PROSTAGLANDIN D_2 ACTS AS A 'PARTIAL ANTAGONIST' IN GUINEA-PIG PLATELETS

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Prostaglandin D_2 (PGD_2) is a potent inhibitor of platelet aggregation in human, sheep and horse plasma but is a weak inhibitor in dog, rabbit and rat plasma (Smith et al., 1974; Whittle et al., 1978). In this study the potency of PGD_2 as an inhibitor of platelet aggregation has been compared to prostacyclin, its stable analogue carbacyclin (Whittle et al., 1980) and the hydantoin prostaglandin, BW245C (Whittle et al., 1983) in platelet-rich-plasma (PRP) from guinea-pig.

Male Halls guinea-pigs (350-450 g) were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and approximately 15 mls of blood was collected from the abdominal aorta using needle puncture (braunula size 1), into plastic Sarstedt neutral tubes containing trisodium citrate (final concentration 0.315%). The blood was centrifuged (3000 r.p.m. for 2 min) at room temperature in Petalfuge I bench centrifuge to obtain PRP. The inhibition of platelet aggregation induced by a submaximal concentration of ADP (2-4 µM) was determined as described previously (Whittle et al., 1978). Dose-inhibition curves were constructed for each prostanoid and the ID_{50} was calculated as the dose required to reduce the aggregation to 50% of its control amplitude.

The ID_{50} for prostacyclin, BW245C and carbacyclin was 0.8 ± 0.2 (n=6), 7.3 ± 3 (n=14) and 18.6 ± 0.6 (n=5) ng ml^{-1} (mean ± s.e. mean) respectively. Thus, guinea-pig PRP provides one of the more sensitive animal platelets to the anti-aggregating actions of these prostanoids.

In contrast, PGD_2 gave a bell-shaped dose-response relationship, giving a maximum inhibition of aggregation of 53.2 ± 6% (n=7) at 200 ng ml^{-1} which was significantly lower at 2000 ng ml^{-1} (29.2 ± 3.4% inhibition, n=8; $P < 0.01$) and 5000 ng ml^{-1} (10.4 ± 3.5% inhibtion, n=7; $P < 0.01$). Furthermore, pre-incubation (1 min) with PGD_2 (200-5000 ng ml^{-1}) caused a dose-related antagonism of the anti-aggregating actions of prostacyclin, carbacyclin and BW245C. Thus the ID_{50} of BW245C was significantly increased 350 fold to 2.5 ± 0.6 µg ml^{-1} ($P < 0.001$) and that of carbacyclin 1000 fold to 20.3 ± 9.8 µg ml^{-1} ($P < 0.05$) in the presence of PGD_2 (2000 ng ml^{-1}).

Previous studies have suggested that BW245C can interact with PGD_2 binding sites on platelets from different species (Whittle et al., 1983; Town et al., 1983). However, in guinea-pig PRP, PGD_2 was found to be only a weak partial inhibitor of platelet aggregation, whereas BW245C acted as a potent full inhibitor of platelet aggregation. Furthermore, PGD_2 acted as a partial 'agonist' and antagonised the inhibitory actions of not only BW245C, but also of prostacyclin and carbacyclin. It is not yet known whether such antagonistic effects of PGD_2 against these prostanoids reflect interactions at the level of the platelet adenylate cyclase.

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IMPLIED INVOLVEMENT OF PROSTAGLANDINS IN THE ANTI-HYPERTENSIVE RESPONSE TO PROPRANOLOL IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

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In normotensive rabbits (Durão & Rico, 1977) and rats (Sugawara & Ozaki, 1980) and in hypertensive man (Durão et al, 1977), the blood pressure lowering effect of (±)-propranolol is prevented by treatment with indomethacin, an inhibitor of endogenous prostaglandin (PG) synthesis. In the present report this interaction has been studied in groups (n=16-25) of conscious, adult female SHR, 7-11 weeks after unilateral adrenalectomy and contralateral adrenal-demedullation (SHR/AD), a model which detects an acute anti-hypertensive response to several β-adrenoceptor antagonists (Buckingham & Hamilton, 1980).

