ON THE EXISTENCE OF TWO SUBCLASSES OF CENTRAL α_2 PRESYNAPTIC ADRENOCEPTORS REGULATING NORADRENALINE AND SEROTONIN RELEASE

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Noradrenergic axon terminals are endowed with alpha-adrenergic receptors mediating a negative autoregulation of noradrenaline (NA) release. It is widely accepted that these presynaptic autoreceptors belong to the $alpha_2$-subtype.

Presynaptic alpha-adrenoceptors have been reported to exist on central serotonergic nerve endings. Also these presynaptic receptors (heteroreceptors), which mediate inhibition of 5-hydroxytryptamine (5HT) release, appear to be of the $alpha_2$-subtype since they can be blocked by yohimbine but not by prazosin (Göthert et al, 1981; Frankhuyzen & Mulder, 1982; Maura et al, 1982).

Noradrenergic autoreceptors can be blocked by the racemic form of the tetracyclic antidepressant mianserin (Baumann & Maître, 1977). In a recent report (Schoemaker et al, 1981) it was found that only the isomer (+)mianserin was an effective antagonist at the $alpha_2$-autoreceptors, whereas (-)mianserin was inactive. In the present study (+)- and (-)mianserin were compared as antagonists of NA respectively at the $alpha_2$-autoreceptors and at the $alpha_2$-heteroreceptors regulating 5HT release.

All the experiments were performed utilizing synaptosomal P_2 fractions prepared from the cerebral cortex of adult male Sprague-Dawley rats (200-250 g). Synaptosomes were labelled either with ^{3}H-NA (0.04 μM) or ^{3}H-5HT (0.04 μM), 10 min at 37°C in Krebs-Ringer medium, and superfused as previously described (Raiteri et al, 1974).

Exogenous NA inhibited in a concentration-dependent way the depolarization-evoked (15 mM KCl) release of both ^{3}H-NA and ^{3}H-5HT. The inhibition was fully counteracted by equimolar concentrations of yoimbine, but was unaffected by prazosin. The inhibitory effect of NA (1 μM) on its own release was fully antagonized by racemic mianserin (2 μM) or by 1 μM (+)mianserin, whereas at this concentration the (-) isomer was ineffective. In contrast, racemic mianserin, (+)mianserin and (-)mianserin were equally effective as antagonists of NA at the $alpha_2$-heteroreceptors regulating 5HT release.

The results suggest that, in the rat cerebral cortex, the presynaptic adrenoceptors mediating respectively inhibition of NA and 5HT release represent two isoreceptors of the $alpha_2$-subtype.

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OESTROGEN MODULATION OF α_2-ADRENOCEPTORS IN RABBIT PLATELET AND UTERUS

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The interesting observation that oestrogen can exert an opposite modulatory effect on the density of α adrenoceptors labelled with ^{3}H-dihydroergocryptine (^{3}H-DHE) in rabbit platelet and uterus (Roberts et al, 1979) is somewhat tempered by the fact that this ligand (a) does not distinguish between α_1 and α_2 adrenoceptors and (b) does not apparently label the same population of α_2 adrenoceptors as the selective ligand ^{3}H-yohimbine (^{3}H-YOH) in human platelets (Daiguji et al, 1981; Motulsky & Insel, 1982). We have carefully reassessed these studies here using the selective α ligands ^{3}H-prazosin (^{3}H-PRAZ) and ^{3}H-YOH as well as ^{3}H-DHE.

Female New Zealand white rabbits were treated for 14 days with 50 µg 17-β-oestradiol/day sc (OT) or vehicle (VT) before exsanguination and removal of the uterus. Washed platelet and uterine homogenates were prepared and used in binding assays with the three radioligands. Incubations were in each case terminated by filtration. Specific binding was defined as that binding displaced by 20 µM phentolamine and the density of binding sites (B_{max}) determined by non-linear saturation analysis.

^{3}H-DHE and ^{3}H-YOH bound specifically with high affinity to platelet and uterine membranes (K_D: ^{3}H-DHE 2.2 and 2.9 nM; ^{3}H-YOH 7.9 and 4.0 nM respectively). There was no significant difference between the K_D values after vehicle or oestrogen treatment in either tissue. However, the maximum density of specific sites identified by these ligands in platelet membranes was significantly different (Table 1), ^{3}H-YOH labelling only 25% of the ^{3}H-DHE sites. On the other hand, in uterine membranes the density of sites for both the ligands were similar. After oestrogen treatment a much larger decrease was seen in platelet with ^{3}H-YOH (>90%) than with ^{3}H-DHE (30%), whilst in uterus ^{3}H-YOH labelled only 50% of the ^{3}H-DHE sites after hormone treatment (Table 1). These differences were not related to the additional labelling of α_1 adrenoceptors by ^{3}H-DHE, since we were unable to identify these sites with ^{3}H-PRAZ in platelets, and uterine α_1 receptors were unaltered by oestrogen (Table 1).

Table 1 Alpha Adrenoceptor Concentrations (B_{max}) in Rabbit Tissues

	B_{max} (fmoles/mg protein)			
	Platelet		Uterus	
Ligand	VT	OT	VT	OT
^{3}H-DHE	184 ± 37	135 ± 20**	78 ± 22	439 ± 117*
^{3}H-YOH	46 ± 17	5 ± 1*	46 ± 6	222 ± 58*
^{3}H-PRAZ	-	-	61 ± 12	58 ± 12

Mean ± SEM (n=3) * $P < 0.05$ VT v OT ** $P < 0.1$ VT v OT

These results suggest that α adrenoceptor modulation by oestrogen is selective for the α_2 subclass and greater in platelet and less in uterus than previously demonstrated with the non-selective ligand ^{3}H-DHE. The nature of the extra ^{3}H-DHE sites is unclear and is presently being investigated.

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GREATER CONTRIBUTION OF SMOOTH MUSCLE α_2-ADRENOCEPTORS TO VASOCONSTRICTOR RESPONSES IN SHR THAN IN WKY RAT TAIL ARTERIES

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Langer and Medgett (1983) recently reported that tail arteries from Sprague-Dawley (SD) rats, contain α_1- and a subpopulation of smooth muscle α_2-adrenoceptors which contribute to the vasoconstrictor effects of exogenous noradrenaline (NA). The present communication compares the effects of the selective α_2-adrenoceptor antagonist RX781094 (Chapleo et al., 1981) and of prazosin on responses to exogenous NA, methoxamine and periarterial electrical stimulation in SHR and WKY tail arteries.

Male SHR or WKY 13-15 weeks (systolic blood pressures 192 $\pm$ 4 mmHg and 138 $\pm$ 3 mmHg ($p<0.05$), respectively) were used. Segments of proximal tail artery removed under pentobarbitone anaesthesia were perfused and superfused with Krebs' solution at 37°C containing cocaine (4 µM) and propranolol (1 µM). Increases in perfusion pressure at constant flow were induced by two frequency-response curves to electrical stimulation (0.3 ms, 15 V, 0.3 - 3 Hz) followed by a concentration-response curve to NA, or methoxamine.

The pD_2 values of NA in SHR and WKY were not different (SHR: 6.44 $\pm$ 0.08 (n=8); WKY : 6.33 $\pm$ 0.10 (n=8)), however the maximum response was significantly ($p<0.05$) higher in SHR (237 $\pm$ 16 mmHg (n=8) ; WKY : 159 $\pm$ 13 mmHg (n=8)). RX781094 (10 nM - 10 µM) caused concentration-dependent rightward shifts of the NA curve in both SHR and WKY, with no depression of the maximum response but did not significantly antagonise responses to methoxamine at less than 0.3 µM ($-\text{Log } K_B = 6.5 \pm 0.09$). In SHR, RX781094 produced significantly greater shifts for NA than in WKY (Table 1). The wide range of $-\log K_B$ values for NA argues against a homogeneous α-adrenoceptor population. It is concluded that, exogenous NA activates both α_1- and α_2-adrenoceptors, but that there is a greater α_2-adrenoceptor component in SHR than in WKY. The log shift values for prazosin (10 nM) against NA were not different (SHR : 1.01 $\pm$ 0.13 (n=3) ; WKY : 0.93 $\pm$ 0.16 (n=4)), but at this concentration prazosin was significantly more potent against methoxamine in SHR (1.77 $\pm$ 0.23 ; n=6 ; $p<0.01$).

Table 1 : Effect of RX781094 on NA responses in SHR and WKY tail arteries.

[RX781094]		10 nM	0.1 µM	1 µM	10 µM
log shift	SHR	0.31 + 0.05	0.39 + 0.05	0.84 + 0.04	1.74 + 0.05
	WKY	0.20 $\pm$ 0.04*	0.21 $\pm$ 0.05*	0.60 $\pm$ 0.07*	1.18 $\pm$ 0.09*
$-\log K_B$	SHR	8.02	7.16	6.77	6.73
	WKY	7.79	6.77	6.45	6.15

n=4-6 ; *$p<0.05$ compared to corresponding SHR value.

The frequency of half-maximal response to electrical stimulation was between 3 and 10 Hz in both SHR and WKY, the maximum response being similar to the respective NA maxima. In SHR, 10 nM RX781094 significantly reduced responses at 0.3 and 1 Hz, whereas in WKY concentrations below 1 µM were without effect. Prazosin (10 nM) almost abolished the responses to stimulation in both SHR and WKY. It may be concluded that, some smooth muscle α_2-adrenoceptors are involved in the responses to sympathetic nerve stimulation in SHR tail arteries.

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CHARACTERISATION OF RAT ADIPOCYTE β-ADRENOCEPTORS WITH (^{125}I)-CYANOPINDOLOL

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The beta-adrenoceptor has been characterised in many tissues from several mammalian species using techniques ranging from direct radioligand-receptor binding studies to classical analysis of functional responses. Evidence to date suggests that there are predominantly two subtypes (beta$_1$ and beta$_2$). However, studies on the nature of the rat lipolytic beta-adrenoceptor have revealed a site that on the basis of antagonist affinities (Harms et al, 1977) and apparent pD$_2$ values of agonists (Arch et al, 1982) differs substantially from other tissues with well defined beta-adrenoceptors. In an attempt to identify this apparently atypical site we have examined the binding characteristics of the specific ligand ^{125}I-cyanopindolol in rat white adipose tissue.

Binding studies were performed on membranes prepared from isolated adipocytes and whole epididymal fat pads in 25 mM Tris-HCl, 154 mM NaCl, pH 7.8, at 37° for 60 min. Incubations were terminated by filtration and specific binding defined with 200 μM (-)-isoprenaline was about 65% in both preparations at 50 pM ^{125}I-cyanopindolol. The dissociation constant (K$_D$) for the two preparations was very similar (33 ± 4, 29 ± 2 pM) whilst the maximal binding capacity (B$_{max}$) was 66.5 ± 3.0 fmole/mg protein (n = 5) in whole fat pad and 53.3 ± 1.4 fmole/mg protein (n = 5) in isolated adipocytes. The order of potency of catecholamines and stereospecificity of the isomers of propranolol was characteristic of a beta-adrenoceptor. Further, the displacement curves of selective beta$_1$ and beta$_2$ drugs displayed characteristics indicative of receptor heterogeneity in both preparations. Analysis of the curves by computer-assisted iterative curve fitting indicated that the selective compounds bound to two sites with affinities expected at typical beta$_1$ and beta$_2$ adrenoceptors. The proportions of beta$_1$ and beta$_2$ sites were different in the two preparations, whole fat pad membranes containing a high proportion of beta$_2$ receptors. The small proportion of beta$_2$ sites on isolated adipocytes, therefore, may reflect contamination by other cell types. In conclusion, we demonstrate that beta-adrenoceptor binding sites can be directly identified in rat white adipose tissue and are predominantly beta$_1$ in nature.

Tissue	Betaxolol (β$_1$-selective) K_i (β$_1$)	Betaxolol (β$_1$-selective) K_i (β$_2$)	ICI 118.551 (β$_2$-selective) K_i (β$_1$)	ICI 118.551 (β$_2$-selective) K_i (β$_2$)	(-)-Propranolol K_i
Lung	3.39 (23%)	297 (77%)	142 (17%)	3.63 (83%)	0.949
Whole fat pad	9.08 (36%)	571 (64%)	169 (40%)	1.53 (60%)	2.28
Isolated adipocyte	11.5 (81%)	956 (19%)	106 (89%)	1.27 (11%)	2.33

Table 1 Affinities of selective and non-selective antagonists at beta-adrenoceptors. K_i values (nM) were calculated by computerised curve analysis routines. Values in parentheses are relative proportions of each receptor subtype.

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DO ELEVATED PLASMA CATECHOLAMINES CONTRIBUTE TO THE DEVELOPMENT OF VENTRICULAR DYSRHYTHMIAS IN THE CORONARY LIGATED RAT HEART?

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Ceremuzynski et al (1969) suggested that the severity of the dysrhythmias which occur following acute myocardial ischaemia is related to plasma catecholamine levels; these elevated levels of catecholamines have been attributed to enhanced adrenal secretion (Kelliher et al, 1975). The present study examined the possible contribution of adrenal catecholamines to the genesis of the ventricular dysrhythmias which occur following coronary artery ligation (CAL) in the rat. CAL was performed on pentobarbitone-anaesthetized rats (Clark et al, 1980); one group of animals were acutely adrenalectomized at least 30 min before CAL, another group were chronically demedullated 5-6 weeks prior to CAL. Dysrhythmias were recorded for 30 min after CAL. Plasma was collected and catecholamines assayed using HPLC. Control adrenaline and noradrenaline levels in non-ligated rats were 5.1 ± 0.1 and 1.2 ± 0.3 pmol/ml respectively.

Table 1 : Effects of CAL on the incidence of premature ventricular contractions (PVC's), ventricular tachycardia (VT), ventricular fibrillation (VF), mortality and plasma catecholamine levels.

	CAL	CAL + Adrenal Demedullation	CAL + Acute Adrenalectomy
n	10	9	10
MABP (mmHg)	93 ± 7	88 ± 5	87 ± 5
Heart Rate (beats/min)	375 ± 10	413 ± 15	378 ± 7
PVC's (total in 30 min)	633 ± 170	546 ± 195	660 ± 216
% incidence VT	100	100	100
VT duration (sec)	44 ± 16	29 ± 10	34 ± 15
% incidence VF	60	44	60
VF duration (sec)	394 ± 233	361 ± 323	640 ± 274
% Mortality	20	11	30
Adrenaline (pmol/ml)	67.3 ± 33.2	0.3 ± 0.08	0.9 ± 0.23
Noradrenaline (pmol/ml)	20.9 ± 12.4	2.3 ± 0.95	3.3 ± 1.17

From Table 1 it can be seen that adrenalectomy or chronic adrenal demedullation did not protect against ischaemically induced dysrhythmias despite the fact that these procedures resulted in very low plasma catecholamine levels. This suggests that elevated plasma catecholamine levels following CAL are not primarily involved in mediating the dysrhythmias of the early phase of acute myocardial ischaemia.

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ICI 118551 A SELECTIVE β_2-ADRENOCEPTOR ANTAGONIST, INHIBITS FENFLURAMINE-INDUCED HYPERTHERMIA IN THE RAT

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We have previously shown that (-)-propranolol was not active following central (i.c.v.) administration against 5-HT-induced rat body shake behaviour when the 5-HT was also injected i.c.v. (Blackburn & Heapy, 1981). We now present evidence that (-)-propranolol and ICI 118551 are active against fenfluramine-induced hyperthermia in the rat, a test that involves the release of endogenous 5-HT.

Intraperitoneal injection of fenfluramine (15mg/kg i.p.) into groups of 8 Alderley Park SPF female rats (180-200g), maintained at an ambient temperature of 27-28°C, caused a submaximal increase in core temperature of approximately 1.4°C. Core temperature was measured by means of a rectal thermistor probe. The dose of antagonist which reduced the response to fenfluramine by 50%(ID_{50}) was determined by linear regression from a log dose v % inhibition of response plot. The potency of a number of standard 5-HT antagonists and β-adrenoceptor antagonists are presented in Table 1.

Table 1 Antagonism of fenfluramine-induced hyperthermia in the rat

Drug	ID_{50} (mg/kg s.c.)	95% confidence limits
Methergoline	0.007	(0.046 - 0.002)
Pizotifen	0.009	(0.04 - 0.001)
Cyproheptadine	0.019	(0.03 - 0.010)
Cinanserin	0.49	(0.21 - 1.13)
(-)-propranolol	0.33	(0.09 - 1.2)
(+)-propranolol	5.72	(1.56 - 20.8)
ICI 118551	0.81	(0.33 - 1.99)
atenolol	5.0	
butoxamine	10.0	

As can be seen from Table 1, the classical 5-HT antagonists are potent inhibitors of fenfluramine-induced hyperthermia. The (-) isomer of propranolol was significantly more potent than the (+) isomer indicating that the antagonism was unlikely to be due to non-specific effects. Since the (-) isomer of propranolol is a non-selective β-adrenoceptor antagonist this result gives no clear indication of the sub-type of β-adrenoceptor that is involved in this response. However, since the selective β_2-adrenoceptor antagonist ICI 118551 (Bilski et al., 1980) was active, and the β_1-adrenoceptor antagonist atenolol was inactive, then these results indicate that the antagonism of fenfluramine-induced hyperthermia is most likely due to an action at the β_2-adrenoceptor site. A direct action on 5-HT receptors seems unlikely since we have shown that ICI 118551 is not a 5-HT antagonist as measured in [^{3}H]-5-HT binding studies (pIC_{50} < 5.0).

