MUSCARINIC ANTAGONISTS SUPRESS PREJUNCTIONAL EFFECTS OF ANTICHOLINESTERASE

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The release of acetylcholine (ACh) from motor nerves is likely to be regulated by presynaptic cholinergic autoreceptors. The nicotinic cholinergic antagonist d-tubocurarine (d-TC) reduces the quantal content of evoked responses at relatively high stimulation rates (Gibb & Marshall, 1984). Various anticholinesterases elevate the miniature endplate potential (MEPP) frequency and spontaneous muscle spike activity. These latter effects can be prevented by simultaneous administration of either d-TC or atropine to the preparation (Carlson & Dettbarn, 1987). Previously we have reported that both eserine and F7-toxin, a toxin with anticholinesterase properties isolated from the green mamba (Dendroaspis angusticeps), increase the duration of MEPP's and that this effect can be antagonized by atropine (Van Wilgenburg et al., 1986, and Gonzalez et al., 1987). The present study was undertaken to examine the effects of the muscarinic antagonists atropine (as a non-selective muscarinic antagonist), pirenzepine (an antagonist with a relatively high affinity for M_1 receptors) and AF-DX 116 (11-2[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one) (with relatively high affinity for M_2 receptors) on the presynaptic effects of eserine at the neuromuscular junction.

Rat phrenic nerve-hemidiaphragm preparations were superfused with Krebs-Ringer solution at 32°C. Cut muscle preparations were used for indirect muscle stimulation. Voltage clamping was performed with two glass micro-electrodes, gain 2000x, ± 15V, holding potential - 70 mV. Recordings were obtained from superficial muscle fibers, since the penetration of the muscarinic antagonists in deeper layers of the preparation was poor.

Superfusion of the muscle preparation with eserine causes an increase in MEPP frequency, an increae in giant MEPP's, spontaneous muscle spike activity and a prolongation of the average MEPP duration. At 1 µmol/l the average duration of the rising time is increased to 174% and of the half decaytime to 231% of the control value. All three antimuscarinic agents reduce the prolongation of the rising and half decaytime in a dose-dependent way. At a concentration of 1 µmol/l AF-DX 116 is more effective than either atropine or pirenzepine. The prolongation by 1 µmol/l eserine is reduced by 1 µmol/l AF-DX 116 to respectively 122% and 138% of the control values. The same results are obtained with atropine and pirenzepine at a 10 - 30 times higher concentration. In contrast to d-TC no significant reduction to the quantal content was found upon high stimulation rates in the presence of the muscarinic antagonists.
The prolongation of the rising and the decay phase of MEPP's by eserine has to be of presynaptic origin, since successive MEPP's from the same motor endplate are either prolonged or have durations close to control values.

It is concluded that autoregulation of ACh release from motor nerve fibres is controled by different processes. Reduction of the quantal content, as caused by d-TC, is not found for muscarinic antagonists. In contrast, it is shown that the duration of MEPP's, as being prolonged by anticholinesterases, like eserine and F7-toxin, can be affected by muscarinic antagonists.

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EFFECT OF TIME AFTER OVARIECTOMY ON UTERINE RESPONSES AND OESTROGEN RECEPTOR STATUS IN THE RAT

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It is accepted that the primary purpose of oestrogen is to increase the size and number of cells in target organs such as the uterus and, in addition, to positively control the oestrogen receptor (OR) population of these tissues. The present study aimed to assess the effect of time after oestrogen withdrawal, by ovariectomy, on uterine responsiveness to oestradiol (E_2) and on OR number and status.

Sexually mature female rats (CD-derived) were ovariectomised under ether and allowed to recover for 3, 6 or 8 weeks prior to experimentation. The effect of a 3 hour treatment with E_2 (0.5μg kg^{-1} i.v.) was assessed in animals anaesthetised with sodium pentobarbitone (60mg kg^{-1} i.p.). E_2-induced uterine blood flow was measured using the radioactive microsphere technique (^{46}Sc; 15μM) and expressed as ml min^{-1} $100g^{-1}$. E_2-induced uterine oedema and the early trophic effect was calculated by measuring uterine wet and dry weight respectively. OR number and state of activation was assessed by an exchange assay (Marshall and Senior, 1987).

Time (3, 6, 8 weeks) after ovariectomy did not affect any of the parameters measured in the unstimulated uterus. Likewise, the uterine hyperaemic and oedematous response to E_2 was similar at all post-ablative stages. However, the early uterotrophic response to E_2 was not observed 8 weeks post ovariectomy (Table 1).

Treatment	Uterine dry weight (mg) measured in time in weeks after ovariectomy		
	3	6	8
Unstimulated control	16±1	16±1	15±1
Stimulated with E_2 0.5μg kg^{-1}	27±1***	25±1***	18±1

***P<0.001 significantly different from unstimulated control.

Table 1 showing the effect of time after ovariectomy on the uterine dry weight response to oestradiol.

No significant change in the intracellular distribution of OR, nor the proportion of activated nuclear OR was noted up to 8 weeks. Similarly, the affinity of E_2 for the receptor was maintained.

Time after ovariectomy affected only the early increase in uterine dry weight induced by E_2 suggesting that this effect of E_2 is mediated via a separate mechanism. Due to the established relationship between E_2-induced uterine growth and OR activation, it may have been anticipated that a diminuition in E_2-stimulated uterine growth would be associated with a reduction in receptor number or affinity. This was not the case which suggests a possible impairment of post-receptor events responsible for eliciting early weight increases 8 weeks following ovariectomy.

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HUMAN PLACENTAL STRIPS - AN IN VITRO MODEL FOR THE STUDY OF MYOFIBROBLAST CONTRACTILITY

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There is presently no widely accepted human tissue to use as an in vitro model for the study of the pharmacological manipulation of myofibroblast activity. Models previously used for this purpose have included wound granulation tissue (Ryan et al, 1974) and capsular tissue surrounding mammary implants (Baker et al, 1981; Ryan et al, 1974). Since both of these tissues possess severe limitations for routine investigations in terms of availability and uniformity other tissue sources have been investigated. The suitability of strips of human full term placentae as a more feasible model for the study of myofibroblasts was investigated.

This tissue was chosen after consideration of the histological studies of Feller et al 1985. Their histological examination, using both electron microscopy, and immunochemistry of the cellular content of the placenta led these authors to conclude that, except for the vascular component and a negligible number of Hofbauer cells, myofibroblasts make up nearly all the cellular constituents of human placental villous stroma.

In tissue superfusion experiments, four strips from each human placentae (n = 6) (approx. 20mm x 2mm x 2mm) were used in Krebs-Henseleit solution (flow rate 2ml min^{-1}) for isometric (1g pre load) recording (Grass FT03C). The effects of a range of agonists were studied by comparing dose/concentration-effect relationships, considering (a) the magnitude of the maximum tension generated (mg) and (b) the duration of the effect (minutes). Agonists administered in bolus doses were barium chloride (1-8mg), potassium chloride (1-8mg), mepyramine (100-800 μg), promethazine (100-800 μg), diphenhydramine (100-800 μg), methapyrilene (100-800 μg), 5-hydroxytryptamine (5HT) (1-20 μg), vasopressin (2.5-5 μg) and papaverine (0.26mM). Attempts were made to use cyproheptadine but solubility problems precluded the use of doses higher than 80μg.

All the agonists used, except papaverine, contracted the placental strips in the superfusion system used. The contractile responses were all dose dependent reproducible and reversible. Barium ions were found to be more potent than potassium ions in causing a contraction of the preparations but the quantities needed to elicit responses were far greater than those reported to stimulate smooth muscle preparations. The four antihistamines tested all proved to be contractile with similar contractile potencies but slight differences in their effects enabled a rank order of effectiveness to be established. Mepyramine > promethazine > diphenhydramine > methapryilene. Of these four agents promethazine had the longest duration of effect, some 2-3 times greater than that of the other agents. The contractile effect of both 5HT and vasopressin were significantly more potent than those for the antihistamines and were both of significantly shorter duration than the antihistamines. However, the concentrations needed to elicit such responses were far in excess of those required for smooth muscle preparations. At a concentration of 0.26mM papaverine progessively relaxed the tissue during the 20 minute contact period.

The results show that the in vitro activity of human placental strips is attributable mainly to myofibroblasts, to be acceptable as a reproducible, relatively uniform and easily available model of human myofibroblasts.

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THE EFFECT OF VASOPRESSIN ON THE ELECTROENCEPHALOGRAM OF RATS IS MEDIATED BY THE VASOPRESSIN V_1 RECEPTOR SUB-TYPE

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It has been previously shown that subcutaneous administration of arginine vasopressin (AVP) causes desynchronisation of the electroencephalogram (EEG) (Ebenezer, 1984; 1987). As vasopressin does not enter the brain from the systemic circulation (see Koob et al., 1986), it is unlikely that it activates the EEG by a direct central action. Moreover, as only doses that increase systemic blood pressure activate the EEG, it has been suggested that the mechanism involved is related to the pressor effect of the peptide (Ebenezer, 1987). Recently, Jard (1981) has described 2 vasopressin receptors, viz., the V_1 and V_2 receptors. The V_1 receptor is involved in the pressor effect of AVP while the V_2 receptor is involved in its antidiuretic effect. The present experiments were designed to determine the nature of the effect of vasopressin on the EEG.

Female Wistar rats (n=8) were used. The methods for chronic recording of EEGs and the subsequent off-line spectral analysis of the data have been described previously (Ebenezer, 1987). The power spectrum was divided into 4 frequency bands viz., 0-3.5Hz, 4-7.5Hz, 8-13Hz, and 13.5-22Hz. On test days the rats were allowed to adapt to the recording chamber for 1h before a 20min pre-drug sample of EEG was recorded. The animals were then injected subcutaneously with either saline followed by another injection of saline; saline followed by AVP (10μg/kg); the V_1 receptor antagonist d$(CH_2)_5$Tyr(Me)AVP (10μg/kg) followed by saline; or d$(CH_2)_5$-Tyr(Me)AVP (10μg/kg) followed by AVP (10μg/kg), and the EEG recorded for a further 60min. Successive injections were given 2min apart, and the rats received all 4 treatments. In a separate experiment, the effect of the V_2 receptor agonist 1-desamino-8-D-arginine vasopressin (dDAVP) (10μg/kg) was tested on the EEG of the rats. Statistical analysis was carried out using analysis of variance and the paired t-test.

AVP (10μg/kg) produced significant decreases ($P<0.05$) in spectral power in all frequency bands when compared with saline control values. Although the peptide caused significant reductions in power throughout the recording period, the most pronounced changes occurred 20 - 40 min after administration. Thus, for example, during this period, the mean power in the 0-3.5Hz frequency band was reduced by 53±7% while that in the 8-13Hz band was reduced by 52±11%. These results are consistent with those described previously (Ebenezer, 1987). In contrast, the V_1 receptor antagonist d$(CH_2)_5$Tyr(Me)AVP (10μg/kg) did not produce any significant change in spectral power but completely blocked the effect of AVP on the EEG. On the other hand, the V_2 receptor agonist dDAVP (10μg/kg), which is devoid of any pressor activity, had no effect on the EEG.

The present results therefore suggest that AVP acts on vasopressin V_1 receptor to produce its effects on the EEG, and thus support the hypothesis that the peptide activates the EEG by virtue of its pressor action.

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CALCULATION OF DISSOCIATION CONSTANTS OF AGONISTS AT CARDIAC β-ADRENOCEPTORS BY USE OF IN VITRO DESENSITIZATION

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Dissociation constants (K_A) or affinities of β-adrenoceptor agonists have been calculated by use of irreversible antagonists (Broadley & Nicholson, 1981) or functional antagonism (Buckner & Saini, 1975). However, both methods have been criticised (Mackay, 1981; Krstew et al. 1984). An alternative method of 'removing' receptors is by desensitization, which we have shown in atria after in vitro exposure to isoprenaline (Broadley & Herepath, 1987). We have used desensitization to calculate the K_A values for (-)-isoprenaline (ISO), (±)-terbutaline (TERB) and (±)-fenoterol (FEN) at atrial β_1-adrenoceptors.

Guinea-pig isolated paced left atria (2Hz, 5ms, threshold voltage + 50%) and spontaneously beating right atria were set up in Krebs-bicarbonate solution containing ascorbic acid (1mM) at 37.5°C gassed with 5% CO_2 in O_2. Increases in left atrial tension and right atrial rate in response to cumulative addition of ISO, TERB or FEN were recorded. At the maximum effect, ISO (10^{-6}M) was left in contact for 8h and pacing stopped. After 8h, pacing was resumed and the tissues washed (x5) for 1h before constructing a second ISO curve. At the maximum effects of TERB or FEN, pacing was stopped and the agonist replaced by ISO (10^{-6}M) for 4h. This was then washed out (x5) for 1h before constructing a second TERB or FEN curve. Pre-incubation curves were corrected from time-matched controls and n≥4. Equiactive concentrations of agonist on individual pre- (A) and post-desensitization (A') curves were plotted as their reciprocals and K_A calculated as (slope-1)/intercept (Furchgott, 1966).

Incubation with ISO for 8h significantly depressed ISO rate and tension maxima to 85.2±5.4 and 68.9±2.3% respectively. Similarly, incubation for 4h caused significant depression of TERB (rate, 74.8±1.5; tension 33.8±2.5) and FEN (rate, 85.6±5.5; tension, 76.0±5.1) maxima. Geometric mean K_A values, determined from experiments where the maximum was depressed below 90%, and EC50 values from uncorrected pre-incubation curves are shown below.

	Right atrial rate		Left atrial tension	
	EC50	K_A	EC50	K_A
Isoprenaline	3.2nM*	57nM	12nM*†	89nM^{NS}
	(1.9-5.4)	(19-170)	(6.5-23)	(15-510)
Terbutaline	2.1μM*	29μM	8.0μM†	25μM^{NS}
	(1.0-4.4)	(8.4-100)	(2.4-26)	(9.9-64)
Fenoterol	0.1μM*	13μM	0.74μM*†	18μM^{NS}
	(0.04-0.2)	(1.9-84)	(0.43-1.3)	(7.7-42)

Differences between EC50 and K_A (*significant $P<0.05$), between left and right atria EC50 (†significant $P<0.05$) and left and right atria K_A (NS, not significant).

