THE CALCIUM PARADOX: SOME CONSEQUENCES OF CHANGING SODIUM ION CONCENTRATION

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Experiments were carried out to investigate the influence of sodium ions on the development of the calcium paradox, the term first used by Zimmerman and Hulsman (1966) to describe the biochemical, physical and functional changes occurring in the heart when calcium is re-introduced to a previously calcium depleted heart. Sodium ions (Na) may play a part in regulating contractility by controlling calcium entry into the cell via the sodium-calcium exchange mechanism (Eisner et al., 1984). Intracellular Na concentration at the onset of calcium (Ca) repletion may, therefore, determine both the extent of Ca entry and ultimately the degree of Ca overload in instances of the calcium paradox phenomenon.

Eighteen isolated whole hearts (NZW rabbits) were perfused with McEwen's solution by the Langendorff method. Each complete experiment lasted only 55 minutes, of which 30 minutes were allowed for the heart to reach a steady state. Thereafter the perfusion sequence was 10 minutes with normal McEwen's (equilibration); 5 minutes with either Ca-free McEwen's (control calcium paradox), or Ca-free McEwen's with the Na content reduced by 50% (low Na series), or Ca-free McEwen's with Na content increased by 50% (raised Na series); and finally 10 minutes with normal McEwen's (Repletion). Although no compensatory osmotic changes were made in those solutions where Na was either increased or reduced, in a small series of experiments (3) with osmolarity corrected by additional sucrose, the protective effect of the solutions with lowered Na was unchanged.

During the depletion period, cardiac arrest occurred after perfusion for 4 minutes with Na-reduced/Ca-free solution, 3 minutes with Ca-free only and after less than one minute with Na-increased/Ca-free solution. Recovery of the hearts which had been subjected to Na reduced/Ca-free was almost complete following the 10 minute recovery time. There was little if any recovery in the Ca-free series and in the Na-increased/Ca-free series there was no recovery at all.

Perfusion with Ca-free and with Na-reduced/Ca-free solutions diminished coronary flow, whereas perfusion with Na-increased/Ca-free caused a small rise in coronary flow. During the repletion period the coronary flow returned to pre-depletion values only in the Na-reduced series of experiments. In the other two series of experiments there was a substantial reduction in the coronary flow on repletion. Analysis of variance was carried out on the data and showed statistical significance ($P < 0.001$)

The extent of myocardial damage was reflected by a change in the colour of the repletion effluent, an event apparently not previously reported. In the case of the control paradox and Na-increased series of experiments, loss of contractile and electrical activity occurred in conjunction with the production of a pale yellow repletion effluent with maximum absorbance at 410 nm on spectrophotometry. During the repletion phase the colour intensity of the effluent rose, reached a peak after about two minutes and then fell in both series of experiments, the colour change being less pronounced in the Na-increased/Ca-free series ($P < 0.05$, $n = 11$).

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COMPARISONS OF THE EFFECTS OF PALMITOYL CARNITINE AND BAY K 8644 ON THE CALCIUM-SENSITIVITY OF THE RAT TAIL ARTERY

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Endogenously occurring lipid metabolites can affect Ca^{2+} channel function in certain conditions (Mir & Spedding, 1986). Palmitoyl carnitine (PC) is a lipid metabolite produced in ischaemic conditions and resembles Bay K 8644 as an activator of calcium channels in some smooth muscle. We have examined its facilitatory actions on Noradrenaline (NA)-induced pressor responses in isolated perfused rat tail artery and also its modulatory actions on the desensitisation of responses (assessed by calcium sensitivity): these actions have been compared with those of Bay K 8644 (BK.) in different O_2 tensions (produced by varying O_2 in the gassing mixture). Earlier we had shown that Bay K 8644 facilitates responses but does not arrest desensitisation (McGrath, Miller & Ugwu, 1987).
Lengths (1-2cm) of the proximal segments of rat tail artery (male Wistar, 300-350g) were perfused at 2-3ml/min with a Krebs' bicarbonate saline ($[Ca^{2+}]$ 2.5mM, PO_2 580-650mmHg, pH 7.2-7.3, at 37°C), and immersed in a similar medium. Vasoconstrictor responses were represented by increases in perfusion pressure. Calcium concentration-response curves (CCRCs) were constructed by reducing $[Ca^{2+}]_o$ to 1uM then activating with NA (3uM). $[Ca^{2+}]_o$ was increased in 6-9 steps from 1uM to 300uM or 5mM using Ca^{2+} buffers (NTA {nitrilotriacetic acid} and EGTA {2.5mM of each}). Construction of each curve in the presence or absence of BK or P.C. (0.1uM), took 45min: six of these were constructed, separated by 15min "rest" intervals in 1uM Ca^{2+}.
PC (0.1μM) and BK (0.1μM) produced similar effects on Ca^{2+} sensitivity, increasing the -log EC_{30} by 0.5 to 1.0 log units for each curve in the series, compared with time controls. This was essentially similar at 95%, 16% and 4% O_2 except that at 16% the controls tended to be more sensitive while sensitivity in PC and BK was the same as at other gas tensions, giving a smaller shift. However, PC responses were of greater magnitude and were better maintained than in controls or in BK. Thus although PC did not prevent the relative loss of sensitivity to extracellular Ca^{2+} which produces desensitisation, even after desensitisation, responses in PC were at least as large as in non-desensitised controls. This suggests that PC exerts other intracellular effects on Ca^{2+} mobilisation or efficiency of contraction which can offset the effects of Ca^{2+}-overload and produce greater facilitation than would Ca^{2+}-channel facilitation alone.
In conclusion, the results show that PC can facilitate pressor responses to NA even in mildly hypoxic conditions and that this is partly attributable to facilitation of Ca^{2+} channel function. We did not investigate whether endogenous PC produced by severe hypoxia could facilitate pressor responses (as it does with KCl-induced responses in rat portal vein; Fasehun, et al., this meeting), since hypoxia virtually abolishes responses to NA.

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EFFECTS OF Ca^{2+} MODULATORS ON THE HYPOXIA-RESISTANT RESPONSE OF THE ISOLATED RAT PORTAL VEIN TO POTASSIUM CHLORIDE

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Hellstrand et al (1977) showed that hypoxia depressed contraction of the rat portal vein to KCl less than that to NA. A similar observation had been made in the rabbit aorta (Shibata & Briggs,1967). We have confirmed this and have now followed this up by investigating the role of Ca^{2+} in KCl-induced contraction in hypoxia, using Ca^{2+} modulators.

Male Wistar rats (245-255 g wt) were used. Isometric contractions were recorded from longitudinal strips of the rat isolated portal vein placed under a resting tension of 1g, in Krebs bicarbonate saline at 37°C and equilibrated with 16% O_2, 5% CO_2, 79% N_2 for 90 min (normoxia). Responses to 5 min exposure to KCl 50 mM were then produced until they were reproducible. The tissues were washed into 'zero Ca' Krebs and after 5 min this was replaced by Krebs containing 0.31 mM Ca^{2+} (this allowed a clearer facilitation by Ca^{2+} facilitators than in higher $[Ca^{2+}]$).

A concentration/response curve for each test drug versus contraction to KCl 50 mM was constructed allowing 15 min equilibration with each concentration of test drug before adding KCl. In other experiments (hypoxia), after an initial equilibration with 16% O_2 and subsequent response to KCl, the gas was then replaced by 95% N_2 (in 5% CO_2 to maintain a constant pH) before constructing the concentration/response curve.

Control responses to KCl 50 mM at 0.31 mM Ca^{2+} in hypoxia were of a similar or higher magnitude to those in normoxic conditions. Bay K 8644 (0.1 nM - 0.3 µM) facilitated the KCl-induced responses in normoxia but this facilitation was suppressed in hypoxia. The maximum facilitation in 16% O_2 and in hypoxia were 120% and 30% respectively. A similar result was obtained for CGP 28392 (10 nM-1 µM), another dihydropyridine facilitator. The hypoxia-resistant response was more resistant to nifedipine than was the response at 16%. Palmitoyl carnitine (10 µM - 100 µM), an endogenous Ca^{2+} facilitator that accumulates during ischaemia (Spedding 1987), had a similar effect to Bay K 8644. POCA (100 µM), an acyl inhibitor, caused about 40% inhibition of the control response to KCl in hypoxia, while the response during oxygenation was hardly inhibited.

We conclude that the resistance to hypoxia of the response to KCl arises because Ca^{2+} channel opening is already facilitated by accumulation of endogenous factors, possibly acyl carnitine. Hence there is a relative ineffectiveness of further facilitators whether they be synthetic Bay K 8644 or exogenous palmitoyl carnitine. This occurs specifically during ischaemic conditions when acyl compounds accumulate and can be prevented by POCA. Thus in ischaemia vasoconstriction may be relatively resistant to both facilitators and blockers of Ca^{2+} channels but should be susceptible to manipulation of acyl carnitine synthesis and metabolism.

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SELECTIVE ANTAGONISM OF THE EFFECTS OF BAY K 8644 AND PALMITOYL CARNITINE IN CHICK HEART BY CALCIUM ANTAGONISTS

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Bay K 8644, a Ca^{2+} channel activator, has selective interactions with calcium-antagonists in smooth muscle in that the inhibitory effects of dihydropyridines are competitively reversed, the inhibitory effects of verapamil and diltiazem non-competitively reversed, whereas the effects of lipophilic class III calcium-antagonists (Spedding, 1985) are not reversed at all. Palmitoyl carnitine, a lipid metabolite which is produced during ischaemia, has been claimed to be an endogenous Ca^{2+} channel activator (Mir & Spedding, 1986), and has similar interactions in smooth muscle with the calcium-antagonists. We have previously shown that palmitoyl carnitine augments contractility in embryonic chick heart cell aggregates and reverses the inhibitory effects of nisoldipine and verapamil (Duncan et al, 1986) at the same concentrations as are required to inhibit [^{3}H]nitrendipine or [^{3}H]verapamil binding to cell membranes (Mir & Spedding, 1986). We have now tested the interaction of Bay K 8644 and palmitoyl carnitine with lipophilic class III calcium-antagonists in chick heart.

Beating of cultured chick heart cell aggregates was measured as described by Duncan & Patmore (1986). Concentrations of the dihydropyridine nisoldipine (0.3 µM) and of the class III calcium antagonists pimozide (2 µM) and lidoflazine (7 µM) were chosen to inhibit the number of aggregates beating by 80 - 90%.

Bay K 8644 (0.01 - 1 µM) reversed the inhibiting effects of nisoldipine, with complete reversal at 1 µM. Palmitoyl carnitine (1-200 µM) reversed the inhibitory effects of nisoldipine with the optimal effect occurring at 200 µM; higher concentrations inhibited beating, presumably due to the detergent effects of the compound. In complete contrast, neither Bay K 8644 (0.01 - 10 µM) not palmitoyl carnitine (1 - 1000 µM) caused any reversal of the inhibitory effects of pimozide (2 µM) or lidoflazine (7 µM).

These results show that Bay K 8644 has similar interactions with the different subgroups of calcium-antagonists in heart as it does in smooth muscle (see also Boddeke et al, 1987), indicating distinct modes of action for the different subgroups (Spedding, 1985). Palmitoyl carnitine has similar selective interactions with the calcium-antagonist subgroups, indicating a distinct site of action at the Ca^{2+} channel.

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THE CYCLIC PEPTIDE BEAUVERICIN PROMOTES CALCIUM INFLUX IN HUMAN PLATELETS AND NEUTROPHILS

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It has been suggested that the cyclic peptide bassianolide may have a selective action in inhibiting receptor-mediated calcium (Ca) influx (Nakajo et al., 1983). We have undertaken studies with the related cyclic peptide beuvericin. Preliminary studies in isolated arteries failed to show any effect of the peptide alone, and did not reveal any interaction with vasoactive agents. However, we have found that beauvericin had unexpected effects on Ca homeostasis in human platelets and neutrophils.

Platelets from normal volunteers were isolated and loaded with the calcium-sensitive photoprotein aequorin by a modification of the method of Vickers and Mustard (1986). Aggregation and luminescence were measured simultaneously using a Chrono-Log PICA lumiaggregometer. Intracellular Ca was estimated from the luminescence signal as described by Johnson et al. (1985). Neutrophils were prepared as described by Boyum (1976). Neutrophils were loaded with aequorin as described by Yamaguchi et al. (1976). As with platelets, aggregation and aequorin luminescence were measured simultaneously. In parallel experiments neutrophil superoxide production was assessed using lucigenin-amplified chemiluminescence.

In platelets beauvericin (B) caused a rapid, dose-dependent rise in intracellular Ca, with maximal response at 10μM B (EC50 3μM). External Ca concentration was 1mM and the maximum intracellular calcium was 20μM: the calcium response to thrombin 0.5U/ml was 14μM. The rise in calcium was accompanied by aggregation at about 5x the rate, for B 10μM, as for thrombin 0.5U/ml. At concentrations of B <6μM aggregation was preceded by shape change. At higher concentrations of B this was obscured by rapid aggregation. The Ca signal and aggregation were halved by replacing the external Ca with 1mM EGTA. In neutrophils (external Ca 0.5mM) the dose-response relationship was similar (max. response at 10μM, but EC50 1μM). Cell aggregation and superoxide release closely paralleled the magnitude of the Ca signal. Maximal superoxide production, at B 10μM, was greater than that induced by 3μM phorbol diester or by 1μM FMLP (chemotactic tripeptide), though the responses to these were variable. The Ca response and superoxide release were virtually abolished by removal of external Ca.

In at least two cell types B causes a dose-related rise in intracellular Ca, largely by influx. This rise is accompanied by physiological responses. B's mechanism of action is obscure. Unlike the calcium ionophore A23187 it does not translocate calcium from an aqueous to an organic phase, though it may interact directly with the plasma membrane. This unusual cyclic peptide may prove a useful probe of membrane structure and function, with particular emphasis on calcium transport.

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EFFECT OF HIGH-DOSE PROPRANOLOL TREATMENT ON CARDIAC HYPERTROPHY IN RESPONSE TO AORTIC COARCTATION OR TO HYPOXIA IN THE RAT

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There is evidence that noradrenaline released from cardiac sympathetic nerve terminals acts on myocardial beta-receptors to induce growth in many of the experimentals models used to study compensatory cardiac hypertrophy (Ostman-Smith, 1981). However attempts at reducing compensatory cardiac hypertrophy with beta-receptor blockade have yielded conflicting results, with Fernandes et al. (1976), Ostadal et al. (1978) and Voelkel et al. (1980) finding that beta-receptor blockade reduced compensatory hypertrophy in models of right and left ventricular hypertrophy, whereas Dennis and Vaughan-Williams (1982) were unable to prevent right ventricular hypertrophy in response to hypoxia with propranolol treatment. The present study was undertaken to study the effect of oral high-dose (80 mg/kg/day) treatment of propranolol on compensatory hypertrophy occurring in the rat in response to a left or right ventricular pressure overload, caused by experimental coarctation of the aorta, or by daily exposure to hypoxia with inspired oxygen of 6%, respectively. The coarctation study was designed so that alterations in cardiac work load caused by the propranolol treatment per se could be accurately assessed.

