H)-DOM can be successfully used to characterise and quantify dopamine D-2 receptors in the hypothalamus.

Huff, R.M. & Molinoff, P.B. (1982) Proc. Natl. Acad. Sci. 79, 7561-7565.
Lazareno, S. & Nahorski, S.R. (1982) Eur. J. Pharmac. 81, 273-285.
List, S.J. & Seeman, P. (1981) Proc. Natl. Acad. Sci. 78, 2620-2624.

EFFECTS OF TUBOCURARINE AND OTHER NEUROMUSCULAR BLOCKING AGENTS ON Ca-ACTIVATED K-CHANNELS IN GUINEA-PIG LIVER CELLS.

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In guinea-pig hepatocytes a variety of receptors, including those for angiotensin II, can elevate $[Ca^{2+}]_i$ which, as in many other cell types, leads to an increase in cell membrane K permeability. This Ca-activated K permeability is blocked by the bee venom peptide apamin. [^{125}I]monoiodoapamin appears to be a specific ligand for these channels. The present results show that tubocurarine and other neuromuscular blocking agents also interact with these channels.

The ability of various agents to inhibit K loss from liver cells in response to angiotensin II was tested using a K^+-sensitive electrode to monitor extracellular K^+ in a suspension of cells (Burgess et al., 1981). Results are presented in table 1(a). The ability of the same agents to inhibit the binding of [^{125}I]monoiodoapamin to isolated guinea-pig hepatocytes was also studied, using methods as in Cook et al., 1983. Briefly, hepatocytes were incubated with labelled apamin, ±competing ligands, for 2 minutes at 37°C in supplemented Eagle's medium. Cell associated [^{125}I]monoiodoapamin was separated by centrifugation through oil. Results are given in table 1(b).

Table 1(a). K release by 10^{-6}M angiotensin II in presence of antagonists.

conc(μM)	Tubocurarine	Atracurium	Pancuronium	Gallamine	Decameth.	Hexameth.
1	83±1	89±5	–	–	–	–
3	50±5	50±4	54,48	78±8	–	–
10	33±1	33±6	–	–	101,90	–
30	15±4	15±1	16,24	19±2	–	–
100	2±1	2±1	–	–	78,71	–
300	–	–	–	–	53	78±6
1000	–	–	–	–	–	46

Table 1(b). Inhibition of [^{125}I]monoiodoapamin binding.

K_I(μM)	9.2±0.4	4.3±0.2	6.6±0.7	16.1±0.6	795±58	982±27

Values for K loss (% of control loss) are either means±s.e.m. for 3 or 4 animals or individual values. K_I values are non-linear least squares estimates based on simple competition for a single class of binding site (s.e.m. from variance of data). The K_d for labelled apamin was taken to be 350 pM (Cook et al., 1983).

In addition to blocking nicotinic receptors, tubocurarine and other neuromuscular blocking agents also block end-plate ion channels (eg; Colquhoun et al., 1979). The ability of these agents to block Ca-activated K channels is probably not, however, simply related to an affinity for either the nicotinic receptor or end-plate channels since we found neither α-bungarotoxin (5μM) nor the end plate channel blockers amantadine (100 μM) and phencyclidine (100μM) (Tsai et al., 1978; Albuquerque et al., 1980) were able to inhibit apamin binding or K loss from liver cells.

The neurotoxicity of apamin is dependent on the presence of two adjacent, positively charged amino acid residues (Granier et al., 1978) and it is speculated that the ability of tubocurarine and related compounds to block Ca-activated K channels may similarly depend on the appropriate spacing of two charged nitrogen atoms.

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Albuquerque, E.X. et al (1980) Mol. Pharmacol. 18, 167-178.
Burgess, G.M. et al (1981) J. Physiol. 317, 67-90.
Colquhoun, D. et al (1979) J. Physiol. 293, 247-284.
Cook, N.S. et al (1983) FEBS Lett. 152, 265-269.
Granier, C. et al (1978) Eur. J. Biochem. 82, 293-299.
Tsai, M-C. et al (1978) Mol. Pharmacol. 14, 787-803.

(^{3}H)-N-(CHLOROETHYL) NORAPOMORPHINE BINDING TO RAT STRIATAL MEMBRANES.

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N-(Chloroethyl)norapomorphine (NCA) causes prolonged inhibition of apomorphine-induced circling and climbing behaviour and ^{3}H-NPA binding to striatal membranes (Costall et al,1980). We now report on the interaction of ^{3}H-NCA with rat striatal membranes.

Washed striatal membranes suspended in 15 mM 2-(N-morpholino)ethane-sulphonic acid NaOH buffer containing 1 mM EDTA were preincubated with displacing agents at 37°C prior to the addition of ^{3}H-NCA (16.3 Ci/mmol; New England Nuclear) and then incubated for a further 20 min. Specific binding was defined as that prevented by 10^{-5} M 6,7-ADTN. The reaction was stopped by rapid vacuum filtration.

Specific binding of ^{3}H-NCA varied according to pH, being maximal between pH 5.7 to 6.3; above pH 7.0 (using Tris-HCl buffer containing 1 mM EDTA) binding was not prevented by 10^{-5} M ADTN. Subsequent experiments were performed at pH 5.9 where specific binding represented 30-35% of total bound.

Non-specific binding of ^{3}H-NCA (4.7 nM) increased in a linear manner with time. Association of specific binding was near maximum after 30 min. By computer curve fitting B_{∞} was 42.2 $\pm$ 3.7 pmol g^{-1} ($\pm$ 1 S.E.M.; n = 3) tissue. The rate constant for binding (K_2) was 3.9 $\pm$ 1.1 x 10^5 M $^{-1}s^{-1}$. At higher concentrations of ^{3}H-NCA B_{∞} was unchanged, showing binding to be saturated. Dissociation of specific binding was slow ($t_{\frac{1}{2}} > 60$ min).

Dopamine receptor agonists with a catechol moiety were potent at preventing binding; pergolide was not (Table 1). Binding was not prevented stereoselectively by the isomers of butaclamol, flupenthixol or sultopride or by sulpiride. Noradrenaline and 5HT were only weakly effective in preventing ^{3}H-NCA binding. Inclusion of compounds into incubates following the addition of ^{3}H-NCA did not cause displacement of the ligand.

Table 1 Inhibition of ^{3}H-NCA binding

	Ki (nM)		Ki (nM)
N,N-propylnorapomorphine	58	cis-flupenthixol	4,400
ADTN	78	trans-flupenthixol	5,000
Dopamine	470	(+)-butaclamol	10,000
Apomorphine	520	(-)-butaclamol	8,000
Pergolide	3,200	(-)-sultopride	> 100,000
Bromocriptine	> 10,000		
Noradrenaline	3,200	(+)-sultopride	> 100,000
5HT	2,000	Sulpiride	> 10,000

Ki was calculated according to Burgen et al (1974)

We conclude that although NCA interacts with dopamine receptors, the specific binding of ^{3}H-NCA observed is irreversible binding to a saturable catecholamine binding site, and not to a dopaminergic binding site.

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Burgen,A.S.V., Hiley,C.R. & Young,J.M. (1974) Br.J.Pharmac. 51,279.
Costall,B. et al (1980) J.Pharmac.Pharmacol. 32, 571.

ELEVATION OF CIRCULATING PROLACTIN CONCENTRATIONS MAY NOT CAUSE STRIATAL DOPAMINE RECEPTOR SUPERSENSITIVITY

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Supersensitivity of striatal dopamine receptors following repeated neuroleptic treatment may be mediated indirectly by elevation of circulating prolactin levels (Hruska et al,1980; Hruska et al,1982). Previously, however, we had been unable to demonstrate any effect of hypophysectomy on the induction of striatal dopamine receptor supersensitivity by high doses of neuroleptic drugs (Jenner et al,1981). We now compare the ability of three neuroleptic drugs which elevate prolactin secretion, but differ in their penetration into brain, to alter behavioural and biochemical indices of striatal dopamine receptor function.

Male Wistar rats (131±2 g at the start of the experiment) were treated for 21 days with either haloperidol, sulpiride or domperidone (all 5 mg/kg i.p.) or an equivalent volume of saline. After 21 days some animals were killed for determination of plasma prolactin concentration. Drug administration was followed by a 3 day drug washout period prior to behavioural and biochemical testing.

In plasma samples taken 1 h after the last injection of haloperidol, domperidone or sulpiride, prolactin levels were increased 6-12 fold by all drug treatments (Table 1). Apomorphine hydrochloride (0.0625-0.5 mg/kg s.c., 15 min previously)-induced stereotyped behaviour was enhanced in haloperidol but not in sulpiride or domperidone-treated animals (Table 1). The number of striatal ^{3}H-spiperone (0.05-1.0 nM; defined using 3 x 10^{-5} M (-)-sulpiride) binding sites (Bmax) was increased by haloperidol treatment, but not by administration of sulpiride or domperidone (Table 1). The dissociation constant (K_D) was unchanged by all treatments.

Table 1 Changes in plasma prolactin concentrations, apomorphine (0.25 mg/kg sc)-induced stereotypy and striatal ^{3}H-spiperone binding after repeated neuroleptic administration

Drug treatment	Prolactin concentration (ng/ml)	Stereotypy score	Bmax (pmoles/g tissue)
Saline	31.7 ± 5.6	2.3 ± 0.2	17.9 ± 1.2
Haloperidol	364.0 ± 51.5*	3.5 ± 0.2*	26.5 ± 1.4*
Sulpiride	191.9 ± 26.4*	2.7 ± 0.2	18.0 ± 1.5
Domperidone	432.1 ± 45.7*	2.5 ± 0.2	19.0 ± 0.9

* $p < 0.05$ compared to saline-treated animals

These findings indicate that neuroleptic-induced elevations in plasma prolactin levels are not solely responsible for the induction of cerebral dopaminergic supersensitivity.

Hruska,R.E. et al (1980) Eur.J.Pharmac. 65, 455
Hruska,R.E. et al (1982) Life Sci. 30, 547
Jenner,P. et al (1981) Eur.J.Pharmac. 76, 31.

COMPARISON OF APOMORPHINE-INDUCED MOTOR BEHAVIOUR IN CLIMBING AND NON-CLIMBING RATS OF THE SAME STRAIN

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Apomorphine-induced climbing in mice is widely used for investigating dopamine agonist and antagonist drugs (Protais et al, 1976; Puech et al, 1976). The use of rats is preferable where biochemical correlates of behavioural experiments are required. However, rats exhibit less consistent climbing behaviour to apomorphine than mice. We now report a comparison of motor behaviour in climbing and non-climbing rats of the same strain.

All apomorphine-induced motor behaviours were investigated following a 1 h acclimatisation period. Climbing and stereotypy were assessed simultaneously at 2 minute intervals from 10-20 minutes after apomorphine. The final score attained by each animal was the sum of the readings taken throughout this 10 minute observation period. Rearing was scored in a similar manner except that readings were taken at 1 min intervals. Activity was measured during the acclimatisation period and up to 30 min following apomorphine administration using automated activity boxes. Spontaneous activity was assessed in a hole board apparatus by counting crosses, dips, and rears exhibited by animals in the first 3 min following introduction.

Female Wistar rats (150-200 g) receiving apomorphine hydrochloride (0.45 mg/kg sc) exhibit a bimodal distribution of climbing response. The animals could be divided into those showing a climbing response to apomorphine (48%) and those showing no climbing at all. Subsequent challenge with apomorphine always elicited the same response in each animal.

Climbing rats exhibited a dose-related increase in climbing and rearing behaviour to apomorphine (0.0625-1.0 mg/kg sc) (Table 1). In contrast, non-climbers did not rear or climb in response to apomorphine. Non-climbing rats were more active than climbers in response to apomorphine; a difference was also apparent in degree of exploratory behaviour exhibited during the acclimatisation period. There was no difference in the ability of apomorphine to induce stereotypy in climbing and non-climbing rats. There was no difference in spontaneous behaviour as assessed using crossing, dipping and rearing behaviour in the hole-board.

Table 1 Comparison of the ability of apomorphine to induce motor behaviour in climbing and non-climbing rats

	ED_{50} values for apomorphine induced behaviour (mg/kg)			
	Climbing	Rearing	Activity	Stereotypy
Climbers	0.16 (0.12-0.22)	0.14 (0.087-0.23)	0.14 (0.11-0.17)	0.15 (0.11-0.20)
Non-climbers	-	-	0.095 (0.088-0.10)	0.12 (0.088-0.16)

95% confidence limits shown in brackets

These data suggest that climbing in the rat in response to apomorphine is dependent on the occurrence of rearing, and that it is an individual response.

Protais,P. et al (1976) Psychopharmac. 50, 1.
Puech,A.J. et al (1976) Eur.J.Pharmac.36, 439.

EFFECT OF FOOD ON THE ABSORPTION OF D-PENICILLAMINE

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D-penicillamine (β,β-dimethylcysteine) is an *in vivo* metabolite of penicillin (Walshe, 1953) which has been shown to have a wide range of biological actions due to its aminothiol properties. It is widely used in the treatment of rheumatoid arthritis, Wilson's disease and cystinuria. The bioavailability of many drugs including some of the penicillins has been shown to be reduced when administered with food (Welling, 1980) and since D-penicillamine binds extensively to proteins and metal ions it is possible that it is similarly affected. Recent work has shown that the absorption of D-penicillamine is reduced when taken with food by 20% in rats (Planas-Bohne, 1972) and by some 33.3% in man (Perrett, 1981). However both these studies employed indirect methods for the measurement of D-penicillamine, the former using radiolabelled drug and the latter by measuring urinary metabolites only. We have developed a sensitive analytical method for the assay of D-penicillamine in plasma which has allowed us to study the drug's pharmacokinetics in man. We present data to show that its absorption is significantly reduced when administered with food which may have a significant influence on the steady state plasma concentration during chronic administration.

