MDL 72222: A POTENT AND HIGHLY SELECTIVE ANTAGONIST AT NEURONAL 5-HYDROXYTRYPTAMINE RECEPTORS

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5-Hydroxytryptamine (5-HT) excites a variety of neurones in the mammalian peripheral nervous system through activation of neuronal 5-HT receptors (Wallis, 1981). Morphine is not a selective antagonist at these receptors although cocaine and a number of its analogues (Fozard et al., 1979) and metoclopramide (Fozard and Mobarok Ali, 1978) show both potency and a degree of selectivity at certain of these sites both in vitro and in vivo (Fozard and Host, 1982). In this report, I describe the 5-HT neuronal receptor antagonist properties of 1αH, 3α, 5αH-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222), the most potent and selective of a series of benzoic acid esters of tropine (Fozard and Gittos, 1983).

Rabbit hearts were perfused by the Langendorf technique at constant pressure with Tyrode solution containing atropine (1.7 μM) at 37°C (Fozard et al. 1979). The Von Bezold-Jarisch effect of 5-HT was quantified in the anaesthetized rat as previously described (Fozard and Host, 1982).

On the rabbit isolated heart MDL 72222 was a potent antagonist of responses mediated through the receptors for 5-HT present on the terminal sympathetic fibres. The threshold for antagonism was approximately 0.1 nM and the pA_2 value estimated by the method of Schild (1947) was 9.27±0.06, n=7. MDL 72222 was also highly selective since responses to the nicotine receptor agonist, dimethylphenylpiperazinum iodide (DMPP), were inhibited only at concentrations more than 1000 times those necessary to inhibit 5-HT (pA_2 = 6.14±0.04, n=6).

In the anaesthetised rat, MDL 72222 produced marked blockade of the Von Bezold-Jarisch effect of 5-HT, 1.25-10 μg/kg i.v., at a dose of 0.1 mg/kg injected intravenously. The ED50 was 0.039±0.007 mg/kg (0.12±0.02 μmol/kg), n=4, and inhibition was selective since doses of MDL 72222 as high as 1 mg/kg (3.2 μmol/kg) failed to alter the response to electrical stimulation of the efferent vagus nerves.

MDL 72222 is only a weak (> μM) and essentially non-selective antagonist of responses mediated by the 5-HT M-receptor present on the cholinergic nerves of the guinea-pig ileum. The compound does not block smooth muscle contractile responses elicited by oxytocin or mediated through 5-HT D-receptors, muscarinic or nicotinic cholinoceptors or histamine H_1-receptors except at relatively high (>>μM) concentrations. Similarly, in a number of radio-ligand binding assays carried out using brain tissue membranes, the displacing effects of MDL 72222 were absent or weak at sites identifying compounds with activity at α_1, α_2 or β-adrenoceptors, $5HT_1$ or $5HT_2$ receptors, benzodiazepine receptors or histamine H_1-receptors.

Thus, MDL 72222 is a potent and remarkably selective antagonist of responses mediated through the 5-HT receptors present on the terminal sympathetic neurones of the rabbit heart and on the neurones subserving the afferent limb of the Von Bezold-Jarisch reflex. The compound should provide a useful means by which responses mediated through such sites can be distinguished.

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RESPONSES OF THE GUINEA-PIG UTERUS TO SYMPATHOMIMETIC AMINES AFTER THE FIRST PREGNANCY

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During pregnancy in the guinea-pig there is a marked decline in noradrenaline levels in fetus-bearing uterine horns (Owman et al, 1975). Loss of sympathetic function is long-lasting, for example, restoration of tyrosine hydroxylase activity of the sympathetic nerves innervating these horns is incomplete even 6 months after parturition (Alm et al, 1979). Pregnancy also leads to a dramatic change in the response of the longitudinal myometrium of the guinea-pig to sympathomimetic amines (Hartley et al, 1983a; Story et al, 1983). Thus although application of noradrenaline, adrenaline and phenylephrine, in contrast to isoprenaline, causes phentolamine-sensitive contraction of both longitudinal and circular myometrium taken from virgin guinea-pigs during the oestrous cycle (Adam et al, 1981; Hartley et al, 1983b), preparations of longitudinal myometrium taken 1-5 days post partum are inhibited by all four amines. These inhibitory effects are blocked by propranolol. The present experiments were undertaken to estimate the duration of the reversal of responsiveness of the longitudinal layer to adrenoceptor agonists following parturition.

Full concentration-response curves for adrenaline, noradrenaline, phenylephrine and isoprenaline were constructed using electrically-stimulated preparations of longitudinal myometrium taken from 10 primiparous guinea-pigs on days 6-10 of the second to ninth post partum oestrous cycles (i.e. 25-130 days post partum).

Isoprenaline produced inhibition of all uterine preparations examined. Some restoration of normal α-adrenoceptor-mediated responsiveness was evident during the second post partum oestrous cycle (days 25-28). Noradrenaline produced phentolamine-sensitive excitatory effects on longitudinal myometrial preparations from 2 of 3 guinea-pigs. Responses to adrenaline and phenylephrine were exclusively inhibitory at this time. The response to phenylephrine as well as that to noradrenaline was restored by the fourth cycle (55 days post partum). The excitatory response to adrenaline was restored by the sixth cycle (90 days post partum).

These experiments indicate that although full restoration of the α-adrenoceptor-mediated excitatory effects of adrenaline, noradrenaline and phenylephrine is relatively slow, it precedes full morphological and functional restoration of the uterine sympathetic nerves.

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CALCIUM REQUIREMENT FOR RECEPTOR-MEDIATED INOSITOL PHOSPHOLIPID BREAKDOWN IN RAT CEREBRAL CORTEX

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Using lithium to amplify the accumulation of inositol phosphates (IP) resulting from agonist-dependent breakdown of phosphatidylinositol (PI) and polyphosphoinositides (PPI), it has recently been possible to pharmacologically characterise several receptor-mediated responses in rat cerebral cortex (Brown and Nahorski, 1983). Since it has been suggested that the hydrolysis of PI and/or PPI may be closely related to Ca^{2+} gating (Michell et al, 1981), we have investigated the Ca^{2+} requirements for inositol phospholipid breakdown stimulated by various agonists.

Rat cerebral cortex slices (350 µM x 350 µM) were preincubated for 1 h at 37° in either a Krebs/Heinsleit buffer, in the same buffer with Ca^{2+} omitted, or with Ca^{2+} omitted and EGTA (0.5 mM) added. They were then transferred to tubes containing 0.18–0.25 µM ^{3}H-myo-inositol for 30 min. after which appropriate agonists were added for a further 45 min. ^{3}H-IP was extracted and separated from ^{3}H-inositol by ion exchange chromatography. ^{3}H-PI was separated from ^{3}H-PPI by thin layer chromatography on silica gel high performance plates. Measurements of calcium contamination in 'calcium-free' buffers were made using a Perkin Elmer 360 atomic absorption spectrometer. In the absence of added Ca^{2+} ions, buffers were found to contain approximately 10 µM Ca^{2+}.

The omission of added Ca^{2+} totally abolished accumulation of ^{3}H-IP due to histamine (10^{-3} M), reduced that due to 5HT (10^{-3} M) by 80% and, to a lesser extent, noradrenaline (10^{-4} M) by 31%, but did not affect carbachol (10^{-4} M) stimulated breakdown. EC_{50} values (when measurable) were, however, unaltered in the absence of added Ca^{2+}. Complete removal of Ca^{2+} by preincubation of slices with EGTA abolished all responses, but the replacement of Ca^{2+} after EGTA preincubations restored responses to normal. Similarly, responses to depolarising stimuli, K^{+} (25 mM) and veratrine (3 x 10^{-5} M), were abolished in the presence of EGTA. Noradrenaline-stimulated breakdown was also antagonised by manganese (IC_{50} 1.7 µM) but not by nitrendipine or nimodipine (upto 30 µM). The calcium ionophore A23187 stimulated hydrolysis with an EC_{50} value of 2 µM, and this response was also blocked by EGTA.

In contrast to the effects on PI and/or PPI breakdown, omission of calcium or preincubation with EGTA (0.5 mM) or manganese (EC_{50} = 230 µM) greatly enhanced the incorporation of ^{3}H-inositol into phospholipids. More than 90% of tritium incorporated was recovered in PI with or without added Ca^{2+}, but the proportion in PPI was enhanced 4-fold by Ca^{2+}.

The results show that different receptors mediating PI/PPI breakdown in rat cortex have quantitatively different calcium requirements, and in the case of carbachol (though not histamine) stimulated hydrolysis, the sensitivity of the response to Ca^{2+} deprivation is less than would be expected if PI breakdown were the result of Ca^{2+} influx. Rigid opinions regarding PI breakdown as either the cause or effect of calcium mobilisation in rat cortex are thus probably inappropriate.

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SOLUBILIZED PUTATIVE CALCIUM CHANNELS: PURIFICATION, GLYCOPROTEIN NATURE AND OTHER PROPERTIES

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Voltage-operated calcium channels which are blocked by nifedipine have been studied in skeletal muscle with electrophysiological methods (Stefani and Chiarandini, 1982). Recently putative calcium channels (PCCs) have been directly identified in guinea-pig skeletal muscle microsomes with the radiolabelled nifedipine derivative [^{3}H]-nimodipine (158 Ci/mmol, Ferry and Glossmann, 1982).

Digitonin (0.25-0.5% w/w) and CHAPS (3-(3-cholamidopropyl)dimethylammonio-1-propansulfonate; 7-10 mM) release PCCs from guinea-pig skeletal muscle microsomes. Released PCCs are precipitated by 10% w/v polyethylene glycol 6000 in the presence of carrier-protein onto GF/C filters. The released PCCs are not cleared from solution by a 50.000 x g centrifugation for 4 hours, and for the CHAPS solubilized PCC sucrose density centrifugation through 5-20% w/w gradients gave a sedimentation coefficient ($S_{20,w}$) of 12.9± 0.2 S (n = 3, mean± s.e.). The PCCs released from skeletal muscle microsomes by CHAPS or digitonin will be referred to as "solubilized PCCs".