In rats with mean gradients over an abdominal coarctation in the range of 16-30 mm Hg the group on control diets showed a 32% increase in left ventricular ratio, whereas the propranolol=treated group showed only a 14% increase; this 65% reduction in hypertrophic response was highly significant ($p < 0.001$). In a second series with a wide range of pressure gradients the regression lines of left ventricular ratio versus mean pressure gradient were significantly different in the two groups (control slope 1.34 $\pm$ 0.12 (SE), propranolol slope 0.67 $\pm$ 0.06, $p < 0.001$). When cardiac work, expressed as the rate x pressure product, was plotted against the left ventricular ratio, the slope of the regression line for the control rats (2.28 $\pm$ 0.16) was still about twice as steep as that for propranolol treated rats (1.08 $\pm$ 0.14; $p < 0.001$). Thus propranolol approximately halved the compensatory cardiac hypertrophy in response to left ventricular pressure overload by a mechanism independent of its effect on cardiac work load.

Rats on control diets exposed to 18 8-hour sessions of breathing inspired oxygen of 6% showed a 59% increase in right ventricular ratio, whereas propranolol-treated rats only developed a 33% increase in right ventricular ratio, a significant reduction of the hypertrophc response ($p < 0.01$).

It is concluded that it is feasible significantly to reduce compensatory cardiac hypertrophy in response to a pressure overload by high-dose oral treatment with a beta-receptor blocker.

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INFLUENCE OF ATRIOPEPTIN III ON RENAL FUNCTION IN DOCA-SALT HYPERTENSIVE RATS

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There is a great deal of evidence which shows that atrial natriuretic peptides, either extracted from atria or synthetized, have a potent action on the kidney causing sodium and water excretion (Schnermann & Briggs, 1987). As yet, no clear concensus has emmerged of the importance of these peptides in the regulation of salt and water balance under physiological or pathological conditions. The intention of this study was to compare the natriuretic and diuretic effectiveness of atriopeptin III in normotensive and DOCA-salt hypertensive rats.

Male Sprague-Dawley rats (270-280 g) were acutely anaesthetized (60 mg/kg sodium pentobarbitone, i.p.) and subjected to right unilateral nephrectomy. Following one week of recovery, rats were maintained on either standard laboratory diet (normotensive controls) or given saline to drink and twice weekly injections of DOCA (15 mg/kg, s.c.) and were used 4-6 weeks later. The animals were again anaesthetized and prepared for measurement of renal function as previously described (Johns, 1985). Five 15 min clearance periods were used, two before and two following a period in which atriopeptin III was administered as a bolus dose, i.v., at the start of the collection. Three doses of atriopeptin III were given, 125, 250 and 500 ng/kg in random order, to each animal.

Systemic blood pressure and renal blood flow in 8 normotensive rats were stable for the duration of the experiments at 140 $\pm$ 2 mmHg and 22.7 $\pm$ 0.3, respectively, and were unchanged by atriopeptin III. Glomerular filtration rate in these animals was 3.4 $\pm$ 0.1 ml/min/kg and was elevated by 19% ($p<0.02$) following 250 ng/kg atriopeptin III. Administration of increasing doses of atriopeptin III caused progressive increases in urine flow, at 42.4 $\pm$ 2.8 µl/min/kg, of 42% ($p<0.05$), 53% ($p<0.01$) and 65% ($p<0.05$), respectively, in absolute sodium excretion, at 6.4 $\pm$ 0.7 µmol/min/kg, of 48%, 70% and 95% (all $p<0.05$), respectively, in fractional sodium excretion, at 1.18 $\pm$ 0.14%, of 47%, 40% and 87% (all $p<0.02$), respectively, while fractional lithium excretion at 31.0 $\pm$ 1.1% did not change. In a group of 8 DOCA-salt animals, systemic blood pressure was 169 $\pm$ 5 mmHg, renal blood flow was 23.2 $\pm$ 1.4 ml/min/kg and glomerular filtration rate was 4.2 $\pm$ 0.1 ml/min/kg and these variables remained stable over the course of the experiment and were not altered by any dose of the peptide. In these hypertensive animals increasing doses of atriopeptin III increased urine flow, at 72.3 $\pm$ 2.7 µl/min/kg, by 46% ($p<0.05$), 85% ($p<0.01$) and 180% ($p<0.01$), respectively, absolute sodium excretion , at 11.2 $\pm$ 0.5 µmol/min/kg, by 48% ($p<0.05$), 109% ($p<0.01$) and 211% ($p<0.001$), respectively, fractional sodium excretion, at 1.69 $\pm$ 0.09%, by 44% ($p<0.05$), 117% ($p<0.01$) and 201% ($p<0.001$), respectively, and fractional lithium excretion, at 35.9 $\pm$ 1.0%, by 10%, 36% ($p<0.01$) and 45% ($p<0.01$).

These data demonstrate that administration of atriopeptin III in this way had minimal effects on blood pressure and renal haemodynamics in both normotensive and hypertensive rats. In the normotensive control animals atriopeptin III caused dose related increases in the output of sodium and water while in the hypertensive animals the magnitude of these responses were approximately doubled. These findings suggest that in the DOCA-salt rat model of hypertension the tubular actions of atriopeptin III are substantially enhanced.

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ADENOSINE CONTRIBUTES TO THE PATHOGENESIS OF RENOVASCULAR HYPERTENSION IN THE RAT: STUDIES WITH CAFFEINE

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We have previously demonstrated that chronic caffeine administration to rats with a 2 kidney 1 clip (2K1C) model of hypertension substantially increased blood pressure (BP), converting moderately severe hypertension to malignant hypertension associated with necrotizing arteriolitis (Ohnishi et al, 1986). One hypothesis to explain this observation is that renal ischemia results in release of not only renin secretagogues to elevate plasma renin activity (PRA), but also adenosine, a potent inhibitor of renin release. Blockade of adenosine receptors with the xanthine, caffeine, could result in unopposed activity of the renin secretagogues and enhanced PRA. This would increase angiotensin II production and augment the hypertensive response.

The present series of studies were designed to further test this hypothesis. We have evaluated changes in arterial PRA, BP and creatinine clearance over time in caffeine treated (0.1% added to drinking water) and vehicle treated rats subjected to the 2K-1C procedure and followed for 6 weeks. We have also measured arterial and renal venous plasma concentrations of adenosine, using a microbore HPLC assay adapted for small samples, at 1, 2 and 3 weeks after surgery in 2K1C and sham operated rats.

Chronic administration of caffeine to 2K1C rats resulted in significant (approximately by 15-20%) elevations in BP from week 2 to week 6 following surgery in comparison to vehicle treated controls. There was a 54% reduction ($P<0.05$) in creatinine clearance in the caffeine treated group by the 6th week, in comparison to no change in the vehicle treated group. PRA was increased by over ten fold between week 1 and 6 after surgery in 2K1C rats treated with caffeine in comparison to vehicle treated rats.

In further groups of rats, arterial plasma concentrations of adenosine one week after renal artery constriction were 902 ± 49ng/ml, falling to 507 ± 84ng/ml by the third week. These values were substantially higher than in sham operated rats after one week (120 ± 17ng/ml) and from renal venous blood from the constricted and nonconstricted kidneys (584 ± 173ng/ml and 506 ± 185ng/ml), respectively, one week after surgery.

These results demonstrate that unilateral renal artery stenosis results in an increase in systemic adenosine levels. We suggest that adenosine is produced by the lungs rather than from the ischemic kidney. Inhibition of adenosine receptors with caffeine is associated with a sustained marked increase in PRA, together with increased BP and decreased renal function. These observations are consistent with the hypothesis that adenosine restrains the hypertensive response to renal artery constriction.

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THE TERMINAL 5-HT AUTORECEPTOR IN THE GUINEA-PIG FRONTAL CORTEX: A PHARMACOLOGICAL ANALYSIS

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Terminal autoreceptors for the neurotransmitter 5-HT have been identified in a number of mammalian species including the rat (Cerrito and Raiteri, 1979) and rabbit (Limberger et al, 1986). In the rat, it has been established that the terminal 5-HT autoreceptor belongs to the 5-HT_{1B} subclass (Engel et al., 1986) and a powerful, competitive antagonist, cyanopindolol, identified (Middlemiss, 1986). The present study reports the presence of a terminal 5-HT autoreceptor in the guinea-pig frontal cortex and describes a preliminary pharmacological analysis of its receptor type.

Slices of the guinea-pig frontal cortex were loaded with [^{3}H]5-HT, superfused and continuously stimulated with Krebs solution containing elevated K^+ ions (30mM) and fluvoxamine (10μM) to prevent the reuptake of 5-HT (for a detailed description of the methodology see Middlemiss, 1986). The inhibition of the K^+-evoked overflow of tritium by 5-HT or the 5-HT_{1A} agonist 8-OH-DPAT was expressed as % of control. The effects of antagonists were assessed after their addition 24 min before the start of the cumulative addition of the agonist, 5-HT (30 to 300nM).

Elevated K^+ ions in the superfusion buffer resulted in a 3 to 4 fold increase in the release of tritium over basal levels. This increased release was completely abolished by the exclusion of Ca^{2+} ions from the medium. 5-HT (30 to 300nM), but not 8-OH-DPAT over the same concentration range, caused a concentration related inhibition of K^+-evoked [^{3}H]5-HT release with a pIC_{25} value of 7.43. The inhibitory effects of 5-HT were attenuated by the 5-HT antagonist, methiothepin (10nM, Table) and by the mixed 5-HT_{1A}/5-HT_{1B} antagonist, cyanopindolol (1μM, Table) but were unaffected by the 5-HT_{1C}/5-HT_2 antagonist, mesulergine (0.1μM) or the 5-HT_3 antagonist, ICS 205-930 (1μM).

	Apparent pA_2 at 5-HT Autoreceptor	
	Guinea Pig	Rat*
Methiothepin	8.20	6.62
Cyanopindolol	6.47	8.30

* Taken from Hibert and Middlemiss (1986), Middlemiss (1986).

These studies have demonstrated the presence of a terminal 5-HT autoreceptor in the guinea-pig with pharmacological characteristics which suggest that it is distinct from 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_2 or 5-HT_3 receptors. This receptor may, however, be similar to the terminal 5-HT autoreceptor in the rabbit (Limberger et al., 1986) owing to the similarity of the pA_2 values for methiothepin and cyanopindolol in the two species.

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CONTROL OF 5-HT METABOLISM IN RAT SCN - A PRIMARY ROLE FOR TERMINAL RECEPTORS?

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Neuronal release of serotonin (5HT) is under the control of a 5HT autoreceptor (Moret, 1985). Using in vivo voltammetry we have established that the nerve terminal 5HT autoreceptor in the rat suprachiasmatic nucleus (SCN) is of the $5HT_{1B}$ sub-type (Marsden & Martin, 1985a). Infusion of the $5HT_{1A}$ receptor agonist DPAT into the dorsal raphe (DRN) does not affect extracellular 5-hydroxyindoleacetic acid (5HIAA) levels in the SCN (Marsden & Martin, 1985b). Since DPAT inhibits DRN unit activity (Wilkinson et al., 1987) and presumably 5HT release, these data suggested that 5HT metabolism in vivo may not always reflect 5HT unit activity. Furthermore, following injection of a pyrogen preliminary data has suggested that 5HT release in the anterior hypothalamus may increase (Martin et al., unpublished data) but 5HT unit activity is unchanged (Fornal & Jacobs, 1987). We therefore examined the possibility that the 5HT autoreceptor may have a stronger influence than 5HT neurone firing rate on 5HT release and metabolism.

Male Wistar rats (295-305 g) were anaesthetised with chloral hydrate (600 mg/kg i.p.) and carbon fibre electrodes (0=20 μm, working electrode) were stereotaxically implanted in the left SCN. Reference and auxilliary electrodes were placed in contact with the dura surface. A 23 g stainless steel guide cannula was implanted 1 mm above the contralateral SCN. Infusions were made via a 31 g injection cannula. A bipolar SNE 100 stimulating electrode (Clark Electromedical Ltd.) was implanted into the DRN. Differential pulse voltammograms were obtained every 5 min and the height of the oxidation peak at +300 mV (peak 3) taken as an index of extracellular 5HIAA concentration (Crespi et al., 1983).

Electrical stimulation (square pulses, 0.5 msec duration, constant current) of the DRN for 10 min resulted in a current and frequency dependent increase in the height of peak 3 recorded in the SCN in the range 50-200 μA and 5-20 Hz. Maximum increases (60±14%, n=4) occurred with stimulation parameters of 200 μA and 20 Hz approximately 30 min after the start of stimulation and therefore 20 min following its cessation. Administration of 0.9% saline (1 μl over 1 min) into the SCN 10 min before stimulation did not affect the response (peak 3 height=150±15%, of control 30 min from start of stimulation, n=5). Infusion of RU 24969, a $5HT_1$ agonist, (10 μg in 1 μl over 1 min) into the right SCN resulted in a rapid, marked decrease in peak 3 height recorded in the left SCN (-80±18% 10 min post infusion, n=5, -95±5%, 40 min post infusion, n=5). Infusion of RU 24969 (10 μg) into the SCN prevented the DRN stimulation induced rise in peak 3 height observed previously. (Peak 3 height 20 min post stimulation and 40 min post RU 24969 = 10±5% of pre-injection control, n=4.)

These data support the view that stimulation of the nerve terminal 5HT autoreceptor in the SCN has a greater influence on the regulation of 5HT release and metabolism than 5HT neurone firing rate.

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IDENTIFICATION OF A 5-HT_1 RECOGNITION SITE IN PIG AND HUMAN BRAIN DIFFERENT FROM 5-HT_{1A}, 5-HT_{1B} AND 5-HT_{1C}

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In radioligand binding studies we have recently identified 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} recognition sites in rat brain membranes using [^{3}H] 8-OH-DPAT (8-hydroxy-[2-N-dipropylamino]-tetralin), [^{125}I]CYP (iodocyanopindolol) and [^{3}H]mesulergine respectively (Hoyer et al, 1985). 5-HT_{1A} and 5-HT_{1C} recognition sites were also identified in pig and human brain membranes (Pazos et al, 1984, Hoyer et al, 1985, 1986a,b), whereas 5-HT_{1B} recognition sites are apparently absent from brain membranes of these species (Hoyer et al, 1985, 1986a). However, detailed autoradiographical studies revealed that in human brain slices [^{3}H]5-HT labels 5-HT_{1A}, 5-HT_{1C} and a third site which is different from the 5-HT_{1B} site (Pazos et al, 1987). In this report we characterise this population of 5-HT_1 sites in pig and human caudatum membranes using [^{3}H]5-HT in the presence of concentrations of 8-OH-DPAT and mesulergine (100 nM each) which prevent binding of the radioligand to 5-HT_{1A} and 5-HT_{1C} sites. Data are expressed as pK_D values (-log mol/l, mean of 2 to 5 experiments). Affinities to other 5-HT_1 and 5-HT_2 sites were determined as described earlier (Hoyer et al, 1985).

