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REDUCED β-ADRENOCEPTOR-MEDIATED VASODILATION IN SPONTANEOUSLY HYPERTENSIVE RATS

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There is evidence that noradrenaline-induced vasoconstriction in the peripheral rat vasculature is reduced by beta-adrenoceptor-mediated vasodilation (Imms et al, 1977). In the perfused rat mesentery (McGregor, 1965) we have shown that the anomalous action of (-)isoprenaline (IPNA) in potentiating noradrenaline-induced contractions in SHR can be explained by a reduced vasodilator capacity (Borkowski & Porter, 1984). The present study investigates this possible reduction in SHR compared to Wistar normotensive rats (WNR) using the selective alpha--1-adrenoceptor agonist (-)phenylephrine (PE).

Male WNR and SHR (250-325g) were anaesthetised with ether and the mesenteric arterial bed removed. This was maintained at 37°C and perfused, via the superior mesenteric artery, at 4 ml/min with Krebs solution (gassed with 95%O_2/5%CO_2) containing EDTA (10mg/l) and ascorbic acid (20 mg/l). Pressor responses to 0.1ml injections of PE (3×10^{-10} mol-1×10^{-5} mol) were measured as increases in perfusion pressure. Responses were calculated as a percentage of the maximal response (R_{max}) in each tissue under control conditions.

Baseline perfusion pressures in SHR (24±2mmHg;n=7) and WNR (20±1mmHg; n=10) were not significantly different; the maximal response to PE of the SHR controls (164±3mmHg; n=7) was higher than that of the WNR controls (121±5mmHg; n=10; $p<0.001$), indicating that the hyperresponsiveness usually associated with SHR vascular tissues was present. The ED_{50} for PE was lower ($p<0.001$) in SHR than in WNR ($2.7\pm0.4\times10^{-8}$ mol and $5.2\pm0.9\times10^{-8}$ mol respectively). The table shows the effect on R_{max} of adding IPNA to the perfusate: significance levels are given relative to the control or (with timolol) to 10^{-6}M IPNA.

	WNR				SHR		
[IPNA]	10^{-8}M	10^{-7}M	10^{-6}M	10^{-6}M	10^{-7}M	10^{-6}M	10^{-6}M
[Timolol]	---	---	---	10^{-7}M	---	---	10^{-7}M
R_{max} (%)	80±6*	50±4***	39±5***	82±5**	81±3**	76±3***	86±4*

n=5-7; * $p<0.05$ **$p<0.01$ *** $p<0.001$

IPNA (10^{-8}-10^{-6}M) caused dose-dependent reductions of R_{max} in WNR. However, IPNA did not significantly alter either the slope of the curve or the ED_{50} for PE, thus acting as a physiological antagonist. In the presence of the beta-adrenoceptor antagonist timolol (10^{-7}M) and IPNA (10^{-6}M), the R_{max} was higher than for IPNA alone, indicating that timolol was able specifically to antagonise the action of IPNA at the beta-adrenoceptor. In SHR-derived tissues, IPNA (10^{-7}M-10^{-6}M) did not significantly affect the control slopes but did shift the ED_{50} to PE rightwards by 0.25 log unit. In a qualitatively similar manner to that seen in WNR, IPNA reduced the R_{max} to PE, and timolol (10^{-7}M) reversed this effect when used to challenge IPNA (10^{-6}M).

The effects of IPNA in reducing the R_{max} were quantitatively greater ($p<0.001$) in WNR than in SHR. The results of this study indicate that in this preparation, in both hypertensive and normotensive rats, IPNA acts via the beta-adrenoceptor as a physiological antagonist of PE-induced vasoconstriction, but that this effect is markedly smaller in the spontaneously hypertensive rat.

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PROTECTION OF THE RAT COLONIC MUCOSA USING A PROSTAGLANDIN E_2 ANALOGUE

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Previous studies have demonstrated that prostaglandins of the E series can protect the rat gastric mucosa against deep necrotic damage induced by topical irritants (Robert et al., 1976; Whittle, 1976; Wallace et al., 1982) and the small intestine from damage induced by indomethacin or prednisolone (Robert, 1975; Lancaster & Robert, 1978). In the present investigation, the effects of the analogue 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$) on ethanol-induced colonic damage have been studied in the rat using macroscopic and histological techniques, as well as by the use of enzyme markers of cellular damage.

Colonic damage was induced by the intracolonic administration of 30% v/v ethanol (0.25 ml), twenty min after intracolonic instillation of the prostaglandin analogue or vehicle (0.5 ml). Ten min after ethanol administration the colon was removed and the damage assessed in a randomized manner, both macroscopically and by histological techniques. In addition, the release into the lumen of an isolated colonic segment, of both the cytoplasmic enzyme, lactate dehydrogenase and the lysosomal enzyme, acid phosphatase was determined using standard spectrophotometric techniques. The colonic segment was incubated *in vitro* in an oxygenated Krebs' solution (37°C) following *in vivo* challenge with ethanol. Release of these enzyme markers *in vitro* has recently been demonstrated to give an index of cellular disruption, particularly of the surface epithelial cells, in the rat gastric mucosa (Whittle & Steel, 1984).

Exposure of the colon to ethanol resulted in the formation of grossly visible regions of hyperemia and hemorrhage. Histologically, the damage was characterized by mucosal vasocongestion, necrosis and complete loss of the surface epithelium. Pretreatment with $dmPGE_2$ (0.2-20 $\mu g\ kg^{-1}$) caused a dose-dependent reduction in colonic injury as measured by all parameters. A significant ($P < 0.05$) reduction of macroscopically-visible damage was observed with $dmPGE_2$ (at doses of 0.2 $\mu g\ kg^{-1}$ and higher). With the higher doses of $dmPGE_2$ the histological signs of damage were significantly reduced and with $dmPGE_2$ (20 $\mu g\ kg^{-1}$) the damage was completely prevented.

Following challenge with ethanol *in vivo*, the colonic segment released significantly ($P < 0.001$) more acid phosphatase and lactate dehydrogenase during a 10 min incubation period than did the colonic segments from control rats receiving only saline *in vivo*. Pretreatment with $dmPGE_2$ (0.2-20 $\mu g\ kg^{-1}$) caused a dose-dependent reduction in the ethanol-induced release of both enzyme markers ($P < 0.001$) with the highest dose of $dmPGE_2$ (20 $\mu g\ kg^{-1}$) reducing enzyme release to control levels.

This study demonstrates that as in the gastric mucosa, local application of $dmPGE_2$ can protect the colonic mucosa from damage induced by a potent topical irritant. Furthermore, in this study, protection of colonic surface epithelial cells as well as deeper mucosal cells from ethanol-induced damage was observed with high doses of the analogue. This protection may be, at least in part, due to an effect of this prostanoid on colonic mucosal vasculature.

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EXAMINATION OF α-ADRENOCEPTORS OF RAT ISOLATED ATRIA

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This study examines the activity of 3 selective α_1-adrenoceptor agonists in the isolated left and right atria of the rat. Left and right atria were set up in Krebs-bicarbonate solution at 30°C gassed with 5% CO_2 in O_2. Tension responses of the paced left atria (2Hz, 5ms pulse width, threshold voltage + 50%) and rate responses of the spontaneously beating right atria were recorded. After a 45-60 min equilibration period, a cumulative concentration-response curve to isoprenaline was constructed. After washout, β-adrenoceptor blockade by propranolol (10^{-6}M) was established and a curve to either cirazoline, methoxamine or phenylephrine obtained. Cirazoline is a full agonist at aortic α-adrenoceptors (Ruffolo & Waddell 1982) yet the right atria exhibited a negative chronotropic response. The left atria yielded positive inotropic responses (EC50, 1.62(0.32 - 8.36)μM) with a maximum 16±7% of that for isoprenaline. Methoxamine exhibited positive inotropic (maximum 41±4%; EC50 34.7(20.5-58.7)μM) and positive chronotropic responses (maximum 18±6%; EC50 5.13(2.37-11.1)μM) in the presence of β-adrenoceptor blockade. Phenylephrine stimulates both α and β-adrenoceptors in rabbit isolated papillary muscles (Schümann 1983). In the present study phenylephrine, in the presence of propranolol, produced monophasic concentration-response curves for increases in rate (EC50 7.24(0.50 - 105)μM) and tension (EC 50 4.78 (2.41-9.51)μM) with maxima of 14±6% and 22±6% respectively relative to isoprenaline.

Phenylephrine was next examined in left atria in the absence or presence of propranolol (10^{-6}M). There was no apparent shift of the concentration-response curve, the geometric mean EC50 values in the absence (2.2(1.62-3.09)μM n=4) and presence of propranolol (3.09(1.06-9.04)μM n=6) not differing significantly ($p>0.05$). Furthermore, the maximum response measured as the tension increase was not significantly different ($p>0.05$) in the absence (0.24±0.03g) and presence (0.18±0.08g) of propranolol. The experiment was repeated with left atria from rats acutely pretreated 18-24h before use with reserpine (5mg kg^{-1} i.p.) to deplete endogenous catecholamines. Again, the EC50 values for phenylephrine in the absence (2.63(1.08-6.38)μM) and presence of propranolol (2.95(1.76-4.95)μM) were not significantly different ($p>0.05$) and the maximum responses (0.41±0.13g and 0.40±0.03g respectively) were also not significantly different. It can therefore be concluded that the positive inotropic response of left atria to phenylephrine is almost entirely due to α-adrenoceptor stimulation, the maximum response being substantially greater than that of any β-adrenoceptor stimulation by it.

The α-adrenoceptors of rat left atria were further characterized by use of the selective α_1-adrenoceptor antagonist prazosin (Davey 1980). Cumulative concentration-response curves to methoxamine were obtained before and in the presence of prazosin (10^{-9}, 3×10^{-9} or 10^{-8}M). The dose-ratios for the rightwards shift of the curves were calculated at the EC50 and corrected from control experiments in which no prazosin was used. The Schild plot slope was 1.07±0.16 and the pA_2 value was 9.05±0.06 (n=11).

This study has demonstrated α-adrenoceptor-mediated increases in tension of the rat left atria. The pA_2 value of prazosin is compatible with this response being mediated via adrenoceptors of the α_1-subtype (Davey 1980).

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A COMPARISON OF PROPRANOLOL AND THE ENANTIOMERS OF PROPRANOLOL GLYCOL ON ISOLATED RAT ATRIA

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Propranolol is extensively metabolised in vivo (Walle and Gaffney, 1972; Vu and Abramson, 1980) but the pharmacological actions of some of the metabolites are not well characterised. The aim of this study was to determine the effects, in comparison with propranolol, of the enantiomers of one such metabolite - propranolol glycol - to block the (±)-isoprenaline-induced increase in force of contraction of the isolated rat atrium.

Isolated rat right atria were set up for isometric tension recording in an organ bath containing Krebs-Henseleit solution at 34°C (Borda et al, 1984). After establishing a control dose-response curve to isoprenaline, propranolol or one of the metabolites under test was added to the bath 1 min prior to the addition of isoprenaline. The change in force of contraction was determined over the next 90 sec. In the absence of isoprenaline no direct action was detected using concentrations in the range 10^{-6} - 10^{-11}M propranolol or 10^{-4} - 10^{-7}M propranolol glycol. For each of the drugs 4 dose-response curves were constructed, the first with isoprenaline alone and the other three with varying concentrations of drug added in addition. From the resulting dose-response curves the EC_{50} values and dose ratios were determined where possible and used in the construction of Schild plots (Arunlakshana and Schild, 1959). The affinity constant for the drugs was also determined. (Bassett, 1971).

TABLE 1

DRUG	SLOPE OF SCHILD PLOT	PA_2	LOG AFFINITY CONST. (K_2)
Propranolol	0.98 ± 0.15	9.12 ± 0.33	9.32 ± 0.27
(S)-Propranolol Glycol	1.01 ± 0.14	6.08 ± 0.17	5.83 ± 0.44

Mean ± S.E.M.

Both propranolol and the (S)-glycol inhibited the isoprenaline effect in a competitive manner, the latter being approx. 1000 times less potent than the parent compound. (Table 1)

In contrast to propranolol the (R)-glycol, even at the lowest concentration studied, showed irreversible antagonism of the isoprenaline effect and therefore no pA_2 value or affinity constant was obtainable. Pretreatment of the preparation with 10^{-7}M propranolol, a concentration blocking some 75% of the isoprenaline effect, did not prevent this irreversible action.

The results of this preliminary study would suggest that, like propranolol, both enantiomers of the propranolol glycol have beta-adrenoceptor blocking activity. Since the 2 enantiomers differ markedly in their actions there may be definite stereochemical requirements for beta adrenoceptor blockade.

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COMPARISON OF THE CARDIOVASCULAR EFFECTS OF CENTRAL AND PERIPHERAL ADMINISTRATION OF INDORAMIN IN ANAESTHETISED RATS

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The selective α_1-adrenoceptor blocker indoramin lowers blood pressure in both animals and man without promoting a reflex tachycardia. The absence of tachycardia has been ascribed to both peripheral and central mechanisms. It has been suggested that indoramin may exert a direct bradycardic effect on the heart due to local anaesthetic properties or anti-arrhythmic activity (Algate et al, 1981; Harron et al, 1984). Indoramin has also been demonstrated to reduce preganglionic sympathetic nerve activity (Ramage, 1982). The cardiovascular effects of indoramin following central administration have now been investigated in comparison with prazosin.

Groups of female rats (n=4) were anaesthetised with α-chloralose and pentobarbitone sodium (80 + 6 $mgkg^{-1}$ i.p) and intubated. Blood pressure (BP) and heart rate (HR) were recorded via the left femoral artery. Drugs were administered by intravenous injection (i.v.) via a femoral vein and centrally by injection (10µl volume) into the lateral cerebral ventricles (i.c.v.) or the cisterna magna (i.c.). Indoramin, prazosin or distilled water vehicle were administered by each of these routes. BP and HR were recorded before and 15,30 or 45 minutes after administration of drugs (predose values were 118 ± 3 mmHg and 397 ± 4 $bmin^{-1}$ n=44).

Data were statistically analysed within groups using a 2-way analysis of variance and between groups using a nested analysis of variance; $p < 0.05$ was taken to be statistically significant.

There was no change in HR following administration of vehicle i.v. and i.c.; in contrast, i.c.v. injection evoked significant increases (13 ± 7 $bmin^{-1}$ after 15 mins). Indoramin, 25µg i.c.v., had no effect on HR at any time point whereas prazosin, 25µg i.c.v., evoked a significant tachycardia compared to the control group 15 mins after dosing (39 ± 13 $bmin^{-1}$). Peripheral administration of indoramin or prazosin, 25µg i.v., did not significantly alter HR, whereas administration of the same dose i.c. evoked significant decreases in HR (maximum falls of 64 ± 9 $bmin^{-1}$ and 48 ± 11 $bmin^{-1}$ respectively). A higher dose of indoramin, 50 µg i.c., did not evoke a greater fall in HR.

Administration of 25 µg indoramin i.c.v. did not significantly alter BP whereas prazosin evoked a significant decrease (maximum fall 33 ± 8 mmHg). Neither indoramin nor prazosin (25µg) evoked a significant change in BP following i.v. injection. Small but significant decreases in BP were evoked, however, after i.c. injection (16 ± 5 mmHg and 33 ± 9 mmHg respectively). In contrast to the HR data above, a higher dose of indoramin (50 µg i.c.) evoked a greater fall in BP (maximum fall 34 ± 7) than that observed following 25 µg i.c.