For each agonist, K_A values were greater than the corresponding EC50 value, significantly except for TERB on left atria. K_A values for rate and tension responses did not differ significantly indicating that the β-adrenoceptors mediating these responses are identical, although each agonist was rate selective.

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COMPARATIVE EFFECTS OF ADENOSINE, L-PIA AND CARBACHOL UPON ISO-PRENALINE RESPONSES OF GUINEA-PIG LEFT ATRIA AND PAPILLARY MUSCLES

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The antiadrenergic effects of P1-adenosine receptor agonists in atrial muscle are mediated by one or a combination of two mechanisms: a decrease in cAMP levels (Linden et al. 1985); and an increase in potassium conductance (Nawrath et al. 1985). The antiadrenergic effects of muscarinic agonists in atrial muscle are mediated by one or a combination of: a decrease in cAMP levels (Linden et al. 1985); an increase in potassium conductance (Nawrath et al. 1985); an increase in cGMP levels (Inui et al. 1979). In ventricular muscle neither P1 nor muscarinic receptor agonism is capable of increasing potassium conductance (Nawrath et al. 1985). We examined the effects of adenosine (AD), the P1 receptor agonist N6-(L-2-phenylisopropyl)-adenosine (PIA) or the muscarinic receptor agonist carbachol (CAR) upon isoprenaline (ISO) responses in guinea-pig left atria (LA) and papillary muscles (PM) to determine any qualitative differences in their actions.

Isolated paced left atria and papillary muscles (2Hz, 5ms, threshold voltage + 50%) were set up in Krebs-bicarbonate solution at 37.5°C, gassed with 5% CO_2 in O_2. Tension responses were recorded. Equiactive concentrations of AD (75μM), PIA (0.52 μM) or CAR (0.17μM) producing approximately 50% reduction of naive left atrial tension were used in atria and ten-fold greater concentrations were used in papillary muscles. Cumulative concentration-response curves to ISO were constructed before and during incubation with AD, PIA or CAR. Pretreatment curves were corrected from controls.

		AD		PIA		CAR	
		absence	presence	absence	presence	absence	presence
LA	EC50 (nM)	34.8	44.0	16.6	139.0*	18.8	91.2*
	% Maximum	100	58.7*	100	28.6*	100	57.1*
PM	EC50 (nM)	10.7	18.2*	19.0	38.8*	17.5	27.3*
	% Maximum	100	119.9*	100	104.7	100	100

EC50 values are geometric mean. *$P<0.05$

In atrial muscle both PIA and CAR produced a large significant increase of the ISO EC50 and a large significant depression of maximum. In contrast, both agents at 10 times the concentration caused only a small but significant increase in ISO EC50 in papillary muscles with no effect on maximum. AD increased ISO EC50 to a lesser extent in atria, possibly due to cellular uptake of AD to which PIA and CAR are not susceptible. The AD induced increase in ISO maximum of papillary muscles may be an intracellular effect such as phosphodiesterase inhibition (Meyer et al. 1984). The differences between PIA and AD are therefore probably not occurring at the P1 receptor. In conclusion, this study offers no evidence that the antiadrenergic effects of muscarinic and P1 receptor agonists are mediated by different sub-receptor mechanisms.

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CHARACTERISATION OF [^{3}H]-KETANSERIN BINDING TO HUMAN PLATELET MEMBRANES

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It has been shown that 5-hydroxytryptamine (5-HT)-induced shape changes and aggregation in human blood platelets can be antagonised by the 5-HT$_2$ receptor blocking agent, ketanserin (DeClerck *et al*, 1984). The binding of [^{3}H]-ketanserin to cat blood platelets was found to be analogous to that at 5-HT receptors located in the central nervous system (Leysen *et al* 1983). The present study was designed to investigate the binding characteristics of [^{3}H]-ketanserin in human blood platelet membranes.

Human blood platelet membranes were prepared by a modification of the method of Neubig & Szamraj (1986) and stored at -20°C. For kinetic binding studies, thawed membrane preparations (100-200 mg platelet protein) were incubated in calcium-free Krebs' buffer for various times, in duplicate, at 37°C in the presence of [^{3}H]-ketanserin (1 nM). Equilibrium binding studies were carried out in triplicate for each experiment. Membranes were incubated with [^{3}H]-ketanserin (0.25 - 4.0 nM) for 30 min. Specific binding was defined as that displaced by unlabelled ketanserin (10 µM).

Binding of [^{3}H]-ketanserin was rapid ($t_{½}$ = 2.5 min) and reversible ($t_{½}$ = 22 min; n = 7). Equilibrium was reached at 20-30 min with a k_{obs} of 0.04 ± 0.023 min^{-1} (n = 7). The dissocation reaction followed first order kinetics with a k_2 = 0.025 ± 0.003 min^{-1} (n = 7). A second order rate constant (k_1) of 0.02 ± 0.003 min^{-1} nM^{-1} was calculated. The kinetically derived value for the equilibrium dissociation constant (K_D = k_2/k_1) was 1.25 nM.

The specific binding of [^{3}H]-ketanserin was saturable and to a single population of binding sites. Scatchard analysis showed a K_D = 1.45 ± 0.58 nM and a B_{max} = 21.26 ± 4.36 fmol/mg platelet protein (n = 5). Hill analysis gave a value for the dissociation constant (K'_D) of 2.09 nM with n_H of 0.98, indicating a lack of co-operativity.

These findings suggest that [^{3}H]-ketanserin provides a ligand suitable for studying the interaction of 5-HT and related compounds with human blood platelet membranes.

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β-ADRENOCEPTOR DESENSITISATION IN CULTURED FIBROBLASTS FROM YOUNG AND OLD DONORS

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The age associated reduction in tissue beta-adrenoceptor responsiveness involves biochemical changes at the beta-adrenoceptor and sites distal to the receptor (Heinsimer & Lefkowitz, 1986). These changes could contribute to the reduced capacity of lymphocyte beta-adrenoceptors to undergo agonist induced desensitisation with ageing in rats (DeBlasi et al, 1987) and the reduced fibroblast beta-adrenoceptor responsiveness reported in two subjects with Werner's syndrome ;a condition of premature ageing (Bannai et al.,1987). We have examined beta-adrenoceptor responsiveness and agonist induced desensitisation in fibroblasts from two healthy young and two old male subjects.

Fibroblasts were cultured in DME/F12 medium supplemented with 10% foetal calf serum in an atmosphere of 95% air, 5% CO_2 and examined at 15-25 population doublings and 90% confluence. Beta-adrenoceptor responsiveness was measured from dose-response curves of (-)isoprenaline ((-)ISO; 1nM-0.1mM) induced adenosine 3',5'-cyclic monophosphate (cyclic AMP) accumulation in intact cells conducted over 5 minutes at 37°C. Beta-adrenoceptor desensitisation was examined following exposure of fibroblasts to (-)ISO (1uM) for 30 minutes at 37°C. Saturation binding using ^{125}I-iodopindolol (^{125}I-PIN; 20-300pM) was conducted in fibroblast membranes at 30° C for 45 minutes to determine beta-adrenoceptor density and iodopindolol dissociation constant (Kd).

Beta-adrenoceptor density (15.9±3.9 vs 5.6±0.9 fmoles/mg protein; $p<0.01$,n=8) and the ^{125}I-PIN (Kd) (178.1±26.6 vs 74±13 pM; $p<0.01$,n=6)were both greater in old compared with young donor fibroblasts. Beta-adrenoceptor responsiveness (Table 1) and the capacity to undergo agonist induced desensitization were similar in the fibroblasts of both donor age groups.

Table 1. Beta-adrenoceptor responsiveness

Donor age(years)		29	32	81	84
Response(max) (pmol cyclic AMP /mg protein)	control	170±14	293±70	237±31	280±157
	desensitized	111±19	150±52	141±16	110±58
desensitized (% control)		65±8	49±8	63±9	48±5

n=4-5; means±S.E.M.

These data demonstrate that cultured fibroblasts obtained from both young and old donors retain beta-adrenoceptor responsiveness and the capacity to undergo agonist induced beta-adrenoceptor desensitisation. Examination of fibroblasts from a larger number of donors is required, however, to confirm the existence of age related differences in beta-adrenoceptor density and antagonist affinity that are apparent in the present study.

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RECEPTOR BINDING AND INTERNALISATION OF ATRIAL NATRIURETIC FACTOR IN CULTURED VASCULAR SMOOTH MUSCLE CELLS

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Atrial natriuretic factor (ANF) lowers blood pressure via natriuresis and diuresis in animals and in humans (Cantin and Genest, 1985; Richards et al, 1985). It also is a potent vasorelaxant, acting directly on smooth muscle tissue in vitro (Currie et al, 1984). In this study we have examined receptor binding and internalisation of ANF in cultured vascular smooth muscle cells (RVSMC) derived from normotensive male Wistar rats.

Cells were cultured from thoracic aorta of 8-10 week old rats after removal of fat and connective tissue, digestion in collagenase (3mg/ml) to remove the adventitia, further digestion in collagenase to remove the endothelium and final dispersion of cells by treatment with fresh collagenase and elastase (0.1mg/ml). The cells were maintained in DMEM supplemented with 5% FCS, 5% Nu-serum, 50 U/ml penicillin/streptomycin, 0.5µg/ml fungizone and 0.1mg/ml gentamycin.

Saturation binding analysis of intact RSVMC at 4°C indicated a single high affinity site for ^{125}I-rANF (1-28) with K_D 1.41 ± 0.2nM (n=5) and Bmax 291 ± 35 fmol/10^6cells (n=5) corresponding to 180,000 sites/cell. Binding of ^{125}I-rANF was displaced by unlabelled rANF (1-28), atriopeptin I (5-25) and by atriopeptin II (5-27) in a dose dependent manner. Examination of the expression of ANF receptors during culture showed that receptor number in whole cells decreased from 400,000 to 100,000 sites/cell despite an increase in cell density over the culture period.

Investigation of the rate and extent of ANF binding showed both to be temperature dependent. At 4°C the maximal level of specific binding occurred after 100 min and remained stable for up to 3hr. At 37°C, specific binding was reduced by 60% and was maximal after 30 min, decreasing thereafter. While the majority of ANF bound at 4 °C was localised on the cell surface (as shown by its complete removal with NaCl/acetic acid, pH 2.5), up to 20% could not be removed at 37°C. In addition the amount of non-dissociable ANF increased in the presence of chloroquine, ammonium chloride, sodium azide and concanavalin A with a concurrent increase in the maximum levels of cell-bound radioactivity.

Specific binding at 4°C, after washing, decreased more rapidly upon subsequent warming of the cells to 37°C than with further incubation at 4°C; up to 75% of the radioactivity released into the medium at 37°C was TCA soluble while at 4 °C all such radioactivity was TCA insoluble. Furthermore 25% of cell bound radioactivity was found in the NaCl/acetic acid resistant compartment at 37°C, whereas it remained on the cell surface at 4°C.

These experiments suggest that the observed temperature dependent decrease in cell bound radioactivity is probably due to internalisation, degradation and elimination of ANF rather than simple dissociation from the cell surface. These processes may be related to the mechanism of down regulation of ANF receptors in RVSMC pretreated with ANF.

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ROLE OF PRE-SYNAPTIC α_2-ADRENOCEPTORS AND P1 PURINOCEPTORS IN TRANSMISSION IN RATS VAS DEFERENS

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Tension responses of isolated desheathed rat vasa deferentia were elicited at 5 minute intervals to challenges of twin pulse field stimulation (TPFS - 0.8msec width, 40V, 3s inter-pulse interval, via parallel platinum electrodes) and digitised at sampling frequency of 100Hz and stored on floppy disc under the control of a BBC microcomputer (Marshall & Sparks, 1981).

The response to the first pulse of a challenge shows a large first peak at 280msec followed by a second smaller peak 650msec after the stimulus. The response to the second pulse attains a greater first peak height with a diminished second phase (Marshall & Spriggs, 1984) which is not fully restored by selective alpha-2-adrenoceptor antagonists (Mallard et al, 1988). The present work compares the effects of clonidine, a selective alpha-2-adrenoceptor agonist with those of 2-chloroadenosine (2CA), a selective P1 purinoceptor agonist both alone and in combination with the respective selective antagonists imiloxan and 8-phenyltheophylline (8PT) on the responses to TPFS.

Clonidine (0.01nM - 10nM, n=6) induced a dose-related inhibition of both responses to the TPFS challenge. At higher concentrations responses were not further depressed but exhibited an increased latency (450msec) to peak tension development. Subsequent relaxation to base line tension was slower and associated with the occurrence of spontaneous activity. In the presence of clonidine (10nM, n=5), imiloxan at low concentrations (1nM - 10nM) had little effect on either response: at higher concentrations (100nM - 5uM) imiloxan induced a full restoration of the first phases of the responses to both pulses, but only a partial restoration of the second phases of each response.

2CA (10nM - 100uM, n=6) induced a dose-related inhibition of both responses to TPFS challenge (97% $\pm$ 22% and 95.2% $\pm$ 27.2% respectively at 100uM), without changing the latency to peak tension development or relaxation to baseline and did not elicit any spontaneous activity. 8PT (10nM - 100uM, n=6) alone, failed to modify the responses to TPFS. In the presence of 2CA (1uM), 8PT induced a dose-related restoration of both the first and second phases of the response to the first pulse (88.9% $\pm$ 11.9% and 96.1% $\pm$ 24.7% respectively at 1uM) and the first phase of the response to the second pulse (95% $\pm$ 8.5% at 1uM). A combination of imiloxan (100nM) and 8PT (10nM - 1uM) failed to produce any distinguishable effects on the responses to TPFS.

The results demonstrate that both clonidine and 2CA can inhibit the NANC and the NA phases of the responses to TPFS. The effects are reversible with imiloxan and 8PT respectively although the inactivity of 8-PT alone suggests that P1 purinoceptors have little role in the interaction between the responses to TPFS. The failure of imiloxan to restore the NA phase of the response to the first pulse at higher concentrations may be attributable to alpha-1-adrenoceptor blocking activity.