TABLE 1: Affinities of drugs to [^{3}H]5-HT recognition sites in pig and human brain in comparison to 5-HT_1- and 5-HT_2-sites

	pig	human	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}	5-HT_2
5-HT	8.13	7.89	8.51	7.63	7.52	5.53
Tryptamine	6.94	7.25	6.77	4.99	7.33	5.95
$5\text{-CH}_3\text{OT}$	8.25	8.01	8.04	6.40	7.36	5.59
5-CT	8.90	8.95	9.53	8.29	6.23	4.66
8-OH-DPAT	6.01	5.90	8.74	4.22	5.24	5.04
Buspirone	4.41	4.45	7.58	3.94	5.08	6.07
RU 24969	7.44	7.57	8.11	8.42	6.52	6.03
(-)Pindolol	4.75	4.79	7.71	7.75	4.23	4.35
(-)Propranolol	4.73	5.36	6.81	7.33	6.75	5.65
Mesulergine	5.86	5.23	6.23	4.88	8.79	8.42
Cinanserin	5.52	5.13	6.09	5.24	6.69	7.66
Ketanserin	5.22	4.54	5.86	5.72	7.01	8.86
Haloperidol	5.37	4.93	5.32	5.06	4.76	6.61

The data indicate that [^{3}H]5-HT labels in pig and human caudatum membranes a 5-HT_1 recognition site which is different from 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites. The pharmacology of this site is very similar in membranes of both species and resembles closely the 5-HT_{1D} site described recently in bovine brain by Heuring and Peroutka (1987). Functional correlates of this site may be found in the cardiovascular and gastro-intestinal systems where many '5-HT_1-like' receptor-mediated effects have been described but not fully characterised (Bradley et al, 1986).

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[^{3}H]-8-OH-DPAT BINDING IN RAT HIPPOCAMPUS AND STRIATUM

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8-Hydroxy-2-(di-N-propylamino) tetralin (8-OH-DPAT) has been widely used to characterise the 5-HT$_{1A}$ receptor subtype. In the rat hippocampus, ^{3}H-8-OH-DPAT labels the postsynaptic 5-HT$_{1A}$ receptor (Hall et al, 1985), but in the rat striatum ^{3}H-8-OH-DPAT labels presynaptic sites which may correspond either to an autoreceptor (Hall et al, 1985) or the 5-HT transporter (Schoemaker & Langer, 1986). We have compared the pharmacological profile of ^{3}H-8-OH-DPAT binding in the rat striatum and hippocampus.

Binding of ^{3}H-8-OH-DPAT was determined according to Peroutka (1986). Briefly, washed and pre-incubated rat striatal or hippocampal membranes were incubated with 1 or 2 nM ^{3}H-8-OH-DPAT respectively, for 20 min at 37°C in the presence of 4mM $CaCl_2$. The reaction was terminated by filtration over Whatman GF/B filters. Non-specific binding was defined using 10µM 5-HT.

In the hippocampus, buspirone, spiperone, spiroxatrine and ergotamine all displaced ^{3}H-8-OH-DPAT binding with high affinity (IC50's in nM: 13.0 ± 1.8; 67.3 ± 18; 8.56 ± 2.6; 4.12 ± 0.53 respectively-all results are means ± SEM from 3-6 experiments). The 5-HT$_{1B}$ agents (-) propranolol and quipazine (IC50's 144 ± 18 and 4170 ± 760nM) and the 5-HT$_{1C}$/5-HT$_2$ agent mianserin (IC50 4560 ± 1100 nM) had low affinity for this site, whereas the 5-HT uptake blockers paroxetine and fluvoxamine had no effect at 10 µM. All of these active agents displaced ^{3}H-8-OH-DPAT binding with slope factors close to unity.

In the striatum, ergotamine displaced 70-80% of specific ^{3}H-8-OH-DPAT binding with high affinity (IC50 6.23 ± 1.2 nM) and slope factor close to unity (1.05 ± 0.28) and had no effect on the residual binding up to 10 µM. Buspirone displaced 80% of striatal ^{3}H-8-OH-DPAT binding with high affinity (IC50 9.76 ± 3.1 nM) and the remainder with low affinity. The ergotamine-resistant component of specific ^{3}H-8-OH-DPAT binding was blocked with high affinity by paroxetine and fluvoxamine (IC50's 63.0 ± 10 and 32.5 ± 2.1 nM respectively, n=3-4). Striatal ^{3}H-8-OH-DPAT binding was blocked with low affinity by mianserin, (-)-propranolol and quipazine (IC50's 1303 ± 470, 512 ± 110 and 1217 ± 330 nM respectively; mean + SEM, n=3-5).

In conclusion, ^{3}H-8-OH-DPAT only labels the postsynaptic 5-HT$_{1A}$ receptor in rat hippocampal membranes. In rat striatal membranes, however, where the density of 5-HT$_{1A}$ sites is relatively low, ^{3}H-8-OH-DPAT appears to label both the 5-HT$_{1A}$ site and the 5-HT uptake site which presumably corresponds to the presynaptic component of ^{3}H-8-OH-DPAT binding in this tissue.

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EVIDENCE THAT THE 5-HT AGONISTS MCPP AND TFMPP CAUSE HYPOLOCOMOTION BY STIMULATING 5-HT1C RECEPTORS

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1-(3-chlorophenyl) piperazine (mCPP), 1-[3-(trifluoromethyl) phenyl] piperazine (TFMPP), and RU 24969 have all been reported to have high affinity for the 5-HT1B receptor (Hamon et al., 1986) and to cause anorexia in freely fed rats, an effect mediated by postsynaptic 5-HT1B receptors in the case of RU 24969 (Kennett et al., 1987). However at anorexigenic doses RU 24969 causes hyperlocomotion (Kennett et al., 1987), whilst TFMPP (Lucki and Frazer 1982) and mCPP cause hypolocomotion. We have now studied the mechanism of this latter effect.

Male Sprague-Dawley rats (200-250 g) were injected i.p. between 10.00 h and 16.30 h with either mCPP or TFMPP, dissolved in saline, and returned to their home cage. 20 min later the rats were placed singly in a wire mesh observation cage for 20 min and activity scored by hand held counter. Both drugs reduced cage crossings and rears whilst grooms were not significantly altered (mCPP, Table 1).

TABLE 1: Effect of mCPP metergoline and mianserin on activity in a novel cage.

Treatment (n)	Case crossings	Rears	Grooms
Saline (8)	62 ± 4	53 ± 3	4.9 ± 0.7
mCPP 2 mg/kg (8)	25 ± 6**	32 ± 5**	9.1 ± 2.6
5 mg/kg (8)	6 ± 1**	4 ± 2**	3.1 ± 0.7
Vehicle + Saline (8)	60 ± 3	51 ± 4	5.1 ± 0.6
Metergoline + Saline (8)	58 ± 5	44 ± 5	4.4 ± 1.0
Vehicle + mCPP (8)	5 ± 2**	4 ± 3**	3.2 ± 0.7
Metergoline + mCPP (8)	45 ± 2^{aa}	40 ± 4^{aa}	7.0 ± 0.5
Vehicle + Saline (8)	53 ± 5	48 ± 5	5.5 ± 0.9
Mianserin + Saline (7)	61 ± 2	38 ± 3	4.1 ± 1.0
Vehicle + mCPP (8)	7 ± 1**	4 ± 1**	4.1 ± 1.3
Mianserin + mCPP (7)	52 ± 5^{aa}	27 ± 6^{aa}	3.7 ± 0.3

Means ± SEM. Significantly different from saline treated group **$p < 0.01$ or from mCPP treated group $^{aa}p < 0.01$ by Dunnetts test following significant ANOVA test.

mCPP-induced (5 mg/kg) hypoactivity was blocked by metergoline (5 mg/kg), mianserin (2 mg/kg) (Table 1), and cyproheptadine (2 mg/kg) given 20 min earlier s.c., all of which have high affinity for the 5-HT1C site (Engel et al., 1986). However the 5-HT1A and 5-HT1B antagonists (-) pindolol (2 mg/kg) (±) cyanopindolol (8 mg/kg) and (-) propranolol (16 mg/kg) had no effect as did the specific 5-HT2 antagonists ketanserin (0.2 mg/kg) and ritanserin (0.63 mg/kg), the 5-HT3 antagonists ICS 205-930 (1 mg/kg), the α2 antagonist Idazoxan (1 mg/kg) and the 5-HT1A, 5-HT2 and DA antagonist spiperone (0.05 mg/kg). TFMPP-induced hypoactivity (5 mg/kg) was also mianserin but not cyanopindolol sensitive. None of the antagonists alone affected activity except spiperone which was inhibitory. The results suggest that mCPP and TFMPP cause hypolocomotion by agonist action at 5-HT1C receptors; RU 24969 has little affinity for these sites (Hoyer et al., 1985).

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FACILITATION BY ALPIDEM AND DIAZEPAM OF THE EVOKED RELEASE OF [^{3}H]-5-HT FROM RAT FRONTAL CORTEX

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In rat cortex, particularly in layer IV, high densities of recognition sites for flunitrazepam (Unnerstall et al., 1982) coexist with high concentrations of GABA (Parnavelas and McDonald, 1983). Varicosities containing 5HT are also present at this anatomical level (Beaudet and Descarriers, 1978). It was therefore of interest to examine in the rat cortex a possible involvement of benzodiazepine (BZ) and GABA receptors in the modulation of 5HT release. Slices of frontal cortex were labelled with ^{3}H-5HT. After 50 min of perfusion at 1 ml/min with Krebs' medium, two periods of electrical stimulation (S_1 and S_2) at 3 Hz, 2 msec, 30 mA, 2 min were applied with an interval of 44 min. Drugs were added either 8 or 24 min before S_2, or when indicated (S_1+S_2), 24 min before S_1. Total radioactivity released during S_1 represented 1.78 ± 0.09% (n=22) of tissue stores, and the ratio (S_2/S_1) between the two periods of stimulation was close to unity (Table 1).

Table 1 Electrically-evoked release of [^{3}H]-5HT from the rat frontal cortex

Drugs	nM	S_2/S_1		
			Ro 15-1788 10 nM (S_1+S_2)	GABA 100 µM (S_2)
Control	-	1.06 ± 0.04 (22)	1.25 ± 0.10 (5)	1.23 ± 0.16 (5)
Diazepam	1	0.94 ± 0.10 (7)	-	1.42 ± 0.10 (4)**
Diazepam	10	1.53 ± 0.08 (13)*	1.14 ± 0.10 (4)	-
Alpidem	1	1.24 ± 0.08 (6)	-	1.78 ± 0.10 (5)***
Alpidem	10	1.70 ± 0.22 (5)*	1.03 ± 0.13 (3)	-

Values are mean ± S.E.M. of ( ) experiments per group. Drugs were added 8 min before S_2.
* $p<0.001$ vs control, ** $p<0.05$ vs diazepam 1 nM, *** $p<0.005$ vs alpidem 1 nM.

The electrically-evoked release (Table 1) but not the spontaneous outflow of ^{3}H-5HT was significantly enhanced by diazepam and the imidazopyridine BZ_1 agonist alpidem (Arbilla et al., 1987). This facilitatory effect was prevented by the receptor antagonist Ro 15-1788 (Möhler and Richards, 1981) (Table 1). Exposure to GABA 100 µM did not modify the evoked release of ^{3}H-5HT (Table 1). Concentrations of GABA higher than 100 µM significantly facilitated the spontaneous release of ^{3}H-5HT (data not shown).

The electrically-evoked release of ^{3}H-5HT was also facilitated by the association of alpidem or diazepam with GABA in concentrations which did not modify transmitter release by themselves (Table 1). The evoked release of ^{3}H-5HT was not modified by diazepam 10 nM (0.95 ± 0.03, n=3) when added 24 min before S_2, indicating rapid desensitization.

In conclusion, the release of ^{3}H-5HT from rat frontal cortex is facilitated by activation of benzodiazepine receptors of BZ_1 subtype through a mechanism that desensitizes very rapidly and is probably associated with GABA receptors.

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NAFION COATED CARBON FIBRE ELECTRODES COMBINED WITH DIFFERENTIAL PULSE VOLTAMMETRY MEASURE 5-HT RELEASE IN VIVO?

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Recently, Adams and his coworkers reported that electrodes coated with the ion-exchange polymer Nafion may be useful for measuring changes in the level of 5-hydroxytryptamine (5HT) in the extracellular fluid of the brain (Brazell et al., 1987). However, their electrodes, used with chronoamperometry, had poor sensitivity and limited ability to differentiate oxidation of 5HT and 5-hydroxyindoleacetic acid (5HIAA) (Brazell et al., 1987). Here, we report that electrically pre-treated carbon fibre electrodes (CFE), electro-coated with Nafion and combined with differential pulse voltammetry (DPV) are capable of measuring basal levels of serotonin (5HT) in the dorsal raphe (DRN) and frontal cortex (FC) with no interference from 5-HIAA or uric acid (UA).

CFE's were manufactured from 30 μm carbon fibres (AVCO Speciality Materials, Mass., USA) and electrically treated as described previously (Crespi et al., 1984). The electrodes were then coated 3 times with Nafion as described by Brazell et al. (1987). No oxidation peaks were observed in vitro in a solution containing ascorbate (AA), dihydroxyphenylacetic acid (DOPAC), 5HIAA and UA in the concentration range 10^{-8}M to 10^{-5}M. However, when DA and 5HT (10^{-8}M) were added, peaks at ~60 mV (Peak A) and ~230 mV (Peak B) were observed. The size of Peak B was dependent on the concentration of 5HT present and was linear in the range tested (10^{-8} to $6x10^{-8}$M). These electrodes were then implanted into the DRN (n=5) or the FC (n=4) of chloral hydrate (300 mg/kg i.p.) anaesthetised rats (Sprague Dawley 260-280 g) and differential pulse voltammograms were recorded every 6 mins. In both regions we were able to record an oxidation peak at 225-230 mV (Peak B) which had an amplitude of ~I nA in the DRN and 0.5 nA in the FC, corresponding to an in vitro concentration of 10^{-8}M and $5x10^{-9}$M 5HT respectively which is similar to the concentrations of extracellular 5HT found with intracerebral dialysis (Marsden et al., 1986).

In both regions studied, local infusions of 5HIAA and UA (10^{-5}M in 2 μl over 20 s) approx. 1 mm behind the CFE did not affect Peak B height, but infusion of 5HT (10^{-6}M in 2 μl/20 s) was associated with a rapid increase in the peak of 60±10% in DRN (mean±S.D.) and of 130±24% in FC, which lasted for 1 scan only suggesting, as already observed in vitro, that there was no adsorbtion of 5HT by the electrode. Similarly, following infusion of KCl (0.1M in 2 μl/20 s) there was a rapid increase in Peak B height of 55±15% in the DRN and 170±60% in the FC (mean±S.D.). Peak height returned to baseline after 20 min. Administration of pargyline, a MAO inhibitor, (100 mg/kg i.p.) was associated with a large increase in Peak B height (80±40%, n=3, mean±S.D.) 20 mins after injection and the peak was still elevated after 60 min.

In conclusion, the data presented indicate that the electrode satisfies many of the criteria required to reach the conclusion that it can monitor extracellular 5HT in vivo. However, further experimentation is required to fully clarify that 5HIAA and UA have no influence on this oxidation peak that we attribute to 5HT.