The location of the β_2-adrenoceptor is likely to be within the CNS since (-)-propranolol and ICI 118551, drugs that readily penetrate the blood brain barrier (BBB) (log p values 3.56 and 3.82 respectively) were active, whereas butoxamine, a β_2-adrenoceptor antagonist that does not readily cross the BBB (log p 2.0), was inactive.

Fenfluramine was donated by Servier Laboratories Ltd.

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THE EFFECTS OF α_2-ADRENOCEPTOR AGONISM AND ANTAGONISM AND OF PG-INHIBITION OF THE RESPONSIVENESS OF THE RAT MESENTERIC ARTERIAL BED

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Alpha-adrenoceptor stimulation and inhibition have been shown to alter the pattern of prostaglandin (PG) release from the perfused mesenteric arterial bed of the rabbit (Pipili & Poyser, 1982a). Moreover, α-adrenoceptor stimulation alters the pattern of PG-release from the normotensive rat mesenteric arterial bed in a different manner to that seen in the genetically hypertensive animal (Pipili & Poyser, 1982b). Before investigating the effect of α-antagonism on PG-release from the normotensive and hypertensive rat preparation, the pharmacological effects of α_1 and α_2 antagonism were looked at as well as those of a PG synthesis inhibitor.

The rat mesenteric arterial bed was prepared as already described (Pipili & Poyser, 1982b). The preparations was perfused with Mc Ewen's solution. Doses to noradrenaline (NA) or phenylephrine (PE) were given as bolus injections and dose-response curves were constructed. Responses to 1μg of NA and 10μg of PE were taken as 100% and all other responses were expressed as % of these. Prazosin, rauwolcine and aspirin were continually present in the perfusion fluid when their effect was studied. UK14304 was given immediately prior to NA or PE.

Prazosin (α_1-antagonist) at 10^{-10}, 10^{-9} and 10^{-8}M shifted the D-R curves to NA and PE to the right in a dose related manner as expected. Rauwolcine (α_2-antagonist) at 10^{-8}, 10^{-7}M shifted the D-R curves to the left and enhanced submaximal responses both to NA and PE by 13.0±3.43% (n=6, $p<0.05$) and 12.11±2.27% (n=6, $p<0.05$) for NA and by 34.16±10.5% (n=5, $p<0.02$) and 38.87±8.5% (n=5, $p<0.05$) for PE. Rauwolcine at 10^{-6}M decreased submaximal responses to NA by 16.34±5.7% (n=6, $p<0.05$) where as it left the PE responses unaffected (2.32±6.12%, n=5). UK14304 (α_2-agonist) (Cambridge, 1981) at concentrations from 5×10^{-7}M, -5×10^{-5}M had no effect by itself but reduced submaximal responses to both NA and PE with $ICSO_6$ of $I\times10^{-5}$ and 5×10^{-6}M respectively. Aspirin (cyclo-oxygenase inhibitor) at 10^{-6}M increased responses to NA but more experiments are needed to confirm these observations and their relevance to a-blockade.

These results suggest that
a) There may be a functional α_2-post-synaptic receptor in the mesenteric arterial bed and this receptor may be inhibitory.
b) Rauwolcine and UK14304 also interact with the α_1-receptor in a form of agonism or antagonism.
c) The α_2-receptor may be coupled to a system which opposes the α_1-mediated stimulation. Occupation of α_2-receptor may alter the balance of this coupling.
d) Prostaglandins play a significant role in the responses to NA in the preparation used.

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THE EFFECTS OF A β_1-SELECTIVE ADRENOCEPTOR ANTAGONIST Ro 31-1118 ON AIRWAYS IN VIVO AND IN VITRO IN THE CAT

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β-adrenoceptor antagonists, even those selective for β_1-adrenoceptor sites, produce bronchoconstriction in asthmatics. Åblad et al (1976) suggest that this is due to β_1-adrenoceptors in the lung mediating bronchodilation. The cat has been proposed as a suitable model for investigating the effects of β-adrenoceptor drugs since the ratio β_1:β_2-adrenoceptors resembles that in man (Bowman and Raper, 1976; Daly and Levy, 1979).

Ro 31-1118, dl-(1-(4-(2-(4-fluorophenethyloxy)ethoxy)phenoxy)-3-isopropylamino--2-propanol hydrochloride) has been shown to be a very selective β_1-antagonist following i.v. administration to the cat (Blaber et al, 1983). Using the method described by Blaber (1982) Ro 31-1118 produced some bronchoconstriction at low doses (with a peak of +30% in airways resistance at 10 $\mu g.kg^{-1}$ i.v.). Doses above 30 $\mu g.kg^{-1}$ did not produce bronchoconstriction. The l-isomer produced a similar response but with the dose-response curve shifted to the left. The d-isomer was inactive. These results are in contrast to those of propranolol which produced bronchoconstriction which was dose related (Blaber, 1982).

The bronchoconstrictor action of histamine has been shown to be potentiated by β-adrenoceptor antagonists in the guinea-pig (MacLagan and Ney, 1979). Histamine (50 $\mu g.kg^{-1}.min^{-1}$ for 2 min) did not produce a significant bronchoconstriction in the anaesthetised cat. However, in the presence of propranolol (30 μg. - 1 $mg.kg^{-1}$ i.v.) histamine produced marked bronchoconstriction related to the dose of propranolol. Ro 31-1118 did not alter the response to histamine. In the cat isolated lung parenchyma strip propranolol also produced potentiation of the response to histamine with a peak effect at 10^{-6}M, in this preparation Ro 31-1118 and its isomers were also inactive.

These results support the suggestion that there are β_1-adrenoceptors in the lungs mediating bronchodilation since both Ro 31-1118 and its l-isomer produced bronchoconstriction whereas the d-isomer did not. However this appeared to be a relatively weak mechanism for bronchoconstriction. The lack of bronchoconstriction at higher doses may have been due to the partial agonist activity of the compound. In contrast to propranolol, histamine bronchoconstriction was not observed following the administration of Ro 31-1118 either *in vitro* or *in vivo*. This suggests that blockade of β_2-adrenoceptors is necessary for histamine bronchoconstriction to be observed in the cat.

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NON-COMPETITIVE ANTAGONISM OF PRESSOR RESPONSES TO NORADRENALINE IN RAT MESENTERIC ARTERY BY PRAZOSIN BUT NOT CORYNANTHINE

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A non-competitive blockade of noradrenaline (NA) induced pressor responses in the rat mesenteric artery by the selective α_1-adrenoceptor antagonist prazosin has been described by Adeagbo (1980). We have compared prazosin with another selective α_1-adrenoceptor antagonist, corynanthine, (McGrath, 1982).

Male Wistar rats (220-300g) were killed by stunning and cervical dislocation. Isolated preparations of the mesenteric artery were prepared as described by Doxey et al (1981) and perfused with Krebs solution containing cocaine (10^{-5}M) and propranolol (10^{-6}M). Dose response curves (DRC) were constructed relating increases in perfusion pressure to constant volume (0.1 ml) injections of NA, given at 5 min intervals. Test DRC's were constructed, in the same preparations as controls, in the presence of corynanthine or prazosin. Slopes were compared by the method described by Cohen et al (1979) Preparations were perfused with antagonist for 30 min prior to the construction of test DRC's. In experiments in which Ca^{++}-free Krebs was used, the preparations were perfursed with Ca^{++}-free Krebs for a minimum of 15 min before eliciting responses to NA.

Corynanthine (2.5×10^{-7} M to 2.5×10^{-5} M) caused a shift to the right of the DRC's for NA with no significant changes in slope ($P > 0.05$) or maximum response ($P > 0.05$). Prazosin (10^{-9} M to 5×10^{-8} M) also caused a shift to the right of the DRC's, but significantly depressed the slopes ($P < 0.05$) and reduced the maximum response; e.g. prazosin (5×10^{-9} M) depressed the maximum by 33.5 $\pm$ 8.6% (n = 10). The pressor responses to NA were markedly slowed by all concentrations on prazosin. The time to peak (T_1) and time to half decay (T_2) for equi-effective doses of NA (around 70% of max) with prazosin (5×10^{-9} M) were: T_1 = 39 $\pm$ 6 sec; T_2 = 71 $\pm$ 9 sec and with corynanthine (2.5×10^{-6} M) T_1 = 14 $\pm$ 1 sec; T_2 = 13 $\pm$ 2 sec. compared with control values of T_1 =13 $\pm$ 1 sec; T_2 = 9 $\pm$ 1 sec, (for all, n = 6). These concentrations of prazosin and corynanthine produced the same log shift (1.0) of the threshold response to NA.

Since constrictor responses of the rat mesenteric artery are resistant to Ca^{++} lack (Adeagbo & Okpako, 1980) we have repeated the above experiments in preparations perfused by Ca^{++}-free Krebs to determine the effect of the antagonists on constrictor responses to NA which are presumably mediated by intracellular Ca^{++} release. Corynanthine had effects similar to those seen in normal Krebs, whereas the effects of prazosin were enhanced, such that 5×10^{-9} M prazosin caused a marked flattening of the DRC and a 65.9 $\pm$ 6.5% ($P < 0.001$) depression of maximum response. Corynanthine (10^{-5} M) was able to reverse completely the depressant effect of 5×10^{-9} prazosin on pressor response in Ca^{++}-free Krebs.

It is concluded that prazosin and corynanthine are acting on the same receptor, but that only prazosin causes a non-competitive depression of NA induced intracellular release of Ca^{++}, this effect therefore appears unrelated to its selectivity for α_1-adrenoceptors.

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NAFAZATROM, PROSTACYCLIN AND ZK36374 SUPPRESS REPERFUSION-INDUCED VENTRICULAR FIBRILLATION IN ANAESTHETIZED GREYHOUNDS

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We have suggested previously (Coker et al., 1981) that thromboxane (Tx) A_2 may be arrhythmogenic whereas prostacyclin may be antiarrhythmic. Inhibition of Tx synthesis is particularly effective in reducing the incidence of ventricular fibrillation (VF) following reperfusion of the ischaemic myocardium (Coker et al., 1982). The aim of the present study was therefore to investigate the effects on reperfusion arrhythmias, of prostacyclin, a more stable analogue ZK36374 (Ciloprost) and nafazatrom (BAY g 6575), a drug which has been reported to increase prostacyclin concentrations in vivo (Vermylen et al., 1979).

Greyhounds, anaesthetised with chloralose, were prepared for occlusion of the left anterior descending coronary artery (LAD). Catheters were placed in the coronary sinus, draining the essentially normal myocardium, and in a local coronary vein draining the area rendered ischaemic by occlusion of the LAD. Plasma concentrations of TxB_2 (the stable metabolite of TxA_2) and 6-keto $PGF_{1\alpha}$ (a stable metabolite of prostacyclin) were measured by radioimmunoassay. Nafazatrom 10 mg/kg was administered orally approximately 30 min prior to anaesthesia (3½ to 4 hr prior to LAD occlusion). Prostacyclin and ZK36374 (5 ng $kg^{-1}min^{-1}$) were infused directly into the coronary circulation via a catheter which had been positioned in the left circumflex coronary artery under fluoroscopic control. Drug infusions were started 15 min prior to LAD occlusion and maintained for the duration of the experiment.

All three drugs markedly reduced the incidence of VF following the release of a 40 min coronary artery occlusion. In the control group 8 out of 9 dogs fibrillated, 7 of them within 1 min of reperfusion whereas the incidence of VF was 1 out of 8 in the prostacyclin group and 1 out of 7 in both the nafazatrom group and in those receiving ZK36374 ($P<0.01$). None of the drugs altered the release of TxB_2 from the ischaemic myocardium. During LAD occlusion the concentrations of 6-keto $PGF_{1\alpha}$ tend to increase in local coronary venous blood. For example, in the control group the pre-occlusion value of 446±91 pg/ml had increased to 797±259 pg/ml 15 min after occlusion and the corresponding values in the dogs receiving ZK36374 were 548±59 and 810±117 pg/ml. The infusion of prostacyclin increased coronary sinus 6-keto $PGF_{1\alpha}$ concentrations from 328±55 to 2057±445 pg/ml and throughout LAD occlusion the local coronary venous concentrations were similar, e.g. 1810±458 pg/ml at 15 min post-occlusion. In the dogs pretreated with nafazatrom the 6-keto $PGF_{1\alpha}$ concentrations were not significantly higher than those in the control group prior to coronary artery occlusion but by 15 min post-occlusion the local coronary venous values had increased from 671±71 to 1477±154 pg/ml. This latter figure is significantly greater than the corresponding values in the control and ZK36374 groups ($P<0.05$). Thus nafazatrom enhances the release of prostacyclin during acute myocardial ischaemia and in contrast to the control group, significant increases in 6-keto $PGF_{1\alpha}$ were also observed in the coronary sinus (553±87 to 845±132 pg/ml 15 min post-occlusion $P<0.05$).

These results suggest that drugs which increase prostacyclin concentrations in the coronary circulation or mimic the actions of prostacyclin can suppress reperfusion-induced VF.

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THE ELECTRICAL AND MECHANICAL RESPONSES OF THE CIRCULAR MUSCLE OF THE GUINEA-PIG ANAL SPHINCTER TO FIELD STIMULATION AND TO DRUGS

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Two types of spontaneous electrical activity were observed during intracellular electrical recording: a) A continuous or intermittent spike discharge (30-40 mV) which was sometimes superimposed on a membrane depolarization; b) A continuous discharge of large spikes (up to 60 mV in amplitude) with no initial preceding depolarization but with a noticeable after-hyperpolarization. There was no significant difference in the membrane potential of each group (-45±3 mV, mean ± S.D., n=114). In both cases the electrical responses were accompanied by development of tone and, usually, mechanical oscillations. Field stimulation (supramaximal, 0.5 msec) using single pulses or trains (5 pulses at 5, 10 and 20 Hz) of stimuli abolished the spikes, hyperpolarized the membrane and relaxed the muscle in the presence or absence of guanethidine ($1-3\times10^{-5}$M) or phentolamine (5×10^{-8}M) and atropine (10^{-6}M). The hyperpolarization and the mechanical relaxation were maintained throughout a prolonged (15 sec, 2 and 5 Hz) period of field stimulation. Spike discharge frequently reappeared during prolonged field stimulation. Tetrodotoxin (5×10^{-6}M) abolished the response to field stimulation without affecting spike discharge. Hexamethonium (3×10^{-5}M) was ineffective. Noradrenaline (3×10^{-6}M) increased the rate of spontaneous discharge, depolarized the membrane and contracted the circular muscle; these effects were inhibited by phentolamine (5×10^{-8}M). Isoprenaline (3×10^{-6}M) and acetylcholine (5×10^{-8}M) did not change the spontaneous electrical or mechanical activity. Adenosine triphosphate (ATP 2×10^{-5}, 2×10^{-4} and 2×10^{-3}M) abolished spike discharge, hyperpolarized the membrane and relaxed the muscle. In the presence of atropine (10^{-6}M) and phentolamine (5×10^{-8}M) the electrical and mechanical responses to field stimulation were unaffected by apamin (10^{-7} and 2×10^{-7}M). The electrical and mechanical effects of field stimulation and of ATP were inhibited by reactive blue -2 (procion blue, 5×10^{-5}M, Kerr & Krantis, 1979) which also abolished spontaneous electrical activity. The results indicate that the circular muscle of the guinea pig anal sphincter has both excitatory α adrenergic and inhibitory non-adrenergic non-cholinergic receptors. There is no evidence of cholinergic mediated responses. Results with apamin suggest that non-adrenergic non-cholinergic responses resemble those in the bovine retractor penis rather than those in the taenia coli (Bowman & Gillespie, 1982).

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ELECTROPHYSIOLOGICAL ACTIONS OF PLATELET ACTIVATING FACTOR ON GUINEA-PIG PAPILLARY MUSCLES

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The platelet activating factor (PAF) is a phospholipid which induces not only platelet aggregation but also chemotaxis and degranulation in polymorphonuclear leukocytes, hypotension and vasopermeability (Blank et al, 1979; Sánchez-Crespo et al, 1982). In addition, PAF itself stimulated Ca^{2+} influx into rabbit platelets (Lee et al, 1981). In the present study we have investigated the effects of PAF on the electrophysiological properties of guinea pig-papillary muscles.