Five healthy volunteers, 3 males 2 females aged 22-42 years, were each given an oral dose (500mg) of D-penicillamine on two occasions. The first was following an overnight fast with no food or milk allowed for two hours after dosing, and the second (a minimum of 8 days later) in the middle of a light breakfast. Blood was collected at 1,2,3,4,6,8, and 24 hours and plasma assayed for total D-penicillamine using a modification of the method of Abounassif and Jefferies (1983). Areas under the curve (AUC) were calculated using the trapezoidal rule.

D-penicillamine was rapidly absorbed from the gastro-intestinal tract with peak plasma concentrations being observed at 2 hours. In the fasting state the peak concentration was 8.04 $\pm$ 0.34μg/ml (mean $\pm$ sem) which was significantly greater ($p<0.02$) than that in the non-fasting state (5.54 $\pm$ 0.43μg/ml). The peak fasting plasma concentrations were significantly higher ($p<0.01$) than those reported by Wiesner *et al* (1981) (4.1 $\pm$ 0.77μg/ml) who administered an 800mg oral dose under similar conditions although in that study it is unclear whether free or total drug was measured. The decline in plasma concentration was biphasic in each individual with an initial decay of 5.19 $\pm$ 0.58 hours followed by a much slower decline of 59.43 $\pm$ 9.06 hours although this last value may be criticised on the basis that the study was not extended past 24 hours. The AUC in the fasting state (83.97 $\pm$ 1.6μg.h/ml) was significantly higher ($p<0.05$) than that in the non-fasting condition (73.19 $\pm$ 2.99μg.h/ml).

Our results indicate that absorption of D-penicillamine is significantly reduced following a light meal. Chronic administration of the drug with food is likely to result in lower plasma concentrations at steady state compared to that obtained when taken in the fasting state. The clinical significance of this in patients with rheumatoid arthritis is being assessed.

Abounassif,M.A. & Jefferies,T.M. (1983) J.Biomed.Pharmaceut.Anal. 1, *in press*
Perrett,D. (1981) J.Rheumatol. Suppl.7, 41
Planas-Bohne,F. (1972) Arzneim Forsch Drug Res. 22, 1426
Walshe,J.M. (1953) Quart.J.Med. 22, 483
Welling,P.G. (1980) Pharm.Int. January, 14
Wiesner, R.H.,Dickson,E.R. *et al* (1981) J.Rheumatol. Suppl.7, 51

ORAL SLOW-RELEASE VERAPAMIL I: A SINGLE DOSE COMPARISON WITH CORDILOX AND ISOPTIN IN 12 HEALTHY VOLUNTEERS

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The antiarrhythmic and vasodilator agent, verapamil, has a short elimination half-life of ($t_{\frac{1}{2}}$) necessitating 3 or 4 times daily dosage therefore , a slow-release formulation (SRV) has been developed.

Single oral doses of 3 preparations of verapamil (240mg) were compared - Cordilox®, Isoptin® and SRV - in random order at weekly intervals in 12 healthy male volunteers. Blood samples were collected at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours after dosage for the measurement of plasma verapamil concentration using an HPLC method (Cole et al, 1981). Pharmacokinetic analysis of the resulting data was achieved using the computer program STRIPE, (Johnston & Woollard, 1983). Statistical comparisons were made using the student's paired t-test.

	CORDILOX		SRV		ISOPTIN
Log time (min)	28 ± 13	P<0.01	52 ± 19	P<0.01	30 ± 12
$t_{\frac{1}{2}}$ Absorption (min)	19 ± 14	P<0.01	39 ± 22	NS	27 ± 14
C max (ng/ml)	359 ± 106	P<0.001	150 ± 48	P<0.05	296 ± 67
T max (hr)	1.4 ± 0.7	P<0.001	3.2 ± 0.8	P<0.001	1.6 ± 0.5
$t_{\frac{1}{2}}$ Elimination (hr)	2.6 ± 0.5	P<0.01	5.0 ± 2.2	NS	3.2 ± 2.2
AUC ($ngml.^{-1}hr$)	1577 ± 417	NS	1433 ± 498		1486 ± 617

Table 1 Mean - SD pharmacokinetic parameters for three verapamil formulations

The mean absorption and elimination $t_{\frac{1}{2}}$ of SRV were longer than Cordilox (P<0.01) and Isoptin (NS) - Table 1.

Table 1 shows that in acute dosage, SRV has a reduced peak plasma concentration and longer apparent $t_{\frac{1}{2}}$ when compared with two conventional preparations, without reducing the AUC. Evaluation of SRV in chronic dosage to volunteers is therefore appropriate.

Cole, S.C.J. et al (1981). J. Chromat., 218, 621-629
Johnston, A. & Woollard, R.C. (1983). J. pharmac. Methods in press

ORAL SLOW-RELEASE VERAPAMIL II: A FIVE DAY CHRONIC DOSE COMPARISON WITH CORDILOX IN 18 HEALTHY VOLUNTEERS

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In single dose studies a slow release formulation of verapamil (SRV) has shown potential pharmacokinetic advantages over conventional preparations. In this study the kinetics of verapamil have been examined at the start and end of a five day period of chronic dosing with both SRV and a conventional formulation. Cordilox^{R} and SRV were compared in 18 healthy male volunteers. Each preparation was given as a 240mg dose 12 hourly for five days. One week was allowed between treatments and their order randomised. Blood samples were taken at 1, 2, 3, 4, 6, 8 and 12 hours at the start of each treatment, before the first dose on days 2, 3 and 4 and at 0, 0.5, 1, 1,5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 24 and 36 hours after the last dose, for plasma verapamil measurement by HPLC (Cole et al 1981). The data were analysed using the pharmacokinetic program STRIPE (Johnston and Woollard 1983) and statistical comparison of the data was by Student's paired t, except for the time to peak values which were compared using Wilcoxan's signed rank test.

	DAY 1		DAY 5	
Mean $\pm$ s.d.	Cordilox	SRV	Cordilox	SRV
Cmax (ng/ml)	279$\pm$122	125$\pm$62	613$\pm$165	341$\pm$162
Tmax (median, hr)	1.0	4.0	1.0	2.0
t_1 absorption (minutes)	----	----	25$\pm$10	50$\pm$40
$t_{\frac{1}{2}}$ elimination (hr)	3.8$\pm$0.9	4.9$\pm$1.8	8.0$\pm$2.2	9.2$\pm$3.6
AUC (ng/ml. hr)	1053$\pm$454	1019$\pm$399	3946$\pm$1208	4044$\pm$1444

Table 1
Mean $\pm$ s.d. pharmacokinetic parameters for verapamil after SRV and Cordilox

The results are summarised in Table 1. Although the half-life of verapamil was increased on day 5 compared with day 1 both for SRV and Cordilox, SRV increased the time to reach maximum concentration and decreased the maximum concentration compared with Cordilox. However, the AUC of both preparations were similar on both day 1 and day 5. Clinical evaluation of the slow-release preparation would therefore seem appropriate.

Cole, S.C.J. et al. (1981). J. Chromat., 218, 621-629
Johnston, A. & Woollard, R.C. (1983). J. pharmac Methods in press

COMPARISON OF THE URINARY EXCRETION OF KETOPROFEN AFTER SINGLE ORAL DOSES OF 'ORUDIS' AND 'ORUVAIL' GIVEN AT NIGHT

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Ketoprofen [2(3-benzoylphenyl)propionic acid], a non-steroidal anti-inflammatory agent, is widely used for the treatment of arthritic symptoms, and when administered in the form of 'Orudis' or 'Profenid' has a short plasma elimination half-life of around 2 h (e.g. Caillé et al, 1978, 1980; Sala et al, 1978; Ishizaki et al, 1980; Stafanger et al, 1981). In order to permit once daily dosing of ketoprofen and reduce the possibility of gastro-intestinal side-effects a controlled release preparation known as 'Oruvail' is now available. We have carried out a preliminary study in two healthy male volunteers of the urinary excretion of ketoprofen following oral administration of single 200 mg doses of 'Orudis' and 'Oruvail'. The treatments were given at 21:00 h with an interval of 1 week, and urine was collected hourly for the first 12 h, 4 hourly until 24 h had elapsed, and then 12 hourly until 48 h after each dose. The amount of free and total ketoprofen (free plus conjugated) in each urine sample was determined by a previously described h.p.l.c.-UV procedure (Kaye et al, 1981).

As shown previously (Upton et al, 1980; Kaye et al, 1981) virtually all of the ketoprofen present in urine was as easily hydrolysable conjugates. The following data were derived from the values of total ketoprofen excreted:

	Subject 1		Subject 2	
	'Orudis'	'Oruvail'	'Orudis'	'Oruvail'
time (h) of peak rate of urinary excretion	4.5	9.5	2.5	10.5
$t_{\frac{1}{2}}$ (h)*	1.8	5.1	2.0	7.9
% of dose recovered in urine				
0-12 h	74.4	42.9	70.6	45.9
12-24 h	4.6	32.0	2.5	21.5
24-48 h	0.5	6.9	0.4	8.3
0-48 h	79.5	81.8	73.5	75.7

*$t_{\frac{1}{2}}$ = apparent elimination half-life over the period during which most of the dose was excreted.

From these limited results it appears that ketoprofen is released more slowly from 'Oruvail' than from 'Orudis', but that it is equally bioavailable from the two formulations. 'Oruvail' is likely to be more suitable than 'Orudis' for once daily dosing since a substantial amount of an 'Oruvail' dose is excreted over the period 12 - 24 h, which is consistent with substantial circulating levels of ketoprofen.

Caillé, G. et al (1978) Ann.pharm.franç. 36, 243-252.
Caillé, G. et al (1980) Biopharm.Drug Disp. 1, 195-201.
Ishizaki, T. et al (1980) Eur.J.clin.Pharmac. 18, 407-414.
Kaye, C.M. et al (1981) Br.J.clin.Pharmac. 11, 395-398.
Sala, G. et al (1978) Farmaco Ed.Prat. 33, 455-460.
Stafanger, G. et al (1981) Scand.J.Rheumatol. 10, 189-192.
Upton, R.A. et al (1980) J.Pharm.Sci. 69, 1254-1257.

COMPARISON OF THE CONCENTRATIONS OF LITHIUM IN PLASMA, SEMEN AND SALIVA

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A number of drugs are secreted into semen in men at various concentrations relative to those in the blood. Salts of lithium are widely used in the treatment of bipolar affective illness and it has been suggested that they disturb some aspects of sexual function.

We investigated the secretion of lithium into semen having shown dose-related inhibition of sperm motility by lithium in vitro (Grech, Ridgway & Pearson, 1983 presented at this meeting).

6 healthy male volunteers between the ages of 18 and 28 were selected. None had received any other medication for 1 week. They took a light breakfast on the morning of the study. A saliva and blood sample were taken prior to administration of the lithium. A dose of 600mg lithium carbonate was taken by mouth and 120ml water which was rinsed round the mouth and then swallowed. The subjects then fasted for 4 hours. Blood, saliva and semen samples were obtained at 1, 2, 4 and 8 hours after the administration of the lithium. The lithium concentration of serum, seminal plasma and saliva was measured by flame photometry.

Concentrations of lithium (mmol/l) in serum, seminal plasma and saliva after oral dose. $\pm$ s.e.m. 6 subjects

	TIME (hrs)				
	0	1	2	4	8
Serum	0	0.11±0.02	0.23±0.02	0.31±0.02	0.29±0.02
Seminal plasma	-	0.05±0.01	0.14±0.02	0.34±0.02	0.40±0.02
Saliva	0	0.15±0.06	0.50±0.07	0.76±0.07	0.69±0.08

Our results (see Table) show that the maximum concentration of the drug in blood occurs at 4 hrs. (0.31±0.02mmol/l). The maximum salivary concentration (0.76±0.07mmol/l) also occured at 4 hours but was 2.5 times greater than that attained in serum. This result is in agreement with earlier reports that salivary lithium concentrations exceed those obtained in serum. Lithium concentration in seminal plasma initially lagged behind the serum levels but by 4 hours had exceeded them and at 8 hours were 1.5 times greater. This supports earlier observations that with long-term lithium therapy, the semen concentrations exceed those in serum.

From our in vitro studies we found that the concentration of lithium inhibiting sperm motility by 50% (EC50) was 63mmol/l. In this study we have shown that concentrations of lithium in seminal fluid after a single therapeutic dose are less than one hundredth of those required to inhibit sperm motility in vitro. Thus any influence that lithium may have on reducing fertility in men on lithium therapy is unlikely to be mediated by an effect on sperm motility.

INHIBITORY ACTION OF LITHIUM ON SPERM MOTILITY AND ITS ANTAGONISM BY CAFFEINE

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Previous reports of studies on the effect of lithium on sperm motility are conflicting. Salts of lithium are widely used in the treatment of bipolar affective illness. We investigated the effect of lithium on sperm motility in vitro since sperm motility is one of the most important variables in evaluating the fertility potential of a semen sample. The effect of caffeine on lithium-induced changes in sperm motility was also studied since caffeine has been shown to oppose the effects of other drugs which inhibit sperm motility (Hong et al 1981).

