COMPARISON OF β-ADRENOCEPTORS IN GUINEA-PIG, RAT AND HUMAN ATRIA

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Cardiac β-adrenoceptors in rat and guinea-pig have been extensively studied, but human cardiac β-adrenoceptors are less well characterized. Adrenoceptors of the β_1-subtype mediate both rate and tension responses of guinea-pig (Lumley and Broadley, 1977) and rat (Bryan et al, 1981) atria. Binding studies have revealed a heterogeneous β_1- plus β_2-adrenoceptor population in human right atrial appendage (Brodde et al, 1983). The present study examines, by use of selective agonists and antagonists, whether both receptor subtypes contribute to the tension response of this tissue.

Rat and guinea-pig right atria (spontaneous: rate response) and left atria (paced: tension response) were set up in Krebs solution at 32°C. Biopsy samples of right atrial appendage were obtained from children undergoing corrective cardiac surgery and transported to the laboratory in warmed Krebs solution. Tension recordings were taken from paced individual pectinate muscles at 32°C. Concentration-response curves were performed to three full agonists, noradrenaline (after phenoxybenzamine pre-incubation) (β_1-selective), fenoterol (β_2-selective) and isoprenaline (non-selective), and three partial agonists, prenalterol (β_1-selective), salbutamol (β_2-selective) and BRL 28410 (non-selective). BRL 28410 is a new β-adrenoceptor agonist which selectively stimulates adipose tissue β-adrenoceptors with low activity at β_1- and β_2-adrenoceptors (Arch et al, 1982). pD_2 and relative intrinsic activity (RIA) values are shown in table 1.

Table 1 Geom. mean pD_2 (50% own max.). Arith. mean RIA in parentheses

	Noradr.	Fenot.	Isopren.	Prenal.	Salb.	BRL 28410
Rat R.A. Rate	7.70(1)	7.32(1)	8.89(1)	7.54(0.82)	6.01(0.94)	4.43(0.69)
Rat L.A. Tension	7.41(1)	7.17(1)	8.39(1)	7.21(0.91)	5.49(0.90)	4.14(0.68)
G-pig R.A. Rate	7.49(1)	7.07(1)	7.73(1)	7.04(0.46)	5.67(0.65)	4.58(0.33)
G-pig L.A. Tension	7.74(1)	6.91(1)	8.08(1)	7.35(0.20)	7.19(0.25)	4.80(0.14)
Human R.A. Tension	7.47(1)	7.76(1)	8.54(1)	7.07(0.14)	6.73(0.89)	4.63(0.32)

The low RIA values for prenalterol and BRL 28410 in human atrium suggest a smaller receptor reserve in this tissue than in rat atrium. The potency of the β_2-selective agonists in human atrium, where the pD_2 for fenoterol is higher than in rat and the RIA for salbutamol is similar to that in rat, must therefore be attributed to the presence of β_2-adrenoceptors. This is supported by selective antagonist data. The pA_2 value for ICI 118, 551 (β_2-selective) in human right atrium was 8.10 with fenoterol as agonist, but only 6.70 with noradrenaline as agonist. Similarly, the pA_2 value for practolol (β_1-selective) was 6.69 with noradrenaline as agonist, but only 5.32 with fenoterol as agonist.

These results suggest that a mixed β_1- plus β_2-adrenoceptor population mediates the inotropic response of the human right atrium to β-adrenoceptor agonists.

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EFFECT OF TERTATOLOL ON PRE- AND POSTJUNCTIONAL β-RECEPTORS IN CANINE BLOOD VESSELS

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Tertatolol (dl [hydroxy-2 't-butylamino-3' propyloxy] -8-thiochromane hydrochloride; Servier R -2395) is a potent inhibitor of responses to beta-adrenergic agonists both in vitro and in vivo (Laubie et al., 1973; Mouille et al., 1977; Chenieux-Guicheney et al., 1978).

The present experiments were designed to investigate the effects of tertatolol on pre- and postjunctional beta-adrenoceptors in isolated blood vessels of the dog.

To examine the effects of tertatolol on postjunctional beta-receptors, segments of canine saphenous veins and coronary arteries were mounted for isometric tension recording in organ chambers filled with aerated (95 % O_2 - 5 % CO_2) physiological salt solution maintained at 37°C. After equilibration the preparations were treated with phentolamine (3×10^{-6}M) and subsequently contractions were evoked in saphenous veins with K^+ (50 mM) and in coronary arteries with prostaglandin $F_{2\alpha}$ (10^{-6}M). When these contractions had stabilized, increasing concentrations of isoprenaline (10^{-8} to 10^{-4}M; saphenous veins) or of noradrenaline (10^{-8} to 10^{-4}M; coronary arteries) were administered to the tissues. The concentration-dependent relaxations thus obtained in the control segments were then compared with those noted in tissues pretreated with either tertatolol (10^{-9} to 10^{-6}M) or propranolol (10^{-9} to 10^{-6}M). Tertatolol, at concentrations which did not affect the contractile responses to either K^+ or prostaglandin $F_{2\alpha}$ inhibited the beta-adrenergic relaxations in both tissues and, as determined by the pA_2-values, had a greater affinity for the postjunctional beta-receptors than propranolol.

To determine the effects of tertatolol on prejunctional beta-adrenoceptors, helical strips of canine saphenous veins were incubated with ^{3}H-noradrenaline (3×10^{-7}M) and then mounted for superfusion to allow detection of the efflux of ^{3}H-noradrenaline and its metabolites. Isoprenaline (2×10^{-6}M) significantly increased the electrical stimulation (2Hz)-induced overflow of both total ^{3}H- and of intact ^{3}H-noradrenaline by acting at prejunctional beta-receptors (see also Verbeuren et al. 1983). Both tertatolol (10^{-7}M) and propranolol (10^{-6}M) inhibited the action of isoprenaline without affecting the control responses to 2Hz-stimulation.

From these experiments, we can conclude that tertatolol is a beta-adrenolytic substance with a high affinity for both $beta_1$- (dog coronary arteries) and $beta_2$- (dog saphenous veins) postjunctional receptors, and that the substance is a potent blocker of prejunctional beta-adrenoceptors in the dog saphenous vein.

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INFLUENCE OF DIFFERENT BACTERIA AND THEIR COMPONENTS ON GUINEA-PIG LUNG β-ADRENOCEPTOR FUNCTION AND NUMBER

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Vaccination of laboratory animals with gramnegative bacteriae like Bordetella pertussis and Haemophilus influenzae leads to a variety of biological effects, among which are the impairment of cholinergic and adrenergic responsiveness within the cardiovascular and respiratory system (Schreurs et al., 1983; De Wildt et al., 1983). These biological effects may be related to the adverse reactions observed after immunization of infants. Recently a new strain of Bordetella pertussis was developed (Sato) with a strongly reduced content of endotoxin (LPS) and lymphocyte promoting factor (LPF). In previous studies with Bordetella pertussis and Haemophilus influenzae we reported that vaccination of guinea-pigs leads to a decreased beta-adrenergic responsiveness and to a decrease in the number of beta-adrenergic binding sites in guinea pig pulmonary tissue (Schreurs et al., 1983). In the present study we investigated the effect of vaccination of guinea pigs with Haemophilus influenzae, two different Bordetella pertussis strains (PG24 and Sato) and two components of these bacteriae (LPF and LPS) on the number and function of beta-adrenoceptors in lung and trachea respectively. Since increased endogenous catecholamines may lead to beta-adrenoceptor desensitization, catecholamine levels were measured in plasma, spleen, thymus, heart, lung and the anterior hypothalamus.

Male guinea-pigs were vaccinated i.p. with the bacteriae or their components 4 days before the experiment. Contraction and relaxation of the isolated trachea l spirals and the number of beta-adrenoceptor binding sites were measured in lung tissue(for references see Schreurs et al., 1983). Catecholamine content of the tissues and plasma were measured radioenzymatically.

Pretreatment of guinea-pigs with the bacteriae and their components does not influence carbachol-induced contraction of the trachea. However, the relaxation induced by isoprenaline after halfmaximal contraction with carbachol was significantly attenuated by 32% for PG24, 23% for Sato, 21% for Haemophilus influenzae and LPS by 29%. LPF, however, was ineffective. The number of beta-adrenoceptor binding sites was also decreased; by 19% for PG24, 25% for Haemophilus influenzae and 23% for LPS, but not by LPF. The Sato vaccine tended to reduce the number of beta-adrenoceptor binding sites, but this was not significant ($p < 0.1$, Student's t-test). Catecholamine concentration in plasma or in the tissues were not affected by any of the above treatments. Although these catecholamine levels appear to indicate that they are not involved in beta-adrenoceptor desensitization, a time-course study with Haemophilus influenzae showed that changes in plasma noradrenaline concentration occur on the 1st and 3rd day after vaccination. So the exact role of catecholamines needs to be further examined.

The present results indicate that bacterial endotoxin but not lymphocyte promoting factor is responsible for the attenuation in the beta-adrenoceptor responsiveness and the reduction in their number in guinea-pig respiratory system after vaccination with gramnegative bacteriae.

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ATYPICAL NATURE OF RAT ADIPOCYTE β-ADRENOCEPTORS: EVIDENCE FROM LIPOLYSIS AND cAMP PRODUCTION

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The precise classification of the rat adipocyte beta-adrenoceptor is unclear. Radioligand binding studies (Bojanic & Nahorski, 1983) have revealed in isolated adipocytes a binding site possessing properties of a typical $beta_1$ receptor, whereas the functional lipolytic response to isoprenaline has been shown to be antagonised by beta-blockers with potencies not expected at either $beta_1$ or $beta_2$ subtypes (De Vente et al, 1980). We have therefore attempted to examine adipocyte responses (lipolysis and cyclic AMP production in cells and membranes) by beta-adrenoceptor agonists and their antagonism by beta-blockers to examine the possible site of action at which the beta-adrenoceptor may be atypical.

For lipolysis and cyclic AMP accumulation experiments, fat cells were isolated and incubated in Krebs-HCO_3 containing 1% BSA. After a 10 min preincubation with antagonist, the agonist was added and incubation terminated after 90 min for lipolysis and 8 min for cyclic AMP accumulation. Free fatty acids released were measured by titration, whilst cyclic AMP was measured by a specific binding protein assay or radioimmunoassay. For adenylate cyclase assays, adipocyte membranes were prepared by hypotonic lysis and incubations performed in the presence of 0.1 mM GTP and an ATP regenerating system.

The results from lipolysis indicate that irrespective of the agonist used (noradrenaline, $beta_1$-selective, or fenoterol, $beta_2$-selective) the pA_2 values of selective antagonists were similar, indicating receptor homogeneity (Table 1). Neither betaxolol or ICI 118.551 had pA_2 values expected at typical beta-adrenoceptors. Experiments on cyclic AMP accumulation in cells and production in membranes yielded results of a similar nature where betaxolol, ICI 118.551 and (-)-propranolol had low pA_2 values, indicating that the atypical beta-adrenoceptor was also involved at the cyclase level. In conclusion, we demonstrate the presence of an apparently atypical beta-adrenoceptor mediating both lipolysis and cyclic AMP generation in rat adipocytes.

Table 1

Effector Response	Agonist	Betaxolol β_1-Selective	ICI 118.551 β_2-Selective	(-)-Propranolol	(+)-Propranolol
Lipolysis	NA	6.02 ± 0.10	5.70 ± 0.06	-	-
		(0.77 ± 0.05)	(1.50 ± 0.11)	-	-
	FEN	5.77 ± 0.19	5.67 ± 0.14	-	-
		(0.90 ± 0.03)	(1.45 ± 0.11)	-	-
c'AMP Accumulation	ISO	6.15 ± 0.20	6.35 ± 0.24	7.35 ± 0.23	6.39 ± 0.27
		(0.84 ± 0.12)	(1.11 ± 0.08)	(1.22 ± 0.18)	(0.98 ± 0.11)
Adenylate Cyclase Activity	ISO	6.15 ± 0.15	6.43 ± 0.16	7.81 ± 0.27	6.78 ± 0.17
		(0.82 ± 0.10)	(0.91 ± 0.15)	(0.68 ± 0.11)	(0.50 ± 0.04)

pA_2 values and Schild slopes (in parentheses) of beta-adrenoceptor antagonists versus the agonists noradrenaline (NA), fenoterol (FEN) and isoprenaline (ISO). Schild plots were calculated by linear regression of four concentrations of antagonist and pA_2 values were estimated by extrapolation of the regression line to a dose-ratio of 2.

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HAEMOPHILUS INFLUENZAE, A TIME-COURSE STUDY FOR THE CHANGES IN β-ADRENOCEPTOR FUNCTION AND NUMBER IN GUINEA-PIG LUNG

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Haemophilus influenzae is a bacterium that can be isolated from the deeper respiratory airways of patients with chronic asthmatic bronchitis. We have previously shown that vaccination of guinea-pigs with this bacterium 4 days prior to the experiment inhibited the function of the beta-adrenergic receptor system in isolated tracheal spirals and reduced the number of beta-adrenoceptor binding sites in lung tissue (Schreurs and Nijkamp, 1982). Moreover the effects could be prevented by an inhibitor of the enzyme dopa-decarboxylase (Schreurs et al., 1982).

In the present study we have investigated the time course of the changes of beta-adrenoceptor function and number over a period of 8 days after vaccination (day 1,3,4 and 8). To investigate a possible etiological relationship between catecholamine levels and loss of beta-adrenoceptor binding sites and function, dopamine, noradrenaline and adrenaline were measured in plasma, some peripheral tissues (spleen, thymus, lung, heart) and the anterior hypothalamus.

Beta-adrenoceptor number was measured by means of a radioligand binding assay, using $|^3H|$-dihydroalprenolol as a ligand, in peripheral lung tissue, while catecholamine levels were measured radioenzymatically. Beta-adrenoceptor function was measured as the isoprenaline induced relaxations of isolated tracheal spirals, that were halfmaximally contracted by carbachol.

Haemophilus influenzae produced no effect on the number of beta-adrenoceptor binding sites on the first day after vaccination. However by 3-4 days there was a ± 25% reduction ($p < 0.05$) compared to non-vaccinated controls. By 8 days the values had returned to control.

The changes in isoprenaline-induced tracheal relaxation followed a similar pattern. A significant decrease in the maximal relaxation produced by isoprenaline was observed only after 3-4 days (± 30%; $p < 0.01$).

Plasma noradrenaline levels on day 1 and 3 after vaccination were significantly increased by 119% and 40% respectively. Interestingly noradrenaline levels in the spleen were decreased by 30-43% (per g tissue) on day 1, 3 and 8 after vaccination. No changes in catecholamine levels were observed in any other tissue.

In conclusion these experiments show that the decrease in beta-adrenoceptor function and number may be related. There appears to be a correlation of these changes to the changes in plasma catecholamine levels. However, turnover studies are required to draw definite conclusions.

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PROPERTIES OF HUMAN CARDIAC β-ADRENOCEPTOR-COUPLED ADENYLATE CYCLASE

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By means of (±)-^{125}iodocyanopindolol binding the coexistence of β_1- and β_2-adrenoceptors (ratio β_1:β_2 = 80:20) in human right atria has been recently demonstrated (Brodde et al., 1983). In order to further characterize the ß-adrenoceptor-adenylate cyclase (AC) system in human right atria, in the present study properties of AC were determined.