Guinea-pig papillary muscles were perfused with Tyrode solution (34ºC) and stimulated at a basal rate of 1 Hz. Intracellular action potentials were recorded with glass microelectrodes (Rodriguez & Tamargo, 1980). Ca^{2+}-dependent slow action potentials were elicited by adding isoprenaline (10^{-6}M) to high K^+ (27 mM)-Tyrode solution and the muscles were stimulated at 0.15 Hz.

PAF, 10^{-11}M, caused a significant shortening in action potential duration (APD) at both 50% (from 97.4 ± 6.0 ms to 58.3 ± 6.5 ms. n=12. $p < 0.01$) and 90% (from 127.5 ± 12.5 ms to 82.4 ± 6.8 ms. $p < 0.01$) level of repolarization. This shortening of the APD was not potentiated when the drug concentration was progressively increased from 10^{-11}M to 10^{-7}M. Moreover, in these range of concentrations, PAF increased the resting membrane potential and the amplitude and maximum rate of rise of the action potential. The shortening of the APD was significantly reduced in papillary muscles perfused with tetraethylammonium (10 mM). Furthermore, PAF (10^{-11}M - 10^{-7}M) did not shortened but prolonged the APD in papillary muscles perfused with a Tyrode solution containing verapamil (10^{-6}M).

PAF did not restore the propagated slow action potentials in depolarized papillary muscles. However, accumulative addition of PAF (10^{-11}M - 10^{-9}M) produced a concentration-dependent increase in the resting membrane potential and in amplitude and maximum rate of rise of the slow action potentials elicited by isoproterenol; the duration of these slow action potentials was also prolonged by PAF. Changes in all these parameters were significant ($p < 0.05$) at concentrations higher than 10^{-11}M. Furthermore, in the presence of PAF, 10^{-10}M, the amplitude of the slow action potential raised from 29.1 to 42 mv per 10-fold change in Ca concentration in the perfusate (0.9 - 9.0 mM).

These results suggest that in guinea pig-papillary muscles PAF increases Ca^{2+} influx via the slow inward current. A possible effect of PAF on K^+ conductance is discussed.

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LYMPHOCYTES, INFLAMMATION AND FIBRIN STUDIES IN AN EXPERIMENTAL FOOT-OEDEMA MODEL OF THE KOCH PHENOMENON

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Inflammation characterised by the appearance of extravascular (e-v) fibrin deposits and oedema has been shown to develop in association with tissue localisation of lymphocytes in rabbit skin homografts (Bach and Jasani, 1976; Bach et al., 1978 and Bullock et al., 1977). The present studies were carried out to investigate whether inflammation and tissue accumulation of re-circulating T lymphocytes induced by M. tuberculosis inoculated into the foot pad of immunised animals (Kostiala et al., 1978) was similarly accompanied by the development of fibrin deposits and oedema.

Heat killed M. tuberculosis, human strains C, DT and PN mixed, was finely ground and freshly suspended (10 mg/ml) in liquid paraffin, B.P. The mixture, widely referred to as Freund's complete adjuvant (FCA), was used for both the immunisation and challenge procedures. Immunisation - on day 0 male COB Wistar rats (Charles River, 200-250 g) were anaesthetised i.p. using methohexitone sodium (40 mg/kg) and inoculated subcutaneously in the scruff with FCA (75 µl). They were then allowed to recover, caged in groups of 6 and allowed food and water ad libitum. Challenge - on day 6 they were re-anaesthetised between 9.00-10.30 hours, inoculated sub-plantar in the right paw with FCA (50 µl), then allowed to recover and were cared for as before, but over sawdust instead of the standard metal grid base. Formation of e-v fibrin deposits was detected microscopically using the method of Roberts (1977) and quantified using ^{125}I-human fibrinogen, B.P., 2-4 µCi (Amersham International, PLC) administered i.v. either 1 h before or 6, 30 or 54 h after challenge. Oedema was measured as the increase in the fresh weight of the inoculated, compared with uninoculated paws, after correction for increase in paw blood content.

Following challenge, formation of e-v ^{125}I-fibrin deposits and foot oedema reached a maximum at 24 h. Thereafter, ^{125}I-fibrin levels ($\pm$ s.e.) progressively declined: 376 $\pm$ 30, 269 $\pm$ 21 and 133 $\pm$ 63 c.p.m. x 10^3 per paw at 24, 48 and 72 h respectively. Oedema persisted unaltered in severity: 1034 $\pm$ 36, 907 $\pm$ 10 and 1144 $\pm$ 116 mg per paw at 24, 48 and 72 h respectively. Fresh ^{125}I-fibrin formation was found to occur between 24 and 48 h and 48 and 72 h in amounts (100 $\pm$ 8 and 101 $\pm$ 15 c.p.m. x 10^3 per paw) sufficient to compensate for that lost via removal through fibrinolysis. Typical pericellular fibrin deposits (Bach et al., 1978) were histologically visible at 24 h through to 72 h. They were associated with the presence in the ev space of lymphocytes, macrophages and PMN leucocytes.

Depletion of the re-circulating pool of T lymphocytes by drainage of thoracic duct lymph was found to inhibit ^{125}I-fibrin and oedema formation by 52 $\pm$ 5 and 43 $\pm$ 7 per cent respectively ($P<0.01$). Warfarin sodium (3 and 10 mg/kg) i.v. 32 h before challenge was found to inhibit ^{125}I-fibrin formation by 45 $\pm$ 8 and 44 $\pm$ 3 per cent respectively ($P<0.01$); n = 6 in all data reported.

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BRONCHIAL HYPERREACTIVITY TO HISTAMINE INDUCED BY HAEMOPHILUS INFLUENZAE VACCINATION IN GUINEA-PIGS

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Noncapsulated forms of the bacterium Haemophilus influenzae often can be isolated from the deeper respiratory airways of patients with chronic asthmatic bronchitis. We previously showed that vaccination of guinea pigs with killed suspensions of this bacterium reduced the in vitro response to β-mimetic stimulation of pulmonary β-adrenoceptors, while the contractile response of isolated tracheal spirals to carbachol was potentiated (Schreurs et al., 1980 a,b). Moreover, the number of β-adrenoceptors as measured by radio-ligand binding was decreased (Schreurs and Nijkamp, 1982). Since all previous effects were shown in in vitro models we evaluated in the present study whether vaccination with H. influenzae results in bronchial hyperreactivity in vivo. Therefore two in vivo models were tested. First, reactions to aerosolized histamine, where the time for asphyxial collapse was recorded. Second, i.v. histamine in anaesthetized, artificially ventilated, guinea pigs, where increases in intratracheal pressure were determined.

Animals exposed to histamine aerosol (0.4%) alone, collapsed in 155 ± 12 s (n=10). However, H. influenzae pretreated guinea pigs (5 x 10^8 killed organisms/100 g b. wt., i.p., 2 days before testing) were significantly more sensitive to histamine and became asphyxial in 123 ± 10 s (n=10, $p < 0.05$). This difference from the controls was also found 4 days after H. influenzae vaccination (151 ± 9 vs. 125 ± 9 s, $p < 0.05$) and gradually disappeared thereafter (day 6: 150 ± 11 vs. 125 ± 8 s, n.s.; day 8: 149 ± 9 vs. 135 ± 11 s, n.s.).

In separate experiments possible protection by isoprenaline or atropine was measured in vehicle treated animals and in 2 days H. influenzae vaccinated animals. Following isoprenaline (30 µg/kg, s.c.) vaccinated animals collapsed significantly faster than vehicle treated animals. However, the degree of inhibition was the same in both groups, so probably the initial difference in histamine sensitivity was maintained. In the model used, bronchoconstriction by aerosolized histamine is mainly mediated by a vagal reflex (Boushey et al., 1980). Interestingly, atropine (1 mg/kg, i.p.) protected much better for asphyxia in 2 days H. influenzae vaccinated animals than in controls (274 ± 71 vs. 104 ± 5 s, respectively, $p < 0.05$). These results therefore suggest a hyperreactivity of the parasympathetic nervous system as a result of H. influenzae vaccination. This is in accordance with in vitro data, where an increased contractile response of isolated tracheal spirals to carbachol was shown (Schreurs et al., 1980 b).

Furthermore, the dose dependent bronchoconstriction established by i.v. histamine (3 - 50 µg/kg) in anaesthetized guinea pigs was significantly potentiated in H. influenzae pretreated animals. Maximal potentiation was seen at 3 µg/kg (controls: 0.5 ± 0.1 and H. influenzae: 3.4 ± 1.4 mm Hg). After isoprenaline (20 µg/kg) again there was a significant difference between the groups although the percentual degree of inhibition was not statistically different.

In conclusion, H. influenzae vaccination results in an increased bronchial sensitivity to histamine in vivo, in which a hyperreactivity of cholinergic pathways seems to be the predominant feature.

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INHIBITORS OF TRANSMETHYLATION DECREASE HISTAMINE RELEASE FROM RAT PERITONEAL AND HUMAN DISPERSED LUNG MAST CELLS

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Increased phospholipid methylation following the cross-linkage of IgE-Fc receptors on rat peritoneal mast cells (RPMC) and leukaemic basophils is an early biochemical event which precedes histamine secretion (Ishazaka et al, 1980; Crews et al, 1981). An essential role for methylation reactions in stimulus-secretion coupling in human basophils and rat mast cells and basophils is indicated by the inhibition of histamine release by compounds which inhibit S-adenosylmethionine-dependent methyltransferases (Morita et al, 1981; Morita and Siraganian, 1981).

We have investigated the importance of methyltransferase reactions in IgE-dependent histamine release from both RPMC and human dispersed lung mast cells (HDLMC). RPMC obtained from male Sprague-Dawley rats were purified to >95% by isopycnic centrifugation through Percoll. Human lung cells containing 2-5% mast cells were dispersed from chopped lung tissue by incubating with pronase and chymopapain. Washed cells were preincubated for 1 h at 37°C with 3-deazaadenosine (DZA), DZA in presence of 100uM L-homocysteine thiolactone (Hcy), or erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA). Cells were challenged with goat anti-human IgE and histamine release, measured fluorimetrically, expressed as net percentage of total.

In RPMC 100uM Hcy alone had no effect on anti-IgE induced histamine release. In the presence of Hcy, DZA (1-500uM) gave dose-dependent inhibition of histamine release by up to 89.1 ± 2.7% (mean ± s.e. mean, n=3). The IC_{50} was 33.4 ± 6.3uM DZA. The combination of DZA and Hcy inhibited maximum histamine release without significantly affecting time course of release (n=3).

In HDLMC, DZA alone (1-1000uM) partially inhibited IgE-dependent histamine release at high concentrations (IC_{50} 534 ± 113uM, n=4). In the presence of Hcy, the IC_{50} for DZA decreased to 32.9 ± 14.3uM and the maximum inhibition was 90.0 ± 2.5% (n=5). The adenosine deaminase inhibitor EHNA (1-1000uM) inhibited histamine release by a maximum of 89.8 ± 1.3% with an IC_{50} of 119 ± 21uM (n=4). In HDLMC the slope of the linear portions of the dose-response curves for the three pharmacological treatments were not significantly different, suggesting that they may have a common mechanism of action. Furthermore, the dose-reponse curves for DZA plus Hcy for RPMC and for HDLMC were almost superimposable.

These results suggest that as in RPMC lipid and possibly protein methylation have an essential role in IgE-dependent release of histamine from HDLMC.

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THE EFFECT OF PASSIVE SENSITISATION ON DEXTRAN INDUCED HISTAMINE RELEASE FROM RAT MAST CELLS

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IgE sensitised rat mast cells selectively secrete histamine when exposed to appropriate concentrations of specific antigen (Ag), antirat IgE serum (Anti IgE) or concanavalin A (Con A). This secretion is associated with cross-linking of IgE fixed to specific receptors in the cell membrane, and each of these secretagogues is potentiated by phosphatidylserine (PS) and antagonised by disodium cromoglycate. Selective release may also be initiated by dextran or compound 48/80. However, whereas dextran induced release is potentiated by PS (Goth et al, 1971) and antagonised by cromoglycate (Hanahoe et al, 1972) release by compound 48/80 is not (Read et al 1977). It is therefore possible that dextran induced secretion shares a common release mechanism with Ag, AntiIgE and Con A. In these experiments this hypothesis has been examined. Rat (Tuck, Wistar) peritoneal mast cells were passively sensitised by the intraperitoneal injection of rat antisera (0.1ml in 4ml of Tyrode solution) against Nippostrongylus braziliensis (PCA titre $\simeq$ 650). Cells were harvested 4 days later by peritoneal lavage with heparinised (10µg/ml) Tyrode solution. Histamine release from these cells was compared with histamine release from control, non-sensitised cells. Sensitisation of the cells exposed to N. braziliensis antiserum was confirmed by comparing release from these cells by Ag or Anti IgE with that from nonsensitised cells.
In the presence of PS (10µg/ml), dextran released histamine in a dose-dependent manner, 10mg/ml releasing approximately 30% of the total histamine content. Sensitisation greatly enhanced dextran induced secretion, 10mg/ml now releasing approximately 60% of the total histamine. When the sensitised mast cells were acid-washed to remove the cell-fixed IgE (Ishizaka and Ishizaka, 1974) release by Ag, Anti IgE and dextran was greatly reduced. Thus, washing the cells in pH 4 Tyrode reduced histamine release by dextran from approximately 60% to 20%. However, histamine release by compound 48/80 (1µg/ml released approximately 60%) was neither enhanced by sensitisation nor reduced by washing at acid pH. These results suggest that perhaps dextran may release histamine from rat mast cells by interacting with cell-fixed IgE.

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THE EFFECT OF POSTNATAL THYROID DEFICIENCY ON GLUCOSE METABOLISM AND ACETYLCHOLINE-SYNTHESIS IN RAT CORPUS STRIATUM

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Consistent with morphological evidence of impaired neuronal arborization in corpus striatum of thyroid deficient developing rats (Lu & Brown, 1977) we found 25-40% deficits in striatal choline transport (ChT) and choline acetyltransferase (ChAT, Kalaria et al, 1981a) suggesting the development of fewer cholinergic nerve terminals than in euthyroid rats. Although ^{14}C-ACh synthesis from ^{14}C-glucose was impaired, $^{14}CO_2$ production was increased in neocortex biopsies in Alzheimer's disease but unchanged in striatum of senescent rats (Sims et al 1981, 1982). We therefore similarly investigated glucose utilization in striatum of thyroid deficient developing rats.

Female Wistar rats with 4 day-old litters were fed propylthiouracil (PTU) (0.3% w/w in 41B meal) ad lib, for 6-7 weeks. Washed striatal slices (0.1 x 0.1 x approx. 1 mm) from 3 or 6 week-olds were incubated with ^{14}C-glucose (2.5 mM; choline chloride 0.2 mM; paraoxon 58 µM; 50 mg tissue) and $^{14}CO_2$ production measured (Sims et al, 1981). ^{14}C-ACh was then recovered from the slices by dipicrylamine extraction in dichloromethane (Gibson & Peterson, 1981).

In 5 independent treatments (approx. 100 neonates total) PTU impaired ^{14}C-ACh synthesis by 25-39%. This effect was statistically significant ($P < 0.05$) on all nine occasions it was assayed using tissue from 3 or 6 week-olds and media containing 5 or 31 mM-K^+ (example single treatment, Table 1). Assays in 5 mM-K^+ indicated the PTU treatments did not affect $^{14}CO_2$ production; 31 mM-K^+ revealed 21-46% deficits, although these effects did not consistently prove significant.

Table 1.

K^+ (mM)	3 week-olds		6 week-olds	
	^{14}C-ACh	$^{14}CO_2$	^{14}C-ACh	$^{14}CO_2$
5	36.69 (2.08;6)	1680 (115;6)	53.14 (1.79;6)	1745 (84;6)
	26.35*(3.30;6)	1785 (50;6)	34.46*(4.52;5)	1771 (118;6)
31	56.45 (4.22;6)	2325 (74;6)	71.36 (3.42;5)	2679 (156;6)
	36.07†(3.99;5)	1846 (232;6)	43.48‡(3.89;6)	2055 (229;6)

Synthesis (cpm/h/mg tissue, counting efficiency 85%), upper figures age matched controls, lower figure PTU treated rats; in parentheses SEM and number of rats sampled, single sample and single assays per rat. U-^{14}C glucose 0.5 Ci/mol.
* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, Student's two tailed t-test.