Fresh semen samples were collected from 6 healthy men. Only samples with a sperm count greater than 15 x $10^6 ml^{-1}$, and a percentage of progressive forward moving sperms higher than 20% were used. The effect of different concentrations of lithium on sperm motility was measured using the transmembrane migration ratio technique (Hong et al 1981). This depends on the ability of spermatoza to migrate across a 5µm millipore membrane during 2 hours incubation at 37°C. Lithium and caffeine were dissolved in phosphate buffered saline at pH7.3. The motility of semen-buffer mixture was used as control and those of semen-drug mixtures were expressed as percentages of the control. Aliquots of semen were mixed with either buffer or drug in the ratio of 2 to 1. When lithium and caffeine were studied together, the volume of each drug was halved so that the ratio could be kept constant. The final concentration of caffeine was kept at 5mM which was optimal for stimulating sperm motility.

Changes in Percentage Sperm Motility Response to Lithium and Lithium with Caffeine (5mM) n = 6

	Lithium concentrations (mM)			
	20	40	60	80
% Motility-lithium ± s.e.m.	82.9±1.69	68.9±2.10	52.7±2.24	35.4±1.55
% Motility-lithium + caffeine (5mM)	103.7±2.30	91.6±2.12	75.7±2.47	61.6±2.53

Our results (see Table) show a dose effect relationship on sperm motility. This inhibitory effect is antagonised by caffeine. The concentration of lithium inhibiting sperm motility by 50% (EC50) is 63mM. Studies on the secretion of lithium into semen (Ridgway, Grech & Pearson, 1983, presented at this meeting) suggest that the concentrations of lithium secreted into seminal fluid after therapeutic doses are one hundred times less than those required to inhibit sperm motility in vitro. We conclude that lithium in therapeutic doses is unlikely to affect sperm motility.

Hong, C.Y. et al (1981) Br.J.clin.Pharmac. 11, 385-387.

COMPARATIVE EFFECTS OF NICARDIPINE AND NIFEDIPINE ON CORONARY ARTERY, MESENTERIC ARTERY AND PORTAL VEIN

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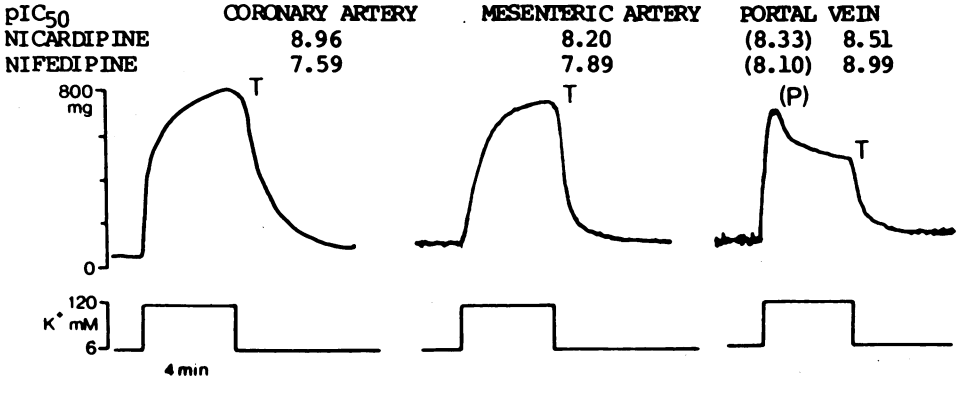
Calcium entry blockers (CEBs) inhibit contractions of vascular smooth muscle via a blockade of inward calcium currents. However, the potency of an individual CEB for different vascular smooth muscle is variable (Vanhoutte, 1981). The present study was conducted to compare the effects of two dihydropyridine CEBs, nicardipine and nifedipine, on potassium-induced contractures of pig coronary artery, guinea-pig mesenteric artery and portal vein.

Spiral strips of each vessel were continuously perfused at 5 $ml.min^{-1}$ with a physiological salt solution (PSS) as described by Patmore & Whiting (1982). The preparations were maintained at 37^0C under an initial tension of 0.5 g and left for 2 h to equilibrate. Contractures were evoked by superfusion for 4 min with 120 mM K^+ PSS as shown in Figure 1. The EC_{50} values shown for K^+ are similar. Portal vein produced both phasic (P) and tonic (T) contractures whilst coronary and mesenteric artery preparations only produced a tonic contracture (Figure 1).

Nicardipine and nifedipine were dissolved in C_2H_5OH to give a stock solution of 3×10^{-3} M and were added to the superfusate to achieve concentrations in the range 10^{-11} to 10^{-7} M. In each preparation the effects of these CEBs were monitored over 0.5 h periods. pIC_{50} values of the CEBs were calculated by regression analysis.



EC_{50} (K^+) 52 mM 58 mM 52 mM

Figure 1

The data show that nicardipine was more potent than nifedipine in antagonising K^+-induced contractures of the coronary artery and nicardipine was more active in the coronary artery than other peripheral vessels. These data confirm the preferential coronary vasodilator profile of nicardipine (Takenaka et al, 1976; Takenaka & Maeno, 1979).

Patmore, L. & Whiting, R.L. (1982) Br.J.Pharmac. 75, 149P.
Takenaka, T. et al (1976) Arzneim.-Forsch. 26, 2172-2178.
Takenaka, T. & Maeno, H. (1979) Jap.J.Pharmac. 29, 163P.
Vanhoutte, P.M. (1981) In: New Perspectives on Calcium Antagonists, ed by G.B. Weiss. Am.Physiol.Soc. 109-121.

DO BENZODIAZEPINES PRODUCE TOLERANCE SUB-CHRONICALLY IN VIVO OR ON THE SINGLE ISOLATED NEURON FOLLOWING EXTENDED ACUTE EXPOSURE?

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Chronic clinical exposure to benzodiazepines has been reported to lead to the development of tolerance (Hillestad et al. 1974) though it is not clear what mechanism(s) underlie this phenomenon. In this study, the acute and sub-chronic actions of the long and short acting benzodiazepines (diazepam and oxazepam) were evaluated on motor incoordination in mice using the accelerating rotarod method (Jones & Roberts, 1968). These effects were related to the drug plasma concentrations of the benzodiazepines which were determined after extraction (Wallace et al. 1979) using HPLC. Sub-chronically treated animals were allowed a 24 hour drug-free period prior to the assessment of benzodiazepine activity and evaluation of plasma concentrations.

In addition, the actions of benzodiazepines on the neuronal firing frequency and pattern of an isolated sensory neuron - crayfish stretch receptor (Roth, 1980) were studied to investigate whether tolerance occurs at the cellular level after extended (30 minute) exposure to these drugs.

A linear relationship was found to exist between rotarod performance and benzodiazepine plasma concentrations in both the drug-naive group and those animals treated daily (50mg/kg i.p.) for 2 weeks. The dose response curves for the latter group were shifted to the right with a consequent three-fold increase in the plasma concentration of diazepam (0.9 up to 2.5μg/ml) and oxazepam (2.2 up to 7.6μg/ml) required to produce a 50% effect on rotarod performance.

At the level of the single neuron (crayfish sensory neuron) diazepam and oxazepam respectively produced graded excitation and depression of the firing frequency under a maintained mechanical stretch stimulus. The peak effect occurred within 2½ minutes of exposure to the drug. Following a 30 minute perfusion with an ED_{50} (2.10^{-4}M) of diazepam or oxazepam, the firing frequency was reduced by 35% of the initial peak effect and this indicated a state of partial short-term tolerance. These findings suggest that benzodiazepine tolerance may occur partially at a functional level (i.e. neuronal membrane) rather than being fully explained by altered biotransformation or solely accounted for by changes in the number of apparent specific binding sites (Rosenberg & Chiu, 1979).

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Hillestad, L. et al. (1974) Clin.Pharmac.Ther. 16, 485-489
Jones, B.J. & Roberts, D.J. (1968) J.Pharm.Pharmacol. 20, 302-304
Rosenberg, H.C. & Chiu, T.H. (1979) Life Sci. 24, 803-808
Roth, S.H. (1980) Fed.Proc. 39, 1595-1599
Wallace, E.J. et al. (1979) Clin.Chem. 25, 1296-1300

BREMAZOCINE PRECIPITATED WITHDRAWAL IN MORPHINE-DEPENDENT RATS: COMPARISON WITH NALOXONE

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It is generally accepted that the in vivo agonist effects of opiates are mediated by subclasses of opiate receptors which have been designated as μ, κ and δ (see Wood, 1982). It is also known that a number of agents which have agonist activity at the κ-receptor, exhibit additional μ-antagonist properties. For example, pentazocine, nalorphine and cyclazocine precipitate an abstinence syndrome in the morphine-dependent dog (Gilbert et al. 1976; Martin et al. 1974). Recently, the benzomorphan analogue bremazocine has been described as a potent κ-receptor agonist (Römer et al. 1980). It was the purpose of the present study to investigate whether bremazocine also possessed μ-antagonist action by attempting to elicit a withdrawal syndrome in morphine-dependent rats.

Rats received twice daily injections of morphine at an initial dose of $20mgkg^{-1}$ which was increased by $20mgkg^{-1}$ on each successive day until a dose of $200mgkg^{-1}$ was reached on the 10th day. On day 11, animals were challenged with either naloxone ($1mgkg^{-1}$) or bremazocine ($5mgkg^{-1}$) and the frequency of 'wet dog' shakes, escape attempts, weight loss, changes in body temperature, and a variety of other signs of withdrawal noted.

Both bremazocine and naloxone precipitated an abstinence syndrome in dependent rats as indicated by a number of withdrawal signs including teeth chattering, ptosis and escape attempts. In addition, these agents produced significant weight loss ($P<0.001$) and hypothermia ($P<0.001$) within two hours of administration. However, unlike for naloxone, bremazocine treated animals appeared to be disorientated and exhibited a bizarre circling motion around the circumference of the observation chamber. This behaviour pattern was very marked and both rapid and uni-directional for individual animals.

The present findings indicate that bremazocine has some μ-antagonist properties and confirms previous observations where this agent elicited jumping behaviour in morphine-dependent mice (Von Voigtlander et al. 1983). We have previously reported that the analgesic profile of bremazocine is comparable to that of pentazocine (Upton et al. 1981) which has been found to have a dopaminergic involvement in its mechanism of action (Hernandez & Appell, 1979). Thus it is conceivable that the observed differences seen in the current study between bremazocine and naloxone may be attributable to a dopaminergic component of the former agent which is not apparent at this dose level in non-dependent animals.

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Gilbert, P.E. et al. (1976) J.Pharmacol.Exp.Ther. 198, 66-82
Hernandez, L.A. & Appell, J.B. (1979) Behav.Neurol.Biol. 26, 384-400
Martin, W.R. et al. (1974) J.Pharmacol.Exp.Ther. 189, 759-771
Römer, D. et al. (1980) Life Sci. 27, 971-978
Upton, N. et al. (1981) IRCS Med.Sci. 9, 614-615
Von Voigtlander, P.F. et al. (1983) J.Pharmacol.Exp.Ther. 224, 7-12
Wood, P.L. (1982) Neuropharmac. 21, 487-497

SELECTIVE AND COMBINED ANTAGONISM OF THE BIPHASIC ANTINOCICEPTIVE ACTIVITY OF MEPTAZINOL USING OPIATE AND CHOLINERGIC ANTAGONISTS

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Meptazinol is a novel partial agonist opiate analgesic (Stephens et al. 1978). In addition to its interaction with opiate receptors a cholinergic component has been implicated in its antinociceptive activity in mice (Bill et al. 1982). In the present study we investigated the effects of meptazinol in the hot plate, writhing, tail clip and tail immersion tests in mice. Using the latter test we have determined the effect of atropine and the two enantiomers of the benzomorphan antagonist (-)Mr 1452 and (+)Mr 1453 on meptazinol antinociception to establish the relative contributions of the opiate and cholinergic components to its antinociceptive activity.

Meptazinol produced dose-related antinociception at doses of 10-40mg/kg in the tail clip test and 1-10mg/kg in the abdominal writhing assay. In contrast a biphasic effect was noted in the hot plate and tail immersion tests. There was a shallow relationship up to 10mg/kg and a much steeper effect at higher doses in both tests. However the slope of the shallow component on the hot plate test was markedly less than that seen in the tail immersion test which is characteristic of partial agonist activity. Atropine (5mg/kg i.p.) significantly antagonised ($P<0.001$) the effects of meptazinol at doses in excess of 10mg/kg, but did not significantly modify the antinociceptive activity on the shallow part of the meptazinol curve. Mr 1452 (5mg/kg i.p.) markedly antagonised ($P<0.001$) the effects of meptazinol up to 10mg/kg but was without antagonistic effect when the dose of meptazinol was increased. However Mr 1453 (5mg/kg i.p.) did not antagonise meptazinol in doses up to 5mg/kg, but thereafter a significant antagonism was observed when the meptazinol dose was increased. As might have been predicted combined atropine and Mr 1452 pretreatment significantly abolished both the shallow and steep components of the meptazinol dose-response curve.

Thus the shallow components of the meptazinol curve represents an opiate effect since it is blocked by Mr 1452 but not Mr 1453. It would appear that the steep component of the curve is cholinergic since it is antagonised by atropine but not by Mr 1452. Whilst it has been shown that (-) isomers of opiate antagonists block opiate analgesia the corresponding (+) isomers have no effect in this respect but are capable of antagonising the analgesic but not hypothermic or tremorogenic actions of oxotremorine (Bensreti et al. 1982). Moreover, Pedigo et al. (1975) demonstrated that (+) isomers of some benzomorphan partial agonists are more potent than their (-) isomers versus i.c.v. administered acetylcholine analgesia. In this study Mr 1453 antagonised the steep component of meptazinol antinociception but was devoid of any blocking effect on the lower antinociceptive dose range.