The dissociation constant of [^{3}H]-nimodipine for digitonin- and CHAPS-solubilized PCCs is in the range 2-3 nM at 37°C in 50 mM TRIS HCl, pH 7.4. As for the membrane bound PCC d-cis-diltiazem at 10 µM (but not l-cis-diltiazem which is the biologically inactive calcium antagonistic diastereoisomer, Nagao et al,1972) increases the density of binding sites (positive heterotropic allosteric regulation) with a K_D of 2-3 nM for [^{3}H]-nimodipine from 560± 50 to 1.750 ±100 fmol/mg of protein (n = 3, mean± s.e.) for digitonin solubilized and from 1.200 ±200 to 2.490± 100 fmol/mg of protein (n = 3, mean± s.e.) for CHAPS-solubilized PCCs. The (+)enantiomer of the nifedipine derivative PN 200 110 (IC_{50}= 8 nM) is 120 fold more potent then the (-)enantiomer for inhibition of specific [^{3}H]-nimodipine binding to solubilized PCCs.

Lectin affinity gels (concanavalin A, lentil lectin and wheat germ agglutinin, all sepharose coupled) quantitatively adsorbed solubilized PCCs but minimal amounts of protein in batch experiments. When 20 mg of digitonin solubilized skeletal muscle microsomal protein was applied to a 7 ml (packed volume) concanavalin A-sepharose column roughly 50% of applied solubilized PCC was retained which could only be eluted biospecifically with 1 M α-methylmannoside allowing a 17-25 fold purification of the solubilized PCC. When similar experiments were performed with CHAPS solubilized PCCs with lentil lectin and wheat-germ agglutinin affinity columns purification factors of 3-6 fold were found.

In conclusion, PCCs can be solubilized from guinea-pig skeletal muscle. The solubilized PCC retains many characteristics of the membrane bound PCC and due to its reversible binding to lectin affinity columns appears to be a glycoprotein.

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CAFFEINE-LIGNOCAINE INTERACTIONS AT THE AVIAN NEUROMUSCULAR JUNCTION

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In a previous communication to this society (Wali,1982), it was reported that the local anaesthetic drug lignocaine may potentiate the contracture responses produced by tetraethylammonium(TEA), while it greatly reduced the contractures and the depolarizations produced by acetylcholine(ACh) in the isolated chick biventer cervicis(BVC) nerve-muscle preparations. Since TEA has been shown to act partly by facilitating the release of calcium ions in the vertebrate skeletal and smooth muscles (Beaulieu & Frank,1967; Haeusler,Kuhn & Thorens, 1980) and that caffeine may act in a similar manner to TEA (Elliott,1981), it was of interest to study the effect of lignocaine on the caffeine-induced responses, in the same preparation, using the moving fluid electrode technique (Fatt, 1950).

Caffeine (0.54-10.8 mM) produced concentration-dependent contractures in the isolated chick BVC muscle. These contractures were unaccompanied by membrane depolarizations. However, the contracture responses produced by caffeine were greatly increased in lignocaine (0.93 mM). The mean ED25s for the contractures produced by caffeine in the control Krebs solution and in Krebs containing lignocaine were: 2.5±0.01 mM and 0.51±0.03 mM respectively (mean±SEM,n=6,$P<0.001$). A dose ratio (test/control) of 0.2:1.0 was obtained. A mean maximum contracture of 0.48±0.02 g tension was produced by caffeine (5.4 mM) in the control Krebs solution and this was increased by 54±1.8% by lignocaine (n=6,$P<0.001$).

Lignocaine (0.93 mM) produced a large contracture-tension in the chick BVC muscle (control tension of 1.5±0.01 g ,n=6), and shifted the concentration-response curve of caffeine to the left, indicating potentiation of the caffeine-induced responses.

It has been shown that caffeine increases the maximal isometric twitch tension by prolonging the plateau of the active state of the stimulated muscle, thereby allowing the muscle more time to develop tension (Sandow & Brust, 1966). In low concentrations (0.05-1.0 mM) caffeine acts primarily on the coupling of the action potential to muscle contraction, and in intermediate concentrations (1-10 mM) produces a contracture response which does not require membrane depolarization (Frank, 1962).
The potentiation, by lignocaine, of the caffeine-induced contractures in the present experiments, could be explained if the 2 agents were acting in a synergistic manner in releasing calcium ions or inhibiting its re-uptake into the sarcoplasmic reticulum (Herz & Weber,1965; Bianchi & Bolton,1967).

It was concluded that lignocaine, in concentrations which completely abolish nerve and muscle action potentials, does not inhibit the caffeine-induced contractures, suggesting that caffeine may produce contractures requiring no membrane depolarization.

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EFFECTS OF METHYLXANTHINES ON ACETYLCHOLINE RELEASE FROM ELECTRICALLY-STIMULATED CORTICAL SLICES

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Much evidence suggests that methylxanthines exert at least part of their pharmacological effects by blocking adenosine receptors (Fredholm, 1980). In a previous study we demonstrated that aminophylline antagonizes the inhibitory effect of adenosine on acetylcholine (ACh) output from electrically stimulated brain slices (Pedata, Antonelli, Lambertini, Beani & Pepeu, 1983). The present investigation concerns the direct effect of caffeine and aminophylline on ACh output from electrically stimulated brain slices.

Rat superfused cortical slices were stimulated at different frequencies according to the method already described (Pedata et al.,1983) and ACh in the perfusate was quantified by bioassay.

Caffeine had no effect on the unstimulated ACh output. Conversely at 0.05 mM concentration it increased and at 0.5 mM it decreased ACh output from stimulated slices as shown in table 1.

Table 1 Effect of caffeine on ACh output (ng/g/min ± S.E.). Drugs added to superfusing Krebs solution 15 min before stimulation cycle.

Drugs	mM	Stimulation frequency in Hz 0	0.2	1	5
none		5.9±0.2	10.4±1.7	33.0±1.7	92.9± 5.8
caffeine	0.05	6.5±0.4	22.1±2.9**	61.2±2.9**	136.4± 9.2**
caffeine + dipyridamole	0.05 0.01	7.2±0.4	4.6±1.9*°	21.3±6.7°	79.2±16.4°
caffeine	0.5	6.8±0.9	4.0±0.7*	20.5±4.8*	50.1±12.8*
caffeine + Ca^{++}	0.5 3.2	6.7±0.7	11.4±3.6	27.8±8.6°	93.5±16.8°

Each value is the mean ± S.E. of 4 - 10 experiments
Statistically significant difference from no drug
* $P < 0.05$, ** $P < 0.01$; from caffeine alone $^{\circ}P < 0.05$

The stimulatory effect of caffeine was antagonized by the addition of dipyridamole which blocks adenosine uptake (Schrader, Berne & Rubio, 1972). The depressant effect of caffeine was antagonized by increasing Ca concentration in the Krebs solution. Caffeine 0.1 mM stimulated ACh output at 0.2 Hz only. Aminophylline tested at the same concentrations as caffeine exerted qualitatively similar effects. Neither drug was active at 0.01 mM. Our results demonstrate a biphasic effect of caffeine on ACh output, and support the hypothesis that, at low concentrations, methylxanthines may stimulate ACh output by antagonizing endogenous adenosine.

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INHIBITION OF NEURONAL UPTAKE OF DOPAMINE BY NOMIFENSINE FACILITATES LATENT DOPAMINERGIC NEUROTRANSMISSION IN THE RAT STRIATUM

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Amphetamine (Amph) inhibits the electrically-evoked overflow of ^{3}H-acetylcholine (Ach) from slices of the rat striatum by promoting the release of dopamine (DA) from a reserpine resistant pool of recently synthetized transmitter (Cantrill et al., 1983). The present experiments were aimed at clarifying whether this inhibitory effect of Amph was partly linked to the inhibition of the neuronal reuptake of DA.

Untreated or reserpine (5 mg/kg, 24 hr) treated rats were used. Striatal slices were labelled with ^{3}H-DA or ^{3}H-choline and superfused with Krebs' solution. Release of ^{3}H-Ach was evoked by two periods of electrical stimulation (S_1 and S_2) at 1 Hz for 2 min (44 min interval). The inhibition of the uptake of ^{3}H-DA by drugs was measured during a 5 min incubation period with 50 nM ^{3}H-DA.

Table 1 : Comparative effects of Amph and nomifensine on the uptake and efflux of ^{3}H-DA and on the electrically-evoked release of ^{3}H-Ach.

		% change from control values			
	µM	^{3}H-DA[a] efflux	^{3}H-DA uptake	^{3}H-Ach overflow[b] (untreated)	(reserpine)
Control		- 2 ± 4		- 21 ± 2	- 19 ± 2
d-Amph	1	+ 74 ± 5	- 34 ± 6	- 41 ± 3[c]	- 50 ± 9[c]
	10	+200 ± 9	- 79 ± 4	- 77 ± 12[c]	- 86 ± 5[c]
Nomifensine	1	+ 9 ± 4	- 59 ± 5	- 61 ± 3	- 30 ± 1*
	10	+ 9 ± 6	- 91 ± 3	- 92 ± 1	- 52 ± 4*

Values are mean ± S.E.M. of 3-9 experiments. * $p<0.001$ when compared with untreated rats. (a) drug induced efflux of ^{3}H-DA expressed as percent of the basal spontaneous outflow. (b) electrically-evoked overflow of ^{3}H-Ach expressed as percent of control stimulation (S_1). (c) from Cantrill et al., 1983.

In untreated rats, within the concentration range that increased the basal efflux of ^{3}H-DA, Amph 1 and 10 µM inhibited the neuronal uptake of ^{3}H-DA (Table 1) and the electrically-evoked release of ^{3}H-Ach (Table 1). The inhibitory effects of Amph on the electrically-evoked release of ^{3}H-Ach were not modified after reserpine treatment (Table 1, Cantrill et al., 1983). In addition nomifensine (Nom) 1 and 10 µM in concentrations that inhibited significantly the uptake of ^{3}H-DA (Table 1) but failed to significantly increase the efflux of ^{3}H-DA (Table 1) has a pronounced inhibitory effect on the evoked release of ^{3}H-Ach. These effects of Nom were reduced but not abolished after reserpine treatment (Table 1). The inhibition by Nom of the electrically evoked release of ^{3}H-Ach was antagonized after inhibition of tyrosine hydroxilase activity with α-methyl-p-tyrosine methyl ester. Similar results to those of Nom were obtained with cocaine in untreated and reserpine pretreated rats. Our results suggest that the inhibitory effect of Amph on ^{3}H-Ach release is brought about by its dopamine releasing action in addition to its inhibitory DA uptake properties. Nom and cocaine by inhibiting the neuronal uptake of DA without actually releasing the transmitter are able to inhibit ^{3}H-Ach overflow even after pretreatment with reserpine. It therefore appears that the latent dopaminergic neurotransmission which is enhanced by Amph can be shown to be operational also under conditions of electrical depolarization provided that the neuronal reuptake of DA is inhibited by drugs.