Strips of human right atria, obtained during open heart surgery, were homogenized in 10 ml ice-cold 1mM $KHCO_3$. The homogenate was centrifuged with 200 g x 10 min at 4°C and the supernatant was centrifuged with 12,000 g x 30 min. The resulting membrane fraction was resuspended in 10 vol. of ice-cold 1mM $KHCO_3$. AC-activity was determined at 37°C as described by Jakobs et al. (1976).

Basal AC-activity was enhanced by Mg^{++}-ions and by guanyl nucleotides like GTP (0.1-100 µM) and Gpp(NH)p (0.01-100 µM) in a concentration-dependent manner; guanyl nucleotide activation was strongly dependent on the presence of Mg^{++}-ions. Kinetics of basal and 10 µM isoprenaline (IPN)-stimulated AC-activity was linear at 37°C up to 15 min; time-course of 10 µM Gpp(NH)p stimulation, however, showed a lag-period of about 3-5 min. In the presence of 100 µM GTP basal AC-activity amounted to 8.7±1.1 pmoles cAMP formed/min/mg protein (N=9).

ß-Adrenoceptor agonists stimulated AC-activity (maximum 2 fold) with an order of potency: IPN>adrenaline = noradrenaline. IPN (10 µM)-stimulated AC-activity was inhibited by propranolol in a stereospecific manner with the (-)-isomer being about 100 times more potent than the (+)-isomer. The β_1-selective antagonist betaxolol was much more potent in inhibiting IPN (10 µM)-stimulated AC-activity than the β_2-selective antagonist ICI 118,551 thus confirming our recent observation that in human right atria β_1-adrenoceptors predominate (Brodde et al., 1983).

The β_2-selective agonists fenoterol and procaterol caused concentration dependent increases in AC-activity; the maximum effect, however, was only 50% of that of IPN. The β_2-selective agonist zinterol, on the other hand, had no stimulatory effect on AC-activity, but inhibited IPN (10 µM)-stimulated AC-activity.

These results demonstrate that the properties of ß-adrenoceptor coupled AC-activity in human right atria are very similar to those recently described in other mammalian cardiac tissues (Minneman et al.,1979)

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THE EFFECT OF THYROID STATE ON THE RESPONSES OF CONSCIOUS DOGS TO PROPRANOLOL, SOTALOL AND NADOLOL

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In addition to β-adrenoceptor antagonism (βAA) propranolol has membrane stabilising activity (MSA) (Barrett, 1970) and sotalol induces prolongation of the action potential duration (PAPD) (Vaughan Williams, 1974) while nadolol has little effect apart from βAA (Lee et al, 1978). We wondered whether thyroid hormones modify either βAA or the other actions of these drugs.

Four dogs were studied while a) euthyroid, b) made hyperthyroid by daily doses of triiodothyronine (3.3 mg/kg by mouth) and c) made hypothyroid by daily doses of carbimazole (1 mg/kg by mouth) **for three weeks**. The three antagonists were infused on different days for 30 mins while recording the ECG from limb leads. The cumulative effects of the antagonists on various parameters of the ECG were compared. Before and after the administration of each antagonist dose response curves for the chronotropic action of isoprenaline were plotted and the dose ratios (DR) calculated. The response curves to isoprenaline, and DR for a given dose of each antagonist were reproducible within narrow limits in each dog, and were unaffected by either carbimazole or triiodothyronine.

Propranolol alone of the antagonists gave a marked dose-dependent and statistically significant increase in the P-R interval, which was independent of thyroid state.

Sotalol alone of the antagonists gave a marked dose-dependent and statistically significant increase in Q-T interval. This action was greatly potentiated in the hyperthyroid state. In euthyroid dogs sotalol lowered heart rate (HR) more effectively than the other drugs for a given isoprenaline DR. The high HR of hyperthyroid dogs was lowered by sotalol, but not by the other antagonists.

Our results suggest the following conclusions: (i) Thyroid state does not influence the cardiac response to β-adrenoceptors agonists or antagonists in the dog. (ii) The PAPD of sotalol is potentiated in hyperthyroidism. (iii) The high HR in hyperthyroid dogs cannot be attributed to sympathetic overactivity since it is refractory to βAA. It is lowered by sotalol, presumably by virtue of PAPD. (iv) Unlike the other drugs, nadolol, even at doses that give an isoprenaline DR greater than 10^5 does not show any ECG disturbance not attributable to βAA.

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EFFECT OF CENTRAL AND PERIPHERAL ADMINISTRATION OF RX 781094 ON BP AND PLASMA NORADRENALINE RESPONSES TO CLONIDINE IN RATS

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Stimulation of central α-adrenoceptors by clonidine results in a decrease in sympathetic nervous activity, hypotension and bradycardia. Clonidine has also been demonstrated to reduce stimulation-evoked release of noradrenaline from various isolated tissues through an action at prejunctional α_2-adrenoceptors (for review, see Starke, 1977). In this study we have investigated whether stimulation of peripheral, prejunctional α_2-adrenoceptors in intact animals by iv clonidine may reduce noradrenaline release independently of central α-adrenoceptor stimulation.

Groups of 10 male Wistar rats (250-350 g) anaesthetised with Inactin (thiobutobarbitone Na 100 mg/kg ip) received the α_2-adrenoceptor antagonist RX 781094 (Idazoxan) (Chapleo et al, 1981) or vehicle (saline) either iv (via a jugular vein) or into a lateral cerebral ventricle (icv, volume 10 µl) as a 5 min infusion 5 min before clonidine (CLON) 5 µg/kg iv. Blood samples (0.35 ml) were withdrawn from the carotid arterial cannula for determination of plasma noradrenaline (NA) and adrenaline (A) by radioenzymatic assay (Brown & Jenner, 1981), 5 min before, and immediately after RX 781094 or vehicle and 5, 10, 30 and 60 min after clonidine 5 µg/kg iv.

In control animals (saline 1 ml/kg iv) mean arterial pressure (MAP) fell by 36.5 ± 4.4 mm Hg 5 min after CLON and heart rate (HR) by 45 ± 5.7 beats/min. Plasma NA fell by 44.8 ± 4% and A by 31.3 ± 9.9% (control NA 0.242 ± 0.03 ng/ml; A 0.053 ± 0.02 ng/ml). After pretreatment with RX 781094 300 µg/kg iv MAP fell by 1.7 ± 1.0 mm Hg and HR by 16.8 ± 2.8 beats/min. Plasma NA rose to 177.3 ± 9% of control after RX 781094, falling by 9 ± 4% 5 min after CLON (control NA 0.23 ± 0.02 ng/ml). A also rose after RX 781094 pretreatment from 0.03 ± .008 ng/ml to .06 ± .02 ng/ml) with no fall occurring after clonidine. In the control group receiving 10 µl saline icv, CLON caused similar falls in MAP and HR as before (32.6 ± 2.9 mm Hg, and 46.8 ± 3.7 beats/min respectively), with concomitant falls in NA and A of 38.3 ± 3.7% and 31.1 ± 11% (control NA .31 ± 0.02 ng/ml; A 0.044 ± 0.01 ng/ml). Plasma NA and A rose after icv RX 781094, but more slowly than after iv RX 781094. After 50 µg RX 781094 icv, when CLON was not given, NA and A reached 212.6 ± 32.7% and 271 ± 69.6% of control values at 60 min (n=8). RX 781094 10 µg icv caused similar blockade of the hypotensive response to CLON as RX 781094 300 µg/kg, but less inhibition of the fall in NA and A (NA 17.4 ± 2.7% from 0.28 ± 0.03 ng/ml; A 24.6 ± 8.6% from 0.05 ± 0.01 ng/ml; $p < 0.05$ in each case, paired 't' test). RX 781094 50 µg icv produced no further inhibition of clonidine hypotension; after this dose clonidine did not reduce plasma NA or A. However, this dose also caused significant attenuation of the initial pressor response to clonidine ($p < 0.05$).

These results suggest that stimulation of peripheral, prejunctional α_2-adrenoceptors by iv clonidine may contribute to the fall in plasma catecholamines observed, whereas the hypotensive response is mediated by central α-adrenoceptor stimulation.

D.H. is an MRC scholar.

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POSTJUNCTIONAL α_1-ADRENOCEPTOR-MEDIATED MODULATION OF CARDIAC SYMPATHETIC NEUROTRANSMISSION IN RATS

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Fairly recently, it has been suggested that in addition to prejunctional α_2-adrenoceptors, prejunctional α_1-adrenoceptors also may modulate the exocytotic release of noradrenaline from sympathetic nerve terminals (Kobinger & Pichler, 1980; Docherty, 1983). The experimental evidence in favour of this view was obtained in pithed rats, in which the cardiac sympathetic efferents were electrically stimulated. However, the effect of α_1-adrenoceptor agonists on the postjunctional β-adrenoceptor mediated chronotropic response has not been studied in detail. In the present study we investigated the influence of α_1-adrenoceptor stimulation on the chronotropic response to sympathetic nerve stimulation (SNS), noradrenaline and to tyramine in pithed normotensive rats.

The animals (350-450 g) were pithed, artificially ventilated and prepared for electrical stimulation of the spinal cord (C7-Th1). The maximal increase in heart rate (beats/min) to SNS (25 S trains of rectangular pulses of 2 MS, 0.2 Hz and 50 V), noradrenaline (1 µg/kg) or to tyramine (10 µg/kg) was measured. The animals were pretreated with saline, cirazoline (100 µg/kg), amidephrine (1000 µg/kg) or with St 587 (1000 µg/kg).

Table 1 — Increase in heart rate**

Pretreatment	SNS	Noradrenaline	Tyramine
Saline	43 ± 4	71 ± 4	82 ± 4
Cirazoline	8 ± 1*	47 ± 4*	39 ± 4*
Amidephrine	2 ± 1*	48 ± 3*	31 ± 3*
St 587	20 ± 3*	54 ± 4*	46 ± 4*

* $p < 0.05$ compared to saline-treated animals
** mean values ± S.E.M. (n=6-9)

The selective α_1-adrenoceptor agonists (Timmermans et al, 1983) inhibited the tachycardia to electrical stimulation of the cardiac sympathetic nerves. However, cirazoline, amidephrine and St 587 also diminished the chronotropic response to noradrenaline and tyramine. From these results it may be concluded that α_1-adrenoceptor agonists can modulate the cardiac sympathetic neurotransmission by a postjunctional mechanism.

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FPL 60278, A NOVEL AGONIST AT PERIPHERAL DOPAMINE RECEPTORS AND β_2-ADRENOCEPTORS: CARDIOVASCULAR EFFECTS IN THE DOG

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Dopexamine (4-(2-{6-[N-2-phenylethylamino]-hexylamino}ethyl)-1,2-benzenediol dihydrochloride) a dopamine (DA) analogue, stimulates peripheral vascular DA_1 and β_2-receptors and has some prejunctional DA_2-receptor agonist activity. It is weakly active at β_1 and inactive at α-adrenoceptors (Brown et al, 1984).

The cardiovascular effects of dopexamine and DA have been compared by iv.infusion (3×10^{-9} - 10^{-7} mol/kg min^{-1}) in dogs. Responses to each agent were rapid in onset, well maintained and recovered within 15 min of terminating infusions.

In pentobarbitone anaesthetised dogs, (n=8, Table 1) dopexamine lowered BP (secondary to reduced peripheral resistance, TPR) and renal vascular resistance (RVR), with enhanced renal blood flow (RBF). A small increase in heart rate (HR) and inotropy (LV $dp/dt.P^{-1}$) occurred, with little change in cardiac output (CO, thermal dilution) and with mesenteric but not femoral vasodilatation. DA (n=4) produced renal and mesenteric vasodilatation, but increased CO and inotropy, without altering TPR. At the highest infusion rate of DA, BP, HR and femoral vascular resistance were elevated.

Table 1 Anaesthetised dog : changes produced by dopexamine

Dose ($mol/kg.min^{-1}$)	BP (mmHg)	HR (bpm)	$dp/dt.P^{-1}$ (%)	RBF (ml/min)	RVR ($mmHg.min.ml^{-1}$)
Control	103±7	158±5	100	71±7	1.66±0.31
3×10^{-9}	-5±2[a]	+5±3[b]	+9±5[b]	+6±2[a]	-0.23±0.07[b]
10^{-8}	-11±2[b]	+14±4[b]	+33±5[b]	+17±5[b]	-0.43±0.08[b]
3×10^{-8}	-35±4[b]	+17±3[b]	+41±10[b]	+13±5[a]	-0.75±0.20[b]
10^{-7}	-44±5[b]	+16±5[b]	+39±18[a]	-15±7[a]	-0.54±0.39[b]

Paired Student's t-test (n=8) a, $p<0.05$; b, $p<0.01$.

The contributions made by DA_2, β and DA_1 receptors in producing some of the responses was assessed in 4 dogs by i.v. administration of the respective antagonists, haloperidol (HP, 50µg/kg each hour), propranolol (P, 0.5mg/kg and 0.25mg/kg.h^{-1}) and bulbocapnine (B, 3mg/kg). Each agonist was given alternately by stepped 5 min i.v. infusion. The dopexamine induced BP fall was inhibited progressively by each antagonist, whereas the increase in HR and inotropy was enhanced by HP but antagonised by P. B antagonised the dopexamine-induced reduction in RVR and reversed the renal vasodilator response to DA.

In conscious dogs (n=3-7), dopexamine produced a dose related small fall in BP and increased inotropy (QA interval; Jackson,1974) and HR. DA also enhanced contractility but raised BP and HR at the highest infusion rate only. Both agents reduced RVR to a similar extent resulting in increased RBF.

Dopexamine therefore has a novel haemodynamic profile, producing afterload reduction and renal vasodilatation as a result of stimulation of DA_1, DA_2 and β-receptors. It is currently undergoing clinical investigation for the treatment of acute heart failure.

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AN EXAMINATION OF PRE- AND POSTSYNAPTIC α-ADRENOCEPTORS IN SPONTANEOUSLY HYPERTENSIVE RATS

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The rat isolated vas deferens can be used to demonstrate pathophysiological changes in the responsiveness of alpha-adrenoceptors, at least with respect to ageing (Docherty & O'Malley, 1983). In that study it was found that there was a reduced sensitivity of presynaptic $alpha_2$-receptors in aged rats. The purpose of the present study was to look for differences between spontaneously hypertensive rats (SHR) and wistar rats (WR) in the sensitivity of alpha-adrenoceptors, employing both the isolated bisected vas deferens and the pithed rat preparations. A reduced density of peripheral postsynaptic $alpha_1$-receptors has been reported in various rat models of hypertension (see e.g. Yamada et al., 1980).

Young adult (3 month old) male SHR and WR were used. Systolic blood pressure was recorded under ether anaesthesia, and was found to be significantly higher ($P<0.05$) in SHR than in WR, with values of 174 $\pm$ 4.8 and 145 $\pm$ 5.4 mmHg (mean $\pm$ s.e. mean, n=4 each), respectively.