These experiments suggest that indoramin exerts a bradycardic effect via a central mechanism and demonstrates the differences between i.c.v. and i.c. administration of α_1-adrenoceptor blockers. Injection into the cisterna magna allows substances to be localized in the region of the brainstem; it appears therefore that a bradycardic action of indoramin may be mediated within this region.

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CHANGES IN VERAPAMIL SENSITIVITY OF RAT AORTA EXPOSED TO CHRONIC HYPOXIA AND NORMOXIA

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Noradrenaline (NA) induced contractions of rat aorta, following exposure to chronic hypoxia, have been found to be less sensitive to verapamil (VER) than normoxic contractions induced prior to or following the hypoxic episode (Downing et al, 1984). These observations suggested that exposure of rat aorta to chronic hypoxia might alter its relative dependence on activator calcium from different sources eg. intra- and extra-cellular. The present experiments were undertaken in order to investigate this phenomenon further.

Circular preparations from male Wistar rats (200-280g) were suspended under a resting tension of 3g at 37°C in Krebs' solution (2.5mM Ca^{2+}) gassed initially with 5% CO_2 in O_2. Reproducible responses to 1μM NA were always obtained at the start of each experiment. In certain tissues, transient responses to NA under Ca^{2+}-free conditions + 0.5mM EGTA were also elicited (EGTA-resistant responses). Preparations were then exposed to acute or chronic hypoxia by gassing with 5% CO_2 in N_2 for 30 min (pO_2 78mmHg) or storing under an atomosphere of 5% CO_2 in N_2 for 70 hr at 10°C (pO_2 39mmHg) respectively. Other preparations were exposed to acute (pO_2 410mmHg) or chronic normoxia (pO_2 390mmHg) by using 5% CO_2 in O_2. Other tissues were exposed to acute hypoxia following 70 hr chronic normoxic storage. The effect of 30 min pre-incubation with 10 μM VER was examined on contractions induced by 1 μM NA under each of these conditions. The relative size of EGTA-resistant contractions exposed to each condition was also determined. Fig 1 shows the sizes of control contractions, EGTA-resistant responses and VER (10 μM) sensitive components relative to normoxic controls obtained at the beginning of each experiment. A large and significant ($p<0.001$) component of the contractions seen following chronic hypoxia was insensitive to VER and yet appeared to be dependent upon Ca^{2+} entry (as judged from the size of the EGTA-resistant responses) Fig 1. Although acute hypoxia and chronic normoxia reduced the overall size of contractions, that component of contractions believed to be due to Ca^{2+} entry (EGTA-sensitive) appeared to be abolished by VER in both cases.

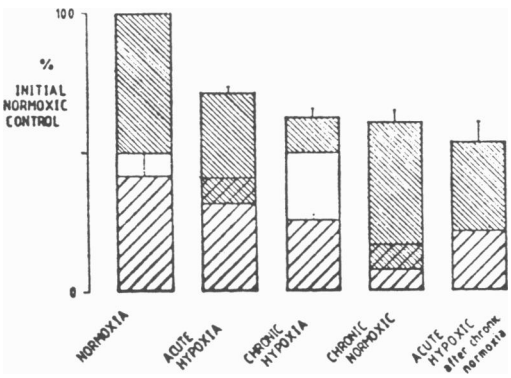

Fig 1 Responses induced by 1 μM NA under hypoxic and normoxic conditions relative to acute normoxic controls (n> 6). Wide hatched areas represent the EGTA-resistant component, close hatched represent the 10 μM VER sensitive response.

Loutzenhiser and van Breeman (1983) have postulated that unstimulated Ca^{2+} entry may occur through VER insensitive 'leak' channels in rabbit aorta. The utilization of such channels appeared to increase following exposure to chronic hypoxia in the rat aorta. It is possible that exposure of the preparations to chronic hypoxia produce changes allowing VER insensitive channels to be 'stimulated' by NA.

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THE EFFECT OF CIMETIDINE AND RANITIDINE ON PARACETAMOL GLUCURONIDATION AND SULPHATION IN CULTURED RAT HEPATOCYTES

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Cimetidine and Ranitidine have been investigated for their ability to interact with sulphation and glucuronidation reactions with paracetamol as substrate in the presence of cultured rat hepatocytes. In untreated cells, rates of glucuronide and sulphate formation in the presence of 10 mM paracetamol remained high in cells maintained in culture for 24h or 48h; the rate of glucuronide formation actually increasing at 48h compared with earlier time-points. Using concentrations of paracetamol of 0.5 mM and 5 mM, neither cimetidine (1 mM) nor ranitidine (1 mM) had any appreciable effects on rates of paracetamol sulphation which was essentially linear over a 24h incubation period. Glucuronidation of paracetamol was followed using 0.5 mM and 5 mM paracetamol and the linearity of conjugate formation remained throughout the 24h incubation period. At a concentration of 0.5 mM paracetamol, both cimetidine (1 mM) and ranitidine (1 mM) caused a 50% inhibition of glucuronide accumulation in the medium. Using 5 mM paracetamol, the inhibition effects of cimetidine and ranitidine decreased to about 75% of control values. At a fixed concentration of paracetamol (1 mM , approximate Km^{app} of the glucuronidation pathway) the formation of glucuronide was examined in the presence of varying concentrations of cimetidine or ranitidine. As the concentration of either was increased, a pronounced decrease in the glucuronide conjugate was observed which was statistically significant in both cases ($p < 0.01$). No adverse effects of cellular viability were noted utilizing enzyme leakage (lactate dehydrogenase) or protein synthesis measurements.

The kinetics of inhibition by ranitidine were studied in more detail (see Table 1). Hepatocytes were incubated with various concentrations of paracetamol (0.5, 0.1, 2.0, 5.0 and 10 mM) for a period of 6h. Km^{app} and $Vmax^{app}$ were obtained by the method of Wilkinson (1961). At 0.25 mM ranitidine the inhibition appeared to be purely competitive. However, at higher concentrations, decreases in $Vmax^{app}$ were noted suggesting a more complex, partial competitive mechanism of inhibition. Estimates of Ki (Ki = 435 ± 134 μM assuming a purely competitive mechanism or 48-160 μM assuming partial competitive inhibition) would suggest that inhibition of glucuronidation by ranitidine may be observable in vivo. Leonard and Dent (1984) have recently shown that ranitidine but not cimetidine potentiates the hepatotoxic effects of paracetamol in rats. The extent to which our present results provide an explanation for those findings will be discussed.

Table 1 The Effect of Ranitidine on Apparent Km and Vmax for Paracetamol Glucuronidation

Ranitidine (mM)	Km^{app} (μM)	$Vmax^{app}$ (n mol.mg protein^{-1}.h^{-1})
0	1052 ± 332	21.5 ± 2.0
0.25	3328 ± 285	21.4 ± 0.8
1.00	3391 ± 244	18.9 ± 0.6
2.00	3729 ± 616	17.9 ± 1.3

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SUBSTANCE P CONTRACTIONS OF GUINEA PIG TAENIA CAECI BY A DIRECT ACTION ON SMOOTH MUSCLE

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The excitatory effect of substance P (S.P.) on the guinea-pig (G.P.) small intestine is a result of both "direct" actions on smooth muscle cells and "indirect" actions on the myenteric plexus (ie. release of acetylcholine; Yau & Youther, 1982). S.P. has been detected in G.P. taenia caeci (Holzer, Emson, Iversen & Sharman, 1981) and has been found to contract this tissue (Dodge & Morton, 1984). It has not been demonstrated, however, whether this action is a combination of both direct and indirect effects (as in the G.P. ileum).

In an analysis of the response of G.P. taenia caeci to electrical field stimulation (EFS), Burnstock, Campbell & Rand (1966) have demonstrated the presence of both pre- and post-ganglionic cholinergic nerves. Responses to cholinergic nerve stimulation are not easy to demonstrate because of the varying tone and other factors contributing towards the contraction (Bennett, 1966). It was therefore decided to use radiolabelling studies as well as conventional methods to show activation of cholinergic nerves.

Strips of G.P. taenia caeci, without attached mucosal layer, or ileum longitudinal muscle-myenteric plexus (LMMP) preparations were incubated in Krebs fluid containing [^{3}H]-choline (2 µCi/ml of 15 Ci/mmol) for 60 min. Tissues were then superfused with Krebs fluid containing hemicholinium (34.8 µM). After 90 min. the tissues were challenged with EFS (0.5 ms, 1.0 Hz, 10 or 30 V, 50 or 60 pulses) or S.P. (0.007 µM superfused for 1 min.).

Radiolabelling experiments have demonstrated release of tritiated material from G.P. taenia caeci on challenge with EFS. This release was less and more inconsistant than that obtained under similar conditions using preparations of G.P. ileum LMMP. Submaximal contractions to S.P. (0.007 µM) were not accompanied by a significant release of labelled material (Table 1).

TABLE 1

	G.P. taenia caeci		G.P. ileum LMMP	
	R%B	FR	R%B	FR
EFS	14.1 ± 7.7	0.146 ± 0.093	98.0 ± 14.1	0.458 ± 0.078
S.P.	1.7 ± 5.1	0.024 ± 0.045		
CONTROL	3.3 ± 4.0	0.030 ± 0.036		

R%B= release as % of basal; FR= fractional release x 100; n=5

Finally, the response of the G.P. taenia caeci to S.P. has been qualitatively analysed: both the peak contraction and the prolonged after-contracture were unaffected by both hyoscine (2.19 µM) and TTX (1.57 µM). Our results indicate that submaximal doses of S.P. contract G.P. taenia caeci by a direct action on smooth muscle cells.

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ANTINOCICEPTIVE ACTIVITY OF INTRAVENOUS AND INTRATHECAL DOPAMINE AGONISTS IN.NAIVE AND SUPERSENSITIVE RATS

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It has been previously reported that the DA agonist apomorphine elevates the thermal tail flick latency (TFL) in barbiturate-anaesthetized rats (Barasi et al., 1984). Moreover, the reputed D2 agonist LY 141865 (Stoof and Kebabian, 1981) produces antinociceptive activity after intracerebral administration whilst the D1 agonist SKF 38393 was inactive in naive mice (Ben-Sreti et al., 1983). The lack of effect of SKF 38393 on nociceptive threshold might be explained by the concept that this compound may only selectively stimulate supersensitive central DA receptors (Setler et al., 1978). Consequently, this study examines the possible antinociceptive actions of apomorphine, SKF 38393 and LY 171555 [the (-) enantiomer of LY 141865] in naive and haloperidol-induced DA supersensitive rats.

Experiments were performed on male Wistar rats (WSP stock) lightly anaesthetized with sodium pentobarbitone (Barasi et al., 1984). Nociceptive sensitivity was determined by measuring the TFL in response to noxious radiant heat. Dopamine supersensitivity was induced after withdrawal from 28-day haloperidol administration in drinking water (50 mg/l). The TFL data following intravenous (i.v.) and intrathecal (i.t.) administration of agonists are summarized as follows:

Tail Flick Latency

Treatment		Route	Naive	DA-Supersensitive
Apomorphine	80 µg/kg	i.v.	Increased	No effect
Apomorphine	75 µg/kg	i.t.	Increased	No effect
SKF 38393	200 µg/kg	i.v.	-	No effect
SKF 38393	75 µg/kg	i.t.	No effect	Increased
LY 171555	80 µg/kg	i.v.	-	No effect
LY 171555	75 µg/kg	i.t.	Increased	Increased

The ability of SKF 38393 to increase the TFL in DA-supersensitive but not naive rats would suggest that greater supersensitivity had developed to D-1 than D-2 systems following chronic exposure to haloperidol. These observations accord with the findings of Setler et al. (1978) who reported that SKF 38393 produced contralateral rotation in rats with nigrostriatal lesions but not in the intact animal. The results show that the induction of DA-supersensitivity abolished the antinociceptive activity of apomorphine probably as a result of a change in the balance of the relative activities of different dopamine receptor systems. Furthermore this work provides some evidence for the characterisation of the dopamine receptor subgroup which may subserve the antinociceptive action of certain dopaminergic agents.

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EVIDENCE FOR A CENTRALLY MEDIATED CARDIOVASCULAR RESPONSE TO 8-HYDROXY-2-(DI-N-PROPYLAMINO)-TETRALIN (8-OH-DPAT) IN THE RAT

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8-Hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) is a putative central 5-hydroxytryptamine (5-HT) receptor agonist (Hjorth et al. 1982) which lowers blood pressure (BP) and heart rate (HR) in both anaesthetized normotensive and conscious spontaneously hypertensive (SH) rats (Fozard and McDermott, 1984). A fall in both BP and HR in conscious animals is suggestive of a central site of drug action. This report describes the results from experiments carried out to explore this possibility. Where appropriate, comparisons have been made with clonidine, a centrally acting hypotensive with negligible affinity for central 5-HT receptors.

Male Sprague-Dawley rats weighing 380-470 g were anaesthetized with pentobarbitone. They were used either as anaesthetized preparations or they were pithed and, in some instances, prepared for stimulation of the whole spinal sympathetic outflow (Fozard, 1982). BP and HR were recorded by standard techniques and i.v. injections were made into a femoral vein. Injections were also made directly into the cisterna magna using volumes < 10 µl with the aid of a stereotaxic frame (Kopf).

In anaesthetized rats i.v. injections of 8-OH-DPAT, 8-128 µg/kg, caused dose-related and sustained (> 75 min at the highest dose) falls in both BP and HR. Responses to clonidine, 2-32 µg/kg, were similar except that dose-related increases in BP preceded the hypotensive responses. In pithed rats, 8-OH-DPAT, 8-128 µg/kg i.v., caused transient (< 5 min) small (+ 45 mmHg at 128 µg/kg) increases in BP accompanied by weak bradycardia (-6 b/min at 128 µg/kg). In contrast to the situation in intact animals, 8-OH-DPAT, 32 µg/kg i.v., did not significantly lower BP in pithed rats with mean blood pressures raised to > 100 mmHg by i.v. infusion of angiotensin II (0.1-0.5 µg/kg/min). Cardiovascular responses to stimulation of the whole spinal sympathetic outflow (0.5-4 Hz ; 5s; 1ms; supramaximal voltage) and phenylephrine, 1-4 µg/kg i.v., were not significantly affected when elicited during a 35 min period starting 5 min after the i.v. injection of 8-OH-DPAT, 32 µg/kg. In contrast, under identical conditions, clonidine, 2 µg/kg, significantly affected the pressor response to spinal stimulation without affecting the response to phenylephrine.

Intracisternal injection of 8-OH-DPAT, 2-10 µg/kg, or clonidine, 0.25-1 µg/kg, gave consistent falls in BP and HR although in the case of 8-OH-DPAT these did not exceed in magnitude or duration the responses to similar doses given i.v. Intracisternal injection of 10 µg/kg of 8-methoxy-2-(N-2-chloroethyl-N,n-propyl)- aminotetralin, a putative, irreversible central 5-HT receptor antagonist (see also Fozard & McDermott, 1984), blocked the cardiovascular response to 8-OH-DPAT, 32 µg/kg, given i.v. ; the same dose given i.v. was without effect.

Thus, even high doses of 8-OH-DPAT do not interfere in the periphery with sympathetic control of cardiovascular function. Coupled with the fact that hypotensive responses are not seen in the BP-supported pithed rat, that 8-OH-DPAT has effects when injected directly to the brain and that the response to systemic 8-OH-DPAT can be blocked by an antagonist injected centrally, a central site of action for 8-OH-DPAT seems probable.