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INTERPRETATION OF CHANGES IN APOMORPHINE-INDUCED STEREOTYPED BEHAVIOUR IN RATS RECEIVING TRIFLUOPERAZINE FOR 15 MONTHS

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Rats treated chronically with trifluoperazine show an enhancement of high-grade stereotypy induced by apomorphine (0.5 mg/kg s.c. or more), but inhibition of low dose (0.125 mg/kg s.c.) stereotypy compared to control rats (Clow et al,1979). We now examine whether low grade stereotypy (locomotion, sniffing) is selectively antagonised by continuous chronic administration of trifluoperazine dihydrochloride (TFP).

Male Wistar rats (205 ± 14 g at the start of the experiment) received TFP (4.4 - 4.9 mg/kg/day) via daily distilled drinking water for 15 months. Age-matched control rats received distilled water only.

After 15 months, while animals continued to receive TFP treatment, stereotypy induced by a high dose of apomorphine hydrochloride (1.0 mg/kg s.c., 15 min previously) was enhanced, and that by a low dose (0.125 mg/kg) inhibited (Table 1). These data confirmed the apparent differential effect of chronic TFP treatment on high and low grade stereotypy.

When animals were withdrawn from TFP treatment for 2 weeks, the dose response curve for apomorphine (0.06 - 0.5 mg/kg)-induced stereotypy was shifted to the left (Table 1). So both high and low grade stereotypy were enhanced showing that underlying changes in all components of the behaviour had occurred.

Spontaneous exploratory locomotion in control and animals treated continuously with TFP, and still on drug, did not differ (Table 1). In control animals, administration of a low dose of apomorphine (0.03125 mg/kg s.c.) reduced locomotor activity. Higher doses of apomorphine (0.0625-0.5 mg/kg s.c.) had no effect on locomotor activity, whilst administration of a high dose (1.0 mg/kg s.c.) increased locomotor behaviour. In contrast, in TFP-treated animals, administration of apomorphine in a range of doses (0.125-0.5 mg/kg s.c.) induced locomotor hyperactivity as compared with responses in control animals and in TFP-treated rats injected with saline (Table 1). Hyperactivity was no longer apparent after administration of the highest dose of apomorphine (1.0 mg/kg s.c.) owing to the induction of rearing behaviour in TFP-treated animals which was not observed in age-matched control animals.

Table 1 Apomorphine (0.125 mg/kg sc)-induced stereotypy and spontaneous and apomorphine (0.125 mg/kg sc)-induced locomotor activity in TFP-treated rats and control animals

	Stereotypy score		Locomotor activity counts in 15 min	
	On drug	2 weeks withdrawal	Spontaneous	Apomorphine
Control	2.5 ± 0.2	2.8 ± 0.2	260 ± 18	214 ± 49
TFP	0.8 ± 0.2*	3.7 ± 0.2*	218 ± 29	382 ± 48*

* $p < 0.05$ compared to age-matched control animals

These findings suggest that one reason for the apparent inhibition of low dose apomorphine-induced stereotypy in TFP treated rats might be the induction of locomotor hyperactivity to apomorphine causing a disruption of stereotypy scores.

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ANTAGONISM BY RX 781094 OF CONTRACTILE RESPONSES TO α_1- OR α_2-ADRENOCEPTOR AGONISTS IN THE TAIL ARTERY OF SHR IN VITRO

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It has recently been reported (Hicks et al., 1983) that vasoconstrictor responses to noradrenaline (NA) are antagonised by low concentrations of the selective α_2-receptor antagonist RX 781094 (Chapleo et al., 1981) in tail arteries of SHR. This communication reports the antagonist effects of RX 781094 on vasoconstrictor responses induced by methoxamine, phenylephrine, TL 99 (Hicks and Cannon, 1980) or NA in SHR tail arteries in vitro.

Male SHR (13 - 15 weeks of age ; systolic blood pressure 190.3 $\pm$ 2.9 mmHg ; tail cuff) were used. Segments of proximal tail artery were removed under pentobarbitone anaesthesia (60 mg/kg i.p.) cannulated at both ends, perfused and superfused with Krebs' bicarbonate containing 2.6 mM Ca^{2+}. Cocaine (4 µM), propranolol (1 µM) and indomethacin (2.5 µM) were routinely present. Increases in perfusion pressure (mmHg) were measured in response to increasing molar concentrations of agonists, before or after incubation with RX 781094, or prazosin (30 min). One concentration of antagonist was employed in each preparation, n=5-8.

The EC_{50} (increase in perfusion pressure by 50% $\pm$ 95% confidence limits) for NA, phenylephrine (PE) or methoxamine (MET) were 0.17 (0.07 - 0.45) ; 0.71 (0.64 - 0.8) and 1.9 (1.34 - 2.72) µM respectively, and the maximum responses obtained to these agonists were similar (for NA : 225 $\pm$ 15 mmHg). TL 99 was less potent (EC_{50} = 5.37 (3.16 - 9.12) µM, and the maximum response was significantly less than for the other agonists (70 $\pm$ 9 mmHg). The vasoconstrictor response curve to TL 99, but not to MET, was significantly reduced in half calcium (Ca^{2+} 1.3 mM) medium.

RX 781094 (0.1 µM) caused a parallel displacement to the right of the vasoconstrictor response curves to PE, TL 99 and NA (-Log K_B values = 7.37 $\pm$ 0.18 ; 7.80 $\pm$ 0.21 and 7.22 $\pm$ 0.05 respectively), but RX 781094 failed to antagonise responses induced by MET at less than 0.3 µM (-Log K_B = 6.5 $\pm$ 0.09).

Prazosin (1 nM) was significantly more potent against MET ($p<0.05$) than NA, or PE (-Log K_B = 9.8 $\pm$ 0.04 ; 9.24 $\pm$ 0.07 ; 9.00 $\pm$ 0.08, respectively), but did not antagonise responses to TL99. The antagonist effects of prazosin (10 nM) against TL99 were non-surmountable.

The differential affinities of prazosin and RX 781094 against a range of α-adrenoceptor agonists strongly suggests that both α_1- and α_2-receptors are present in tail arteries of SHR. Although mixed α_1/α_2 receptor agonist effects have been reported in vivo for PE (McGrath, 1981) the high potency of RX 781094 against responses to this agonist in vitro suggest an α_2-receptor component in the responses to PE but not MET in the tail artery of SHR. TL99 may not be a selective α_2-receptor agonist in vitro in this preparation.

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DIFFERENTIAL EFFECT OF D-600 ON CONTRACTIONS OF RAT AND GUINEA-PIG AORTA BY DIFFERENT α_1-ADRENOCEPTOR AGONISTS

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Recent studies in pithed rats suggest that selective α_1-adrenoceptor agonists can be subdivided into two classes, one triggering vasoconstriction insensitive towards calcium entry blockade (cirazoline, phenylephrine and methoxamine), the other mediating vasoconstriction which is inhibited by calcium antagonists (St 587, Sgd 101/75; De Jonge et al, 1981, 1983; Thoolen et al, 1983). In order to further establish this division of selective α_1-adrenoceptor agonists, the contractile responses of cirazoline, noradrenaline (NA), St 587 and Sgd 101/75 and the influence of calcium entry blockade by D-600 on rat and guinea-pig isolated aorta were analysed. The effect of reducing the receptor number by dibenamine on the contractile responses to NA and the effect of D-600 in rat aorta were studied as well. Helically cut strips from male Wistar rats (300-350 g) or male guinea-pigs (300-350 g) were suspended in oxygenated 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 (rat aorta) or 2 g (guinea-pig aorta). Agonists were added cumulatively to the organ bath, isometric contractions were recorded and expressed as percentage of the maximal response by NA (3-10 μM) in the absence or presence of D-600. Values ± S.E.M. for pD_2 and intrinsic activity (i.a.) of NA, cirazoline, St 587 and Sgd 101/75 on rat isolated aorta amounted to 8.2 ± 0.08 and 1, 7.6 ± 0.1 and 0.90 ± 0.04, 7.1 ± 0.14 and 0.76 ± 0.05, 6.4 ± 0.09 and 0.92 ± 0.04, respectively. D-600 (10 nM-10 μM) caused parallel rightward shifts of the dose-response curves of NA and cirazoline with a minor suppression of the maximal response (maximally 20%). In contrast, D-600 strongly and dose-dependently reduced both slope and maximum of the dose-response curves of St 587 and Sgd 101/75. After 5 min exposure of rat aorta to dibenamine (0.1 μM) the dose-response curve of NA was shifted 10-fold to the right with a 25% reduction of the maximal response. Also under these circumstances the contractile responses to NA were not abolished by D-600 (0.1-10 μM). In guinea-pig aorta NA and cirazoline acted as full agonists (pD_2 = 7.2 ± 0.1 and 7.0 ± 0.08, respectively), whereas St 587 and Sgd 101/75 were partial agonists (pD_2 = 6.1 ± 0.13 and 4.8 ± 0.27; i.a. = 0.60 ± 0.02 and 0.39 ± 0.07, respectively). All contractile responses were virtually unaffected by D-600 (10 μM).
These results show, that NA and selective α_1-adrenoceptor agonists contract rat isolated aorta via different processes, dependent on and independent of calcium influx. The differential sensitivity towards calcium entry blockade of contractions elicited by NA and cirazoline on the one hand and St 587 and Sgd 101/75 on the other hand cannot be solely explained by partial agonism of the latter two substances. It is proposed, that two subtypes of α_1-adrenoceptors exist on rat aorta, whereas only one type is present on guinea-pig aorta.