Striatal ^{14}C-glucose metabolism in thyroid deficient developing rats more closely resembles that in senescence than that of neocortex in Alzheimer's disease. Impaired ^{14}C-ACh synthesis is consistent with impaired ChAT activity and ChT in striatum of hypothyroid rats. That K^+-stimulated production of $^{14}CO_2$ may be impaired raises the possibility of reduced intraterminal glucose metabolism and acetylCoA synthesis. However the conversion of ^{3}H-choline into ACh in striatal slices is not affected by neonatal thyroid deficiency (Kalaria et al, 1981b) suggesting the cholinergic nerve terminals that develop are unimpaired in capacity for acetylation of choline. Deficits in ChAT activity, ChT and ^{14}C-ACh synthesis therefore seem most likely to result from the development of fewer terminals.

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CROSS TOLERANCE BETWEEN ETHANOL, MORPHINE AND BARBITURATES IN THE ELECTRICALLY-STIMULATED VAS DEFERENS FROM C57B1 AND LACG MICE

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C57Bl mice prefer ethanol solutions to water when offered a choice and are relatively insensitive to its CNS depressant effects (Unwin & Taberner, 1982). In LACG mice ethanol produces aversion and also has more potent CNS effects. The present work has compared the potency of ethanol in inhibiting electrically-induced contractions of the isolated vas deferens from control and ethanol-tolerant mice of either strain.

Adult C57Bl and LACG mice from inbred colonies were used throughout. Mice were rendered tolerant to ethanol by a chronic drinking schedule (Unwin & Taberner, 1980). Single vasa were set up for stimulation in modified Krebs solution using essentially standard procedures (Hughes et al, 1975).

Morphine ($5x10^{-7}$M-$4x10^{-6}$M), met-enkephalin ($2x10^{-10}$M-$2x10^{-9}$M), sodium barbitone ($2x10^{-4}$M-$8x10^{-4}$M), and ethanol (100mM-800mM) all produced dose dependent inhibition of evoked contractions that was reversible on washout of drug. In control LACG mice, however, ethanol produced an increase in amplitude of the contractions.

Vasa deferentia from ethanol-tolerant C57Bl mice were tolerant to the effects of both ethanol and morphine. The ID_{50} for ethanol was 366mM in control mice compared to 566mM in ethanol tolerant mice. In LACG mice made behaviourally tolerant to ethanol there was no evidence of tolerance to ethanol or morphine on the vas deferens. However, tolerance to morphine was evident in vasa deferentia from LACG mice previously made dependent upon morphine (by depot injection) or sodium barbitone (by chronic feeding). All doses of morphine were less effective on vas deferens from barbiturate-treated mice, suggesting some degree of tolerance, although the differences were not significant. The inhibitory effects of met-enkephalin ($4x10^{-10}$M-10^{-9}M) were significantly enhanced in both ethanol tolerant LACG and C57Bl mice.

Cross tolerance between morphine and ethanol has already been shown in the guinea pig ileum, although the drugs are recognised to have different sites of action (Mayer et al, 1980). The present work confirms these findings in a preparation in which morphine is thought to act presynaptically to inhibit noradrenaline release (Hughes et al, 1975). The changes observed in ethanol tolerant animals imply an alteration in membrane function associated with opiate receptors in the vas deferens induced by chronic ethanol consumption.

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THE DOPAMINERGIC SYSTEM AND SPONTANEOUS OROFACIAL DYSKINESIA IN AGED RATS DURING VERY LONG-TERM NEUROLEPTIC TREATMENT

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There is conflicting evidence as to whether neuroleptic treatment for substantial periods of a rats adult lifespan is associated with enduring dopamine (DA) receptor blockade and the emergence of persistent spontaneous orofacial dyskinesia possibly analogous to clinical tardive dyskinesia (Clow et al, 1980; Gunne et al, 1982; Crow et al, 1982; Waddington et al, 1982, 1983). We describe here studies on the effects of 6 month neuroleptic treatments in aged animals, as both spontaneous and tardive dyskinesia may be more serious with advancing age (Smith & Baldessarini, 1980).

Young (3 months, 400 g) and old (1 year, 700 g) male Sprague-Dawley rats were treated via drinking water with haloperidol (HAL, 0.9-2.4 mg/kg/day) or fluphenazine (FPZ, 0.8-2.0 mg/kg/day). After 6 months of treatment they were assessed for spontaneous behaviour and then for stereotypy responses to 0.15 mg/kg s.c. apomorphine (APOM). Neuroleptics were subsequently withdrawn and striata assayed for ^{3}H-spiperone binding (0.8 nM) 11 days later. At the 6 month point stereotypy to APOM was markedly and indistinguishably antagonised ($P < 0.01$) in all neuroleptic groups. Similarly, they were subsequently shown to have indistinguishable increases in striatal ^{3}H-spiperone binding (+29-51%, $P < 0.05$).

There was a weak trend towards decreases in both control binding and neuroleptic-associated changes with ageing. The pattern of prevalence of orofacial dyskinesia distinguished ($P < 0.05$) these groups: young controls 14%; HAL 8%; FPZ 38%; aged controls 50%; HAL 27%; FPZ 50% ($N = 10-14$). The principle effects contributing to heterogeneity were the raised prevalence in young non-butyrophenone-treated animals (Waddington et al, 1982,1983) and a generalised effect of age in increasing the overall prevalence of dyskinesia ($P < 0.05$), such that in aged animals neuroleptic effects were less prominent.

The emergence of spontaneous orofacial dyskinesia was independent of both enduring functional DA receptor blockade and associated DA receptor supersensitivity, and was more prevalent generally in aged animals. This further supports the propositions (Crow et al, 1982; Waddington et al, 1982, 1983) that dyskinesia thought to emerge as a consequence of long-term neuroleptic treatment may (i) not have a DAergic pathophysiology and (ii) be contaminated by non-neuroleptic-associated factors, especially in ageing populations.

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BIOLOGICALLY-MEANINGFUL STIMULI ACTIVATE BOTH OPIOID AND NON-OPIOID ENDOGENOUS ANALGESIA MECHANISMS IN MALE RATS

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It is now widely recognised that exposure to certain forms of environmental stimulation (e.g. footshock, cold water swim) can activate endogenous analgesia mechanisms in rats and mice (Bodnar et al, 1980). Furthermore, the biological relevance of these findings is suggested by recent reports that male mice exhibit opiate-like analgesia in response to conspecific attack (Miczek et al, 1982; Rodgers & Hendrie, 1983). Intriguingly, similar experience fails to induce post-encounter analgesia in male rats (Rodgers & Hendrie, 1982). As inter-male fighting in rats is of a highly ritualized nature, we have argued that this apparent discrepancy in results may relate to variation in the stimulus factors involved rather than species differences in endogenous analgesia mechanisms per se. To test this hypothesis, the present study examined whether male rats would develop opiate-like analgesia as a result of exposure to non-ritualized attack, such as that characteristic of lactating conspecifics.

36 adult virgin female rats (250-350 g) and 72 naive adult male rats (300-400 g), from Bradford University hooded Lister colony, were used as subjects. At the beginning of the experiment, 36 mating pairs were formed, with the remaining males (n=36, intruders) housed in like-sex groups of 4/cage. On the day of parturition, breeding males were removed and females left undisturbed (with litters) until testing. All animals were maintained on a reversed L/D cycle, in a temperature-controlled environment (24±1°C) with food and water freely available. On test days (post-partum day 9), baseline tail-flick latencies were established for male intruders, using the radiant heat method. Ten minutes prior to their introduction into the females' cages, males were injected (i.p.) with saline, 0.1, 1.0 or 10.0 mg/kg naloxone hydrochloride (n in each condition = 9). Immediately following the ten-minute encounter period with resident females, tail-flick latencies were re-established for male intruders.

Analysis of variance indicated significant main effects for drug ($F(3,32) = 3.2$, $p < 0.05$) and time ($F(1,3) = 30.4$, $p < 0.05$). Within-groups comparisons (correlated t-tests) revealed significant post-encounter elevations in tail-flick latency in all groups (saline, $p < 0.001$; 0.1 mg/kg, $p < 0.02$; 1 mg/kg, $p < 0.02$; 10 mg/kg, $p < 0.001$). However, between-groups comparisons (Dunnett's tests) indicated that the analgesic reaction in animals treated with low doses of naloxone was significantly less than that in saline-treated controls (0.1 mg/kg v saline - $p < 0.025$; 1 mg/kg v saline - $p < 0.05$). These data demonstrate that exposure to biologically-meaningful stimuli activates endogenous analgesia mechanisms in male rats. However, the profile of partial naloxone antagonism suggests that this form of environmentally-induced antinociception in rats has both opioid and non-opioid components.

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PREVENTION AND AMELIORATION OF NEUROLOGICAL DEFECTS IN ACUTE EXPERIMENTAL DIABETES BY SORBINIL, AN ALDOSE REDUCTASE INHIBITOR

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Treatment of diabetic rats with the aldose reductase inhibitor ICI 105552 prevents the development of reduced axonal transport and motor nerve conduction velocity (MNCV), defects which are characteristic of untreated acute experimental diabetes (Tomlinson, Holmes & Mayer, 1982). The present study was designed to examine possible reversal and prevention of these defects using a stucturally different aldose reductase inhibitor and to relate the effects to nerve sorbitol and *myo*-inositol concentrations. Male Wistar rats (290-310 g) were made diabetic by injection of streptozotocin (50 mg/kg i.p.). The first series of experiments comprised three groups; controls, diabetic untreated and diabetic given daily sorbinil (25 mg/kg p.o.) throughout the period of diabetes. MNCV (sciatic) was measured the day before streptozotocin and again 3 weeks later. After three weeks' diabetes accumulation of choline acetyltransferase (ChAT) activity, proximal to a 24 h ligature applied to the left sciatic nerve, was measured as an index of axonal transport. Blood glucose and sciatic nerve concentrations of sorbitol and *myo*-inositol were also measured. All procedures are described elsewhere (Tomlinson et al., 1982). The results (see Table) show that sorbinil prevented the development of sorbitol accumulation and maintained normal MNCV, ChAT accumulation and nerve *myo*-inositol without affecting the severity of diabetes. In a second series of experiments diabetic rats were untreated for three weeks to allow development of the defects. One group were then treated with sorbinil (25 mg/kg/day p.o.) for three weeks whilst the other remained untreated. MNCV was measured weekly, the other variables after 6 weeks' diabetes. The untreated diabetics showed the defects seen in the 3 week group (see Table). The other group showed reduced MNCV after 3 weeks (-5.6±0.9 m/sec; $p<0.01$), which was later reversed after 3 weeks' sorbinil (+4.0±1.3 m/sec; $p<0.05$). Sorbinil treatment also reversed the reductions in ChAT accumulation and nerve *myo*-inositol and returned nerve sorbitol to normal levels. These findings show that aldose reductase inhibition prevented and ameliorated neurological defects in acute diabetes and that the effects may be related to either nerve sorbitol or *myo*-inositol levels.

Mean values (±SEM) after 3 or 6 weeks' diabetes (number of rats in brackets)

Treatment	MNCV (m/sec)	ChAT accumulation (n mol ACh/h/nerve)	Sorbitol (n mol/mg nerve)	*myo*-Inositol (n mol/mg nerve)	Blood glucose (m mol/l)
Controls (12) (non-diabetic)	+2.2±1.5	5.7±0.5	0.08±0.01	2.30±0.10	6.3±0.2
		**		*	
3 wk Diabetic untreated (11)	-6.2±0.7**	2.8±0.4	1.56±0.22	1.47±0.10	22.5±1.4
		**		*	
3 wk Diabetic + sorbinil (6)	+3.5±1.0*	7.1±0.6	0.06±0.03	2.28±0.12	27.5±1.2
6 wk Diabetic untreated (9)	-9.3±1.5**	3.6±0.3	1.48±0.14	1.73±0.17	25.8±0.9
		**		*	
6 wk Diabetic + sorbinil (6)	see text	6.1±0.4	0.15±0.05	2.68±0.34	29.1±1.9

*$p<0.05$; **$p<0.01$ by either paired (MNCV changes) or unpaired (other data) t-tests.

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EFFECTS OF NORADRENALINE INFUSION ON CARDIAC OUTPUT AND ITS REGIONAL DISTRIBUTION IN CONTROL AND STREPTOZOCIN-DIABETIC RATS

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Several studies have demonstrated increased vascular reactivity to noradrenaline (NA) in tissues isolated from diabetic rats e.g. rat aorta (Cseuz et al, 1973), and perfused mesentry (Callingham et al, 1982). Pithed diabetic rats, however, were found to be less sensitive to the pressor effects of NA (Foy & Lucas, 1976). This contrast may be due to low cardiac output (CO) in NA stimulated diabetic rats, reduced NA sensitivity in non-mesenteric vascular beds, or endogenous factors opposing NA. It was decided to investigate these possibilities using the microsphere method for estimating CO and its regional distribution.

Male Wistar 200-300g rats received 55mg Kg^{-1} streptozocin or vehicle only i.v. and were used 14 days later. Diabetes was confirmed in the former group by body weight and blood glucose measurement on the test day. Animals were pithed under ether and respired with O_2 (1 $ml.100g^{-1}$,50 cycles min^{-1}). Cannulae were placed in the left ventricle via the right carotid for microsphere injection in the right femoral artery for blood withdrawal and pressure recording and in the right femoral vein for NA infusion. CO and its regional distribution were estimated before, and 5 min after beginning infusion of 1.16 x 10^{-8}mol NA Kg^{-1} min^{-1} using 15μ dia ^{46}Sc and ^{99}Tc labelled microspheres. Plasma NA was estimated by HPLC with electrochemical detection using dihydroxybenzylamine as the internal standard. A 2 ml blood sample was taken from the ventricular cannula for this purpose, and for blood glucose estimation, at 5.5 min, during continued NA infusion.

Mean blood pressure reached a plateau in both groups within 5 min of NA infusion. The pressor reponse was smaller in the diabetic group (Table 1). Mean plasma NA and blood glucose concentrations at 5.5 min in control and diabetic groups were 0.92 and 1.16 x 10^{-7} $mol.L^{-1}$ and 10 and 48 $mmol.L^{-1}$ respectively. NA induced vasoconstriction in several areas e.g. both intestines was reduced in diabetic rats. In other areas e.g. the hind leg, responses were similar in both groups (Table 1).

Table 1 Mean blood pressure changes and responses in 2 areas to NA infusion in control (C) and diabetic (D) rats.

		Pre NA		+NA		Change	
		C	D	C	D	C	D
Mean b.p. (mm Hg)		53±6	56±7	134±6	100±14[a]	81±6	44±10[b]
Small intestine	%CO	14±1.3	14±1.8	16±1.3	26±2.9[c]	2±1.9	12±1.8[b]
	R	6.2±0.8	5.6±0.7	13±1.5	6.5±1.1[c]	6.4±1.9	0.9±1.1[a]
Left hindleg	%CO	3.4±0.2	3.6±0.3	2.6±0.2	2.3±0.3	-0.7±0.2	-1.3±0.4
	R	25±3	24±3	72±5	71±14	47±4	47±3

data: mean s.e. mean a P<0.05 b P<0.01 c P<0.005
n = 7 in both groups. Resistance (R) units are $mmHg.min.ml^{-1}$

These results are a marked contrast to those of several in vitro studies and suggest that the constrictor effects of NA, particularly in mesenteric vessels may be opposed by factors not present in vitro and that this effect is greater in streptozocin diabetic rats than in controls.

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INFLUENCE OF CAPTOPRIL ON RENAL ON RENAL FUNCTION DURING RENAL NERVE STIMULATION IN THE RAT

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It is now recognised that renal sympathetic nerves innervate both the vasculature and tubules of the kidney (DiBona, 1982). Recent studies in the rat have shown that during adrenergically mediated renal vasoconstriction (Arundell & Johns, 1982; Ball & Johns, 1982), the regulation of glomerular filtration rate was dependent upon an intact renin-angiotensin system. The present study was conducted to investigate whether the other major influence of the renal nerves, on tubular sodium reabsorption, was also dependent on the generation of angiotensin II.

The left kidney of sodium pentobarbitone anaesthetised male Sprague Dawley rats (340 - 400g) was exposed by a midline abdominal incision and the left ureter cannulated. The renal nerves were isolated and prepared for stimulation (15V, 0.2 msec). Renal blood flow was measured using a non-cannulating flow transducer and electromagnetic flowmeter and glomerular filtration rate was estimated by the clearance of inulin. Control values were taken as a mean value of the two clearance periods before and after stimulation and were compared to the value obtained during stimulation.