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PERIPHERAL EFFECTS OF ENANTIOMERS OF 3-(3-HYDROXYPHENYL-N-n-PROPYLPIPERIDINE) (3-PPP) ON α RECEPTORS IN RAT

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The two enantiomers of the synthetic dopaminergic compound 3-(3-hydroxyphenyl)N-n-propylpiperidine (3-PPP) have been shown to differ in their dopaminergic properties at the central nervous system level (HJORTH,1983). Additionnally, an unusual peripheral spectrum of activity on dopamine receptors has also been reported for the (+) and (-) enantiomer (GOLDBERG et al.1983). Although data on CNS seem to exclude any interaction of 3-PPP with α adrenergic receptors, we have investigated its peripheral cardiovascular interactions with α_1 and α_2 receptors on the pentobarbital anaesthetized pithed rat model.

The intravenous administration of cumulative doses of (-) and (+) 3-PPP resulted in a dose-dependent increase in systolic (BPs) and diastolic blood pressure. Although threshold doses were the same, the slope of the two curves differed markedly : (+)3-PPP was much more potent than (-)3-PPP, the increase in BPs produced by 1 mg/kg being 62.9 ± 6.0 mmHg and 15.0 ± 0.9 mmHg for each drug respectively The dose-response curve to (+)3-PPP was unaffected by the α_2 antagonist yohimbine (500 μg/kg) but was greatly depressed by the α_1 antagonist prazosin (100 μg/kg). Conversely, the dose-response curve to (-)3-PPP was only slightly displaced to the right by yohimbine (500 μg/kg), prazosin (500 μg/kg) or the combination of both antagonists. The responses to (-)3-PPP were neither antagonized by 5 HT antagonists methysergide (50 μg/kg) and ketanserine (50 μ/kg) nor by the histamine H_1 antagonist mepyramine (100 μg/kg) and H_2 antagonist cimetidine (500 μg/kg)

The intravenous administration of clonidine and phenylephrine produced dose-dependent increases in BPs. The dose-response curve to clonidine was significantly displaced to the right by (-)3-PPP (1 mg/kg) but not by (+)3-PPP (1 mg/kg). The dose-response curve to phenylephrine was only slightly displaced to the right by (-)3-PPP (1 mg/kg).

Thus, in addition to their dopaminergic properties, the enantiomers of 3-PPP show different peripheral α-adrenergic activities : (+)3-PPP is a pure α_1 agonist while (-)3-PPP is a partial agonist of α_1 and α_2 receptors but also shows some α_1 and α_2 antagonist properties.

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DESENSITIZATION OF HUMAN NEUTROPHIL CYTOSOLIC CALCIUM CHANGES AND DEGRANULATION TO PAF-ACETHER AND FMLP

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The chemotactic tripeptide formylmethionylleucylphenylalanine (FMLP) and the phospholipid PAF-acether (1-O-hexadecyl-2-O-acetyl-3-phosphorylcholine) both activate polymorphonuclear leukocytes (PMNs) and cause PMN aggregation, migration, superoxide generation and degranulation (Becker, 1976; Vallone & Goetzl, 1983). Here we investigate whether FMLP and PAF induce degranulation of human PMN by a common mechanism and determine their effects on the mobilisation of internal calcium using the calcium-sensitive fluorescent indicator quin-2.

Suspensions of human PMN (10^8/ml, ca. 97% pure) were prepared from the blood of healthy adult donors by dextran sedimentation and density gradient centrifugation and loaded with the indicator by incubation at 37°C for 20 min with 100 μM quin-2 acetomethoxy ester (Lancaster Synthesis) in 10 mM Hepes buffer pH 7.4. The suspension was than diluted tenfold and incubated for a further 40 min. After washing the cells and resuspending in the same buffer at 10^7 cells/ml, fluorescence changes were monitored in a Perkin Elmer MPF-4 spectrophotofluorimeter with stirring at 37°C, with excitation at 339 nm and emission 492 nm. Exocytosis was determined by assaying the release of the granular enzymes β-glucuronidase (β-G; using spectrophotfluorimetry and 4-methylumbelliferyl-β-D-glucuronide as substrate, pH 5.0) and lysozyme (L; using spectrophotometry and a suspension of Micrococcus lysodeikticus as substrate).

FMLP and PAF-acether both induced release of β-G and L in a concentration-dependent manner with ED_{50} values of 0.04 μM and 0.03 μM, respectively. The maximal enzyme release (expressed as percent of the total cellular enzyme content) was greater for FMLP than PAF (34.8 ± 0.8% compared to 28.5 ± 1.1% for lysozyme at 10 μM stimulating agent, and 23.3 ± 3.6% compared to 14.3 ± 0.6% for β-glucuronidase, both $P < 0.001$). With both agents (FMLP 1 μM; PAF-acether 10 μM) release of enzymes was complete within 1 min.

FMLP and PAF-acether also both increased cytosolic levels of Ca^{++}, which reached a maximal level in about 30 sec.

PMNs pretreated with 10 μM FMLP did not respond to a second exposure to this stimulus applied 3 min later with respect to the increase in cytosolic calcium level. However, degranulation, although reduced, did occur under these conditions of repeated FMLP stimulation. Similar desensitization of the calcium response occurred with lower doses of FMLP but needed repeated treatments. Cells desensitized to FMLP responded normally to PAF-acether in terms both of intracellular calcium changes and enzyme release.

A similar pattern of responses was observed using 10 μM PAF-acether in that complete desensitization of the calcium response occurred to the agent itself but not to FMLP, whereas enzyme release was only partially reduced.

We conclude that FMLP and PAF-acether act via different mechanisms (receptors?) to increase intracellular calcium levels. Moreover, as the desensitization of the calcium response is not accompanied by an equal reduction in the degranulation response, we infer that an additional intracellular process may mediate the coupling between the initiating stimulus and exocytosis.

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DIFFERENCES BETWEEN ENDOGENOUS AND RADIOLABELLED CATECHOLAMINE RELEASE FROM SUPERFUSED RAT BRAIN SLICES

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The properties of catecholamine release from brain slices are usually studied by prelabelling the tissue with radiolabelled amine and then monitoring the release of this radiolabel evoked by various stimuli (e.g. Mulder, 1982). However, there is little direct evidence that labelled amine release is an accurate reflection of endogenous amine release, since (a) the radiolabel may not distribute evenly within endogenous releasable pools, (b) endogenous release may have a newly synthesised component, and (c) in some brain areas the radiolabel may be taken up and metabolised to other amines.

We have made direct comparisons between labelled and endogenous catecholamine release from superfused rat brain slices. The slices (from striatum or hypothalamus) were prelabelled by incubation with ^{3}H-DA (0.1 μM), then superfused (0.5 ml/min) and 2 min samples collected as described previously (Nahorski & Strupish, 1981). Catecholamines were quantified by HPLC with electrochemical detection and the radioactivity present in fractions collected from the detector cell was monitored.

Two successive 1 min 50 mM K^+ pulses, 40 min apart, released similar amounts of endogenous DA from striatal slices but the second K^+ pulse released 50% less ^{3}H-DA than did the first. A K^+ gradient (K^+ rising from 5 to 53 mM over 30 min) released relatively more ^{3}H-DA compared to endogenous DA at the lower K^+ (5–25 mM) than the higher K^+ (25–53 mM) concentrations. K^+ (pulse or gradient) induced endogenous DA release was greatly reduced in the presence of 50 μM alpha-methyl-p-tyrosine (alpha-MPT) without any major effects on ^{3}H-DA release. Alpha-MPT also produced similar reductions in K^+-induced endogenous DA release from non-prelabelled slices.

Both basal DA release and the release induced by a 5 μM veratrine pulse were greatly increased in the presence of 10 μM d-amphetamine, but the effects of amphetamine were 3–4 times greater on endogenous DA release than on ^{3}H-DA release. Neither basal nor veratrine-stimulated DA release in the presence or absence of amphetamine was decreased by alpha-MPT in experiments in which prelabelling had occurred. However, DA release from non-prelabelled slices was decreased 50% by alpha-MPT.

Hypothalamic slices prelabelled with ^{3}H-DA released both ^{3}H-DA and ^{3}H-NA in response to a 50 mM K^+ pulse, and the amount of ^{3}H-NA formed increased with time so that a second K^+ pulse 40 min later released mainly ^{3}H-NA. In contrast, the ratio between endogenous DA and NA release was the same for the two K^+ pulses.

These results indicate that labelled and endogenous amine release do not always occur in parallel, and that one major cause of discrepancy is the release of newly synthesised amine. The studies on amphetamine effects on DA release also suggest that other factors (e.g. endogenous release from unlabelled pools) may also cause differences between endogenous and labelled release, and that the prelabelling process itself may alter the pools contributing to subsequent endogenous release. In the light of these studies, the assumption that labelled amine release provides an accurate marker for endogenous release should be reconsidered.

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EFFECT OF BAY K 8644 IN GUINEA PIG ATRIAL AND VENTRICULAR MUSCLE FIBRES

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BAY K 8644 is a new dihydropyridine derivative which stimulates Ca entry into cardiac and smooth muscle (Schramm et al., 1983) and seems to behave as a Ca channel activator at the chromaffin cell membrane (Garcia et al., 1984). In the present communication the electrophysiological effects of this compound were studied on isolated guinea-pig atrial and ventricular muscle fibres.

Preparations were superfused with Tyrode solution (34ºC) and stimulated at a basal frequency of 1 Hz. Transmembrane action potentials (AP) were recorded with glass microelectrodes. In some experiments following the equilibration period the papillary muscles were perfused with high-K (27 mM) Tyrode solution and stimulated at a frequency of 0.12 Hz.

In both isolated atrial and ventricular muscle fibres BAY K8644 (10^{-9} M-10^{-6}M) did not modify the amplitude and Vmax of the AP or the resting membrane potential. In isolated atrial fibres BAY K8644 (10^{-8}M-10^{-6}M) significantly shortened the action potential duration at 50% of repolarization (APD_{50}), whereas the duration at 90% of repolarization (APD_{90}) was shortened only at 10^{-6}M (from 130.0 ± 5.5 ms to 112.2 ± 6.2 ms. n=10. $P < 0.05$). In papillary muscles BAY K8644 (10^{-8}M) significantly shortened the APD_{50} (from 105.1 ± 13.1 ms to 59.3 ± 9.2 ms. n=12. $P < 0.01$) and APD_{90} (from 142.5 ± 13.7 ms to 91.2 ± 11.2 ms. $P < 0.01$). This shortening disappeared when increasing concentrations of the drug were added to the perfusate, so that at 10^{-6}M, the APD_{50} and APD_{90} values were similar to control values (108.8 ± 10.8 ms and 146.0 ± 12.2 ms, respectively. $P > 0.05$). The shortening of the APD was also suppressed in papillary muscles pretreated with tetraethylammonium (10 mM) or with verapamil (10^{-6}M). The changes in APD_{90} were accompanied by similar changes in the duration of the effective refractory period (ERP), so that the ERP/APD ratio was not altered by the drug.

In papillary muscles perfused with high-K Tyrode solution the resting membrane potential fell to -42.3 ± 1.1 mV (n=7). Under these conditions, BAY K8644 (10^{-7}M) rapidly (within 3-5 min) induced slow APs with the following characteristics: Vmax of 9.1 ± 1.1 V/s, AP amplitude of 67.3 ± 1.9 mV, APD_{50} of 90.0 ± 3.4 ms and APD_{90} of 125.3 ± 8.9 ms. These slow APS were suppressed with verapamil (10^{-6}M).

These results confirm that in guinea pig atrial and ventricular muscle fibres BAY K8644 increases Ca influx via the slow inward current.

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EFFECT OF PROPAFENONE ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF SHEEP VENTRICULAR MUSCLE AND PURKINJE FIBRES

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Propafenone (P) is a new antiarrhythmic drug effective against chronic supraventricular and ventricular tachycardia and tachycardias associated with the Wolff-Parkinson-White syndrome (Schlepper & Olsson 1983). In the present communication we have studied the electrophysiological effects of P on isolated sheep ventricular muscle (VM) and Purkinje fibres (PF).

Preparations were obtained at the local slaughterhouse, placed in a chamber perfused at 7 ml/min with Tyrode solution (37ºC) and stimulated at a frequency of 1 Hz. Transmembrane action potentials (AP) were recorded with conventional glass microelectrodes.

In PF and VM, P (0.01-10 uM) decreased dose-dependently the amplitude and maximum rate of depolarization (MRD) of the AP and at concentrations between 1 and 5 uM the effects were accompanied by a progressive decrease of the resting membrane potential (RMP). At 1 and 5 uM P significantly reduced conduction velocity in PF and decreased anterograde as well as retrograde conduction at the PF-VM junction. Moreover, at 1 uM P consistently decreased the MRD at any given level of RMP and shifted the membrane responsiveness curve downward and to the right. At 10 uM the RMP was shifted to -52.7 ± 4.9 mV (PF) or to -63.8 ± 3.0 mV (VM) and all fibres became inexcitable within 5 min. P (0.1-5 uM) also significantly shortened in PF the action potential duration measured at 50% (APD_{50}) and 90% (APD_{90}) of repolarization, whereas in VM P (1 and 5 uM) did not significantly modify the APD_{50} but significantly prolonged the APD_{90}. At concentrations up to 1 uM the changes induced in the APD_{90} in PF and VM were followed by parallel changes in the duration of the effective refractory period (ERP), but at concentrations higher than 1 uM P made the ERP long as compared to APD_{90}, increasing the ERP/APD ratio.

P also decreased the spontaneous frequency in PF perfused with Tyrode solution containing 2.7 mM K. This effect was due to a progressive decrease in the slope of phase 4 depolarization and to a shift of the threshold potential to less negative values.

From these results it can be concluded that the mechanisms responsible for the antiarrhythmic action of P in therapeutic concentrations are thus complex but most of them can be explained by a reduction in Na conductance.

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CALCIUM ENTRY BLOCKING AGENTS: COMPARATIVE POTENCY USING RAT AORTA AND TAENIA CAECUM PREPARATION IN VITRO

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Kent et al (1982) have proposed that rat isolated aortic strips can be used to differentiate between vasodilators that act by inhibiting the influx of extracellular calcium and those acting against intracellular calcium. The former type of agent are identified by their greater potency as inhibitors of contractions evoked by barium-induced (Ba) membrane depolarisation and calcium entry relative to their lesser potency in attenuating contractions evoked by phenylephrine (PHE), an effect mediated largely by the release of calcium from inside the cell. We have now adapted this method for the evaluation of calcium slow channel blocking agents (CEB). The results obtained were compared with those from parallel experiments using the guinea pig taenia caeca preparation as described by Spedding (1982).

Rat thoracic aortae were cut into spiral strips (weighing 29± 0.4mg, n=24) and guinea pig taenia caecae into 2 cm lengths (weighing 28± 0.9mg, n=24). Both tissues were set up in a 10 ml organ bath, bathed in physiological fluid maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Krebs solution (Ca^{++} 2.5mM) was used in the case of aortic strips and for taenia the fluid was Ca^{++} and Mg^{++}-free but rich in potassium (40 mM). An initial resting tension of 1 gm was applied in either case. Aortic preparations were contracted by adding barium chloride (1mM). Taenia caecae were contracted by addition of calcium chloride (3 mM). When a sustained plateau had been obtained the agents to be studied were added to produce a series of cumulatively increasing concentrations. The relaxations produced were expressed as a percentage of the evoked contraction and the results subjected to linear regression analysis. Calculations were made of the $-\log_{10}$ molar concentration needed to produce 50% inhibition (pIC50 means± s.e.m. from 5-7 preparations) and the ratio antilog.(pIC50 Ba - pIC50 PHE). The results are shown in the Table.