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INTERACTION OF PHORBOL-12, 13-DIBUTYRATE AND FORSKOLIN WITH THE PRESYNAPTIC INHIBITORY 5-HT AUTORECEPTOR IN RAT HYPOTHALAMIC SLICES

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There is increasing evidence that second messengers are implicated in the modulation by presynaptic autoreceptors of the Ca^{++}-dependent exocytotic release of neurotransmitters. A negative coupling between presynaptic α_2-adrenoceptors and adenylate cyclase was described in rat brain slices (Schoffelmeer et al, 1986 ; Galzin et al, 1987). Activation of α_1 or $5HT_2$ postsynaptic receptors has been shown to stimulate phosphoinositide turnover in brain slices (Fisher and Agranoff, 1987). The present experiments were carried out to examine the effects of phorbol-12,13-dibutyrate, which activates protein kinase C, and forskolin, a diterpene which activates adenylate cyclase, on the electrically-evoked release of [^{3}H]-5HT and on the 5HT autoreceptor-mediated inhibition of release in rat hypothalamic slices.

Male rats (180-200 g) were killed by decapitation and hypothalamic slices prepared immediately. The endogenous stores of 5HT were labelled with 0.1µM [^{3}H]-5HT for 30 min. The slices were superfused with Krebs'solution at 37°C. Two periods (S_1 and S_2) of electrical stimulation were applied (3 Hz, 2 msec, 20 mA, 2 min) with an interval of 44 min. Drugs were added 20 min before S_1 or S_2. Exposure to phorbol-12,13-dibutyrate (0.01 to 10µM) 20 min before S_2 increased in a concentration-dependent manner the electrically-evoked release of [^{3}H]-5HT, without affecting the spontaneous outflow of radioactivity (maximal increasing effect 315 % at 10µM). When phorbol-12, 13-dibutyrate (0.1 and 1µM) was added to the medium 20 min before S_1, the control ratio S_2/S_1 was not different from unity (S_2/S_1 = 1.07 $\pm$ 0.06 n = 3, and S_2/S_1 = 0.98 $\pm$ 0.10, n = 3 respectively in the presence of 0.1 and 1µM phorbol-12, 13-dibutyrate). The 5HT receptor agonist 5-methoxytryptamine (5-MeO-T) inhibited in a concentration-dependent manner the electrically-evoked release of [^{3}H]-5HT (IC_{50} = 0.56µM). In the presence of phorbol-12,13-dibutyrate (0.1 or 1µM), the concentration-effect curve for 5-MeO-T was significantly shifted to the right (IC_{50} for 5-MeO-T = 1.32µM and 12.6µM, in the presence of 0.1 and 1µM phorbol ester respectively). The 5HT receptor antagonist methiothepin increased in a concentration-dependent manner the electrically-evoked release of [^{3}H]-5HT, and this effect was significantly less pronounced in the presence of phorbol-12,13-dibutyrate (S_2/S_1 = 2.28 $\pm$ 0.12, n = 5 for methiothepin 1µM, $p<0.001$ when compared to the ratio in the presence of 0.1µM phorbol-12, 13-dibutyrate : S_2/S_1 = 1.43 $\pm$ 0.13, n= 5).

Similar experiments were carried out in the presence of forskolin, an activator of adenylate cyclase which increased by itself the electrically-evoked release of [^{3}H]-5HT (Galzin et al, 1987). When forskolin (1µM) was added to the medium 20 min before S_1, the control ratio S_2/S_1 was : 0.86 $\pm$ 0.03, n=10. Under these conditions, the inhibitory effect of 5-MeO-T on the electrically-evoked release of [^{3}H]-5HT was potentiated, with a significant shift to the left of the concentration-effect curve (IC_{50} = 22nM in the presence of 1µM forskolin).

These results support the view that the modulation of the release of [^{3}H]-5HT by presynaptic inhibitory 5HT autoreceptors may involve an interaction with the phosphoinositide cycle and protein kinase C-dependent mechanisms. Moreover, activation of adenylate cyclase potentiates the inhibitory effects of 5-MeO-T on [^{3}H]-5HT release and it remains to be clarified if this effect is related to the protein kinase C-dependent modulation of transmitter release.

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CALCITONIN GENE-RELATED PEPTIDE INHIBITS A DISTINCT NEURAL PATHWAY SUPPLYING INTESTINAL CIRCULAR MUSCLE

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In the guinea-pig digestive tract, calcitonin gene-related peptide (CGRP) is present both in intrinsic (enteric) and extrinsic (primary afferent) neurones. The localisation of CGRP-containing nerve endings to intestinal nerve plexuses and muscle layers (Costa et al., 1986) suggests a mediator role of this peptide in the control of intestinal motility. Previous work (Barthó et al., 1987) has shown that the predominant effect of CGRP on the longitudinal muscle of the guinea-pig small intestine (GPSI) is a relaxation caused by a direct action on the muscle. In the present study the motor effects of CGRP on the circular muscle (CM) of the GPSI were examined.

The experiments were made on segments of GPSI, 6-7 cm in length, which bathed in Tyrode solution at 37 °C; the mechanical activity of the CM was recorded under isotonic conditions. CGRP (3-100 nM) elicited a series of phasic contractions, the maximal amplitude of which amounted to about 40 % of the contraction caused by a maximally effective concentration of carbachol (6 µM). The contractile effect of CGRP waned within 1 min; it was prevented by tetrodotoxin (0.3 µM) or atropine (0.7 µM).

To study the enteric ascending reflex contraction of the CM, an intraluminal balloon was inflated to a diameter of 7.5 mm and the ensuing contraction of the CM on the side orally to the balloon was recorded. This contraction was entirely due to activation of a neural reflex since it was abolished by tetrodotoxin (0.3 µM). CGRP (0.3-100 nM) had no effect on the ascending reflex contraction.

When atropine (0.7 µM) was added to the bath, the ascending reflex contraction was first reduced to less than 5 % of the control response. However, following a 60 min presence of atropine, the reflex contraction recovered to about 25 % of the original response. This "atropine-resistant" reflex contraction was abolished by tetrodotoxin (0.3 µM), hexamethonium (130µM), or the tachykinin antagonist "spantide" (30 µM). From these observations it would appear that both preganglionic cholinergic and postganglionic tachykinin-containing neurones are involved in the "atropine-resistant" reflex contraction of the CM. CGRP (0.3-30 nM) inhibited this "atropine-resistant" reflex contraction in a concentration-dependent manner, the highest concentrations of CGRP leading to a nearly complete abolition of the reflex. The concentrations of CGRP causing a 50 % reduction of the reflex were in the range of 1-3 nM.

These findings can be summarized as follows. (1) CGRP exerts a weak excitatory action on cholinergic motoneurones supplying the CM. (2) Since in the absence of atropine the ascending reflex contraction is not affected by CGRP it is inferred that the activity of the circular muscle, unlike that of the longitudinal muscle (Barthó et al., 1987), is not suppressed by CGRP. (3) Hence, the inhibitory effect of CGRP on the "atropine-resistant" reflex contraction of the CM suggests that CGRP can selectively inhibit a distinct enteric nervous pathway supplying the circular muscle.

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REGIONAL HAEMODYNAMIC EFFECTS OF RAT CALCITONIN GENE-RELATED PEPTIDE (RAT-CGRP) IN CONSCIOUS, UNRESTRAINED, BRATTLEBORO RATS

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Bolus doses or i.v. infusions of human α-CGRP cause hypotension in conscious rats (Craig et al., 1986; Marshall et al., 1987). The hypotensive response to CGRP infusion is maintained, but the regional haemodynamic effects of this manoeuvre are unknown. Now we have investigated the cardiovascular effects of a 1 h infusion of rat-CGRP (6 nmol rat^{-1} h^{-1}) in 9 conscious, male, Brattleboro rats instrumented with pulsed Doppler probes (Haywood et al., 1981) and intravascular catheters. Animals were anaesthetized with sodium methohexitone (60 mg/kg i.p.) and had miniaturized pulsed Doppler probes sutured around superior mesenteric and left renal arteries and the distal abdominal aorta. Animals were allowed at least 7 days to recover by which time those that went through the experimental protocol were healthy and gaining weight and had acceptable signals from all 3 probes. The day before the experiments animals were briefly re-anaesthetized (sodium methohexitone, 40 mg/kg i.p.) and had intra-arterial (distal aorta via the caudal artery) and intravenous catheters implanted. The experiment consisted of a 30 min baseline recording of B.P., HR and regional Doppler shift signals, after which the CGRP infusion was started and continued for 1 h. All variables were monitored continuously during infusion and for 1 h after infusion. Changes in regional vascular resistances (%) were calculated by dividing mean B.P. by mean Doppler shift (Haywood et al., 1981).

Infusion of rat-CGRP caused a sustained fall in mean B.P. (resting 112±3; 5 min 70±4; 60 min 71±2 mmHg) and a tachycardia (resting 341±8; 5 min 475±17; 60 min 425±22 beats/min). The table shows the corresponding changes in regional vascular resistances.

Table. Changes (%) in renal, mesenteric and hindquarters vascular resistances with rat-CGRP infusion (mean (s.e.m.))

	Renal	Mesenteric	Hindquarters
5 min	-22 (4)*	+10 (10)	-44 (5)*
60 min	+3 (8)	+43 (11)*	-50 (3)*

* denotes a significant change from baseline ($P<0.05$).

These results indicate that the sustained hypotensive effect of intravenous infusion of rat-CGRP is against a background of a changing regional haemodynamic profile. Within 5 min of the onset of infusion of rat-CGRP there were significant renal and hindquarters vasodilatations, but no change in mesenteric resistance. After 60 min infusion, renal resistance was back to baseline levels, but the hindquarters vasodilatation was similar to that seen at 5 min. Since at 60 min there was a significant mesenteric vasoconstriction, it is possible that the persistent hypotension was contributed to by a reduction in cardiac output.

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EFFECTS OF BRETYLIUM ON ELECTRICAL ACTIVITY IN SYMPATHETIC POSTGANGLIONIC NERVE TERMINALS

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Recently a method has been developed in which the relationship between the nerve terminal action potential and transmitter release from sympathetic postganglionic nerve terminals can be studied (Brock & Cunnane, 1987). This approach has now been used to investigate the mechanism of the inhibitory action of the adrenergic neurone blocker, bretylium, on sympathetic neurotransmission.

Bretylium, at concentrations (10^{-6} - 3 x 10^{-6}M) known to inhibit transmitter release in many sympathetically innervated tissues (Haeusler et al, 1969), had marked effects on the configuration of the nerve terminal action potential in the isolated vas deferens of the guinea-pig (Fig. 1). Evoked transmitter release was only abolished when bretylium induced local failure of action potential propagation. Pretreatment of tissues with the uptake1 inhibitor, desipramine, markedly reduced the inhibitory effects of bretylium.

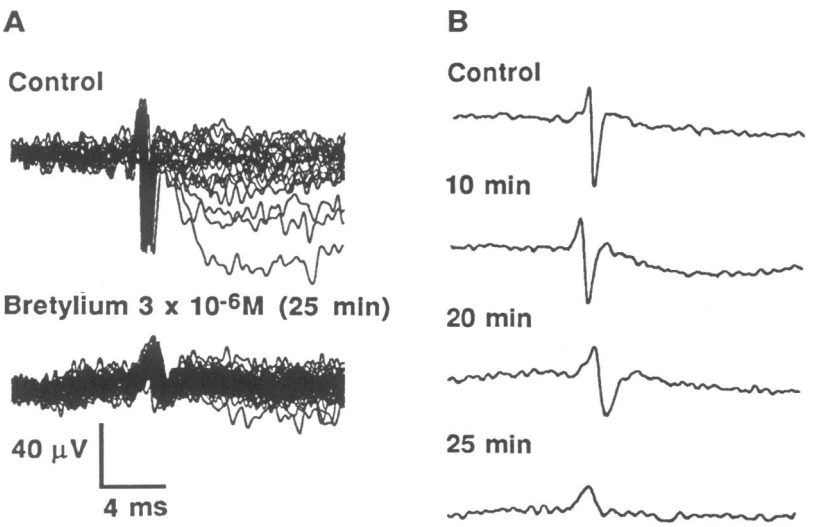

Figure 1. Effects of bretylium (3 x 10^{-6} M) on evoked electrical activity recorded extracellularly from the guinea-pig vas deferens (A) The nerve terminal action potential and evoked release (50 pulses, 1 Hz) (B) averages of 20 records at different times during the application of bretylium.

These results support the view that bretylium is accumulated in sympathetic nerve terminals by neuronal uptake1, where it inhibits transmitter release presumably by acting as a local anaesthetic.

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THE EFFECTS OF A NOVEL ACE-INHIBITOR/β-ADRENOCEPTOR BLOCKING AGENT ON PLASMA RENIN ACTIVITY IN THE DOG

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A consistent finding after angiotensin converting enzyme (ACE) inhibition, is a significant elevation in plasma renin activity (PRA). Although it is not clear by which mechanism(s) ACE-inhibition evokes this renin response, it has been demonstrated that this elevation is sensitive to β-adrenoceptor blockade (Pickering et al., 1982). We have therefore examined the effects of a novel dual acting ACE-inhibitor/β-adrenoceptor blocking agent BW B385C (Allan et al., 1987) on the control of PRA following acute administration to anaesthetised or conscious dogs.

In anaesthetised dogs (n=9) i.v. infusion of enalapril (total dose 2 $mgkg^{-1}$) significantly reduced plasma ACE-activity (PAA), by 80%, and diastolic blood pressure (DBP) (114 ± 7 to 93 ± 5 mmHg), without changing LVdP/dt or heart rate (HR). Enalapril also significantly increased PRA (6.6 ± 1.3 to 15.3 ± 2.8 ng Al ml^{-1} hr^{-1}). In a second group (n=6) i.v. infusion of the same dose of enalapril, in combination with the β-adrenoceptor blocking agent, atenolol (total dose of 1 $mgkg^{-1}$) caused similar reductions in PAA (77%) and DBP (114 ± 5 to 99 ± 5 mmHg). In addition this combination also caused significant reductions in LVdP/dt (4500 ± 403 to 2375 ± 240 mmHg sec^{-1}) and HR (179 ± 6 to 136 ± 3 bt min^{-1}). However, unlike enalapril alone, this combination significantly reduced PRA (8.2 ± 2.2 to 2.6 ± 1.1 ng Al ml^{-1} hr^{-1}). In a third group (n=8) i.v. infusion of BW B385C (total dose 2 $mgkg^{-1}$) caused significant reductions in PAA (78%), DBP (121 ± 3 to 96 ± 5 mmHg), LVdP/dt (5078 ± 430 to 3375 ± 177 mmHg sec^{-1}) and HR (187 ± 11 to 161 ± 5 bt min^{-1}). These effects were comparable to those produced by the combination. In 5/8 animals, BW B385C caused no change in PRA (4.4 ± 0.3 to 4.2 ± 0.6 ng Al ml^{-1} hr^{-1}) but in the remaining 3 animals there was a marked increase (6.7 ± 0.4 to 38.9 ± 4.3 ng Al ml^{-1} hr^{-1}).

In a further study in Na^{+}-depleted conscious dogs (n=4) enalapril (1 $mgkg^{-1}$ i.v.) caused a significant increase in PRA (3.2 ± 0.5 to 23.5 ± 1.8 ng Al ml^{-1} hr^{-1}). Using a cross-over design, in these same animals, BW B385C (1 $mgkg^{-1}$ i.v.) was found to produce a similar effect (2.5 ± 0.5 to 35.1 ± 12.9 ng Al ml^{-1} hr^{-1}). No haemodynamic measurements were made in these animals.