Vasa deferentia were bisected and isometric contractions were obtained in prostatic portions to single pulse field stimulation (supramaximal voltage,0.5ms) at intervals of 5 min. The effects of the $alpha_1$-agonist amidephrine and the $alpha_2$-agonists xylazine and clonidine were assessed against stimulation-evoked contractions. Xylazine and clonidine produced concentration-dependent inhibitions of the isometric contraction to a single pulse, but there was no significant difference in agonist potency between WR and SHR : e.g. IC_{50} values of 7.00 $\pm$ 0.15 and 7.09 $\pm$ 0.09 for xylazine in WR and SHR, respectively (mean and 95% confidence limits, -logM).

The $alpha_1$-agonist amidephrine produces concentration-dependent potentiations of the isometric contraction to a single stimulus in prostatic portions of the rat vas (Docherty, 1983). Both the maximum potentiation produced by amidephrine and the potency were significantly reduced in SHR ($P<0.01$) with maximum responses of 222 $\pm$ 20.4 and 170 $\pm$ 2.4 % of control (mean $\pm$ s.e. mean) and EC_{50} values of 6.51 $\pm$ 0.15 and 6.21 $\pm$ 0.14 (mean and 95% confidence limits,-logM) for WR and SHR, respectively.

In pithed rats, pressor dose-response curves were constructed for xylazine and amidephrine, but no significant differences were found in potencies or maximum pressor responses for either agonist between WR and SHR.

In conclusion, there was a reduced sensitivity and reduced maximum responsiveness of postsynaptic $alpha_1$-receptors in the vas deferens but not in the vasculature of SHR. These findings may reflect a reduction in density of peripheral postsynaptic $alpha_1$-adrenoceptors which would result in demonstrable changes in responsiveness only in tissues where there are few spare receptors.

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SELECTIVITY OF WY 26392 AND YOHIMBINE FOR PRE-JUNCTIONAL α_2-ADRENOCEPTORS IN THE ANAESTHETISED DOG

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It has been previously reported that Wy 26392 is a potent and selective antagonist at α_2 adrenoceptor sites, both in the pithed rat (Pierce and Waterfall, 1982) and in isolated tissue preparations (Lattimer et al., 1982). We have now determined the selectivity and potency of Wy 26392 in comparison with yohimbine at pre-junctional α_2 adrenoceptors in the anaesthetised dog.

Beagle dogs of either sex (9-15kg) were anaesthetised with pentobarbitone (35 $mgkg^{-1}$ and $6mgkg^{-1}hr^{-1}$ i.v.) and artificially respired with room air ($25min^{-1}$; S.V. $17mlkg^{-1}$). Animals were bilaterally vagotomised and atropinised ($1.0mgkg^{-1}$). Blood pressure and heart rate were recorded from a femoral artery and drugs administered via a femoral vein. The chest was opened at the second intercostal space, the right ansa subclavia nerve isolated and placed over a pair of shielded platinum electrodes. The ansa subclavia was stimulated every 2 min for 1 min periods at 0.25 to 2.0 Hz, 1 msec duration and supramaximal voltage. Following each series of stimulations, phenylephrine (PE, $10\mu gkg^{-1}$ i.v.) was administered. Control stimuli were repeated until a stable response was obtained, and then clonidine ($15\mu gkg^{-1}$ and $2\mu gkg^{-1}hr^{-1}$) was administered, 15 mins later the frequency response curve and PE injections were repeated. Subsequently doses of Wy 26392, yohimbine or saline vehicle were administered at 30 min intervals with a 15 min equilibration period prior to nerve stimulation.

Clonidine produced a frequency related inhibition of the tachycardia evoked by nerve stimulation. The doses of the antagonists required to inhibit the responses to clonidine and PE by 50% were obtained graphically (ID_{50}). A selectivity ratio for the α_2 adrenoceptor was expressed as ID_{50} PE/ID_{50} clonidine.

Bolus doses of PE evoked pressor responses of 60.0 ± 1.5 mmHg (n = 20). Wy 26392 and yohimbine produced 50% inhibitions of this response at doses of 1.1 ± 0.22 $mgkg^{-1}$ (n = 7) and 0.95 ± 0.17 $mgkg^{-1}$ (n = 9) respectively. Wy 26392 potentiated the responses to nerve stimulation above pre-clonidine control levels at 30-100 μgkg^{-1}, yohimbine produced no potentiation over the dose range employed.

Table 1 ID_{50} values for Wy 26392 and yohimbine versus clonidine

Frequency Hz	Wy 26392		Yohimbine	
	ID_{50} Clonidine μgkg^{-1}	Selectivity	ID_{50} Clonidine μgkg^{-1}	Selectivity
0.25	12.0 ± 3.8	91.7	69.5 ± 30.0	13.7
0.5	5.5 ± 1.9	200.0	27.1 ± 19.0	35.1
1.0	3.3 ± 0.3	333.0	7.3 ± 2.4	130.0
2.0	3.9 ± 0.6	282.0	27.3 ± 22.0	34.8

Values are mean ± s.e.mean (n = 4-5)

In a further series of experiments the effects of Wy 26392 and yohimbine on nerve stimulation in the absence of clonidine were assessed. Wy 26392 produced a significant potentiation, ($p<0.01$, unpaired 't' test) at a threshold dose of $10\mu gkg^{-1}$, yohimbine produced no potentiation over the dose range employed.

Wy 26392 and yohimbine are both potent and selective antagonists at pre-junctional α_2 adrenoceptors in the dog.

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ALFUZOSIN (SL77.499), A NEW ANTIHYPERTENSIVE AGENT WITH A PERIPHERAL SITE OF ACTION: I. IN VIVO PHARMACOLOGICAL STUDIES

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Alfuzosine (SL 77.499) hydrochloride (SL 77.499-10) is the N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)methylamino]propyl]tetrahydro-2-furancarboxamide hydrochloride. Similarly to prazosin, it possesses the 4-amino-6,7-dimethoxyquinazolinyl moiety. In this communication we will present the results of some studies performed to assess the pharmacological profile of alfuzosine as a potential antihypertensive agent.

Adult spontaneously hypertensive rats (SHR) were prepared for the measurement of blood pressure from a catheter inserted into the caudal artery during a brief period of ether anesthesia and used to study the effects of orally administered placebo, alfuzosine (3.0 mg/kg) and prazosin (1.0 mg/kg). In pentobarbital anesthetized SHR, the blood pressure effects of alfuzosine (25.0 µg/kg) and clonidine (7.5 µg/kg) injected i.v. or into the lateral cerebral ventricle (i.c.v.) were compared. Dose-pressor response curves to cirazoline and UK-14,304 were determined in pithed SHR pretreated with either i.v. saline, alfuzosine (0.01-0.3 mg/kg) or prazosin (0.01-0.3 mg/kg). In the same preparation, the effects of alfuzosine (1.0 mg/kg, i.v.) and prazosin (0.3 mg/kg, i.v.) were studied on the clonidine (5.0 µg/kg, i.v.)-induced inhibition of the heart rate which had been elevated (100 beats/min) by sustained electrical stimulation of the spinal cord. Conscious dogs were lifted on their hind legs for 60 sec during which carotid artery blood pressure was measured before and after oral alfuzosine (0.3 mg/kg) or prazosin (0.1 mg/kg). In pentobarbital anesthetized dogs the effects of i.v. propranolol (0.3 mg/kg), methylatropine (0.3 mg/kg), cimetidine (0.5 mg/kg) plus promethazine (1.0 mg/kg), diclofenac (1.5 mg/kg) and bilateral vagotomy on the blood pressure decrease produced by alfuzosine were also studied.

In conscious SHR, alfuzosine (3.0 mg/kg p.o.) and prazosin (1.0 mg/kg p.o.) produced a similar long lasting decrease in blood pressure (20-25% of base-line value). Heart rate was either unchanged or, in the alfuzosine group, slightly decreased. In anesthetized SHR, i.c.v. injected alfuzosine, in contrast to i.v. administration and to i.c.v. clonidine, produced a minor decrease in blood pressure. In pithed SHR alfuzosine, like prazosin, shifted to the right and in a parallel manner the dose-pressor response curve to cirazoline (α_1-adrenoceptor agonist) but not to UK-14,304 (α_2-adrenoceptor agonist). Alfuzosine (1.0 mg/kg, i.v.), but not prazosin (0.3 mg/kg, i.v.) antagonized slightly (22 %) the inhibition of neural sympathetic tachycardia evoked by clonidine. In anesthetized dogs, the decrease in blood pressure produced by alfuzosine was not affected by bilateral vagotomy or by antagonists of vascular postjunctional β_2-adrenoceptor, histamine H_1 and H_2 or muscarinic-receptors or by blockade of cyclooxygenase with diclofenac. In conscious dogs, the increase in blood pressure accompanying the rapid change from the normal position to standing on hind legs was inhibited by alfuzosine whereas it was reversed to a depressor response by prazosin.

These results indicate that alfuzosine is an antihypertensive agent which, like prazosin (Cavero & Roach, 1980), blocks preferentially the postjunctional vascular α_1-adrenoceptor subtype. However, alfuzosine affects significantly less than prazosin the pressor response to postural changes. This difference may be of potential therapeutic interest for a member of this pharmacological class.

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ALFUZOSIN (SL 77.499), A NEW ANTIHYPERTENSIVE AGENT WITH A PERIPHERAL SITE OF ACTION: II. IN VITRO PHARMACOLOGICAL STUDIES

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Alfuzosine, like prazosin, is a dimethoxyquinazolinyl derivative which decreases blood pressure in spontaneously hypertensive rats by a peripheral mechanism apparently compatible with the blockade of postjunctional vascular α_1-adrenoceptors (Cavero et al, this meeting). This communication describes the effects of alfuzosine on isolated vascular preparations, on several radioligand bindings, and on the release of ^{3}H-noradrenaline.

Rabbit pulmonary artery strips were bathed in a salt solution. A dose-response curve to phenylephrine was determined before and 30 min after exposure to alfuzosine or prazosin. The rat vas deferens was prepared for studying the effects of alfuzosine and prazosin on the clonidine-induced inhibition of the twitch response. From the results of these studies pA_2 values were calculated using a Schild plot. Rabbit aortic strips were incubated for 30 min in a solution containing 1.0 µM of phenoxybenzamine and then exposed to propranolol (1.0 µM) and contracted with KCl or $CaCl_2$ before the addition of cumulative concentrations of alfuzosine, prazosin or papaverine. The effects of alfuzosine were studied on ^{3}H-prazosin and ^{3}H-clonidine binding to rat cerebral membranes (Langer and Pimoule, 1982) and on the electrically-evoked release of ^{3}H-noradrenaline from slices of rabbit hypothalamus (Galzin et al, 1982).

In rabbit pulmonary artery strips the pA_2 of alfuzosine and prazosin against phenylephrine were 7.95 $\pm$ 0.34 and 9.29 $\pm$ 0.05, respectively. In the rat vas deferens the pA_2 of alfuzosine, prazosin and idazoxan against clonidine were 6.2 $\pm$ 0.1, 6.1 $\pm$ 0.09 and 8.07 $\pm$ 0.13 respectively. Prazosin in a concentration up to 0.1 mM (limit of solubility) did not relax rabbit aortic strips contracted with KCl or $CaCl_2$. In contrast, the pEC_{50} of alfuzosine and papaverine against KCl contraction were 3.11 $\pm$ 0.06 (n=7) and 4.48 $\pm$ 0.08 (n=7), respectively. The same results were obtained in preparations contracted with $CaCl_2$. The IC_{50} of alfuzosine on ^{3}H-prazosin binding was 15 nM while on ^{3}H-clonidine binding it was 600 nM. These values were 3 nM and 7000 nM, respectively, for prazosin. Alfuzosine did not exhibit direct interactions at dopamine (DA-2), 5HT-2 and muscarinic cholinergic receptor sites. Exposure to alfuzosine (0.01 to 1.0 µM before S_2) produced only a small increase in the electrically-evoked release of ^{3}H- noradrenaline from slices of the rabbit hypothalamus. In the same range of concentrations idazoxan increased the overflow of ^{3}H- noradrenaline in a concentration-dependent manner. Prazosin (0.01 to 1.0 µM) was inactive on the electrically-evoked release of ^{3}H-noradrenaline, but increased the spontaneous outflow of radioactivity at 1.0 µM.

These results indicate that alfuzosine is a relatively selective antagonist of the α_1-adrenoceptor subtype. Unlike prazosin but similarly to papaverine, alfuzosine relaxes aortic strips contracted with either KCl or $CaCl_2$. This property may be of therapeutic relevance.

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ENHANCEMENT BY α_2-ADRENOCEPTOR AGONISTS OF RESPONSES MEDIATED VIA α_1-ADRENOCEPTORS

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Post-junctional α_2 adrenoceptors have been reported to mediate vasoconstrictor responses in a number of vascular preparations (Timmermans and Van Zwieten, 1981). There are, however, few reports of contractile responses of non-vascular smooth muscle preparations to α_2 adrenoceptor agonists. This communication describes the effects of α_2 adrenoceptor agonists upon the contractile responses of smooth muscle in vascular and non-vascular preparations.

Cats (2-2.5kg) were anaesthetised with pentobarbitone (30mg/kg i.t. and 6mg/kg/hr i.v.) and the femoral veins, a femoral artery and trachea cannulated. The preparation was bivagotomised, ganglion and β-blocked (1mg/kg chlorisondamine and 1mg/kg propranolol i.v.). The nictitating membrane (NM) was attached to a Grass FT03 isometric transducer and a tension of 10-12g applied. Blood pressure, heart rate and NM tension were recorded on a Grass polygraph. Dose response curves to phenylephrine (PE, 1-30μg/kg i.v.) or angiotensin II (AII, 0.03-0.3 μg/kg i.v.) were constructed. Either clonidine (1-10μg/kg i.v.) or the selective α_2 adrenoceptor agonist UK14304 (0.3 or 3μg/kg i.v.; Cambridge, 1981) was then administered and the dose response curves repeated.

PE evoked dose dependent contractions of the NM (Table 1) and an increase in diastolic blood pressure (DBP, max increase 109.9 ± 4.4 mmHg). Following the administration of clonidine (n = 4) or UK14304 (n = 4) the response of the NM was enhanced (Table 1). In contrast the increase in DBP was not significantly affected (max increase 102.3 ± 3.7 and 97.5 ± 6.5 mmHg in the presence of clonidine and UK14304 respectively). Administration of the α_2 adrenoceptor antagonist Wy 26392 (30μg/kg i.v.; Pierce and Waterfall, 1982) in the presence of UK14304 reduced the response of the NM to PE (89 ± 6% and 21 ± 6% reduction of responses to 3 and 30 μg/kg respectively). AII (n = 4) evoked dose dependent increases in NM tension (Table 1) and DBP (max increase 110 ± 4 mmHg), administration of UK14304 did not alter these responses.