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CROMAKALIM AND CALCIUM MOVEMENTS IN VASCULAR SMOOTH MUSCLE

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The K^+ channel opener cromakalim evokes a hyperpolarisation and relaxes vascular smooth muscle (Hamilton et al, 1986). Bray et al (1987) have demonstrated that the noradrenaline (NA) contraction in rabbit aorta is electrically quiescent, the tonic phase (dependent on extracellular Ca^{2+}) being more sensitive to inhibition by cromakalim than the phasic component (dependent on intracellular (ic) Ca^{2+}). The effect of cromakalim on the tonic phase of NA contraction has been investigated in models of Ca^{2+} influx, release, uptake and Na^+/Ca^{2+} exchange. De-endothelialised rabbit aortic rings were placed under 1 g tension in normal Krebs and prepared for isometric recording of tone. Cromakalim relaxed the tonic phase of NA (1 μM) contraction in a dose-related manner with a threshold of 0.3 μM and a maximum relaxation of 38.0 ± 6.6% at 3 μM (n = 4). Pre-treatment with verapamil (1 μM) and KCl (65 mM), but not verapamil alone, prevented the inhibition by cromakalim.

In O-Ca^{2+} and EGTA (1 mM), NA (10 μM) evokes a phasic contraction (ic Ca^{2+} release) and subsequent administration of Ca^{2+} (2.5 mM) restores a tonic contraction (receptor operated Ca^{2+} influx) (Hester et al, 1985). Pre-treatment of preparations with cromakalim (3 μM, 20 min) affected neither the phasic nor tonic component of NA contraction.

In Ca^{2+} uptake studies, aortic rings were Ca^{2+} depleted by incubation in O-Ca^{2+}, EGTA (1 mM) and repeated dosing with NA (10 μM) (Karaki, 1987). Tissues were then washed in a O-Ca^{2+} solution without EGTA and reloaded with Ca^{2+} (1 mM) for 10 min in the presence or absence of cromakalim (20 min prior to Ca^{2+}). Following this loading period tissues were bathed in O-Ca^{2+} and EGTA. Subsequent NA administration evoked a phasic response. Tissues incubated in the presence of cromakalim exhibited a reduced NA contraction (38.8 ± 6.8% of control) when compared with vehicle (61.8 ± 4.5% of control) ($\bar{x}$ ± sem, n = 4, $p<0.05$ t-test).

Experiments to investigate Na^+/Ca^{2+} exchange were performed on guinea-pig aortic spirals suspended in normal Krebs solution under 1 g tension. Exposure to O-KCl Krebs containing verapamil (10 μM) and phentolamine (10 μM) evoked a contraction (Ca^{2+} influx) of 0.98 ± 0.06 g (n = 16), developing over 3 hrs. Addition of KCl (5.4 mM) relaxed (Ca^{2+} efflux) this tissue (Bova et al, 1988). Administration of cromakalim (20 min prior to O-KCl or KCl re-addition) affected neither the peak nor magnitude of contraction or relaxation.

In conclusion cromakalim evokes a hyperpolarisation of vascular smooth muscle and inhibits the tonic NA contraction. In vascular tissue this is associated with reduced Ca^{2+} uptake to depleted tissue. Under our experimental conditions this reduced Ca^{2+} influx evoked by cromakalim is not due to actions at receptor operated channels, voltage operated channels or the Na^+/Ca^{2+} exchanger. The precise mechanism of action remains to be elucidated.

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INFLUENCE OF KCl CONCENTRATION ON THE INHIBITORY ACTIVITY OF CROMAKALIM AGAINST CONTRACTIONS TO VARIOUS SPASMOGENS

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The anti-hypertensive drug, cromakalim (CRK) (BRL 34915) inhibits contractions to serotonin (5HT) (Buckingham et al., 1984), angiotensin II (AII) (Cook et al., 1987), noradrenaline (NA) and low, but not high, KCl concentrations (Clapham & Wilson, 1987) in isolated blood vessels. The spasmolytic activity of CRK against NA contractions in rabbit aorta can be inhibited by concurrent exposure to KCl 40mM (Bray et al., 1988). The present experiments investigate the relationship between KCl concentration and the spasmolytic activity of CRK against contractions to NA, 5HT, histamine (HIS) and AII.

Rings of rabbit mesenteric artery (RMA) or abdominal aorta (RAA) were prepared for isometric tension recording as described previously (Clapham & Wilson, 1987). In RMA (n=5), CRK 1μM relaxed contractions to 20, 30, 40 & 65mM KCl by 85±8%, 85±7%, 42±5% and 14±3% respectively. In the same tissues, relaxations by CRK 1μM of contractions to NA 0.1mM were 92±2%, 84±2%, 89±1% and 77±7% respectively. When the same tissues were contracted by NA 0.1mM in the presence of 20, 30, 40 and 65mM KCl respectively, relaxations by CRK were reduced to 64±7%, 42±7%, 13±3% and 2±1%. In separate experiments where the Ca^{2+} channel blocker, PY 108-068 (0.1μM), was present, KCl did not modify contraction, but the spasmolytic effect of CRK was similarly inhibited.

In RAA (n=5), it was also possible to obtain stable contractions to 5HT, HIS and (to a lesser extent) AII, by using maximal concentrations of the spasmogens . CRK 1μM was much less effective against NA 0.1mM in this tissue, but, as in RMA, spasmolytic activity was [KCl] sensitive (Table 1). CRK was much more effective against contractions to 5HT 30μM and HIS 0.1mM. Again the activity was [KCl] sensitive. CRK was most effective against contractions to AII 0.3μM. These relaxations were reduced, but not abolished, by increasing [KCl].

Table 1. Mean % relaxation (± s.e.mean) by CRK 1μM of contractions to NA, 5HT, HIS and AII in RAA in the absence and presence of different [KCl].

Spasmogen.	20mM KCl	30mM KCl	40mM KCl	65mM KCl
NA	14 ± 3%	12 ± 3%	13 ± 1%	18 ± 3%
NA+KCl	9 ± 1%	7 ± 2%	5 ± 1%	0 ± 0%
5HT	83 ± 8%	70 ± 11%	82 ± 1%	79 ± 3%
5HT+KCl	49 ± 6%	33 ± 6%	29 ± 12%	4 ± 2%
HIS	79 ± 5%	73 ± 3%	54 ± 10%	65 ± 8%
HIS+KCl	51 ± 5%	30 ± 1%	28 ± 10%	9 ± 2%
AII	94 ± 2%	92 ± 5%	91 ± 2%	95 ± 3%
AII+KCl	76 ± 3%	44 ± 9%	32 ± 7%	39 ± 9%

Contractions to 20, 30, 40 and 65mM KCl alone in these tissues were relaxed by 76±2%, 47±2%, 22±6% and 1±1% respectively by CRK 1μM. As before, PY 108-068 did not influence the inhibition of cromakalim-induced relaxation.

The results demonstrate that relaxation by CRK of contractions to spasmogens is sensitive to the presumed smooth muscle cell depolarisation produced by increasing concentrations of KCl.

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AN INVESTIGATION INTO THE INHIBITORY ACTION OF CROMAKALIM IN RABBIT ISOLATED MESENTERIC ARTERY

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Cromakalim is a potent vasorelaxant in a number of isolated vascular tissues (Hamilton et al., 1986; Cain & Nicholson, 1988). This study was therefore undertaken to determine the mechanical, biochemical and electro-physiological effects of cromakalim in the fine arterial branches of the mesenteric bed.

The mesenteric bed was removed from male half lop rabbits (2.5-3.5 Kg). A segment of the mesenteric bed containing one branch was cannulated and perfused with a MOPS buffered physiological salt solution(PSS) at 37 °C. Noradrenaline (NA, 0.1-30μM) added in a cumulative fashion to the PSS caused concentration dependent increases in perfusion pressure. Concentration-response curves were constructed and the pD_2 calculated to be 5.34±0.11 (n=6). Cromakalim (0.1-10μM) was found to reduce an established 10μM NA(EC_{70}) induced increase in perfusion pressure in a concentration dependent manner. Complete inhibition of the NA effect was not, however, achieved.

In the ^{86}Rb efflux study segments of mesenteric artery of approximately 2 cm in length and 0.5 mm in diameter were impaled on fine needles and loaded with ^{86}Rb (5μCi.ml^{-1} for 90 min). The rate of ^{86}Rb efflux was determined at 2 min intervals. Cromakalim (0.4-10μM) significantly increased the ^{86}Rb efflux from these tissues, for example, cromakalim (10μM) increased the mean basal ^{86}Rb efflux rate coefficent measured between the 18th and the 26th minutes of efflux from 0.88±0.08 %min^{-1}(n=6) to 1.32 ±0.11 %min^{-1} (n=5).

The membrane potential of small segments of the mesenteric artery was determined using glass microelectrodes (impedence 50-100 MΩ) and was found to be -64.6±1.3(n=31). The addition of cromakalim caused a concentration-dependent hyperpolarization of the cell membrane (Table 1). NA (3 & 10μM) depolarized the cell membrane by 4.8±1 and 9±0.8 mV, respectively (n=5-7).

Table 1

	Cromakalim (μM)		
	0.4	2	10
Δ Membrane Potential (mV)	5.5±0.9(9)	9.8±1.2(8)	15.1±1.4(8)

Data represent mean±s.e. mean(number of observations)

The results obtained in this study show that the NA induced increase in perfusion pressure is associated with a depolarisation of the smooth muscle cell membrane. Cromakalim was found to inhibit the NA effect on perfusion pressure at concentrations which were also found to hyperpolarize the smooth muscle cell membrane and significantly increase ^{86}Rb efflux from this tissue. These findings suggest that the vasorelaxant effect of cromakalim is a consequence of the opening of ^{86} Rb-permeable potassium-channels.

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HAEMODYNAMIC AND PHARMACOLOGICAL MECHANISMS OF THE HYPOTENSIVE EFFECTS OF CROMAKALIM IN RATS : BLOCKADE BY GLIBENCLAMIDE

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Cromakalim belongs to a novel class of pharmacological agents which appear to produce vasorelaxation by activating an outward K^+ current (Cook, 1988). In the anaesthetized rat, the hypotensive effects of cromakalim do not require the presence of an operational sympathetic nerve drive and are enhanced by inhibition of angiotensin converting enzyme (Donato Di Paola *et al.*, 1987). This communication reports the results of studies aimed at the haemodynamic and pharmacological characterization of the hypotensive activity of cromakalim.

Male normotensive rats (Sprague-Dawley : 220 - 250 g, n=5-10/group) were anaesthetized with pentobarbitone (55 mg/kg i.p.), respired artificially and prepared for the measurement of blood pressure (carotid artery) and i.v. drug administration. Cromakalim (5 µg/kg/min, 20 min) was studied in rats pretreated with i.v. methylatropine (0.3 mg/kg), BW 755C (5.0 mg/kg : cyclo and lipoxygenase inhibitor), 59227 RP (0.3 mg/kg : PAF receptor antagonist), glibenclamide (20 mg/kg), promethazine (1.0 mg/kg), propranolol (0.75 mg/kg) or enalapril (0.3 mg/kg). Additional experiments were performed in bivagotomized rats with carotid arteries ligated and in pithed rats with blood pressure elevated to pre-pithing values with an i.v. infusion of vasopressin (PI + VAS). Cromakalim was also studied in rats in which miniaturized pulsed Doppler flow probes were placed around the abdominal aorta (exit from the diaphragm to estimate cardiac output), superior mesenteric and left renal arteries and distal aorta (hindquarter). Results are given as means ± S.E.M.

Cromakalim produced a fall in mean carotid artery blood pressure reaching a maximum at the end of the infusion (-40±2 mmHg from baseline value of 122±3). At this time, systemic, hindquarter, mesenteric and renal resistances decreased by 35±3, 45±5, 27±3 and 19±3%, respectively. The hypotensive response to cromakalim was not changed by bilateral vagotomy with concurrent carotid artery ligation or by PI + VAS. Furthermore, it was not affected by pretreatment with BW 755C, methyltropine, 59227 RP, propranolol or promethazine. In contrast, enalapril enhanced (ΔMAP : -66±3 mmHg) and glibenclamide abolished the decrease in blood pressure produced by cromakalim.

These results indicate that haemodynamically the hypotensive activity of cromakalim can be accounted for by a fall in systemic resistance. The blood pressure lowering effect of cromakalim was not mediated by various classical receptors (muscarinic, H, β_2), prostaglandin release or vagal reflexes. Its enhancement by enalapril suggest that cromakalim activates the renin-angiotensin system (Donato Di Paola *et al,* 1987). Finally, in contrast to the finding of Wilson *et al.* (1988), glibenclamide was found to block the cromakalim-induced hypotension. Thus, the latter compound may activate K^+ channels which resemble pancreatic β cell or cardiac ATP-sensitive K^+ channels of which glibenclamide is a potent antagonist (Schmid-Antomarchi *et al,* 1987).

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EFFECTS OF DOPAMINE ON HUMAN CARDIAC ACTION POTENTIAL CHARACTERISTICS

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One mechanism by which dopamine improves cardiac performance in patients with heart failure, is through a positive inotropism mediated by ß-adrenoceptor stimulation (Rajfer & Goldberg, 1982). The aim of this study was to compare the in vitro cardiac electrophysiology of dopamine with isoprenaline, on isolated human ventricular tissue, obtained from patients undergoing cardiac transplantation.

Papillary muscles were dissected from the left ventricular myocardium and transferred to the laboratory in cooled Krebs (4°C). The tissue was superfused with Krebs at a rate of 6 mls.min^{-1} at 37°C. The composition of the Krebs was as follows (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 5.0, Na pyruvate 2.0, ascorbic acid 0.3, 0.5 mMoles of Na EDTA prechelated equimolar $CaCl_2$, and 2.5 mM $CaCl_2$. Krebs solution was gassed with 95% O_2/5% CO_2 to give a pO_2 of 648 ± 5 mmHg and a pH of 7.4.

Muscles were electrically field stimulated at 0.2 Hz. Action potential recordings were made using standard microelectrode techniques. The action potential parameters measured were the action potential amplitude (APA), the maximum rate of depolarisation (MRD), as well as the action potential duration to 50% and 90% repolarisation (APD_{50}, APD_{90}). In addition, the force of contraction (T) was also recorded. Single cell impalements were maintained throughout the experiment whenever possible, otherwise satisfactory reimpalements were obtained. All drugs were added cumulatively and given an equilibration time of ten minutes. The following protocol was used: Prior to impalement, tissues were given a washout period of at least three hours. Once a stable impalement had been obtained, muscles were exposed to 10^{-7}M and 10^{-6}M isoprenaline. The tissues were then washed out for an hour before dopamine was added.