The control of renin release is complex, and several other mechanisms besides the renal sympathetic innervation (via β-adrenoceptors) have been identified (Keeton and Campbell, 1981). Our data appears to reflect this complexity. In those animals where BW B385C (and an ACE-inhibitor/β-adrenoceptor blocking agent combination) does not increase PRA, then it may be concluded that the renal sympathetic innervation provides the major contribution to the elevation of PRA associated with ACE-inhibition. However, in those animals where PRA is increased, the opposite conclusion may be drawn. Clearly further studies are needed.

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THE PRECLINICAL PHARMACOLOGY OF A NOVEL DUAL-ACTING ANTI-HYPERTENSIVE AGENT, BW B385C

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The pharmacological properties of a novel dual acting angiotensin converting enzyme (ACE) inhibitor/β-adrenoceptor blocking agent, BW A575C have been previously described (Allan et al., 1987). This compound consists of a mixture of diastereoisomers and therefore biological activity may reside within different isomers of the hybrid components. The present study describes the preclinical pharmacology of BW B385C, the *S,S,S,S.* diastereoisomer derived from the aforementioned mixture.

In vitro studies using a partially purified preparation of ACE obtained from rabbit lung demonstrated BW B385C to have an inhibitory potency (IC_{50}) of 1.2 ± 0.18nM. In a guinea-pig right atrial preparation, BW B385C produced a competitive blockade of heart rate responses to isoprenaline with a pK_b of 6.7 ± 0.08. Intravenous administration of BW B385C to conscious rats and dogs produced a significant rightwards displacement of both angiotensin I induced pressor responses and isoprenaline induced heart rate responses. A comparison of dose ratios obtained for inhibition of both responses demonstrated that in both species BW B385C was approximately 10 times more active as an ACE inhibitor than as a β-adrenoceptor blocking agent. In the conscious dog BW B385C administered orally produced a similar profile of dual activity as that observed when the drug was administered intravenously (see Table 1).

Table 1 Dose ratios and 95% confidence limits for inhibition of angiotensin I-induced pressor responses (ACE) and isoprenaline induced tachycardia (β) in conscious dogs and rats following pretreatment with BW B385C.

	ACE	β	ACE/β ratio
Dog			
1 mg kg^{-1} i.v.	111 (80-156)	10.1 (7.5-14.9)	11
10 mg kg^{-1} p.o.	103 (74-144)	8.4 (5.6-12.3)	12.3
Rat			
1 mg kg^{-1} i.v.	29.5 (20-42.7)	3.1 (2.0-4.8)	9.5

In a series of experiments designed to evaluate the chemical stability of BW B385C, the technique of fast atom bombardment-mass spectroscopy (FAB-MS) has been used. In this study, BW B385C was administered intravenously (1 mg kg^{-1}) and orally (10 mg kg^{-1}) to conscious dogs. Plasma samples were obtained at timed intervals (0-6h) after drug administration and analysed directly using FAB-MS by appropriate molecular ion monitoring of the parent molecular species and its chemical templates. The results of this study demonstrated that the parent molecule appeared predominantly in the plasma following either intravenous or oral administration with very little cleavage to the hybrid templates (approximately 5%).

In conclusion, BW B385C displays dual activity as an ACE inhibitor with β-adrenoceptor blocking properties both in vitro and in vivo. The agent is orally active and there is no evidence to support substantial metabolism to separately active templates following either intravenous or oral administration.

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EXPRESSION OF DES-ARG^9-BK SENSITIVE (B1) RECEPTORS IN VIVO AND IN VITRO BY ANGIOTENSIN CONVERTING ENZYME INHIBITORS

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It has been reported that an i.v. injection of 10 µg of bacterial lipopolysaccharide (LPS) from E.Coli to rabbits induces in 5-20 h a hypotensive response to the selective B1-receptor agonist des-Arg^9-BK, an effect not observed in control animals (Regoli et al., 1981). Endotoxins can activate the kinin system (Rothschild and Castania, 1968). An infusion of bradykinin (BK) and captopril (CAPT) can induce a similar effect to that seen with endotoxin (Akinosi and Whalley, 1986). This study compares the effect of enalapril (ENAL) and teprotide (TEP) with CAPT, BK and LPS on the hypotensive response to the selective B1 receptor agonist des-Arg^9-BK in vivo and the responsiveness of the B1-receptor containing tissue, the rabbit aorta in vitro.

Male New Zealand White rabbits (2 - 2.5 kg) were treated with either: LPS from E.Coli, 10 µg i.v.; CAPT, 5 mg kg^{-1} i.v.; TEP, 5 mg kg^{-1} s.c.; ENAL, 5 mg kg^{-1} i.v.; BK, 5 µg min^{-1} i.v. for 30 min. 18-20 h later the rabbits were anaesthetised and blood pressure recorded (Akinosi and Whalley, 1986). Dose response curves were produced to BK, des-Arg^9-BK, angiotensin I (AI) and acetylcholine (ACh). Separate groups of pretreated rabbits were killed, the aorta removed, and vascular rings (intact) prepared and mounted under 2g T in 4 ml baths containing Kreb's solution at 37°C and bubbled with 95% O_2/5% CO_2. After 1 h equilibration concentration effect curves were constructed to either des-Arg^9-BK or 5-HT.

	CONT	BK infusion	CAPT	ENAL	TEP	LPS
a) in vivo						
BP	65.6 ± 1.8	71.1 ± 6.1	57.9 ± 3.8	57.9 ± 7.3	63.1 ± 2.8	59.5 ± 8
d-A9	>1 x 10^{-7}	5.9 ± 2.8* x10^{-8}	2.8 ± 0.6* x10^{-8}	4.9 ± 1.8* x10^{-8}	6.5 ± 4.3* x10^{-8}	6.4 ± 2.1* x10^{-9}
ACh	3.2 ± 1.0 x 10^{-10}	2.1 ± 0.6 x10^{-10}	2.3 ± 0.6 x10^{-10}	6.9 ± 1.4 x10^{-10}	5.1 ± 1.4 x10^{-10}	9.3 ± 7 x10^{-10}
b) in vitro						
d-A9	0.032±0.01	0.330±0.06*	0.256±0.05*	1.004±0.04*	0.611±0.1*	1.065±0.1*
5-HT	2.53 ±0.3	3.26 ±0.4	2.65 ±0.2	3.08 ±0.1	2.72 ±0.1	3.45 ±0.1

The results are shown in the table. Resting BP's (mm Hg) are shown for each group. The in vivo results represent the dose (moles) of des-Arg^9-BK (d-A9) or ACh to produce a 15% fall in BP. Responses to BK or AI were not significantly different between the groups. The in vitro data represent the gT pull by des-Arg^9-BK or 5-HT. * = $P < 0.05$, significantly different from control, $n = 4$-15. The B1 receptor antagonist des-Arg^9-Leu^8-BK at 20 µg kg^{-1} min^{-1} produced significant ($P < 0.05$) inhibition of responses to des-Arg^9-BK but not ACh in vivo and at 10^{-5}M significantly ($P < 0.05$) inhibited des-Arg^9-BK but not 5-HT in vitro.

These results demonstrate that various angiotensin converting enzyme inhibitors have the capacity to selectively express Kinin B1-receptors in vivo. This may reflect their capacity to elevate endogenous kinin levels and may be consistent with the observed incidence of skin eruptions in man (Luderer et al, 1982)

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THE INTERACTION BETWEEN α- AND β-ADRENOCEPTORS IN THE RAT ISOLATED THORACIC AORTA AND THE ACTION OF PRAZOSIN

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We have reported that prazosin exerts an unsurmountable inhibition of the initial component of contractions elicited by noradrenaline (NA) in the rat thoracic aorta (Downing et al., 1981). In preliminary experiments, we noted that both propranolol and phentolamine were capable of reversing the effect of prazosin. We have, therefore, investigated the interaction and relative importance of α- and β-adrenoceptors in this unusual action of prazosin.

Helical strips of the thoracic aorta from Male Wistar rats (220-250g) were prepared as previously described (Downing et al., 1981). Following pretreatment with 1μM phenoxybenzamine for 30 min, preparations were repeatedly washed and a response to 20mM KCl elicited. NA was added cumulatively and the relaxation produced expressed as a percent of the KCl contraction. This was repeated 60 min after exposure to 1μM propranolol. In another series of experiments, strips exposed to 5nM prazosin were contracted with a supramaximal concentration of NA (100μM) in the absence and the presence of increasing concentrations of either propranolol or phentolamine. The time to 30% of maximum (T_{30}) was determined for each contraction. The effect of propranolol was also studied in aortic strips from 6 month old rats, since the function of vascular β-adrenoceptors is impaired in old rats (Fleisch et al., 1970).

NA (0.1μM - 100μM) effected a concentration-dependent relaxation of KCl -induced contractions in phenoxybenzamine-treated strips and the maximum relaxation (62.2±4.4%, n=8) was abolished by 1μM propranolol. Contractions to 100μM NA in the presence of 5nM prazosin were characterized by a delay in the onset of responses (T_{30} = 153±17 sec, n=9; compared to 20±2 sec, n=5, in control preparations). Propranolol (0.01μM - 10μM) and phentolamine (0.01μM - 1μM) reduced T_{30} in a concentration-dependent manner, but the slope of the concentration-effect curves differed; thus, 0.01μM propranolol produced a greater reduction of the prazosin induced delay than 0.01μM phentolamine ($p<0.05$), while the opposite was observed for 1.0μM propranolol and 1.0μM phentolamine. Furthermore, although responses to 100μM NA in the presence of 1μM phentolamine and 5nM prazosin were biphasic, those elicited in the presence of 10μM propranolol and 5nM prazosin were not. Finally in 6 month old rats propranolol did not affect the prazosin-induced delay (T_{30} = 86±9 sec, n=4) of responses to 100μM NA.

Based upon the above differences, propranolol and phentolamine appear to act at β- and α-adrenoceptors respectively, to influence the action of prazosin on NA contractions in the rat aorta. Moreover, although NA stimulates β-adrenoceptors on the rat aorta, it is only in the presence of prazosin (by virtue of its ability to selectively impair the initial component of contractile responses; Downing et al., 1985) that the consequence of concomitant stimulation of the functionally antagonistic α- and β-adrenoceptors becomes apparent.

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CHARACTERISATION OF THE 5-HT_1-LIKE RECEPTOR MEDIATING RELAXATION OF PORCINE VENA CAVA

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The aim of this study was to further characterise the 5-hydroxytryptamine (5-HT) receptor mediating relaxation of isolated rings of neonatal porcine abdominal vena cava (Trevethick, et al., 1986).

Isolated rings of vena cava were mounted for isometric recording and contracted with α-methyl-5-HT, histamine or U46619 at concentrations giving 50-75% of the maximum response. Relaxant agonist potencies compared to 5-HT were then determined as decribed by Trevethick et al (1986). Antagonists were examined against the relaxant effect of 5-carboxamidotryptamine (5-CT) in preparations where tone was induced by histamine or U46619. Results are arithmetic means ± s.e.m. of 'n' observations.

Agonist potencies for relaxation of the porcine vena cava and the potency of methysergide as an antagonist were similar to those previously reported for isolated cat saphenous vein and guinea-pig ileum preparations (Feniuk et al., 1983; 1984). Thus, 5-CT (EC_{50} 4±3 nM; n=48) was some 20-times more potent than 5-HT (EC_{50} 100±70nM; n=9), whereas 2-methyl-5-HT, n,n-dipropyl-5-CT, 8-OH-DPAT and GR43175 (Humphrey et al, 1987) were either weakly active or inactive at concentrations up to 10μM. The relaxant effect of 5-CT (but not that of PGE_2) was antagonised in a concentration-dependent manner by methiothepin, methysergide and spiperone, but not by 1μM cyanopindolol (Table 1). The 5-HT_3-receptor antagonist, GR38032 (Brittain et al, 1987), ketanserin, GR43175 and 8-OH-DPAT were also inactive as antagonists of 5-CT (each at 1μM).

Table 1: Antagonism of 5-CT-induced relaxation of isolated rings of porcine vena cava contracted with U46619 or Histamine

Antagonist	pA_2	Slope	n
Methiothepin	9.00 (±0.21)	1.50 (±0.85)	5
Methysergide	7.94 (±0.16)	0.95 (±0.11)	4
Spiperone	7.52 (±0.27)	0.87 (±0.11)	4
Cyanopindolol	<6.0	-	4

These results suggest that the 5-HT_1-like receptor mediating relaxation of porcine vena cava is similar to that in cat saphenous vein and guinea-pig ileum, but is different from the $5HT_1$-like receptor mediating contraction of dog saphenous vein (Humphrey et al, 1987) and the currently described $5HT_1$ ligand binding sites (Heuring & Peroutka, 1987).

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DOWN-REGULATION OF RAT CORTICAL β-ADRENOCEPTORS BY THE PUTATIVE ANTIDEPRESSANT BTS 54 524: CONTRIBUTION OF METABOLITES

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BTS 54 524 (N-1-(1-(4-chlorophenyl)cyclobutyl)-3-methylbutyl-N,N-dimethylamine HCl,H_2O) has an acute pharmacological profile consistent with an antidepressant effect, probably due to monoamine uptake inhibition (Buckett et al, 1987a). It also causes rapid and potent down-regulation of rat cortical β-adrenoceptors after oral administration for only three days (Buckett et al, 1987b). We now report the contribution of two metabolites, the secondary (BTS 54 354) and primary (BTS 54 505) amines, to this activity by studying both their ability to induce β-adrenoceptor down-regulation and their acute pharmacological profile.

Putative or standard antidepressants were examined for their acute ability to reverse reserpine-induced hypothermia in mice, to prevent reserpine ptosis in rats and to increase the mobility of mice in the Porsolt test, a possible model of depression. The effect of 3 days oral administration of the compounds on rat cortical β-adrenoceptors was evaluated as previously described (Buckett et al, 1987b) and they were also examined for their ability to block ^{14}C-monoamine uptake into rat cerebral tissues in vitro.

BTS 54 524, and its secondary (BTS 54 354) and primary (BTS 54 505) amine metabolites, each potently down-regulated cortical β-adrenoceptors after three days of oral administration to rats (Table 1). The compounds showed similar potent activity in acute behavioural tests predictive of potential antidepressant activity. However, the three compounds showed a differential ability to inhibit in vitro noradrenaline (NA), 5-hydroxytryptamine (5HT) and dopamine (DA) uptake with BTS 54 354 and BTS 54 505 being up to 100-fold more active than BTS 54 524 (Table 1). Standard antidepressants were generally weaker than the BTS compounds in the in vivo tests (Table 1).