Table 1 Contractile response of the cat NM to i.v. administration of PE and AII

Agonist	Change In Nictitating Membrane Tension (g, Mean ± SEM)				
	Control	Clonidine (μg/kg i.v.)		UK 14304 (μg/kg i.v.)	
		3	10	0.3	3
PE. 3μg/kg	0.14 ± 0.03	0.6 ± 0.3	1.7 ± 0.5	0.6 ± 0.03	3.1 ± 0.5
PE. 30μg/kg	2.43 ± 0.3	3.8 ± 0.5	4.9 ± 0.6	4.7 ± 0.3	7.6 ± 0.9
AII 0.3μg/kg	0.8 ± 0.4	- ± -	- ± -	0.4 ± 0.1	0.5 ± 0.2

Clonidine and UK14304 themselves evoked increases in DBP (max increase 66.7 ± 1.7 and 60.8 ± 6.7 mmHg respectively) and NM tension (max increase 1.8 ± 0.3 and 1.5 ± 0.3g respectively). Both parameters returned to pre-dose values before the dose-response curves to PE and AII were constructed. In subsequent studies these responses to clonidine and UK14304 were markedly reduced by prazosin (30μg/kg iv).

Thus, the response of the cat NM to the preferential α_1 adrenoceptor agonist PE is potentiated by prior administration of α_2 adrenoceptor agonists, in contrast the pressor response to PE was not significantly changed. The potentiation is markedly reduced by the α_2 antagonist Wy 26392. These results suggest that in some tissues, although postsynaptic α_2 adrenoceptors do not mediate responses *per se* they facilitate the response mediated via α_1 adrenoceptors.

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POTENTIATION OF NORADRENALINE-INDUCED VASOCONSTRICTION BY (-)-ISOPRENALINE IN SHR MESENTERIC ARTERIES

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Imms et al (1974) have suggested that peripheral vascular β-adrenoceptors might modulate vasoconstrictor responses to intra-lumenal noradrenaline (NA), and we have reported the presence, in the normotensive rat mesenteric arterial bed, of a vasodilator β-adrenoceptor population which is physiologically antagonistic with respect to vasoconstrictor α-adrenoceptors (Borkowski & Porter, 1983). The present study set out to examine β-adrenoceptor-mediated responses in the mesenteric arteries from spontaneously hypertensive (SHR) rats.

Male SHR rats (200-400g) were anaesthetised with ether, the mesenteric arterial bed removed as described by McGregor (1965), kept at 37°C and perfused, via the mesenteric artery, at a rate of 4.0ml min^{-1} with Krebs solution (gassed with 95% O_2/5% CO_2) to which EDTA (10 $mgdm^{-3}$) and ascorbic acid (20 $mgdm^{-3}$) had been added. Pressor responses to bolus injections of NA (0.01-100μg) in volumes of 0.1 ml were measured as increases in peak perfusion pressure. Test drugs were dissolved in the Krebs solution. Group sizes of six preparations were used in all cases and responses calculated as a percentage of the maximum control response for a particular tissue. Statistical analysis was performed using the paired Student's t-test (significance accepted where $p<0.05$) and computer-assisted curve fitting.

Timolol (10^{-7}M), added to the perfusate, significantly ($p<0.001$) elevated the maximal pressor response to NA, while (-)isoprenaline (10^{-4}M) suppressed NA-induced vasoconstriction, indicating the presence of a vasodilator β-adrenoceptor population. Moreover, the (-)isoprenaline (10^{-4}M)-induced suppression of NA-induced vasoconstriction was attenuated by timolol (10^{-7}M).

However, the NA-induced pressor responses were significantly ($p<0.05$) elevated, and leftward shifts in the log dose-response curves observed, in the presence of lower doses of (-)isoprenaline (10^{-7}M - 10^{-5}M). This observation might be due to (-)isoprenaline occupying β-adrenoceptors, without evoking a vasodilator response, yet reducing the total adrenoceptor pool available to exogenous NA and thus increasing its concentration at the vasoconstrictor α-adrenoceptors. This was supported by the observation that the potentiation of NA-induced responses by (-)isoprenaline (10^{-5}M) was abolished in the presence of the α_2-adrenoceptor antagonist rauwolscine (10^{-7}M).

These results suggest that although a vasodilator β-adrenoceptor population exists in SHR, its function as a physiological antagonist of α-adrenoceptor-mediated vasoconstriction is compromised when compared to that in normotensive rats, in which (-)isoprenaline (10^{-7}M - 10^{-4}M) suppressed NA-induced responses and shifted the log dose-response curves to NA, to the right (Borkowski & Porter, 1983).

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COMPLEX INHIBITORY EFFECTS OF 6-FLUORONORADRENALINE ON THE TWITCH RESPONSE OF THE RAT VAS DEFERENS AT DIFFERENT FREQUENCIES

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6-Fluoronoradrenaline (6FNA) inhibits the electrically-evoked release of ^{3}H-noradrenaline or ^{3}H-5HT from slices of the rabbit and rat hypothalamus (Galzin et al., 1983), these inhibitory effects of 6FNA are blocked by α_2-adrenoceptor antagonists. The twitch response elicited by low frequency stimulation in the rat vas deferens is also inhibited by 6FNA (Shepperson et al., 1981). These results suggest that 6FNA might be a useful agonist with which to study prejunctional α_2-adrenoceptor mechanisms. Differences between phenylethylamines and imidazolines in terms of their α_2-adrenoceptor effects, have been exemplified by Ruffolo et al. (1977) and Mottram (1982). We have therefore re-evaluated the effects of 6FNA in the rat vas deferens preparation using the selective α_2-adrenoceptor antagonist idazoxan (RX 781094 ; Chapleo et al., 1981).

Prostatic segments of rat vas deferens (Sprague-Dawley, 300 - 350g) were set up using a modification of the method of Drew (1977), in which the Krebs' bicarbonate with no Mg^{2+} ions contained cocaine (1 µM) propranolol (1 µM) and prazosin (0.3 µM). The preparations were stimulated at 40 V, 2 ms pulse width at either 0.01 Hz or 0.1 Hz. Inhibitory dose-response curves to 6FNA or clonidine were made before or after the α_2-adrenoceptor antagonist idazoxan, n=4-8 preparations. The tension developed in response to stimulation at 0.01 or 0.1 Hz was 1.97 $\pm$ 0.19 g, and 1.24 $\pm$ 0.19 g, respectively. 6FNA (3 nM - 30 µM) caused a concentration dependent inhibition of the twitch at 0.1 Hz (IC_{50} 1.18 $\pm$ 0.23 µM) and was significantly ($p<0.05$) more potent at this frequency than at 0.01 Hz (IC_{50} 3.94 $\pm$ 0.7 µM). However at 0.01 Hz 6FNA caused an initial potentiation of the twitch (22.3 $\pm$ 3%), prior to the secondary inhibitory effects, which were accentuated by idazoxan. This potentiation of the twitch was also observed with NA, or amidephrine at 0.01 Hz. Neither the potentiation nor the inhibitory effects of 6FNA were modified by treatment with indomethacin (2.6 µM, 1h, n=4), pargyline (10 µM, n=4), or half calcium medium (1.3 mM). However after pretreatment with reserpine (2.5 mg/kg s.c., 24 h) the twitch potentiating effects of 6FNA were markedly increased (154%) at 0.1 Hz. Idazoxan was a competitive antagonist of clonidine at 0.01 Hz (pA_2 7.7 ; slope 0.94, r=0.96). Idozaxan (0.1 µM) antagonised the inhibitory responses to 6FNA (-log KB 8.01 $\pm$ 0.25 ; log shift 1.2 $\pm$ 0.24), however, no further antagonism of 6FNA was observed by idazoxan 1 µM. In reserpinised rats, or in controls at 0.1 Hz the effects of 6FNA were also resistant to blockade with idazoxan (0.1 and 1 µM).

The differential antagonist effects of idazoxan against 6FNA and clonidine are not related to endogenous catecholamines, nor to the potentiating effects of 6FNA. The results may relate either to the differences known to exist between the α_2-adrenoceptor affinity of phenylethylamines and imidazolines (Ruffolo et al., 1977 ; Mottram, 1982), or to an influence of these agonists on the unknown mediator of the twitch in the rat vas deferens. The inhibition of the twitch of the rat vas deferens by 6FNA is therefore more complex than the classical stimulation of the presynaptic inhibitory α_2-adrenoceptors.

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DIFFERENCES IN THE EFFECTS OF UPTAKE-1 BLOCKERS ON RESPONSES OF RAT VAS DEFERENS TO SINGLE PULSE STIMULATION

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Rat vasa deferentia were stripped and suspended in 5ml tissue baths containing Krebs solution gassed with 5% carbon dioxide in oxygen and maintained at 37 degrees centigrade. The tissues were subjected to a resting tension of 0.5 grammes and equilibrated for 30-45 minutes before experimentation was commenced. Tension responses to single pulse field stimulation(SPFS-0.8msec width,40v every 5min via parallel platinum electrodes) were digitised at a sampling frequency of 50Hz and and stored on floppy disc under the control of a microcomputer(Marshall & Sparks,1981).
We report a detailed analysis of the the effects of 4 potent noradrenaline uptake-1 blockers over the time course of the response to SPFS. Protryptyline,desmethylimipramine,metaraminol & cocaine with ID50's respectively of 13nM, 13nM, 77nM & 380nM in rat heart(Iversen 1957) were investigated. Three concentrations of each blocker(10nM, 100Nm & 1uM) were used. Three successive consistent control responses were obtained before each concentration of blocker was administered. The drug effects were analysed first by meaning and scaling the control and treated responses at each of the sampled points and plotting the results in grammes. Secondly the control response immediately preceding a treated response was subtracted from it at each sampled point and the resulting difference meaned with similar data from other tissues and plotted.

Responses to SPFS reveal an early tension peak 300ms after the stimulus followed by a second peak at 600ms which is in agreement with Mcgrath et al(1978). The second peak was not always discrete sometimes being evident only as a shoulder on the falling phase of the first peak. Rarely was the second peak larger than the first.
Cocaine the least potent uptake-1 blocker on rat heart produced the the greatest overall potentiation of the vas responses (101%). The effect was dose related and the maximum difference from control occured 600ms after the stimulus. Metaraminol produced a smaller (35%) dose-related overall increase in tension with a maximal difference from controls occuring at 320ms after the stimulus. Protryptyline and desmethylimipramine (10nM,100NM) elicited small overall (6%) increases with the the maxmal differences from controls occuring at 680 and 320ms repectively. At 1uM both of these latter drugs inhibited the later phase of the response.
The differences reported highlight the value of the detailed analysis of drug effects on the time course of the responses to SPFS that the computer technique affords and draw attention to some subtle differences between uptake-1 blockers which may give insight into multiple mechanisms of action.

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INHIBITORY DOPAMINE RECEPTORS AND α_2-ADRENOCEPTORS ON SYMPATHETIC NEURONS INNERVATING THE CARDIOVASCULAR SYSTEM OF THE RAT

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The involvement of presynaptic α_2-adrenoceptors and dopamine receptors in the regulation of the release of noradrenaline is supported by ample experimental evidence (for reviews see Langer, 1980; Starke, 1981; Lokhandwala & Barrett, 1982). We studied the effects of the selective dopamine agonist N,N-di-n-propyldopamine (DPDA), the mixed α_2-adrenoceptor/dopamine receptor agonist B-HT 920 and the selective α_2-adrenoceptor agonist B-HT 933 on stimulation evoked pressor responses and tachycardia in pithed normotensive rats.

Male, normotensive Wistar rats (230-270 g) were pithed, artificially ventilated and prepared for electrical stimulation of the spinal cord at the level Th5-L4 and C7-Th1 to increase diastolic pressure and heart rate, respectively (rectangular pulses, 2 ms, 20V, pulse trains of 25 s). DPDA (30 and 100 µg/kg/min, i.v.), B-HT 920 (1, 3 and 10 µg/kg/min, i.v.) and B-HT 933 (0.6 and 1 mg/kg/min, i.v.) reduced the stimulation-induced increase in diastolic pressure. The effect of DPDA was blocked by haloperidol and sulpiride (0.3 mg/kg of each), but not by yohimbine (1 mg/kg). The inhibition by B-HT 933 was antagonized by yohimbine and not by sulpiride. B-HT 920 was without effect after pretreatment with the combination of yohimbine and sulpiride. Sulpiride alone partially diminished this inhibition, but yohimbine alone was ineffective. The stimulation-induced increase in heart rate was reduced by B-HT 920 but not by DPDA. The B-HT 920-induced inhibition was not affected by sulpiride. Yohimbine was an effective antagonist.

The results confirm that B-HT 920 possesses dopamine-agonistic effects in contrast to B-HT 933 (Andén et al., 1982, 1983) and they indicate that both α_2-adrenoceptors and dopamine receptors modulate the sympathetic neurotransmission in the vascular smooth muscle of the pithed rat. The cardiac sympathetic neurotransmission is modulated by α_2-adrenoceptors but not by dopamine-receptors. This observation is in agreement with the results of Hicks & Cannon (1979) and Cavero et al. (1981). Activation of prejunctional α_2-adrenoceptors more effectively inhibits the sympathetic neurons to the heart than to the resistance vessels.

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ADRENALINE ENHANCES VASOCONSTRICTOR RESPONSES EVOKED BY ADRENERGIC NERVE STIMULATION IN THE RAT ISOLATED PERFUSED KIDNEY

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Adrenaline has been shown to enhance the release of noradrenaline from vasomotor adrenergic nerves (Majewski, 1983). This effect could lead to an increase in amplitude of vasoconstrictor responses due to adrenergic nerve activity and could also contribute to the development of hypertension (Majewski, 1981). In a previous study we have shown that adrenaline can enhance vasoconstrictor responses evoked by sympathetic nerve stimulation in the pithed rat (Borkowski and Quinn 1983). In the present study we have investigated the effect of adrenaline on vasoconstriction induced by sympathetic nerve stimulation and by noradrenaline in the rat perfused kidney, as it has been suggested that this organ has a pivotal role in the maintenance of blood pressure. (Coleman et al., 1975).

Isolated kidneys from normotensive female Wistar Kyoto rats (4-6 months of age) were prepared as described by Collis and Vanhoutte (1977). The kidneys were perfused at a constant flow (6 ml/min) with Krebs solution (37°C) containing ascorbic acid (10 mg/l), EDTA (1 mg/l) and dextran (AV.mol.wt.81,600, 36g/l). The perfusion pressure of the kidneys was measured. Vasoconstrictor responses were evoked by electrical stimulation of the renal nerves via platinum electrodes using parameters that activate adrenergic nerves (Collis and Vanhoutte, 1977) (0.5-5 Hz, 12v, 1 msec, 10 sec trains). Responses were also evoked by close intra-arterial injection of noradrenaline (6.25 - 200 ng is 20 µl of saline).

Continuous infusion of adrenaline (10^{-9}-5×10^{-9}M) had no significant effect on basal perfusion pressure of the kidneys. A higher dose of adrenaline (10^{-8}M) increased basal perfusion pressure by 8 ± 1.4 mmHg,(n=6). Adrenaline infusion (2.5×10^{-9}-10^{-8}M) caused a dose-related enhancement of vasoconstrictor responses evoked by renal nerve stimulation. Responses induced by exogenous noradrenaline were only significantly enhanced by 10^{-8}M adrenaline. After cessation of the adrenaline infusion (5×10^{-9}M) the enhancement of the vasoconstrictor responses to renal nerve stimulation persisted for at least 25 minutes.