Neither isoprenaline or dopamine produced any marked effects on the cardiac action potential characteristics, although dopamine slightly enhanced the plateau phase. However, isoprenaline caused a significant increase in the force of contraction, whereas dopamine produced only a small, non-significant, increase.

Table 1. The effects of dopamine (D) on action potential characteristics of human ventricular tissue. (Figures represent means ± SEM, n=4, *n=6).

GROUP	APA (mV)	MRD (v.s^{-1})	APD_{50} (ms)	APD_{90} (ms)	T (mg)*
Control	109±3	250±70	265±12	356±18	131±33
10^{-5}M D	105±4	233±68	269±21	364±23	124±28
10^{-4}M D	110±5	204±61	293±27	378±22	146±34
10^{-3}M D	111±5	245±66	283±29	363±24	165±37

Similar results on the action potential have been obtained previously with both isoprenaline and epinine (n-methyl dopamine) in human ventricular myocardium (Gristwood & Rothaul, 1987). The lack of a significant inotropic effect with dopamine may be due to decreased ß-adrenoceptor responsiveness of diseased cardiac tissue in vitro (Ginsburg et al, 1983).

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P_1-PURINERGIC, α_2-ADRENERGIC AND K-OPIATE RECEPTOR AGONISTS SHOW A MUTUAL ANTAGONISM ON RECEPTOR MEDIATED DECREASES IN $[Ca^{2+}]_i$

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We have previously reported that α_2-adrenergic and k-opiate receptor agonists reduce evoked release of [^{3}H] noradrenaline (NA) (McWilliam and Campbell, 1988) and also the concentration of free $[Ca^{2+}]_i$ (Adamson et al, 1987) in rat brain synaptosomes. Subsequent studies have demonstrated a mutual antagonism of the above effects when an α_2 adrenergic and K-opiate agonist are co-incubated (McWilliam and Campbell, 1987; Adamson et al. 1988). To establish whether this mutual antagonism between two presynaptic receptors is a general phenomenon, we expanded our investigation to include purinergic agonists which have been reported to interact with α_2-adrenoceptors (Allgaier et al, 1987).

Rat cortical synaptosomes were prepared on discontinuous percoll density gradients and $[Ca^{2}]_i$ was measured using the fluorescent indicator fura-2 (Adamson et al, 1988).

We report here that the P_1-purinergic agonist N^6-(R-phenylisopropyl)-adenosine (PIA) reduces $[Ca^{2+}]_i$ in a dose dependent manner in the range 0.1-1.0uM ($p<0.001$, paired randomization test) with a maximum effect of 24.5%. However, when this agonist is co-incubated with either an α_2 adrenergic agonist or a K-opiate agonist a mutual antagonism of the effects of these two drugs on the depression of $[Ca^{2+}]_i$ is observed. These mutually antagonistic effects can be reversed if a specific antagonist of either P_1-purinergic, α_2-adrenergic or the K-opiate receptors is included in the relevant incubation (Table 1).

TABLE 1

Drug Combination/Conc(uM)	% Change in $[Ca^{2+}]_i$	Significance from Control
PIA(1)	-22.6	$p \leq 0.008$
U50, 488H(1)	-14.1	$p \leq 0.015$
Clonidine(1)	-19.8	$p \leq 0.004$
		Significance from values obtained with agonists alone
PIA(1)+Caffeine(1)	+ 9.6	$p \leq 0.001$
U50, 488H(1)+naloxone(20)	+10.8	$p \leq 0.001$
Clonidine(1)+idazoxan(2)	+ 0.8	$p \leq 0.001$
PIA(1)+Clonidine(1)	+20.9	$p \leq 0.001$
PIA(1)+Clonidine(1)+idazoxan(2)	-18.4	N.S.
PIA(1)+Clonidine(1)+Caffeine(1)	-15.9	N.S.
PIA(1)+U50, 488H(1)	+18.7	$p \leq 0.001$
PIA(1)+U50, 488H(1)+naloxone(20)	-13.0	N.S.
PIA(1)+U50, 488H(1)+Caffeine(1)	-19.6	N.S.

We conclude that this mutual antagonism which we have observed using these presynaptic systems may be a general phenomenon and may be a new regulatory mechanism at nerve endings.

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MPP^+ HAS DIFFERENT EFFECTS TO PARAQUAT AND MENADIONE IN RAT BRAIN MICROSOMES

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1-Methyl-4-phenylpyridinium (MPP^+) the neurotoxic metabolite of MPTP is structurally related to paraquat. Paraquat cell toxicity may involve its redox cycling to produce toxic oxygen free radicals. In contrast MPP^+ does not appear to be a potential redox cycler at least in liver microsomes (Frank et al, 1987). However, no comparison has been undertaken in brain tissue where MPTP (and MPP^+) exert their toxic effects. We now compare the ability of MPP^+ and paraquat in rat brain microsomes to accept electrons compared to the effects of menadione.

Rat brain microsomes were incubated for 60 min at 37°C with NADPH (0.1-10 mM) alone or with paraquat (6 mM) or menadione (6 mM) or MPP^+ (6 mM). Lipid peroxidation was assessed using malondialdehyde (MDA) formation measured by the thiobarbituric acid (TBA) assay (Ohkawa et al, 1979). In addition the effect of paraquat (0.1-2.5 mM) menadione (0.0025-0.05 mM) or MPP^+ (5-25 mM) on the rate of NADPH oxidation in brain microsomes was determined.

Table 1 Effects of NADPH stimulated lipid peroxidation and NADPH oxidation in rat brain microsomes.

		TBA reactive products (nM/mg protein)			Rate of NADPH oxidation (nM/mg protein/min)
Paraquat	(6 mM)	2.41 ± 0.21*	Paraquat	(1.0 mM)	17.28 ± 5.71*
Menadione	(6 mM)	0.04 ± 0.14*	Menadione	(0.01 mM)	16.91 ± 5.04*
MPP^+	(6 mM)	4.63 ± 0.49	MPP^+	(10 mM)	1.77 ± 1.65
NADPH	(1 mM)	4.50 ± 0.3+	NADPH	(0.2 mM)	2.10 ± 3.54
Basal		1.80 ± 0.07			

* $p < 0.05$ compared to NADPH group
\+ $p < 0.001$ compared to basal values

NADPH (0.1-10 mM) increased the formation of TBA reactive products in brain microsomes in a concentration dependent manner. Menadione (6 mM) and paraquat (6 mM) inhibited the NADPH induced increase in TBA reactive products in brain microsomes. In contrast MPP^+ (6 mM) did not alter the increase at any NADPH concentration. The incubation of menadione (0.0025-0.05 mM) or paraquat (0.1-2.5 mM) with rat brain microsomes increased the rate of NADPH oxidation in a concentration dependent manner. In contrast MPP^+ (5-25 mM) did not alter the rate of NADPH oxidation.

The results suggest that paraquat and menadione can accept electrons from NADPH but that MPP^+ does not. The mechanism of neurotoxicity of MPP^+ appears to be different from that of paraquat.

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INVESTIGATION OF THE TRANSDUCTION MECHANISM FOR MUSCARINE-INDUCED CURRENTS IN AMPHIBIAN SYMPATHETIC NEURONES

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The transduction mechanisms involved in the generation of muscarinic outward currents in C-neurones and muscarinic inward currents in B-neurones were studied in Rana pipiens sympathetic ganglia using the whole cell patch clamp technique (see Selyanko et al., 1988 for methods and basis of identification of isolated B- and C-cells). Inclusion of the non-hydrolysable GTP analogue, GTPγS (50 μM) in the patch pipette prolonged both inward and outward currents induced by muscarine. Inclusion of the protein kinase C inhibitors, H-7, 1-(5-isoquinolinylsulphonyl)-2-methyl-piperazine, 50 μM) or gold sodium thiomalate (GST, 50 μM) failed to antagonize the two muscarinic currents. The muscarine-induced outward current in C-cells could not be mimicked by extracellular application of the protein kinase C activator, phorbol-12-myristate-13-acetate (PMA, 2-5 μM). This muscarinic outward current was attenuated, however, by PMA. The phorbol ester also produced an inward current as a result of M-current supression in both B- and C-cells and reduced the muscarine-induced inward current in B-cells. These effects were not antagonized by H-7 or GST suggesting that protein kinase C is neither involved in the production of outward and inward muscarinic currents nor is it involved in the effects of PMA. The inclusion of inositol triphosphate (IP_3, 0.5 mM), or high Ca^{2+} (approx. 1 μM; 4.4 mM EGTA:4 mM Ca^{2+}) in the patch pipette failed to mimic the inwardly rectifying K^+ current or the M-current supression produced by muscarine. Also, as mentioned in the accompanying abstract, (Selyanko et al., 1988) muscarinic responses were more readily recorded when cyclic AMP was included in the patch-pipette. It is concluded that although G-proteins may be involved in the transduction mechanisms for both responses, neither adenylate cyclase inhibition, nor the second messengers Ca^{2+}, IP_3 or diacylglycerol seem to be involved in the generation of muscarinic responses in amphibian sympathetic ganglion neurones. Furthermore, in terms of their sensitivity to drugs which affect second messenger systems, there were striking similarities between the outward current induced by muscarine and that induced by adrenaline. Ca^{2+}, PMA and IP_3 also failed to mimic the effect of adrenaline. It is therefore possible to suggest that the two agonists not only share the same potassium channels (Selyanko et al., 1988) but they may share the same transduction mechanism (see North, 1986).

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MUSCARINE- AND ADRENALINE-INDUCED CURRENTS IN PATCH-CLAMPED AMPHIBIAN SYMPATHETIC GANGLION NEURONES

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The effects of muscarine and adrenaline on neurones in Rana pipiens sympathetic ganglia were studied by the whole-cell patch clamp technique. Neurones were dissociated using trypsin (Sigma Type III, 3 mg/ml, 50 min, 37°C) followed by collagenase (Sigma Type Ia, 1 mg/ml, 25 min, 37°C). External solution contained (mM) NaCl, 117, KCl, 2, $MgCl_2$, 2, $CaCl_2$, 2, Hepes/NaOH pH 7.2, 5 and d-glucose, 10. Internal solution contained (mM) KCl, 110, NaCl, 10, $MgCl_2$, 2, $CaCl_2$, 0.4, EGTA, 4.4, Hepes/KOH pH 7.2, 5 and d-glucose 10. Drugs were applied using the "U-tube" technique (Krishtal & Pidoplitchko, 1980). The dissociated cells were categorized as "small" and "large." The "small" cells had a mean input capacitance (C_{in}) of 20.7±1.6pF (n=22, range 9-35pF) and lacked a fast, transient outward potassium current (A-current). The mean C_{in} of large cells was 33.3±9.4pF (n=16, range 20 to 56pF). Both cell types exhibited the outwardly rectifying M-current (Adams et al., 1982). Drug responses were more readily observed when 100 μM cyclic AMP ± 100 μM GTP was included in the patch pipette. The effects of 10 μM muscarine and 10-100 μM adrenaline were examined on 16 large cells. Muscarine supressed the M-current in all cells and thereby produced an inward current. A similar effect of adrenaline was seen in 5 of these 16 cells. In contrast, these agonists failed to affect the M-current in small cells but instead produced an outward current (with muscarine in 15 and adrenaline in 14 out of 22 small cells). This current displayed marked inward rectification and its reversal potential corresponded well with the potassium equilibrium potential. Luteinizing hormone releasing hormone (10 μM) supressed the M-current in all small and large cells tested. Since there are both small C-cells and larger B-cells in amphibian sympathetic ganglia (Dodd & Horn, 1983a) which exhibit hyperpolarizing and depolarizing responses to muscarine, respectively (Adams et al., 1982; Dodd & Horn, 1983b), it is concluded that the small cells are C-cells and the large cells are B-cells. Outward currents produced by muscarine and adrenaline were not additive (an occlusion effect). Also, both agonists produced more than 50% inhibition of the M-current, suggesting that some M-channels must be sensitive to both adrenaline and muscarine. These data indicate (i) that muscarine and adrenaline produce an outward current in C-cells or an inward current as a result of M-current supression in B-cells and (ii) that both agonists share the same potassium channels to generate either inward or outward currents.

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SINGLE PULSE-EVOKED RELEASE OF NEUROTRANSMITTERS FROM RABBIT BRAIN IS NOT SUBJECT TO AUTOINHIBITION

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The autoreceptor hypothesis predicts that autoreceptor antagonists should increase transmitter release only when the "biophase" concentration of endogenous transmitter is high enough to activate the autoreceptors. Hence, the antagonists should increase the release elicited by appropriate trains of pulses but not (or almost not) the release elicited by a single pulse (Starke, 1987).

Slices of rabbit brain cortex (NA) or caudate nucleus (DA and ACh) were preincubated with [3H]-NA, [3H]-DA or [3H]-choline and then superfused (see Limberger et al., 1986). Four stimulation periods were applied consisting either of single pulses (18 mA; 2 ms; Valenta et al., 1988) or of trains of 4 or 8 pulses at 1 Hz (S1-S4). The stimulation-evoked overflow of tritium was taken to reflect action potential-evoked transmitter release. Increasing concentrations of agonists or antagonists were added from S2 to S4. Experiments on [3H]-NA release were carried out in the presence of desipramine 1 μM. In single pulse experiments, the overflow of tritium evoked at S1 amounted to 0.19 % of the tissue tritium content. Yohimbine 0.01-1 μM did not change whereas clonidine 1-100 nM reduced this release. When the stimulation periods consisted of 4 pulses, the evoked tritium overflow at S1 also was 0.19 %. Yohimbine 0.01-1 μM now increased the release of [3H]-NA by maximally 253 %. Experiments on [3H]-DA release were carried out in the presence of nomifensine 1 μM. In single pulse experiments, the overflow of tritium evoked at S1 amounted to 0.40 %. Sulpiride 0.01-1 μM did not change whereas quinpirole 0.01-1 μM reduced this release. When the stimulation periods consisted of 4 pulses, the evoked overflow at S1 amounted to 0.50 %. Sulpiride 0.01-1 μM as well as domperidone 1-100 nM now increased the release of [3H]-DA by maximally 240 %. Experiments on [3H]-ACh release were carried out in the presence of physostigmine 1 μM. In single pulse experiments, the overflow of tritium evoked at S1 amounted to 0.18 %. Atropine 10 and 100 nM increased this release by 25 and 60 %. When the stimulation periods consisted of 8 pulses, the evoked overflow at S1 amounted to 0.35 %. Atropine 10 and 100 nM now increased the release of [3H]-ACh by 30 and 250 %. In the absence of physostigmine, atropine 1-100 nM increased neither the release of [3H]-ACh elicited by a single pulse nor the release elicited by trains of 8 pulses.