In saline-infused animals (n = 6), renal blood flow was 1.70 ± 0.11 ml min^{-1} $100g^{-1}$ and was reduced by 15% during renal nerve stimulation. Glomerular filtration rate was unaltered from a control value of 0.39 ± 0.03 ml min^{-1} $100g^{-1}$ whilst the control values of absolute and fractional sodium excretion of 1.60 ± 0.27 µmol min^{-1} $100g^{-1}$ and 2.49 ± 0.50% were reduced by 36.5 ± 5.0% and 34.0 ± 5.8% respectively. In a second group of animals (n = 7), the angiotensin converting enzyme inhibitor captopril was infused i.v. at a rate (0.38 mmol kg^{-1} h^{-1}) which did not alter b.p. but abolished the systemic pressor and renal vasoconstrictor responses to i.v. angiotensin I (77 pmol). Control values of renal blood flow (2.41 ± 0.15 ml min^{-1} $100g^{-1}$) and glomerular filtration rate (0.52 ± 0.02 ml min^{-1} $100g^{-1}$) were significantly greater ($P < 0.01$) in captopril-infused than saline-infused animals. Absolute and fractional sodium excretion values of 1.91 ± 0.17 µmol min^{-1} $100g^{-1}$ and 2.07 ± 0.12% respectively were similar to those observed in the saline-infused animals. Renal nerve stimulation, causing a 15% reduction in renal blood flow, decreased glomerular filtration rate by 15.6 ± 1.8% ($P < 0.001$). Reduction in absolute sodium excretion of 30.7 ± 4.2% was similar to that observed in the saline-infused animals. Fractional sodium excretion, a variable independent of changes in the filtered load, was reduced by 18.3 ± 4.4%, a response significantly less ($P < 0.05$) than that observed in the absence of captopril.

These observations support the view that angiotensin II is involved in the regulation of glomerular filtration rate during modest nerve mediated reductions in renal blood flow, possibly by modifying efferent arteriolar tone. They also show that an intact renin-angiotensin system is necessary for the full expression of the renal nerves on tubular sodium reabsorption.

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EFFECT OF SARALASIN ON BLOOD PRESSURE, AFTER ADRENALECTOMY, OF RATS WITH ISOLATION-INDUCED HYPERTENSION

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We have shown that male Wistar rats housed individually in glass metabolism cages become hypertensive within 5 days (Gardiner & Bennett, 1977). Animals kept under these conditions for 6 weeks remain hypertensive; at this stage adrenalectomy causes a fall in BP, but BP rises again within 4 weeks of the operation (Bennett & Gardiner, 1982). We have now investigated the possible involvement of angiotensin II in the hypertension seen in isolated, adrenalectomized rats.

Male Wistar rats (body weight 240 - 260 g) were housed individually in glass metabolism cages ("Metabowl", Jencons) for 6 weeks prior to bilateral adrenalectomy (n=6) or sham operation (n=6). Subsequently, all animals (still housed individually) were given 1% saline to drink. Four weeks after operation rats were anaesthetized with sodium methohexitone (60 mg/kg i.p.) and catheters implanted in the abdominal aorta for BP recording and in the right jugular vein for drug administration. The experiments (performed 5 h later, when the animals were fully conscious) involved infusion of saralasin (11 nmol/kg/min; 0.6 ml/h) for 240 min, while BP and heart rate were recorded continuously.

The results are summarized in the table. There were no significant differences between the BP or heart rates in the 2 groups before saralasin. At the end of the experiment, the pressor response to angiotensin II (21 nmol) was inhibited by more than 90%.

Table: Effect of saralasin infusion on BP and heart rate (mean ±s.e.mean)

	Sham-operated			Adrenalectomized		
	Systolic BP	Diastolic BP	Heart Rate	Systolic BP	Diastolic BP	Heart Rate
Rest	178 ± 3.7	107 ± 4.4	356 ± 14	167 ± 3.4	102 ± 3.2	368 ± 6
Saralasin infusion (120 min)	185 ± 6.4	118 ± 8.2	345 ± 12	114 ± 15.6*	70 ± 11.6*	366 ± 10
Saralasin infusion (240 min)	196 ± 6.5*	127 ± 7.5*	339 ± 17	99 ± 13.7*	65 ± 8.2*	355 ± 10

*Significantly different ($P<0.05$) from resting values (Wilcoxon test for pair differences).

The present results indicate that the high blood pressure seen 4 weeks after adrenalectomy of rats with isolation-induced hypertension might involve angiotensin II-mediated mechanisms that are not important in the maintenance of the high blood pressure in the intact animals.

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THE REVERSAL BY PYRIDOSTIGMINE OF SOMAN INDUCED NEUROMUSCULAR BLOCKADE IN PRIMATE RESPIRATORY MUSCLE

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The effectiveness of carbamate pretreatment as an adjunct to atropine therapy in protecting against poisoning by the organophosphorus anticholinesterase soman has been attributed to the ability of the carbamate to protect a proportion of acetylcholinesterase (AChE) from irreversible inhibition by soman. After poisoning spontaneous decarbamoylation produces sufficient free AChE to restore normal function (Berry & Davis, 1970). Dirnhuber & Green (1978), using anaesthetised rhesus monkeys, reported that pyridostigmine pretreatment effectively reversed soman induced neuromuscular blockade in tibialis anterior and soleus muscles. They concluded that, on the assumption that respiratory diaphragm (DIA) and intercostal (ICM) muscles would respond similarly to hind limb muscles, the protective effect of pyridostigmine at the neuromuscular junction may play a role in survival against soman poisoning. The objective of the present work was to substantiate this claim by determining the effectiveness of pyridostigmine in reversing soman induced neuromuscular blockade in vitro in primate respiratory muscles.

Human ICM, rhesus monkey and marmoset respiratory muscles were prepared for stimulation using techniques previously described (Wolthuis et al, 1981; Smith & Wolthuis, 1982; Bullbring, 1946). Tetanic responses were tested at 15 min intervals using various stimulation frequencies including 25 and 50 Hz. Pyridostigmine or saline (control) were added to the tissue bath followed 30 min later by soman. Thirty min later all compounds were washed out from the tissue bath and neuromuscular function monitored for up to 2.0 h. Finally a second dose of soman, twice the concentration of the first was added to the bath and neuromuscular function retested 15 min later.

In the absence of pyridostigmine, soman 50 nM (marmoset DIA), 400 nM (marmoset ICM) 200 nM (rhesus DIA & ICM) and 500 nM (human ICM) inhibited 25 and 50 Hz tetanic responses by >90% within 30 min; no recovery of tetanic function was observed following washout. In both rhesus monkey and marmoset DIA and ICM pretreatment with 1×10^{-7}M pyridostigmine restored 25 and 50 Hz tetani to 80% of the control value. Addition of a second soman dose reinhibited the restored tetani suggesting that AChE reactivation was responsible for the recovery. In human ICM 1×10^{-6}M pyridostigmine inhibited 25 and 50 Hz tetani by 10 to 20% but was the lowest concentration effective at reversing soman induced blockade; this concentration restored 25 and 50 Hz tetani to 70 and 25% of control values respectively. A second dose of soman completely reinhibited the restored tetani.

The results obtained in primate respiratory muscle substantiate the theory that the protective effect of pyridostigmine at the neuromuscular junction may play a major role in survival against soman poisoning and suggest that pyridostigmine pretreatment would be effective in man.

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EFFECT OF THE RENIN INHIBITOR H77 ON THE PRESSOR RESPONSES TO INJECTED RAT, PIG AND DOG RENINS IN VIVO

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H77 (H-His-Pro-Phe-His-NH-$\overset{CH_2CH(CH_3)_2}{\overset{|}{C}}$H-$CH_2$-Leu-Val-Tyr-OH) is a renin substrate analogue which is a potent inhibitor of the conversion of renin substrate to angiotensin I by dog renin in vitro. H77 produces a hypotensive response in sodium-depleted conscious dogs. It is also a less potent inhibitor of rat and human renins in vitro (Szelke et al., 1981). However ID_{50} values for H77 against injected renins in vivo have not been reported. In the present study we have measured the inhibitory activity of a sample of H77 synthesised at ICI against the pressor responses to injected rat, pig and dog renins in rats anaesthetised with Inactin (120mg/kg i.p.) and treated with pentolinium (20mg/kg i.p.). We have also measured the inhibitory activity of H77 against the pressor responses to injected dog renin in chronically catheterised conscious dogs.

The partially purified renins were obtained from rat, pig and dog kidneys. They contained no angiotensin I because of the extraction procedures used. The renin and angiotensin I were injected at doses producing sub-maximal pressor responses of 30 to 40mmHg. The dose of angiotensin I used was 200ng/kg and of pig renin 0.3iu/kg (calibrated using MRC standard pig renin 65/119). Rat and dog renins were not calibrated due to lack of suitable standards. H77 at infusion rates up to 50mg/kg/hour was without effect on the pressor response to angiotensin I in the rat (1.7 ± 17.5% change in pressor response after 27 min H77 infusion, n = 6). In the rat the effects of at least 3 different infusion rates of H77 were investigated for each type of renin injected. In the dog 6 different infusion rates of H77 were used. From these data the following ID_{50} values for H77 have been calculated: rat renin in the rat - 40.0mg/kg/hr; pig renin in the rat - 1.1mg/kg/hr; dog renin in the rat - 0.53mg/kg/hr; dog renin in the dog - 0.06mg/kg/hr.

Hence in vivo the rank order of potency for H77 against the three injected renins was dog>hog>rat. These results are in agreement with the in vitro data of Szelke et al. (1981). Although H77 was a potent inhibitor of dog renin injected into rats, it was even more potent as an inhibitor of dog renin injected into dogs.

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HYPOTHERMIA-INDUCED SUPERSENSITIVITY TO PARTIAL AGONISTS AT β_1- AND β_2-ADRENOCEPTORS

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Hypothermia induces supersensitivity to orciprenaline of tissues containing β_1-adrenoceptors but not β_2-adrenoceptors (Williams & Broadley, 1982). Supersensitivity can be demonstrated not only by a decrease in EC_{50} value of full agonists but also by an increase in the maximum response to a partial agonist. This study examines the effect of hypothermia on the maxima of partial agonists on a variety of guinea-pig tissues containing β_1- and/or β_2-adrenoceptors.

Tissues were set up in Krebs-bicarbonate solution gassed with 5% CO_2 in oxygen and containing metanephrine (10μM) and phentolamine (5μM) at 38°C or 30°C. After equilibration, a cumulative isoprenaline dose-response curve was constructed, followed by washout and a dose-response curve to the partial agonists salbutamol or ritodrine (n>4). The isoprenaline EC_{50} values of 7.1 (5.5-9.2)nM and 8.4 (3.0-23.4)nM on spontaneously beating right and paced left (2Hz, 5ms pulse width, threshold voltage + 50%) atria at 38°C were significantly ($P<0.05$) greater than the values at 30°C of 0.65 (0.11-3.7)nM and 0.12 (0.02-0.7)nM respectively. Also, the maximum responses to salbutamol at 38°C of 45.8±3.9% and 14.1±5.2% relative to isoprenaline for right and left atria respectively, were significantly ($P<0.01$) less than the values of 76.7±8.4% and 48.2±7.3% obtained at 30°C. Similarly, for tracheal spiral and K^+-contracted (100mM) ileum, supersensitivity was demonstrated with the isoprenaline EC_{50} values of 6.81 (0.6-72.2)nM and 75.1 (54.1-104)nM at 38°C being significantly greater ($P<0.001$) than the values of 0.15 (0.1-0.3)nM and 18.4 (9.2-35.9)nM at 30°C. Neither salbutamol nor ritodrine were partial agonists on these tissues.

In contrast, no supersensitivity was observed with uncontracted lung strips or K^+-depolarized vas deferens or uterus. The isoprenaline EC_{50} values of 2.50 (1.4-4.6) nM, 29.9 (6.9-125.7)nM and 15.8 (4.6-52.2)nM of lung, vas deferens and uterus at 38°C, were not significantly different from the values of 4.08 (0.7-25.7)nM, 20.0 (4.9-80.6)nM and 24.3 (4.0-150.6)nM at 30°C. Similarly, there was no difference between the maximum responses to ritodrine of lung, vas deferens and uterus at 38°C (74.8±1.8%, 61.3±3.6% and 83.3±2.2%) and at 30°C (65.1±5.2%, 66.0±4.5% and 74.0±3.1%).

The responses of the heart (Zaagsma et al., 1979) and intestine (O'Donnell & Wanstall, 1975) are mediated via β_1-adrenoceptors, while the responses of the lungs (Siegl et al., 1979), uterus (O'Donnell et al., 1978) and vas deferens (Von Euler & Hedqvist, 1975) are mediated via β_2-adrenoceptors. The responses of the trachea are mediated via both β-adrenoceptor subtypes (O'Donnell & Wanstall, 1979). The results of this study therefore provide further evidence to suggest that β_1-adrenoceptors, but not β_2-adrenoceptors, are temperature-dependent.

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CALCITONIN IN HAEMORRHAGIC SHOCK

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The hypotension observed after haemorrhagic or endotoxic shock, has been shown to be exacerbated by morphine (Chance et al, 1982) and reversed by naloxone (Faden & Holaday, 1979). We have studied the effects of calcitonin, a centrally acting antinociceptive agent (Bates et al, 1981), on the blood pressure in anaesthetised, normotensive, haemorrhaged and pithed rats.

Sprague-Dawley rats (150-350g) were anaesthetised with i.p. urethane (1.4g kg^{-1}). The jugular vein and carotid artery were cannulated for drug administration and blood pressure recording respectively. Vehicle (0.154M NaCl, with 1mg ml^{-1} BSA) or drugs (leucine enkephalin (LE), naloxone and salmon calcitonin (sCT)), were administered on a latin-square system.

Separate groups of animals were bled by withdrawing arterial blood, until the mean arterial pressure (MAP) was reduced by 20%. After a 20 minute stabilisation period, vehicle or drugs (naloxone or sCT) were administered. The MAP was monitored for 60 minutes thereafter. The change in MAP was determined from the post-haemorrhage value, and statistical analysis was undertaken by Student's t test.

In normotensive animals, LE produced a significant, ($p < 0.05$) transient hypotension. Pretreatment with naloxone (10mg kg^{-1}), administered, i.p., 30 minutes prior to LE, completely abolished the responses to LE. sCT (0.01-100 i.u. kg^{-1}) was without effect on the blood pressure of normotensive animals.

Table 1 Change in MAP, after drug treatment in haemorrhaged animals (mean ± SE MEAN, *p < 0.05

Treatment	Change in MAP (mm Hg) - Post-Haemmorrhage 5 Minutes	15 Minutes	60 Minutes
Vehicle	+4.3 ± 3.3	-3.8 ± 2.9	-3.5 ± 3.6
sCT (0.1 i.u. kg^{-1})	+2.6 ± 3.3	+1.8 ± 2.4	+6.4 ± 0.87*
sCT (1 i.u. kg^{-1})	+7.0 ± 3.2*	+6.6 ± 3.0*	+7.0 ± 2.1*
sCT (10 i.u. kg^{-1})	+16 ± 4.8*	+15.8 ± 4.2*	+11.4 ± 6.2
Naloxone	+5.7 ± 2.9	+11.0 ± 3.8*	+17.2 ± 2.8*

As shown in Table 1 Naloxone produced a rise in MAP, which was significantly different at 15 minutes, and was sustained for 60 minutes. sCT (0.1-10 i.u. kg^{-1}) produced a dose-dependent increase in MAP, at 5 minutes which was sustained for 60 minutes. In pithed animals, sCT (10 i.u. kg^{-1}) produced no observable effect, although these animals were responsive to a direct-acting vasoconstrictor such as vasopressin (1.5-300mU kg^{-1}).

We have shown that sCT exerts a pressor effect in rats rendered hypotensive by haemorrhage. There was no effect observed with sCT in normotensive animals. The absence of an effect in the pithed animal indicates that the pressor action of sCT in haemorrhage is probably not by direct peripheral vasoconstriction.

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ADENOSINE RELAXES THE GUINEA-PIG ISOLATED AORTA BY INTERACTING WITH A CELL SURFACE A_2 RECEPTOR AND WITH AN INTRACELLULAR SITE

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Cell surface adenosine receptors have been divided into A_1 and A_2 sub-types on the basis of the relative potencies of a series of adenosine analogues(Bruns et al.,1980;Brown & Collis,1982;Collis,1983). At the A_1 receptor 5'-N-ethylcarboxamide adenosine(NECA) and 5'-N-cyclopropylcarboxamide adenosine(NCPCA) are of similar potency to L-N^6-phenylisopropyl adenosine(L-PIA) and N^6-cyclohexyladenosine(CHA). L-PIA is about 100 times more potent than D-PIA at the A_1 receptor. By contrast, the 5'-substituted analogues are significantly more potent than the N^6-substituted compounds at the A_2 receptor. L-PIA is only 3-5 times more potent than D-PIA at this receptor. In addition to these two types of cell surface receptors, an intracellular "P site" with which adenosine, 2-chloroadenosine and 9-β-D xylofuranosyl adenosine may interact has also been identified (Londos and Wolff, 1977). The 5' and N^6 substituted adenosine analogues are not agonists at this site (Daly,1982). In the present study we have investigated the nature of the receptors that mediate adenosine evoked relaxation of the guinea-pig aorta.