Hence it is suggested that the biphasic antinociceptive activity of meptazinol in the tail immersion test has an opiate and cholinergic involvement and the results also support the hypothesis of reciprocal stereospecificity between opioid and cholinergic analgesia with respect to opioid antagonists.

Ben-Sreti, M.M. et al. (1982) Arch.Int.Pharmacodyn. et Ther. 256, 219
Bill, D.J. et al. (1982) In: Recent Adv.Clin.Exp. Study of pain. Proc. BRA and IASP April Bristol
Pedigo, N.W. et al. (1975) J.Pharm.Exp. 143, 845
Stephens, R.J. et al. (1978) Gen.Pharmac. 9, 73

DIFFERENTIAL INTERACTIONS OF FOUR ANTIDEPRESSANTS WITH OPIATE AND NON-OPIATE INDUCED ANTINOCICEPTION

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There is a case for combining antidepressants with opiate analgesics such that there is a sparing effect permitting a reduction in dosage of the narcotic required to produce effective analgesia (Beaumont, 1973). In this context it has been shown, in animal studies, that (+) oxaprotiline [(+)OXA)], nomifensine [NOM] and clomipramine [CLOM] potentiate the antinociceptive activity of morphine (Gonzalez et al. 1982a & b). In the present study we have included two other opioid agents; etorphine and D-ala^2-D-leu^5 enkephalin (DADL) and the non-opiate analgesics clonidine and oxotremorine to explore whether a similar interaction pattern exists for these agents. The above antidepressants including mianserin [MIAN], which possesses a diverse range of activity in respect of neurotransmitter function, were combined with the analgesics and the antinociceptive activity assessed using the mouse tail immersion test (48°C).

Table 1. Mouse Tail Immersion Test: Combination of analgesics with Antidepressants

	(+)OXA	(-)OXA	NOM	CLOM	MIAN
Morphine	↑	0	↑	↑	↓
Etorphine	↑	0	↑	↑	↓
DADL	↑	0	↑	↑	↓
Oxotremorine	0	0	↓	0	0
Clonidine	↑	0	↓	↑	0

↑ = Potentiation; ↓ = Inhibition; 0 = No Interaction

Predictably etorphine and DADL produced the same pattern of potentiation by (+)OXA, NOM and CLOM as with morphine. However MIAN antagonised the antinociceptive activity of all three opioid agents and (-)OXA, which is inactive as an inhibitor of synaptic noradrenaline reuptake (Mishra et al. 1982) produced no interaction.

The cholinergically mediated analgesia produced by oxotremorine was antagonised by NOM but was unaffected by the other antidepressants tested. Clonidine antinociception was potentiated by (+)OXA and CLOM, antagonised by NOM, whilst (-)OXA and MIAN were without effect.

The data presented emphasises the mechanistic differences associated with analgesia produced by opioids, cholinergic stimulants and imidazolines such as clonidine. It is suggested that the mechanisms subserving the three classes of analgesia may be differentiated using their interaction patterns with antidepressants.

Beaumont, G. (1973) J.Int.Med.Res. 1, 435
Gonzalez, J.P. et al. (1982a) In: Depression, Far Horizons (ed: E.Murphy) Cambridge Med.Pub. p.82
Gonzalez, J.P. et al. (1982b) Br.J.Pharmac. 76, 272p
Mishra, R. et al. (1982) Life Sci. 30, 1747

SELECTIVE EFFECTS OF DOPAMINERGIC MODIFIERS ON ANTINOCICEPTION PRODUCED BY DIFFERENT OPIOID RECEPTOR AGONISTS

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It has been suggested that altered central DA function may modulate the activity of morphine (Iwamoto & Way, 1979). However, both pharmacological and biochemical studies indicate that opioid receptors in the CNS are not homogeneous (Iwamoto & Martin, 1981) and that various opioid receptor agonists differentially affect striatal DA turnover (Wood et al. 1980). In view of this the present study set out to determine whether these differences are reflected in a converse way such that DA modification may selectively affect opioid antinociception.

Antinociceptive activity was assessed in the mouse (GB1 variants of an ICI derived strain 18-20G) tail immersion test (48°C) in a way which has previously been described (Sewell & Spencer, 1976). Groups of 10 male mice were pretreated intraperitoneally at either -30 min (haloperidol) or 0 min (apomorphine) then given submaximal doses of morphine (2.5mg/kg s.c.), D-ala^2-D-leu^5-enkephalin (DADL) (1.0µg/animal i.c.v.), Ethylketocyclazocine (EKC) (0.5mg/kg s.c.) or SKF 10,047 (7.5mg/kg s.c.). The antinociceptive effect of each opioid either alone or in the presence of each dopaminergic agonist or antagonist was then assessed over a period of 100 min and the results are summarized in table 1. None of the dopaminergic agents per se affected baseline nociceptive latencies to any significant degree ($P>0.05$).

Table 1. Effect of DA receptor agonists and antagonists on opioid antinociception in the tail immersion test (48°C)*

Treatment (mg/kg)	Morphine	DADL	EKC	SKF 10,047
Saline vehicle	127 ± 16	81 ± 11	96 ± 8	62 ± 9
apomorphine (1.0)	95 ± 10[a]	44 ± 7[a]	105 ± 11	69 ± 6
(5.0)	75 ± 8[a]	31 ± 7[b]	89 ± 8	75 ± 12
haloperidol (0.3)	152 ± 11	126 ± 13[a]	117 ± 10	84 ± 11
(3.0)	190 ± 22[a]	144 ± 10[b]	123 ± 9	90 ± 12

*Values are mean % antinociceptive effect ± S.E. (Sewell & Spencer, 1976)
[a]$P<0.05$, [b]$P<0.01$ compared to appropriate controls.

As can be seen, apomorphine significantly attenuated morphine and DADL antinociception and haloperidol markedly augmented the activity of these two agents. In contrast, neither haloperidol nor apomorphine produced any significant change in EKC or SKF 10,047 antinociceptive activity. These data suggest that a dopaminergic mechanism is more likely to play a role in the antinociception produced by morphine (µ) and DADL (δ) and this does not apply to EKC (κ) or SKF 10,047 (σ). This is totally consistent with the observation that µ- and δ- but not κ-agonists affected DA turnover through an opioid mechanism which was not shared by SKF 10,047 (Wood et al. 1980).

Iwamoto, E.T. & Martin, W.E. (1981) Med.Res.Rev. 1, 440
Iwamoto, E.T. & Way, E.L. (1979) Adv.Biochem.Psychopharmacol. 20, Loh, H.H. (ed) p357
Sewell, R.D.E. & Spencer, P.S.J. (1975) Neuropharmacology 15, 683
Wood, P.L. et al. (1980) J.Pharmacol. Exp.Ther. 215, 697

INTERACTION BETWEEN CLONIDINE AND α-METHYL NORADRENALINE IN THE PRESENCE OF THE NON-COMPETITIVE ANTAGONIST BENEXTRAMINE

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Benextramine blocks post-junctional α-adrenceptors in a non-competitive and irreversible manner (Melchiorre et al, 1978). The pre-junctional effects of clonidine in guinea pig heart are also blocked competitively, though this blockade appeared to be slowly reversible (Belleau et al, 1982). The effects of benextramine on pre-junctional α_2-adrenoceptor mediated responses of field stimulated guinea pig ileum have been investigated. Tissues were set up according to the method of Drew (1978) and bathed in Kreb's solution containing 3 μM cocaine, 1 μM propranolol and 0.3 μM prazosin to inhibit neuronal uptake and to block β and α_1-adrenoceptors respectively. Benextramine in concentrations between 0.1 and 10 μM was added to tissues for 30 mins before washout. No potentiation of the twitch response, as normally seen with competitive antagonists of α_2-adrenoceptors, was observed. A dose-dependent inhibition of the twitch response by benextramine was however recorded and this is attributed to its muscarinic receptor blocking activity (Benfey et al, 1979). Benextramine produced a non-competitive blockade of clonidine, B-HT 920 and α-methyl noradrenaline. Benextramine was equipotent as an antagonist against clonidine and B-HT 920, however it required a seven-fold increase in the concentration of benextramine to produce an equivalent blockade of α-methyl noradrenaline.

Cumulative doses of clonidine invariable failed to produce a complete inhibition of the twitch response in guinea pig ileum, with a maximum inhibition of 80-95%, in accord with a previous report by Drew (1978). On the other hand cumulative doses of α-methyl noradrenaline invariable completely abolished the twitch response. If clonidine (10 μM) was added to the tissue following complete inhibition of the twitch response by α-methyl noradrenaline, then a reversal of the inhibitory effect of α-methyl noradrenaline was seen, at least up to the 80-90% level to which clonidine inhibits the twitch. This suggested an antagonistic effect of clonidine against α-methyl noradrenaline. To investigate this further, increasing concentrations of clonidine (0.3 μM to 3 μM) were used to antagonise the cumulative dose-response effect of α-methyl noradrenaline in field stimulated guinea pig ileum pre-treated with 1 μM benextramine for 30 mins. Under these conditions the differential non-competitive blocking effect of benextramine ensures an effective blockade of the inhibitory effect of clonidine whilst leaving the inhibitory effect of α-methyl noradrenaline almost intact. Results showed that clonidine produced a competitive blockade of α-methyl noradrenaline.

It is concluded that the results presented, showing a differential blocking effect of benextramine between clonidine/B-HT 920 and α-methyl noradrenaline, coupled with the ability of clonidine to antagonise the effect of α-methyl noradrenaline whilst its agonist effects are blocked, provides further evidence in support of the previous observations (Ruffolo et al, 1979; Mottram, 1982) that phenethylamines and imidazolines may interact differently with α_2-adrenoceptors.

Belleau, B. et al (1982) Br.J.Pharmac. 75, 617-621.
Benfey, B.G. et al (1979) Can.J.Physiol.Pharmacol. 57, 41-47.
Drew, G.M. (1978) Br.J.Pharmac. 64, 293-300.
Melchiorre, C. et al (1978) J.Med.Chem. 21, 1126-1132.
Mottram, D.R. (1982) Br.J.Pharmac. 75, 138P.
Ruffolo, R.R. Jr. et al (1979) J.Pharm.Exp.Ther. 211, 74-79.

THE BILIARY EXCRETION AND ENTEROHEPATIC CIRCULATION OF CATECHOL OESTROGEN METABOLITES OF ETHYNYLOESTRADIOL IN RAT AND MAN

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Catecholoestrogens are major metabolites of natural and synthetic oestrogens (Ball & Knuppen, 1980). Their formation in vivo has toxicological implications since they are readily oxidized to reactive intermediates by hepatic microsomes in vitro (Nelson et al. 1976; Maggs et al., 1983) and the intermediates bind irreversibly to microsomal and soluble protein. We have shown that 2-hydroxyethynyloestradiol (2-OHEE$_2$) is a biliary metabolite of i.v. and p.o. dosed ethynyloestradiol (EE$_2$) in rats and women, respectively (Maggs et al., 1982; Breckenridge et al., 1983). During further work, we have studied the formation of de-ethynylated catechol metabolites of EE$_2$ in rats and women, and the enterohepatic circulation (EHC) of EE$_2$'s metabolites in rats.

Two female Wistar rats were dosed i.v. with 5µg/kg (80µCi/kg) [6,7 - ^{3}H]EE$_2$ (^{3}H-EE$_2$) and bile collected for 3h. Pooled bile (0.5 ml, 4.5µ Ci) was infused into the duodena of 4 anaesthetized female rats over 30 min and bile collected for 5h. Bile from donor and recipient rats was incubated with enzymes in the presence of ascorbate, and the deconjugated metabolites were analysed by reversed-phase and diol-phase h.p.l.c. Four-hour bile samples collected via T-tubes were obtained over 24h from two women (M.H., age 75 yrs; S.P., 47 yrs) dosed p.o. with 50µg (80µCi) ^{3}H-EE$_2$. The samples were hydrolysed with enzymes and analysed by h.p.l.c.

The recipient rats excreted 15 ± 6%($\bar{x}$ ± S.D.) of the infused ^{3}H in bile. Glucuronylated 2-OHEE$_2$ was the principal biliary metabolite: 2-OHEE$_2$ represented 41 ± 3% ($\bar{x}$ of 3 analyses) and 46 ± 3% (n = 4) of the aglycone ^{3}H with donor and recipient rats, respectively. Neither 2-hydroxyoestrone (2-OHE$_1$) nor 2-hydroxyoestradiol (2-OHE$_2$) were observed amongst the aglycones. The proportion of EE$_2$ was slightly lower in the recipient rats' metabolite profile - 5 ± 1% v. 8 ± 0% for the donor bile - whereas the proportion of 2-methoxyethynyloestradiol was unchanged: 24 ± 2% (donor) v. 26 ± 3%.

Both women excreted 2-OHEE$_2$ in bile, but at notably different rates relative to EE$_2$ (Table 1). 2-OHE$_1$ and 2-OHE$_2$ were not found in human bile.

Table 1 Diol-hplc analysis of deconjugated EE$_2$ & 2-OHEE$_2$ from human bile (% ^{3}H)

	M.H.		S.P.	
Bile Collections(h)	EE$_2$	2-OHEE$_2$	EE$_2$	2-OHEE$_2$
0 - 4	67	8	50	12
4 - 8	55	2	35	13
8 - 12	48	3	35	13
12 - 16	42	3	28	18
16 - 20	47	3	29	16

Thus, 2-OHEE$_2$ appears to be the sole biliary catecholoestrogen metabolite of EE$_2$ in female rats and humans, and may undergo EHC.