The adrenaline (5×10^{-9}M) induced enhancement of vasoconstrictor responses evoked by renal nerve stimulation was abolished by the β_2 selective adrenoceptor antagonist ICI 118551 (3×10^{-8}M) but was unaffected by the β_1 selective antagonist atenolol (10^{-6}M). Cocaine (3×10^{-5}M) reduced but did not abolish the facilitatory effect of the adrenaline infusions (5×10^{-9}M) on responses to renal nerve stimulation. However, in the presence of cocaine there was no persistant facilitation of the responses after cessation of the adrenaline infusion.

These results indicate that adrenaline, at levels similar to those found to occur in rat plasma during stress (McCarty and Kopin, 1978), selectively enhances vasoconstrictor responses evoked by adrenergic nerve stimulation in the rat perfused kidney. This effect appears to be mediated by a β_2 adrenoceptor. The persistent nature of this effect may be due to transport of adrenaline into the adrenergic neurone, and its subsequent release when the nerves are activated.

PQ is an SERC - CASE student in conjunction with ICI plc.

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THE D_1 DOPAMINE ANTAGONIST SCH 23390, BUT NOT A D_2 ANTAGONIST, BLOCKS RESPONSIVITY TO THE D_1 AGONIST *R*-SK & F 38393

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The forebrain D_1 dopaminergic site has been considered a receptor looking for a function, as the D_2 receptor appears prepotent in dopamine-mediated behaviours such as stereotypy (Laduron, 1982). Non-stereotyped behaviours, such as grooming, induced by the D_1 agonist SK & F 38393 (2,3,4,5,-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine) are stereospecific for its *R*-enantiomer which stereoselectively displaces ^{3}H-piflutixol binding to D_1 sites (Molloy & Waddington, 1983a, b; O'Boyle & Waddington, 1983a, b). We have now compared the actions of SCH 23390 (*R*-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol; Hyttel, 1983) and metoclopramide on such dopaminergic receptors and behaviours, using male Sprague-Dawley rats.

Their relative affinities for binding sites for 0.3 nM ^{3}H-piflutixol and 0.1 nM ^{3}H-spiperone were determined in rat striatum (O'Boyle & Waddington, 1983a, b; 1984). Rats were challenged with *R*-SK & F 38393 or apomorphine and behavioural responses assessed using a rapid sampling behavioural check-list technique and a stereotypy scale (Molloy & Waddington, 1983a,b; 1984). SCH 23390 potently and selectively displaced the binding of ^{3}H-piflutixol while metoclopramide selectively displaced ^{3}H-spiperone (Table).

	IC_{50} (nM)		$\frac{^3\text{H-PIF (D1)}}{^3\text{H-SPIP (D2)}}$
	^{3}H-piflutixol	^{3}H-spiperone	
SCH 23390	0.72 ± 0.23	1,640 ± 420	0.0004
Metoclopramide	> 100,000	330 ± 120	> 303

Mean ± S.E.mean (n = 3).

Grooming and sniffing induced by 20 mg/kg s.c. *R*-SK & F 38393 were antagonised ($P < 0.05$) by 0.1-0.5 mg/kg SCH 23390. 1.0-5.0 mg/kg metoclopramide was without effect, but antagonised ($P < 0.05$) stereotypy induced by 0.5 mg/kg s.c. apomorphine. This is consistent with these responses to *R*-SK & F 38393, and their antagonism by SCH 23390, being D_1-mediated. However, SCH 23390 was also able to antagonise ($P < 0.05$) stereotypy responses to apomorphine. Also, rearing ($P < 0.05$) and locomotion (weakly) induced by *R*-SK & F 38393, in contrast to grooming and sniffing, were antagonised by metoclopramide. We suggest (i) a behavioural role for D_1 receptors can be demonstrated and in the past may have been too hastily dismissed, and (ii) the ability of a selective D_1 antagonist to exert physiological but not pharmacological antagonism of classically D_2-mediated responses indicates a complex functional interaction between the two sites.

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METOCLOPRAMIDE-INDUCED STEREOTYPED BEHAVIOUR IN THE GUINEA-PIG DOES NOT DEPEND ON THE RELEASE OF DOPAMINE IN THE BRAIN

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Metoclopramide causes repetitive snout-rubbing in the pig and a high dose (78 mg/kg, s.c.) of metoclopramide induces stereotyped muzzle-rubbing behaviour in the guinea-pig (Ely, Rumble & Sharman, 1978). In the guinea-pig this response can be antagonized by the simultaneous administration of apomorphine (8 mg/kg, s.c.) or by atropine (5 mg/kg, s.c.) given 10 min before (Rodriguez del Camino & Sharman, 1981) and pretreatment with haloperidol results in a greatly increased response.

In order to study the possible involvement of cerebral dopaminergic neuronal systems in this response to metoclopramide, guinea-pigs were pretreated with reserpine (2.5 mg/kg, s.c. 24 h), α-methyl-p-tyrosine (200 mg/kg i.p. 3 h) or a combination of reserpine (2.5 mg/kg, s.c. 24 h) and α-methyl-p-tyrosine (250 mg/kg, i.p. 2 h). The response was unaffected when these drugs were given alone and was enhanced following the combined treatment. The concentration of dopamine in the striatum was reduced to 2% of its control value by the latter treatment and the concentrations of the acid catabolites of dopamine, 4-hydroxy-3-methoxyphenylacetic acid and 3,4-dihydroxyphenylacetic acid were below the limit of detection.

The stereotyped muzzle-rubbing behaviour was antagonized by pretreatment with diazepam (2.5 mg/kg, i.p. 30 min); muscimol (2 mg/kg, s.c., 30 min) also prevented the stereotyped behaviour. However, muscimol at a lower dose (31 μg/kg, s.c. 30 min), enhanced the response to metoclopramide.

Physostigmine (1 mg/kg, s.c. 30 min together with atropine methylbromide 4 mg/kg, s.c., 90 min) caused the stereotyped behaviour to occur with a lower dose of metoclopramide (39 mg/kg, s.c.).

It is concluded from these results that the stereotyped muzzle-rubbing induced in guinea-pigs by metoclopramide does not depend on the release of dopamine from dopaminergic neurons in the brain. It is probable that, in order for the behaviour to occur, certain dopamine receptors need to be blocked or not stimulated.

The behaviour also appears to involve cholinergic and GABA-ergic cerebral mechanisms and it is possible that the property of metoclopramide to increase the release of acetyl choline in the periphery (Fosbraey & Johnson, 1980), if a similar effect occurs in the brain, might help to explain the stereotyped response.

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DOPAMINERGIC BEHAVIOUR IN THE RABBIT AFTER ACUTE AND CHRONIC PRE-TREATMENT WITH DESIPRAMINE, TRIMIPRAMINE, SULPIRIDE AND FLUPENTHIXOL

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In two previous communications (MIGNOT and SAVINI, 1980, 1982) we have shown that dopaminergic drugs (apomorphine, methoxamine and dextro-methamphetamine) produced in the rabbit typical and easy measurable reactions, peculiar stereotypies (foot jerks), increase in locomotor activity (LA) and turning behaviour (rotations). Methoxamine and apomorphine induced mostly foot jerks (blocked by pimozide) whereas dextro-methamphetamine produced predominantely rotations (blocked by sulpiride).

In order to find out which kind of changes occur in dopamine receptor sensitivity in the rabbit, new assays were performed by using chronically given: sulpiride for blocking D_2 and D_4 receptors (SEEMAN, 1982), α-cis-flupenthixol a ligand for D_1 and D_2 receptors and desipramine which seems to modify post-synaptic receptors in the mesolimbic dopaminergic projection (SPIRAKI and FIBIGER, 1981).

Batches of 9 fulvous Burgundy rabbits received IM either as acute or as chronic (once daily during 12 consecutive days) the following drugs : saline, desipramine HCl (25 µMol/kg), trimipramine mesilate (25 µMol/kg), α-cis-flupenthixol di-HCl (25 µMol/kg) and sulpiride (25 µMol/kg). Animals were challenged with apomorphine HCl (5 µMol/kg, IM) and dextro-methamphetamine HCl (12,5 µMol/kg, IV) at day -5 and then three days after the acute or the end of the chronic administration, these tests being continued once a week.

In acute experiments none of the compounds produced any modification of the response intensity when apomorphine or dextro-methamphetamine were administred. After 12 days chronic administration, the effects were different : Apomorphine induced stereotypies (foot jerks) were not modified by any of the four given psychotrops. Dextro-methamphetamine produced foot jerks were inhanced by desipramine ($P = 0,05$) but not changed by the two neuroleptics and by trimipramine. Apomorphine induced turnings were augmented during 3 weeks after cessation of sulpiride treatment ($P = 0.05$) ; desipramine and trimipramine enhanced turning behaviour for about one or two weeks ($P = 0.05$ and 0.01). Flupenthixol did not produce any effect.
Methamphetamine produced turnings, were enhanced by sulpiride during two weeks ($P = 0.01$), and during one week by desipramine ($P = 0.05$) and by trimipramine ($P = 0.01$). Flupenthixol had no significant effect.

These results have shown that (in the rabbit) sensitivity modifications appear after chronic treatment only. Sulpiride seems to be more active on D_2 receptors, their sensitivity being enhanced in meso-limbic system. Desipramine acts on mesolimbic system (increase in turnings) and also on nigro-striatal area enhancing the score of stereotypies (foot jerks). Trimipramine acts on the meso-limbic system only. Flupenthixol inhibits both of these sorts of effets, because it blocks both D_1 and D_2 receptors.

At the present time, assays concerning destruction of parts of neo-striatum by kainic acid are in progress, in order to better analyse the possible role of DA receptors in the rabbit brain.

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THE ACQUISTION OF TOLERANCE TO THE DEPRESSANT EFFECT OF VASOPRESSIN ON SPONTANEOUS MOTOR ACTIVITY

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Since the initial report by De Wied (1971) that vasopressin may improve memory retention and recall in a conditioned avoidance experiment, a large number of studies has been conducted to investigate the effects of vasopressin on various aspects of behaviour. However, most of these studies consist of 'single shot' experiments in which the experimental animals are only given a single dose of vasopressin before their behaviour is assessed, and little attempt has been made to look at the behavioural effects of repeated administration of vasopressin. The present study was designed to investigate the effects of chronic exposure to vasopressin on the spontaneous motor activity of rats.

Spontaneous activity was measured in a 'grid-floor, short-circuit type' activity box described previously (Ebenezer, 1983). Sixteen male Wistar rats (wt 275 - 330g) were randomly divided into 2 equal groups. Rats in the control group received saline (0.09% w/v) whilst rats in the test group received arginine vasopressin (AVP) (1.0 IU/Kg). AVP and saline were administered subcutaneously once daily over a period of 9 days. The spontaneous activity of the rats in each treatment group was measured on alternate days (i.e. days 1, 3, 5, 7 and 9) immediately after their respective treatments. Rats were placed singly in the activity box and the activity scores were read at 5 min intervals over a 30 min period. All experiments were carried out between 4 - 9 pm. Analysis of variance was used to analyse the activity data.

Rats in the AVP treated group showed lower activity scores compared with control rats during the first 2 measurement trials (i.e. days 1 and 3) ($p < 0.01$), with the most pronounced depression occurring during the first 10 - 15 min. By trial 3 (i.e. day 5) this depressant effect was no longer apparent and similar results were obtained during the last 2 trials (i.e. days 7 and 9).

In order to establish whether the tolerance to the depressant effect of AVP was due to repeated administration of the drug or repeated experience of the experimental situation (see Robbins, 1978) a further experiment was carried out. Male rats (n = 8 in each group) with no previous experience of the experimental situation were given 16 subcutaneous injections of saline or AVP (1.0 IU/Kg) over a period of 8 days. On day 9 the rats were given either saline or AVP (1.0 IU/Kg) according to their treatment schedule. Their activity was then measured as described above. Analysis of the activity data showed no significant difference between control and AVP treated rats.

The results from this study thus indicate that rats acquire a tolerance to the depressant effect of AVP on spontaneous motor activity which is dependent on repeated exposure to the drug rather than repeated experience of the experimental situation. It is not known at present whether this acquisition of tolerance is due to increased metabolism of AVP or to other central or peripheral changes caused by repeated exposure to the drug.

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BEHAVIOURAL EVIDENCE FOR ALTERED BRAIN 5-HYDROXYTRYPTAMINE FUNCTION FOLLOWING REPEATED IMMOBILISATION IN RATS

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Brain 5-hydroxytryptamine (5HT) and dopamine (DA) are involved in responses to acute immobilisation (Joseph & Kennett, 1983; Bliss & Ailion, 1971). However, their involvement in prolonged stress is less well established. Responses to drugs affecting 5HT and DA systems have therefore been examined in rats after repeated immobilisation.

Male Sprague-Dawley rats (200-260 g) were immobilised on wire grids for 2 h per day (light phase) for 7 days. 24 h later, rats were put singly in observation cages and 60 min later given (i.p.) saline, d-amphetamine, p-chloroamphetamine (pCA) or 5-methoxy-N,N-dimethyltryptamine (5MeODMT). 5HT- and DA-dependent responses were recorded by a 'blind' observer as described by Dickinson & Curzon (1983).

Previously immobilised rats given saline showed decreased sniffing (Control (C) 7.3(4-12) n = 9, immobilised (I) 4.9(2-11) n = 10, mean (range), $p < 0.05$, Mann-Whitney) whereas grooming (C; 2.0(0-5), I; 6.6(1-12), $p < 0.02$) and body shakes (C; 0.5(0-1), I; 1.9(0-7), $p < 0.05$) were increased. No other apparent behaviour was altered. When given amphetamine (3 mg/kg) all rats exhibited classical DA-behaviours, none were significantly altered. Responses to the 5HT releaser pCA and the 5HT agonist 5MeODMT are remarkable as immobilisation increased forepaw treading and tremor but not two other 5HT-behaviours (head weaving, hind limb abduction). Immobilisation also increased backward walking due to pCA (Table 1).

Table 1. Behavioural responses, mean (range) induced by pCA and 5MeODMT.

Drug (mg/kg)	n		Tremor	Head Weaving	Limb Abduction	Forepaw Treading	Backward Movement
pCA(4)	(10)	I	1.7(0-5)	6.4(0-13)	7.2(3-12)	9.0(2-14)**	1.0(0-4)
" "	(10)	C	1.3(0-3)	6.5(0-15)	7.1(1-11)	4.7(0-11)	0.2(0-1)
pCA(10)	(10)	I	6.9(0-11)**	9.5(1-16)	11.4(8-14)	11.0(3-18)	2.5(0-7)*
" "	(10)	C	3.0(0-7)	12.2(4-16)	11.6(0-17)	8.8(3-13)	0.5(0-2)
5MeODMT(5)	(8)	I	3.3(1-6)*	1.4(0-3)	5.6(2-10)	6.1(4-9)**	-
" "	(9)	C	1.3(0-3)	1.0(0-3)	4.3(2-9)	3.2(1-5)	-

*$p < 0.05$, **$p < 0.02$, 2 tailed Mann-Whitney U test, Immobilised(I), Control(C).