Table 1 Activity of compounds in in vivo models indicative of antidepressant efficacy and against 14C-monoamine uptake in vitro

Compound	β-binding (dose;Bmax decrease)	Reserpine prevention (ED50)	Reserpine reversal (ED50)	Porsolt test (LED)	In vitro uptake inhibition NA (IC50)	5HT (IC50)	DA (IC50)
BTS 54 524	1.8; 21%	0.6	1.8	10	2.2	477	11.0
BTS 54 354	1.8; 19%	0.6	4.1	10	0.097	2.9	0.14
BTS 54 505	3.3; 23%	1.1	4.0	10	0.08	4.3	0.36
Nomifensine	10; 26%	1.1	2.2	10	0.18	>20	0.24
Imipramine	10; 19%	10	17	30	0.7	2.1	18.0
Dothiepin	100; 15%	159	67	100	1.5	4.6	8.1

β-Binding: oral dose (mg/kg) given for 3 days prior to β-adrenoceptor analysis, Bmax decrease $P<0.05$. Reserpine prevention and reversal values are oral doses (mg/kg) required to inhibit the effect of reserpine by 50% (n=8-10 animals/dose). Porsolt test value is the lowest effective oral dose (mg/kg) to increase mobility by 50% compared with concurrent controls (n=10-15 mice/dose). NA, 5HT and DA (in vitro uptake) values are the IC50 concentrations (μM) required to inhibit 14C-monoamine uptake by 50% (n=3-4 experiments).

BTS 54 524 and two of its metabolites exhibit a similar pharmacological profile apart from marked differences in potency as monoamine uptake inhibitors in vitro. The potent NA reuptake blocking activity of the secondary and primary amine metabolites probably contributes to the rapid and potent down-regulation of rat β-adrenoceptors induced by the putative antidepressant BTS 54 524.

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RAT FRONTAL CORTICAL $GABA_B$ AND $5\text{-}HT_2$ BINDING SITES FOLLOWING CHRONIC ORAL ADMINISTRATION OF TWO ANTIDEPRESSANT DRUGS

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Increases in the number of frontal cortical $GABA_B$ binding sites have recently been reported in rodents following chronic antidepressant administration (Lloyd et al., 1985; Suzdak and Gianutsos, 1987). In our initial investigations we found that chronic administration of desmethylimipramine (DMI) or zimelidine (ZIM) did not alter the number or affinity of $GABA_B$ binding sites in the rat whole cortex (Cross and Horton, 1986). We have now extended this study to investigate the effects of higher doses of these two drugs on $GABA_B$, and for comparative purposes on $5HT_2$ binding sites in the rat frontal cortex.

Male Wistar rats were dosed via gastric tube twice daily for 21 days with DMI, ZIM (5 and 10mg/kg) or vehicle, or given a single dose of drug (10mg/kg). The animals were killed 24h after the final dose and frontal cortices were immediately dissected. $GABA_B$ binding was performed as previously described (Cross and Horton, 1986). For $5HT_2$ binding, frontal cortices previously frozen at -20°C were prepared and assayed as described by Leysen et al., 1982 using 8 concentrations (0.1-5nM) (^{3}H)-ketanserin. The maximal number of binding sites (Bmax) and equilibrium dissociation constants (K_D) were determined on tissue from individual animals using non-linear regression.

Both chronic DMI and ZIM significantly reduced the number of frontal cortical $5HT_2$ binding sites but had no effect on $GABA_B$ sites at either dose (Table 1). The affinity of $5HT_2$ and $GABA_B$ binding sites were unaltered by acute or chronic administration of either drug. A single dose of DMI did not affect the number of $5HT_2$ or $GABA_B$ sites, whereas ZIM decreased $5HT_2$ but not $GABA_B$ sites.

Table 1: Effects of DMI and ZIM on Bmax of $5HT_2$ and $GABA_B$ binding sites.

TREATMENT GROUPS	$5HT_2$		$GABA_B$	
	DMI	ZIM	DMI	ZIM
Vehicle-treated controls	289±13		1.07±0.07	
5mg/kg x 2/day for 21 days	220±7*	240±13	1.09±0.11	1.17±0.20
10mg/kg x 2/day for 21 days	186±8***	226±11**	1.01±0.14	1.19±0.12

Bmax = fmole/mg protein for $5HT_2$ and pmole/mg protein for $GABA_B$. Results are means ± s.e.m. For single dose n=3-4; for chronic dosing n=6-8. Significant differences from controls are denoted by * P<0.05, ** P<0.01, *** P<0.001 (Student's t test).

These results substantiate and extend our initial findings and indicate that the previously reported increases in frontal cortical $GABA_B$ binding sites may not be as consistent an effect of chronic antidepressant administration as originally thought.

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ABSENCE OF A CIRCADIAN RHYTHM IN THE BMAX OF [^{3}H]-IMIPRAMINE BINDING IN PLATELETS FROM HEALTHY VOLUNTEERS DURING WINTER

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The high affinity binding sites for [^{3}H]-imipramine (IMI) present in brain and platelets are associated with the neuronal uptake for serotonin and may represent a biological marker in depression (Langer et al., 1987). Comparisons between Bmax of [^{3}H]-IMI binding in platelets of untreated severely depressed patients and control volunteers carried out in different laboratories showed some discrepancy. We reported recently on the existence of a circadian rhythm of the Bmax of [^{3}H]-IMI binding in blood platelets of rabbits maintained on a long photoperiod (14 h light-10 h dark) (Galzin & Langer, in press). The existence of a circadian rhythm of 5HT uptake in the blood platelets of normal controls (Wirz-Justice and Richter 1979), and the recent report on the purification of a plasmatic glycoprotein which may act as an endogenous modulator of platelet [^{3}H]-IMI binding and serotonin uptake (Abraham et al., 1987) prompted the present study, which was carried out in order to compare day and night values of [^{3}H]-IMI binding parameters in blood platelets of healthy volunteers.
Blood samples from normal volunteers (7 male, 28 to 37 years old ; mean age : 33 $\pm$ 1), in good health and free of drugs, were collected on a 24 h cycle, at 07.00, 12.00, 19.00, 02.00 and 07.00 h (GMT) the next morning. All the samples were obtained between December 12th 1986 and February 6th 1987. Platelet pellets were prepared immediately after blood sampling, according to the method of Poirier et al. (1984). [^{3}H]-IMI binding assays were performed according to Langer et al. (1980) but with 10 uM fluoxetine to define non-specific binding. Binding experiments on samples obtained from one control were carried out in parallel the same day.

Table 1 [^{3}H]-IMI binding parameters in blood platelets from healthy volunteers on a 24 h cycle.

Time of sampling (GMT)	n	Bmax (fmol/mg/prot)	Kd (nM)
07.00	7	622 $\pm$ 37	0.37 $\pm$ 0.03
12.00	7	772 $\pm$ 68	0.43 $\pm$ 0.05
19.00	7	636 $\pm$ 56	0.35 $\pm$ 0.05
02.00	6	753 $\pm$ 91	0.57 $\pm$ 0.13
07.00	6	749 $\pm$ 67	0.39 $\pm$ 0.04

No significant differences were found in the parameters of [^{3}H]-IMI binding in blood platelets of healthy volunteers obtained between December and February. These results are in contrast with the pronounced differences found between night and day values for the Bmax of [^{3}H]-IMI in blood platelets of rabbits maintained on a long photoperiod (Galzin and Langer, in press). A similar study in control volunteers during summer is necessary to validate the conclusion that [^{3}H]-IMI binding in human platelets does not show circadian variations.

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COMPARISON OF α_2- AND β-ADRENOCEPTOR FUNCTION IN MALE AND FEMALE RATS AND EFFECTS OF REPEATED ADMINISTRATION OF ECS OR DESIPRAMINE

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Cortical β-adrenoceptors and α_2-adrenoceptor mediated hypoactivity (sedation) are both reduced in male rats by repeated administration of desipramine (DMI; Heal et al, 1983) or electroconvulsive shock (ECS; Heal et al, 1981). Since oestradiol treatment has been shown to modify the effects of DMI on adrenergic responses in female rat brain (Bristow et al, 1986), we have now compared α_2- and β-adrenoceptor function in male and female rats and the effects of repeated DMI or ECS administration on these.

Adult CD rats (males 75 - 100g; females 100 - 125g) were used throughout. ECS (110V, 1s) was given to halothane anaesthetised rats once daily for up to 10 days. DMI (5 mg/kg ip) or saline was injected twice daily for up to 14 days. Hypoactivity was rated on 4 parameters (passivity, tactile responsiveness, posture and gait) as described by Heal et al (1981). Cortical β-adrenoceptor binding was measured using [^{3}H]-dihydroalprenolol (0.4 - 4nM) with specific binding determined by 200μM isoprenaline. Results were analysed by Wilcoxon's 2-tailed rank order test, Student's unpaired "t" test or 2-way analysis of variance for 2 independent variables combined with Tukey's test, as appropriate.

Dose-response curves to clonidine (0.05 - 1.0 mg/kg) showed that female rats were significantly ($P<0.01$) less sedated than males. To obtain equivalent hypoactivity responses for subsequent studies, clonidine was given 0.5 mg/kg to females and 0.2 mg/kg to males. Repeated DMI treatment attenuated ($P<0.01$) clonidine hypoactivity in male rats after 4 days, but required 9 days to reduce ($P<0.01$) this response in females. Furthermore, the maximum attenuation observed after 14 days DMI injection was also greater ($P<0.01$) in males (42%) than females (27%). Although hypoactivity responses were decreased after ECS x 2 in both sexes, the maximal decrease observed after ECS x 7 was again greater ($P<0.01$) in males (76%) than females (58%). There was no difference in the numbers of cortical β-adrenoceptors in male and female rats and these were decreased by DMI treatment in both sexes. This reduction was rapid and was observed after 2 as well as 14 days of DMI injection. β-Adrenoceptors were also decreased in both sexes by ECS x 10. However, this reduction was not observed after 2 days of ECS.

In conclusion, α_2-adrenoceptor mediated hypoactivity was more pronounced in male rats than females, but in males this response was more easily reversed by repeated administration of DMI or ECS. In contrast, these treatments produced identical effects on cortical β-adrenoceptors in both sexes. The study, therefore, demonstrates sex differences in some aspects of adrenergic function and, furthermore, that male and female rats do not respond identically to antidepressant treatment.

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CHRONIC LITHIUM, CARBAMAZEPINE AND HALOPERIDOL, BUT NOT PHENYTOIN STIMULATE ^{86}Rb UPTAKE BY MOUSE BRAIN P_2 FRACTIONS

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Previous in vitro studies have shown increased erythrocyte Na/K-ATPase activity and cation transport in patients taking long-term lithium as treatment for manic-depressive psychosis (Hokin-Neaverson et al. 1976). This action may be important in mediating the effect of the drug. However, in animals given lithium the enzyme activity is reduced when assessed as the rate of formation of inorganic phosphate from ATP in stored synaptosome membranes (Swann et al. 1980). In order to investigate further this discrepancy between peripheral and central tissues, we have developed a technique to assess directly cation transport across the membrane of intact synaptosomes via Na/K-ATPase. We have used this technique to study the effect of lithium and other psychotropic drugs on central cation transport.

Mice were pre-treated for 14 days as follows: LiCl in 0.9% NaCl 3mmol/kg s.c. b.d.; carbamazepine in propylene glycol 0.25-0.50 g% in the diet; haloperidol in 0.9% NaCl 0.5mg/kg i.p. o.d.; phenytoin in EtOH/H_2O 20mg/kg i.p. b.d. In each experiment control animals were treated with the appropriate vehicle solution. Control and treated animals were killed in pairs by decapitation. Tissue from the paired animals was processed together throughout. The synaptosome-rich P2 fraction was prepared by the method of Gray & Whittaker,(1962). Cation transport was assessed as the rate of glycoside-sensitive uptake of ^{86}rubidium (^{86}Rb) into synaptosomes suspended in K^+-free phosphate buffer at 37°C. After 5 min preincubation ^{86}Rb was added and the incubation was stopped after a further 5min. Synaptosomes were separated by filtration, washed in ice-cold 0.32M sucrose, and ^{86}Rb was measured by liquid scintigraphy.

^{86}Rb uptake was calculated as the percentage uptake of total extracellular isotope per milligram protein in 5min. The results for treated animals are shown in Table 1 (median, interquartile range (IQR) n=10) as percentages of the median value for control animals in each experiment. The IQR for control animals is shown separately.

Table 1 Effect of psychotropic drugs on ^{86}Rb uptake into synaptosomes

Drug	Treated	Control	P-value
Lithium	139% (130-148)	(96-115)	0.002
Carbamazepine	119% (109-142)	(87-119)	0.03
Haloperidol	110% (105-115)	(90-103)	0.05
Phenytoin	106% (91-115)	(95-109)	NS
			(signed-rank test)

Conclusions:1. Treatment with lithium for 2 weeks stimulates cation transport via Na/K-ATPase in mouse brain. 2. This property is shared by carbamazepine & haloperidol, but not by the anticonvulsant phenytoin. 3. It is not possible to know whether stimulation of cation transport is the primary mechanism by which these drugs act in manic-depressive psychosis, or whether this stimulation is secondary to their established effects on monoamine neurotransmitter function.

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SUBSTRATE CONCENTRATION GRADIENT AND THE POTENCY OF UPTAKE INHIBITORS IN SLICES OF THE RAT CEREBRAL CORTEX

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We recently reported that the rat cerebral cortex possesses an extraneuronal O--methylating system for (±)-^{3}H-Isoprenaline (ISO), the uptake component of which is similar to uptake$_2$ described in other preparations (Trendelenburg and Wilson 1986). However, as the IC_{50} of recognised uptake$_2$ inhibitors (eg: O-methylated ISO - OMI) was approximately 10-fold greater than that noted at peripheral uptake$_2$ sites, we have investigated the possibility that very active "sites of loss" lead to steep concentration gradients for the substrate within the slice and this contributes to the unexpectedly low potency of the uptake inhibitors (see: Trendelenburg 1984).

Slices or cubes of the cerebral cortex were obtained from male Wistar rats (240-300g, reserpine-pretreated) as previously described (Trendelenburg and Wilson 1986). Preparations were exposed to either 50nM ^{3}H-ISO or 50nM (±) -^{3}H-NA and 30µM U-0521 (an inhibitor of catechol-O-methyltransferase) for 30 min and 10 min, respectively, and the metabolites ^{3}H-OMI and ^{3}H-DOPEG (3,4-dihydroxyphenylglycol) were determined as described by Henseling (1983).

The rate of ^{3}H-OMI formation in 0.25mm thick slices (10.0 ± 0.6 pmol/g/min, n=8) was significantly greater ($P<0.05$) than that observed in 0.25mm cubes of the cerebral cortex (7.0 ± 0.6pmol/g/min,n=7). Furthermore, there was no evidence for differences in the effectiveness of OMI as an inhibitor of ^{3}H-OMI formation in the two preparations - the IC_{50} was approximately 50µM. 0.25mm thick slices exposed to 50nM ^{3}H-NA produced 40.2 ± 4.2pmol/g/min (n=6) ^{3}H-DOPEG (accounting for 95% of the total metabolite formation). Desipramine effected a concentration--dependent inhibition of 90% of the total ^{3}H-DOPEG formation. The IC_{50} for desipramine was 6.7 ± 0.5nM (n=8), a value similar to that reported for desipramine at uptake$_1$ sites (Bonisch et al.,1984).