	AORTA			TAENIA
	Ba	PHE	RATIO	Ca
nicardipine	9.7 ± 0.06	8.0 ± 0.41	45.7	9.2 ± 0.12
nitrendipine	9.4 ± 0.12	7.5 ± 0.39	87.1	9.4 ± 0.08
nifedipine	9.1 ± 0.05	7.4 ± 0.13	50.1	9.0 ± 0.18
verapamil	7.2 ± 0.11	6.5 ± 0.21	5.0	7.4 ± 0.06
diltiazem	6.9 ± 0.07	5.9 ± 0.39	9.8	7.1 ± 0.11
papaverine	6.0 ± 0.16	6.6 ± 0.34	0.3	5.5 ± 0.06
lidoflazine	4.6 ± 0.63	4.6 ± 0.36	1.1	5.3 ± 0.16
cinnarizine	4.6 ± 0.44	4.1 ± 0.49	3.7	5.3 ± 0.18
prazosin	< 4	10.3 ± 0.09	---	---
yohimbine	< 4	6.6 ± 0.04	---	---

Good agreement was obtained for CEB activity between the aorta (BA) and taenia (CA) preparations, perhaps reflecting a similarity in the calcium channels.The dihydropyridines studied exhibited the greatest selectivity (Ba/PHE) for inhibition of external calcium. However, the selectivity of agents that potently block alpha-1 adrenoceptors (like prazosin but unlike yohimbine) in addition to inhibiting calcium should be assessed using other methods.

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NICARDIPINE LOWERS CORONARY AND TOTAL PERIPHERAL VASCULAR RESISTANCE IN ANAESTHETISED DOGS

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Nicardipine inhibits the influx of extracellular calcium through membrane slow channels and is more potent in blood vessels than in cardiac muscle in vitro (Clarke et al 1983). Nicardipine is also more potent in attenuating potassium-evoked contractions in isolated coronary arterial tissue than in other peripheral vessels (Eglen et al 1983). We have now studied the haemodynamic effects of intravenous (iv) nicardipine in anaesthetised dogs with particular reference to its effect on coronary and peripheral vascular resistance.

Mongrel dogs of either sex and weighing 12-15 kg were anaesthetised with pentobarbitone sodium (initially 35 mg/kg and then 5 mg/kg/hr, iv) and ventilated artificially. Recordings were made of the following cardiovascular parameters:- aortic and pulmonary arterial blood pressure, cardiac left ventricular pressure, integrated heart rate (HR), left descending coronary arterial blood flow (CBF) and ascending aortic blood flow (ABF). These were displayed using a Beckman Dynograph and the analogue electronic signals digitized by means of a Buxco Datalogger and subsequently processed on-line using an IBM Personal Computer (model XT). The latter apparatus allowed calculation of the following derived variables:- mean, systolic and diastolic aortic pressures (MAP, SAP, DAP), left ventricular stroke volume (SV), end diastolic (LVEDP)and systolic pressure, contractility (dp/dt/P), left ventricular minute work (LVMW), rate pressure product and coronary and total peripheral vascular resistance (CVR, TPR). Nicardipine (in cumulatively increasing doses) was infused via a saphenous vein. The baseline values (mean ± s.e.m.) and the most important percentage changes (Δ%) measured after 15 min of nicardipine infusion at each dose in 6 dogs are shown in the Table.

Parameter	Baseline value	Δ% during nicardipine infusion (ug/kg/min iv) 0.1	0.3	1.0	3.0
CBF	41.3 ± 12.1 ml/min	24 ± 8	47 ± 13	69 ± 15	98 ± 11
CVR	3.5 ± 0.8 mmHg/ml/min	-16 ± 6	-30 ± 7	-42 ± 7	-61 ± 2
ABF	1.9 ± 0.2 l/min	9 ± 6	17 ± 10	45 ± 25	53 ± 11
TPR	54.4 ± 5.2 mmHg/l/min	-5 ± 4	-12 ± 8	-28 ± 10	-48 ± 6
MAP	98.4 ± 4.7 mmHg	3 ± 2	-2 ± 2	-6 ± 4	-23 ± 5
HR	147.7 ± 9.4 beats/min	4 ± 1	7 ± 2	13 ± 5	12 ± 4

Changes in control animals did not exceed ± 10% of their initial mean values. Nicardipine (3.0 ug/kg/min) increased the initial stroke volume of 12.8± 1 ml/beat by 37± 8% and this effect contributed greatly towards the increased ABF since HR did not change substantially. At the same time and dose level, nicardipine lowered the DAP of 85± 4 mmHg by -30± 5% but the SAP of 123± 8 mmHg decreased only by -13± 4% thereby increasing the pulse pressure. No major changes occurred in contractility or LVEDP. Nicardipine also increased LVMW of 3.5± 0.3 kg.m/min by 52± 15% and any greater oxygen supply needed to support this should readily be met by the marked increase in coronary blood flow. These findings together with those of in vitro studies indicate that nicardipine potently relaxes arterial smooth muscle to produce vasodilatation and that the coronary vascular bed is particularly sensitive in this respect.

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FITTING A LOGISTIC CURVE TO DOSE RESPONSE CURVES USING ALLFIT ON AN APPLE IIE

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De Lean et al's (1976) ALLFIT program enables logistic curves to be fitted to experimental points and has the advantage that two curves can be fit to two sets of data points simultaneously with common, or fixed, slope or maximum. This program has been adapted to run on an Apple IIE.

Each set of data can refer to either a single experimental dose-response curve or can be the mean of several. Data can be added by hand or from a previously created data file (Rees, Roberts et el, 1984). It is then inspected visually in either plot or tabulated form to permit removal of outliers. Once accepted the mean values are stored for input into ALLFIT.

When two sets of data have been stored in this way ALLFIT is run, common or fixed parameters being defined by the user. The mean data is then displayed on the monitor together with the fit line. If the fit is unacceptable the process can be repeated. A hardcopy of the accepted result can then be obtained on an Imagewriter.

Various parameters can then be calculated, depending on the type of assay, such as the dose ratio, the ED50 ratio, the apparent dissociation constant of an antagonist (using the Gaddum or Schild equation), the apparent dissociation constant of a partial agonist (Roberts, 1984), the intrinsic activity of a partial agonist. A hard copy table of these results is then produced.

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IS THE ANTAGONISM BY ADENOSINE OF RESPONSES TO BOTH ISOPRENALINE AND HISTAMINE IN GUINEA PIG ISOLATED ATRIA MEDIATED THROUGH cAMP?

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In addition to the negative inotropic and chronotropic properties of adenosine in mammalian atria, it has more recently been shown to also attenuate the cardiac actions of histamine and catecholamines (Baumann et al., 1981). The proposed mechanism for this effect is an interaction of adenosine with a purine receptor (A1), which results in inhibition of adenylate cyclase and reduced formation of cAMP (Endoh et al., 1983). The action of histamine on the guinea-pig left atria is however H1-receptor-mediated and cAMP-independent (Levi et al., 1982). It was therefore of interest to compare the antagonism of histamine and isoprenaline by adenosine in guinea-pig isolated atria.

Atria were suspended in Krebs-bicarbonate solution at 38°C gassed with 5% CO_2 in O_2. Rate responses were obtained from spontaneously beating right atria and inotropic responses from paced left atria (2Hz, 5ms, threshold voltage + 50%). Cumulative dose-response curves to isoprenaline (in presence of 10^{-5}M metanephrine) or histamine were constructed 3 min after the addition of adenosine (7.5×10^{-5}M). At the maximum responses, adenosine deaminase (0.3U/ml) was added to destroy the adenosine. Dose-response curves in the absence of adenosine were also obtained. Increases in rate or tension were expressed as a percentage of the response seen on addition of adenosine deaminase and the mean values (n=5-8) calculated.

Adenosine (7.5×10^{-5}M) exerted negative inotropic and chronotropic effects, the resting tension and rate being reduced by 55.5±1.6 and 16.1±3.9% respectively. In its presence, the maximum rate responses to histamine and isoprenaline were slightly, but significantly ($P < 0.05$), reduced. Isoprenaline was reduced by 7.7% from 103.3±1.3% of the maximum in the presence of deaminase to 95.3±2.3%. Histamine was reduced by 8.4% from 100.8±0.7% to 92.3±1.4%. There was also a small rightward shift of the dose-response curves, the isoprenaline EC_{50} increasing from $3.61(2.37\text{-}5.48) \times 10^{-9}$M to $5.31(2.33\text{-}12.07) \times 10^{-9}$M (not significant), whilst the histamine curve shifted significantly ($P < 0.05$) from $1.35(0.72\text{-}2.56) \times 10^{-6}$M to $2.75(2.39\text{-}3.17) \times 10^{-6}$M. On the left atria adenosine (7.5×10^{-5}M) significantly ($P < 0.05$) decreased the maximum responses to both agonists. Isoprenaline was reduced by 34.6% from 103.8±2.2% to 67.9±3.6%, whilst histamine was reduced by 47.2% from 130.0±9.0% to 68.6±6.1%. There was also a small, but significant ($P < 0.05$), rightward shift of the dose-response curves in both cases. The isoprenaline EC_{50} was $6.44(3.92\text{-}10.58) \times 10^{-9}$M in the absence and $17.35(10.56\text{-}28.59) \times 10^{-9}$M in the presence of adenosine. The corresponding values for histamine were $1.53(0.99\text{-}2.36) \times 10^{-6}$M and $2.50(2.11\text{-}2.99) \times 10^{-6}$M respectively.

The fact that responses to histamine and isoprenaline in the left atria are antagonised by adenosine in a similar manner suggests an action of adenosine other than inhibition of cAMP formation.

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INFLUENCE OF PHENOXYBENZAMINE, NIFEDIPINE AND D 600 ON VASOCONSTRICTION ELICITED BY ENANTIOMERS OF SK&F 89748A.

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In pithed rats and in rat isolated aorta vasoconstriction via postjunctional α_1-adrenoceptor activation can be mediated by mechanisms dependent on and independent of calcium influx (Timmermans et al., 1983; Beckeringh et al., 1984). Recently, it has been put forward that phenoxybenzamine (PBZ) selectively impairs the calcium influx independent vasoconstrictor mechanisms after α_1-adrenoceptor stimulation (Timmermans et al., 1984). In order to further investigate the effect of PBZ on calcium-influx independent- and dependent contractions after α_1-adrenoceptor activation, we have studied the interaction between the α_1-adrenoceptor agonists d- and l-SK&F 89748A (DeMarinis & Hieble, 1983; Timmermans et al., 1984), PBZ and the calcium entry blockade (CEB) elicited by nifedipine or D 600 in pithed rats and in rat isolated aorta. Male Wistar rats (200-250 g), anaesthetized with hexobarbitone (150 mg/kg, i.p.), were pithed and respired with room air. Dose response (DR) curves were constructed 15 min after i.a. treatment with nifedipine (1-3 mg/kg) and/or phenoxybenzamine (3-1000 µg/kg, i.v., -1 hr). Maximal increases in DBP (mm Hg) were measured. Helically cut strips of thoracic aorta were set up as described previously (Beckeringh et al., 1984). If necessary the strips were exposed to phenoxybenzamine (10^{-6} M) for 6 or 10 min. After repeated washings for 45 min, conc. response curves were constructed. In pithed rats, injection of d- and l-SK&F 89748A in the presence of nifedipine (1 mg/kg) resulted in a slight depression of the maximal increase of DBP. PBZ dose-dependently reduced the maximal response and shifted both DR curves to the right. After PBZ treatment, at low doses (3-10 µg/kg) which by itself did not displace the DR curves, nifedipine induced a considerable depression of the maximal increase of DBP and a rightward shift of the DR curves. After 30 µg/kg of PBZ, the susceptibility to inhibition by nifedipine of the vasoconstriction to both agonists was maximally increased. Except for a slight difference in potency (l->d-, 7x) no difference between the enantiomers existed. In rat isolated aorta both the enantiomers proved partial agonists (pD_2, E_{max}: l-; 7.50, 88.1% (NE=100%), d-; 6.85, 93.6%). Contractile responses to the l-isomer were significantly more susceptible to CEB with D 600. PBZ significantly increased the CEB of D 600. Even with low doses of D 600 present, a remarkable decrease of the maximal response was seen. PBZ treatment eliminated the differential effect of D 600 to d- and l-SK&F 89748A. The results show that both in vivo and in vitro α_1-adrenoceptor -mediated constriction of vascular smooth muscle to both enantiomers of SK&F 89748A is markedly more sensitive towards CEB after treatment with PBZ. The differences between the inhibitory effects of CEB on SK&F 89748A mediated responses in pithed rats and in rat aorta point toward differences in calcium utilization between constriction in both preparations. The finding that after irreversible α_1-adrenoceptor blockade by PBZ in rat aorta the difference in susceptibility to inhibition by D 600 between both enantiomers has disappeared, is in support of the hypothesis that PBZ preferentially affects the calcium influx independent component in α_1-adrenoceptor mediated vasoconstriction.

We thank SK&F Laboratories (Philadelphia, U.S.A.) for the generous gift of SK&F d- & l-89748A.

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SELECTIVITY OF DIFFERENT MUSCARINIC AGONISTS FOR RECEPTORS IN THE HEART, URINARY BLADDER AND SYMPATHETIC GANGLIA IN THE PITHED RAT

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Several authors have concluded both from pharmacological and from radioligand binding experiments that muscarinic receptors do not consist of a homogenous population and that they should be divided into at least two different subtypes (review: Burgen, 1983). Pharmacological experiments have identified the atrial muscarinic receptor to belong to the M_2-type, whereas in the sympathetic ganglia the M_1-receptor type is found (Burgen, 1983). In order to investigate the differential properties of the muscarinic receptors in the heart, bladder and ganglia, and to investigate the selectivity of various muscarinic agonists (McN-A-343 (4-m-chlorophenylcarbamoyloxy)-2-butynyl-Trimethylammonium), pilocarpine, carbachol, oxotremorine, arecoline, β-methylcholine and acetylcholine (ACh)) we studied the effects on heart rate, bladder pressure and diastolic pressure in the pithed rat after pretreatment with the following antagonists: pirenzepine (0.1 mg/kg, M_1-selective, Hammer and Giachetti, 1982), dexetimide (1 mg/kg, mixed $M_1 + M_2$, Wilffert et al., 1983) and gallamine (1 mg/kg and 10 mg/kg, cardioselective, Burgen, 1983). Male normotensive Wistar rats (200-350 g) were pithed and artificially ventilated with room air. I.v. pretreatment with antagonists was performed 15 min before i.a. injection of the agonists (gallamine -5 min). In each animal a complete dose-response curve was obtained after pretreatment with atenolol (10 mg/kg). In the experiments with ACh and β-methylcholine the rats were also pretreated with physostigmine (0.1 mg/kg). Pirenzepine more strongly attenuated the increase in blood pressure than the increase in bladder tension or decrease in heart rate after i.a. injection of the various agonists. On the other hand, dexetimide more strongly inhibited the increase in bladder tension and the bradycardia than the increase in diastolic pressure to the various agonists. Gallamine more effectively blocked the increase in heart rate than the increase in bladder tension, whereas the increase in diastolic pressure was hardly influenced. The following orders of potencies were obtained for the agonists: decrease in heart rate: β-methylcholine (full agonist) = carbachol (full) > ACh (full) > oxotremorine (full) >> pilocarpine (partial agonist) = McN-A-343 (partial). Increase in bladder pressure: carbachol (partial) > β-methylcholine (full) = ACh (full) > oxotremorine (partial) > arecoline (partial) >> McN-A-343 (partial) = pilocarpine (partial). Increase in diastolic pressure: McN-A-343 (full) > pilocarpine (partial) >> arecoline (partial); carbachol, oxotremorine, β-methylcholine and ACh displayed virtually no effect. These results show that McN-A-343 and pilocarpine selectively stimulate M_1-receptors in sympathetic ganglia, whereas the other agonists preferentially activate M_2-receptors in heart and bladder.