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Ball, P. & Knuppen, R. (1980) Acta endocr. Copenh. 93 Suppl. 232, 1-128.
Breckenridge, A.M. et al (1983) Br. J. Pharmac. 78, 63P.
Maggs, J.L. et al (1982) Xenobiotica 12, 657.
Maggs, J.L. et al (1983) J. steroid Biochem., in press.

HYPERPYREXIC INTERACTIONS BETWEEN TRANYLCYPROMINE, OPIOID COMPOUNDS AND DOXEPIN IN RABBITS

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Severe hyperpyrexia was previously reported to occur after administration of centrally acting drugs to rabbits chronically treated with the monoamine oxidase inhibitor, pargyline (Fahim et al 1972). In this study we have examined the possible interactions between tranylcypromine, opioid drugs and doxepin in rabbits. Attempts were also made to modify the interactions by various compounds. Hyperpyrexia was used as a marker for the interaction. Rise in core temperature was was measured using electrical thermocouples supplied by Sierex Ltd. Drugs were given per Kg body weight as indicated. Groups of male rabbits (each weighing 2Kg, n=8) were pretreated with tranylcypromine sulphate(10mg,s.c.) for 5days. On the 5th day each of the following drugs or its vehicle was administered to one group of rabbits 20min after the last dose of tranylcypromine, pentazocine(10mg, i.v.), dextromethorphan(15mg, orally),dextropropoxyphene hydrochloride(37mg,i.v.), tilidine(25mg,i.v.)and doxepin(25mg,s.c.) Core temperature was monitered over a period of 3hrs. In other groups of tranylcypromine-pretreated rabbits(n=4), indomethacin(10mg,s.c.) or their vehicles were administered 30min before injecting the compounds under test. Statistical significance was calculated using unpaired 't' test. Rabbits treated with tranylcypromine, indomethacin, cyproheptadine, haloperidol, opoid compounds or doxepin did not show any significant change in core temperature compared with vehicle-treated control rabbits. Administeration of dextropropoxyphene, pentazocine, dextromethorphan and doxepin significantly elevated core temperature by 2.0± 0.2, 2.6 ± 0.1, 3.7 ± 0.4 and 3.9 ± 0.3 °C, respectively ($P<0.05$). Some of the rabbits died within 20 - 120 min after the injection of pentazocine, doxepin and dextromethorphan. Tilidine was ineffective. The hyperpyrexia was successfully prevented by pretreatment with haloperidol. Neither cyproheptadine nor indomethacin protected the rabbits against the hyperpyrexia. The results indicate that neither prostaglandins nor 5-hydroxytrptamine were involved in the hyperpyrexia. The effectiveness of haloperidol suggests that the hyperpyrexia was mediated via the activation dopamine receptors. This may be a result of combined inhibition of monoamine oxidase enzyme by tranylcypromine and the potentiation of dopamine through the prevention of uptake by the effective drugs. Alternatively, the effective drugs may have sensitized the dopaminergic receptors. The ineffectiveness of tilidine may be due to failure to affect dopamine uptake or receptors. Based on these findings, we think it is important to note that administration of opioid compounds and/or doxepin to patients chronically taking tranylcypromine may possibly result in such toxic interaction.

Fahim,I. et al (1972) Br.J.Pharmac. 46, 416p.

EFFECTS OF INDOMETHACIN, PIROXICAM AND SELECTED PROSTANOIDS ON GASTRIC ACID SECRETION IN VITRO

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Although the inhibitory effect of prostanoids on gastric acid secretion is well known, the prostanoid receptor mediating this effect has not been characterised, and the role of endogenous prostaglandins in controlling acid secretion is unclear (Main & Whittle, 1975; Frame & Main, 1980). The purpose of the present study was to use an isolated preparation of gastric mucosa to (a) determine the antisecretory potencies of a number of prostanoid agonists and relate their rank order of activity to those of the prostanoid receptor classification proposed by Kennedy et al. (1982), and (b) investigate the effects of the cyclo-oxygenase inhibitors, indomethacin and piroxicam, on acid secretion.

All experiments were carried out in the rat isolated gastric mucosa preparation (Main & Pearce, 1978). The serosal surface was bathed with a modified Krebs solution (gassed with 95% O_2/5% CO_2) to which all drugs were added. The mucosal surface was perfused at 0.5 ml/min with an unbuffered solution (gassed with 100% O_2) and the pH of the effluent perfusate was continuously monitored.

Sequential concentration-response curves for histamine were constructed on mucosae in Krebs, or in Krebs containing indomethacin (2.7×10^{-6}M) or piroxicam (3.0×10^{-6}M). In normal Krebs, concentration-related increases in acid secretion were elicited by histamine from 5×10^{-6}M to 3×10^{-4}M, with a maximum increase in output over basal secretion of 29.7 ± 5.3 nmol H^+/min/cm^2. Indomethacin and piroxicam had no effect on basal secretion, but markedly potentiated the secretory response to histamine. Histamine was effective at concentrations of 5×10^{-7} to 3×10^{-5}M, eliciting maximum responses of 101.6 ± 10.8 and 129.9 ± 15.2 nmol H^+/min/cm^2 in the presence of indomethacin or piroxicam respectively.

Subsequent experiments on the antisecretory effects of prostanoids were carried out in the presence of indomethacin (2.7×10^{-6}M). Four consecutive secretory responses to a submaximal concentration of histamine (10^{-5}M) were obtained in each mucosa; the prostanoid was added serosally 15 min before the third dose of histamine and the % change in secretion calculated between the 2nd and 3rd histamine responses. The prostanoids were usually tested at 3 concentrations in 6 preparations at each concentration, and inhibitory EC_{50} values calculated. Ranked in order of potency, the EC_{50} values in μM with 95% confidence limits for these prostanoids were: 16,16-dimethyl PGE_2 0.011 (0.008 - 0.017), PGE_2 0.088 (0.054 - 0.14), $PGF_{2\alpha}$ 2.7 (2.1 - 3.4), U-46619 7.2 (4.5 - 10.0), PGD_2 8.9 (5.8 - 35.0), PGI_2 > 10. The relative inactivity of PGI_2 is surprising but may be due to its instability in aqueous solutions (Johnson et al., 1976). With the exception of PGI_2, this rank order of potency is very similar to that reported for other PGE-sensitive preparations, and suggests that inhibition of acid secretion by prostanoids in the rat is mediated by an "EP" receptor (Kennedy et al., 1982).

Frame, M.H. et al. (1980). Br. J. Pharmac., 69, 171 - 178.
Johnson, R.A. et al., (1976). Prostaglandins, 12, 915 - 928.
Kennedy, I. et al. (1982). Prostaglandins, 24, 667 - 689.
Main, I.H.M. & Pearce, J.B. (1978). J. Pharmacol. Meth., 1, 27 - 38.
Main, I.H.M. & Whittle, B.J.R. (1975). Br. J. Pharmac., 53, 217 - 224.

CHANGES IN T_3, T_4 AND TSH RAT PLASMA LEVELS FOLLOWING ELECTROCONVULSIVE SHOCK

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Several studies on the endocrinological effects of electroconvulsive treatment have failed to demonstrate changes in the plasma levels of thyroid hormones and TSH immediately or shortly (up to 6 hours) after the last electroconvulsive shock (ECS)(Reichlin and O'Neal,1962; Ryan et al,1970;Thorell et al,1973; Kirkegaard,1975; Ylikorkala et al,1976). The present study examines T_3, T_4 and TSH levels in rats up to four days after the last treatment.

40 Albino Wistar female rats were used for these experiments.They were divided into 5 groups: 1 control group (C) and four experimental (E) groups; E1 received a single ECS, with the rats killed one hour afterwards; E2, E3 and E4 received daily ECS for 8 days and the rats were killed 1 hour, 48 hours and 96 hours respectively after the final treatment. The ECSs(90V,1s, 50Hz) were applied bilaterally through flat electrodes placed on parieto-temporal regions and the animals were always killed at the same time of day (13.00 hours). T_3, T_4 and TSH levels were estimated by radioimmunoassay (Amersham International plc). The results (table 1) were compared by variance analysis followed by student t test. T_3 and T_4 levels are decreased one hour after single (E1) or repeated (E2) ECS. 96 hours after the last ECS (E4) T_4 is no different from control (C) and T_3 goes up ($p < 0.001$) but does not reach control values. TSH, which is unchanged in E1 and E2, decreases 48 and 96 hours after the last ECS (assay sensitivity = 0.7μU).

Table 1: T_3, T_4 and TSH levels after single or repeated ECS ($\bar{x} \pm$ SE of mean)

		T_4 ($nmol \times l^{-1}$)	T_3 ($nmol \times l^{-1}$)	TSH ($mU \times l^{-1}$)
Control	(C)	23.43 ± 1.49	1.04 ± 0.07	1.91 ± 0.12
Single ECS	(E1)	16.11 ± 1.49 [a]	0.79 ± 0.08 [a]	2.02 ± 0.12
Repeated ECS:	(E2)	14.26 ± 1.49 [c]	0.23 ± 0.07 [c]	1.70 ± 0.12
	(E3)	21.74 ± 2.11	0.43 ± 0.10 [c]	1.16 ± 0.16 [b]
	(E4)	23.26 ± 2.11	0.76 ± 0.10 [a]	0.88 ± 0.16 [c]

[a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.001$ compared with control.

In summary, the above data show transient changes in T_4 and more lasting in T_3 and a delayed effect of ECS on TSH which is not present immediately after treatment, but is evident at 48 and 96 hours after the final ECS treatment.

Kirkegaard, C. et al (1975) Arch.Gen.Psychiat. 32, 1115-1118
Reichlin, S. & O'Neal, L. (1962) J.Clin.Endocrin. 22, 385-388
Ryan, R.J. et al (1970) J.Clin.Endocrinol.Metabol. 30, 51-58
Thorell, J.I. et al (1973) The Lancet p43
Ylikorkala, O. et al (1976) Clin. Endocrinol. 5, 571-574

BOMBESIN-INDUCED GROOMING IN RATS: AN ANIMAL MODEL FOR EVALUATING SYSTEMIC ANTIPRURITIC AGENTS

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Standardized animal models for preclinical evaluation of systemic antipruritic agents are presently unavailable (Savin, 1980). I.c.v. administration of bombesin, a tetradecapeptide originally isolated from frog skin (Anastasi et al, 1971), causes dose-related excessive grooming (scratching) in rats (Brown et al, 1977; Gmerek & Cowan, 1982a). In this study, we have explored the use of bombesin-induced scratching in rats as a model for preclinical screening of potential antipruritic agents.

Male, Sprague Dawley rats (180-200 g) were implanted stereotaxically with stainless steel cannualae in the right lateral cerebral ventricle. Five to seven days later, rats (n=6-8) were placed singly in Plexiglas observation boxes (22 cm long; 18 cm wide; 25 cm high) and pretreated s.c. or i.p. with vehicle or test compound 15 min before a standard i.c.v. dose of bombesin (0.10 μg in 3-4 μℓ saline). Rat behaviour was then monitored for 5 sec, every 20 sec, for 30 min with the help of a microcomputer (Murray et al, 1981). Results were calculated as percent of the maximum number of grooming episodes (Gmerek & Cowan, 1982b). A 50 values (and 95% confidence limits) were determined by linear regression analysis.

Methdilazine and trimeprazine, two phenothiazines that are approved in the U.S.A. for use in pruritus, attenuated bombesin-induced scratching at behaviourally nondepressant doses. Subcutaneous A 50 values were 6.2 (4.4-8.8) mg/kg and 8.0 (5.2-12.3) mg/kg, respectively. Chlorpromazine, a third phenothiazine antihistamine, was also active with a s.c. A 50 value of 0.9 (0.3-1.6) mg/kg. Diphenhydramine (25 mg/kg, i.p.), mepyramine (20 mg/kg, s.c.), hydroxyzine (100 mg/kg, s.c.), cyproheptadine (10 mg/kg, s.c.) and cimetidine (10 mg/kg, i.p.) had no marked effects. We therefore conclude that histamine is not necessarily involved in bombesin-induced scratching. Indeed, bombesin does not cause a release of histamine from mast cells obtained from Sprague Dawley rats (Sydbom, 1982).

The phenothiazine nucleus appears to confer selectivity to methdilazine, trimeprazine and chlorpromazine as antagonists of bombesin since haloperidol was not effective at behaviourally nondepressant doses (<0.5 mg/kg, s.c.; see also Gmerek & Cowan, 1982a).

In summary, a need exists for animal models that will facilitate evaluation of systemically administered antipruritics. We have described a model that may be useful in the preclinical screening and discovery of systemically active antipruritic agents, particularly those directed against histamine-independent pruritus.

Anastasi, A. et al (1971) Experientia 27, 166
Brown, M. et al (1977) Science 196, 998
Gmerek, D.E. & Cowan, A. (1982a) Life Sci. 31, 2229
Gmerek, D.E. & Cowan, A. (1982b) Pharmac. Biochem. Behav. 16, 929
Murray, R.B. et al (1981) Pharmac. Biochem. Behav. 15, 135
Savin, J.A. (1980) Br. J. Dermatol. 103, 113
Sydbom, A. (1982) Ag. Actions 12, 91

INCREASED CHOLINERGIC RESPONSES WITH BRL 20627 IN RAT ISOLATED STOMACH: COMPARISON WITH METOCLOPRAMIDE

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BRL 20627 [(2α,6β,9aα)-(±)-4-amino-5-chloro-2-methoxy-N-(octahydro-6-methyl-2H-quinolizin-2-yl)benzamide hydrochloride] and metoclopramide (Mcp) are benzamides which both increase gastric emptying and motility. Unlike Mcp, BRL 20627 is potentially devoid of the side effects associated with dopamine antagonism in the central nervous system (McClelland et al, 1983). Since Mcp may affect gastric motility at least partly by increasing the release of acetylcholine (ACh) in the gut, we have looked for a similar action of BRL 20627 using a stomach preparation previously used to study the effects of Mcp (Anderson et al, 1977; McClelland & Sanger, 1982).