5HT, DA and metabolites measured by HPLC in the striatum, cortex, hippocampus, hypothalamus and midbrain were comparable in non-drug treated control and immobilised groups. Brain pCA concentrations, 30 min after injection measured fluorimetrically (adapted from Axelrod, 1954) were also comparable. The above biochemical and behavioural data indicate that the increased brain 5HT function is probably mediated postsynaptically. It is of interest that two of these increased 5HT-responses (forepaw treading, backward movement) also require or are increased by DA whereas head weaving and hind limb abduction which were not altered are inhibited by the DA-agonist apomorphine (Curzon et al., 1979; Dickinson & Curzon, 1983). The above effects of repeated immobilisation could be indices of either adaptive or non-adaptive responses to stress.

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BEHAVIOURAL EFFECTS OF 8-HYDROXY-2-(DI-N-PROPYLAMINO) TETRALIN, A PUTATIVE 5-HT_{1A} RECEPTOR AGONIST

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Administration of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) to rats induces a behavioural syndrome including hyperlocomotion, reciprocal forepaw treading, head weaving and a flat body posture consistent with agonist activity at central 5-HT receptors (Hjorth et al., 1982). In vitro studies have shown that 8-OH-DPAT has high affinity for a subtype of the 5-HT_1 recognition site designated 5-HT_{1A} (Middlemiss and Fozard, 1983). In the present report the behavioural effects of 8-OH-DPAT have been further investigated and evidence for an involvement of the 5-HT_{1A} site in the syndrome is given.

Male Sprague-Dawley rats (190-230 g) were allowed 5 min in an observation cage (1 animal per cage) before subcutaneous injection of 8-OH-DPAT. Observation began 3 min after injection and forepaw treading, flat body posture and head weaving scored using a ranking scale (0=absent, 1=equivocal, 2=present, 3=intense) during 45 sec every 3 min for a total of 15 min. Ambulation was scored simultaneously by counting the number of quadrants of the cage entered during each observation period. Antagonists were injected subcutaneously 30 min before 8-OH-DPAT.

In untreated animals 8-OH-DPAT induced the four components of the syndrome in a dose related manner at doses of 0.03-2.5 mg/kg. A submaximal dose of 0.125 mg/kg was chosen for routine evaluation. Pretreatment with reserpine (0.1-5 mg/kg s.c. 18 h before testing) caused dose-related inhibition of ambulation and head weaving, but left forepaw treading and the flat body posture unaltered. Most components of the syndrome were also reduced by prazosin (0.5-1 mg/kg), haloperidol (0.0125-0.25 mg/kg) and sulpiride (5-30 mg/kg) consistent with catecholaminergic neurons having an important role in the expression of the behavioural response to 8-OH-DPAT in untreated animals.

In animals treated 18 h previously with reserpine (1 mg/kg) the residual behavioural syndrome of flat body posture and forepaw treading was not altered by haloperidol (0.05-0.25 mg/kg) and was resistant to blockade by prazosin (1.0 mg/kg) suggesting that under these circumstances neither dopamine receptors nor α_1-adrenoceptors are essential to the expression of the response. Ketanserin (2.5-5 mg/kg) was similarly ineffective, rendering a key role for 5-HT_2 receptors unlikely. In contrast, these behaviours in the reserpinized rat were effectively inhibited stereospecifically by (-), but not (+),-pindolol (2-4 mg/kg), which shows both high affinity for, and stereoselectivity at, the 5-HT_1 recognition site (Nahorski & Willcocks, 1983), and by spiperone (0.05-0.25 mg/kg), which discriminates between the 5-HT_{1A} and 5-HT_{1B} recognition sites (Pedigo et al., 1981 ; Middlemiss & Fozard, 1983).

Thus, the 8-OH-DPAT behavioural syndrome in normal rats is a complex response having both catecholaminergic and non-catecholaminergic components. In reserpinised animals, the residual effects of forepaw treading and a flat body posture may reflect the behavioural consequences of stimulation of the putative 5-HT_{IA} receptor.

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MICROINJECTION OF METHYL-β-CARBOLINE-3-CARBOXYLATE INTO NUCLEUS RAPHE DORSALIS REDUCES SOCIAL INTERACTION IN THE RAT

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Methyl-β-carboline-3-carboxylate (βCCM) binds with high affinity to benzodiazepine receptors (Braestrup & Nielsen, 1981) and when given intravenously to rats is anxiogenic as assessed by the Vogel conflict test (Corda et al, 1983). Thiebot et al, (1982) have reported that microinjections of chlordiazepoxide into the Nucleus Raphe Dorsalis (NRD) produce anxiolytic effects. In the present study βCCM was microinjected into the NRD and significantly decreased scores in the social interaction test; suggesting that it is strongly anxiogenic.

Male Wistar rats were anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.) and a steel guide cannula was implanted at an angle of 24° from the vertical, with the tip 6mm away from the NRD. One week later a fine glass needle (70-90 μ o.d.) was introduced into the guide cannula to project 6mm into the NRD (Azami et al, 1980). 0.5 μl volumes were injected over a 2 minute period.

Two hours before testing, an implanted and a normal rat were placed together into an open field for 10 minutes. A microinjection of either saline or βCCM in saline was given 5 minutes before the test. The animals were then replaced in the open field for 10 minutes and the duration of social interaction of the treated animal and the locomotor activity were recorded. Four days later the procedure was repeated with the same pairs such that all animals received both saline and βCCM. Pontamine Sky Blue was then microinjected for histological verification of the microinjection site.

The social interaction scores of implanted animals which received saline did not differ from a group of 6 unoperated controls. The effects of βCCM were determined using a paired t test between individual saline and βCCM scores. 10 ng of βCCM reduced social interaction by 54% ($n = 4$, $P<0.05$); 1 ng βCCM by 40% ($n = 6$, $P<0.05$); 100 pg βCCM by 53% ($n = 5$; $P<0.01$); 10 pg βCCM by 32% ($n = 7$). There was no significant change in locomotor activity. 25 microinjection sites were 0.5 mm or further from NRD. None of these animals showed any reduction in social interaction. This observation indicates that it is unlikely that these very small doses of drug are having effects by diffusion or transport to a distant site. (Clarke et al, 1983).

These results, in conjunction with the results of Thiebot et al, suggest that the NRD is a particularly sensitive site for benzodiazepine receptor ligands and the expression of their anxiety effects. An investigation of the effects of NRD lesions on the anxiety response to systemically administered βCCM is presently being conducted.

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BENZODIAZEPINE CONTRAGONISTS CAUSE KINDLING

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It is well recognised that with chronic administration, tolerance develops to the actions of the benzodiazepines. Recently, a group of drugs have been described which are benzodiazepine receptor ligands with pharmacological effects opposite to the benzodiazepines (Cowen et al., 1981). These have been termed "contragonists" or "inverse agonists". One such compound, FG 7142 (N'-methyl-β-carboline-carboxamide) has been shown to be anxiogenic in man (Dorow et al., 1983) and proconvulsant in animals (Petersen et al., 1983). We were interested in whether tolerance developed to the effects of contragonists and now report that for the proconvulsant effects of FG 7142 the obverse obtains.

FG 7142 was suspended in distilled water with one drop of Tween 40 in 10 mls. A dose of 40 mg kg^{-1} (4 mg ml^{-1}) was injected i.p. into male CDI mice (32 - 38 g). This dose lowered seizure threshold to pentylenetetrazol (PTZ) significantly in naive mice without producing overt seizures (controls, 54 ± 4; FG 7142, 30 ± 2, mean ± S.D., $P < 0.001$, measured using an i.v. infusion of PTZ; Nutt et al., 1980). This proconvulsant action is lost by 90 min after injection. Two treatment schedules were used (a) three times per day (10 a.m., 4 p.m. and 10 p.m.) and (b) one per day (8 - 10 p.m.). Control mice received Tween vehicle. No significant differences in weights between control and FG 7142 treated mice were found following treatment.

In schedule (a), by day 3 all animals were showing brief myoclonic jerks of the head and neck. On day 4 generalised seizure activity was noted (see table), which consisted of loss of righting reflex with clonic activity of both fore and hind limbs and sometimes tonic extension of the fore limbs. In general, this was observed once after each injection and rapid recovery was seen after 10 - 20 sec. In one mouse on days 5 and 6 two episodes were observed. The number of mice showing these seizures increased to 6/8 by day 6. After day 6 injections were stopped for 6 days then the mice given a further dose on day 12, when they still showed increased sensitivity to the convulsant effects of the drug. This suggested that pharmacological kindling had occurred.

Schedule (b) produced essentially the same findings, but with a slower onset (see table).

Table 1

(a) 3 x day

Day:-	1	2	3	4	5	6	12
1st Injection	0	0	0	2	3	6	
2nd Injection	0	0	0	1	2	5	5
3rd Injection	0	0	0	0	3	3	

(b) 1 x day

Day:-	1	2	3	4	5	6	7	8	9	10	11	12
	0	0	0	0	1	3	2	3	4	5	3	**6**

No. of mice out of groups of 8 showing full seizures.

These findings show that, in contrast to the benzodiazepines, the effects of the contragonist increased with repeated administration, using two different time schedules. This phenomenon, which is kindling or inverse tolerance, shows certain similarities to the effects of daily picrotoxin treatment (Nutt et al., 1982). Whether the underlying mechanism has a pharmacodynamic or pharmacokinetic basis is presently being investigated.

We thank Ferrosan for supplying FG 7142.

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THE ANTICONVULSANT EFFECT OF PROTEIN SYNTHESIS INHIBITORS

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Non-toxic doses of the protein synthesis inhibitors (PSI) have been shown to produce a variety of behavioural and pharmacological effects. For instance, they inhibit the behaviours produced by treatments indirectly activating dopamine receptors such as amphetamine and L-dopa plus an MAOI, without affecting responses produced by agonists such as apomorphine (Green et al, 1976). One such inhibitor, cycloheximide (Cx), has been shown to have anticonvulsant properties (Green et al, 1976) we now report some further studies on this phenomenon.

The seizure threshold in male (Sprague-Dawley derived) rats of 180+10g were measured using an i.v. infusion of pentylenetetrazol (PTZ) ($10 mgml^{-1}$ in 0.9% w.v. saline) (Nutt et al, 1980). Cycloheximide ($1 mgml^{-1}$ in saline) at doses of 1 and $5 mgkg^{-1}$ was given i.p. 2h pre-infusion. Emetine $50 mgkg^{-1}$ ($25 mgml^{-1}$ in saline) was given i.p. 3h pre-infusion. Anisomycin $30 mgkg^{-1}$ ($5 mgml^{-1}$ in pH 6.5 saline/HCL) was given i.p. 1h pre-infusion. Cx in a dose-dependent fashion and anisomycin both showed anticonvulsant activity towards PTZ. Emetine in a dose which inhibits protein synthesis to an extent equivalent to about $2 mgkg^{-1}$ Cx (Grahame-Smith, 1972) had no effect (see table). To assess whether the anticonvulsant effects of Cx were mediated via an action at the benzodiazepine receptor the effects of the competitive antagonist Ro 15-1788 (Hunkeler et al, 1981) were assessed. Ro 15-1788 $10 mgkg^{-1}$ i.p. ($2 mgml^{-1}$ suspension in Tween 40/distilled water) was given .5h pre-infusion of PTZ. This treatment did not alter the seizure thresholds in the control group and neither did it alter the Cx-induced threshold rise. Regional brain GABA concentrations were measured using the method of Baxter (1972) 3 hours after Cx ($10 mgkg^{-1}$ i.p.) no differences were found as compared with control values (controls; cortex 0.332±0.028, hippocampus 0.456±0.022, striatum 0.526±0.056; Cx, cortex 0.325±0.035, hippocampus 0.425±0.060, striatum 0.512±0.034; results are GABA concentrations (mgg^{-1} wet weight) given as mean ± s.e.m. with n=6).

It therefore seems that not all PSIs show anticonvulsant effects perhaps implying that there is some differential inhibition of protein synthesis or that other biochemical mechanisms are involved. The latter possibility is supported by the report that isocycloheximide, an isomer without protein synthesis inhibitory properties, also has behavioural effects such as sedation (Squire & Barondes, 1973).

TREATMENT	SEIZURE THRESHOLD ($mgkg^{-1}$ PTZ)	P.VALUE
Vehicle	35 ± 4 (6)	0.01
Cycloheximide ($1 mgkg^{-1}$)	42 ± 1 (6)	0.01
Cycloheximide ($5 mgkg^{-1}$)	48 ± 4 (5)	0.05
Emetine ($50 mgkg^{-1}$)	34 ± 3 (6)	
Vehicle	30 ± 3 (4)	0.05
Anisomycin ($30 mgkg^{-1}$)	40 ± 8 (6)	
Ro 15-1788 ($10 mgkg^{-1}$)	34 ± 2 (6)	0.01
Cx ($1 mgkg^{-1}$) & Ro ($10 mgkg^{-1}$)	42 ± 3 (6)	

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ALLOSTERIC AND NON-SPECIFIC INTERACTIONS OF BARBITURATES WITH THE ACETYLCHOLINE RECEPTOR FROM TORPEDO

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Recently we have shown that pentobarbitone binds specifically to a site on the nicotinic acetylcholine receptor from Torpedo (Miller et al, 1982). This raises two questions: (1) what is the functional significance of this site, and (2) do barbiturates also exert a non-specific, general anaesthetic-like action on this membrane?

AChR-rich membranes were prepared from electroplaques of freshly killed Torpedo by differential and sucrose gradient centrifugation. [^{3}H]Acetylcholine (ACh) binding was determined at pH 7.0 by filtration after pre-incubation with 0.1mM diisopropylfluorophosphate. On the other hand, [^{14}C]amobarbitone (AmB) (6μM) binding was determined by centrifugation assay and nondisplaceable binding was estimated in the presence of excess AmB (1mM).

At fixed concentrations of [^{3}H]ACh, such that its receptor was half occupied, low concentrations of barbiturates caused a decrease in binding. This decrease plateaued at intermediate concentrations ($\simeq$ 1mM), but at higher barbiturate concentrations a new action was manifested in a steep increase in [^{3}H]ACh binding.

The decrease in [^{3}H]ACh binding attained at the plateau varied from twenty to seventy per cent depending on the barbiturate, its magnitude increasing in the order secobarbitone < phenobarbitone = pentobarbitone < butabarbitone < AmB. The concentration dependence of this decrease in binding followed mass action kinetics and half maximal effects (EC_{50}) were in the range 17-280μM (EC_{50}s were in the order: Amb < secobarbitone < pentobarbitone < phenobarbitone < butabarbitone). In the same concentration range these agents also completely displaced specific [^{14}C] Amb binding in a manner that followed mass action kinetics. The half displacement concentrations (IC_{50}) correlated closely ($r = 0.998$) with the EC_{50}, suggesting that these barbiturates can modulate ACh binding by themselves binding to an allosteric site on the receptor-ionophore complex.