The results show that the release of NA and DA upon a single pulse is not subject to presynaptic autoinhibition. In contrast, marked autoinhibition develops during short trains of pulses. In the case of ACh, rapid breakdown prevents autoinhibition even during short pulse trains. Blockade of acetylcholine esterase allows accumulation of sufficient endogenous ACh to activate presynaptic autoreceptors.

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VARIATIONS IN SUSCEPTIBILITY OF SPONTANEOUS DEPOLARISING SHIFTS TO MAGNESIUM IN THE MOUSE NEOCORTICAL SLICE PREPARATION

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The mouse and rat neocortical slice preparations (Burton et al 1987; Harrison & Simmonds 1985) have been used in pharmacological studies of the responses of central neurones to the excitatory amino acids. Before these experiments can commence the slices must be bathed in Mg (1mM) containing artificial cerebrospinal fluid (ACSF) for a suitable period followed by a 30-60 minute period where the slice is bathed in Mg free ACSF, these ions being omitted since they suppress responses to N-methyl-D-aspartate (Harrison & Simmonds 1985). Here we have investigated the suppression of spontaneous depolarising shifts, commonly seen in the neocortical slice preparation, by changes in Mg concentration.

Male MF1 mice were killed by cervical dislocation, decapitated and the brain quickly removed into ice cold Mg containing ACSF. Coronal sections of neocortex (500μM thick) were prepared and from these tissue wedges (approximately 1mm wide) were taken and transferred to a two compartment bath perfused with Mg containing ACSF (2.5ml/min) at room temperature (24°C). The tissues were arranged such that the cortical tissue was contained almost entirely in one chamber of the bath and the ventral margin of the cortex passed through a greased slot so that the corpus callosum was entirely contained in the other compartment. Both compartments were perfused with Mg containing ACSF for the first hour after which the experimental ACSF was introduced. The DC potential between the two compartments was continously monitored via Ag/AgCl electrodes and a high impedance amplifier

If the cortical chamber was bathed in Mg containing ACSF, spontaneous depolarising shifts (DS) never occurred in up to 6 hours superfusion. If, however, after a suitable period the Mg containing ACSF was substituted by Mg free ACSF then spontaneous depolarising shifts with or without rhythmic after potentials occurred in about 60% of the tissue wedges, in the 30-60 minutes after the commencement of washout of Mg. These depolarising shifts gradually develop in amplitude and frequency over the next 60-120 minutes until they are fairly consistent with respect to both parameters. The Mg content of the cortical chamber was then gradually increased in discrete increments (200μM or 400μM) with each Mg concentration being allowed to equilibrate for 30-60 minutes. If the Mg content was raised in this way then DS could still be observed, albeit at a low frequency and amplitude, at Mg concentrations in excess of 2mM. When the high Mg concentration ACSF was washed out with Mg free ACSF, the DS reappeared over the Mg washout time (10-15 minutes) compared with 30-60 minutes needed for initiation of DS followed by the further period needed for full development.

These results suggest that a change in the properties of the neocortical slice occur during initial washout of Mg and subsequent development of DS discharges which alters the response of the slice to magnesium ions.

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PROTECTION BY PURINES AGAINST QUINOLINIC ACID NEUROTOXICITY

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Quinolinic acid is a selective excitant at the N-methyl-D-aspartate (NMDA) receptor (Stone & Burton, 1988). It is also a kainic acid like neurotoxin in the hippocampus producing a preferential degeneration of pyramidal cells. This selectivity is also shown by kainic acid but not by NMDA itself. In addition both kainate and quinolinate require intact afferent projections in order to manifest toxicity, again unlike NMDA and ibotenic acids. These observations suggest some presynaptic component in the toxic mechanism of quinolinate and kainate, but not NMDA or ibotenate.

Recently we have shown that quinolinate has a potent releasing action on glutamate and aspartate in the rat cerebral cortex in vivo (Connick & Stone, 1988). We have therefore attempted to modulate quinolinate toxicity by inhibiting the (presynaptic) release of endogenous excitatory acidic amino acids using purines such as R-N6-phenylisopropyladenosine (R-PIA). Such purines are known to inhibit glutamate release from the hippocampus (Dolphin & Archer, 1983; Corradetti et al 1984).

Male Wistar rats (200-250g) were anaesthetised with pentobarbitone (60mg/kg i.p.) and placed in a stereotaxic apparatus. Injections (1µl) of 120mM quinolinic acid, either alone or with R-PIA (100ng) were made into the dorsal hippocampus (2.5mm posterior to bregma, 2.5mm lateral to the central suture and 3mm down from the pial surface). The animals were allowed to recover for 4 days before histological examination of the brains. Sections surrounding the injected area were assessed (double blind) using an arbitrary scale of 0 to 10 (0= no damage; 10= total obliteration).

Quinolinic acid (120mM) consistently produced total obliteration of the hippocampus (10.0 ± 0.0, n=7). When R-PIA was coinjected with quinolinate, however clear protection was observed (3.5 ± 1.65 n=4 $p<0.001$). Damage was only observed to pyramidal cells in the CA1 and CA3 areas of the hippocampus. Some degree of protection against 100mM quinolinate lesions was observed with i.p. administration of 0.01mg/kg R-PIA, although increasing the dose of R-PIA did not cause a corresponding increase in the protection observed.

The results are consistent with the involvement of a presynaptic component in quinolinate toxicity. Work is now in progress to assess the potential for modulation of responses to NMDA and other neurotoxins by purines.

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TOXICITY OF DL αAMINOADIPATE TO STRIATAL CELLS *IN VITRO* AND *IN VIVO*

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The putative gliotoxin DL-α-aminoadipate (DLαAA) causes a transient depletion of glial cells *in vivo* (Olney *et al*, 1980), and *in vitro* (Huck *et al*, 1984), although Straffan and Crutcher (1987) recently reported a lack of effect towards hippocampal astrocytes *in vivo*. The aim of this study was to determine whether DLαAA was gliotoxic to striatal astrocytes either *in vitro* or *in vivo*, and whether the consequent alteration in glial cell function modified the ability of known neurotoxins to cause degeneration of striatal neurones.

Coronal slices of rat brain were prepared using a Campden Vibroslice. Each slice (0.5mm thick) was placed in an open superfusion chamber and perfused with oxygenated Krebs' Bicarbonate Medium at 30° for periods up to 3 hours. Inclusion of known neurotoxins in the incubation medium caused a time-and dose-dependent loss in the viability of striatal cells, as indicated by light microscopy of thionin-stained sections. For instance, widespread striatal degeneration was visible after 40 minutes incubation with 100μM kainate, whereas quinolinate and N-methyl-D-aspartate (NMDA) required a concentration of 500μM, and L-glutamate, 1mM. Cortical cells showed some resistance to these doses of kainate, quinolinate and NMDA, but not to L-glutamate. DL-2 Amino-5-phosphonovaleric acid (APV), 1mM, provided protection of striatal cells against both NMDA and quinolinate, but not kainate or glutamate. Inclusion of DLαAA (1-3mm) for periods of up to 1 hour caused no alteration in the morphology of striatal neurones, nor in glial glutamine synthetase (GS) activity. Increased doses of DLαAA coupled with longer incubation times led to widespread neuronal degeneration within the striatum. The high-affinity uptake of [^{3}H]L-glutamate into synaptosomes was significantly reduced. Co-incubation of DLαAA (1-3mM) with NMDA (500μM) or quinolinate (500μM) provided complete protection of striatal neurones against degeneration by these compounds which, in the absence of any observed alteration in GS activity by DLαAA, is probably due to a direct antagonism of amino acid receptors. However, pre-incubation of slices with either APV or DLαAA followed by a 10-minute washout before addition of quinolinate (500μM) reduced the ability of APV, but not DLαAA, to protect striatal neurones from degeneration.

For the *in vivo* studies, DLαAA (100μg/2μl) was injected directly into the striatum of anaesthetised rats. Six hours after the injection there was a significant reduction in striatal GS activity in the ipsilateral, compared to the contralateral striatum (1.22±.06 (7) and 1.67±.10(7) nmolglutamine/mg protein/hr, respectively). The neurones within the striatum all appeared quite normal, but there was a notable absence of glial proliferation around the site of injection. By 24 hours after DLαAA, GS activity had returned to normal. Thus, direct injections of DLαAA into the striatum yielded changes in the glial-associated enzyme, without any alteration in neuronal morphology. Preliminary evidence from slices prepared from previously-injected striata reveals that the ability of neurones to survive incubation *in vitro* is not attenuated, and that the toxicity of kainate in these slices is reduced.

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THE EFFECTS OF MK-801 UPON LOCAL CEREBRAL GLUCOSE UTILISATION IN CONSCIOUS AND IN HALOTHANE-ANAESTHETISED RATS

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MK-801 is a highly potent and selective non-competitive N-methyl-D-aspartate(NMDA) receptor antagonist (Wong et al. 1986). MK-801 induces heterogenous changes in local cerebral glucose use in conscious rats, most notably in the limbic system and in neocortex (Kurumaji et al. 1988). General anaesthesia remains a necessary feature of many in vivo electrophysiological investigations despite profound changes in synaptic transmission and metabolic process which anaesthesia effects. We examined the influence of halothane anaesthesia on the alterations in function related glucose use which are associated with MK-801 administration.

The experiments were carried out on 20 male Sprague Dawley rats (300-500g). Rats in the conscious group were fitted with a loose plaster cast around the pelvis and lower abdomen. Animals in the halothane-anaesthetised group were mechanically ventilated with a gaseous mixture (0.5% halothane in 70% N_2O/30% O_2) to yield arterial carbon dioxide tension 35-40mmHg . The measurements of local cerebral glucose utilisation were initiated 10 mins. after the administration of either MK-801 (0.5mg/kg, i.v.) or saline, and conducted in a manner similar to the [^{14}C] 2-deoxyglucose technique described previously (Sokoloff et al. 1977).

The intravenous administration of MK-801 in conscious animals results in increased glucose use in the limbic system and in decreases in the cerebral cortices. In halothane-anaesthetised rats, the administration of MK-801 failed to change the rate of glucose use in the hippocampal region. In the posterior cingulate cortex, the pattern of response to MK-801 was reversed with halothane anaesthesia. In the cerebral cortices, the reductions in glucose use following MK-801 in anaesthetised animals (approx. 15%) were less marked compared to those in conscious animals (approx. 35%).

Table 1 Effects of MK-801 (0.5mg/kg) upon Cerebral Glucose Utilisation

	Conscious		Halothane-Anaesthetised	
	Saline (n=4)	MK-801 (n=5)	Saline (n=5)	MK-801 (n=6)
Limbic System				
Hippocampus Mol. Layer	74 ± 2	94 ± 2*	96 ± 3	94 ± 7
Entorhinal Cortex	55 ± 3	98 ± 4*	92 ± 6	84 ± 8
Post. Cingulate Cortex	97 ± 6	125 ± 7*	99 ± 11	64 ± 7*
Cerebral Cortex (Layer IV)				
Auditory Cortex	133 ± 6	85 ± 7*	97 ± 4	79 ± 4*
Sensory-Motor Cortex	97 ± 3	65 ± 5*	72 ± 2	62 ± 2*

Data are presented as umol.100g^{-1} min^{-1} (Mean ± SEM) * $P < 0.05$ for the comparison between saline-treated and MK-801-treated animals (Student's t-test).

The functional consequences in vivo of blockade of NMDA receptors were remarkably modified by the halothane anaesthesia. The divergent responses to MK-801 emphasises the difficulties in the extrapolation of data from anaesthetised rats to conscious rats.

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COMPOUNDS ACTIVE AT PCP BINDING SITES INHIBIT NMDA-STIMULATED GUANYLATE CYCLASE ACTIVITY

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Receptors for excitatory amino acids (EAAs) can be divided into three classes for which NMDA, quisqualate and kainate are potent and selective agonists. The best characterised subtype is the NMDA-preferring receptor. This subtype is the only one for which selective antagonists have been described. It has been demonstratated recently that compounds active at phencyclidine (PCP) binding sites are potent non-competitive blockers of NMDA receptors (Lodge, D., 1987). We have studied the effects of such compounds on the synthesis of cGMP induced by NMDA and other EAAs in cerebellar slices.

Cerebellar slices were prepared by hand from 8-day old rats. The slices were preincubated in Krebs-HCO_3 buffer at 37°C. After 120 min, the EAAs were added for a further 4 min. The studied antagonists were added 3 min before the EAA. The reaction was terminated by replacement of the medium with 2 ml 95% ethanol. Cyclic GMP was measured by RIA (NEN kit).

cGMP levels (basal 7.8 $\pm$ 0.4 pmol/mg prot) could be increased dramatically by NMDA (10^{-4} M: 121.4 $\pm$ 5.2 pmol/mg prot) and also by glutamic acid (10^{-3} M: 61.0 $\pm$ 10.8 pmol/mg prot) and by kainic acid (10^{-3} M: 35.8 $\pm$ 4.8 pmol/mg prot). Quisqualic acid was inactive. These findings are in agreement with previously published results (Garthwaite, 1982). The synthesis of cGMP produced by NMDA was antagonised by phencyclidine and other compounds active at the PCP binding site. MK 801 was the most active (Table 1).

	phencyclidine	MK 801	ketamine	NANM	APV	DαAA
NMDA 10^{-4}M	6.54 ± 0.27	7.7 ± 0.13	5.52 ± 0.1	5.96 ± 0.26	5.19 ± 0.05	3.64 ± 0.2
Glutamate 10^{-3}M	6.26 ± 0.15	7.64 ± 0.1	5.12 ± 0.3	5.9 ± 0.22	3.00 ± 0.11	2.97 ± 0.03

Table 1: Activities of NMDA antagonists. The data are represented as the pIC50 values $\pm$ SEM obtained from linear regression analysis of inhibition curves (n = 5).

The glutamate response could also be inhibited by PCP ligands with similar IC50s. This was in contrast to the results obtained with competitive NMDA antagonists like 2APV and DαAA to which the glutamate response was less sensitive than the NMDA response. The synthesis of cGMP induced by kainate was blocked by both competitive (APV) and non-competitive (phencyclidine) NMDA antagonists.