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EFFECT OF CAPTOPRIL AND NIFEDIPINE ON THE NORADRENALINE-INDUCED VASOCONSTRICTION IN CATS

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Mainly as the result of studies on pithed animals, there is little doubt that postjunctional α_1 - as well as α_2 -adrenoceptors mediate vasoconstriction to intravenously administered noradrenaline (Timmermans & van Zwieten, 1981). Vasoconstriction to postjunctional α_2 -adrenoceptor stimulation appeared to be significantly diminished by calcium-entry blockers like nifedipine (Van Meel et al, 1981) or angiotensin-converting enzyme inhibitors like captopril (De Jonge et al, 1982). The present study was designed to investigate the involvement of a postjunctional α-adrenoceptor mediated mechanism in the hypotensive action of nifedipine and captopril in cats.

Male cats (2-4 kg) were anaesthetized with α-glucochloralose (60 mg/kg, i.p.). Arterial blood pressure was measured directly from the cannulated left iliac artery. The hypertensive response to noradrenaline (0.1, 0.3 and 1.0 µg/kg) administered via the left femoral vein was studied in intact and pithed cats, pretreated with saline, nifedipine (10, 30 and 100 µg/kg) and captopril (0.1, 0.3 and 1.0 mg/kg). The increase in resistance to flow (mm Hg. min. 100 g/ml) in the lower part of cat right hind limb to noradrenaline (0.1, 0.3 and 1.0 µg/kg) administered via the caudal artery was studied in saline, nifedipine (100 µg/kg) and captopril (1 mg/kg) pretreated cats (Folkow, 1952).

Nifedipine and captopril dose-dependently diminished mean arterial pressure in both pithed and intact cats ($P < 0.01$, paired t-test). Nifedipine significantly ($P < 0.01$, unpaired t-test) diminished the increase in mean arterial pressure to i.v. noradrenaline in both intact and pithed cats. Captopril inhibited the hypertensive response to noradrenaline in pithed cats, whereas converting enzyme blockade had no effect on the hypertensive response to noradrenaline in intact cats. Intraarterially administered noradrenaline produced a dose-dependent increase in resistance to flow in the lower part of cat right hind limb. In contrast with captopril, nifedipine significantly inhibited the vasoconstriction to noradrenaline in cat hind limb.

The results suggest that the hypotensive action of nifedipine may be, at least in part, attributed to a functional inhibition of postjunctional α-adrenoceptor triggering. In contrast, the hypotensive activity of captopril may be unrelated to the attenuating effect of converting enzyme inhibitors on the postjunctional α-adrenoceptor mediated vasoconstriction as observed in pithed animals.

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INDICATIONS FOR DIFFERENT LOCATIONS OF POSTJUNCTIONAL α_2-ADRENOCEPTORS IN THE RAT DEMONSTRATED BY β_2-ADRENOCEPTOR MEDIATED VASODILATATION.

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Activation of vascular α- and β_2-adrenoceptors gives rise to vasoconstriction and vasodilatation, respectively. It has recently been suggested that different populations of α_1-adrenoceptors exist, the effect of which is differentially affected by β_2-adrenoceptor mediated vasodilatation (Wilffert, 1983). Both subtypes of α_1-adrenoceptors can be stimulated selectively by different agonists (Timmermans and Van Meel, 1983; Wilffert, 1983). The present study was designed in order to gain insight into the interaction between β_2-adrenoceptor mediated vasodilatation and α_2-adrenoceptor mediated vasoconstriction.

Male normotensive Wistar rats (200-300 g) were anaesthetized with hexobarbitone-sodium (150 mg/kg, i.p.), pithed and artificially ventilated. Catheters were introduced into the right jugular vein and the left common carotid artery for the administration of drugs and the measurement of arterial pressure, respectively. Drugs were dissolved in saline, prazosin excepted, which was dissolved in 5% (w/v) glucose solution. Agonists and antagonists were injected in a volume of 0.5 and 1.0 ml/kg, respectively.

The selective α_2-adrenoceptor agonist B-HT 920 (Kobinger and Pichler, 1981; Van Meel et al, 1981) induced an increase in diastolic pressure, which was dose-dependently attenuated by the selective β_2-adrenoceptor agonist salbutamol (0.01-1 mg/kg). This effect was antagonized by the selective β_2-adrenoceptor blocking agent ICI 118,551 (0.3 mg/kg). The vasoconstriction to the selective α_2-adrenoceptor agonist UK 14,304 (Van Meel et al, 1981) and that induced by tyramine after pretreatment of the animals with prazosin (0.1 mg/kg) was also diminished by salbutamol (1 mg/kg). The pressor effect of intravenously administered noradrenaline in pithed rats pretreated with prazosin (0.1 mg/kg) was not reduced by salbutamol (1 mg/kg). The vasopressor response to intravenously administered adrenaline in rats pretreated with prazosin (1 mg/kg) and ICI 118,551 (0.3 mg/kg) was more pronounced than that observed in rats pretreated with prazosin (1 mg/kg) only.

These results show that intravenously administered B-HT 920, UK 14,304, adrenaline and neuronally released noradrenaline activated α_2-adrenoceptors mediating an increase in diastolic pressure which was attenuated by β_2-adrenoceptor mediated vasodilatation. In contrast the vasoconstriction to α_2-adrenoceptor stimulation by intravenously administered noradrenaline was not affected by salbutamol. This may suggest that there exist two populations of α_2-adrenoceptors, one located in the synapse and the other outside the synapse.

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EFFECTS OF HISTAMINE ON THE RAT ISOLATED VAGUS INNERVATED STOMACH STRIP

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Although the effects of histamine on tissue responses to sympathetic nerve stimulation have been extensively studied (Marshall 1981), there have been few reports of histamine mediated modulation of responses to parasympathetic nerve stimulation. In the present study we have examined the effects of histamine on the response of the rat isolated stomach strip to vagal stimulation.

Stomach and oesophagus were removed from male albino rats, 190-210g. Circular muscle strips were cut from the gastric corpus to include the oesophagus. The vagi were isolated and stimulated (square wave pulses, 0.5 ms duration, supramaximal voltage). Contraction of the stomach strip was measured isotonically.

Stomach strips contracted in a frequency related manner in response to vagal stimulation 0.2 - 20Hz. Contractions were rapid in onset and usually reached a maximum after less than 60 seconds of stimulation. Maximal responses were seen after stimulation at 5 - 10Hz, and contractions were abolished by the muscarinic receptor antagonist atropine, 0.3μM.

Stomach strips contracted in response to carbachol, EC_{50} 0.33±0.07μM (mean ± s.e. mean, n=12) and to histamine, EC_{50} 19.4±2.5μM(n=5). Consecutive cumulative response curves to histamine were not reproduceable due to tachyphylaxis. In separate tissues, responses to histamine were inhibited non-competitively by the histamine H_1-receptor antagonist mepyramine, 0.3μM, maximal responses being reduced by 43%, but were not affected by the histamine H_2-receptor antagonist cimetidine, 100μM, or by atropine, 0.3μM. Histamine, 1 and 10μM, had no effect on the response to vagal stimulation. Histamine 10μM also had no effect on the cumulative response curve to carbachol.

The selective histamine H_2-receptor agonist dimaprit 100μM had no effect on the resting tone of the tissue or on the cumulative response curve to carbachol. However dimaprit significantly inhibited ($P<0.05$) responses to stimulation at 1, 2, 5 and 20Hz. Cimetidine, 100μM, significantly inhibited responses to stimulation at 0.2, 0.5, 1 and 2Hz, and abolished the inhibitory effects of dimaprit.

Mepyramine, 0.3μM, significantly potentiated the response to 2Hz stimulation, but had no effect at other frequencies. In the presence of mepyramine, 0.3μM, histamine 10μM significantly inhibited the response to 10Hz stimulation. However, histamine 100μM had no effect at any frequency of stimulation. In the presence of cimetidine, 100μM, histamine, 10μM, had no significant effect on the response to stimulation.

Histamine has been shown to cause contraction of the rat stomach strip, an effect antagonized in a non-competitive manner by mepyramine. The effect of dimaprit and it's antagonism by cimetidine suggests there may by an H_2-receptor through which agonists can inhibit responses to vagal stimulation, although this interpretation is not supported by the lack of effect of histamine in the presence of mepyramine.

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ANTIPYRINE METABOLITE KINETICS IN PREGNENOLONE-16α-CARBONITRILE TREATED RATS

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Antipyrine clearance or half-life is widely used as a quantitative index of hepatic mixed-function oxidase activity *in vivo* in both man and animals. These parameters are sensitive to both phenobarbitone (PB)- and polycyclic hydrocarbon (PCH)-type enzyme induction. It has been recently demonstrated in rats (Rhodes and Houston, 1983) that by measuring the kinetics of formation of antipyrine individual metabolites, PB and PCH-type induction may be discriminated. Thus antipyrine metabolite kinetics provides an animal model system that can detect not only quantitative but qualitative changes in hepatic mixed-function oxidase activity *in vivo*. In order to evaluate further the comprehensiveness of antipyrine metabolite kinetics we have investigated the influence of pregnenolone-16α- carbonitrile (PCN) pretreatment. PCN like PB and PCH is known to induce characteristic forms of cytochrome P-450 (Lu and West, 1979) a major component of the mixed-function oxidase system.

Determination of metabolite formation kinetics requires both information on the total elimination rate of parent drug (elimination half-life or clearance) and the characterisation of the metabolic pattern (fractions of the dose metabolised by a specific primary pathway). To this end, male Sprague-Dawley rats received (N-methyl-^{14}C)-antipyrine (50 mg/kg; 10 μCi/kg; i.p.) and were housed in all-glass metabolism cages which allowed collection of $^{14}CO_2$ and urine. The half-life of antipyrine was estimated from the $^{14}CO_2$ exhalation rate (CER)-time profile. Urinary metabolite pattern for 4-hydroxy (4H)-, 3-hydroxymethyl (3HM)- and nor (N)-antipyrine was determined by h.p.l.c. following conjugate hydrolysis and chloroform extraction. Twelve rats were studied on 2 occasions 1 week apart, 6 of these animals received daily i.p. injections of PCN (80 mg/kg) for 4 days prior to the second test. Liver weights, as a percentage of body weight determined 24 hours after the last antipyrine test, were statistically significantly higher in the PCN (5.2 ± 0.3%) than in the control group (3.6 ± 0.3%).