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α_1-ADRENOCEPTOR MEDIATED CONTRACTION BY B-HT 920 AND ST 587 IN RAT PERFUSED HINDQUARTERS

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Various studies have shown that vasoconstriction via vascular postjunctional α-adrenoceptors in vitro can be modified by several spasmogens (Day and Moore, 1976; Lues and Schümann, 1984). In the present study we have studied the effect of several spasmogenic agents on vasoconstriction by the selective α_2-adrenoceptor agonist B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-(4,5)-azepine) and the selective α_1-adrenoceptor agonist St 587 (2-(2-chloro-5-trifluoromethylphenylimino)-imidazolidine) in rat perfused hindlimbs. Male Wistar rats (180-250 g) were anaesthetized with hexobarbitone-Na (150 mg/kg, i.p.), artificially respirated, pithed and subsequently prepared. Cannulation of abdominal aorta and vena cava inferior was performed between ileac bifurcation and renal vessels, ileolumbar vessels being ligated. Immediately thereafter abdominal blood flow was interrupted to allow cannulation and perfusion was started. Hindquarter flow was measured at the venous side by means of a drop-counter device. Temperature was maintained at 37°C. Perfusion pressure was held at 60 cm H_2O. Agonists were introduced via a rubber sleeve proximal to the aorta cannula in a volume of 0.5 ml/kg. Perfusion fluid was gassed with carbogen and composed of: 118 mM Na^+, 5.88 K^+, 2.52 Ca^{++}, 1.64 Mg^{++}, 24.88 HCO_3^-, 1.18 $H_2PO_4^-$, 1.64 $SO_4^=$, 11.1 glucose. Agonists were evaluated with respect to their potency and intrinsic activity in reducing hindlimb perfusion flow (% of initial value). St 587-induced reduction of flow was only 20 ± 4.4% (mean ± S.E.M., n=5) at 10^{-5} mol/kg and desensitization occurred rapidly. B-HT 920 did not influence flow up to 3.10^{-5} mol/kg. Adding angiotensin II (A II, 10^{-9}-10^{-6} M, K^+ (20-40 mM) or PGF2α (5.10^{-7}-10^{-6} M) to perfusion fluid did not increase potency or intrinsic activity of both agonists. After treatment of the rats with reserpine (5 mg/kg/day, i.p., 2 days), B-HT 920 only slightly reduced flow (5±2%, mean S.E.M., n=4), whereas after 3-4 day-treatment with reserpine (day 1: 5 mg/kg, day 2-4: 3 mg/kg/day) the intrinsic activity of B-HT 920 was enhanced further (E_{max}=37±4%, ED_{50} was 3±5.10^{-6} mol/kg, mean ± S.E.M., n=6). For St 587 the same pattern was observed: after 3-4 days reserpine treatment E_{max} was 45±4%, ED_{50} was 6±4.10^{-8} mol/kg, mean ± S.E.M., n=8, although desensitization still occurred. Mean initial flow (5-10 ml/min) was not significantly affected by reserpine pretreatment. The α-adrenoceptors involved in the effects of B-HT 920 and St 587 were studied by adding prazosin (3.10^{-8} M) or rauwolscine (10^{-6} M) to the perfusion fluid. For B-HT 920 dose ratio's (DR) of 7±4 and 67±15 (mean ± S.E.M., n=6) were calculated for rauwolscine and prazosin perfused hindquarters, respectively. For St 587 no significant shift could be observed following rauwolscine, whereas for prazosin DR > 100.
It is concluded that in rat perfused hindquarters A II, K^+ nor PGF2α increase the potency or intrinsic activity of B-HT 920 and St 587. Upon pretreatment with reserpine for several days, however, hindquarter contractility to B-HT 920 and St 587 was greatly improved. Both contractions obviously are mediated via α_1-adrenoceptors.

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180P

NALOXONE INDUCED DESENSITISATION OF PERIVASCULAR NOCICEPTORS IN THE ISOLATED PERFUSED RABBIT EAR

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Functional single unit recordings have been made from fine filaments of the auricular nerve in response to bolus arterial injections of 0.3-10µg acetylcholine (ACh) in isolated perfused rabbit ears. The response to ACh in non-myelinated afferents (conduction velocities 0.8-1.5 m/sec) arising from perivascular nociceptors consisted of short bursts of impulses lasting less than 20 seconds and reached peak frequencies of 6-10 impulses/sec. These responses could be repeated at 5 minute intervals with no signs of tachyphylaxis.

The opiate antagonist naloxone, at several concentrations in the range 1-100µg/ml, was perfused through the ear for periods of 15 minutes. At concentrations above 7.5µg/ml naloxone caused a dose-related inhibition of sensory receptor responses to ACh (IC_{50} 45µg/ml). In contrast, indomethacin up to 100µg/ml did not affect receptor responsiveness and morphine showed modest effects only in the concentration range 100-200µg/ml (IC_{50} 167µg/ml)

Naltrexone showed a similar profile to naloxone in inhibiting responses to ACh. The stereoselectivity of the response was examined using the optical isomers MR2266 and MR2267. The potent opioid antagonist MR2266 was found to be 30 fold more potent than its isomer MR2267 which suggests that a major component of the inhibitory effect observed is receptor mediated despite the relatively high perfusion concentration required to inhibit C-fibre responses to ACh. Low concentrations of naloxone were shown to antagonise the inhibitory effects of morphine.

These results are consistent with previous reports of peripheral opiate receptors sited on sensory neurones (Ferreira, 1981; Smith, 1984) and with the observations that agents which are antagonists in other systems are capable of eliciting agonist responses at this site. The isolated perfused rabbit ear represents a novel neurophysiological model for the study of these phenomena.

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EXCITATORY AMINO ACID AGONISTS AND ANTAGONISTS ALTER K^+-EVOKED, BUT NOT BASAL, RELEASE OF [^{3}H]-DOPAMINE FROM RAT STRIATAL SLICES

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L-Glutamate was reported to increase the spontaneous release of ^{3}H-dopamine, whether added exogenously (Roberts & Sharif, 1978; Roberts & Anderson, 1979) or formed within the tissue from ^{3}H-tyrosine (Giorguieff et al, 1977). We set out to characterise the excitatory amino acid receptors responsible for this effect.

Striatal slices (1.0 mm) from male Wistar rats were incubated with ^{3}H-dopamine (50 nM) and superfused with Krebs bicarbonate medium containing pargyline (4×10^{-5}M) at 37°C. After 30 mins the superfusate was collected as 4 minute fractions and the percentage of total tissue radioactivity released during each fraction calculated. Release was expressed as total release above basal for the three fractions after exposure to drug, A; or as the ratio of potassium chloride (25 mM) evoked release (2nd K^+ pulse/1st K^+ pulse), A'/A. Compounds tested on spontaneous ^{3}H-dopamine release were the excitatory amino acid agonists D,L-glutamic acid, D,L-aspartic acid, kainic acid, quinolinic acid, quisqualic acid and N-methyl-D-aspartic acid; and the putative excitatory amino acid antagonists L-glutamic acid diethyl ester, D-α-amino adipic acid and 2-amino-4-phosphono-butyric acid. All were tested at 200 uM, except kainic acid (100 uM).

None of the compounds examined increased the spontaneous release of ^{3}H-dopamine ($P > 0.05$, n = 4-7). However, D-α-amino adipic acid inhibited spontaneous ^{3}H-dopamine release (A = -0.19 ± 0.08, n = 4 compared with control, 0.07 ± 0.05, n = 6; $p < 0.05$). Inclusion of potassium chloride (25 mM) increased release approximately 70-fold (A = 4.825 ± 0.55, n = 4 compared with control).

Table 1 Effects of excitatory amino acid agonists and antagonists on K^+-evoked striatal release of ^{3}H-dopamine

Treatment	Concentration (uM)	n	A'/A
Control	-	6	0.705 ± 0.056
Kainic acid	100	7	1.032 ± 0.107*
L-glutamic acid	200	7	0.971 ± 0.085*
D-α-aminoadipic acid	200	4	0.480 ± 0.014*

* $P < 0.05$ compared with control, Student's t test

L-Glutamate (200 uM) and kainic acid(100 uM), but not L-aspartic acid (200 uM) enhanced the potassium chloride (25 mM) evoked release of ^{3}H-dopamine. Potassium evoked release was decreased in the presence of D-α-amino adipic acid (200 uM).

In contrast to previous reports we find no consistent effect of excitatory amino acid agonists and antagonists on spontaneous release of ^{3}H-dopamine from rat striatal slices. However, potassium evoked release is subject to modulation, suggesting a role for excitatory amino acids in the control of active striatal dopaminergic neuronal function.

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METHOD FOR THE ELECTRICAL STIMULATION OF POSTGANGLIONIC CARDIO-ACCELLERATOR SYMPATHETIC NERVE FIBRES IN PITHED RATS

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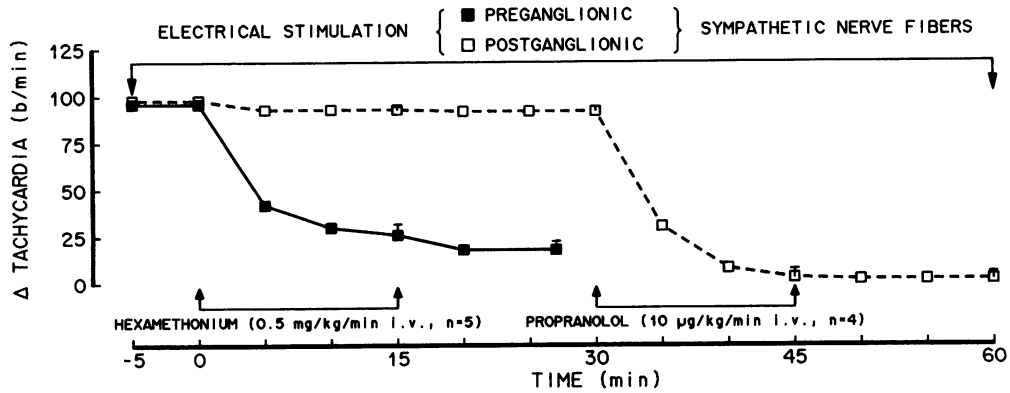
The electrical stimulation of the thoracic portion of the spinal cord in pithed rats is a widely used technique for studying the role of prejunctional α_2-adrenoceptors on sympathetic responses (Drew, 1978, Cavero et al. 1980). However, its shortcoming is that it does not allow to distinguish between sites of action at sympathetic ganglia and/or postganglionic nerve terminals. This communication illustrates the successful achievement of a selective stimulation of postganglionic sympathetic cardioaccelerator nerve fibers in pithed rats.

Male rats (Sprague-Dawley, Charles River Laboratories) were anaesthetized with sodium pentobarbitone, pithed and placed under artificial respiration. The left carotid artery was cannulated for blood pressure and heart rate measurements. Drugs were injected via the left femoral vein. The rat was layed on its left side and the thorax opened after cutting the second and third right ribs. A small bipolar platinum electrode was placed on nerve fibers visible on the ventral side of the descending vena cava below the thymus gland. Sustained electrical stimulation was delivered using 0.3-0.4 Hz, 2 ms and 20 V. The effects of a 15 min i.v. infusion of hexamethonium (0.5 mg/kg/min), propranolol (10 µg/kg/min) and clonidine (0.5 µg/kg/min) were studied on heart rate responses to this stimulation. In a group of rats the thoracic spinal cord was stimulated (0.3-0.4 Hz, 1 ms and 60 V) through the metal pithing rod and hexamethonium administered.

Sustained electrical stimulation of either the thoracic cord or fibers on the ventral site of the descending vena cava, with the above parameters produced stable increases in heart rate of approximately 100 beats/min. Only the effects due to cord (preganglionic sympathetic nerve fibers) stimulation were blocked by hexamethonium. However, the responses to the stimulation of the other nerve fibers (postganglionic) were inhibited by propranolol (see figure) and clonidine. Idazoxan antagonized the effects of the latter agonist.



In conclusion, these results indicate that the nerve fibers located on the ventral side of the descending vena cava are of sympathetic nature and are located postganglionically. Furthermore, they appear to be endowed with prejunctional α_2-adrenoceptors.

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EFFECTS OF RO 15-1788 ON CAUDATE-EVOKED INHIBITION OF NEURONES IN THE RAT SUBSTANTIA NIGRA

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The imidazodiazepine Ro15-1788 has been shown to exhibit weak benzodiazepine (BZ) agonist as well as antagonist properties in a range of behavioural tests (see Vellucci & Webster, 1983). This dual action makes Ro15-1788 a useful tool for studying the possible involvement in GABAergic transmission of any endogenous ligand for the BZ receptor. Whilst electrophysiological studies *in vitro* have shown that Ro15-1788 (Skerritt & Macdonald, 1983) and its desfluoro analogue Ro14-7437 (Krespan *et al*, 1984) can augment the inhibitory effects of exogenous GABA, the latter authors also noted that low concentrations of both compounds tended to reduce electrically-evoked inhibition of hypothalamic and hippocampal neurones. We have therefore studied the effects of different doses of Ro15-1788 on the GABA-mediated inhibition of neurones in the substantia nigra zona reticulata (SNzr) produced by stimulation of the caudate nucleus (CN) (Collingridge & Davies, 1981).

Male Sprague-Dawley rats (250-300g) were maintained on 1-1.5% halothane and extracellular unit recordings obtained with a tungsten microelectrode lowered into the SNzr. Recording sites were located by histological examination. Stimulation of the ipsilateral CN consisted of square-wave pulses of 0.3ms width delivered at 1-2Hz through a bipolar electrode. The current used was just suprathreshold (150-325 μA). The effect on cell firing was measured from peristimulus-time histograms (PSTHs) constructed from 64 or 128 sweeps of 250ms duration. Recordings were made from one cell in each rat. After determining the control period of inhibition, PSTHs were produced 2, 6 and 12 minutes following Ro15-1788 (5 mg/kg i.v.). 15 minutes after this first injection a further 25 mg/kg Ro15-1788 was administered and the duration of inhibition again determined. Results are shown in Table 1.

Table 1. Effects of Ro15-1788 on the duration of electrically-evoked inhibition

Treatment	Control	5mg/kg Ro15-1788			25mg/kg Ro15-1788		
Time(min)		2	6	12	2	6	12
Inhib'n.(ms)	17.7±2.2	10.6±1.5*	11.8±0.9*	13.5±2·1**	20.0±2.8	16.3±2.5	16.8±3.7

Figures represent mean±s.e.mean (*p<0.02, **p<0.01, paired t-test. n=10).

5 mg/kg Ro15-1788 increased (by 30%) basal firing rate in 5 out of 10 cells whilst the 25 mg/kg dose reduced (25%) the firing rate of 6 cells (2 increased, 2 unaffected). The number of sweeps used for each PSTH was therefore adjusted to maintain the number of spikes sampled. It can be seen that whereas 5 mg/kg Ro15-1788 produced a significant decrease in the duration of inhibition this effect was reversed by 25 mg/kg Ro15-1788. 1 mg/kg Ro15-1788 reduced the inhibitory pause in 3 out of 4 cells ($p<0.05$). In 5 further cells the Tween 80 vehicle (30μl per 10ml distilled water) had no effect.