These results support previous data showing that activation of NMDA receptors stimulates guanylate cyclase, and imply that the effects of glutamic and kainic acids are also mediated by an NMDA receptor. The blockade of NMDA receptors by PCP ligands seen in electrophysiological studies can also be observed in this biochemical model.

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SOME EFFECTS OF AMITRIPTYLINE AND IDAZOXAN ON ADRENOCEPTOR NUMBER IN RABBIT BRAIN

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The tricyclic antidepressants are widely employed in the treatment of depression, but their mode of action remains controversial (Charney et al, 1981). Changes in adrenoceptor number in rat brain after antidepressant treatment have been reported with consistent down regulation of beta adrenoceptors, but up- or down-regulation or no changes in alpha receptor number have been observed (Charney et al, 1981). In addition, coadministration of alpha$_2$-adrenoceptor blockers with antidepressants has been reported to increase the rate and magnitude of the decrease in beta adrenoceptor number (Johnson et al, 1980; Wiech et al, 1980).

The effects of treatment with amitriptyline (A) (30 mg/kg/day intraperitoneal) and idazoxan (I) (4.9 mg/kg/day intravenous) alone or in combination on alpha$_2$ and beta-adrenoceptor numbers in the rabbit brain were studied. Rabbits (n=6-12) received vehicle (V) for 6 or 21 days (d), A for 21 d or I alone or in combination with A for 6 d. At the end of the experiment animals were killed, brain membranes removed and dissected into fore and hindbrain. The tissues were prepared by centrifugation and homogenisation and membranes incubated with [^{3}H] yohimbine (alpha$_2$ ligand) or [^{3}H] dihydroalprenolol (beta ligand) for 25 mins at 25^{o}C in final volume of 1 ml.

In the hindbrain, a significant reduction in [^{3}H] yohimbine binding was observed after 21 days A (Table). However, neither A + I nor I alone had any effect on binding. No significant changes in [^{3}H] yohimbine binding were observed in the forebrain with any of the treatments. Neither A nor I alone or in combination altered [^{3}H] dihydroalprenolol binding in either fore or hindbrain. There were no treatment effects on K_D.

TABLE

	[^{3}H] yohimbine				[^{3}H] dihydroalprenolol			
	Forebrain		Hindbrain		Forebrain		Hindbrain	
	Bmax	K_D	Bmax	K_D	Bmax	K_D	Bmax	K_D
V 21d	124±16	8±1	98±22	8±3	31±17	1.2±0.9	51±23	0.7±0.5
A 21d	116±44	12±4	45±11*	7±2	26±10	0.9±0.6	45±16	0.8±0.5
V 6d	110±47	9±3	109±47	12±4	35±18	1.9±1.0	51±13	0.7±0.3
I 6d	134±57	10±5	125±38	12±4	32±14	2.0±1.3	50±19	1.0±0.6
A+I 6d	95±33	8±4	91±26	9±2	25±11	1.6±1.1	50±19	1.0±0.5

Results are mean ± SD, Bmax fmoles/mg protein, KD nM
* $P < 0.001$, using t-test or ANOVA as appropriate.

Thus chronic amitriptyline treatment may modify alpha$_2$-adrenoceptor number in rabbit brain but, in contrast to reports in rat (Scott et al, 1983; Wiech et al, 1980) we have no evidence for down-regulation of beta adrenoceptors. Changes may occur in rabbit brain in localised areas or with longer treatment or higher doses. These results, however, emphasise the danger of generalising findings with receptor binding across species and tissues.

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ANTIDEPRESSANT DRUGS, INCLUDING SIBUTRAMINE HCl, AND ECS DO NOT ALTER α_1-ADRENOCEPTOR BINDING IN RAT CORTICAL MEMBRANES

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Although administration of antidepressants and electroconvulsive shock (ECS) is consistently reported to down-regulate cortical β-adrenoceptor binding or function (Sulser and Mobley 1981), their effects on α_1-adrenoceptors is controversial. For instance, Stockmeier et al (1987) reported that antidepressant treatment did not increase cortical α_1-adrenoceptors in contrast to Maj et al (1985). We now report that neither ECS nor antidepressants alter [^{3}H]-prazosin binding to rat cortical α_1-adrenoceptors.

Adult male CD rats (100-125 g) were used. Drugs were given i.p. once daily for 1, 3 or 10 days; sibutramine HCl (BTS 54 524, 3 mg/kg), desipramine (DMI, 10 mg/kg) pargyline (10 mg/kg), amitryptyline (10 mg/kg) or water (1 ml/kg). In addition, halothane anaesthetised rats received a single ECS (200V, 2s) or 5 ECS over 10 days. Controls were given halothane. 24h after the final treatment corticies were removed, and stored at -80°C. Six-point saturation binding analyses were performed on membranes from individual rats using [^{3}H]-prazosin (0.1 - 5.0 nM). Specific binding was defined by 5 μM phentolamine. Correlation for Scatchard plots was $r \geqslant 0.95$.

Neither acute nor chronic antidepressant drugs or ECS significantly altered the number of α_1-adrenoceptors (Table 1). None of the treatments altered the Kd value for [^{3}H]-prazosin binding (control = 0.5 ± 0.02 nM, n = 38).

Table 1

Treatment	Bmax (fmol/mg protein)		
	1 day	3 days	10 days
Control	257 ± 19 (8)	250 ± 9 (12)	279 ± 14 (18)
Sibutramine HCl	256 ± 15 (8)	256 ± 10 (15)	281 ± 16 (14)
Desipramine	249 ± 8 (8)	264 ± 8 (15)	294 ± 11 (17)
Pargyline	257 ± 9 (8)	270 ± 13 (14)	291 ± 12 (18)
Amitryptyline	283 ± 13 (8)	292 ± 11 (15)	323 ± 13 (17)
Halothane	240 ± 6 (10)		292 ± 12 (16)
ECS	229 ± 7 (10)		325 ± 15 (17)

Results are Bmax ± s.e. mean (n).

The above data contradict Maj et al (1985), but agree with other reports that antidepressant administration does not alter α_1-adrenoceptor number (Stockmeier et al, 1987) or function (Heal, 1984; Kendall and Nahorski, 1987). Thus our results do not support the view that changes in α_1-adrenoceptors in experimental animals reflect the clinical efficacy of antidepressant treatments.

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LESIONING OF CENTRAL 5-HT NEURONES DOES NOT PREVENT THE ATTENUATION OF CLONIDINE SEDATION IN MICE BY REPEATED ECS

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Hypoactivity (a distinct form of sedation) is induced in rodents by low doses of clonidine and this α_2-adrenoceptor mediated response is attenuated by repeated administration of electroconvulsive shock (ECS) (Heal et al, 1981). It has recently been shown that the decrease in rat cortical β-adrenoceptors induced by ECS treatment was prevented by destruction of central 5-HT neurones (Nimgaonkar et al, 1985). We have, therefore, determined whether intact 5-HT neurotransmission was also essential for the attenuation of α_2-adrenoceptor function by ECS administration.

Male C57/Bl/60la mice (25-30g) were pretreated with desipramine (5 mg/kg i.p.) and were then given an intracerebroventricular injection of 5,7-dihydroxy-tryptamine (5,7-DHT; 75 µg) or saline-ascorbate vehicle (4 µl), as described by Heal and Philpot (1987). After 2 weeks, head-twitches to 5-methoxy-N,N-dimethyl-tryptamine (2 mg/kg i.p.) were taken as an in vivo index of 5-HT denervation (head-twitches in 6 min ± s.e. mean: 5,7-DHT lesioned = 20.4 ± 0.9, n = 23; sham lesioned = 8.2 ± 0.5, n = 30; $P<0.01$). Subsequent HPLC analysis showed a mean 5-HT depletion of 65% ($P<0.001$) in the brains of 5,7-DHT treated mice. Groups of 5,7-DHT and sham lesioned mice were given either an ECS (200V, 2s) under halothane, or halothane alone, 5 times over 10 days. Hypoactivity responses to clonidine (0.1 mg/kg) were measured on 5 behavioural parameters (passivity, tactile responsiveness, posture, gait and body sag) (Heal et al, 1981). Hypoactivity responses were higher in 5,7-DHT lesioned mice (total score in 60 min ± s.e. mean: 5,7-DHT lesioned = 30.5 ± 0.7, n = 23; sham lesioned = 24.8 ± 0.8, n = 29; $P<0.01$) as previously reported by Heal and Philpot (1987). Repeated ECS attenuated clonidine hypoactivity in the 5,7-DHT and sham lesioned mice by 26% ($P<0.01$) and 36% ($P<0.01$), respectively. Therefore, although 5-HT has a permissive role in the down-regulation of β-adrenoceptors by ECS (Nimgaonkar et al, 1985), intact 5-HT neurotransmission does not appear to be a prerequisite for the attenuation of α_2-adrenoceptor function. However, it should be noted that mice were used in this study, while Nimgaonkar et al (1985) employed rats and there may be neuroanatomical differences between species. Although the depletion of 5-HT was incomplete in this study, it was sufficient to directly influence clonidine mediated responses, as reported by Heal and Philpot (1987), and markedly enhance 5-HT_2 mediated head-twitches.

The pharmacological mechanisms underlying the reduction of α_2- and β-adrenoceptor function by ECS are unclear at present, but these probably do not involve the inhibition of noradrenaline (or 5-HT) reuptake (Minchin et al, 1983) or alterations in noradrenaline turnover (Nimgaonkar et al, 1986). However, the present results suggest that these mechanisms are probably not identical, because the former is independent of intact 5-HT neurotransmission, while the latter is not.

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GM-1 GANGLIOSIDE TREATMENT OF RATS WITH A UNILATERAL 6-HYDROXY-DOPAMINE-INDUCED NIGROSTRIATAL LESION

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A reduction in apomorphine-induced circling occurs following GM-1 ganglioside treatment of rats with unilateral diencephalic hemitransection (Agnati et al, 1983) or unilateral electrolytic lesion of substantia nigra (Jackson et al, 1987), suggesting recovery of dopaminergic function. Such lesions do not selectively damage dopamine neurons so we have investigated the effects of repeated GM-1 treatment on forebrain dopamine function in rats with a unilateral 6-hydroxy-dopamine (6-OHDA)-induced lesion of the nigrostriatal dopamine system.

Male Sprague-Dawley rats (330-350 g) received 6-OHDA (8 ug/2 ul) lesions of the caudate nucleus (A 0.8; L 3.5; V 6.0 mm) (Pellegrino et al, 1979). Rats were treated with either GM-1 (20 mg/kg i.p.) or vehicle for 3 days prior to surgery and for 35-36 days post-operatively. Rotational responses to apomorphine (1.0 mg/kg s.c.) were measured up to 30/31 days post-operatively. Animals were killed between days 36-37 post-operatively for histological and biochemical analysis.

Table 1 Effect of GM-1 treatment on forebrain dopamine function

Treatment	Side of brain	Total turns after apomorphine		Striatal ^{3}H-dopamine uptake (pmol/mg/hr)	Accumbens dopamine (pmol/mg)
		Day 14/15	Day 30/31	Day 36/37	Day 36/37
GM-1 (20 mg/kg i.p)	Intact	386 ± 51**	439 ± 65	401 ± 55	104 ± 10
	Lesioned			84 ± 19*	49 ± 4*
Saline (1 ml/kg i.p)	Intact	545 ± 58	568 ± 74	396 ± 58	107 ± 11
	Lesioned			128 ± 27*	69 ± 7*

* $p < 0.05$ (intact compared with lesioned side); mean ± S.E.M.; n = 11-15
** $p < 0.05$ (GM-1 treatment compared with saline); mean ± S.E.M.; n = 17-22

Rats treated with GM-1 showed reduced contraversive circling in response to apomorphine (1.0 mg/kg s.c.) between 3 and 22 days post-operatively ($p < 0.05$, repeated measures ANOVA) but this was no longer evident at day 30/31 ($p > 0.05$). Lesion-induced changes in striatal ^{3}H-dopamine uptake or accumbens levels of dopamine, DOPAC, HVA and noradrenaline were unaltered by GM-1 treatment compared to saline treated control animals ($p > 0.05$, two way ANOVAs). Lesion-induced losses of midbrain dopamine (TH-positive) cell bodies at 36-37 days post-operatively, in GM-1 and saline-treated rats respectively, were: A9, 11 and 8% of intact side; A10, 95 and 98%; A8, 50 and 53%. GM-1 treatment did not cause any preservation of dopamine cell bodies.

GM-1 ganglioside treatment caused early post-operative reduction of apomorphine-induced contraversive circling, but by days 30-37 post-operatively there were no behavioural, biochemical or immunocytochemical indicators of GM-1-induced dopaminergic recovery. GM-1 treatment may delay secondary degeneration or enhance the initial rate of spontaneous recovery following a 6-OHDA lesion.

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CHANGES IN STIMULATED DOPAMINE EFFLUX AND UPTAKE IN THE RAT BASAL GANGLIA DURING SENESCENCE: IN VIVO VOLTAMMETRIC DATA

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Although motor deficits are often apparent in senescent rats (Marshall and Altar, 1986), biochemical studies have sometimes failed to reveal any decrease in striatal dopamine (DA) content or synthesis (Watanabe, 1987). However, tissue levels and synthesis do not necessarily reflect the functional release of neurotransmitters. We therefore measured stimulated DA efflux into the extracellular fluid (ECF) as a more direct index of DA function.

Experiments were conducted, under chloral hydrate anaesthesia, in two groups of male Wistar rats : old (mean age: 25.1±0.8 months) and young adults (3.8±0.1 months). Carbon fibre voltammetric electrodes were implanted into the caudate (CPu) and nucleus accumbens (Acb) while a bipolar stimulating electrode was located in the MFB. Reference (Ag/AgCl) and auxiliary electrodes were placed on the skull. Fast cyclic voltammetry (Stamford et al, 1986) was used to measure the efflux and uptake of DA in CPu and Acb in response to electrical stimulation of the MFB (50Hz, 200μA r.m.s.). Two different stimulus train lengths (2s and 10s) were used to investigate, respectively, the rate of DA efflux and the size of the releasable DA pool. The results are shown in the Table.