The CER half-life decreased from 133 ± 24 to 85 ± 5 min and this was accompanied by an increase in the maximum CER attained from 0.11 ± 0.02 to 0.15 ± 0.02% dose/min following PCN pretreatment. These changes were statistically significant ($P<0.01$ by paired t test) and indicative of enzyme induction. The urinary recovery of antipyrine and 3HM decreased significantly ($p<0.025$), 3.5 ± 0.6 to 2.7 ± 0.2 and 27.8 ± 4.0 to 21.1 ± 2.6% of dose, respectively. In contrast 4H recovery increased (26.8 ± 3.7 to 32.1 ± 4.1%, of dose, $p<0.001$) and N recovery remained unaltered (19.5 ± 2.2 and 21.3 ± 2.5% of dose). No statistically significant changes in any of the above parameters were observed in the control group.

On average each of the 3 metabolite formation rate constants were increased following PCN pretreatment - 4H 198 ± 47, N 185 ± 57 and 3HM 128 ± 36% of control. These changes are similar to that previously reported for PB (Rhodes and Houston, 1983). Thus induction by PCN and PB can be distinguished from that by PCH pretreatment. However the data reported herein indicate that PB- and PCN-type induction cannot be resolved adequately from each other by use of antipyrine metabolite kinetics.

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α_1-ADRENOCEPTOR SUBTYPES IN VASCULAR SMOOTH MUSCLE OF THE PITHED RAT: DISCRIMINATION BY CALCIUM ENTRY BLOCKADE

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Log dose-vasopressor response curves in pithed normotensive rats obtained for selective α_1-adrenoceptor agonists, such as (-)-phenylephrine, methoxamine, St 587 and cirazoline, are steep and reach their maximum (increase in diastolic pressure) above 110 mm Hg (Van Meel et al, 1981; De Jonge et al, 1981). However, Sgd 101/75 (Coates et al, 1982), showing all characteristics of a preferential agonist of (vascular) α_1-adrenoceptors with respect to the interference of selective antagonists, had a shallow log dose-pressor effect curve and a lower maximum, similar to all selective agonists of α_2-adrenoceptors, like B-HT 920, B-HT 933 and UK-14,304 (Thoolen et al, 1983). This observation has prompted us to evaluate the effect of calcium entry blockade on the Sgd 101/75 induced vasoconstrictor response, since calcium entry blockers markedly reduce the vasoconstriction evoked by α_2-adrenoceptor stimulation (for review see Van Zwieten et al, 1982).

Male Wistar rats (200-250 g), anaesthetized with hexobarbitone (150 mg/kg, i.p.) and mongrel cats of either sex (2.5-4 kg) under chloralose (60 mg/kg, i.p.) anaesthesia were pithed and respired with room air. Single doses of agonists were injected i.v. 15 min after i.v. treatment with (-)-verapamil (0.1-3 mg/kg) or nifedipine (0.1-3 mg/kg). Maximal increases in diastolic pressure were measured. In both animal preparations, nifedipine and (-)-verapamil profoundly and dose--dependently reduced both slope and maximum of the log dose-increase in diastolic pressure response curve to Sgd 101/75. Following 0.1 mg/kg of nifedipine, the maximal pressor effect (105 $\pm$ 5 mm Hg, n=6) of Sgd 101/75 was diminished to 23 $\pm$ 5 mm Hg in the cat and after 1 mg/kg to 31 $\pm$ 4 mm Hg in the rat preparations.

As a result of this finding we (re)evaluated the sensitivity of the pressor responses to methoxamine, (-)-phenylephrine, cirazoline and St 587 to calcium entry blockade by (-)-verapamil (0.1-3 mg/kg) and nifedipine (0.1-3 mg/kg). It was found that the vasoconstriction to methoxamine, (-)-phenylephrine and cirazoline was relatively unaffected by these calcium entry blockers. Virtually parallel, 3- to 5-fold rightward shifts only of the log dose-pressor effect curves were determined following treatment with the highest doses of the antagonists. In contrast, the increase in diastolic pressure caused by St 587 was markedly influenced by (-)--verapamil and nifedipine. Both calcium channel blockers reduced slope and maximum of the log dose-vasoconstrictor response curve to this selective α_1-adrenoceptor agonist in a dose-dependent fashion.

The results suggest that α_1-adrenoceptor-mediated vasoconstriction *in vivo* (pithed normotensive rat), which is characterized by its sensitivity to blockade by prazosin and its relatively insensitivity to antagonism by yohimbine/rauwolscine, can be subdivided into distinct processes which are differentially affected by calcium entry blockade. Cirazoline, (-)-phenylephrine and methoxamine probably initiate contraction without an entry of extracellular calcium ions, whereas Sgd 101/75 and St 587 contract vascular smooth muscle via a mechanism which seems primarily governed by an influx of extracellular calcium. Two distinct types of postjunctional (vascular) α_1-adrenoceptors may be postulated to explain these findings.

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REDUCTION BY AMILORIDE OF RENAL MAGNESIUM CLEARANCE IN MAGNESIUM-LOADED RATS DURING FRUSEMIDE DIURESIS

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The K-sparing diuretic amiloride may also exert Mg-sparing properties. Both amiloride and triamterene have been shown to reduce urinary Mg excretion ($U_{Mg}V$) in conscious saline-loaded rats (Devane & Ryan, 1981a). Acute administration of amiloride to congestive heart failure patients receiving frusemide resulted in reduced urinary Mg excretion and increased plasma and lymphocyte Mg (Ryan et al, 1981) Amiloride has been shown to reduce the fractional excretion of Mg (FE_{Mg}) during clearance studies in rats (Devane & Ryan, 1980) and a dose-response relationship has been established for the Mg-conserving action of amiloride during frusemide diuresis in rats (Devane & Ryan, 1981b). The action of amiloride on renal handling of Mg could involve either enhanced reabsorption or reduced renal secretion of Mg though the evidence for the existence of a Mg secretory mechanism in the kidney is conflicting. We have investigated the effects of amiloride on Mg excretion during conditions most likely to stimulate Mg secretion.

Male Wistar rats were anaesthetized with thiobutobarbitone sodium (100 mg/kg i.p. 'Inactin'). Donor rat plasma was infused into the right jugular vein (2.4 ml/h for the first hour; thereafter 0.4 ml/h). A modified Ringer solution containing 15 mM $MgSO_4$ and 0.6 µCi (^3H)-inulin/ml was infused at a rate of 60 ml $kg^{-1}h^{-1}$. The left ureter was cannulated. After 100 min equilibration, frusemide (1 mg $kg^{-1}h^{-1}$) was added to the infusion solution and, after 40 min, three control 15 min urine collections were made. Amiloride hydrochloride (2 mg $kg^{-1}h^{-1}$) was then added to the infusion solution and, following 15 min equilibration, three further 15 min urine collections were made. Statistical analysis was by paired Student's t test.

Time-control experiments indicated that kidney function parameters were stable over the period of investigation with the exception of a slight decrease in urinary volume ($p<0.001$). Amiloride infusion prevented the fall in urinary volume observed in the time-control group. Amiloride increased the fractional excretion of Na (FE_{Na}; $p<0.05$), while FE_K ($p<0.001$), FE_{Mg} ($p<0.05$) and FE_{Ca} ($p<0.05$) were all reduced. Both plasma K ($p<0.01$) and Mg ($p<0.05$) were increased by amiloride. The FE_{Mg} was not greater than 100% and therefore no conclusive evidence for Mg secretion was indicated in these experiments. Comparison of these results with those obtained in non Mg-loaded rats (Devane & Ryan, 1981b) show that amiloride reduced FE_{Mg} by the same extent in both Mg-loaded and non Mg-loaded rats. These findings provide additional evidence that amiloride exerts Mg-sparing properties. Further experiments are required to establish whether the Mg-sparing action of amiloride is a result of enhanced reabsorption or reduced secretion.

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TRANSFER OF PARACETAMOL, AMPICILLIN AND CAFFEINE INTO BREAST MILK

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Transfer of drugs into breast milk is likely to be governed by their physical and chemical properties. We have recently reported to the Society our findings on the effect of milk pH on the transfer of salicylic acid from blood (Bennett *et al* 1982). We now present data on the transfer into breast milk of an alcohol (paracetamol), a base (caffeine), and an amphiphilic compound (ampicillin).

Volunteers 2-8 months post-partum who were in the process of weaning their babies participated in the study. Breast milk was collected using an electrically driven pump. Both breasts were emptied at the commencement of the study following which each volunteer received a single dose either of paracetamol (1.0g) or ampicillin (0.5g); caffeine was administered as dietary coffee to volunteers who took ampicillin. Repeated samples of milk were taken from the same breast during the subsequent four hours; venous blood samples were drawn to coincide with milk samples. In an additional study, random paired blood and milk samples were taken from breast-feeding mothers who had taken paracetamol for pain relief 2-9 days post-partum. All drugs were measured by HPLC. The results appear below.

MILK : PLASMA CONCENTRATION RATIOS
(mean ± SEM; n = pairs of samples)

Vol. No.	PARACETAMOL	Vol. No.	AMPICILLIN	CAFFEINE
1	1.43 ± 0.16 (n=7)	5	0.058 ± 0.014 (n=4)	1.08 ± 0.21 (n=4)
2	1.32 ± 0.08 (n=6)	6	0.174 ± 0.070 (n=5)	0.48 ± 0.13 (n=3)
3	0.89 ± 0.20 (n=7)	7	0.110 ± 0.030 (n=3)	1.06 ± 0.29 (n=4)
4	1.32 ± 0.16 (n=5)	8	0.087 ± 0.032 (n=3)	1.53 ± 0.26 (n=3)
single random samples	0.95 ± 0.16 (n=9)			

No correction was made for plasma protein binding which for the compounds studied is low.

The very limited transfer of ampicillin into milk is predictable for a drug which contains chemical groups (acidic pKa 2.5; basic pKa 7.2) that are ionised at all body and milk pH values. Paracetamol (pKa 9.5), lipid soluble and unionised at physiological pH, transfered readily into milk which has a higher lipid content than blood. The weak base caffeine (pKa 0.8) would be expected to undergo ion trapping if the pH of milk at the point of transfer is more acidic than that of blood (Bennett *et al* 1982) but this was not found consistently in the present study.