The reduction in electrically-evoked inhibition produced by low doses of Ro15-1788 is similar to that observed by Krespan *et al* (1984) and, as suggested by these authors, may be explained by antagonism of an endogenous BZ-like compound that could be co-released with GABA. The trend towards enhancement of inhibition with a higher dose of Ro15-1788 supports the concept that this compound is a weak partial agonist at the BZ receptor.

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EXCITATORY AMINO ACID INDUCED CHANGES IN CHICK RETINAL CYCLIC GMP LEVELS

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Recent evidence from neurochemical and neuropharmacological studies suggests that excitatory amino acids such as glutamate and aspartate may have a neurotransmitter role within the retina of several species (Ariel et al, 1984; López-Colomé & Somohano, 1984; Marc & Lam, 1981 and Neal, 1982). We have in turn, examined the ability of these compounds to increase the concentration of chick retinal cyclic GMP (cGMP), a neurochemical system that has already been demonstrated as being convenient for assessing excitatory amino acid receptors in the rat cerebellum (Foster & Roberts, 1981).

Retinas free from pigment epithelium were removed from one to two day old chicks in ice cold Krebs-bicarbonate medium, transferred to one maintained at 37 °C for 15 minutes preincubation, following which drug additions were made as appropriate. Tissue cGMP levels were determined as previously described (Foster & Roberts, 1981).

Kainic acid (KA), N-methyl-D-aspartic acid (NMDA) and quisqualic acid (QA) all produced a dose-related calcium-dependent increase in retinal cGMP levels with EC_{50}s of 14 μM, 56 μM and 19 μM respectively, with the response peaking within 5 minutes of drug exposure and then declining. A number of excitatory amino acids and analogues such as L-glutamate, L-aspartate, dihydrokainate, α-ketokainate and α-allokainate displayed similar profiles but possessed a much reduced potency. AMPA and quinolinic acid were ineffective.

The responses to KA, NMDA and QA (tested at their EC_{50} concentrations) were attenuated by excitatory amino acid antagonists (Table 1).

Agonist	Antagonist (IC_{50})				
	(-)APV	cis 2,3 PDA	γDGG	GDEE	GAMS
KA	N.E.	400 μM	N.T.	90 μM	280 μM
NMDA	550 μM	N.E.	300 μM	*	N.T.
Quis.	N.E.	N.E.	460 μM	N.E.	N.T.

* reduced by approx. 50% at 300 μM.
N.E. = not effective.
N.T. = not tested.

These results indicate that activation of retinal excitatory amino acid receptors leads to a marked increase in retinal cyclic GMP levels. This event may be of physiological relevance in the visual process.

Dr A.M. López-Colomé was a visiting scientist from the Department of Neuroscience, CIFICE, UNAM, Mexico.

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B-HT 920 IS A DOPAMINE AGONIST CAUSING LITTLE OR NO MOTOR HYPERACTIVITY, BUT IS IT A SELECTIVE AUTORECEPTOR AGONIST?

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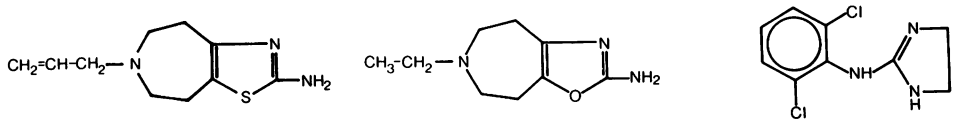
B-HT 920 differs from the structurally related B-HT 933 (α_2 agonist) and from clonidine because it exhibits predominantly dopamine (DA) agonism in the CNS (Anden et al., 1982; 1983). These authors reported that the type of DA agonism exhibited by B-HT 920 was selective for autoreceptors. We have examined effects of B-HT 920 to see if its profile is typical or atypical of DA agonists.

B-HT 920 B-HT 933 **(azepexole)** clonidine



Effects of drugs on locomotor activity (following i.p. treatment with saline, reserpine 5 mg kg^{-1}, or spiperone 0.2 mg kg^{-1}) and on oesophageal temperature were measured in male mice (C. River UK. CD1, 25-30g). Specificity was assessed by the ability of i.p. spiperone or idazoxan (0.02-0.2 mg kg^{-1} and 1.6 mg kg^{-1}; DA- and α_2-antagonists respectively) to reverse the effects. Presynaptic DA agonism was assessed by measuring inhibition of striatal L-DOPA synthesis in male rats (H&C UK. CFY, 175-300g) pretreated with g-butyrolactone (to inhibit dopaminergic nerve impulses and remove postsynaptic feedback) and NSD 1015 (to inhibit L-DOPA decarboxylation). Emetic effects were measured in female beagle dogs (12-14kg).

B-HT 920 (0.2, 0.01), B-HT 933 (10.0, 7.7) and clonidine (0.5, 0.04) caused hypothermia and hypolocomotion in saline pretreated mice (doses mg kg^{-1} s.c. to cause 3° hypothermia and 50% inhibition of locomotion respectively). These effects of B-HT 920 were reversed by spiperone but not by idazoxan. Conversely the effects of B-HT 933 and clonidine were antagonised by idazoxan but not by spiperone. Striatal L-DOPA accumulation was potently inhibited by B-HT 920 (ED_{50} 0.082 mg kg^{-1} s.c.) but clonidine was inactive at 8.6 mg kg^{-1} s.c., and B-HT 933 was only weakly inhibitory at 21.4 mg kg^{-1}s.c. Hyperactivity was not observed with B-HT 920 (0.3-3.0 mg kg^{-1} s.c.) in saline pretreated mice but some reversal ($p<0.01$) of reserpine-induced, but not spiperone-induced, hypolocomotion was observed. This suggests very little potential for action at postsynaptic dopamine receptors mediating hyperlocomotion. B-HT 920 also provoked emesis (ED_{50} 0.017 mg kg^{-1} s.c.).

These results confirm that B-HT 920 is a dopamine agonist rather than an α_2 agonist. The type of DA agonism appears atypical because it does not include marked hyperactivity with increasing doses, however, it appears typical in other respects. B-HT 920 is a potent emetic and it causes marked hypothermia. The emetic effect does not rule out autoreceptor selectivity as DA receptors mediating emesis, though not necessarily located presynaptically, may be pharmacologically similar to those mediating inhibition of locomotion and L-DOPA accumulation (Brown & Campbell, 1984). If B-HT 920 is selective, then hypothermia may also be mediated by receptors pharmacologically similar to DA autoreceptors. Alternatively, if the hypothermia indicates DA agonism of a more general nature, B-HT 920 would then require explanation in terms other than lack of postsynaptic DA agonism.

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IS CHRONIC ADMINISTRATION OF THE BENZODIAZEPINE RECEPTOR LIGAND FG 7142 ANXIOGENIC?

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In a previous communication to the Society we reported that the β-carboline benzodiazepine (BDZ) receptor ligand FG 7142 (N-methyl-β-carboline-3-carboxamide) caused a progressive sensitisation to generalised seizures (kindling) on repeated administration (Little and Nutt, 1984). In this study we have investigated whether chronic FG treatment alters the behaviour of mice in an animal test of anxiety. The paradigm used was the 4-plate test (Boissier et al. 1968) in which exploratory behaviour in a novel environment is suppressed by footshock. Furthermore, the effect of this FG treatment on the anxiolytic activity of chlordiazepoxide (CDP) was determined along with the effect on unpunished exploratory behaviour as a control for non-specific sedative effects.

Male CD1 mice (22-26 g, Charles River) were dosed with 40 mg kg^{-1} FG 7142 (4 mg ml^{-1} in saline/Tween) or vehicle i.p. once daily for 12 days. Six days after the last treatment, mice received CDP (5 mg kg^{-1}) or saline: 30 min later the mice were placed singly in a perspex cylinder with a quadrant grid floor. Following a familiarisation period of 15 sec, plate crossing results in a footshock of 0.3 mA for 0.5 sec. The number of shocks received in a 1 min period was recorded. A separate group of FG and vehicle treated mice were dosed with saline and subjected to an identical test procedure except that foot shock was omitted. In this instance the number of plate crossings was recorded in the 1 min session.

During punished exploratory behaviour, the FG/saline group received fewer shocks as compared with the vehicle/saline group (mean ± SEM, vehicle/saline 8.1 ± 6.7, FG/saline 6.7 ± 0.4, $p<0.02$ Mann-Whitney 'u' test, n = 10). CDP pretreatment significantly increased the number of shocks received in the punished phase, this response releasing (anxiolytic) effect occurring to a similar degree in both groups (vehicle/CDP 11.3 ± 0.9, FG/CDP 10.2 ± 1.0, n = 10). Unpunished exploratory behaviour was unaffected by repeated FG administration (vehicle/saline 13.4 ± 1.8, FG/saline 13.4 ± 1.8, FG/saline 12.8 ± 1.2, n = 5).

The reduced exploration of the FG/saline animals receiving shock as compared with vehicle/saline controls is consistent with raised anxiety in the FG group. This suggests kindled mice have increased susceptibility to both seizures and anxiety although their sensitivity to CDP appears to be unchanged. The lack of effect on unpunished crossings suggests that the effect on punished crossings is not a consequence of non-specific sedation.

We thank Ferrosan for FG 7142 and Roche for CDP.

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Little & Nutt 1984 Br J Pharmac 81, 28P

KINDLING WITH FG 7142 AND BENZODIAZEPINE-GABA RECEPTOR FUNCTION

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In previous communications to the Society we described kindling produced by the β-carboline benzodiazepine (BDZ) receptor ligand, FG 7142 (N-methyl-β-carboline-3-carboxamide, FG) (Little & Nutt, 1984). We have now investigated whether GABA-BDZ receptor function is changed in mice that had been given FG 40 mg kg^{-1} or Tween i.p. once daily for 12 days. All testing was done on day 19, after 6 drug-free days. A challenge dose of FG 40 mg kg^{-1} on day 19 produces seizures in about 5/8 mice. When kindled groups were given 10, 20, and 80 mg kg^{-1} doses of FG, 0/8, 4/8 and 5/8 respectively convulsed, as compared with 0/8 of the Tween groups given these 3 doses of FG ($p \geq 0.6$, $p<0.038$ and $p<0.013$ respectively, Fischer's exact test). This suggests a widening of the in vivo 'u' shaped dose response curve of FG (see Little et al. 1984).

Seizure thresholds were measured using i.v. infusions of bicuculline, pentylenetetrazol (PTZ) and the convulsant β-carboline DMCM, at a rate of 1.1 ml min^{-1}. Thresholds in kindled mice were not different from controls. In addition the anticonvulsant effect of flurazepam FLZ (10 mg kg^{-1}) and muscimol (2 mg kg^{-1}) were tested by giving each drug 15 min before a PTZ infusion. The effects of both drugs were unaltered in the kindled mice.

The sedative effects of FLZ were assessed using automated activity counts of pairs of mice in a new cage, for 1 hour. Kindled mice were significantly less sedated by the 20 mg kg^{-1} dose (acute saline controls: 1706 ± 191 (5)).

Previously we had shown the hypothermic effect of 40 mg kg^{-1} FG was unaltered after kindling. A similar lack of difference was demonstrated with 10 and 20 mg kg^{-1} FG. However, the hypothermic effect of muscimol, 1 mg kg^{-1} was significantly attenuated. Temperatures (mean ± SEM) pre-injection and 30 min post-injection: Controls 37.4 ± 0.2 → 34.9 ± 0.3; Kindled 37.7 ± 0.1 → 35.8 ± 0.2* *$p<0.05$-v-controls at 30 min n = 8.

Convulsion Thresholds (mg kg^{-1})	Control (Tween)			FG 7142 Kindled		
Pentylenetetrazol (2.5 mg ml^{-1})	39	± 1	(6)	38	± 1	(6)
Bicuculline (0.05 mg ml^{-1})	0.41	± 0.02	(7)	0.42	± 0.02	(7)
DMCM (500 μg ml^{-1})	3.5	± 0.2	(8)	3.5	± 0.3	(7)
FLZ 10 mg kg^{-1} i.p./PTZ infusion	107	±11	(6)	104	±12	(8)
Muscimol 2 mg kg^{-1} i.p./PTZ infusion	47	± 5	(7)	51	± 4	(6)
Locomotor Activity						
Flurazepam 20 mg kg^{-1} i.p.	382	±70	(5)	654	±62*	(4)
Flurazepam 40 mg kg^{-1} i.p.	262	±58	(5)	279	±82	(5)

Numbers are mean ± SEM with (n). *$p<0.05$ Mann Whitney 'u' test-v-controls.

These data suggest that kindling does not alter seizure susceptibility to drugs other than FG 7142. However, the reductions in the sedative effect of flurazepam and the hypothermic effect of muscimol may suggest a reduction of function in the receptors subserving these actions.

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Little & Nutt, 1984, Br J Pharmac. 81: 28
Little et al. 1984, Br J Pharmac. in press

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IS FG 7142 MEDIATED KINDLING DUE TO CHANGES IN EFFICACY AT THE BENZODIAZEPINE/GABA RECEPTOR COMPLEX?

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Chronic treatment of mice with the proconvulsant benzodiazepine ligand FG 7142 (N-methyl-β-carboline-3-carboxamide) caused sensitisation to its effects, resulting in the production of convulsions (Little and Nutt, 1984). This "kindling" treatment also produced behavioral changes suggestive of increased anxiety (Jeevanjee et al., this meeting). We now report biochemical studies which indicate that the coupling between GABA and benzodiazepine receptors may be altered in animals so kindled.

Male CD1 mice (30-35g) were injected i.p. with 40 mg kg^{-1} FG 7142 (suspended in Tween 80, 1 drop in 10 ml distilled water) for days 1-12. This treatment has been shown to produce sensitisation to the effect of FG 7142 lasting over a month after cessation of drug treatment (Little et al., 1984). The binding studies in vivo were carried out on day 23 and the binding studies in vitro on day 35 (in separate groups of mice).

Binding studies in vivo were carried out using ^{3}H-flunitrazepam (^{3}H-FNZ) (Jeevanjee et al., 1984). Whole brain total homogenate radioactivity was unaltered in FG 7142 kindled mice compared to Tween treated controls (mean ± s.e.m., control 2316 ± 74 kindled 2454 ± 91 dpm x 10^2 g^{-1} wet weight, n=6). Equally, specific whole brain particulate binding was unchanged (control 1030 ±81, kindled 1046 ± 41 dpm x 10^2 g^{-1}). Non-specific binding was measured using a separate group of control and kindled mice pretreated with 20 mg kg^{-1} clonazepam p.o. and represented 15% of membrane bound radioactivity. These data suggest that FG 7142 treatment did not change benzodiazepine receptor binding or access of benzodiazepine to the brain and that no residual FG 7142 remained in the CNS.

Binding studies in vitro were carried out in cortex, hippocampus and cerebellum (Martin and Doble, 1983), using a single ligand concentration of 0.5 nM ^{3}H-ethyl-β-carboline-3-carboxylate (BCCE) or 0.5 nM ^{3}H-FNZ. No consistent differences were found in any region between control and kindled animals. However, ^{3}H-FNZ binding studies carried out in the presence of 100 μm GABA and 150 mM NaCl (conditions which increase the affinity of the benzodiazepine receptor for agonist ligands, Mohler and Richards, 1981) showed that there was a significant decrease in this facilitation in the kindled animals in cerebellum (control 17,473 ± 1,119, kindled 13,677 ± 899 dpm mg $protein^{-1}$), cortex (control 42,638 ± 3,331, kindled 29,305 ± 2 ,119 dpm/mg protein) and hippocampus (control 45,670 ± 1,297, kindled 38,366 ± 2,328 dpm/mg $protein^{-1}$). Each of these effects were significant at the 5% level, n=6 throughout.