On the other hand, the concentration dependence of the increase in [^{3}H]ACh binding was much steeper than expected for mass action and the effect did not plateau even in saturated barbiturate solutions. It was characterized by determining the threshold concentration (ThC) at which each barbiturate caused a significant ($p < 0.01$), but arbitrary, increase in [^{3}H] ACh binding of thirty per cent. ThC was in the range 2-9mM (AmB < secobarbitone < pentobarbitone < phenobarbitone = butabarbitone) and correlated well ($r = 0.965$) with the barbiturates' octanol/water partition coefficients. This effect on [^{3}H]ACh binding was reversible, ruling out membrane lysis and suggesting a non-specific mechanism. Indeed, this action of the barbiturates, unlike that at lower concentrations, resembles that of the alcohols which also increase [^{3}H]ACh binding with a steep concentration dependence.

Thus, barbiturates can modify the conformation of the acetylcholine binding site by two mechanisms. At sub-clinical concentrations they interact with a specific allosteric site, whereas at high concentrations they act non-specifically by a mechanism which remains to be elucidated. This second action is shared with alcohols and volatile agents and thus correlates better with their action as general anaesthetics.

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EFFECT OF EXCITATORY AMINO ACIDS ON (^{3}H)-ACETYLCHOLINE RELEASE FROM THE RABBIT RETINA

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ACh is believed to be a transmitter substance in most if not all vertebrate retinae (Neal, 1983, for review). In the rabbit, the retinal cholinergic neurones form a small sub-population of amacrine cells, the cell bodies of which are distributed more or less equally in the inner nuclear ganglion cell layers.

The bipolar cells, which provide the main excitatory input to the cholinergic amacrine cells may use glutamate and/or aspartate as their transmitters. There is also increasing evidence that the photoreceptor transmitter is one or both of these amino acids. In the present experiments, we have used the eye-cup preparation in anaesthetised rabbits to study the effects of excitatory amino acids and some antagonists on the release of [^{3}H] ACh from cholinergic amacrine cells. The methods have been described previously (Cunningham & Neal, 1983).

Glutamate and aspartate applied locally to the retina at concentrations of 1-5 mM, progressively blocked the light evoked release of [^{3}H] ACh, but strikingly increased the resting release. Quisqualate and kainate produced similar effects on ACh release, but at much lower concentrations (2-8 μM). Kainate differed from the others in that it first potentiated the light evoked release of [^{3}H] ACh (at 4 μM) before abolishing it (at 8 μM). N-methyl-D-aspartate (NMDA) had no effect on ACh release at concentrations up to 0.25 mM. Higher concentrations (0.5-1 mM) progressively reduced the light evoked release of ACh but had no effect on the resting release. It is not possible in these experiments to determine the exact site of drug action, but as the amplitude of the E.R.G. b-wave was not significantly reduced, it is probably that the excitatory amino acids act directly on the cholinergic amacrine cells.

The excitatory amino acid antagonists piperidinedicarboxylic acid (PDA), γ-D-glutamylglycine (DGG) and DL-2-amino-4-phosphonobutyrate (APB) blocked the light evoked release of ACh by different mechanisms. APB has been shown to block the light evoked release of ACh by mimicing the photoreceptor transmitter i.e. by hyperpolarizing the depolarizing bipolar cells (Neal et al., 1981). In contrast, PDA does not affect the E.R.G. b-wave and must therefore be blocking transmission between the depolarizing bipolar cells and the cholinergic amacrine cells. This result is consistent with the idea that aspartate or glutamate is the bipolar cell transmitter and supports recent electrophysiological findings in the mudpuppy retina (Slaughter & Miller, 1983). The effects of kainate on the resting release of [^{3}H] ACh were blocked by PDA, DGG and APB, while quisqualate was antagonised only by PDA. APB abolished the action of aspartate on resting ACh release but did not reduce the effect of glutamate.

Our results support in a general way the idea that the bipolar cell transmitter is glutamate/aspartate and indicates that the cholinergic amacrine cells may possess receptors for excitatory amino acids.

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CHOLINERGIC FUNCTION IN DEVELOPING RAT BRAIN CELL CULTURES: EFFECTS OF THYROID HORMONE

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Brain cell culture has become a useful tool for studying CNS neurotransmitter function and offers some advantages over in vivo studies. An important advancement was the development of serum-free culture conditions enabling the study of hormone action on nerve cells (see Atterwill, Kingsbury & Balázs, 1983). We have now used mixed, three-dimensional reaggregate cultures from foetal rat brain to examine the effects of tri-iodothyronine (T_3) on several aspects of cholinergic neurone development and function. It is known from both in vivo (Kalaria et al, 1981) and in vitro (Honegger & Lenoir, 1980) studies that thyroid hormone affects the development of central cholinergic neurones.

Reaggregate cultures were prepared from 16-day foetal rat whole brains as previously described (see Atterwill et al, 1983) by mechanical dissociation of cells from the tissue. Aggregates were formed and maintained by rotation in Delong culture vessels (70-80 rpm) in 9% CO_2 plus 95% humidified air. The culture media used were as previously described (see Atterwill et al, 1983) and contained either 10% foetal calf serum (S^+) or were serum-free (S-) containing the supplement of Bottenstein & Sato (1979). T_3 (30nM) was added to the S- cultures after two days in vitro (2 DIV) and every 48h thereafter. Aggregates were harvested at various times up to 1 month and choline acetyltransferase (ChAT), muscarinic receptors, and [^{3}H]choline uptake (ChU) measured.

The ChAT activity of reaggregates cultivated in S+ conditions was considerably higher than in the S- cultures. Chronic T_3 treatment of the S- aggregates was found to enhance the developmental rise of ChAT activity in agreement with previous work (Honegger & Lenoir, 1980). Similarly, specific [^{3}H]QNB binding in the S+ aggregates increased between 9 and 14 DIV, and showed similar levels of binding to that in vivo at an equivalent postnatal age (Kalaria et al, 1981). However, in the absence of serum (S-) no such developmental increase was seen and receptor levels were lower. As in the case of ChAT, T_3 treatment restored [^{3}H]QNB binding to the level found in the S+ aggregates.

A detailed study of in vitro [^{3}H]choline uptake in the reaggregates revealed that the uptake, although energy-dependent, was completely Na^+-independent and was not associated with measurable conversion to ACh (under any of the three culture conditions, or at any age). Approximately 14% of the accumulated radiolabel was converted to phosphorylcholine. Kinetic analysis of ChU at 12 and 22 DIV revealed only a low-affinity uptake system (K_t app. = 10-20μM, Vmax = approx. 900 fmols/min/mg protein) together with a large, non-saturable diffusional component, which were not affected by T_3 treatment. At low choline concentrations, hemicholinium-3 (10^{-5}M) did inhibit ChU (approx. 70% reduction).

These data show firstly that S- reaggregate cultures with suppressed cholinergic synaptogenesis (in terms of ChAT and muscarinic receptors) can be stimulated by thyroid hormone treatment. Secondly, ChU does not appear to be a marker for cholinergic neuronal function in these cultures (under our conditions), is not affected by T_3 and may reflect non-neuronal ChU.

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SUBTYPES OF NEURONAL 5-HYDROXYTRYPTAMINE ($_5$-HT) RECEPTORS AS IDENTIFIED BY COMPETITIVE ANTAGONISTS

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Although 5-HT receptors are widely distributed throughout the peripheral nervous system their classification has been hindered by the lack of selective competitive antagonists (see Wallis, 1981). Thus in 1979 we started a programme to develop selective agonists and antagonists for these receptors. The bioassays used were: the rabbit vagus nerve (Neto, 1978), the Langendorff heart and the guinea pig ileum (Fozard et al, 1979). Contractions of the rat uterus and displacement of ^{3}H-5-HT and ^{125}I-LSD from rat cortex membranes were used to monitor the affinity of compounds for smooth muscle D-receptors and for central 5-HT_1 and 5-HT_2 binding sites respectively (Engel et al, 1983).

α-methyl-5-HT and 2-methyl-5-HT are selective agonists for smooth muscle and peripheral neuronal 5-HT receptors respectively (see Table). Interestingly, although only 3 times less potent than 5-HT itself at peripheral neuronal receptors, 2-methyl-5-HT is about 300 and 60 times less potent than 5-HT at 5-HT_1 and 5-HT_2 binding sites in the cortex, indicating that peripheral and central neuronal 5-HT receptors are different.

	LOCATION OF 5-HT RECEPTORS					
	Peripheral Neurones			Smooth Muscle	Brain Cortex	
COMPOUND	Vagus	Heart	Ileum	Uterus	5-HT_1	5-HT_2
					(-log K_D)	
Agonists[a]						
5-HT	100	100	100	100	8.5±0.1	7.1±0.1
α-CH_3-5-HT	<0.1	<2	<10	95±4	6.1±0.0	6.7±0.1
2-CH_3-5-HT	40±7	27±3	27±5	<0.1	6.0±0.0	5.2±0.1
Antagonists[b]						
Compound I	15.4±1.4[c]	10.1±0.1[c]	9.0±0.1[c]	<5.0	<5.0	<5.0
Compound II	8.8±0.2[d]	10.0±0.1[d]	7.6±0.1[d]	<5.0	<5.0	<5.0

The results are the means ± S.D. of 3-6 individual experiments.
[a]Potency on vagus, heart, ileum and uterus relative to 5-HT (=100); [b]pA_2 values on vagus, heart, ileum and uterus; [c,d]values different from each other, $P<0.001$.

A large series of highly potent and selective competitive antagonists for the 5-HT receptors on peripheral neurones were also made. Two examples are given in the Table: (3α-homotropanyl)-1-methyl-5-fluoro-indole-3-carboxylic acid ester (Compound I) is a competitive antagonist of neuronal receptors in the vagus, heart and ileum, but has widely differing pA_2 values for each of these tissues, showing that the receptors are different. Compound I is extremely potent and relatively selective on the vagus, whereas Compound II (N-desmethyl-3α-homotropanyl)-1H-indole-3-carboxylic acid ester) is selective for neuronal 5-HT receptors in the heart. Neither compound is active on the 5-HT smooth muscle receptors in the uterus or on 5-HT_1 and 5-HT_2 binding sites in the cortex.

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ICS 205-930: A HIGHLY SELECTIVE AND POTENT ANTAGONIST AT PERIPHERAL NEURONAL 5-HYDROXYTRYPTAMINE (5-HT) RECEPTORS

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Gaddum and Picarelli (1957) were the first to demonstrate 5-HT receptors on peripheral neurones. Since then it has become clear that 5-HT receptors are widely distributed over the peripheral nervous system (see Wallis, 1981) although, until recently, no selective antagonists were available.

Here we present the pharmacology of ICS 205-930 ((3α-tropanyl)-1H-indole-3-carboxylic acid ester), a potent and highly selective antagonist for neuronal 5-HT receptors on 'C' fibres in the vagus nerve and on sympathetic nerve terminals in the heart. Metoclopramide and MDL 72222 have been included for comparison (Fozard and Host, 1982; Fozard, 1983). The methods used for assessing the affinity of compounds to neuronal 5-HT receptors *in vitro* and *in vivo* have been described previously (Neto, 1978; Fozard and Host, 1982).

Compound	Rabbit Vagus pA_2	Rabbit Heart pA_2	Guinea Pig Ileum pD'_2	Von Bezold-Jarisch Effect K_i (μg/kg i.v.)
Metoclopramide	7.3 ± 0.2	7.1 ± 0.0	3.9 ± 0.0	183 ± 15
MDL 72222	7.9 ± 0.2	8.9 ± 0.1	6.2 ± 0.2	39 ± 7[b]
ICS 205-930	10.2 ± 0.3	10.6 ± 0.1	7.9 ± 0.1[a]	0.37 ± 0.03

Values are the means ± s.e.mean of 3-7 individual experiments
[a]pA_2 value; [b]Data from Fozard (1983)

As the Table shows, ICS 205-930 is a potent, competitive antagonist at neuronal 5-HT receptors in the vagus, heart and ileum. Despite the fact that the 5-HT receptors in these 3 tissues are different (Donatsch et al, 1984), ICS 205-930 had approximately the same affinity for the receptors on vagal 'C' fibres as for those on sympathetic nerve terminals in the heart. Although it had a much lower affinity for the 5-HT receptors on parasympathetic nerves in the ileum, it still behaved as a true competitive antagonist in this tissue. Both MDL 72222 and metoclopramide were considerably less potent than ICS 205-930 on all types of neuronal receptor and behaved as non-competitive antagonists in the ileum. As would be anticipated from its pA_2 value on vagal 'C' fibres, ICS 205-930 was about 100 and 500 times more potent than MDL 72222 and metoclopramide respectively in inhibiting the 5-HT induced Von Bezold Jarisch effect *in vivo*.

ICS 205-930 does not possess affinity for 5-HT D-receptors on uterine smooth muscle or for 5-HT_1 or 5-HT_2 binding sites in rat cortex. ICS 205-930 failed to displace radioligands from α_1, α_2, β_1 or β_2 adrenoreceptors, D_1 or D_2 dopaminergic receptors, muscarinic cholinergic receptors or benzodiazepine binding sites at concentrations below 10^{-5} M.

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THE INHIBITORY EFFECTS OF NEURONAL 5-HYDROXYTRYPTAMINE (5-HT) RECEPTOR ANTAGONISTS ON EXPERIMENTAL PAIN IN HUMANS

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When applied to a blister base on the human forearm 5-HT causes pain (Keele and Armstrong, 1964). Since this pain cannot be inhibited by antagonists of 5-HT smooth muscle receptors such as bromolysergic acid diethylamide, we investigated the effects of two neuronal 5-HT receptor antagonists (Donatsch et al, 1984).

Double blind studies were conducted on 7 healthy male volunteers aged 32-55. Blisters were produced on the medial aspect of the forearm as previously described (Keele and Armstrong, 1964). Increasing concentrations of 5-HT (10^{-8} - 10^{-5} M) were applied to the blister base in Krebs ringer solution every 2 minutes. The subjects registered the pain experienced during each 2 minute interval by moving a lever graduated on a 0-3 rating scale (0 = no pain, 1 = slight, 2 = moderate, 3 = severe pain). This was coupled to a linear integrator that then recorded the area under the "pain intensity-time curve". The concentration of 5-HT was increased every 2 minutes until no further pain was produced. In this way, a cumulative dose-response curve for 5-HT could be constructed. Afterwards, the 5-HT was washed off for at least 40 minutes before incubating the blister base with either 10^{-8} M (3α-tropanyl)-1H-indole-3-carboxylic acid ester (compound I) (4 volunteers) or 10^{-7} M N-(3α-homotropanyl)-1-methyl-indole-3-carboxylic acid amide (compound II) (3 volunteers) for 20 minutes. These compounds are competitive antagonists for neuronal 5-HT receptors, with pA_2 values on the rabbit vagus nerve of 10.2 and 9.3 respectively (Donatsch et al, 1984). Increasing concentrations of 5-HT in combination with these antagonists were then applied and the pain response recorded as previously until a maximum response had again been obtained. Subsequently, the 5-HT and antagonist were washed off.