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ATENOLOL DOSE-RESPONSE IN MODERATE AND SEVERE HYPERTENSION.

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Atenolol is a relatively B_1-selective adrenoceptor antagonist used in the treatment of hypertension. In mild hypertension 100 mg or less may exert the maximum hypotensive effect (Jeffers et al., 1977), but one study in moderately severe hypertensives found the optimal daily dose of atenolol to be 200 mg (Hansson et al., 1975). The aim of this study was to prove or disprove an additional hypotensive effect from increasing the dose of atenolol to 200 mg in patients who remained hypertensive (MAP > 110 mm Hg) while taking atenolol 100 mg (± other antihypertensive drugs). 34 patients were studied. After a run-in period they were randomly allocated to receive 100 mg or 200 mg of atenolol in a parallel group design. The 100 mg group also received a matching placebo and the study was double blind. Three replicate supine BP measurements (Hawksley random zero) were made at each visit and patients were seen twice on each treatment. The patients in each group were well matched for age, renal function, initial BP and number of drugs taken (table). There were no significant differences in the falls of BP from run-in values between the two doses of atenolol (table).

From the standard deviation of the differences in blood pressure obtained during the run-in period (Freestone et al., 1982) the power of the study to detect a 6.7 mm Hg difference in MAP (≡ 10/5 mm Hg) between the two doses was predicted to be > 80% (Altman, 1980). We conclude that there is no important additional hypotensive effect from increasing the dose of atenolol above 100 mg even in moderate and severe hypertension.

Table. Mean (s.e. mean) baseline characteristics and change in blood pressure from run-in values with atenolol 100 mg and 200 mg

	Atenolol 100 mg	Atenolol 200 mg
M : F	11 : 6	9 : 8
Age (years)	53.9 (2.4)	55.5 (1.9)
Creatinine (μmol/l)	103.8 (4.5)	99.3 (5.3)
No. of drugs taken	2.4	2.6
Initial MAP (mm Hg)	123.1 (2.0)	124.1 (1.5)
Δ supine systolic BP (mm Hg)	-9.5 (3.1)	-8.7 (2.4)
Δ supine diastolic BP (mm Hg)	-2.8 (1.4)	-3.7 (1.0)
Δ supine MAP (mm Hg)	-5.0 (1.9)	-5.4 (1.2)

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RECTAL ABSORPTION OF CLOBAZAM: A POTENTIAL ROUTE FOR TREATMENT OF CONVULSIONS

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Treatment of fits by i.v. anticonvulsants is sometimes difficult and absorption of i.m. anticonvulsants is erratic (Dhillon et al, 1982). Rectal administration may be an alternative route for giving anticonvulsants (Dhillon et al, 1982). We report that a rectal solution of the anticonvulsant 1,5-benzodiazepine, clobazam, can achieve adequate plasma concentrations sufficiently rapidly for the treatment of fits.

Six normal subjects (2F, weights 56.2-86 kg, ages 26-45y) were given (balanced crossover) clobazam (30 mg) as a single, 0800h, dose of each of 3 formulations: oral capsule, suppository, rectal solution (10 ml in polypropylene syringe with soft polyethylene applicator). After dosing, venous blood (5 ml) was taken at 0, 5, 10, 20 min, then, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 24, 30, 96 and 164h. The rectal solution was absorbed rapidly with virtually no lag time (Figure 1) to give plasma clobazam concentrations above 200 ng/ml which are likely to be effective for treatment of epilepsy (Cano et al 1981). Peak plasma clobazam concentrations were greater with capsule than with solution ($p < 0.01$) and for the solution than suppository ($p < 0.05$) (respectively, 702.5 ± 183.6, 410.4 ± 110.8, 281.6 ± 86.6) (mean ± SD, ng/ml) (p values from analysis of variance and Duncan's multiple comparison test). Bioavailability represented by trapezoidal $AUC_{0-\alpha}$ was similar for capsule, solution and suppository (13275 ± 2913, 13742 ± 3468, 12190 ± 3489, mean ± SD, ng ml^{-1} h) as was clobazam terminal half-life (31.7± 11.7, 32.3 ± 11.8, 32.9 ± 11.1 mean ± SD, h). Effective plasma concentrations of clobazam were sustained for 6h after the rectal solution; the major, active, metabolite N-desmethylclobazam was detectable from 24-164h after administration. Clobazam rectal solution gave, rapidly, adequate plasma concentrations which were sustained for 6h after dosing and so could be rapidly effective and long lasting in treatment of fits.

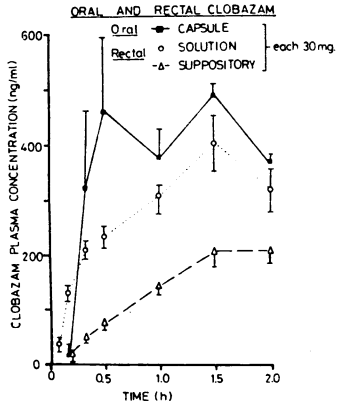

Figure 1. Plasma clobazam concentrations for 2h after 3 formulations of clobazam (values mean ± s.e.m.)

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EFFECTS OF ETHANOL AND CIMETIDINE ON THE METABOLIC ACTIVATION OF PARACETAMOL IN MAN

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Inhibition of the metabolic activation of paracetamol could be an adjunct to the treatment of paracetamol poisoning with N-acetylcysteine. Cimetidine inhibits the oxidative metabolism of some drugs and may protect against paracetamol hepatotoxicity in animals (Mitchell et al, 1981; Donn et al, 1982). It has been thus been proposed as an antidote for paracetamol poisoning (Abernethy et al, 1982; Jackson, 1981) but it is not known whether it inhibits the oxidative metabolism of paracetamol in man. Ethanol has variable effects on paracetamol metabolism and toxicity in animals depending on whether it is given chronically or acutely. We have studied the effects of cimetidine and acute ethanol on the metabolism of paracetamol in 20 healthy male volunteers (mean age and weight 37yr and 72Kg). Ten took ethanol in moderation only occasionally and 10 were heavy drinkers according to the Michigan Alcoholism Screening Test. The fasting volunteers took 20 mg/Kg of paracetamol orally on 3 separate occasions at least a week apart, once alone, once one hour after 800mg of oral cimetidine followed by 3 x 400mg doses 4 hourly and once 0.5 - 1.0h after 0.6g/Kg of ethanol (70^{o} whisky) followed by 0.1 - 0.16g/Kg hourly for 8h. Paracetamol and its metabolites in plasma and urine were estimated by HPLC and plasma ethanol by GLC.

Contrary to expectations, cimetidine had no effect on the urinary excretion of paracetamol metabolites. In contrast, the metabolic activation of paracetamol was strikingly reduced by ethanol as shown by a highly significant reduction in the urinary excretion of the mercapturic acid and cysteine conjugates. The effects of cimetidine and ethanol on paracetamol metabolism were the same in the occasional and heavy drinkers.

Mean (±S.D.) % urinary recovery of paracetamol and metabolites in 10 occasional and 10 heavy drinkers with and without cimetidine or ethanol

	CONTROL	CIMETIDINE	ETHANOL
Paracetamol	3.8 ± 1.5	5.6 ± 1.8	6.2 ± 4.7
Glucuronide conjugate	58.2 ± 7.2	57.3 ± 6.9	57.5 ± 6.8
Sulphate conjugate	28.7 ± 4.5	28.5 ± 5.4	33.3 ± 4.7*
Mercapturate conjugate	5.3 ± 2.3	4.9 ± 2.6	1.8 ± 1.0*
Cysteine conjugate	4.0 ± 2.0	3.7 ± 1.7	1.2 ± 0.7*

*P=<0.001

There was no correlation between the mean plasma ethanol concentrations (73 and 94mg/dl for the occasional and heavy drinkers respectively) and reduction in the excretion of mercapturic acid and cysteine conjugates. The mean plasma paracetamol half life was significantly prolonged with ethanol but not cimetidine (control 2.25, cimetidine 2.20 and ethanol 2.53h, P=<0.001). There were corresponding differences in the 0-8h AUC's (control 59.2, cimetidine 63.9 and ethanol 69.2 µg/ml/h). These results provide no basis for the proposed use of cimetidine for paracetamol poisoning in man whereas ethanol which is often taken by self-poisoners may have a significant protective action.

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FACTORS AFFECTING THE CUTANEOUS RESPONSE TO INTRADERMAL INJECTION OF PLATELET ACTIVATING FACTOR IN MAN

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Platelet activating factor (PAF-acether) is a phospholipid which has been chemically characterized (1-0-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) and recently synthesized (reviewed by Vargaftig et al, 1981). It is released by 1g E-dependent mechanisms from basophils and alveolar macrophages and from activated neutrophils and platelets. In addition to being a platelet activating agent it has been shown to be a powerful bronchoconstrictor and inducer of cutaneous inflammatory responses in experimental animals. In man it produces a biphasic cutaneous response (Basran et al, 1982).

Consistent with the two component hypothesis of acute inflammation, histamine and bradykinin-induced cutaneous inflammation can be potentiated by Prostaglandin E_2 (a vasodilator) and inhibited by both αadrenoreceptor agonists (vasoconstrictor) and βadrenoreceptor agonists (antipermeability). To test the validity of this hypothesis in man we have studied the effect of Prostaglandin E_2, salbutamol and phenylephrine on the early component of the PAF-induced cutaneous response.

Eight volunteers (aged 21 - 28) were studied. Wheals were induced by intradermal injection of a fixed volume of test solution (50 µl). To ensure that the measurements were made without bias the solutions were coded and assigned randomly to marked sites on the volar surface of the forearm. Measurement of the skin thickness before and 12 minutes after intradermal injection together with two perpendicular diameters of the wheal allowed the wheal volume to be calculated by a method previously described (Basran et al, 1982).

To test for interaction between PAF (P) and Prostaglandin E_2 (E), the following solutions were injected (dose/site): 1) P 50 ng, 2) E 500 ng, 3) P 50 ng + E 500 ng, 4) P 100 ng, 5) E 1000 ng. In a separate experiment to test the effect of adrenoreceptor agonists on PAF responses, PAF was given either alone (P 100 ng) or mixed with salbutamol 100 µg or phenylephrine 15 µg.