In conclusion, our observations do not support the view that substrate concentration gradients for ^{3}H-ISO in slices of the rat cerebral cortex contributes to the high IC_{50} values for OMI at the uptake site. Firstly, the metabolizing capacity of the preparation was greater in slices than in cubes. Secondly, OMI inhibited ^{3}H-OMI formation in both preparations similarly. Thirdly, the activity of desipramine was not affected by the 4-fold greater rate of substrate uptake by the deaminating system - with the caveat, as observed for isolated blood vessels (Henseling 1983; Trendelenburg 1984), that the biophase for uptake$_1$ and uptake$_2$ sites are bound to differ.

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KINETIC STUDIES OF HISTAMINE H_1-RECEPTOR MEDIATED AUGMENTATION OF CYCLIC AMP ACCUMULATION IN GUINEA-PIG BRAIN SLICES

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Histamine can elevate cyclic-AMP levels in brain slices by two mechanisms. It can stimulate adenylate cyclase directly via H_2-receptors or it can act through H_1-receptors to augment the cyclic-AMP responses to H_2- or adenosine A_2-receptor stimulation (Hill et al., 1981; Al-Gadi & Hill, 1987). Both calcium ions and the products of inositol phospholipid breakdown have been implicated as mediators of the H_1-receptor response (Al-Gadi & Hill, 1987; Hollingsworth et al., 1985). However, in order to demonstrate that the cyclic-AMP response to H_1-receptor stimulation is related to the H_1-receptor-mediated response of these second messengers, it will be necessary to show that the time courses of the two responses are consistent. We describe here the time course of cyclic-AMP changes in slices of guinea-pig cerebral cortex in response to adenosine A_2- and histamine-H_1 receptor stimulation.

Slices labelled with ^{3}H-adenine were incubated in a 20 ml glass vial containing Krebs-Henseleit medium gassed with O_2/CO_2 (95:5) at 37°C and stirred magnetically at a rate sufficient to buoy up the slices. At various times before and after agonist addition, 300 µl aliquots of the slice suspension were taken for determination of cyclic AMP by the method described previously (Brown et al., 1987). In all experiments where histamine was used as an agonist, the H_2-receptor antagonist tiotidine (30 µM) was included in the incubation medium to eliminate the H_2-receptor-mediated cyclic-AMP response.

Addition of adenosine (0.1 mM) to cerebral cortical slices resulted in a rapid accumulation of ^{3}H cyclic-AMP. A new steady state level (47.2±9.6 fold over basal, n=6) was achieved within 10 min (half time, $t_{0.5}$ = 1.5±0.1 min, n=6) and maintained for at least a further 20 min. Addition of adenosine deaminase (ADA, 1.2 units/ml) during the steady state caused a return of the cyclic-AMP content to basal levels with a similar $t_{0.5}$. If histamine (1 mM) was added 10 min after adenosine (i.e. during the adenosine steady state) the ^{3}H-cyclic-AMP level rose to a new steady level (increasing from 53.8±5.4 to 139.7±18.9 fold over basal, n=7), again within 10 min. The initial rate of cyclic-AMP accumulation following histamine addition was similar to that following adenosine, but the $t_{0.5}$ was longer (4.0±0.1 min, n=8). Rapid removal of the H_1-receptor stimulus by the addition of mepyramine (10 µM), either 1 min after histamine addition or after 10 min (i.e. during the histamine steady state), caused cyclic-AMP content to fall to a level similar to that obtained with adenosine alone This fall occurred within 20 min ($t_{0.5}$ = 5 min). Addition of ADA during the steady state with histamine and adenosine caused cyclic-AMP content to fall to basal levels within 10-13 min. This fall was faster and had a shorter $t_{0.5}$ (3 min) than that following mepyramine addition.

This study shows that both accumulation and loss of cyclic-AMP following changes in adenosine and histamine H_1-receptor stimulation occur with half times of a similar order of magnitude. In addition, they show that the continued presence of histamine is required to maintain the augmentation of the adenosine response. These results provide a basis for studies to assess the roles of putative second messengers in the augmentation of cyclic-AMP responses in this tissue.

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582P

EVIDENCE THAT CCK MIMICS SATIETY, BUT DOES NOT INDUCE MALAISE OR DECREASE DIET PALATABILITY

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Peripherally administered cholecystokinin octapeptide (CCK) reduces feeding, but the mechanism of this effect is controversial. Some suggest that CCK produces or enhances inhibitory (satiety) signals during the course of a meal (Kraly et al, 1978) while others argue that CCK induces a malaise similar to that produced with LiCl (Swerdlow et al, 1983) or reduces diet palatability (Waldbillig and O'Callaghan, 1980). A novel maze procedure is described here that may aid in the resolution of this controversy. The maze consists of 7 sequential T-mazes, the centre of each choice point being 12" from the previous one. Both goal boxes at each choice point are baited with food pellets, but the amount of pellets increases by one with each successive choice point. After consuming the pellets in the first goal box chosen, the rat is removed, and placed in the start box for another trial until 10 trials have been completed. Goal boxes are rebaited before each trial. Rats therefore balance out reward delay and distance travelled against reward magnitude when choosing a goal box to enter. Pilot experiments indicated that food deprivation decreases the distance that rats will run before choosing a goal box, thereby resulting in a reduction in the number of pellets consumed. This is presumably due to an increasing importance of reward delay with increasing deprivation.

Ten male Sprague-Dawley rats were given 10 trials under each of the following conditions: 1) LiCl (0.15 M, 1.0 ml/100 g, i.p. 30 min prior to testing), 2) saline (0.15 M, 1.0 ml/100 g, i.p., 30 min prior to testing), 3) CCK (8.0 ug/kg, 10 min prior to testing), 4) saline (0.9%, 1ml/kg, 10 min prior to testing), 5) prefeeding of 15 g of food for 1 hour prior to the session, 6) rewarding responses with less-preferred food pellets. Each treatment was separated by at least one week, with baseline testing on days between tests, except for the cases of the vehicle injections, which were conducted on the days prior and subsequent to the relevant test days. Both prefeeding and CCK increased the distance rats travelled before choosing a goal box ($F(1,9)=6.56$, $P < 0.05$; $F(1,9)=11.09$, $P < 0.01$, respectively) and increased latencies to leave the start box (prefeed: $F(1,9)=5.54$, $P < 0.05$; CCK: $F(1,9)=6.36$, $P < 0.05$). Both of the latter treatments increased the time to run to the goal box by an equivalent amount, but only the effect produced by CCK was statistically significant ($F(1,9)=10.2$, $P < 0.025$). None of the other treatments produced significant alterations in any of the measures of maze performance. Thus, in this situation, CCK produced effects that were similar to those produced by satiety, but not by LiCl or by replacement of the reward with a less-preferred diet. These data support the suggestion that CCK mimics satiety, and argue against either a malaise or palatability interpretation of the mechanism of CCK's inhibition of feeding.

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CHARACTERIZATION OF "PERIPHERAL" TYPE CCK RECEPTORS IN RAT BRAIN USING [^{3}H]-L-364,718

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Cholecystokinin (CCK) is present in both the mammalian peripheral and central nervous system where it binds to specific sites with high affinity for CCK and related peptides (Innis and Snyder, 1980). Central binding sites differ from peripheral receptors in having relatively high affinity for desulphated CCK (dCCK) and gastrin. However, certain areas within rat brain such as the area postrema (AP), the nucleus tractus solitarius (NTS) and the interpeduncular nucleus (IPN) have low affinity for dCCK suggesting they resemble peripheral CCK receptors despite their central location (Moran et al, 1986).

^{3}H-L-364,718 (1-methyl-3-(2-indoloyl)amino-5-phenyl-3H-1,4 benzodiazepin-2-one) is an antagonist which displays high affinity and selectivity for peripheral CCK receptors (Chang et al, 1986; Evans et al, 1986). In the present study we have used ^{3}H-L-364,718 and a close analogue L-365,031 (1-methyl-3-(4-bromobenzoyl)-amino-5-phenyl-3H-1,4-benzodiazepin-2-one) to visualize by autoradiography peripheral CCK receptors within the AP, NTS and IPN and to characterize them using radioligand binding techniques.

For each binding assay, the brains from 13 rats were removed and the AP, NTS and IPN dissected out. The combined tissue was homogenized in 10mM Tris-HCl pH 7.4 and following centrifugation, the pellet was resuspended (0.4mg protein/ml) for assay in 20mM HEPES pH 6.5 containing 150mM NaCl, 5mM $MgCl_2$, 1mM EGTA and 0.025% bacitracin. The tissue was incubated with 0.2nM ^{3}H-L-364,718 at 20°C for 60 min with or without unlabelled test compound and the assay terminated by filtration. Autoradiography was performed using slide mounted cryostat sections. These were incubated with 5nM ^{3}H-L-364,718 under the same conditions as described for homogenates and then washed 4 times for 2 min each.

^{3}H-L-364,718 binding was localized in the AP, medial aspects of the NTS and the IPN, and was displaced by 1μM L-365,031 or sulphated CCK. In homogenates of these areas, binding was saturable and of high affinity (Bmax 20.3 $\pm$ 4.0 fmol mg protein n = 3; pKd 9.85 $\pm$ 0.27, n = 4) and displaced by compounds previously shown to be active at pancreatic CCK receptors. Sulphated CCK-8 (pIC_{50} 7.8) was approximately equipotent with caeurulein (pIC_{50} 7.4) and L-365,031 (pIC_{50} 7.9). Desulphated CCK was only weakly active (pIC_{50} 5.7) and gastrin, CCK-4 and CCK-6 were essentially inactive (pIC_{50} < 4). Sites labelled by ^{3}H-L-364,718 were sensitive to GppNHp (100μM) which shifted the affinity of CCK-8 from pIC_{50} 7.7 $\pm$ 0.03 to pIC_{50} 6.5 $\pm$ 0.12 in the presence of GppNHp and increased the Hill slope from 0.5 $\pm$ 0.06 to 0.9 $\pm$ 0.17 (n=3).

In conclusion, these data extend the observations of Moran et al (1986) and show the existence of CCK receptors in the CNS, termed CCK-A receptors by Moran and colleagues which resemble those in the periphery.

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BLOCKADE OF CHOLECYSTOKININ (CCK)-INDUCED ANOREXIA IN THE RAT BY THE CCK-A RECEPTOR ANTAGONIST L-365,031

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It is well established that injection of cholecystokinin octapeptide (CCK) decreases food intake in animals and man (Smith, 1984). However, the identity and location of the receptors which mediate CCK anorexia is controversial and both central and peripheral sites of action have been proposed. Recent ligand binding studies have suggested the existence of 2 subtypes of CCK receptor termed CCK-A (generally found in the periphery) and CCK-B (brain sites) (Moran et al., 1986). The novel non-peptide CCK antagonist L-365,031 (1-methyl-3-(4-bromobenzoyl)-amino-5-phenyl-3H-1,4-benzodiazepin-2-one) selectively acts on CCK-A sites located in peripheral tissues and in certain discrete regions of the rat brain (i.e. area postrema, nucleus tractus solitarius (NTS) and interpeduncular nucleus (IPN) (Campbell et al, 1987). The present communication reports the effects of L-365,031 on ad libitum food intake and on CCK-induced anorexia. All studies were conducted on male Sprague-Dawley rats (300-400g body weight). In the first experiment the effects of L-365,031 (1.0-8.0 mg/kg sc) on food intake during a 4 h daytime test in ad libitum fed animals were examined. In the second experiment the effects of L-365,031 (30 min pretreatment) on anorexia induced by 8 µg/kg CCK (i.p. immediately before a 30 min test) in 17h food deprived rats were examined.

Table 1 Effects of L-365,031 on CCK Anorexia in the Rat

	FOOD INTAKE (g/30 min)		
	Saline	CCK	%Saline/Vehicle Control
Vehicle	6.06±0.59	2.59±0.39***	42.7
L-365,031 1 mg/kg	6.97±0.46	3.13±0.31**	51.6
L-365,031 2 mg/kg	5.47±0.23	3.81±0.48*+	62.8
L-365,031 8 mg/kg	6.10±0.57	4.92±0.55++	81.2

Data are mean ± s.e. of 6-10 rats per group.
Significant difference between Saline/Vehicle and other treatments: P<0.02, **P<0.01, ***P<0.001. Significant difference between Vehicle/CCK and L-365,031/CCK treatments: +P<0.10, ++P<0.01.

L-365,031 (2 and 4 mg/kg) given alone significantly increased ad libitum food intake during a daytime test but had no effect on food intake in 17h food deprived rats. CCK anorexia was dose dependently antagonized by L-365,031 treatment (2 way ANOVA interaction, $F(5,84) = 3.56$, $P<0.01$, see Table 1). These data suggest that CCK anorexia may be mediated by CCK-A receptors located in the gut and/or the AP, NTS and IPN. Interestingly, lesions of the AP or NTS block CCK anorexia (Van der Kooy, 1984; Crawley and Schwaber, 1984).

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EFFECTS OF CHOLECYSTOKININ AND PENTAGASTRIN ON NEURONAL ACTIVITY IN THE VENTROMEDIAL NUCLEUS OF THE RAT HYPOTHALAMIC SLICE PREPARATION

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A number of groups have suggested that the ventromedial nucleus (vmn), an area possessing a dense population of receptors for cholecystokinin (CCK) (Day et. al. , 1986) may be involved in the regulation of feeding(Kow & Pfaff, 1985).CCK itself is a potent excitant of neurones located in the vmn (Kow & Pfaff,1986) and the finding that some central nervous system CCK receptors differ from their peripheral counterparts (Boden & Hill, 1986 ; Clark et. al. , 1986)prompted an investigation of the effects of the sulphated octapeptide CCK-8S and the smaller CCK analogue pentagastrin on neuronal activity in coronal slices containing this region.

Extracellular recording revealed that CCK-8S (0.1-1.0uM) produced a concentration dependent increase in firing rate ,which was mimicked by pentagastrin. CCK-8S induced excitation was decreased by 80% in the presence of the peripheral CCK receptor antagonist 30 uM L-364,718 (donated by Merck, Sharp and Dohme), a concentration reflecting its binding affinity to CNS sites (Chang & Lotti,1986), and confirming its action as an antagonist at central CCK receptors.Intracellular recordings from neurones contained within the ventromedial nucleus revealed two populations with comparable resting membrane properties but differing neuronal activity. The first group (n=20) fired spontaneous action potentials of less than 2ms duration which were followed by a long lasting (80-150 ms) after hyperpolarisation (a. h. p.) of some 20mV at resting membrane potential. The second group (n=11) only fired action potentials in response to depolarising current injection and were otherwise silent.Action potentials from these neurones possessed an a. h. p. of similar magnitude but much shorter in duration (20-50ms.) than that found in spontaneously active neurones.

Addition of 0.5uM CCK-8S to 13 spontaneously active neurones produced a small depolarization (3.4 +/- 1.8 mV) concommitant with a large increase in firing rate in all cases, but the peptide was without effect when tested on 4 silent neurones. Pentagastrin (0.5 uM) depolarised 6 out of 7 spontaneously active neurones (2.9 +/- 1.7 mV) and also increased action potential firing. These data suggest the existence of at least two types of neurone within the vmn only one of which is responsive to CCK, and that the CCK receptor found in the rat ventromedial nucleus resembles that found in other brain regions and not that in peripheral tissues.