Preincubation of the blister base with either of the neuronal 5-HT antagonists caused a substantial parallel right shift in the pain dose response curve in all 7 individuals. To show that this effect of the antagonists was a real one and did not merely reflect a general decrease in the sensitivity of the blister base to painful stimuli with time, dose-response curves for bradykinin (10^{-8} - 10^{-6} g/ml) were performed before, during and after incubation of the blister with the antagonists. The three dose-response curves for bradykinin were virtually superimposable and the maximum response similar at each time point. This shows that the sensitivity of the blister did not decrease during the course of the experiment and that, in contrast to the effects on 5-HT responses, compound I and II did not inhibit bradykinin-induced pain. A specific densitization of the blister base to the painful effects of 5-HT did not occur since 5 of the 7 individuals regained their sensitivity to 5-HT after washing out the antagonist.

Assuming competitive antagonism to occur between the antagonists and 5-HT under these conditions, the "in vivo pA_2 values" obtained for compounds I and II were 11.2 $\pm$ 0.8 and 9.7 $\pm$ 0.4 respectively, which are similar to the in vitro pA_2 values obtained on the vagus nerve. Thus these compounds prevent 5-HT induced pain in humans by increasing the threshold concentration required to produce an effect.

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PANURAMINE: A SELECTIVE INHIBITOR OF 5-HT UPTAKE

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A deficit in 5-HT levels in the CNS has been linked with some subtypes of depression, especially those involving mood disturbances. This has led to the development of drugs which selectively increase the amount of 5-HT in the synapse by blocking re-uptake. In the present study we describe the neurochemical profile in the rat of the selective 5-HT uptake inhibitor, panuramine (Wy 26002; Moser et al, 1981).

Uptake and release of monoamines were measured in vitro as described elsewhere (Wood and Wyllie, 1983; Ennis and Cox, 1982). Binding of ^{3}H -5HT and ^{3}H -spiroperidol to rat cortical membranes was studied using the method of Peroutka and Snyder (1979). 5HT levels were measured spectrophotometrically (Curzon and Green, 1970). 2 hr after administration of para-chloroamphetamine (pCA) (20mgkg^{-1} ip.) or vehicle. Rats were pretreated with panuramine at various times prior to p-CA.

In vitro, panuramine was a potent and selective inhibitor of the 5-HT uptake system. (Table 1). Kinetic analysis indicated that the inhibition was non-competitive with an inhibitory affinity constant (K_I) of 22nM. Neither spontaneous or K^+-evoked release of ^{3}H -5HT was affected by pretreatment with panuramine. The addition of 10nM unlabelled 5-HT produced a 40% inhibition of K^+-evoked tritium release, reflecting autoreceptor-mediated modulation of 5-HT release, which was also unaffected by panuramine pretreatment. Panuramine in concentrations up to 10^{-5}M had no effect on ^{3}H -5-HT or ^{3}H -spiroperidol binding thus giving no evidence of an effect on 5HT, or $5HT_2$ receptors. In vitro panuramine was therefore selective for the 5HT uptake system and had little effect on other components of serotonergic transmission.

Parachloroamphetamine causes depletion of5HT, dependent on its transport into serotonergic nerve terminals via the 5-HT uptake system. This effect can be reduced by uptake inhibitors (Fuller and Molloy, 1974). Panuramine was a potent inhibitor of p-CA-induced 5-HT depletion (ED_{50} 14.8 mgkg^{-1}, p.o.)which reflects the ability of the drug to inhibit 5-HT uptake in vivo. When considered in relation to the high doses required to alter catecholaminergic function (Moser et al, 1981) the in vivo data is consistent with the in vitro selectivity of panuramine.

Table 1: IC_{50} values (nM) S.E.M. for the inhibition of 5-HT, l-NA and DA uptake in rat synaptosomal preparations.

Compound	5HT	l-NA	IC_{50}NA/IC_{50}5HT	DA
Panuramine	22 ± 4 (4)	848 ± 371 (3)	39	$>10^{-5}$
Zimelidine	206 ±18 (4)	6380 ± 813 (3)	31	$>10^{-5}$
Imipramine	212 ±34 (5)	77 ± 12 (5)	0.4	$>10^{-5}$

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THE ASSOCIATION BETWEEN SEROTONIN UPTAKE AND ANTIDEPRESSANT BINDING SITES

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There is circumstantial evidence to suggest that the action of imipramine is at a site closely associated with, yet distinct from, the serotonin re-uptake site (Langer et al. 1980; Paul et al, 1981). Much of this information has been obtained from radioligand characterisation of the interaction of ^{3}H - imipramine with its binding site. We describe here another approach involving kinetic analysis (Wood and Wyllie, 1981) of the interaction of tricyclic antidepressants with the 5-HT transport system. In addition to the standard agents, imipramine, zimelidine and fluoxetine, the interactions of two more selective inhibitors, panuramine (Wy 26002); Moser et al, 1981) and Wy 26157 (Broadhurst et al, 1983) are described.

The carrier-mediated transport of 5-HT is a multicomponent system involving at least three steps:- binding to the carrier, translocation across the membrane and release from the recycling of the carrier (Wood and Wyllie, 1982). Reflecting the structural requirements of the system, was the finding that 5-HT was a potent inhibitor, whereas catecholamines were weak inhibitors, of ^{3}H - 5-HT uptake. On short incubation periods, 5-HT was found to be a competitive inhibitor of its own transport and also inhibited the initial binding to the carrier. The structurally related serotonin analogue, Wy 26157, also displayed the profile of competitive inhibitor.

In contrast, imipramine was not a competitive inhibitor of ^{3}H - 5HT uptake and did not result in substantial displacement from the carrier binding site. This pattern of inhibition would be consistent with an interaction of imipramine at a site closely associated with, yet distinct from, the 5-HT uptake site. Panuramine, which interacts with the ^{3}H - imipramine binding site was a non-competitive inhibitor of 5-HT uptake.

It is pertinent to note however, that both zimelidine and fluoxetine resulted in competitive inhibitory profiles, as described previously by Wong et al (1977) and Ross et al, (1975). It is enigmatic if an interaction of these compounds with the imipramine binding site is a prerequisite for antidepressant action, that qualitatively different effects on ^{3}H - 5-HT uptake were achieved.

In summary, within the limitations of this type of analysis, the non-competitive nature of the interaction of imipramine and panuramine would indicate that the imipramine binding site and the 5-HT uptake site were closely associated, yet distinct. The competitive interaction observed with zimelidine and fluoxetine would be better explained if the two sites were in fact one and the same. It remains, however, that the imipramine binding site is intimately associated with serotonergic nerve terminals.

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THE SUBCELLULAR SITE OF ACTION OF IMIPRAMINE

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The recently discovered high affinity binding site for imipramine is considered to be associated with the serotonin re-uptake site (Langer et al, 1980; Paul et al, 1981). Subcellular fractionation, however, has failed to demonstrate a selective localisation of {^{3}H}-imipramine binding sites on nerve terminals (Laduron et al, 1982), and an intraneuronal site of action, on synaptic vesicles, has been postulated for imipramine (Daniels et al, 1980; Okada et al, 1979). We have studied the subcellular distribution of {^{3}H}- imipramine and its long-acting derivative, {^{3}H}-2-nitroimipramine in an attempt to substantiate this proposal.

Subcellular fractions were prepared according to Whittaker (1965). The composition of the subcellular fractions was determined by morphological and biochemical analysis. In addition to enzyme markers, the subcellular distributions of 5-HT and reserpine were determined. {^{3}H}- imipramine was found to be associated with the nuclear (P_1, 60% of total) and synaptosomal fraction (P_2, 40% of total). A specific, ie. displaceable, association was observed only in the synaptosomal fractions (P_2 and P_2B). After further fractionaction of the P_2 fraction into its component organelles, {^{3}H}- imipramine was associated mainly with the synaptic vesicle fraction and to a lesser extent with synaptic membranes. A similar distribution pattern was observed after both in vivo and in vitro administration of {^{3}H}- imipramine. The proportion of {^{3}H}- imipramine associated with synaptic membranes was increased when a rapid subcellular fractionation scheme was used, and was reduced if an excess of unlabelled imipramine was included in the incubation. In contrast, the slowly dissociating derivative {^{3}H}-2-nitroimipramine was selectively associated with synaptic membranes. It is possible therefore that the {^{3}H}- imipramine associated with synaptic vesicles arises from redistribution of radiolabel during the fractionation procedure.

Ten days after 5,7-dihydroxytryptamine treatment (5, 7-DHT) brain 5-HT levels were reduced by 50% in mice which had received 20μg free base 5,7-DHT icv and by 34% in rats which had received 80μg free base 5-7,D-HT. This treatment, however, did not affect the absolute levels or subcellular distribution of {^{3}H}-imipramine administered in mice. In contrast, in rats which had received 20μg 5.7 DHT base icv the {^{3}H}-imipramine associated with synaptosomes after in vitro incubation with slices and subsequent subcellular fractionation, was reduced by 38%. The distribution pattern of {^{3}H}- imipramine was unaffected.

These results suggest that both in vivo and in vitro, {^{3}H}- imipramine is associated with synaptic membranes from which it rapidly dissociates. The observation that 5,7-DHT reduced this association in vitro is consistent with a localisation on serotonergic terminals, but the failure of 5,7-DHT to affect the in vivo association is apparently at variance with such a localisation.

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PROCONVULSANT ACTIONS OF SELECTIVE α_2-ADRENOCEPTOR ANTAGONISTS

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Recent work has shown that α_2 adrenoceptors can influence the seizure threshold of rodents. The α_2 antagonist yohimbine has been reported to be proconvulsant (Lloyd and Worms, 1982), whereas clonidine and other α_2 agonists appear to possess anticonvulsant properties (Papanicolaou et al., 1982). In this communication we describe the effects of yohimbine, and the more selective α_2 antagonists Wy26392 (Lattimer et al., 1982) and RX781094 (Doxey et al., 1983), on chemically-induced convulsions in mice.

Male Olac MF1 mice (weighing 28-32g) were used. The α_2 antagonists were administered orally 1h before challenge with convulsant. Solutions of convulsant in 0.9% w/v NaCl (pentylenetetrazol 12mg.ml.$^{-1}$, bicuculline 0.1 mg.ml.$^{-1}$ or strychnine sulphate 0.1 mg.ml.$^{-1}$) were infused at a constant rate (0.15 ml.min.$^{-1}$) into a tail vein, and the time to onset of seizures was measured.

All three α_2 antagonists consistently decreased the latency of pentylenetetrazol- or bicuculline - induced tonic convulsions (to 74-55% of control values) over the following dose ranges (mg.kg.$^{-1}$ p.o. of the hydrochloride salts): yohimbine 1-16; RX781094 2-32; Wy26392 30-90. The latency of pentylenetetrazol clonic seizures was reduced (to 85% of control values) only by the highest dose of yohimbine. Both RX781094 and yohimbine significantly decreased the latency of bicuculline clonic seizures (to 83% and 77% of controls respectively) at the highest doses used. In contrast to the marked proconvulsant effects of the α_2 antagonists, the noradrenaline uptake inhibitor desipramine (5-15 mg.kg.$^{-1}$ i.p., 30min. predose time) did not modify pentylenetetrazol - induced convulsions. Strychnine - induced tonic seizures were unaffected by either RX781094 or Wy26392, whereas yohimbine produced a small but significant (113-120% of control values) increase in latency at 4 and 16mg.kg.$^{-1}$. Since strychnine acts predominantly on the spinal cord to induce seizures, the lack of effect of the more selective α_2 antagonists suggests that their proconvulsant action is supraspinal. The slight anticonvulsant effect of yohimbine against strychnine may be non-specific and unrelated to α_2 adrenoceptor blockade.

A large body of literature evidence implicates central catecholamines in the mechanism(s) controlling the seizure threshold. However, a consistent finding is that catecholamine depletion is proconvulsant. Such observations (together with the lack of effect of a noradrenaline uptake inhibitor (desipramine) in our experiments) are difficult to reconcile with a presynaptic site of action of α_2 antagonists - which would result in an increased central noradrenergic tone. The possibility that the proconvulsant action of α_2 antagonists is mediated postsynaptically must therefore be considered.

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A COMPARISON OF THE EFFECTS OF SELECTIVE α-ADRENOCEPTOR AGONISTS ON RENAL TUBULAR SODIUM REABSORPTION IN THE RABBIT

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It is well recognised that stimulation of renal tubular alpha-adrenoceptors, during either increased renal nerve activity or infusion of alpha-adrenoceptor agonists, results in an antinatriuresis (DiBona, 1982). In a recent study we have obtained evidence which suggests that renal tubular alpha-1-adrenoceptors mediate the antinatriuresis of renal nerve stimulation (Hesse & Johns, 1983). The aim of the present study was to further investigate the subtype(s) of alpha-adrenoceptors influencing renal tubular sodium handling in the rabbit by using alpha-adrenoceptor agonists of varying selectivity.

The left kidney of sodium pentobarbitone anaesthetised Carolina rabbits (2.0-3.5 Kg) was approached retroperitoneally, the renal nerves sectioned and the ureter cannulated. A cannula placed in a lumbar branch of the renal artery allowed administration of alpha-adrenoceptor drugs directly into the artery. Alterations in basal values of renal sodium excretion were measured during 15 min. periods of agonist infusion at dose rates which caused minimal or no changes in renal haemodynamics:

Table 1 Effect of Alpha-adrenoceptor agonists on renal function.

Dose(ng/kg/min)	NA (40±10) n = 7	PHE (90±50) n = 7	MET (100±50) n = 5	GUA (60±40) n = 10	UK14304 (90±30) n = 7
RBF(ml/min/kg)	- 0.5± 0.3	- 0.7± 0.4	+ 0.2±0.9	- 0.1±0.1	- 0.5± 0.1*
GFR(ml/min/kg)	- 0.2± 0.1	- 0.2± 0.1	- 2.8±1.3	+ 0.1±0.1	- 0.3± 0.1*
V(ul/min/kg)	-80.3±17.5‡	-119.1±47.4*	-33.0±6.0‡	+25.1±8.9‡	+11.8±12.9
ENa(umol/min/kg)	-10.0± 2.8‡	- 10.6± 4.6*	-30.2±8.1‡	+ 3.1±0.8‡	+ 0.5± 2.0
FENa (%)	- 2.2± 0.6‡	- 2.3± 0.7‡	-27.9±6.9‡	+ 0.7±0.3‡	+ 0.7± 0.6

RBF, Renal blood flow; GFR, Glomerular filtration rate; V, urine flow rate; ENa, rate of sodium excretion; FENa, Fractional sodium excretion. NA, noradrenaline; PHE, phenylephrine; MET, methoxamine; GUA, guanabenz; n = number of experiments. * $p<0.05$; ‡ $p<0.02$; ‡ $p<0.01$ using paired Student's 't' test on the changes from basal values.

The results showed that, at the dose levels infused, the nonselective alpha-adrenoceptor agonist, noradrenaline, and the selective alpha-1-adrenoceptor agonists, methoxamine and pheylephrine, increased sodium reabsorption while the alpha-2-adrenoceptor agonists, UK14304 (Cambridge, 1981) was without effect, and guanabenz decreased it. These experiments demonstrate that the alpha-adrenoceptor mediating renal tubular sodium reabsorption is of the alpha-1- subtype and support previous evidence obtained from nerve stimulation studies (Hesse & Johns, 1983).

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