The volume of the wheal induced by the mixture of P 50 ng and E 500 ng (95±18 µl; mean, SE mean) was significantly greater than (i) the algebraic sum of the individual responses to P 50 ng and E 500 ng (32.5±9 µl; $P<0.0025$), (ii) the response to P 100 ng (37±12 µl; $P<0.0005$) and (iii) the response to E 1000 ng (21±3.8 µl; $P<0.005$). PAF responses were attenuated by both salbutamol (52%; $P<0.02$) and phenylephrine (43%; $P<0.05$).

These responses demonstrate a synergistic interaction between PAF and PGE_2. Both α- and βadrenoreceptor agonists inhibit the cutaneous responses induced by PAF. These findings in man support the validity of the two component hypothesis of acute inflammation. Furthermore, they reaffirm the anti-inflammatory effect of salbutamol repeatedly observed in experimental animals (Svensjo et al, 1977).

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PHARMACODYNAMIC COMPARISON OF N-DESMETHYLCLOBAZAM WITH N-DESMETHYLDIAZEPAM

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In equipotent anxiolytic doses, the 1,5-benzodiazepine, clobazam impairs psychomotor performance less than the 1,4 benzodiazepine, diazepam (Hindmarch, 1979). Chronic doses of clobazam and diazepam result in accumulation of active N-desmethyl metabolites. The separation of anxiolytic from psychomotor-impairing effects may be greater for N-desmethylclobazam than for clobazam (Fielding and Hoffman, 1979). Therefore we compared the effects of N-desmethylclobazam (norclobazam) with N-desmethyldiazepam (nordiazepam) upon psychomotor performance.

Six normal subjects (3F, age 23-42, weight 50-68 kg) were given blind (balanced crossover design), single, morning (0800h) doses of norclobazam (30 mg) or nordiazepam (15 mg) or lactose placebo on 3 occasions each 2 weeks apart. The following tests were done 0, 1, 2, 3, 6 and 8h after dosing: visual analogue self-rating scale of drowsiness (VASD) and concentrating ability (VASC), critical flicker fusion test of vigilance (CFF), maze drawing test of co-ordination (M) and finger tapping on a morse code key (FTT)(testing dominant hand), digit symbol substitution test (DSST) of cognitive ability. Each subject was familiarised with the tests before the study. Evaluation of results was by analysis of variance followed by pairwise comparisons; statistically significant results were taken as those with $p < 0.05$ or less.

The VASC and VASD showed greater sedation and inability to concentrate with nordiazepam compared with placebo and norclobazam from 1-6h (significant at 1 and 3h) (effect most marked at 1h); norclobazam did not differ from placebo. Both drugs increased the time needed to complete the M-test (significant at 1, 2 and 6h); times returned to placebo values by 8h. Norclobazam impaired CFF only at 3h, but nordiazepam impaired CFF throughout (significant at 1, 3, 6 and 8h). Nordiazepam impaired FTT from 1-4h (significant at 1, 2h); norclobazam impaired FTT only at 3h. Neither drug changed the values or errors obtained in the DSST although there was a tendency for more errors after nordiazepam. In conclusion, this study showed that like the parent compounds, the N-desmethyl metabolites of clobazam and diazepam differ in their effects on psychomotor performance with greater impairment caused by nordiazepam at the doses given.

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ETHNIC DIFFERENCES IN PARACETAMOL METABOLISM: A COMPARATIVE STUDY IN SCOTLAND AND GHANA

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Ethnic differences in drug metabolism are well recognised (Kalow, 1982) but little is known of paracetamol in this respect. It is extensively conjugated with glucuronic acid and sulphate but a minor potentially toxic route of metabolism involves microsomal oxidation followed by glutathione conjugation. The latter reaction yields mercapturic acid and cysteine conjugates which are excreted in the urine (Mitchell et al., 1974). The overall rate of paracetamol metabolism varies little between individuals but there are considerable differences in the extent of glucuronide versus sulphate conjugation. The 24h urinary excretion of paracetamol and its metabolites following a single oral dose of 1.5g was compared in 66 medical students in Accra, Ghana (50M, 16F; mean age 23.1yr) and 57 medical students, nurses and doctors in Edinburgh (45M, 12F; mean age 23.5yr). Subjects taking oral contraceptives or chronic medication were not included in the data analysis. Urine paracetamol and its glucuronide, sulphate, mercapturic acid and cysteine conjugates were estimated by HPLC (Prescott et al., 1979).

The proportional excretion of paracetamol glucuronide and sulphate did not differ significantly between Accra and Edinburgh and the mean glucuronide/sulphate ratios were virtually identical (1.98 and 1.99 respectively). In contrast, 12 Edinburgh women who were taking oral contraceptives, had a significantly higher mean ratio (3.30; $P<0.001$) with glucuronide and sulphate recoveries of 64.4% and 21.7% respectively.

Striking and highly significant differences were observed in the proportions excreted as mercapturic acid and cysteine conjugates with 5.2% recovered as these metabolites combined in the Ghanaians compared with 7.9% in the Scots. Thirty seven (56%) of the Ghanaians excreted less than 5% in this form while only 5 (9%) of the Scots did so.

Mean (± S.D.) % recovery of paracetamol and its metabolites following an oral dose of 1.5g in 66 subjects in Accra and 57 in Edinburgh

	ACCRA	EDINBURGH
Paracetamol	4.6 ± 2.0	5.2 ± 2.0
Glucuronide conjugate	58.1 ± 8.7	56.5 ± 7.6
Sulphate conjugate	32.1 ± 7.7	30.5 ± 6.4
Mercapturate conjugate	2.3 ± 1.3*	4.1 ± 1.6
Cysteine conjugate	2.9 ± 1.5*	3.8 ± 1.5

*P=<0.002

In both groups there was a highly significant correlation between the fractions excreted as mercapturic acid and cysteine conjugates but the mercapturic acid/cysteine ratio in the Ghanaians was significantly less than in the Scots (0.85 compared with 1.13, $P=<0.001$). Sex, smoking and alcohol had no obvious effect on paracetamol metabolism in either group.

The reduced excretion of mercapturic acid and cysteine conjugates in the Ghanaians implies less metabolic activation of paracetamol and hence a greater safety margin. The capacity for microsomal oxidation and possibly acetylation is thus reduced in Ghanaians but the relative contributions of genetic and environmental factors is unknown.

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SODIUM SALICYLATE IN POSTOPERATIVE DENTAL PAIN

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A previous study (Seymour & Rawlins, 1982) showed that the analgesic efficacy of aspirin in postoperative dental pain was dose related (600mg to 1200mg), and that there was a significant correlation between analgesia and plasma salicylate concentrations. In order to investigate whether this correlation was causal, and whether salicylate itself is analgesic, we have conducted a double-blind, placebo-controlled study in patients undergoing bilateral removal of their lower third molars.

24 patients (12 females) aged 19-32 years, with similar bilateral impacted lower third molars, agreed to participate in the study which had received prior approval from the Health Authority Ethics Committee. Surgery was carried out by one operator (R.A.S.) under local anaesthesia (2ml lignocaine, 20mg/ml) and four weeks elapsed between the two operations. When complete return of lingual and mental nerve sensations had occurred the patients received either sodium salicylate orally, or placebo. 12 patients (6 females) were given 537mg salicylate (as the sodium salt), and the remaining 12 received 1074mg salicylate. These solutions are equi-molar with 600 & 1200mg aspirin. Patients thus received salicylate and placebo in random order and acted as their own controls. Venous blood was withdrawn from an indwelling catheter at 0, 15, 30, 45, 60, 90, 120, 180, 240 and 300 mins. after the oral dose. The patients recorded their pain experience using a visual analogue scale after each blood sample had been taken. For each time, analgesia scores were calculated from the difference between placebo and sodium salicylate. Plasma concentrations of salicylate were measured by high performance liquid chromatography (Lo & Bye, 1980).

After 537mg sodium salicylate patients reported virtually the same pain as after placebo. Following 1074mg sodium salicylate, patients reported slightly less pain than after placebo, however, at no time point did the difference reach conventional levels of statistical significance ($P>0.05$). Peak plasma concentrations of salicylate were observed between 30-45 mins. after dosing.

It is concluded that 537mg and 1074mg sodium salicylate has no analgesic effect in postoperative dental pain. The correlation observed in our previous study (Seymour & Rawlins, 1982) appears to have been fortuitous.

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KINETICS AND EFFICACY OF HIGH-DOSE METOCLOPRAMIDE USED IN THE PREVENTION OF CYTOTOXIC-INDUCED NAUSEA AND VOMITING

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Nausea and vomiting are amongst the most troublesome adverse effects of cytotoxic drug therapy, for which anti-emetic drugs are often ineffective. Recently Gralla et al (1981) described a regimen using high doses of metoclopramide (10mg/kg body weight in five divided doses over 9 hours) which provided effective prophylaxis against vomiting. At conventional doses, metoclopramide has been shown to have dose-dependent kinetics. (Bateman et al, 1980; Graffner et al, 1979). Furthermore we have demonstrated that metoclopramide accumulates when given by the regimen recommended by Gralla. (Taylor & Bateman, 1983). We have therefore used a simplified infusion regimen to investigate the pharmacokinetics and plasma concentration-effect relationship of this drug when given in high doses.

Six patients (age range 33-61 years) were included in the study. They were all receiving standard chemotherapy for non-Hodgkin's lymphoma consisting of cyclophosphamide, adriamycin, vincristine, bleomycin and prednisolone, a potent emetic regimen. Three dosage regimens of metoclopramide were administered to the patients in random order during consecutive courses of chemotherapy. A bolus injection of 1, 2 or 3 mg/kg body weight was given over 15 minutes followed by a constant i.v. infusion for 8 hours. The infusion rates were 10, 20 and 25 mg/h respectively for patients under 50 kg and 15, 25 and 30 mg/hr for patients over 50 kg. Venous blood samples for estimation of plasma metoclopramide (Bateman et al, 1981) were obtained at intervals for at least 24 hours after the start of therapy. Nausea and drowsiness were assessed using visual analogue scales and the number of vomits was counted.