Rings of guinea-pig thoracic aorta were mounted for isometric tension recording in organ baths containing Krebs solution aerated with 95% O_2, 5% CO_2, at 37°C. The rings were sub-maximally contracted with noradrenaline(2×10^{-6}M) and purines were added cumulatively to the bath contents to evoke relaxation. The noradrenaline evoked contraction was stable for the period required for the construction of the purine dose response curve.

The 5'-substituted analogues NECA and NCPCA were approximately 100 times more potent than the N^6-substituted compounds L-PIA and CHA. D-PIA evoked contractions that reversed to relaxation in the presence of cocaine (10^{-5}M). In the presence of cocaine, L-PIA was approximately 3 times more potent than D-PIA. Relaxations evoked by these analogues were not altered by the purine transport inhibitor dipyridamole (10^{-5}M), but were antagonised by the adenosine receptor antagonist 8-phenyltheophylline (10^{-5}M).

Adenosine and 2-chloroadenosine differed from the 5'-substituted analogues in two respects. They evoked greater maximal relaxation and their responses were blocked to a lesser extent by 8-phenyltheophylline. These differences between responses evoked by adenosine or 2-chloroadenosine and the 5'-substituted analogues were abolished in the presence of dipyridamole (10^{-5}M). 9-β-D xylofuranosyl adenosine evoked small relaxations that were blocked by dipyridamole but were unaffected by 8-phenyltheophylline.

These results indicate that adenosine can evoke relaxation of the aorta by interacting with two different sites. Low concentrations interact with a cell surface A_2 adenosine receptor. High concentrations also interact with an intracellular site which exhibits some of the characteristics of the P-site. D-PIA has an additional contractile effect which may be related to its structural similarity to D-amphetamine (Collis, 1983).

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SUBSTANCE P EFFECTS ON INOSITOL PHOSPHOLIPIDS, (^{3}H)-SUBSTANCE P BINDING AND CONTRACTION IN GUINEA-PIG ILEUM

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A wide variety of cell surface receptors which raise intracellular Ca^{++} levels also cause the hydrolysis of inositol phospholipids (Ptd Ins) in the cell membrane. Although the role of this hydrolysis is unknown, it offers a useful method for monitoring such functional receptors. Substance P has been reported to stimulate the incorporation of ^{3}H-inositol into Ptd Ins of the rat parotid gland with an EC_{50} of 1.7×10^{-8} M (Hanley et al, 1980), but similar studies in other tissues have been hampered by the low sensitivity of the available methods. This problem has recently been largely overcome by the development of a more sensitive assay (Berridge et al, 1982) which has been used in the present study.

Briefly, 350 µm slices of rat hypothalamus or guinea-pig ileum longitudinal muscle were pre-labelled with ^{3}H-inositol. After washing, they were transferred to incubation baths containing Ca^{++}-free Krebs (ileum) or Ca^{++}-containing Krebs (hypothalamus). The incubation medium also contained 12 mM Li^{++} which blocks inositol-1-phosphatase, and so causes its substrate to accumulate following Ptd Ins hydrolysis. Tissue was exposed to peptide agonists for 30 min and ^{3}H-inositol-1-phosphate was subsequently extracted and separated by ion exchange chromatography.

The EC_{50} values for substance P in rat hypothalamus and guinea-pig ileum were 6.0×10^{-8} M and 2.2×10^{-8} M respectively, and are therefore in close agreement with that reported on the rat parotid. Further, the structure-activity relationship for this response in these three, functionally distinct, tissues was similar (Table 1), suggesting that the same sub-type of substance P receptor is present. The rank order of potency of agonists closely resembles that reported for SP-P receptors (Lee et al, 1982). Although the relative potencies observed on Ptd Ins hydrolysis agree well with those reported on ileum contraction (Table 1), the actual potencies are approximately one order of magnitude lower on contraction; thus the EC_{50} for substance P on contraction is 1.4×10^{-9} M (Lee et al, 1982). One plausible explanation for these data is the presence of spare receptors for contraction but not for Ptd Ins hydrolysis, which is more likely to be an event closely associated with receptor activation.

Preliminary binding data for ^{3}H-substance P on guinea-pig ileum longitudinal muscle revealed a single binding site with a K_D value of 1.85×10^{-9} M, and a Hill coefficient not significantly different from 1. IC_{50} values of substance P analogues will be presented and compared with potencies obtained on the above systems.

Table 1. Potencies of substance P analogues relative to substance P (=1) on various test systems

	Ileum contraction	Ptd Ins hydrolysis in ileum	Ptd Ins hydrolysis in rat hypothalamus
Substance P	1	1	1
Eledoisin	0.9	0.78	2.07
Physalaemin	1	0.84	-
Substance P methyl ester	0.95	1.29	0.98

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SUBSTANCE P AND THE REGULATION OF MESOCORTICAL AND MESOLIMBIC DOPAMINE NEURONES IN RAT BRAIN

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Neurones of the ascending mesotelencephalic dopamine (DA) system may be resolved into sub-populations not only on the basis of the particular forebrain areas they innervate but also according to their pattern of response to a variety of pharmacological and physiological manipulations. For example, mild footshock stress in the rat causes a marked acceleration of activity in the mesocortical DA neurones innervating prefrontal cortex, while the response is less pronounced in forebrain regions such as nucleus accumbens innervated by mesolimbic DA neurones and there are no changes in nigrostriatal DA or cortical noradrenaline or serotonin metabolism (see Bannon and Roth, 1983).

Substance P (SP) containing terminals establish afferent contact with mesocortical and mesolimbic DA cells or with non-DA elements in their vicinity in the ventral tegmentum. An involvement of this SP innervation in the stress response has been suggested on the basis of reduced SP content in the ventral tegmental area and interpeduncular nucleus after a regimen of footshock stress comparable to that affecting DA activity. In parallel with its lack of effect on nigrostriatal DA metabolism, mild footshock stress appears to leave SP levels in substantia nigra unaltered (Lisoprawski et al., 1981). If SP terminals in the ventral midbrain are critical to the effects of stress on DA neurone activity it follows that such effects (i) should be mimicked in unstressed animals by pharmacological stimulation of SP receptors and (ii) should be prevented in stressed animals by inactivation of SP released endogenously.

(i) To investigate the effects of SP receptor stimulation in the region of the mesocortical and mesolimbic DA cell bodies of the ventral tegmental area in rat brain, bilateral infusions of DiMeC7 (4 μg/μl), a metabolically stable analogue of SP (Eison et al., 1982), were made into this area through 30 gauge injection cannulae lowered through 23 gauge guide cannulae stereotaxically implanted in male Sprague-Dawley rats. The animals were then placed in activity cages equipped with photocells for 20 min following infusion and were killed at the end of this period. Brain tissue was rapidly dissected, frozen and stored for subsequent HPLC determinations of DA and DOPAC. Compared with artificial CSF (vehicle) alone, DiMeC7 elicited increased locomotor activity in treated rats, as described previously (Eison et al., 1982), and produced a significant elevation of DOPAC levels in the forebrain (80% increase in prefrontal cortex, 30% increase in nucleus accumbens) with little change in dopamine concentrations in these areas; a profile of DA neurone activation closely resembling that seen after mild footshock.
(ii) To attempt to evaluate the role of endogenous SP release in the enhanced utilisation of DA associated with stress, discrete bilateral infusion of a monoclonal antibody to SP were delivered to the ventral tegmental area as described above. DA and DOPAC levels were measured 20 min later after an intervening period of mild footshock (0.20 mA : 160 ms on-off). The effects of SP antibody under shock and baseline conditions will be discussed.

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INTERACTIONS BETWEEN FORSKOLIN AND VIP IN THE REGULATION OF PROLACTIN RELEASE AND CYCLIC AMP CONTENT OF GH_3 PITUITARY CELLS

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The investigation of intracellular mechanisms mediating hormone release is often complicated by the heterogeneous cell populations present in glandular tissues. GH_3 pituitary tumour cells grown in culture provide a homogeneous cell population and a useful model system for investigating the control mechanisms involved in prolactin release. Various peptides have been reported to modify prolactin release from GH_3 cells such as TRH (Martin & Tashjian, 1977) and VIP (Gourdji et al, 1979). This laboratory has previously reported results supporting an involvement of cAMP in the control of prolactin release by a variety of agents including VIP (Guild & Drummond, 1982). Forskolin, a diterpene of the labdane family, activates adenylate cyclase via a non receptor-mediated mechanism and causes a marked increase in the level of intracellular cAMP in a number of tissues (Seamon et al, 1981). Low concentrations of forskolin are reported to potentiate greatly hormonal activation of adenylate cyclase. We have investigated this phenomenon in GH_3 cells using VIP as the hormonal stimulant.

GH_3 cells were grown in monolayer culture at 37°C under 95% air/5% CO_2in Ham's F10 Medium supplemented with foetal calf serum (2.5% v/v), horse serum (15% v/v), benzyl penicillin (100 iu/ml) and streptomycin (100 µg/ml). The cells were suspended in a balanced salt solution (BSS) at 37°C for experiments, washed twice to remove prolactin from the medium and exposed to forskolin and/or VIP. The incubations were terminated by the addition of 4 volumes of BSS at 4°C. The cells were spun down and prolactin levels in the medium measured by radioimmunoassay. The cAMP was extracted from the cell pellet by sonication and boiling and measured by protein binding assay.

Forskolin produces a concentration-dependant rise in intracellular cAMP levels (EC_{50} = 0.3µM) and increase in prolactin (EC_{50} = 0.1µM). The maximal rise in prolactin release was 2-fold and the maximal rise in cAMP was 2.7 fold. The phosphodiesterase inhibitor, IBMX (1mM) greatly augmented the rise in cAMP elicited by forskolin. IBMX (1mM) alone was sufficient to stimulate prolactin release maximally and no further increases were seen when forskolin was added. VIP produced a concentration-dependant rise in intracellular cAMP levels (EC_{50} = 3nM) and increase in prolactin release (EC_{50} = 0.2nM). In both cases a 2-fold increase was seen. When a dose of forskolin (0.1µM), which on its own produced only a small rise in cAMP, was used in conjunction with VIP the rise in cAMP elicited by VIP was greatly augmented. However no shift in the dose response curve for VIP effects upon intracellular cAMP levels or prolactin release was observed.

The results obtained using forskolin and VIP would support the involvement of cAMP in the control of prolactin release from GH_3 cells, and indicate that a 2-fold rise in cAMP is sufficient to activate cAMP-dependant prolactin release fully. The results also confirm the finding of Seamon et al (1981) that forskolin potentiates the hormonal activation of adenylate cyclase.

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MONITORING ASCORBATE AND MONOAMINE METABOLITES IN FREELY MOVING RATS, USING VOLTAMMETRY: SOME NEW DEVELOPMENTS

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We have recently developed a number of procedures to enhance the catechol, 5-hydroxyindole and methylated catechol signals obtained using microprocessor-based linear sweep voltammetry and carbon paste electrodes. These modifications include eliminating the ascorbate signal in alternate voltammograms, subtracting the background current, and correcting for circadian changes. Using this new approach, we have monitored changes in the rat striatum and hippocampus after the administration of the alpha-2 antagonist, yohimbine.

Male Sprague-Dawley rats (250-400 g initial weight) were stereotaxically implanted with four carbon paste electrodes as described previously (1). The animals were given at least 2 days to recover before being placed in their recording cages. Five voltammograms per electrode were recorded continuously before each experiment and averaged to give the "background voltammogram"; subsequent recordings were made at 12 min intervals. The sweep ranges were, alternately, -200 to +500 mV and +30 to 480 mV at a sweep rate of 5 mV/s. The ascorbate oxidation potential was 80 mV (with respect to the Ag-AgCl reference); the catechol, 130 mV; the 5-hydroxyindole (principally 5HIAA), 220 mV; and the methoxycatechol peak was at 380 mV. Yohimbine (10 mg/kg i.p.) caused a steep rise in ascorbate in the striatum between 1 and 3 h after injection, followed by a fall to near baseline levels. In the hippocampus, however, there was an immediate fall in ascorbate which reached a minimum after 1 h; the baseline value was restored by 3 h. The 5HIAA current did not change for at least 1 h after injection in either brain regions; this signal then increased in the striatum while it decreased in the hippocampus. After these initial differences in the 5HIAA response, both regions showed similar changes in the level of this metabolite. In the striatum, both the DOPAC and homovanillic acid currents increased after the administration of yohimbine; their time courses were parallel and did not return to baseline until 18 h post injection. There were no observable changes in the current at the oxidation potentials of the catecholamine metabolites in the hippocampus.

These results show that yohimbine causes changes in a wide variety of compounds, each with its individual time course. It is doubtful, however, whether this technique is sensitive enough to detect noradrenaline metabolites in the hippocampus.

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UPTAKE OF (^{3}H)-TAURINE BY THE RABBIT RETINA: EFFECT OF LIGHT STIMULATION

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Taurine is the most abundant amino acid in the retina where it is relatively concentrated in the photoreceptors. However, the function of taurine in the retina is unknown. It may be a transmitter released from a small sub-population of amacrine cells but it probably has a more generally important role in the maintenance of the photoreceptors. This is suggested by the photoreceptor degeneration that occurs in cats fed on a taurine free diet (Hayes, Carey & Schmidt, 1975).

As part of a study on the mechanisms involved in regulating photoreceptor taurine levels, we have examined the characteristics of {^{3}H}-taurine uptake by the isolated rabbit retina. The effects of light on taurine uptake have also been studied.

Rabbit retinas quickly accumulated {^{3}H}-taurine and tissue : medium ratios of 60 : 1 were attained after 60 min incubation at 37°C. Under these conditions no metabolites of {^{3}H}-taurine were detected in the tissue. Non-linear regression analysis of kinetic data revealed that taurine was accumulated by separate high and low affinity transport processes, the kinetic parameters being Km_h = 93 ± 12 μM, Vm_h = 72 ± 7; Km_l = 8.8 ± 5 mM, Vm_l = 274 ± 79 nmol/min/g wet weight respectively. The properties of the high and low affinity taurine uptake processes were very similar. Both were temperature sensitive, particularly between 25° and 37°C, sodium and chloride dependent, inhibited by metabolic inhibitors and were able to produce a net uptake of taurine. Thus, taurine uptake by the retina is mediated by active transport processes. The specificities of the high and low affinity uptake processes were also similar both processes being inhibited by β-alanine, guanidinoethylsulphonate and GABA but not by α-alanine or glycine. Hypotaurine selectively inhibited the high affinity uptake process for {^{3}H}-taurine.

Exposure of retinas to continuous light did not affect either the high or the low affinity uptake of {^{3}H}-taurine compared with dark adapted controls. However, flickering light (0.5 - 30 Hz, 25% duty cycle) reduced the high affinity accumulation of {^{3}H}-taurine by as much as 50%. The reduction in {^{3}H}-taurine accumulation was apparently due to a localised decrease in uptake (or possibly an increase release) by the photoreceptors because the same reduction was found when synaptic transmission in the retina was blocked by exposure to medium containing high Mg/low Ca. High Mg/low Ca did not itself affect taurine accumulation.

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MET-ENKEPHALIN LEVELS IN THE RAT POSTERIOR PITUITARY GLAND FOLLOWING HAEMORRHAGE

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It has been shown (Rossier et al, 1979) that following five days 2% saline imbibition in rats, which is a profound posterior pituitary hormone-releasing stimulus, that there was a 60% decrease in the immunoreactive Leu-enkephalin levels concomitant with the 80% decrease in posterior pituitary hormone levels, however the β-endorphin levels remained unchanged. Various other reports (Clarke, Wood, Merrick & Lincoln, 1979; Bicknell & Leng, 1982) have implicated opioid compounds in the control of release of posterior pituitary hormones, both in vivo and in vitro.

We have examined the levels of immunoreactive Met-enkephalin in the posterior pituitary gland of rats subjected to 1% body weight haemorrhage, a stimulus which has been shown to cause vasopressin release (Ginsburg & Smith, 1959), and also levels in sham operated and untreated control rats.

Adult male Wistar rats, bodyweight 400-500 g, were anaesthetised with urethane 1.25 g/kg I.P. and carotid cannulae inserted. The animals were subjected to a haemorrhage of 1% body weight equivalent and one minute later were decapitated. The brain and posterior pituitary were rapidly dissected out and the posterior pituitary and a small portion of frontal cortex from each rat were processed for radioimmunoassay of Met-enkephalin using a method based on that described by Clement-Jones, Lowry, Rees & Besser (1980), which is highly specific for Met-enkephalin.