	Caudate nucleus		Nucleus accumbens	
	Adult	Old	Adult	Old
Peak DA efflux per 2s stim (μM)	9.93±1.72	2.22±0.81	4.48±0.65	2.94±0.54
(P)	(0.002)		(0.17)	
Peak DA efflux per 10s stim (μM)	30.46±2.71	13.00±2.69	25.03±2.97	11.95±1.76
(P)	(0.002)		(0.004)	
DA uptake rate (μM/s)	3.28±0.38	2.04±0.44	2.44±0.32	1.56±0.23
(P)	(0.072)		(0.074)	
DA uptake/efflux ($\frac{\mu M/s \times 100}{\mu M \text{ peak}}$)	10.68±0.96	15.51±1.42	9.66±0.35	13.42±1.50
(P)	(0.014)		(0.052)	

All values are means ± s.e.m. (n = 5-8). P values (adult v old) calculated by Mann Whitney U test.

The clearer effects were observed in CPu. Peak DA efflux (2s train) was significantly reduced in old rats in CPu but not in Acb. Peak DA efflux on 10s stimulations was decreased in both CPu and Acb of the aged group. The rate of DA uptake showed much variation. The apparent decline with age was not significant in either CPu or Acb. However the rate of uptake in relation to DA efflux was elevated in the CPu of the old rats. A similar, though not quite significant, trend was observed in Acb.

In conclusion, the data indicate that the rate of DA efflux and the size of the releasable DA pool are significantly reduced in the CPu of aged rats. The data also show that DA uptake is proportionally more dominant during aging. These results are consistent with motor deterioration during senescence.

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EFFECTS OF SELECTIVE 5-HT RECEPTOR ANTAGONISTS ON THE ANORECTIC EFFECT OF (+)-FENFLURAMINE

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The recognition of multiple receptor subtypes for serotonin (5-HT) (Bradley et al., 1986) raises the possibility of one or more 5-HT receptor subtypes in the anorectic effects of several compounds, including (+)-fenfluramine. The aim of the present series of experiments was to attempt to antagonize the reduction of palatable food consumption in non-deprived rats produced by 3.0 mg/kg i.p. of (+)- fenfluramine. A series of 5-HT receptor antagonists was tested.

Male blackhooded rats housed under standard laboratory conditions were familiarized with a palatable sweet mash made up as follows: 50 ml sweetened condensed milk, 150 ml ground rat maintenance diet, and 200 ml distilled water. Drugs were administered either i.p. (metergoline and xylamidine) or s.c. (all other antagonists) 30 min prior to (+)- fenfluramine, which was itself injected i.p. 30 min before the 30 min test of palatable food consumption. One exception was that xylamidine was injected 2.5h before (+)- fenfluramine. Metergoline was ultrasonically dispersed in 0.3% Tween, cyanopindolol was dissolved in 2-3 drops of glacial acetic acid and made up to volume with 0.9% saline, ritanserin was dissolved in a vehicle containing 4-5 drops of lactic acid in 20% propylene glycol (pH5). All other drugs were dissolved in 0.9% saline. At least 6 animals were tested at each antagonist dose. Data were analysed by ANOVA for independent groups and Dunnett's t test.

Table 1. Effect of 5-HT receptor antagonists on (+)- fenfluramine-induced anorexia

Antagonist	Dose range (mg/kg)	Minimum effective[a] dose (mg/kg)	5-HT receptor[b] selectivity
Metergoline	0.3-3.0	0.3	$5\text{-HT}_1/5\text{-HT}_2$
Methiothepin	0.03-0.3	0.1	$5\text{-HT}_1/5\text{-HT}_2$
Cyanopindolol	0.3-3.0	3.0	$5\text{-HT}_{1A}/5\text{-HT}_{1B}$
Ritanserin	0.3-3.0	0.3	5-HT_2
Mianserin	0.3-10.0	no effect	$5\text{-HT}_{1C}/5\text{-HT}_2$
ICS 205930	0.3-3.0	no effect	5-HT_3
Xylamidine	1.0-10.0	no effect	peripheral 5-HT

[a] To attenuate (+)-fenfluramine anorexia ($p < 0.05$, Dunnett's).
[b] From Hoyer (1988) and Fuller et al., (1988)

Metergoline, methiothepin, ritanserin and cyanopindolol antagonized (+)-fenfluramine-induced anorexia, whereas mianserin, xylamidine and ICS 205 930 did not. The results indicate that both 5-HT_1 and 5-HT_2 receptor subtypes may be involved in the anorectic effect. They are consistent with a recent report that ketanserin reversed the anorectic effect of (+)-fenfluramine (Hewson et al., 1988), but emphasize that (+)-fenfluramine's effect on food intake does not depend exclusively on activation of 5-HT_2 receptors.

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EFFECTS OF 5-HT_{1A} RECEPTOR LIGANDS ON EXPLORATORY BEHAVIOUR IN SOCIALLY ISOLATED RATS

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Recent experimental studies have suggested that drugs acting at the 5-HT_{1A} receptor may be of benefit in the treatment of anxiety (Traber & Glasser, 1987). As benzodiazepine anxiolytics such as chlordiazepoxide have been shown to reverse the deficit in exploration seen in socially isolated rats (Morinan & Parker, 1986), it was decided to investigate the effects of some 5-HT_{1A} receptor ligands on this behaviour. In this study, the partial agonist buspirone, and the agonists 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) and dipropyl-5-carboxamidotryptamine (DP-5CT) were used.

Immediately after weaning, male Wistar rats were isolated for 21 days. On the morning of day 22, isolates were given a s.c. injection of saline (controls), buspirone (0.5-2.0 mg/kg), 8-OHDPAT (0.05-0.1 mg/kg), or DP-5CT (0.1-0.5 mg/kg). Thirty minutes later each animal was given a 5 min trial in the elevated X-maze, and the number of rears, arm entries and locomotor activity counts recorded.

None of the drugs tested increased exploration in the X-maze, although at the higher doses, both buspirone and DP-5CT significantly reduced exploratory behaviour (Table 1).

Table 1 Exploration in the elevated X-maze after drug treatment

	BUSPIRONE			8-OHDPAT		DP-5CT		
Dose(mg/kg)	0.5	1.0	2.0	0.05	0.1	0.1	0.2	0.5
Rears	84	45*	12*	78	61	100	103	69
Entries	108	52*	59	109	113	109	124	60
Activity	96	43*	26*	80	86	104	109	49*

Values represent the means (n=6-8) expressed as a % of the controls (= 100%). The control values (mean ± s.e.m., n=43) were 10.7 ± 0.8 for rears, 3.6 ± 0.4 for entries, and 368 ± 38 for activity. *$P < 0.05$ compared to controls.

In similar studies to this one, where exploration is used to assess anxiety, neither buspirone nor 8-OHDPAT have anxiolytic activity (File et al, 1987; Moser et al, 1988). However, in other tests buspirone has been shown to have a significant anxiolytic effect (Guy & Gardner, 1985; Kehne et al, 1988; Moser et al 1988). Therefore, the anxiolytic activity of 5-HT_{1A} receptor ligands may be related to the type of test procedure employed, and in the maze exploration used in this study, these drugs appear to be inactive.

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SEROTONERGIC INHIBITION OF AUDITORY EVOKED RESPONSES RECORDED IN THE RAT HIPPOCAMPUS

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Sensory evoked responses recorded in the rat hippocampus have been reported to reflect activity in afferent neuronal pathways which were relatively modality non-specific (Brankack & Buzsaki, 1986; Deadwyler et al., 1981). Previous work has shown that a middle latency component of the auditory evoked response in rats implanted with epidural electrodes can be inhibited by systemic administration of the 5-HT precursor 5-hydroxytryptophan (Concu et al., 1978), apparently by activation of 5-HT1 receptors (O'Connor et al., 1987). Since 5-HT exerts inhibitory effects on electrically evoked field potentials in the hippocampus which are mimicked by buspirone (Rowan & Anwyl, 1986) the present study compared their effects on auditory evoked responses recorded from the hippocampus of alert rats by means of depth electrodes. The effect of the 5-HT1 receptor agonist RU24969 (5-methoxy-3(1,2,3,6-tetrahydro-4-pyridimyl-H-indole, Green et al., 1984) was also examined.

Rats (200-250g) were anaesthetised with pentobarbitone (60 mg/kg i.p.) prior to surgical procedures. Stainless steel wire electrodes attached to a cannula were placed in the stratum radiatum of the CA1 region of the hippocampus for depth recordings of auditory evoked responses. Animals were allowed at least 5 days recovery before recordings were taken. Acoustic clicks (86 dB SPL) were presented binaurally and the average of 64 responses was taken. Typical auditory evoked responses contained a negative peak at 18 ms followed by a positive peak at 29 ms and a second negative peak at 54 ms. The location of the electrodes and cannula was confirmed using histological techniques.

Local injection of 5-HT (10 µg in 0.5 µl distilled water) directly into the hippocampus reduced the amplitude of the positive and second negative peaks significantly at 5 mins (to 53±11% and 67±12% control respectively, mean±s.e. mean, $P < 0.05$). There was also a significant latency increase in these two peaks at this time by 3.1±0.8 ms and 9.2±3.9 ms respectively, $n = 5$; $P < 0.05$). Intrahippocampal application of 1 µg of buspirone mimicked these effects, for example, it produced a reduction in the amplitude of the positive peak of the auditory evoked response (to 68±10% control at 5 min, $n = 4$; $P < 0.05$). Injection of 3 mg/kg i.p. of this compound also produced inhibitory effects (to 64±15% control for the positive component at 30 min, $n = 4$; $P < 0.05$). Similarly RU24969 (1 mg/kg, s.c.) reduced the amplitude of this peak to 57±18% ($n = 3$, $P < 0.05$). None of the compounds affected the early negative peak.

The present data provide direct evidence for a serotonergic modulation of the later but not the early components of auditory evoked responses recorded in the hippocampus. The finding that buspirone and RU24969, two compounds known to stimulate 5-HT1 receptors, mimic these effects is consistent with the view that 5-HT1 receptors may be involved.

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BUSPIRONE REDUCES PERFORMANCE OF PASSIVE AVOIDANCE AND SPATIAL LEARNING TASKS IN THE RAT

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Buspirone is an anxiolytic drug which has a high affinity for 5-HT1A binding sites (Peroutka, 1985) and which mimics the inhibitory effect of 5-HT on neuronal firing apparently via 5-HT1A receptors (Rowan & Anwyl, 1986) in the rat hippocampus. Both anxiolytics and 5-HT1 receptor activation have been reported to impair passive avoidance (Gray, 1982; Ogren, 1985) and spatial (McNaughton & Morris, 1987; Hunter et al., 1987) learning. Since performance of these tasks is considered to be dependent on hippocampal function, we have studied the effect of buspirone on passive avoidance learning in a Light-Dark Box and spatial learning in the Morris Water Maze.

The doses of buspirone chosen for these studies (1-2 mg/kg i.p.) were found not to affect the activity of the experimental animals (male Wistar rats,200g) in a novel hole-board apparatus over a 180s period or during the first 180s of exposure to a two-compartment Light-Dark Box.

Passive avoidance learning was studied by measuring the step-through latency 24 hr after receiving a mild(0.5 mA, 5s) uncontrollable shock in the dark compartment of the Light-Dark Box (Ogren, 1985). This latency was found to be significantly ($P < 0.05$) shorter for the buspirone (1 mg/kg) group (95s, median, n = 12) when injected prior to both training and test sessions than for the saline treated group (300s, n = 10). A similar decrease in latency (63s, n = 6) was obtained when buspirone (1 mg/kg) was given prior to the test session only,but not when injected before the training session (300s, n = 5).

Spatial learning was examined using a test (McNaughton & Morris, 1987) where the latency to find the location of a hidden platform in a circular water maze was observed over a period of 4 days during which each animal had 13 training trials. Whereas 1 mg/kg buspirone had no apparent effect, the latency to find the platform on the 5th, 9th and 13th trials was increased from 43±11, 26±16 and 10±2s (mean ± s.e. mean, n = 12) respectively for saline controls to 105±21, 64±15 , and 34±8s respectively for buspirone (2 mg/kg, n = 12) treated rats. On transfer testing on the fourth day, quadrant bias (23±1s per min, n = 12 for controls compared with 16±1 per min, n = 12 for the buspirone group) and annulus crossings (3.6±0.5, n = 12, for controls compared with 1.6±0.5, n = 12 for the buspirone group) were both found to be significantly ($P < 0.05$) affected. These doses of buspirone were without effect when given only prior to the transfer test and not during the acquisition stage (n = 6 for each dose).

Buspirone at apparently non-sedative doses impaired both passive avoidance learning and spatial learning. The results indicate that a relatively low dose of buspirone affects the response to an aversive stimulus whereas at a higher dose the acquisition of spatial memories is altered.

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THE EFFECTS OF CLOMIPRAMINE AND FLUOXETINE ON THE 5-HTP-INDUCED HEAD TWITCH IN THE MOUSE

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The administration of the 5HT precursor 5-hydroxytryptophan (5HTP) causes head twitches in the mouse due to elevated central 5HT levels, the response being antagonised by the 5HT antagonist methysergide (Corne et al, 1963). More recent studies have indicated that central $5HT_2$ receptors are important in the head twitch syndrome (Lucki & Minugh-Purvis, 1987). This model has been used to investigate central activity of antidepressants (Goodwin et al, 1984). In this study the effects of two antidepressants known to block neuronal uptake of 5HT were monitored, clomipramine and fluoxetine.

Male albino mice 25 - 30g were dosed intraperitoneally (i.p.) with 5HTP, 300mgkg^{-1}, this being found to be the optimal dose to cause head twitching. The antidepressant drugs were administered 30 minutes prior to the 5HTP, also by i.p. injection. All drugs were dissolved in 0.9% saline. Head twitches were counted over a ten minute period from 35 - 45 minutes after administration of 5HTP.

Clomipramine was found to inhibit 5HTP-induced head twitches in a dose-dependent manner. In contrast, fluoxetine was found to cause a dose-dependent increase in the number of twitches (Table 1.).

Table 1. The effects of clomipramine and fluoxetine on head twitch No.