Mean plasma concentrations, calculated from AUC, achieved during the period of the infusion were 441 $\pm$ 63 ng/ml, 856 $\pm$ 145 ng/ml and 1198 $\pm$ 151 ng/ml at the three dosage levels studied. Steady state was obtained for the duration of the infusion in 9 out of the 18. In those in whom steady state was not achieved, the infusion rate was probably inappropriate to the patients weight. Plasma concentrations declined mono-exponentially after the infusion period with a mean half-life of 5.9 $\pm$ 0.39 h. Plasma clearance (mean $\pm$ SEM) was 25.4 $\pm$ 4.8 L/h. Neither half-life nor clearance were dose-related.

High-dose metoclopramide prevented vomiting during 14 of the 18 courses of cytotoxic therapy, and no patient vomited more than twice. Neither vomiting nor self-rated nausea were related to the dose regimen or plasma concentration of metoclopramide. Significant drowsiness occurred during 9 of the 18 courses but again this did not appear to be dose-related. Other side-effects notes were diarrhoea in two patients and minimal extra-pyramidal signs in one patient. These preliminary results suggest that metoclopramide has linear kinetics when administered at high doses, and that it should therefore be possible to achieve predictable steady state concentrations with this anti-emetic drug.

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ADRENOCEPTOR BLOCKING ACTIVITY OF A NEW α AND β BLOCKING AGENT INFENDOLOL (LQ 31-341) IN NORMAL HEALTHY VOLUNTEERS

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Animal studies have shown that Infendolol (I) 1-((3-Chloro-2-methyl-1H-indol-4-yl)oxy) -3- ((2-phenoxyethyl)-amino)-2-propanol-hydrogenmalonate, a new indol derivative, is an alpha and beta adrenoceptor blocking drug. There is evidence that the alpha 2 blocking activity is greater than alpha 1 blocking action. (Müller-Schweinitzer 1980). The present study was designed to assess beta and any alpha 1 blockade in healthy human volunteers and to compare (I) with labetalol (L) which has well established alpha 1 and beta blocking effects in man (Richards & Prichard, 1979).

A preliminary study was done using 100 mg (I) in three healthy normal volunteers to assess the duration of action. Submaximal exercise heart rate (HR) and blood pressure (BP) response to cycle ergometer exercise was measured serially over 8 hours. Maximum inhibition of end exercise HR occurred 2 and 3 hours post dose. In the second phase of the study, placebo, 50 mg and 100 mg (I) were given in a randomised double-blind design to three healthy volunteers and subsequently placebo and 200 mg (I). Measurement of HR and BP response were made during infusion of phenylephrine to produce a systolic rise of approximately 40 mm Hg 2 hours after drug dosage. A dose dependant shift of the phenylephrine dose response curve to the right was demonstrated in 2 of the 3 subjects. The greatest shift occuring with 200 mg dosage (I). Therefore, in the third phase of the study, 6 healthy male volunteers aged 21-26 years received randomised, double-blind single doses of (I) 200 mg, (L) 400 mg and placebo on three separate occasions, at least one week apart. HR and BP was measured at rest supine, after one minute tilt, during Valsalva's manoeuvre, during phenylephrine and isoprenaline infusions (administered in random order between volunteers) and after two minutes of submaximal exercise on a cycle ergometer. Observations commenced 110 mins post dose and were completed by about 200 mins post dose.

(I) but not (L) significantly ($P < 0.05$) inhibited the rise in heart rate on tilt. (I) and (L) significantly ($P < 0.01$) reduced the rise in systolic blood pressure on exercise compared with placebo (17, 30 and 56 mm Hg rise for (I), (L) and placebo respectively. (I) had a significantly greater effect than (L) ($P < 0.05$). (I) (-8 mm Hg) significantly reduced the fall in diastolic pressure compared to placebo (-21 mm Hg) ($P < 0.05$), (L) (-16 mm Hg) did not. End exercise heart rate was reduced by 14% with (L), 118 compared to 136 beats/min on placebo and by 22% with (I), 106 beats/min. The reduction with (I) but not (L) was significant ($P < 0.05$). The isoprenaline requirement to increase heart rate by 25 beats/min averaged was 1.5, 22.8 and 118.6 μg with placebo, (L) 400 mg and (I) 200 mg respectively, (I) significantly greater than (L) ($P < 0.05$), whereas the phenylephrine requirement to produce 25 mm Hg systolic rise in blood pressure was 200, 575 and 219 μg with placebo, (L) and (I) respectively, (L) significantly greater than (I) ($P < .001$). Thus, 200 mg Infendolol was five times more potent than 400 mg labetalol as regards its beta blocking effect but showed a small and relatively insignificant alpha 1 blocking action in man. This is in accord with weak alpha 1 blocking activity in animals. We did not measure its alpha 2 blocking action in man.

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DOSE AZATHIOPRINE SUPPRESS THE MIXED LYMPHOCYTE REACTION (MLR) IN THE SAME WAY AS 6-MERCAPTOPURINE (6-MP)?

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How azathioprine and its metabolite 6-MP suppress immune responses is unknown. Using the MLR (transplantation reaction in a test-tube), Al-Safi & Maddocks (1982; 1983) found azathioprine more inhibitory (34%) than 6-MP. This difference in activity was not due to 5-mercapto-1-methyl-4-nitroimidazole, a metabolite released when azathioprine is split to 6-MP, as it had no effect on the MLR. Moreover, addition of the de novo purine pathway metabolite 5-amino-4-imidazole carboxamide reversed 6-MP inhibition(54%) but not azathioprine inhibition of the MLR, suggesting that 6-MP was inhibiting de novo purine synthesis. Here we present further evidence for a difference in the mode of action of azathioprine and 6-MP on the MLR.The purine nucleosides, inosine and adenosine almost completely reversed 6-MP inhibition of the MLR (Table 1). In contrast, inosine had no effect on azathioprine inhibition and only a slight (13-29%) reversal occurred with low concentrations of adenosine (1-20μM).

Table 1 Effect of adenosine and inosine on azathioprine and 6-MP inhibition of the MLR

Nucleoside	(μM)	% Reversal of inhibition ± SEM (n) Azathioprine (36 μM)	6-MP (100 μM)
Adenosine	1	25 ± 9 (6) p<0.02	13 ± 4 (8) p<0.02
	10	29 ± 9 (6) p<0.025	18 ± 4 (8) p<0.005
	20	13 ± 5 (10) p<0.02	44 ± 8 (8) p<0.005
	100	0.4 ± 0.2(12) NS	86 ± 7 (30) p<0.001
	200	0.4 ± 0.3(12) NS	-
	400	-0.2 ± 0.2(12) NS	74 ± 15 (8) p<0.005
Inosine	1	2 ± 6 (6) NS	14 ± 4 (8) p<0.02
	50	-	19 ± 3 (12) p<0.001
	100	-	48 ± 3 (12) p<0.001
	200	-	77 ± 4 (12) p<0.001
	500	0.6 ± 0.4(12) NS	93 ± 5 (12) p<0.001
	1,000	0.2 ± 0.3(12) NS	91 ± 6 (12) p<0.001

Adenosine (100 μM) added to the MLR after 2, 12, 24, 48, 72 and 96h caused significant (p<0.001) reversal of 6-MP (100 μM) inhibition by 69%, 58%, 51%, 21%, 8% and 3% respectively. Addition of 100, 200 and 400 μM of adenosine to the MLR 2h before azathioprine (36 μM) resulted in no reversal of its inhibition. Guanosine at 1-700μM or at 1-150 μM caused no reversal of azathioprine or 6-MP inhibition respectively. The main conclusions of this study are:

1. Adenosine and inosine reversed 6-MP inhibition.
2. Adenosine at 1-20 μM caused slight reversal of azathioprine inhibition and inosine had no effect.
3. The reversal of 6-MP inhibition decreased when addition of adenosine was delayed.
4. Guanosine had no effect on azathioprine or 6-MP inhibition of the MLR.
5. These results suggest that azathioprine acts differently to 6-MP in inhibiting the MLR.

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β-ENDORPHIN AND PUERPERAL PSYCHIATRIC SYMPTOMS

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We have previously shown a relationship between serum prolactin (PRL) and puerperal symptoms of depression, anxiety and tension (George et al 1980) and between serum PRL and plasma B-endorphin (BEP) immunoreactivity (George and Wilson 1982). In this study, we have investigated BEP levels and T.S.H. levels in relation to psychiatric symptoms in the early puerperium.

42 women aged 15-38 (mean age 26) were selected at random after having a normal vaginal delivery. Psychiatric ratings (using the modified Present State Examination) of Cooper et al (1977) and blood samplings were carried out on the 2nd, 4th and 6th days post-partum. 38 complete sets of blood samples and ratings were obtained. Bendorphin was assayed by the R.I.A. method of Wardlaw and Frantz (1979) and T.S.H. by the R.I.A. method of Golstein and Vanhaelst (1973). Table 1(a) shows that there was a statistically significant decline in BEP levels over the study period while the fall in T.S.H. levels was not significant.

Examination of the correlation co-efficients between plasma BEP and the psychiatric symptoms of depression, anxiety and tension showed significant correlations over the study period while plasma T.S.H. was unrelated to symptomology. The results suggest that puerperal psychiatric symptoms may be related to specific endocrine changes rather than to general alterations in pituitary function.

Table 1(a) Plasma BEP and T.S.H. levels $\pm$ S.D. during the study period

	Day 2	Day 4	Day 6
BEP $pg.ml^{-1}$	101.7 ± 14.2*	87.4 ± 10.9**	50.7 ± 9.7
T.S.H. $uU.ml^{-1}$	6.7 ± 0.9	6.1 ± 0.8	5.9 ± 0.8

Table 1 (b) Coefficients of correlation (r) between psychiatric symptoms and BEP concn.

	Day 2	Day 4	Day 6
Anxiety	r = 0.47**	r = 0.49**	r = 0.41**
Tension	r = 0.51+	r = 0.45**	r = 0.32*
Worries	r = 0.46**	r = 0.45**	r = 0.42**
Depression	r = 0.41**	r + 0.36*	r = 0.28 N.S.
Sig level	p = 0.05*	p = 0.01**	p = 0.001+

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