Strips of rat gastric fundus were cut parallel to the longitudinal muscle. Consistent, 'just-maximal' contractions were obtained with electrical field stimulation (EFS; bipolar 0.5ms rectangular pulses; 5Hz rate; 20s train; 80-120V/cm [maximum current 1A]; 10min cycle). The contractions could be prevented with tetrodotoxin 0.1μM or atropine 1.4μM, indicating predominant cholinergic activation. BRL 20627 0.3-28μM increased the EFS-induced contractions, whereas BRL 20627 282μM caused inhibition; there were no effects on resting muscle tone. In Table 1 the results are compared with our earlier data for Mcp (McClelland & Sanger, 1982). There was no difference between the effects of 0.003-2.8μM BRL 20627 or Mcp ($P>0.4$; Mann Whitney U-test). BRL 20627 28μM was less effective than Mcp 28μM ($P<0.05$), but this high concentration is considerably greater than the maximum detected in blood plasma after a therapeutic dose of Mcp (approx. 0.3μM; Bateman et al, 1978).

Table 1: % Increase (medians; semiquartile ranges in parenthesis) in contractions to EFS *$P<0.05$, **$P<0.01$ Wilcoxon matched pairs comparison with control

Drug μM	BRL 20627 (n=8)	Metoclopramide (n=8)
0.003	11 (4 to 15)*	12 (4 to 15)
0.03	16 (-1 to 30)	19 (3 to 24)*
0.3	41 (11 to 49)*	43 (16 to 70)*
2.8	78 (28 to 124)*	99 (59 to 139)**
28.0	41 (19 to 94)*	169 (89 to 320)**
282.0	-100 (-100 to -89)**	-70 (-89 to -21)*

As with Mcp, submaximal contractions to ACh (30s contact; 10min cycle; contractions approx. 50% maximum) were not significantly affected by 0.003-282μM BRL 20627 ($P>0.05$, Wilcoxon test; n=6) although in 5 of the 6 experiments with 282μM the contractions to ACh were reduced. Similarly, BRL 20627 did not affect the relaxations caused by stimulation of non-adrenergic, non-cholinergic inhibitory neurones. These relaxations were revealed by EFS in the presence of atropine 1.4μM and $BaCl_2$ 0.48mM (to block cholinergic contractions and to raise muscle tone, facilitating detection of relaxations) and were respectively 100 (93-100)% and 98 (95-100)% of controls with BRL 20627 2.8 and 28μM ($P>0.05$, Wilcoxon test; n=6 each). BRL 20627 may therefore increase ACh release in rat stomach, but without the side effects predicted for drugs which block dopamine responses (McClelland et al, 1983).

Anderson, J.D. et al (1977) J.Pharm.Pharmac. 29, 53P
Bateman, D.N. et al (1978) Br.J.clin.Pharmac. 6, 401-407
McClelland, C.M. et al (1983) This meeting
McClelland, C.M. & Sanger, G.J. (1982) Br.J.Pharmac. 77, Proc.Suppl. 539P

BRL 20627: A NON-DOPAMINERGIC BLOCKING STIMULANT OF GASTRIC MOTILITY

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The gastric motility stimulant activity of a novel benzamide BRL 20627, [(2α,6β, 9aα)-(±)-4-amino-5-chloro-2-methoxy-N-(octahydro-6-methyl-2H-quinolizin-2-yl)-benzamide HCl], has been compared to that of metoclopramide hydrochloride (Mcp) in the rat. Comparison has also been made of dopaminergic blocking activity.

Conscious male Wistar rats (200-500g) with a chronic gastric fistula were fasted overnight and individually restrained. Gastric motility was assessed from the mean amplitude of pressure waves recorded via the gastric fistula pre and post treatment. Rats with a low pre-treatment basal motility (mean amplitude <4mm Hg) were selected for use. BRL 20627, similarly to Mcp, increased the amplitude of phasic waves. Over an effective dose range stimulation in each animal was of an all or none nature with an increase in mean amplitude of 95 ± 16% (s.e. of mean) in responding animals. However, the number of animals responding with a significant increase ($P<0.05$) was dose related. ED_{50} values (significant increase in 50% of animals; Litchfield & Wilcoxon, 1949), showed BRL 20627 to be equipotent to Mcp by the s.c. [ED_{50} 0.8 (0.3-2.5, 95% confidence limits) and 1.0 (0.3-2.7)mg Kg^{-1}, respectively] and intragastric route [ED_{50} 1.7 (0.6-5.1) and 1.5 (0.5-4.7)mg Kg^{-1}, respectively]. At 50mg Kg^{-1} s.c. both compounds inhibited gastric motility. Gastric emptying in the fistula rat, as assessed by the recovery of a liquid test meal (Hunt & Knox, 1962) 10 minutes after instillation into the stomach, was significantly increased by both BRL 20627 and Mcp at 10mg Kg^{-1} s.c. (Table 1).

Table 1: Effect of BRL 20627 and Mcp on the gastric emptying of a 5ml test meal (33mM Citric acid) in the gastric fistula rat

Treatment 15 mins prior to test meal	n	% test meal emptied in 10 mins (mean ± se mean)	Significance Student's 't' test
Vehicle	6	36.3 ± 2.1	
BRL 20627 10mg kg^{-1} s.c.	7	55.0 ± 6.4	$P<0.05$
Vehicle	6	42.6 ± 1.5	
Mcp 10mg kg^{-1} s.c.	6	57.3 ± 3.1	$P<0.01$

Mcp promotes prolactin release by antagonism of dopamine mediated mechanisms (Carlson et al, 1977). In the non-stressed rat, Mcp produced a significant ($P<0.01$) elevation in the mean plasma immunoreactive prolactin level over the dose range which was stimulatory on the gut (0.25-10mg Kg^{-1} s.c.). In contrast BRL 20627 in doses below 10mg Kg^{-1} s.c. did not significantly elevate prolactin levels.

As a central dopamine antagonist, assessed by inhibition of apomorphine induced climbing in the mouse (Protais et al, 1976), BRL 20627 [ED_{50} 25 (20.7-30.3)mg Kg^{-1} s.c.] was considerably less potent than Mcp [ED_{50} 0.75 (0.62-0.90)mg Kg^{-1} s.c.].

It can be concluded that BRL 20627 has a stimulatory effect on basal gut motility in the rat comparable to that of Mcp but in the apparent absence of dopamine antagonist activity. It can be predicted that BRL 20627 would be less likely than Mcp to elevate plasma prolactin or induce extrapyramidal reactions in man.

Carlson, H.E. et al (1977) Proc.Soc.Exp.Biol.Med. 154, 475-478
Hunt, J.N. & Knox, M.T. (1962) J.Physiol. 163, 34-45
Litchfield, J.T. & Wilcoxon, F. (1949) J.Pharmacol. 96, 99-113
Protais, P. et al (1976) Psychopharmacol. 50, 1-6

PHENYLBIGUANIDE MIMICS THE BEZOLD-JARISCH EFFECT OF 5-HT IN THE RAT

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Fastier et al. (1959) reported that both phenylbiguanide (PB) and 5-hydroxytryptamine (5-HT) produce abrupt falls in heart rate and blood pressure in the cat. These effects are analogous to the Bezold-Jarisch (B-J) reflex and are produced by several chemical substances which activate sensory neurones in the heart (Paintal, 1973). However, we have recently shown that PB mimics the effects of 5-HT on the rat isolated vagus nerve (VN) and superior cervical ganglion (SCG) preparations (Fortune et al., 1983). It is therefore of interest to investigate the possibility that both 5-HT and PB may activate a similar 5-HT receptor to induce the B-J reflex.

Male, Sprague-Dawley (C. River CD) rats weighing 250-325g, starved for 18h, were dosed orally with drugs or vehicle (n=8 per group). After 1h the animals were anaesthetised with sodium pentobarbitone (50mg/kg ip). Heart rate was recorded from the ECG, and PB, 5-HT or vehicle (saline) were given as bolus injections into the jugular vein.

Intravenous administration of 5-HT (1-100ug/kg) and PB (1-300ug/kg) produced abrupt and transient (5-90s), dose-related falls in heart rate, 15-320 and 10-248 beats/min respectively. These effects were abolished by bilateral vagotomy ($p<0.01$, Dunnett's test), and reduced in a dose-dependent manner by atropine (0.1-3mg/kg po) ($p<0.05$-$p<0.01$), indicating that the bradycardia resulted from increased vagal tone.

Metoclopramide (1-30mg/kg po) also produced a significant dose-related inhibition of the falls in heart rate induced by submaximal doses of 5-HT (30ug/kg iv) and PB (100ug/kg iv) ($p<0.05$-$p<0.01$), whereas methysergide (10mg/kg po) was ineffective. It was notable that haloperidol (5mg/kg po), prazosin (5mg/kg po) and propranolol (5mg/kg po), as expected, did not modify responses to 5-HT and PB. The B-J effects of 5-HT and PB therefore appear similar to their interaction with the 5-HT neuronal receptor. In vitro 5-HT induced depolarisations of the VN and SCG are distinct from 5-HT effects on many smooth muscle preparations by being competitively antagonised by metoclopramide (pA_2 = 6.60 and 6.25 respectively) and not modified by methysergide ($1x10^{-4}M$) (Ireland et al., 1982, 1983).

In summary, the results indicate that there is a close similarity in the mechanisms by which PB and 5-HT induce the B-J reflex in rats. It is suggested that the effects of PB and 5-HT may be mediated through the same 5-HT receptor subtype.

Fastier, F.N. et al. (1959) Br.J.Pharmac. Chemother. 14, 527-535.
Fortune, D.H. et al. (1983) Br.J.Pharmac. Soc. (submitted).
Ireland, S.J. et al. (1982) Br.J.Pharmac. 75, Proc. Suppl. 16P.
Ireland, S.J. et al. (1983) Br.J.Pharmac. 78, Proc. Suppl. 68P.
Paintal, A.S. (1973) Physiol. Rev. 53, 159-227.

PREFERENTIAL ANTAGONISM OF ELEDOISIN BUT NOT SUBSTANCE P IN THE RAT SUPERIOR CERVICAL GANGLION.

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Substance P (SP) and related tachykinins depolarise the rat superior cervical ganglion (SCG) in vitro (Brown et al., 1982). Several SP analogues with D-amino acid substitutions have been proposed as antagonists of SP (Bjorkroth et al., 1982) and one of these, [D-Pro^2, D-Tryp^{7,9}]SP, selectively antagonises eledoisin on the hemisected neonatal rat spinal cord (Briggs et al., 1982). Agonist actions of these putative SP antagonists have also been demonstrated (Leander et al., 1981; Hawcock et al., 1982). It was therefore of interest to test [D-Arg^1, D-Pro^2, D-Tryp^{7,9}, Leu^{11}]SP, RPTTL-SP), [D-Pro^2, D-Tryp^{7,9}]SP (PTT-SP), [D-Pro^4, D-Tryp^{7,9,10}]SP (4-11) (PTTT-SP) for agonist activity in the rat SCG and for their ability to antagonise SP and eledoisin. Male rats were decapitated, the SCG rapidly dissected out and desheathed under a microscope. The membrane potential of cell bodies in the ganglion was recorded extracellularly as described by Brown et al. (1982).

RPTTL-SP, PTT-SP and PTTT-SP, $3x10^{-5}$M, all exhibited weak agonist activity which was not sustained. Subsequent applications of these compounds produced much reduced responses, presumably due to desensitisation. To test for antagonist activity, the compounds were superfused at $3x10^{-5}$M for 10 min before and during application of concentrations of SP, eledoisin and muscarine producing matched responses.

Table 1: Effect of putative SP antagonists on responses to SP, eledoisin and muscarine in rat SCG

	Mean % change (+ s.e.) in agonist response			
Compound	SP ($6x10^{-7}$M)	Eledoisin ($6x10^{-8}$M)	Muscarine ($6x10^{-8}$M)	n
RPTTL-SP	+1.7 (6.8)	-65 (2)	+19 (6.5)	7
PTT-SP	+4 (4)	-42 (6)	+5 (5)	7
PTTT-SP	+20 (15)	-10 (7)	+2 (6)	5

The results (Table 1) show that these analogues did not antagonise SP on the SCG. PTTT-SP also did not antagonise eledoisin. However, RPTTL-SP and PTT-SP did reduce responses to eledoisin, although it is impossible to say whether this is due to receptor antagonism or desensitisation.

Bjorkroth, V., Rosell, S., Xu J-C., & Folkers, K. (1981) Acta Physiol Scand 116 167-173.
Briggs, I., Jamieson, A., & Walsh, S., 1982. Regulatory Peptides 4 (6) 357.
Brown, J.R., Hawcock, A.B., Hayes, A.G., Hill, R.G. & Tyers, M.B. (1982). J. Physiol. 334 91-92P.
Hawcock, A.B., Hayes, A.G., Tyers, M.B. (1982). Eur. J. Pharmacol. 80 135-138.
Leander, S., Hakanson, R., Rosell, S., Folkers, K., Sandler, F. & Tornqvist, K. (1981) Nature 294 467-469.