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NEUROKININ-3 RECEPTORS ARE LINKED TO INOSITOL PHOSPHOLIPID HYDROLYSIS IN THE GUINEA-PIG ILEUM

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The original subdivision of tachykinin receptors into 'SP-P' (NK-1) and 'SP-E' (NK-2) based on the rank order potencies of tachykinins in a variety of smooth muscle preparations (Lee et al., 1982) has more recently been extended to include a third tachykinin receptor subtype - the 'SP-N' (NK-3) receptor identified on myenteric plexus neurones of the guinea-pig ileum (Laufer et al., 1985). Substance P and related tachykinins stimulate inositol phospholipid hydrolysis in both NK-1 and NK-2 systems (Watson & Downes, 1983; Bristow et al., 1987). We here present evidence in support of NK-3 receptor-mediated inositol phospholipid hydrolysis.

Experiments were performed on cross-chopped slices of guinea-pig ileum longitudinal muscle strips essentially as previously described (Watson & Downes, 1983). The mammalian tachykinins substance P, neurokinin A and neurokinin B produced a concentration-dependent accumulation of total [^{3}H]-inositol phosphates with similar maximal responses and mean (n=3) EC_{50} values (concentration inducing 50% of the maximal response) of 87, 84 and 28 nM respectively. The selective NK-1 agonist substance P methyl ester (Watson et al., 1983) and the selective NK-3 agonist suc-[Asp^6,MePhe^8]-SP$_{(6-11)}$ (senktide: Wormser et al., 1986) also stimulated [^{3}H]-inositol phosphate formation with mean (n=7) EC_{50} values of 50 and 0.54 nM, and maximum responses of 50.69 ± 0.96 and 45.64 ± 1.17% relative to substance P (10 μM) respectively. When added together, maximally effective concentrations of substance P methyl ester (10 μM) and senktide (1 μM) were fully additive, producing 93 ± 4.4% of the response to 10 μM substance P (n=5) suggesting the existence of two distinct populations of tachykinin receptors coupled to inositol phospholipid hydrolysis in this preparation. In contrast, substance P and neurokinin B, which show little discrimination between these receptor subtypes, were not additive.

The substance P antagonist [D-Pro^4,D-Trp^{7,9,10}]-SP$_{(4-11)}$ (15 μM), which alone did not alter basal [^{3}H]-inositol phosphate levels, produced an 8.6-fold shift to the right of the substance P methyl ester concentration-response curve for inositol phosphate accumulation (n=4), but had no effect on the response to senktide. This result is in agreement with previous findings that [D-Pro^4, D-Trp^{7,9,10}]-SP$_{(4-11)}$ is an antagonist at the NK-1 receptor (believed to be responsible for tachykinin-induced contraction of the guinea-pig ileum) but not at the NK-3 receptor which mediates acetylcholine release from myenteric plexus neurones (Featherstone et al., 1986).

It is concluded that activation of NK-3 receptors results in the hydrolysis of inositol phospholipids in the guinea-pig ileum longitudinal muscle-myenteric plexus preparation.

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IN VITRO AND IN VIVO PROFILE OF THE OPIOID ANTAGONIST NORBINALTORPHIMINE

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The use of selective opioid antagonists is of critical importance to the effective determination of the receptor selectivity of opioid agonists. Recently, Portoghese et al. (1987) have reported the synthesis of two potent opioid antagonists, binaltorphimine and norbinaltorphimine (norBNI), selective for the κ receptor. In the present study, the in vitro and in vivo profile of norBNI (synthesised in Dept. Chemical Research, Glaxo) has been characterised.

Isolated segments of guinea-pig ileum (GPI) or vasa deferentia (hamster - HVD, mouse - MVD, rabbit - LVD, rat - RVD) were set up as previously described (Hayes et al., 1986). In the MVD preparation, norBNI (30-min pretreatment) produced a dose-dependent antagonism of the effects of the κ agonist U50488H, the μ agonist DAGO and the δ agonist DPDPE with pA_2 values (95% C.L.) of 10.2 (9.5-10.5), 7.6 (7.4-7.9), and 7.8 (7.7-8.0), respectively (n=4). Schild analyses gave slopes of unity. Similar results were obtained for the antagonism of κ effects in LVD and GPI, of μ effects in GPI and RVD, and of δ effects in the HVD. The results confirm that in vitro norBNI is a potent selective κ antagonist.

In in vivo experiments, the effect of norBNI was investigated on opioid-induced changes in urine output in the water-loaded rat (Skingle et al., 1985) and in a number of antinociceptive tests (Tyers, 1980). NorBNI (3mg/kg s.c.) co-administered with agonists, antagonised the κ diuretic effect (measured at 4hr) of U50488H (Table; n=5). However, norBNI (3mg/kg s.c.) also antagonised the μ antidiuretic effect (measured at 2 hours) of morphine (n=5).

	DOSE	MEAN URINE OUTPUT (ml±s.e.)		
DRUG	mg/kg	CONTROL	DRUG ALONE	DRUG + norBNI
U50488H	10	3.4±0.2	8.6±0.4	5.6±0.3
MORPHINE	10	3.6±0.2	0.1±0.1	3.2±0.6

In the acetylcholine-induced mouse abdominal constriction test, norBNI (3mg/kg s.c.), co-administered with the agonist (30 min pretreatment) failed to antagonise the antinociceptive effect of U50488H (dose ratio (95% C.L.): 1.5 (0.9-2.7), n=6). NorBNI alone, 10-30mg/kg s.c., produced a naloxone-insensitive inhibition of the number of abdominal constrictions. ICV administration of norBNI (30μg/mouse; 30min pretreatment) also failed to antagonise the effect of icv co-administered U50488H, 0.3-3μg/mouse, (dose ratio: 1.6 (0.8-7.1), n=8). Similarly in the paw pressure test in the rat norBNI (3mg/kg s.c., 30min pretreatment) produced no shift of the U50488H dose-response curve (dose ratio: 1.3 (0.8-2.2) n=6).

In conclusion, although norBNI has the profile of a potent, selective κ antagonist in vitro, this antagonist profile is not maintained in the rat or mouse in vivo.

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CHRONIC CEREBROVENTRICULAR INFUSION OF NEUROTENSIN ANTISERUM ENHANCES MESOLIMBIC DOPAMINE FUNCTION IN THE RAT

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The study of the physiological relationships between central neuropeptide and neurotransmitter systems, is generally restricted by the lack of substances which selectively deplete the endogenous levels of the peptides or block their receptors. The administration of selective neuropeptide antisera provides a means of examining transmitter interactions in animals with depleted extracellular peptide levels (Lighton et al., 1986). We describe results from a study in which a purified gamma-globulin fraction of a neurotensin (NT) antiserum (NT-AS) has been cerebrally infused, in order to investigate the relationship between extracellular levels of this peptide and central dopamine (DA) function (Nemeroff and Cain, 1985).

Anaesthetised male Sprague-Dawley rats (230-270g) were bilaterally implanted with i.c.v. cannulae. Osmotic mini-pumps (14 day infusions, 0.5ul/h, 2 pumps/rat) containing NT-AS or control serum (C-AS; purified fractions) were implanted interscapularly and connected to the i.c.v. cannulae. After 6 and 13 days of infusion, and 6 days after pumps had been disconnected, rats were placed in photocell boxes to monitor gross activity and scored for stereotypy. The DA agonist, n-propylnorapomorphine (NPA, 0.2mg/kg,i.p.) was given after one hour acclimatisation, and activity monitored for a further 2 h. On day 21, 22 or 23, each rat was anaesthetised with halothane and implanted stereotaxically with a carbon fibre electrode into the left accumbens (ACB) for in vivo voltammetry. DOPAC levels were measured at 5 min intervals, and the effect of successive 0.5ul infusions into the ipsilateral ventral tegmentum (VTA) of saline, NT(1ug) and haloperidol (2.5ug) were determined (see Ford et al., 1987).

In both groups of rats, after 6, 13 and 20 days, the injection of NPA induced stereotyped behaviours which were unaffected by NT-AS infusion. The hyperactivity produced by NPA was, however, significantly enhanced in the NT-AS rats after 6 but not 13 or 20 days ($p<.05$, c.f. C-AS, n=9). The voltammetrically-measured ACB DOPAC levels were elevated by the intra VTA injections of NT and haloperidol but not saline; NT injection into the NT-AS rats increased DOPAC (as % of stable pre-injection levels) by three times as much as compared to C-AS rats (increase of 91.1 +/- 6 NT-AS, 32.9 +/- 9 C-AS; $p<0.01$, n=5), while the haloperidol response was unaffected by NT-AS.

It is concluded that the chronic immunological inactivation of endogenous NT in the rat brain results in alterations of both DA- and NT-receptor-mediated responses. This suggests that NT does play an important physiological role in regulation of mesolimbic function. This study demonstrates further that the technique of cerebral infusion of selective neuropeptide antibodies may be successfully employed for the elucidation of endogenous neuroregulatory functions.

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DES-ENKEPHALIN-γ-ENDORPHIN BLOCKS DOPAMINERGIC HYPERACTIVITY IN RATS AND MARMOSETS

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The hyperactivity which results from a slow persistent infusion of dopamine into the rat or primate mesolimbic nucleus accumbens is sensitive to blockade by neuroleptic agents (Costall et al, 1982; Barnes et al, 1987). In the present studies we show that the dopamine response can also be antagonised by the neuropeptide des-enkephalin-γ-endorphin (DEγE, β-endorphin-[6-17], Org 5878), which has neuroleptic-like activity in some experimental models and appears to be antipsychotic without extrapyramidal effects in schizophrenic patients (van Ree et al. 1986).

The studies used male Sprague-Dawley rats (300-325g) and male and female common marmosets (Callithrix jacchus) (350-400g). Each were subject to standard stereotaxic surgery to implant chronically indwelling intracerebral guide cannulae for subsequent infusion of dopamine (25μg/24h, 0.48μl/h, 13 days) into the nucleus accumbens. Locations were confirmed on completion of the studies. Dopamine infused into rat nucleus accumbens caused biphasic hyperactivity with maxima between days 3-4 and 10-11 (224±23 counts/60 min, measured in individual photocell cages, compared with 99±10 counts/60 min following vehicle infusion). This hyperactivity was antagonised by DEγE at 100, 250 and 500μg/kg given s.c. t.d.s. (responses reduced to means of 98±10, 86±9 and 103±10 counts/60 min respectively, $P<0.001$). In the common marmoset the dopamine infusion caused a single phase of hyperactivity (290±33 counts/60 min, measured in cages equipped with 4 infra-red photocell units, compared with vehicle responses of 63±6 counts/60 min). This hyperactivity was significantly antagonised by DEγE at doses of 25μg/kg s.c. t.d.s. (peak responding reduced to 180±21 counts/60 min, $P<0.001$), 50 and 100μg/kg s.c. t.d.s. (responses indistinguishable from vehicle, $P<0.001$). Both rats and marmosets used in these studies were preselected according to their responses to the dopamine agonist (-)N-n-propylnorapomorphine [(-)NPA]: all animals were initially high responders. Following infusion with dopamine, without treatment with DEγE, both rats and marmosets changed their activity status to low activity: this change in responsiveness to (-)NPA was prevented in animals where the dopamine hyperactivity was antagonised by DEγE (for e.g. in the marmoset responding to (-)NPA was initially 337±43 counts/60 min, after dopamine infusion this was reduced to 227±31 counts/60 min, but in those animals receiving DEγE, 50-100μg/kg t.d.s., responses to (-)NPA remained at 333±39 counts/60 min, $P<0.001$).

In a further series of experiments rats were subject to an intra-accumbens dopamine infusion suppressed by haloperidol (0.15mg/kg i.p. b.d.). Within 2 days of ceasing this treatment, and the dopamine infusion, a marked rebound hyperactivity developed (273±28 counts/60 min, vehicle 98±10 counts/60 min, $P<0.001$). This rebound hyperactivity could be suppressed by DEγE at doses of 100 and 50μg/kg s.c. t.d.s. Thus, in addition to suppressing a dopamine infusion effect similarly to neuroleptic agents, but without sedative potential or any alteration in motor performance to values below control levels, DEγE is also effective to control a hyperactivity response which follows a 13 day treatment with haloperidol. This profile of activity supports the use of DEγE in the treatment of schizophrenia.

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THE DESIGN OF SALMETEROL, A LONG-ACTING SELECTIVE β_2-ADRENOCEPTOR AGONIST

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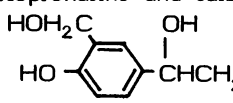
Contraction of bronchial smooth muscle in asthma is rapidly reversed by inhalation of a selective β_2-adrenoceptor agonist such as salbutamol (Ia) but its duration of action is only about 4 hours. There is, therefore, need for a longer-acting inhaled bronchodilator for maintenance treatment of the disease and to control nocturnal asthma.

We thought that this problem might be solved by making a β_2-agonist which persists in the vicinity of β_2-adrenoceptors for at least 12 hours and that this might be achieved by modifying salbutamol to obtain a drug with much greater affinity for its receptors because of increased exo-receptor binding (Brittain et al, 1981). More precisely, the energy of activation for the disengagement of the new drug from its active site would have to be high enough for this to be improbable at body temperature. The required drug was expected to be a larger molecule than salbutamol and to have a slower onset and a much slower offset of action. These ideas were tested by varying the N-substituent in saligenin ethanolamines, and certain N-aralkyloxyalkyl analogues of salbutamol were found to be unusually long-acting β_2-stimulants. Salmeterol (Ib) was chosen for detailed evaluation. In this paper, its actions on guinea-pig tracheal muscle are compared with those of isoprenaline and salbutamol.



1(a) R = $-C(CH_3)_3$

1(b) R = $-CH_2CH_2CH_2CH_2CH_2CH_2OCH_2CH_2CH_2CH_2-C_6H_5$

1

Guinea-pig isolated tracheal strips were contracted by superfusion with Krebs solution containing $PGF_{2\alpha}$ (2.9 µM). The absolute potency of each agonist was determined from concentration-effect curves obtained by infusing it in graded concentrations into the superfusion fluid, the infusion being continued until a steady state response was achieved. Infusion of the agonist was then discontinued and the recovery of the muscle tone observed during continued superfusion with the Krebs/$PGF_{2\alpha}$ solution. Isoprenaline had an EC_{50} of 5 nM; the equipotent concentration for salbutamol (isoprenaline = 1) was 2.0 (95% confidence limits: 1.1-5.6, n = 7) and for salmeterol 1.1 (0.5-3.3, n = 6). The rates of onset of action (times for 50% onset for EC_{50} concentrations) were isoprenaline 1.1 min, salbutamol 1.9 min and salmeterol 9.0 min and the corresponding rates of offset of action (times for 50% recovery from EC_{50} concentrations) were 1.7 min, 3.7 min and 18->24 min.

The relaxant effect of salmeterol was antagonised by sotalol, a highly specific β-adrenoceptor blocker, a result that confirms β-agonist activity. Sotalol 10 µM added to the Krebs/$PGF_{2\alpha}$ solution after salmeterol treatment, also increased the rate of offset of β-stimulant activity because full muscle tone was re-established within 6 min. However, the relaxant effect of salmeterol reasserted itself within 90 min after the sotalol had been removed from the superfusing solution. Thus the persistent action of salmeterol is not due to irreversible binding to β_2-adrenoceptors but possibly to strong exo-receptor binding that localises the drug in the vicinity of these receptors.

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