Results were expressed as a ratio of the amount of Met-enkephalin in ng per pituitary gland to the amount found in μg/gram of frontal cortex for each animal. The untreated control animals showed a mean ratio of 26.5 (n=4), the sham operated animals had a mean ratio of 22.2 (n=5) and the animals exposed to haemorrhage had a mean ratio of 24.7 (n=9). None of these values were significantly different.

It is possible that changes in enkephalin levels would only be seen with the prefound stimulus used by Rossier et al (1979), it is also possible that Leu-enkephalin and not Met-enkephalin is involved in these changes. Another explanation might be associated with the findings of Bicknell & Leng (1982) that in vitro opioid effects are seen with oxytocin release, which does occur during saline imbibition, rather than vasopressin release which is mainly associated with the haemorrhage stimulus.

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REGIONAL CHANGES IN RAT BRAIN TRH CONTENT FOLLOWING ELECTRO-CONVULSIVE SHOCK

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Following repeated electroconvulsive shock (ECS) rats display enhanced responses to both 5-hydroxytryptamine (5-HT) and dopamine (DA) agonists (see Green, 1980). It has been suggested that these changes in monoamine function might be associated with the therapeutic efficacy of electroconvulsive therapy in depression (Green, 1980). In view of the close association of various neuropeptides with monoamine neurotransmitters, we have now examined changes in one peptide known to co-exist with 5-HT in rat spinal cord (Gilbert et al, 1982).

Table: Effect of repeated ECS on TRH content of rat brain

	TRH Concentration (pg TRH/μg prot)		Significance
	Control	ECS	p
N. Accumbens	0.38 ± 0.04 (16)	0.22 ± 0.03 (17)	< 0.01
Septum	0.77 ± 0.11 (8)	0.71 ± 0.14 (7)	N.S.
Hypothalamus	2.53 ± 0.21 (16)	2.36 ± 0.25 (16)	N.S.
Suprachiasmatic nucleus	1.61 ± 0.16 (16)	1.68 ± 0.25 (16)	N.S.
Raphé (Median & Dorsal)	0.27 ± 0.03 (16)	0.20 ± 0.03 (15)	N.S.
Lumbar Spinal Cord	0.99 ± 0.08 (16)	0.60 ± 0.05 (16)	< 0.001
Thoracic Spinal Cord	0.57 ± 0.21 (7)	0.56 ± 0.05 (10)	N.S.

Results expressed as mean ± s.e. mean with number of determinations in brackets.

Male Sprague-Dawley derived rats (Charles River, Kent) were given 5 ECS (125V, 1s, 50Hz) over 10 days (Mon,Wed,Fri,Mon,Wed) through ear-clip electrodes whilst anaesthetised with halothane. Controls received halothane and electrode placement but no current was passed. Twenty-four hours after the final treatment both groups were killed and various brain regions were dissected out on ice. TRH was measured as described by Bennett et al (1981). Following repeated ECS there was a marked decrease in the TRH content of the n. accumbens and lumbar spinal cord but no significant changes in any other region examined (Table). No changes in regional brain TRH content were seen 24 h after a single ECS (data not shown).

Neither the concentration of 5-HT in the lumbar spinal cord nor the rate of 5-HT synthesis (as measured by 5-HT accumulation after a monoamine oxidase inhibitor) was observed to change after repeated ECS. Whole brain 5-HT concentration and turnover has previously been shown to be unaltered by repeated ECS (see Green, 1980).

Repeated ECS enhances dopamine-mediated responses in the n.accumbens (Heal & Green, 1978) and 5-HT-mediated behaviour, which may be hind-brain or spinally mediated (Deakin & Green, 1978). Whilst it is therefore of interest that the peptide changes occur specifically in these two regions, it is premature to suggest an association between the peptide change and ECS-induced alterations in monoamine-mediated behaviours.

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PROPORTIONATE CHANGES OF DOPAMINE TURNOVER DUE TO PARTIAL INHIBITION OF SYNTHESIS WHEN MEASURED IN BRAIN AND CSF

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We have previously shown that 5HT turnover can be determined, using the probenecid method, in the CSF of conscious freely moving rats and is a good indicator of brain 5HT turnover (Sarna et al, 1982). We have now validated the use of CSF to obtain an index of DA turnover concurrently from the increase of HVA and DOPAC after probenecid.

Sprague-Dawley rats were implanted with polyethylene catheters in the cisternal space as previously described (Sarna et al, 1982). Three days later they were given either vehicle or the decarboxylase inhibitor DL-α-monofluoromethyl DOPA (MFMD) 100 mg/kg p.o. (Merrell International). After 16 h, CSF samples (30 µl) were taken, rats killed and brains removed before and 30 and 60 min after probenecid 200 mg/kg, i.p. Free HVA and DOPAC concentrations were determined in CSF, striatum and rest of brain (minus cerebellum) by HPLC. Total HVA and DOPAC were estimated following a modification of the acid hydrolysis procedure of Dedek et al, (1979). The linear increase of total HVA + DOPAC over 60 min after giving probenecid was used as a measure of DA turnover. These increases were 2-4 times greater than those of the free acids.

Table 1. DA turnover by probenecid method: comparison of brain and CSF.

	Turnover values			
	CSF ($nmol.ml^{-1}.h^{-1}$)	Striatum ($nmol.\ g^{-1}.h^{-1}$)	Rest of Brain ($nmol.\ g^{-1}.h^{-1}$)	Whole Brain ($nmol.\ g^{-1}.h^{-1}$)
Saline (n = 22)	1.74 ± 0.09 r = 0.98	11.39 ± 2.29 r = 0.76	1.70 ± 0.20 r = 0.89	2.02
MFMD (n = 21)	0.82 ± 0.09 r = 0.91	2.61 ± 2.45 r = 0.24	0.75 ± 0.22 r = 0.62	0.81
% decrease	53	77	56	60

Turnover values are means ± S.D. Nos. of rats in brackets. r values are correlation coefficients (sum of metabolites vs time after giving probenecid).

MFMD caused very comparable decreases of DA turnover whether measured in CSF or in the extrastriatal part of the brain. The decrease of turnover in the striatum was appreciably greater. The decrease in whole brain (as calculated from the mean striatal and extrastriatal values) compares reasonably with the decrease as measured in CSF. These findings thus validate the use of rat CSF to provide an index of central DA turnover.

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EVIDENCE FOR THE INVOLVEMENT OF BRAIN GABA AND SEROTONIN SYSTEMS IN THE ANTI-CONFLICT EFFECTS OF CHLORDIAZEPOXIDE IN RATS

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Shock-induced suppression of feeding or drinking used in conditioned conflict procedures has become a widely used test for anxiolytic activity in animals. Benzodiazepines consistently increase punished responding (Sepinwall & Cook, 1980), and there is a high correlation between these anti-conflict effects and reported clinical potency in man (Lippa et al, 1978). Stable baselines may be reinstated and different treatment combinations tested in the same animals. Varied components may also be used, to assess simultaneously effects on rewarded, non-rewarded, and punished responding (Tye et al, 1979).

This paradigm was used to test the hypotheses that behavioural effects of benzodiazepines are mediated either by increased brain GABA (Costa et al, 1975) or by decreased serotonin activity (Wise et al, 1972).

CDP (2.5, 5.0 and 10.0 mg/kg i.p.) dose-relatedly increased punished and time out responding in rats, but increased rewarded responding in an inversely dose-related manner. Punished responding was enhanced by chronic treatment to a rate which remained stable between 9 - 19 injections.

The GABA transaminase inhibitor ethanolamine-O-sulphate (EOS), given chronically in drinking water (5.0 mg/ml), increased punished responding linearly to a high stable level after 2 - 3 weeks. Rewarded and time out responding were less substantially increased. CDP given with EOS dose-relatedly increased time out and punished responding substantially above the rates found with either treatment alone, with the increase in punished responding being particularly massive. The GABA antagonist picrotoxin blocked the increase in punished and time out responding found with EOS and CDP alone, and reduced to single treatment levels the increases obtained with EOS + CDP.

The tryptophan hydroxylase inhibitor, p-chlorophenylalanine (PCPA; 100 mg/kg x 3) linearly increased punished responding for the first week of treatment. CDP with PCPA selectively and significantly increased punished responding above the rates for either treatment alone, but the increases were not as substantial as those with EOS + CDP. The 5-HT reuptake inhibitor Wy 25093 reduced increases in time out and punished responding under CDP, and the precursor 5-hydroxytrytophan (5-HTP) counteracted increases in punished responding under PCPA and PCPA + CDP, but also substantially reduced rewarded responding.

These results provide evidence that both increased GABA and decreased 5-HT transmission are involved in the anti-conflict effects of CDP, since EOS and PCPA both mimicked and potentiated effects of CDP, whilst picrotoxin, Wy 25093, and 5-HTP counteracted them. Findings that picrotoxin antagonized increases in punished responding brought about by PCPA alone and with CDP, whilst Wy 25093 reduced the increases obtained with EOS and EOS + CDP indicate that the two systems may interact in mediating anti-conflict activity.

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THE ROLE OF GABA IN THE LEPTAZOL INDUCED EPILEPTOGENIC EEG OF THE ANAESTHETIZED RAT

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The slow i.v. infusion of leptazol (0.5M, 15μl min^{-1}) in urethane anaesthetised rats produces a developing epileptogenic EEG of distinct phases. Control activity (phase a) changes to arousal (b), followed by high amplitude, low frequency waves (c). Spikes appear (d), become predominant as background activity falls (e), and then group with increasing periods of interspike silence (f), leading to full body convulsion. Clonazepam (0.79μmol kg^{-1}, i.v.) was the most effective anticonvulsant tested against leptazol, and specifically prolonged phase (c), delaying the appearance of spikes (Kent & Webster, 1983). Since leptazol may act as a GABA antagonist (Scholfield, 1979) and as the benzodiazepines are believed to augment GABA function, we have tried to establish if the leptazol induced phase (c) is dependent on GABA.

Male Sprague-Dawley rats (300-350g) were anaesthetised with urethane (6ml kg^{-1}, 25% w/v, i.p.). The EEG was recorded from screw electrodes in the skull over one parietal cortex and from platinum electrodes in a cortical cup placed on the other parietal cortex. It was displayed after amplification (Devices 3160, time constant 0.2s) on a pen recorder (Washington, 400 MD/2). Drugs were either infused i.v. (Palmer injector, 6130) or applied, in artificial CSF, to the cortex by superfusion (CS, 33μl min^{-1}).

Infusion (i.v.) of the GABA antagonists bicuculline (0.5mM, 30μl min^{-1}) and picrotoxin (10mM, 15μl min^{-1}) produced spiking like leptazol (phases e+f) but phase (c) was absent. Bicuculline (CS), in a concentration (10μM) which did not produce spiking, significantly ($P<0.05$, n=4) reduced the duration of the leptazol (c) phase. In contrast, GABA (10mM, CS) significantly ($P<0.05$, n=4) prolonged phase (c) and abolished phase (d). Clonazepam (CS) did not prolong phase (c) even at 0.10mM (n=6).

Leptazol (CS) did not produce a phase (c), although spikes (phase e) eventually developed at 0.05-0.5M. Bicuculline and picrotoxin (both at 50-500μM, CS) also only produced spiking (no phase c) as when given i.v.. Addition of GABA (10mM) to the superfusate had little effect on leptazol-induced spiking, but increased background activity to give an EEG pattern more characteristic of phases (c+d).

These results show that cortically applied GABA can modify the EEG both during the i.v. infusion of leptazol when it prolonged phase (c) and during cortical application of leptazol when it actually converted spiking (phase e) to phase (c/d)-type activity. This suggests that the (c) phase of the leptazol EEG is associated with increased GABA function in the cortex, possibly due to activation of cortical inhibitory mechanisms in response to some other, perhaps subcortical, action of leptazol rather than to GABA antagonism. This idea is supported by the inability of the GABA antagonists bicuculline and picrotoxin to produce phase (c) activity, either i.v. or CS. Thus, it appears that GABA, released in the cortex by an initial stimulant effect of leptazol, can delay the development of spiking and thus produces wave-like activity (phase c). Further experiments are required to explain why clonazepam can potentiate this effect when given i.v. but not when applied to the cortex.

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KINETIC CHARACTERISTICS OF GABA UPTAKE BY SYNAPTOSOMES PREPARED FROM FROZEN, POST MORTEM HUMAN BRAIN

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The kinetic characteristics of the high affinity GABA uptake system of rat synaptosomes are well established. We have previously shown that metabolically active synaptosomes can be prepared from rat and human brain up to 24h post mortem (Dodd et al 1979, Hardy et al 1982). We have also shown that active preparations can be isolated from fresh brain and human brain (biopsy) pieces after these have been slow frozen to -70C, stored for up to 1 month, then rapidly thawed (Hardy et al 1983).

We report that synaptosomes isolated from post mortem (5-15h pm delay), frozen (1 day - 1 month) human brain possess a high affinity (Km = 12μM) uptake system for GABA. This uptake system is inhibited by nipecotic acid ([GABA] = 20μM, [nipecotic acid] = 100μM, inhibition = 90%; Figure 1). Such preparations from post mortem, frozen human brains may therefore be used to study the effects of neuroactive drugs on GABA uptake in human tissue.

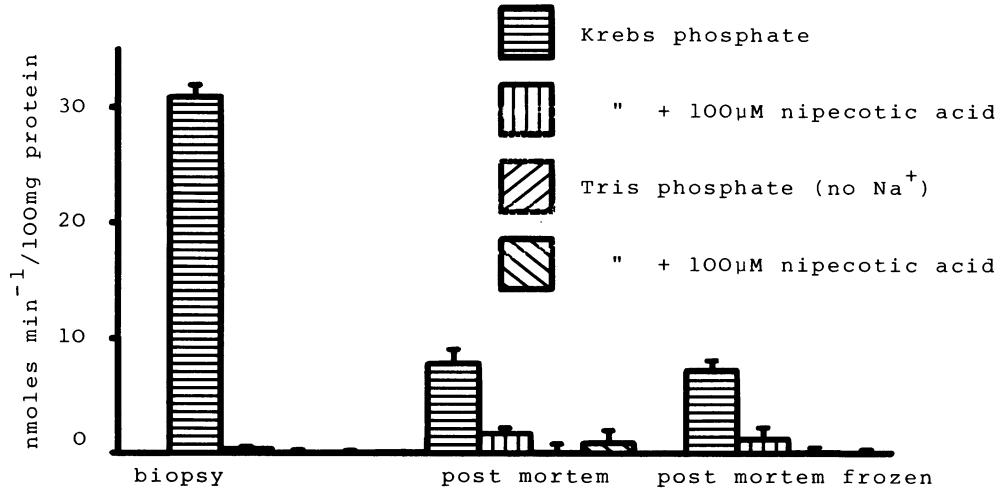

Figure 1 Uptake of ^{3}H GABA by human synaptosomes prepared from a biopsy specimen, post mortem brain used fresh (p.m. delay = 5h) and post mortem brain used frozen; effects of nipecotic acid and incubation in Na^{+} free media. (n = 3-6 determinations ± s.e.m.)

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THE GABA-MIMETIC ACTIVITY OF ETHYLENEDIAMINE IS DEPENDENT UPON PHYSIOLOGICAL CONCENTRATIONS OF HCO_3^- IONS

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Ethylenediamine (EDA) is a GABA-mimetic at $GABA_A$ and $GABA_B$ receptors (Bowery et al, 1982, see also Hill & Bowery, 1981). Thus high concentrations (>100 μM) will potentiate ^{3}H-diazepam binding to rat brain synaptic membranes in Tris-buffers ($GABA_A$ effect) (Davies et al, 1982; Morgan & Stone, 1982) and will depress the evoked release of ^{3}H-noradrenaline from sympathetic nerve terminals ($GABA_B$ effect (Bowery, 1982). EDA will also displace ^{3}H-GABA from binding sites on whole brain membranes. However, its potency is significantly enhanced if Krebs-Henseleit solution (KHS) is used as the incubation solution instead of Tris-buffers. By contrast, the potency of the agonists, isoguvacine, muscimol and GABA are unaffected (Bowery et al, 1982). Evidence is now presented that shows the potency of EDA and GABA receptors is dependent upon physiological concentrations of HCO_3^- ions.

$GABA_A$ and $GABA_B$ receptor binding assays were performed as previously described using rat brain crude synaptic membranes (Bowery et al, 1982; Hill & Bowery, 1981). ^{3}H-Diazepam binding (2 nM) was performed at 20°C (20 min incubation) using a filtration assay with lorazepam (1 μM) to determine the non-specific portion of bound ^{3}H-diazepam. The pH of all buffers was 7.4.