These data suggest that in FG 7142 kindled animals there are no overt changes in the basal binding characteristics of the ligands used, though GABA stimulation of benzodiazepine binding in vitro was significantly reduced. There is considerable evidence to suggest that such a measure provides an indication of the efficacy linkage between the benzodiazepines and the GABA receptor (e.g. Mohler and Richards, 1981). This is consistent with the suggestion that kindling induced by FG 7142 may be associated with changes in the coupling of the GABA-benzodiazepine receptor complex which could underlie the sensitisation observed.

We thank Ferrosan for FG 7142, the Wellcome Trust for finance and Janet Baker and Geoffrey Wright for technical assistance.

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β-ADRENOCEPTOR ANTAGONISTS AND BLOCKADE OF THE 5-HT AUTORECEPTOR IN RAT FRONTAL CORTEX

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Using ligand binding techniques, it has been repeatedly demonstrated that some β-adrenoceptor antagonists, including propranolol, have stereoselective affinity for the 5-HT_1 but not the 5-HT_2 recognition site in rat cerebral cortex (Middlemiss et al., 1977 ; Nahorski and Willcocks, 1983 ; Middlemiss, 1984). Recent studies have demonstrated that propranolol is also a reasonably potent and stereoselective antagonist at the 5-HT autoreceptor in the rat frontal cortex (Middlemiss, 1984). The present communication extends this work by describing the effects of a number of other β-adrenoceptor antagonists at the 5-HT autoreceptor of the rat frontal cortex.

Slices of the rat frontal cortex were loaded with [^{3}H] 5-HT, superfused and continuously stimulated with Krebs solution containing elevated K^+ ions (25 mM) and either paroxetine (3.2 μM) or citalopram (20 μM) to inhibit 5-HT uptake (for detailed methodology see Middlemiss, 1984). Antagonists were added 24 min before the start of the cumulative addition of the agonist, 5-HT (30 nM to 1 μM). The inhibition of the K^+ evoked overflow of tritium by 5-HT was expressed as % of control and was calculated as previously described (Middlemiss, 1984).

At a concentration of 1 μM, the (-)isomer of pindolol was without effect on basal or K^+ evoked overflow of tritium but attenuated the inhibitory effects of 5-HT with an apparent pA_2 of 6.66. In contrast, (-)-alprenolol caused a significant enhancement of K^+ evoked but not basal tritium overflow (+34%) and attenuated the inhibitory effects of 5-HT (apparent pA_2 6.82). At 1 μM, (+)-pindolol and (+)-alprenolol caused an increase in the basal outflow of tritium. Neither the β_1 selective adrenoceptor antagonist atenolol (10 μM) nor the β_2 selective adrenoceptor antagonist ICI 118551 (0.3 μM) showed antagonist activity at the 5-HT autoreceptor.

In ligand binding studies, propranolol, pindolol and alprenolol show stereoselective affinity for the 5-HT_1 but not the 5-HT_2 recognition sites in rat brain membranes (Middlemiss et al., 1977 ; Nahorski and Willcocks, 1983 ; Middlemiss, 1984). In contrast atenolol and ICI 118551 are essentially inactive on both recognition sites (Middlemiss et al., 1977 ; Middlemiss, unpublished observations). These experiments thus lend support to the view (Engel et al., 1983) that the 5-HT autoreceptor may bear a pharmacological resemblance to the 5-HT_1 recognition site. The data also highlight a paradox previously noted (Middlemiss, 1984), namely that significant antagonism of the 5-HT autoreceptor does not necessarily result in an increase in evoked transmitter overflow.

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THE RELEASE OF ENDOGENOUS DOPAMINE FROM SUPERFUSED RAT STRIATAL SYNAPTOSOMES IS POTENTIATED BY ACETYLCHOLINE

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A large number of studies have been focused on the release of dopamine (DA) from striatal tissues. In almost all of these studies the release of radiolabeled DA was analyzed. It has been reported that the basal release of ^{3}H-DA newly synthesized from ^{3}H-tyrosine in rat striatal slices (Giorguieff et al, 1977) and the release of previously taken up ^{3}H-DA evoked by electrical stimulation from rabbit caudate slices (Helmreich et al, 1982; Lehmann and Langer, 1982) or by K^{+}-depolarization from rat striatal synaptosomes (Raiteri et al, 1982) can be potentiated by acetylcholine (ACh) through the activation of receptors of the muscarinic type. In the present investigation the release of endogenous DA and its modification by ACh were studied utilizing rat striatal synaptosomes in superfusion.

Synaptosomes (P_2 fractions) were prepared from adult male Sprague-Dawley rats and superfused with a tyrosine-containing (10 µM) Krebs-bicarbonate medium at 37° C in an apparatus provided with 20 parallel superfusion chambers (Raiteri et al, 1974). The DA present in the superfusate fractions and that remaining in the synaptosomes at the end of superfusion was measured according to Peuler and Johnson (1977).

Table 1 shows that depolarization with 15 mM KCl evoked a release of DA which was totally Ca^{2+}-dependent. Also the basal release was, although in part, Ca^{2+}-dependent. Exogenous ACh, added at 10 µM to the superfusion medium, enhanced (41%) the K^{+}-evoked DA release. Atropine (0.1 µM) totally counteracted the action of ACh.

Table 1

	ng DA/mg protein/min	% of total DA
Basal	0.719 ± 0.110	0.62 ± 0.10
15 mM KCl	2.573 ± 0.174	2.18 ± 0.28
Basal (Ca^{2+}-free)	0.454 ± 0.087	0.40 ± 0.08
15 mM KCl (Ca^{2+}-free)	0.576 ± 0.087	0.54 ± 0.12
15 mM KCl + 10 µM ACh	3.344 ± 0.110°	2.83 ± 0.18
15 mM KCl + 10 µM ACh + 0.1 µM Atropine	2.610 ± 0.120°°	2.21 ± 0.20

° $p < 0.01$ vs 15 mM KCl (calculated as K^{+}-evoked minus basal release)
°° not significantly different from 15 mM KCl

The present data give further support to the idea that DA terminals in the corpus striatum are provided with muscarinic receptors whose activation leads to an enhancement of the release of DA evoked by depolarization.

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EFFECT OF DOPAMINE D-1 RECEPTOR SELECTIVE DRUGS ON DOPAMINE RELEASE AND METABOLISM IN RAT BRAIN IN VIVO

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It is widely believed that dopamine receptors autoregulate dopaminergic neurotransmission in the brain. In support of this idea we have recently shown, using a novel brain dialysis method, that dopamine agonists reduce, while dopamine antagonists enhance, dopamine release and metabolism in the rat brain in vivo (Zetterström & Ungerstedt 1984; Zetterström et al. 1984). On the basis of biochemical studies two types of dopamine receptor, D-1 and D-2, have been identified in rat striatum (Kebabian & Calne 1979). However, due to the lack of selective drugs we have been unable to fully evaluate the selective role of the D-1 and D-2 receptors in mediating changes in dopamine release and metabolism in vivo. Recently, a selective D-1 receptor agonist, SKF 38393, and antagonist, SCH 23390, have been described (O'Boyle & Waddington 1984). Here we report the effects of these drugs on dopamine release and metabolism in the rat striatum in comparison to the D-1/D-2 agonist apomorphine and the D-2 selective antagonist sulpiride.

Sprague-Dawley rats (270-300 g) were anaesthetized with halothane throughout the experiment. Dialysis probes (2 mm dialysis loops glued into inlet and outlet cannulae) were unilaterally implanted into striatum and perfused continuously (2 µl/min) with ringer solution. Perfusate samples were collected every 20 mins and analysed for dopamine, DOPAC and HVA in a single run using HPLC-ED. Drugs were administered after an 80-100 min control period and measurements were continued for a further 120 mins.

Apomorphine (0.05 mg/kg s.c.) caused a maximal 40 % (N=4) decrease in striatal dopamine release 60 min post drug, and this effect was accompanied by a 29 % and 30 % decrease in DOPAC and HVA respectively. In comparison, SKF 38393 (10 mg/kg s.c.) decreased dopamine release to 65 % (N=5) of control values 80 mins after injection with DOPAC (-20%) and HVA (- 12%) decreasing over the experimental period. Sulpiride (250 mg/kg s.c.) increased dopamine release by 60 % (N=5) whereas DOPAC and HVA increased 62 % and 46 % resepctively over the time course. In contrast, SCH 23390 (0.5 and 5 mg/kg s.c.) had no effect on either release or metabolism of dopamine. However, SCH 23390 (0.5 mg/kg s.c.) completely antagonized the decrease in dopamine release and metabolism induced by SKF 38393 (10 mg/kg s.c.) but had little effect on the action of apomorphine (0.05 mg/kg s.c.).

The results indicate that stimulation of D-1 and D-2 receptors can produce a decrease in dopamine release and metabolism in rat striatum. While the D-2 action is probably presynaptic it is unclear whether the D-1 effect is mediated via a pre- or postsynaptic mechanism.

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PROCONVULSANT ACTION OF α2-ADRENOCEPTOR ANTAGONISTS IN MICE: POSSIBLE INVOLVEMENT OF α1-ADRENOCEPTORS

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Selective α2-adrenoceptor antagonists such as idazoxan and yohimbine have recently been reported to lower seizure threshold to chemical convulsants in mice (Fletcher and Forster, 1984). Idazoxan and yohimbine are known to increase central noradrenergic activity in rodents (Walter et al, 1984) and the possibility exists that this effect may underlie their proconvulsant action. In the presence of an α2-adrenoceptor antagonist, endogenously released noradrenaline could act at α1- and β-adrenoceptors, but not α2-adrenoceptors, located at postsynaptic sites. This study provides some evidence for an involvement of α1-adrenoceptors in the proconvulsant action of idazoxan and yohimbine on pentylenetetrazol (PTZ) - induced seizures in mice.

Seizure thresholds were determined in male OMI mice (24 - 30g). PTZ was given by constant-rate infusion (12 mg/ml; 0.1 ml/min) into a tail vein, and the time to onset of tonic seizure was measured. Seizure threshold dose of PTZ was calculated from seizure latency and mouse body weight. Groups of 10-12 fasted mice were used and drugs were administered either p.o. or i.p. 30 min before PTZ infusion.

Both idazoxan (1.25 - 20 mg/kg,p.o.) and yohimbine (1.25 - 10 mg/kg,p.o.) reduced the tonic convulsant dose of PTZ. The proconvulsant effect was not clearly dose-related with either drug and became less pronounced as the dose was increased. Peak reductions in PTZ threshold dose (vehicle controls: 115 ± 9 mg/kg,i.v.) occurred after 2.5 - 10 mg/kg idazoxan (35 - 43 % reduction) and 5 mg/kg yohimbine (30%). The proconvulsant effect of idazoxan (5 mg/kg,p.o.) was antagonized in a dose-related manner by the selective α1-adrenoceptor antagonist prazosin (1 and 3 mg/kg,i.p.), but was unaffected by the β-adrenoceptor antagonist propranolol (3 mg/kg,i.p.). Prazosin (3 mg/kg,i.p.) also antagonized the proconvulsant action of yohimbine (5 mg/kg,p.o.). Prazosin alone caused a small increase (10 - 20%) in PTZ threshold dose whereas propranolol alone was without effect.

The α1-adrenoceptor agonist St 587 (De Jonge et al, 1981) caused a marked dose-related (1-30 mg/kg,i.p.) reduction in PTZ seizure threshold. After 30 mg/kg, St 587 lowered the seizure threshold by 64%, a significantly greater proconvulsant effect than that observed after idazoxan and yohimbine. Prazosin (3 mg/kg,i.p.) caused a 6-fold rightward shift in the dose-proconvulsant response curve for St 587, indicating the involvement of α1-adrenoceptors in the seizure response.

These results indicate that activation of central α1-adrenoceptors can potentiate PTZ-induced seizures in mice. The proconvulsant action of idazoxan and yohimbine could result from an increased stimulation of α1-adrenoceptors by noradrenaline released through antagonism of presynaptic α2-adrenoceptors.

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ANTAGONIST POTENCY OF RX 821002 AND ITS ENANTIOMERS AT α1- AND α2-ADRENOCEPTORS IN RATS

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RX 821002 (2-(2-(2-methoxy-1,4 benzodioxanyl))2-imidazoline hydrochloride) is a more potent and selective α2-adrenoceptor antagonist than the parent antagonist idazoxan (Doxey et al., 1984a). RX 821002 is a mixture of two enantiomers. RX 821019 (R-isomer) and RX 821020 (S-isomer) have been prepared (as hydrochloride salts) and their activities at central and peripheral α1- and α2-adrenoceptors have been determined and compared to RX 821002.

All experiments were performed in male Sprague-Dawley rats (weighing 250 - 350 g). Antagonist potencies of the separate isomers and the racemic mixture at peripheral prejunctional α2-receptors in the vas deferens and postjunctional α2-receptors in the vasculature of pithed rats were determined as the dose (μg/kg,i.v.) required to produce a 2-fold shift (DR2) of the dose-response curve to UK-14,304 (Doxey et al., 1983). Potency at postjunctional α1-receptors was similarly determined against the pressor responses to cirazoline. Activity at central α2-receptors was assessed as the antagonist dose reversing by 50% (AD50; μg/kg,i.v.) the maximal hypotensive and mydriatic responses to 20 and 300 μg/kg UK-14,304 given i.v. to pentobarbitone-anaesthetised rats (Berridge et al., 1983). Affinity (Ki; nM) at α1- and α2-sites was determined from the ability to displace saturable 3H-prazosin and 3H-idazoxan from rat cerebral cortex membranes (Doxey et al., 1984b).

RECEPTOR SYSTEM	TEST	ANTAGONIST POTENCY OR AFFINITY			STEREO-SELECTIVITY
		RX 821002 (RS)	RX 821020 (S)	RX 821019 (R)	
PRE α2-	VAS DEFERENS	1·9±0·5	1·0±0·2	544±168	544
POST α2-	DIASTOLIC B.P.	8·0±2·4	4·7±1·4	972±453	207
CENTRAL α2-	HYPOTENSION	5·9±0·4	2·9±0·5	1620±160	559
CENTRAL α2-	MYDRIASIS	5·0±0·5	2·0±0·03	1836±119	918
CENTRAL α2-	BINDING †	0·8±0·1	0·4±0·1	256±59	640
POST α1-	DIASTOLIC B.P.	176±19	58·9±11·5	12792±1735	217
CENTRAL α1-	BINDING †	66±16	27·0±8·2	6763±573	250

Table 1. Antagonist potency or affinity of RX 821002, RX 821019 and RX 821020 at peripheral and central α1- and α2-receptors. + Ki values (nM); all other potency (DR2 and AD50) values are expressed in μg/kg,i.v.

The S-isomer (RX 821020) was found to be a much more potent antagonist than the R-isomer (RX 821019) in all the *in vivo* test systems studied. (No agonist actions for these compounds were found). A similar marked stereoselectivity was observed in the binding studies. In contrast, Dabiré et al., (1983) reported only a 3-fold separation in the activities of the enantiomers of idazoxan at pre α2-receptors in the rat isolated vas deferens; they also suggested a difference in central and peripheral α2-receptors. Our findings with the stable enantiomers of RX 821002 illustrate (a) the marked stereospecific nature of α2-adrenoceptors and (b) the pharmacological similarity of central and peripheral α2-receptors in rats.