ALPHA-ADRENOCEPTOR MEDIATED ANTINOCICEPTION AND SEDATION IN THE RAT

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Recent evidence from Paalzow and Paalzow (1982) suggests that clonidine-induced antinociception is mediated by two or more receptor systems. Earlier studies have implicated both α_1- and α_2-adrenoceptors (Reddy et al., 1980; Paalzow and Paalzow, 1976) in antinociception, although there is general agreement that clonidine-induced sedation is an α_2-adrenoceptor mediated effect (Drew et al., 1979). The present series of experiments compares the antinociceptive and sedative effects of compounds with varying α-adrenoceptor selectivities.

Nociceptive pressure thresholds were determined for the hindpaws of weanling rats (AH hooded, male, 50-70g) using an 'analgesymeter' (Ugo Basile). Rotarod reaction latencies were determined for the same rats immediately after antinociceptive testing. Testing, which was done blind, was carried out 10 min after intracerebroventricular (icv) administration of the α-adrenoceptor agonists.

In vitro agonist potencies at α_1- and α_2-adrenoceptors were determined using the rat (AH hooded, male 270-350g) anococcygeus muscle and field-stimulated vas deferens preparations. Anococcygeus muscles were incubated in Krebs bicarbonate solution containing 6-hydroxydopamine ($1x10^{-3}$M) and ascorbic acid ($1x10^{-4}$M) at 4°C for 16-20h. The muscles were then mounted in organ baths and RX 781094, $6x10^{-7}$M, added to the bathing fluid. Prostatic vasa deferentia were field stimulated with monophasic supramaximal pulses, 0.5 ms wide, delivered at 0.1 Hz; prazosin, $6x10^{-7}$M, was added to the bathing fluid.

Table 1 : The effects of α-adrenoceptor agonists in vivo and in vitro

	ED50 (95% confidence limits) ug/rat icv		EC50 (uM ± s.e.)		
agonist	Paw Pressure	Rotarod	Anococcygeus m. (α_1)	Vas deferens (α_2)	Potency ratio
ST 587	2.2 (0.7-5.7)	>90	0.21 (±0.003)	>1	<0.21
Methoxamine	4.1 (1.9-8.7)	>30*	0.70 (±0.06)	3.0 (±0.6)	0.23
CP 18,534	3.3 (1.0-9.6)	>30*	0.108 (±0.016)	0.0114 (±0.003)	9.4
Clonidine	1.9 (1.2-3.0)	13.3 (5.6-33.2)	0.14 (±0.02)	0.0055 (±0.001)	25
UK 14,304	1.0 (0.4-2.2)	4.0 (1.8-10.1)	>200	0.004 (±0.00006)	>1000

* Shallow dose response curve (approx. 60% clonidine maximum)

All α-adrenoceptor agonists tested were antinociceptive agents suggesting that antinociception may be mediated by both α_1- and α_2-adrenoceptors. In contrast, sedation was most marked in those compounds with higher α_2-adrenoceptor selectivity suggesting that sedation is predominantly an α_2-adrenoceptor effect.

Drew, G.M. et al (1979) Br. J. Pharmac., 67, 133-141.
Paalzow, H.M. & Paalzow, L.K. (1976) Naunyn-Schmiedeberg's Arch. Pharmac., 292, 229-126.
Paalzow, H.M. & Paalzow, L.K .(1982) J Pharmac. Exp. Ther. 223, 795-800.
Reddy, S.V.R. et al.(1980) J. Pharm. Exp. Ther. 213, 525-533.

EVALUATION OF ETHYLCHOLINE MUSTARD AZIRIDINIUM ION (ECMA) AS A SPECIFIC NEUROTOXIN OF BRAIN CHOLINERGIC NEURONES

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Post-mortem studies have established that ascending cholinergic projection to cortex and hippocampus degenerate in Alzheimer's disease (Perry, 1980). However, no experimental model of the illness exists. ECMA is a toxic choline analogue which is recognized by the high affinity choline uptake site on cholinergic neurones. The use of this compound as a selective cholinergic neurotoxin to mimic the neurochemical changes of Alzheimer's disease has been proposed (Fisher et al, 1982).

To evaluate the neurotoxic effects of ECMA, varying concentrations of the drug were stereotaxically microinjected into the region of the substantia innominata/ medial globus pallidus of rat brain. This area contains the cholinergic cell bodies which project to neocortex, whereas the cholinergic projection to the hippocampus arises from the septal nuclei (Fibiger, 1982). Stereotaxic coordinates were developed using dye injections. Animals were anaesthetized with 'Avertin' and unilateral microinjections of 1 µl made via a 30 guage cannula. Rats were killed 3-10 days post-operatively and left and right neocortex and hippocampus removed for assay of choline acetyltransferase, a biochemical marker of cholinergic nerve terminals. Other animals were re-anaesthetized and perfused for acetylcholine esterase (AChE) and cresylviolet histological staining of brain sections to evaluate damage to cholinergic neurones and non-specific effects of the injection.

Doses of ECMA in excess of 50 nmole caused all animals to die within 24 hours. 14.6, 7.3, 0.73, 0.073 nmole ECMA caused equal depletions (up to 50%) of CAT in ipsilateral neocortex but not in hippocampus which receives cholinergic projections from the septal nuclei. 0.0073 nm ECMA was ineffective. Injection of the non-cyclized form of the compound (7.3 and 0.073 nmole) was also effective in depleting cortical CAT content. Histology revealed greatly reduced AChE staining in ipsilateral neocortex sharply delineated from intact staining of contiguous cingulate and temporal cortices and of hippocampi. However, all effective doses of ECMA caused large areas non-specific necrosis around the injection site.

It is concluded that cholinergic neurones of the rat substantia innominata/medial globus pallidus project specifically to neocortex and not to limbic cortical areas. In agreement with previous studies (Caulfield et al, 1983), the present results suggest that selective destruction of cholinergic cell bodies cannot be achieved with ECMA.

We are grateful to Salford Fine Chemicals for preparation of ECMA precursor.

Caulfied, M.P. et al (1983) Br. J. Pharmac. (in press)
Fibiger, H.C. (1982) Brain Res. Rev. 4, 327
Fisher, A. et al (1982) J. Pharmac. exp. Ther. 222, 40
Perry, E.K. (1980) Age and Aging 9, 1

THE EFFECT OF REPEATED ELECTROCONVULSIVE SHOCK ON THE ANTICONVULSANT AND HYPOTHERMIC RESPONSE TO THIP

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Repeated electroconvulsive shock (ECS) causes an enhancement of behavioural responses mediated by 5-HT and dopamine and an increase in the number of 5-HT_2 receptors (see Green & Nutt, 1983). In addition, repeated ECS elevates GABA levels in some brain regions (Bowdler et al., 1983). In the present study we have measured the hypothermic and anticonvulsant effects of 4,5,6,7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol (THIP), a GABA agonist (Krogsgaard-Larsen et al., 1977), after ECS in order to assess whether the change in GABA levels was associated with a change in GABA function.

Adult male Sprague-Dawley derived rats were given ECS (125 V, 1 sec, 50 Hz sinusoidal via earclip electrodes) once daily for 10 days. Control rats had the electrodes applied but no current was passed. Twenty-four hours after the last treatment the rectal temperature of each animal was taken. The rats were then injected i.p. with saline or THIP (10 mg/kg) and 30 min later their rectal temperature was again measured. Immediately afterwards seizure threshold to the GABA antagonist drug, pentylenetetrazol (PTZ), was measured using an i.v. infusion method (see Nutt et al., 1980).

Repeated ECS caused an increase in basal rectal temperature which was not significantly reduced by THIP (Table). In the handled group THIP produced a lowering of temperature comparable to that seen in naive animals.

Seizure threshold following THIP was not different in the ECS group compared with the handled group (ECS x 10 plus THIP, 42 ± 3, n=8; handled x 10 plus THIP, 42 ± 6, n=6, all values in mg PTZ/kg, and are mean ± S.D.). In both groups THIP significantly elevated seizure threshold compared to saline treated, handled x 10 controls (30 ± 3 mg PTZ/kg, n=5, mean ± S.D.; significantly different from both THIP-treated groups $P < 0.01$ 2-tailed t-test).

TABLE. The effect of repeated ECS on basal temperatures and the hypothermic effect of THIP

	Rectal temperature, °C	
	Before THIP	30 min after THIP (10 mg/kg)
Handled x 10	37.4 ± 0.4	36.2 ± 0.9* (6)
ECS x 10	38.1 ± 0.4†	37.8 ± 0.5 (8)

Values are mean ± S.D. with the number of animals in brackets.
*$P < 0.05$ compared with before THIP, paired t-test.
†$P < 0.01$ compared with handled x 10, unpaired t-test.

These findings demonstrate that repeated ECS does not alter the anticonvulsant effect of THIP. By contrast, the hypothermic effect of THIP was markedly attenuated in the ECS-treated animals. However interpretation of this finding is complicated by the fact that the basal temperature in the ECS-treated animals was significantly elevated. Both these phenomena could be explained by a decrease in GABA receptor sensitivity in the thermoregulatory centres. However another possibility is that ECS enhances the 5-HT system in these centres (see Vetulani et al., 1981) which may override the effect of THIP.

Bowdler J.M. et al. (1983). J. Neural Trans. 56 3
Green A.R. & Nutt D.J. (1983). In D.G. Grahame-Smith et al. (Eds), Psychopharmacology, Vol. 1/1 pp. 1-37, Excerpta Medica, Amsterdam
Krogsgaard-Larsen P. et al. (1977). Nature 268 53
Nutt D.J. et al. (1980). Neuropharmacology 19 1017
Vetulani J. et al. (1981). Eur. J. Pharmac. 76 81

KETANSERIN IN DOCA/SALINE- AND RENAL HYPERTENSIVE RATS

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Renal hypertensive-, DOCA/saline- and spontaneously hypertensive rats (SHR) are supersensitive to the vasopressor effect of serotonin (Haeusler and Finch, 1972). This observation has led to speculations about a role of serotonin in the pathogenesis of arterial hypertension (Vanhoutte, 1982). The discovery of the "serotonin antagonist" ketanserin, a drug with antihypertensive properties (De Cree et al, 1981) seems to support this hypothesis. However, the antihypertensive effect of ketanserin in SHR is due to antagonism of vascular α_1-adrenoceptors (Fozard, 1982; Kalkman et al, 1982). We now report on the blood pressure lowering potency of ketanserin in DOCA/saline- and renal hypertensive rats.

Blood pressure of conscious animals was measured from the carotid artery (DOCA/saline rats) or abdominal aorta (renal hypertensive rats). DOCA/saline rats when provided with an indwelling aorta catheter all died within two days after the surgical intervention due to an extreme water retention. Ketanserin was administered via a cannulated jugular vein. Ketanserin (0.03-30 mg/kg) was injected in a cumulative way.

In both hypertension models doses up to 0.3 mg/kg of ketanserin did not alter blood pressure. Results are shown in table 1.

Table 1. Doses of ketanserin (i.v.); blood pressure (mean ± S.E.M.)

	0 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	
Normotensive	109 ± 5	109 ± 4	98 ± 4	77 ± 2	75 ± 3	(n=5)
DOCA/saline	167 ± 6	158 ± 7	137 ± 9	113 ± 6	96 ± 9	(n=6)
Renal hypertensive	135 ± 9	132 ± 7	127 ± 7	117 ± 7	115 ± 7	(n=6)

In renal hypertensive rats the average decrease in diastolic pressure amounts to 20 mm Hg only. Ketanserin antagonizes vascular serotonin receptors in doses of 0.03 mg/kg and higher; for antagonism of vascular α_1-adrenoceptors a dose of 0.3 mg/kg is required (Kalkman et al, 1982). From the results of Haeusler and Finch (1972) a hypotensive effect of ketanserin is expected at low (antiserotonergic) doses. As can be seen from the table, both potency and efficacy of ketanserin in the two hypertension models is limited. The results are therefore not in favour of the hypothesis that ketanserin lowers blood pressure by a serotonin antagonistic activity.

De Cree, J. et al (1981) Clin. Sci. 61, 473s
Fozard, J.R. (1982) J. Cardiovasc. Pharmacol. 4, 829
Haeusler, G. and Finch, L. (1972) Naunyn-Schmiedeberg's Arch Pharmacol. 272, 101
Kalkman, H.O. et al (1982) J. Pharmacol. Exp. Ther. 222, 227
Vanhoutte, P.M. (1982) T.I.P.S. 3, 370

EFFECTS OF FORSKOLIN, ISOPRENALINE AND IBMX ON TENSION RESPONSES AND CYCLIC NUCLEOTIDE LEVELS IN GUINEA PIG LUNG

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Although both cyclic AMP and cyclic GMP have been implicated in the relaxation of airway smooth muscle (Katsuki & Murad, 1977) it is generally accepted that β-adrenoceptor-mediated relaxation occurs via a cyclic AMP-dependent mechanism. We report here the results from a study in which we have compared the effects of forskolin, a direct activator of adenylate cyclase, with those of isoprenaline and 3-isobutyl-1-methyl xanthine (IBMX) on the magnitudes and time courses of relaxation and on the levels of cyclic AMP and cyclic GMP in guinea pig lung.

Sub-pleural lung strips were suspended in Krebs-Henseleit solution at 30°C and bubbled with 5% CO_2 in O_2. Responses to forskolin, isoprenaline and IBMX in KCl-contracted (75 mM) tissue were recorded by conventional methods. After a fixed contact time each lung was rapidly frozen in liquid N_2 and assayed for cyclic nucleotides using standard kits (Radiochemical Centre, Amersham). The results are expressed as mean ± s.e. mean of 3-5 determinations, levels of significance with respect to control values being set at $P<0.05$. Cyclic nucleotide levels are expressed in pmol/mg protein.