When each of the individual ionic constituents of KHS was added to 50 mM Tris-HCl, pH 7.4, only $NaHCO_3$ (25 mM) enhanced the potency of EDA at $GABA_A$ receptors. The other ions had little or no effect. Thus, the IC_{50} for inhibition of ^{3}H-GABA binding was reduced from >100 μM in Tris-HCl alone to 2.7 ± 0.28 μM (n=3) in the presence of $NaHCO_3$. $KHCO_3$ (25 mM) produced similar results (IC_{50} EDA 1.8 ± 0.49 μM (n=3). Addition of $NaHCO_3$ or $KHCO_3$ to Tris-HCl containing 2.5 ml $CaCl_2$ also reduced the IC_{50} for inhibition of ^{3}H-baclofen binding to the $GABA_B$ receptor from >100 μM to 1.06 ± 0.18 μM and 1.07 ± 0.12 μM (Na and $KHCO_3$ respectively: n=3 in both cases). The potency of GABA at either receptor was unaffected by the presence of the anion.

In Tris-HCl alone GABA (100 μM) potentiated ^{3}H-diazepam binding by 126 ± 1.2% above basal (ED_{50} 0.53 ± 0.02 μM (n=3). By contrast, EDA (up to 10^{-2} M) stimulated binding by only 68.4 ± 4.3% (n=3; ED_{50} = 560 μM) when compared with GABA (100 μM) in the same experiment. In KHS or Tris-HCl plus $KHCO_3$ (25 mM) the maximum effect of EDA was significantly raised when compared to the GABA control (= 100%). (Maximum potentiation by EDA was 89.9 ± 9.0% of GABA control in KHS: 89.4 ± 5.9% in Tris-HCl plus $KHCO_3$:n=3 in both cases). Moreover, in contrast to the observations of Morgan et al, 1982, the potency of EDA was also increased (ED_{50} 20 μM in KHS; 10 μM in Tris + $KHCO_3$, cf. 560 μM in Tris-HCl alone). The potency of GABA was unaffected by HCO_3^- ions. The effects of GABA and EDA were antagonised by bicuculline-methobromide (20 μM). Dose-ratios of 8.8 and 17.7 were obtained for GABA and EDA respectively. This finding concurs with earlier results (Perkins et al, 1981) showing EDA to be twice as sensitive to blockade by bicuculline as GABA. In summary, these data show the activity of EDA at GABA receptors to be dependent upon HCO_3^- ions.

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BEHAVIOURAL SUPERSENSITIVITY IN RESPONSE TO AMPHETAMINE FOLLOWING PROLONGED TREATMENT WITH OXYPERTINE

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Neuroleptic drugs induce dopamine receptor blockade and this has been postulated as their therapeutic action (Snyder et al 1974). Their prolonged use in man can produce tardive dyskinesia which has been postulated to be due to dopamine supersensitivity following prolonged receptor blockade (Tansy and Baldessarini, 1976). Treatment of this dopaminergic hyperactivity syndrome is very difficult because further receptor blockade with neuroleptics would lead eventually to further supersensitivity and a worsening of the symptoms. Oxypertine, which is an antipsychotic drug that acts mainly as a catecholamine depleter (Bak and Hasler, 1968; Anden and Fuxe, 1971) has been proposed as a treatment for tardive dyskinesias (Freeman et al, 1980). Withdrawal from prolonged haloperidol treatment as a model for dopaminergic supersensitivity has been used by us before in biochemical studies (Chiu et al, 1978 and 1981). In these experiments we look for evidence of behavioural supersensitivity in response to amphetamine on withdrawal from prolonged Oxypertine treatment (4 mg/Kg. i.p. twice daily during ten days) and compare this to the effect of haloperidol withdrawal (1.25 mg/Kg. i.p. daily during ten days).

74 Albino Sprague-Dawley male rats (Charles River U.K. Ltd., London) were used for these experiments. Following the Oxypertine or haloperidol pretreatment, they received one injection of either saline or amphetamine (4 mg/Kg.) either two, four or six days after the last Oxypertine dose or two days after the last haloperidol dose and the rat stereotyped (repetitive) motor behaviour (SB) was recorded in a hole board apparatus for four minutes every twenty minutes during one hour. In all the groups (Table 1), amphetamine produced an increase in SB ($p<0.01$) that is highest in the haloperidol group ($p<0.01$) compared with any other group. SB in response to amphetamine is also higher in the two days after Oxypertine group than in control ($p=0.05$, Mann-Whitney Test) and at least two animals out of ten in each of the three Oxypertine groups behaved in a supersensitive fashion giving a SB score ≥ 100.

In summary we can conclude: first that Oxypertine given at doses that block SB (Palomo and Russell, 1982) can produce supersensitivity to amphetamine but to a lesser extent than haloperidol does, and, second, that if tardive dyskinesias are due to dopamine supersensitivity, Oxypertine is not without risk of producing this syndrome.

Table 1: Stereotyped behaviour (SB) ($\bar{x} \pm$ SE of mean, $n \geq 5$) in response to Amphetamine

	Control	After Oxypertine			After Haloperidol
		(2d. after)	(4d. after)	(6d. after)	(2d. after)
Saline	0.9 ± 0.40	1.5 ± 0.27	0.5 ± 0.29	0.8 ± 0.20	1.0 ± 0.45
Amphetamine	11.1 ± 5.87	53.6 ± 20.91	54.4 ± 20.39	38.1 ± 17.75	174.4 ± 37.24

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CORTICAL SLOW POTENTIALS RECORDED IN RATS ANAESTHETIZED WITH URETHANE ARE AFFECTED BY NICOTINE

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The Contingent Negative Variation (CNV) Walter et al, 1964) is a slow negative wave that can be recorded from the scalps of conscious human subjects during the interval between a warning stimulus (S_1) and a response stimulus (S_2). Slow potentials (SPs) similar to the CNV have also been described in the conscious rat (Pirch, 1978; Ebenezer, 1982a). Recently we have demonstrated in our laboratory that CNV-like SPs are generated in rats anaesthetised with urethane. This paper describes the technique used to evoke SPs in rats anaethetised with urethane, and the effect of nicotine on the magnitude of these SPs.

Male rats that were previously implanted with silver/silver chloride electrodes (see Ebenezer, 1982a) were anaesthetised with urethane (1.2 - 2g/Kg). Tail-electrodes consisting of two stainless steel pins were inserted into the end of the tail of the rat (3 cm apart) and an earth electrode (another stainless steel pin) was inserted into the base of the tail. The body temperature of the rat was maintained at 37 ± 1°C, and EEGs were recorded by means of a.c. amplifiers (time-constant 15 s) and a pen recorder. Rats were given three habituation sessions to S_1 (1400Hz, 100msec, 70db tone). Thereafter S_1 was paired with S_2 (a 1.0 s, 50Hz, 0.7 - 1.5mA tail shock). The current of the tail shock was kept constant for any particular rat. The S_1 - S_2 interval was gradually increased (over a number of training sessions) from 0.5s to 3.0s. Fifteen trials were given to a rat in a single session. Intertrial intervals varied between 30 - 90 s. Intersession intervals were at least 5 min. The EEG data were averaged off-line on a PDP8 computer, and the area of the SPs calculated.

Presentation of the Tone (S_1) alone evoked a slight negative shift of the EEG which habituated with repeated presentations of the tone. However, when S_1 was paired with tail-shock (S_2) a negative shift developed during the S_1 - S_2 interval. When the SP response was established, presenting the warning stimulus (S_1) alone, without reinforcing it with tail shock, resulted in rapid extinction of the negative SP shift.

One of the characteristics of the CNV and rat SPs is their sensitivity to psycho-active drugs (see Ashton et al, 1980; Pirch, 1978). Previous work on conscious rats has shown that nicotine (0.4mg/Kg) is essentially depressant on the magnitude of the SPs (Ebenezer, 1982b). Rats that had developed steady SPs under urethane were given nicotine (0.4mg/Kg, s.c.) or saline, and SPs measured 0 - 10min, 15 - 25min and 30 - 40min after administration. Nicotine caused significant decreases in the mean SP areas of the rats ($p < 0.05$) during each measurement interval post-drug compared with the mean pre-drug SP area. Saline did not affect the SP responses in control animals.

The results indicate that SPs can be evoked under urethane anaesthesia, and that these SPs are similarly affected by nicotine (0.4mg/Kg) as those evoked in the conscious rat. It is suggested that this preparation may prove to be useful in SP experiments that were hitherto difficult or impossible to carry out in the conscious rat.

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DUAL COMPONENTS OF CIRCLING MEDIATED BY NIGRAL DOPAMINE RECEPTOR STIMULATION IN THE RAT

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Unilateral injection of dopamine agonists into substantia nigra produces circling behaviour in rats with an ipsilateral 6-hydroxydopamine (6OHDA) lesion of the medial forebrain bundle (MFB) but apparently not in intact animals (Kozlowski et al,1980). We now characterise the postural asymmetry produced by unilateral nigral injection of dopamine or apomorphine.

Unilateral injection of dopamine (25-50 ug in 0.5 ul 0.9% saline) into zona reticulata of rats pretreated with nialamide (25 mg/kg 1 h previously) produced dose-dependent contraversive rotation lasting up to 1 h. Circling was most intense from the central region of zona reticulata but less marked in ventral and lateral areas bordering on crus cerebri. Injections into the dorsal layer of zona compacta produced either weak ipsiversive posturing and circling or a mixture of ipsiversive and contraversive posturing and circling. Unilateral injection of dopamine (5-50 ug) or apomorphine (2.5-10 ug in 0.5 ul 0.9% saline) into zona reticulata of rats with a prior 6OHDA lesion of the ipsilateral MFB enhanced contraversive rotation. Doses of dopamine (5 or 12 ug) or apomorphine (5 or 10 ug) ineffective in the intact rat induced marked contraversive rotation in the 6OHDA lesioned animal. Dopamine (50 ug) injected into zona compacta of animals with a 6OHDA lesion produced contraversive, but not ipsiversive, circling. Haloperidol (0.1 mg/kg ip 1 h previously) reduced contraversive rotation induced by unilateral injection into central zona reticulata of dopamine (50 ug) in the intact rat and apomorphine (10 ug) in animals with a prior 6OHDA MFB lesion. Dopamine (50 ug) injected into zona compacta of haloperidol treated animals produced contraversive, but not ipsiversive, circling. Electrolytic lesions of the internal capsule rostral to nigra or kainic acid lesions of caudate and globus pallidus attenuated contraversive rotation produced by injection of apomorphine (10 ug) into zona reticulata of animals with a prior 6OHDA lesion of the MFB.

Table 1 Circling produced by intranigral injection of dopamine or apomorphine

Injection site		Maximum rate of rotation (turns/min)	
	Other treatment	Dopamine (50 ug)	Apomorphine (10 ug)
Zona reticulata	-	11.5±1.7 C	2.2±0.6 C
	6OHDA	22.2±2.8* C	20.5±3.3* C
	Haloperidol	6.5±2.1* C	-
	Haloperidol + 6OHDA	-	4.4±1.3+ C
Zona compacta	-	1.7±0.4 I	-
	6OHDA	3.8±1.7* C	-
	Haloperidol	6.3±2.6* C	-

C = contraversive rotation; I = net ipsiversive rotation; * $p < 0.05$ compared to dopamine alone; + $p < 0.05$ compared to 6OHDA alone

The ipsiversive rotation produced by intranigral administration of dopamine agonists is dependent on the nigrostriatal pathway. Contraversive rotation involves afferent input from striatum and/or globus pallidus.

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ANABASINE AND CYTISINE: NICOTINE-LIKE BEHAVIOURAL EFFECTS IN A DRUG-DISCRIMINATION PROCEDURE

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The nicotine analogues anabasine and cytisine displace nicotine from a binding site on rat brain membranes and may be active behaviourally in a T-maze drug-discrimination task (Romano et al, 1981). The ability of the analogues to produce nicotine-like behavioural effects has now been examined further with a conventional two-bar, drug-discrimination procedure. Groups of 6-8 rats were trained according to Garcha et al (1982) and all drugs were administered subcutaneously. An additional, quantal bar-selection index was obtained by defining the selected bar as the one which the rat first pressed 10 times in a session.

Nicotine increased the percentage of drug-appropriate responding in a dose-related manner from 0.7 ± 0.4% after saline to 95.6 ± 1.4% after 0.4 mg/kg of nicotine, the initial training dose (these quantitative scores are means ± sem). The percentages of rats selecting the drug-appropriate bar were 0% and 100% after saline and nicotine (0.4 mg/kg) respectively. Anabasine (1.0-4.0 mg/kg) increased the percentage of drug-appropriate responding in a dose-related manner to 59.8 ± 12.7%, with 50% of rats selecting the drug-appropriate bar. Garcha et al (1982) obtained similar results with cytisine. These partial effects prompted further investigations in rats retrained to discriminate a lower, 0.1 mg/kg dose of nicotine. The animals responded reliably, as shown by scores of 1.3 ± 1.2% and 94.4 ± 2.4% after saline and nicotine (0.1 mg/kg) respectively, with 0% and 100% of rats selecting the drug-appropriate bar. Anabasine (0.5-6.0 mg/kg) now increased the percentage of drug-appropriate responding to 74.6 ± 4.8%, with up to 100% of rats selecting the drug-appropriate bar. Cytisine (0.125-2.0 mg/kg) increased drug-appropriate responding to 73.6 ± 6.9%, again with up to 100% of rats selecting the drug-appropriate bar.

In rats trained with nicotine (0.4 mg/kg), mecamylamine administered 30 min before tests blocked the effect of cytisine. The percentage of drug-appropriate responding was reduced from 48.1 ± 10.0% after cytisine (2.0 mg/kg) alone to 14.8 ± 6.7% after mecamylamine (0.9 mg/kg) plus cytisine. Hexamethonium (1.0-9.0 mg/kg), which penetrates poorly to the CNS, did not block the response to cytisine. Thus, the effects of the ganglion-blocking drugs on the response to cytisine were similar to their well-established effects on the response to nicotine.

The present results provide the clearest evidence to date that the analogues tested can produce nicotine-like discriminative responses, although their effects were not fully equivalent to those of nicotine itself. It is concluded that there is some correlation between the behavioural effects of the analogues and their actions at the binding site for nicotine on rat brain membranes: such a correlation supports the view that this cholinergic site may be a pharmacologically relevant receptor.

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(^{3}H)-KETANSERIN BINDING TO 5-HT$_2$ RECEPTORS IN HUMAN BRAIN

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Until recently binding studies of 5-hydroxytryptamine (5-HT) receptors have relied upon the use of relatively unspecific ligands such as (^{3}H)LSD and (^{3}H)spiperone as well as (^{3}H) 5-HT. However, the development of (^{3}H)ketanserin has provided a ligand of high specificity and affinity for the 5-HT$_2$ receptor subtype (Leysen et al, 1982). These receptors are implicated in many behavioural effects of 5-HT and have a relatively high affinity for many neuroleptic drugs. We have initiated a study of 5-HT$_2$ receptors using (^{3}H)ketanserin binding to human cortical tissue obtained post-mortem, in order to assess the effects of some neuroleptic drugs and to attempt to clarify the contradictory reports suggesting 5-HT$_2$ receptor abnormalities in schizophrenic brain.

Tissue homogenates were prepared by a simplified procedure involving a single centrifugation step and incubations were performed in Tris buffer (50 mM, pH 7.7) following the method of Leysen et al (1982). Displacement by d-LSD at 10^{-6} M was used to define specific binding.

Scatchard analysis of specific (^{3}H)ketanserin binding to five preparations of human cortex yielded a mean K_D value of 0.47 ($\pm$ 0.06 s.e. mean) nM and maximum binding of 10.2 ($\pm$ 1.2 s.e. mean) pmol/g tissue. Using a sub-saturating concentration of (^{3}H)ketanserin, a range of compounds were tested for their ability to displace binding; K_I values are summarized in Table 1.

Table 1. K_I values (nM) for the displacement of (^{3}H)ketanserin binding to human frontal cortex

Ketanserin	0.35 $\pm$ 0.05
5-HT	280 $\pm$ 50.0
d-LSD	2.3 $\pm$ 1.5
Pipamperone	1.23 $\pm$ 0.28
Pirenperone	0.61 $\pm$ 0.22
Chlorpromazine	2.0 $\pm$ 0.4
Thioridazine	26.0 $\pm$ 10
Haloperidol	31.0 $\pm$ 5.0
Fluphenazine	4.0 $\pm$ 1.1
Clozapine	3.7 $\pm$ 0.2
Propranolol	1280 $\pm$ 350
Dopamine	>10000

The pattern of binding displacement indicates that (^{3}H)ketanserin binds to 5-HT$_2$ receptors. It is notable that the neuroleptic drugs studied have a relatively high affinity for this binding site; for several of the drugs this is at least an order of magnitude higher than their affinity for the spiperone-labelled dopamine D_2 sites. It is, however, unlikely that this site is directly involved in the antipsychotic action of neuroleptics since these results do not indicate any correlation with antipsychotic potencies.

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