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CAN SUPERSENSITIVITY FOLLOWING CHRONIC CATECHOLAMINE DEPLETION BE FULLY EXPLAINED BY AN INCREASE IN β-ADRENOCEPTOR NUMBER?

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Several investigators have reported an increase in B-adrenoceptor binding sites following chronic catecholamine depletion with 6-hydroxydopamine (6-OHDA) (Yamada, Yamamura & Roeske, 1980) or reserpine (Tenner, Mukherjee & Hester, 1980). This study examines the relevance of this receptor change to the increased B-adrenoceptor responsiveness observed with depleted tissues.

Guinea-pigs were pretreated with 6-OHDA (460 $mgKg^{-1}$ in 6 doses over 20 days) while rats were pretreated with reserpine (1 $mgKg^{-1}$ day^{-1} for 7 days). Papillary muscles were removed from treated and control animals and set up in a Krebs solution gassed with 5% CO_2 in O_2, at 32°C, in the presence of metanephrine (10 M). Tissues were paced at 1 Hz (pulse-width 5 mS; threshold voltage + 50%) and equilibrated for 20 min before construction of a cumulative dose-response curve to isoprenaline. The remaining ventricular tissue was assayed for [^{3}H] dihydroalprenolol (DHA) binding sites using 200 M isoprenaline to calculate non-specific binding. Pretreatment of rats with reserpine resulted in a significant reduction of the isoprenaline EC_{50} from 18.6 (8.2 - 42.0) to 4.3 (2.8 - 6.7)nM. Similarly pretreatment of guinea-pigs with 6-OHDA resulted in a reduction of the isoprenaline EC_{50} from 5.2 (4.1 - 6.6) to 2.0 (1.2 - 3.3)nM. DHA binding experiments revealed that the B-adrenoceptor number in the rat was increased from 25.9 $\pm$ 3.1 to 36.6 $\pm$ 3.2 fmole mg^{-1} protein by reserpine and from 43.1 $\pm$ 6.7 to 65.4 $\pm$ 8.3 fmole mg^{-1} protein by 6-OHDA in the guinea-pig.

The following equation is derived to calculate the fractional increase in receptor number (y) required to explain the difference in EC_{50} between control (A_1) and pretreated (A_2) tissues : $y = \frac{A_2\ (Kd + A_1)}{A_1\ (Kd + A_2)}$ where Kd is the dissociation constant of isoprenaline for the B-adrenoceptor. Using a Kd of 5nM determined previously by pharmacological methods (Broadley & Williams, 1983), it can be calculated that an 80% increase in receptor number is required to explain the observed supersensitivity. Since the increase was found to be only 40-50% it is concluded that an increase in B-adrenoceptor number alone, is unable to explain fully the cardiac supersensitivity observed following chronic reserpine or 6-OHDA pretreatment.

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EFFECTS OF β-ADRENOCEPTOR AGONIST AND ANTAGONIST ON CLONIDINE AND α-METHYLDOPA WITHDRAWAL SYNDROMES IN RATS

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Sudden cessation of stimulation of α_2-adrenoceptors by clonidine in rats evokes an overshoot of heart rate (HR) and lability of blood pressure (BP), due to short lasting blood pressure upswings (Thoolen et al., 1983). Stimulation of presynaptic α_2- and β_2-adrenoceptors decrease and increase, respectively, the intracellular cAMP level by inhibiting respectively stimulating the membrane bound adenylate cyclase (AC). An increased activity of AC during the clonidine withdrawal syndrome might be induced by the long term reduction of the cAMP level during clonidine treatment. In order to study whether the clonidine withdrawal symptoms could be attenuated or potentiated after concomitant treatment with a β-adrenoceptor agonist or antagonist, respectively, we have investigated the effects of continuous infusion and withdrawal of clonidine together with salbutamol, propranolol or saline, and of α-methyldopa (αMD) together with propranolol or saline. Furthermore, in order to discriminate between central and peripheral mechanisms, involved in the clonidine withdrawal syndrome, we also studied the effects on HR and BP of s.c. and i.c.v. infusion of clonidine and s.c. infusion of oxymetazoline. The effects of i.v. and i.c.v. infusion and withdrawal of α-methylnoradrenaline (αMN) on HR and BP were also studied. Conscious rats with permanently indwelling aortic catheters were used. Infusions were performed with Alzet osmopumps. Administration of yohimbine (3 mg/kg, i.p.) to normotensive rats (NR) on the 7th day of oxymetazoline infusion (300 µg/kg/day, s.c.) induced a severe tachycardia (450± 5 bpm); BP upswings did not occur. Cessation of a 7-day i.c.v. infusion with clonidine (30 µg/kg-day) to NR caused maximal 10.9± 0.5 upswings h^{-1}, 8 h after clonidine withdrawal, whereas 8 h after withdrawal of a s.c. infusion with clonidine (30 µg/kg/day) 1.9±0.5 upswings h^{-1} occurred. No withdrawal tachycardia was observed after the i.c.v. or s.c. infusion. Concomitant infusion of salbutamol (12 mg/kg/day, s.c.) and clonidine (300 µg/kg/day,s.c.) in NR attenuated the clonidine withdrawal tachycardia from 433±8 to 380±13 bpm ($p<0.05$), without an effect on the incidence of the upswings. Concomitant infusion of propranolol (18mg/kg/day,s.c.) and clonidine (100µg/kg/day, s.c.) in NR aggravated the HR overshoot from 319±11 to 420±13 bpm ($p<0.05$) and increased the upswings frequency from 4.3±0.4 to 13.3±1.4 h^{-1} ($p<0.05$), 8 h after withdrawal. No difference existed in the isoprenaline-induced tachycardia in pithed NR, 8-14 h after cessation of the treatments mentioned above compared to animals treated with saline. After withdrawal of αMN (300µg/kg/day,i.c.v.) or αMD (200mg/kg/day, intragastric) in SHR a maximal frequency of upswings of 10.3±1.4 respectively 5.6± 0.9 h^{-1} occurred 26 and 8 h, respectively, later; no withdrawal tachycardia was observed. During a 7-day infusion with αMN (1mg/kg/day,i.v.) in SHR tachycardia (464 ±15bpm) occurred; BP was not affected. After αMN withdrawal HR returned to pre-infusion values; no BP upswings occurred. Concomitant 7-day infusion with αMD (200 mg/kg/day, intragastric) and propranolol (18mg/kg/day,s.c.) in SHR increased the maximum incidence of upswings, 8 h after αMD withdrawal to 9.5±0.8 h^{-1}; no withdrawal tachycardia occurred. It is concluded that the clonidine withdrawal tachycardia is mainly due to cessation of stimulation of presynaptic cardiac α_2-adrenoceptors. The interaction of presynaptic α_2- and β_2-adrenoceptors seems to suggest the involvement of the AC/cAMP system. Withdrawal of αMD or αMN evokes upswings,but no tachycardia. On the basis of the present results an explanation for this finding cannot be given. It may, however, be speculated that desensitization of postjunctional β-adrenoceptors, due to long term stimulation by αMN, plays a role.

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A COMPARISON OF THE BINDING OF [^{3}H](+) SKF 10047 AND [^{3}H]PCP IN RAT BRAIN MEMBRANES

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Behavioural and biochemical studies have provided evidence that the psychotomimetic effects of phencyclidine (PCP) and those of the so called 'sigma opiates' such as N-allylnormetazocine (SKF 10047) and cyclazocine are mediated through a common receptor (Holtzman, 1982; Quirion et al., 1981). The present study compares the binding properties of the sigma selective (+) isomer of SKF 10047 with those of PCP in order to investigate further this hypothesis. Binding studies were carried out in rat brain membranes (Alderley Park strain) using 15nM [^{3}H](+)SKF 10047 or 5nM [^{3}H]PCP. Specific binding was defined using 100μM PCP for [^{3}H](+)SKF 10047 binding and 100μM (±)SKF 10047 for [^{3}H]PCP binding. The reaction (45 minutes at 25°C) was terminated by filtration through Whatman GF/C filters pretreated with polyethylenimine to reduce filter binding

[^{3}H]PCP and [^{3}H](+)SKF 10047 showed no significant binding to the traditional opiate sites, mu, delta and kappa, as demonstrated by the finding that naloxone (0.1mM) produced no displacement of either ligand. Further studies showed that the total binding of [^{3}H]PCP displaced by (±)SKF 10047 was similar to the total binding of [^{3}H](+)SKF 10047 displaced by PCP. Scatchard analysis showed that both ligands bound to a similar number of sites (Bmax). This evidence would be compatible with a single binding site for both ligands. However, on comparing the potencies of a range of opioid compounds, with known sigma activity, to displace the specific binding of [^{3}H]PCP and [^{3}H](+)SKF 10047 a poor correlation was observed (r=0.30) (Table 1).

These data provide preliminary evidence for the existence of more than one binding site for [^{3}H]PCP and [^{3}H](+)SKF 10047.

Table 1 Ki values for some standard compounds displacing [^{3}H]PCP and [^{3}H](+)SKF 10047

Compound	*Ki (μM) [^{3}H](+)SKF 10047	[^{3}H]PCP
Bremazocine	0.012	1.7
Pentazocine	0.16	4.0
(±) SKF 10047	0.50	3.0
Cyclazocine	0.58	1.3
Ethylketocyclazocine	0.92	540
PCP	1.1	0.2
+U50488	1.9	3700
Ketocyclazocine	13	8.5
Tifluadom	20	>10000

*Ki's mean of at least two separate experiments performed in triplicate
+Trans-3,4-Dichloro-N-Methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide

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THE EFFECT OF OXYPERTINE ON DOPAMINE, DOPAC AND HVA IN THE RAT STRIATUM

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We have previously reported behavioural evidence for a depleter mode of action for oxypertine (Palomo & Reid, 1983). From our behavioural data it seems that oxypertine may be an amine depleter acting on the reserpine resistant (amphetamine releasable) pool of amines but has no effect on the reserpine releasable one (Palomo & Reid, 1984). However, receptor binding studies have suggested that oxypertine may also be a dopamine receptor blocker (Nakahara & Uchimara, 1980), while behavioural data (Palomo *et al*, 1984) and *in vivo* studies in the cardiovascular system (Combe & Geddes, 1984) failed to demonstrate a dopamine antagonism. Here we have observed the effects of oxypertine on brain dopamine and metabolite levels and also the effect of reserpine (an amine depleter acting on the vesicular pool of amines) and haloperidol (a dopamine receptor blocker).

30 albino male Sprague Dawley rats were used in these experiments. They received i.p. dose of drug solvent or oxypertine (4,8,16 mg/kg) or reserpine (5 mg/kg) or haloperidol (1 mg/kg) and the rat motor behaviour was recorded for 4 min, 90 min later, to confirm previously recorded behavioural observations. The animals were killed immediately by decapitation, the brains removed and the striatal samples stored at -80^{0}C until analysed. Dopamine and its metabolites were estimated by HPLC-ECD based on the method of Taylor *et al*, (1983).

The results in Table 1 show that oxypertine, unlike reserpine, failed to produce a reduction in the level of dopamine in the striatum. Levels of DOPAC were increased but levels of HVA were decreased after oxypertine treatment whereas both dopamine metabolites were increased after haloperidol treatment.

Table 1 Striatum levels of dopamine, DOPAC and HVA (ng/mg protein) $\bar{X}$±S.E.M. n=5

	DOPAMINE	DOPAC	HVA
Control (solvent)	333±23.9	65±4.1	51±2.3
Oxypertine 4mg/kg	397±98.2	186±55.8*	23±4.3**
Oxypertine 8mg/kg	382±69.8	172±41.6*	17±7.5**
Oxypertine 16mg/kg	449±54.6	271±20.6**	22±4.7**
Reserpine 5mg/kg	12±2.5**	103±12.3*	63±6.8
Haloperidol 1mg/kg	264±44.1	169±9.9**	116±14.8**

*,**: $p<0.05$, $p<0.01$ respectively (student t-test)

The above data is in agreement with the postulated mechanism of action for oxypertine as an intraneuronal depleter (hence increasing DOPAC but not HVA) on the reserpine resistant pool of amines. As this pool accounts for less than 10% of the amine content in the neurone (Arbilla *et al*, 1984) it is not surprising that oxypertine fails to produce a significant change in the level of dopamine.

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SR 41378, A NEW POTENTIAL HYPNOTIC DRUG WITH ANTICONVULSANT PROPERTIES

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SR 41378, 3-(4' hydroxy-piperidinyl)-6-(2,4-dichlorophenyl)-pyridazine, is a new, chemically original,hypnotic drug which exhibits anticonvulsant properties, and which appears as potent as pentobarbitone (PB).
Female Swiss mice (CD_1, Charles River, France ; 19-23 g) and male Sprague Dawley rats (CD COBS, Charles River, France ; 250-260 g), were used in this study. The effect of SR 41378 and PB on motor functions was investigated using the traction and rota-rod tests (Boissier et al, 1961). The narcotic effect of these drugs was assessed by measuring the latency and duration of loss of righting reflex in mice, after oral (p.o.) or intravenous (i.v.) administration. Antagonism of metrazol (105 mg/kg, i.p.), bicuculline (0.9 mg/kg, i.v.) and maximal eletroshock (MES)-induced seizures was assessed as described by Chambon et al (1984). The hypnotic effect was investigated in the rat model of experimental insomnia described by Michaud et al (1982).

Table 1

Test Drug	ED50's (mg/kg) Narcosis i.v.	Narcosis p.o.	Traction p.o.	Rota-rod p.o.	Metraz.[a] clonic p.o.	MES tonic p.o.	Bicuc.[a] tonic p.o.
SR 41378	14.5	105	24[b]	32	18	31	8.5
PB	24	52	22[b]	29	49	15	21

[a] Metraz. = metrazol ; Bicuc. = bicuculline ; [b] Pretreatment time : 30 min

As shown in Tab. 1, SR 41378 induced narcosis after either i.v. or p.o. administration in mice. The mean duration of sleep were 30 min at 15 mg/kg, i.v., and 6 hours at 100 mg/kg, p.o.. PB was less effective than SR by i.v. route (duration : 20 min at 25 mg/kg), and more active but of shorter duration (31 min at 50 mg/kg) after p.o. administration. Both drugs were equiactive in the traction and rota-rod tests ; however, in the former model, the effect of SR 41378 was much longer lasting than that of PB (time 3 h : ED50s = 34 mg/kg and > 40 mg/kg, respectively). In the rat model of insomnia, both SR 41378 and PB (15 and 30 mg/kg, p.o.) increased slow wave sleep above the control level during the first 2 hours (controls : 11 ± 10 min ; SR 15 : 30 ± 14* ; SR 30 : 43 ± 13** - PB 15 : 13 ± 8 ; PB 30 : 55 ± 17**) and 4 hours of recording (controls : 69 ± 25 min ; SR 15 : 100 ± 39 ; SR 30 : 134 ± 18** - PB 15 : 57 ± 40 ; PB 30 : 151 ± 20**).
In addition, SR 41378 (30 mg/kg, p.o.) resynchronized the sleep-wakefulness cycle in these animals. Finally, SR 41378 was more active than PB as an anticonvulsant (Tab. 1), and its activity lasted much longer than that of PB. These data indicate that SR 41378 is a new potential hypnotic drug which appears to be, in these experimental conditions as potent as PB, although with a longer duration of action. Finally, it must be noted that SR 41378 exhibits strong anxiolytic properties in a conflict test in the rat (A. Perio et al, This meeting).

* $p < 0.05$; ** $p < 0.01$ vs controls (unpaired Student's t test)

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