Drug treatment	no. of twitches in 10 mins ± sem.
5HTP 300mgkg^{-1}	42.3 ± 6.4
5HTP 300mgkg^{-1} + clomipramine 5mgkg^{-1}	22.5 ± 4.2
5HTP 300mgkg^{-1} + clomipramine 10mgkg^{-1}	10.5 ± 3.1**
5HTP 300mgkg^{-1} + fluoxetine 5mgkg^{-1}	46.1 ± 5.8
5HTP 300mgkg^{-1} + fluoxetine 20mgkg^{-1}	64.3 ± 6.9**

n = 10 p 0.01 significantly different from 5HTP alone **

Although both drugs have similar effects on 5HT reuptake these results indicate differences in the central effects of these drugs on seratoninergic mechanisms. Fluoxetine is a potent inhibitor of 5HT uptake but has no effect on central 5HT receptors (Hall et al, 1984). Clomipramine also inhibits 5HT uptake but also blocks central $5HT_2$ receptors (Tang & Seeman, 1980). As both drugs increase 5HT levels at the synaptic cleft both would be expected to enhance 5HTP-induced head twitches. This study shows that clomipramine inhibits head twitches. This is possibly due to blockade of central $5HT_2$ receptors.

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THE EFFECTS OF THE ENKEPHALINASE INHIBITOR ACETORPHAN ON SPONTANEOUS LOCOMOTOR ACTIVITY

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Increased spontaneous locomotor activity (SLA) has been observed after administration of opioid peptides (Broekkamp et al. 1979; Stinus et al. 1980) and this effect has been shown to be blocked by naloxone suggesting an opioid receptor involvement. The enkephalinase inhibitor acetorphan (ACET) also increases SLA when administered intravenously (Giros et al. 1987; Michael-Titus et al. 1987) so it was the purpose of this study to examine further the locomotor activity induced by acetorphan after peripheral and central administration.

Male mice (GB1 variants of the ICI strain) weighing 20-25g were used throughout. Cumulative locomotor activity was measured using paired photocell cages (5 animals/cage) and animals were allowed a habituation period of 1hr on the day prior to the experiment and immediately before experimentation.

Results showed that ACET administered intraperitoneally (ip) produced a dose related increase in SLA over the dose range 50-150mg/kg ip, there being a significant difference ($P<0.05$) compared to vehicle treated animals 1.5hrs after injection at doses from 100mg to 150mg/kg ip but not at 50mg/kg. Similarly, ACET administered intracerebroventricularly (icv) produced a dose related increase in SLA over the dose range 3-50µg/animal. This effect was more rapid in onset and a significant ($P<0.05$) difference compared to controls was noted at 1hr for doses above 3µg/animal. D-ala^2-D-Leu^5-enkephalin (DADL) 0.1µg/animal (icv) produced a significant increase in SLA and 1hr pretreatment with ACET 50mg/kg ip caused a significant (27.4%, $P<0.05$) increase in the DADL response - both responses being significantly different from vehicle or ACET treatments alone. The enhanced response of DADL combined with ACET was reversed by naltrexone 0.1mg/kg ip, there being an 80.2% ($P<0.05$) decrease in SLA to a level which was not significantly different from controls. Naltrexone at this dose displayed no significant effect on SLA when administered alone.

We conclude that these stimulant effects are opioid mediated since the effect of the enkephalin analogue DADL is enhanced by ACET, and this effect can be blocked by the opioid antagonist naltrexone. It has been proposed (Michael-Titus et al. 1987) that locomotor hyperactivity induced by ACET may result not only from protection of local enkephalins but may also be linked with mesolimbic dopaminergic neurones.

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DIFFERENTIAL DEVELOPMENT OF OPIOID AND NON-OPIOID STRESS-INDUCED ANTINOCICEPTION IN INFANT RATS

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There is now ample evidence that exposure to environmental stress can reduce pain sensitivity in laboratory animals. For example, swimming stress produces antinociception in adult rats via opioid and/or non-opioid mechanisms depending on the duration of swimming time (Terman et al., 1986). There have been relatively few reports, however, of stress-induced antinociception in developing animals, and accordingly, we have examined this phenomenon in 20- and 25-day old rat pups using 3 or 10 min warm water swims as the stress procedure and employing the opioid antagonist naloxone to determine the opioid/non-opioid nature of the stress-induced responses.

Experiments were performed between 13.00 h and 17.00 h on litters of Wistar rat pups containing 8 animals (male and female). Each litter was divided into 4 treatment groups. One group received 0.9% saline i.p. and remained in the home cage as a control group. The other 3 groups were injected i.p. with saline or naloxone (dose volume 0.1ml/20g rat) and 10 min later placed in 20°C water for 3 or 10 min. Antinociception was assessed using the tail immersion test (50°C) immediately before drug administration and 1, 5, 10, 15, 30, 45 and 60 min following the swim stress. Each measure of aninociception represents the mean of 10 observations determined from 5 litters on at least 2 separate days and treatment group means were statistically compared using analysis of variance and the Dunnett's test.

A 3 min swim at 20°C produced significant antinociception in the 20- and 25-day old pups (Table 1) which was evident for 15 min and attenuated by 1 and 10 mg/kg doses of naloxone. A 10 min swim did not significantly increase tail immersion latencies in the 20-day old rats. However, 25-day old animals subjected to a 10 min swim at 20°C displayed naloxone-insensitive antinociception which was maintained for over 30 min following the swim-stress. The tail immersion latencies of unstressed 20- and 25-day old rats were not significantly altered by the highest dose of naloxone (10mg/kg) used in this study.

Table 1.

Tail immersion latencies (s) of infant rats measured 5min after swimming stress

Age	Control	3 min swim	Control	10 min swim
20 days	3.8 ± 0.3	7.4 ± 0.6*	3.4 ± 0.3	2.9 ± 0.4
25 days	3.3 ± 0.2	6.8 ± 0.5*	3.5 ± 0.2	5.4 ± 0.3*

Values represent means ± s.e. mean; n = 10; * $P < 0.05$ compared to controls.

The results in 25-day old rats support the observations in adult animals that short swims produce opioid mediated stress-induced antinociception whereas longer swims induce anti-nociception via non-opioid mechanisms. Moreover, the lack of antinociceptive response to a 10 min swim in 20-day old pups suggests that these non-opioid pain modulatory systems may develop more slowly than those dependent on endogenous opioids, which appear to be fully functional in this age group.

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AN INVESTIGATION OF ANTICONVULSANT TOLERANCE TO ZOLPIDEM IN MICE

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It has been reported that repeated administration produces modest tolerance to the behaviour suppressing effect of zolpidem in rats (Sanger & Zivkovic, 1987). It has also been reported that mice treated repeatedly with a related imidazopyridine, alpidem, showed no tolerance in protecting against pentylenetetrazol (PTZ)-induced convulsions, but the same authors (Morel et al, 1987) found that after repeated administration alpidem was about 3 times less active against supramaximal electroshock. However, the PTZ test used was not the slow intravenous infusion of PTZ that has been described (Meldrum, 1986) as "probably the most sensitive and accurate system" for evaluating anticonvulsant tolerance, and which has proved valuable for showing differences between benzodiazepines (BDZs) as regards both the degree and speed of onset of tolerance (Garratt et al, 1988). We have used this latter test to study anticonvulsant tolerance to zolpidem and compare it with tolerance to the BDZ diazepam.

Zolpidem (ZPM) and diazepam (DZP) were dissolved in vehicle and given S.C. 15 min (ZPM) and 45 min (DPZ) before testing with PTZ (See Gent et al, 1984). Groups of 5 mice (adult male Tuck No. 1, 25-35 g) were used. The minimum convulsant dose (MCD) required to elicit a clonic convulsion in each animal was measured and protection was calculated as the difference between the mean MCD for experimental and control groups.

Table: Protection (mg/kg PTZ) following repeated, 12 hourly, administration of zolpidem and diazepam

Period of dosing:		Acute	24h	48h	4d	8d	16d
ZOLPIDEM $1.5\ mg.kg^{-1}$	mean	81.3	82.9	51.4	61.2	52.4	59.1
	s.e.	5.2	1.6	4.4	5.2	6.1	6.7
DIAZEPAM $1.5\ mg.kg^{-1}$	mean	60.3	33.2	-	38.6	30.6	-
	s.e.	2.7	1.9	-	1.7	2.9	-

In the zolpidem study while protection was unchanged at 24h it was significantly reduced by 48h ($P < 0.01$, single classification analysis of variance), though it did not fall further thereafter. With diazepam both the rate (protection down from 60.3 ± 2.7 to 33.2 ± 1.9 by 24h, $P < 0.01$) and extent of tolerance appeared greater than with zolpidem. Our findings are broadly in line with the observations of Sanger and Zivkovic (1987) concerning the development of modest tolerance to the behaviour suppressing effects of zolpidem in rats.

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LOCAL CEREBRAL GLUCOSE UTILIZATION AFTER TOLERANCE TO THE SEDATIVE AND ANXIOLYTIC ACTIONS OF DIAZEPAM

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Benzodiazepines have a variety of actions including sedative, anxiolytic, muscle relaxant and anticonvulsant effects. Tolerance to these effects occurs at different rates (File 1985; Pratt et al., unpublished) Thus, tolerance to sedation occurs after about 3 days treatment in rats and tolerance to the anxiolytic effect after about 3 weeks. This study was designed to identify brain structures involved in tolerance to the various behavioural effects of diazepam (DZP) using the quantitative ^{14}C-2-deoxyglucose technique (Sokoloff et al. 1977) in conscious male hooded rats.

Rats were randomly assigned to each of four groups. Groups 1 and 2 received daily injections of vehicle (1% Tween 20 in saline i.p.) for 28 days. A third group received vehicle for 25 days followed by 3 daily injections of DZP (5 mg/kg i.p.) and the fourth group received DZP (5 mg/kg i.p.) daily for 28 days. On the test day measurements were initiated 10 min after the administration of vehicle (Group 1; controls) or DZP (0.3 mg/kg i.v. Groups 2-4) and the rate of glucose utilization determined in 66 brain regions. DZP administered to drug-naive rats depressed glucose utilization in a variety of structures, especially limbic structures and those involved in auditory and visual processing (Table 1).

Table 1. Effect of diazepam upon glucose utilization

Structure	Vehicle (n=5)	Diazepam (0.3 mg/kg i.v.) Acute DZP (n=5)	3 days DZP (n=6)	28 days DZP (n=4)
Dorsal Tegmentum	83±4	69±1*	82±4+	77±2
Dorsal Raphe	79±4	66±1*	76±3+	74±2
Mammillary Body	90±6	72±4*	81±2	92±4+
Subiculum	82±5	66±2*	73±2	76±2
Visual Cortex IV	95±6	79±3*	86±3	93±1
Auditory Cortex IV	116±7	97±3*	117±4+	122±6+
Infralimbic Cortex	88±6	74±2*	83±2	88±4+
Inferior Colliculus	146±11	116±7	157±6+	144±8

Data are presented as mean glucose use (μmol/100g/min)±S.E.M. * $p < 0.05$ compared to vehicle, + $p < 0.05$ compared to acute DZP, Bonferroni test.

Tolerance to the DZP-induced depression of glucose use in the dorsal tegmentum, dorsal raphe, auditory cortex and inferior colliculus was apparent after 3 days pretreatment with DZP, suggesting that these structures may be involved in the sedative effects of the drug. In contrast, tolerance to the depressant effects of DZP upon glucose use in some limbic structures (e.g. mammillary body and infralimbic cortex) was not apparent until after 28 days pretreatment (Table 1) implicating these structures in the anxiolytic effects of DZP.

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EFFECTS OF TRAINING IN A WATER MAZE ON PERFORMANCE IN A TEST BOX DIVIDED INTO BLACK AND WHITE COMPARTMENTS

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The present studies assessed behaviour of rats in the Morris water maze concurrently with behaviour in a box separated into dark and light compartments. The water maze was used as a test of spatial learning in which a rat can locate an island by means of distal, visual cues, and can escape onto the island (Morris, 1981). The dark:light box was used to measure rat aversion to a brightly lit environment, a response which is reduced by known anxiolytic agents (Costall et al. 1987).

Male Lister Hooded rats (250-300g) were trained in the water maze using the protocol described by Morris (1984), that is, each rat was subject to 6 escape trials per day, the aim being to locate the submerged island. The latency to escape onto the island, swim speed and % time spent in the island quandrant were noted for each trial. This was repeated on 3 consecutive days. Immediately after each trial rats were placed in a two-compartment box, escape from the averse, white brightly-lit area into the dark section being enabled by means of an opening in the partition between the two compartments. The basis of this test is that the less anxious the animal the more it will venture into the normally averse environment whilst the more anxious the animal the more it will remain in the black, darkened section (Costall et al., unpublished). Behaviour of the animals was assessed from videotape. Measures taken included the numbers of rears and crossings of lines marked on the floor of each compartment.

In addition to those animals which had been trained in the water maze, the behaviour of control groups of rats was assessed in the black:white box. One group was placed in warm water before testing to assess the effects of being wet whilst the other group consisted of normal animals and showed the effects of normal habituation to the test box. All animals were subject to testing in the black:white box on 3 consecutive days, 6 sessions/day.

After 3 days of training in the water maze latency to escape decreased from 48.8 $\pm$ 10.2 to 10.7 $\pm$ 0.5 s ($P<0.01$) and the % time spent in the island quadrant increased from 27.0% to 61.3% ($P<0.01$). The swim speed remained constant throughout at 24.6 $\pm$ 0.86 cm s^{-1}.

Normal control rats exhibited 10.2 $\pm$ 0.6 rears/5 min the white section of the box on day 1, and 12.8 $\pm$ 1.9 rears/5 min in the white on day 3. In contrast, the rats which had been subject to testing in the water maze spent most time in the black, darkened area of the box, exhibiting 3.3 $\pm$ 1.6 rears/5 min in the light compartment on day 1, and 4.3 $\pm$ 0.9 rears/5 min on day 3 ($P<0.01$ compared to normal rats). This increased aversion of rats to the light after training in the water maze could not be explained purely in terms of stress caused by the process of wetting, since wet rats exhibited 8.2 $\pm$ 0.8 rears/5 min in the white compartment on day 1 and 11.1 $\pm$ 1.8 rears/5 min in the white on day 3 ($P>0.05$ compared to normal control animals on day 3).

The data indicates that animals subject to training in the Morris water maze exhibited a profile of behaviour similar to that seen when animals are treated with anxiogenic agents such as the β-carbolines (Costall et al. unpublished). It may therefore be concluded that training in the Morris water maze is accompanied by stress, and it is important that this factor is taken into consideration when effects of drug treatments on performance in the water maze are analysed.

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