All three drugs relaxed KCl-depolarised lung strips. Forskolin (100μM) and IBMX (1 mM) produced comparable, slow relaxations requiring longer than 3hr for maximal effect (forskolin: 7.4 ± 0.6mN; IBMX: 5.9 ± 0.5mN, $P<0.001$). Forskolin responses were accompanied by time-dependent elevations in cyclic AMP (from 14.4 ± 3.4 to 555.7 ± 101.8 after 180 min, $P<0.001$). Cyclic GMP levels were unaffected by forskolin. IBMX time-dependently elevated the levels of both cyclic AMP and cyclic GMP (after 180 min: cyclic AMP-from 14.4 ± 3.4 to 30.9 ± 7.1, $P<0.01$; cyclic GMP-from 4.18 ± 0.15 to 21.5 ± 2.5, $P<0.001$). Relaxations to isoprenaline (1μM) were by comparison rapid (< 15 min) but only amounted to about 26% of the maximum forskolin response measured (1.9 ± 0.1 mN, $P<0.05$). These relaxations were accompanied by increases in the levels of both cyclic nucleotides that peaked after about 2 min (cyclic AMP- from 14.3 ± 3.4 to 44.0 ± 6.5, $P<0.001$; cyclic GMP- from 4.18 ± 0.15 to 10.6 ± 2.9, $P<0.05$).

Forskolin, isoprenaline and IBMX all produced relaxation responses that were associated with increases in the levels of cyclic AMP. These data are, therefore, consistent with the hypothesis that cyclic AMP may mediate smooth muscle relaxation in guinea pig lung. It should be emphasised, however, that there is no unifying concentration-effect relationship between the levels of cyclic AMP in this tissue and the associated relaxations produced by the different drugs. Cyclic GMP appears not to be involved in the relaxation process initiated by forskolin.

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Katsuki, S. & Murad, F. (1977) Mol. Pharmacol., 13, 330.

TOXIC INTERACTIONS BETWEEN TRANYLCYPROMINE AND DOPAMINERGIC DRUGS IN RABBITS

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Drug interactions of high pharmcological significance occur after administration of some centrally acting drugs to monoamine oxidase inhibitors (MAO I's)- pre-treated rabbits (Fahim et al 1972). In this study we have examined the possible interaction between tranylcypromine and dopaminergic drugs in rabbits. We have also studied the modification of the interaction by various drugs. Hyperpyrexia was used as a marker for the interaction. Rise in core temperature was measured using electrical thermocouples supplied by Sierex Ltd. Drugs were given per kilogram body weight s.c. as indicated.

Groups of male rabbits (each weighing 2kgm) (n=8) were injected with tranylcypromine sulphate (10mg) for five successive days. On the 5th day each of the following drugs or its vehicle was administered to one group of rabbits 20 min. after the last dose of tranylcypromine. The drugs were nomifensine(50mg), bromocriptine(100mg), apomorphine(10mg) and ergometrine(100µg). The core temperature was monitered over a 3h period. In other groups of tranylcypromine-pre-treated rabbits (n=4) indomethacin(10mg), cyproheptadine hydrochloride(5mg), haloperidol(2mg) or their vehicles were administered 30min before the injection of the dopaminergic drugs. Statistical significance was calculated using unpaired 't' test.

Rabbits treated with dopaminergic drugs, tranylcypromine, indomethacin, cyproheptadine or haloperidol alone did not show any significant change in core temperature compared with vehicle treated control animals. Administration of bromocriptine, apomorphine and nomifensine significantly elevated the core temperature by 3.9 ± 0.2, 4.2 ± 0.1 and 4.7 ± 0.2 °C respectively ($P<0.05$). Some of the rabbits died within 60min of injection of dopaminergic drugs. Ergometrine was ineffective. The hype rpyrexia was completely pre vented by haloperidol. Neither indomethacin nor cyproheptadine protected the rabbits against hyperpyrexia.

These results suggest that neither prostaglandins nor 5-hydroxytryptamine were involved in the hyperpyrexic reaction. The effectiveness of haloperidol suggests that hyperpyrexia was mediated via excessive activation of the dopamine receptors. This may be due to the combined inhibition of MAO enzyme and dire ct activation of the receptors. Alternatively, it could be due to impairment of biotransformation of the dopaminergic drugs by tranylcypromine. The ineffectiveness of ergometrine may be due to its partial agonistic action: acting both as agonist and antagonist. The results indicate a possible hazard in administering dopaminergic drugs to patients being chronically treated with tranylcypromine.

Fahim,I. et al (1972) Br.J.Pharmac. 46, 416p.

α_1-ADRENOCEPTOR AGONISM OF CIRAZOLINE AND ST 587 ON RAT ISOLATED AORTA

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The α-adrenergic receptor system on rat isolated aortic smooth muscle is not well characterized. Controversy exists on the question whether α_1- or α_2-adrenoceptors, a mixed population or a population of as yet unclassified α-adrenoceptors mediate contractions of this preparation (Ruffolo et al., 1982; Randriatsoa et al.,1981). The existence of two populations of α_1-adrenoceptors on rat aorta has recently been suggested (Ruffolo et al., 1982; Beckeringh et al., 1983). Because of the differential effects of calcium entry blockade on contractions elicited by cirazoline and St 587, these two substances may be selective agonists for the two subtypes (Beckeringh et al., 1983). In order to further investigate the interaction of cirazoline and St 587 with α-adrenoceptors on rat aorta, we have analysed the antagonism by prazosin and yohimbine of the contractions elicited by the two agonists.

Helically cut strips of male Wistar rats (300-350g) were suspended in Krebs-Henseleit solution containing 1 µM dl-propranolol, 10 µM cocaine and 20 µM cortisol at 37 °C under a resting tension of 800 mg. Agonists were added cumulatively to the organ bath in the absence or presence of different concentrations of antagonist. Contractions were recorded isometrically and expressed as percentage of the maximal response to noradrenaline (3-10 µM). Antagonism was analysed by means of Schild-plots according to Arunlakshana and Schild (1959).

Table 1 Characteristics of Schild-plots of prazosin and yohimbine against cirazoline and St 587 on rat aorta

	prazosin			yohimbine		
	n	slope	pA_2	n	slope	pA_2
cirazoline	23	-1.14±0.10	9.62±0.19	15	-1.07±0.15	6.78±0.16
St 587	35	-0.99+0.14	9.89+0.24	36	-0.81+0.10	7.08+0.13

The slopes and pA_2 values calculated from the Schild-plots are listed in Table 1 and are expressed as mean values ± S.E. The calculated slopes were not significantly different ($p > 0.05$) from unity. The difference in pA_2 values for prazosin and yohimbine demonstrate that cirazoline and St 587 stimulate α_1-adrenoceptors in rat aorta. No significant difference ($p > 0.05$) existed between the pA_2 values of prazosin against cirazoline and St 587 nor between the pA_2 values of yohimbine against the two agonists.

On the basis of the antagonism by prazosin and yohimbine, the α - adrenoceptor which is stimulated by cirazoline and St 587 on rat aorta is characterized as the α_1-subtype. If different populations of α_1-adrenoceptors exist on rat aorta, these subtypes cannot be distinguished using prazosin as antagonist.

Arunlakshana, O. & Schild,H.O. (1959) Br. J. Pharmacol. 14, 48
Beckeringh, J.J. et al (1983) This meeting
Randriatsoa, A. et al (1981) Eur. J. Pharmacol. 75, 57
Ruffolo, R.R. et al (1982) J. Pharm. Exp. Ther. 221, 309

A COMPARISON OF LIGNOCAINE AND TOCAINIDE ELIMINATION IN CHRONIC LIVER DISEASE

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Lignocaine (L) is a well established drug for the control of ventricular arrhythmias. Dosage schedules are based on its marked first-pass hepatic clearance and in patients (pts) with impaired hepatic function these schedules must be modified to avoid the development of high plasma L concentrations, leading to central nervous toxicity. The L analogue tocainide (T) has similar electrophysiological properties but does not undergo first-pass metabolism and is approximately 40% excreted unchanged by the kidney. It may, therefore,be a more appropriate drug than L for the treatment of pts with concomitant hepatic disease. The aim of this study was to investigate the elimination kinetics of L and T in this clinical setting to assess the implications for dosage with T in patients with hepatic dysfunction.

Nine pts (5M, 4F) mean $\pm$ SD age 53.1 $\pm$ 10.0 yr with chronic stable liver disease gave informed consent to the study which was approved by the Edinburgh Royal Infirmary Ethical Committee. Five had biopsy proven cirrhosis, whilst in three the diagnosis of cirrhosis was based on typical clinical features and biochemical tests of hepatic function; in the remaining case the diagnosis of cirrhosis was based on biochemical tests and hepatic angiography. The severity of liver disease was graded A-C (most severe) according to the criteria of Pugh et al, 1973. At the start of the study the pts were classified as 1A, 4B and 4C. Pts received oral doses of L (400 mg) and T (200 mg) in random order, each treatment being at least 4 days apart. Frequent blood samples were collected up to 24 h (L) or 36 h (T) for the measurement of plasma L & T using a selective high-performance liquid chromatographic method. Urine was collected for 48 h following each treatment for drug measurement. Drug concentration data were analysed using the interactive pharmacokinetic program STRIPE (Johnston & Woolard 1983).

Mean observed peak plasma concentrations were 2.37 $\pm$ 0.68 mg/l (L) and 1.37 $\pm$ 0.37 mg/l (T) and the mean elimination half-lives were 5.8 $\pm$ 2.9 h (L) and 21.8 $\pm$ 5.6 h (T). L half-lives correlated with prothrombin time ratio, $r = 0.64$ ($p < 0.05$) but there was no correlation between L and T half-lives ($r = 0.24$). The mean fraction of the dose excreted unchanged in urine over 48 h was 3.8 $\pm$ 2.6% (L) and 41.1 $\pm$ 9.8% (T). Mean peak L concentration and elimination half-life were substantially greater than those reported for healthy volunteers receiving oral L (Perucca & Richens, 1979), the half-life being prolonged more than four-fold, consistent with diminished hepatic clearance. Whilst there was a modest increase in the mean half-life of T compared with volunteer studies (of the order 40%) peak T concentration and urinary excretion of T were not affected (Lalka et al., 1976). One pt experienced mild nausea 90 min after T and 3 pts experienced mild dizziness, one with drowsiness, shortly after receiving L. No other adverse effects were noted or reported.

These data indicate that, in contrast with L, T elimination kinetics are not markedly altered in pts with chronic hepatic failure. Moreover, the poor correlation between L and T elimination half-lives suggests that T elimination would be less prone to changes in hepatic blood flow in pts with congestive cardiac failure or shock.

Johnston, A & Woolard,A(1983) J. Pharmacol. Meth. (in press)
Lalka, D. et al., (1976) Clin. Pharmacol, Ther. 19 757.
Perucca, E. & Richens, A. (1979) Br. J. Clin. Pharmacol. 7 201.
Pugh, R.M.H. et al., (1973) Br. J. Surg. 60 646.

THE EFFECT OF ALCOHOLIC CIRRHOSIS ON THE BIPHASIC O-DEETHYLATION OF 7-ETHOXYCOUMARIN IN HUMAN LIVER MICROSOMES

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Studies in animals and man have shown that the O-deethylation of 7-ethoxycoumarin follows biphasic Michaelis-Menten kinetics. Furthermore, the two components are probably dependent upon different cytochrome P450 forms (Boobis et al, 1981). Measurement of these components might therefore be a useful probe in the investigation of different cytochrome P450 species in man. As it has been suggested that not all cytochrome P450 dependent monoxygenases are impaired in subjects with alcoholic cirrhosis (Farrell et al, 1979), we have measured the activities of the high and low affinity components of 7-ethoxycoumarin O deethylase (EOC) in liver from subjects with alcoholic cirrhosis and in controls.

Measurements of EOC activity were undertaken at 1 uM and 1000 uM substrate concentrations. Preliminary experiments using human wedge liver biopsies showed that at 1 uM, 79% of measured activity was attributable to the high affinity phase, whereas at 1000 uM, 85% of measured activity was attributable to the low affinity phase. Activity was measured using the method of Greenlee and Poland (1978). Results are shown below EOC activity is expressed as pmol 30H coumarin formed/mg microsomal protein/min.

	High affinity component	Low affinity component
Normals	9.43 ± 2.37 (n = 7)	111.3 ± 9.2 (n = 10)
Alcoholic Cirrhosis	3.27 ± 1.18* (n = 9)	60.9 ± 11.6** (n = 8)
	*$p < 0.05$	**$p < 0.01$

In the 1 uM substrate concentration experiments, microsomal protein content per incubation was similar, in both groups (78.9 ± 17.5 ug/incubation for normals and 83.4 ± 15.5 ug/incubation for alcoholic cirrhosis).

The reduction of both components of EOC activity in alcoholic cirrhosis is further evidence that there is a widespread impairment of cytochrome P450 dependent enzyme system in patients with alcoholic cirrhosis.

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Boobis, A.R. et al (1981) Biochem.Pharmacol. 30: 2451-2486
Greenlee, W.F. and Poland, A. (1978) J.Pharmacol. Exp. Ther. 205: 596-605
Farrell, G.C. et al (1978) Clin.Pharmacol.Ther. 26: 483- 492