Mean arterial pressure (MAP) and heart rate were recorded continuously for 3 hours from an indwelling aortic catheter, and drugs or drug vehicle (0.9% w/v saline) were administered i.v. or i.p. via indwelling cannulae. Results were analysed by Student's 't' test for unpaired data; values of $p<0.05$ were considered significant.

In SHR/AD, (±)-propranolol, 3.3 or 10 μmol/kg i.v., produced an immediate significant increase in MAP (8-16%) and reduction in heart rate (11-15%). As the bradycardia progressively diminished, a prolonged significant fall in MAP (~8%) ensued. The time of onset of this latter effect was 50 minutes for the lower dose and 120 minutes for the higher dose. By contrast, (+)-propranolol, 10 or 30 μmol/kg i.v., produced inconsistent changes in MAP and a transient (<30 minutes) dose-dependent bradycardia (6-19%), presumably attributable to the drug's membrane stabilizing property (Howe & Shanks, 1966). Simultaneous administration of indomethacin, 5 mg/kg i.p., virtually abolished the anti-hypertensive response to (±)-propranolol, 3.3 or 10 μmol/kg i.v. Pretreatment (1 hour) with nabumetone (50 mg/kg p.o.), a non-steroidal anti-inflammatory drug with weaker PG synthesis inhibitory activity than indomethacin (Boyle et al, 1982), only slightly delayed the onset (to 80 minutes) of the blood-pressure lowering response to (±)-propranolol, 3.3 μmol/kg i.v. Indomethacin, 5 mg/kg i.p., given after the anti-hypertensive response to (±)-propranolol, 3.3 μmol/kg i.v., had become established, restored MAP to baseline, but also increased blood pressure in controls such that a significant net anti-hypertensive effect was maintained.

Increasing the dietary intake of polyunsaturated fatty acids of SHR/AD with cod liver oil (1 ml/rat p.o. daily for 21-22 days) did not influence baseline MAP but hastened the onset and increased the magnitude (~2-fold) of the anti-hypertensive response to (±)-propranolol, 10 μmol/kg i.v.

The results imply an involvement of PGs in mediating the anti-hypertensive response to (±)-propranolol in SHR/AD.

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MODULATION OF ARACHIDONIC ACID METABOLISM IN IMMUNE MODELS OF CELL INFILTRATION

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Some products of the lipoxygenase pathway of arachidonic acid metabolism (viz LTB_4, 5-HETE) are known to posses potent chemotactic activity for inflammatory cells (Palmer et al, 1980). In vivo studies with the dual cyclooxygenase/lipoxygenase inhibitor BW 755c have demonstrated reduced cellular infiltration in subcutaneously implanted sponges (Higgs et al, 1980) and in carrageenin pleurisy (Blackham & Norris, 1982) but the significance of lipoxygenase products in immunologically - induced cell recruitment has not been investigated fully. In the present study the effects of single and dual inhibitors of cyclooxygenase and lipoxygenase pathways were compared on a reversed passive Arthus reaction (RPAR) in rats and on delayed - type hypersensitivity reaction (DTHR) in guinea-pigs.

Female, Cobb Wistar rats (70-100g) and female, Hartley guinea-pigs (300-350g) were used and the inflammatory lesions were produced in the pleural cavity of both models using procedures described previously (Blackham & Woods, 1979, Keogh et al 1981). All compounds were administered orally. Cell infiltration (predominantly PMNs)was measured after 4hr in the RPAR and was reduced by dosing 1hr before challenge with BW 755c, timegadine and phenidone (IC_{50} 25mg/kg, 38mg/kg and 70mg/kg respectively). Indomethacin (0.3 - 3mg/kg) failed to reduce cell infiltration although naproxen showed significant inhibitory activity (34%) at 3mg/kg. None of these compounds reduced pleural exudate formation to a significant level. In contrast, dexamethazone (1mg/kg) inhibited both cell infiltration (52%) and the oedematous response (86%).

The 48hr cellular response in the DTHR was largely monocytic and was unaffected by modulation of the cyclooxygenase and lipoxygenase pathways using indomethacin (10mg/kg) and BW 755c (50mg/kg) administered 1hr before challenge, 5hr, 21hr, 29hr and 45hr after challenge. However, prednisolone (40mg/kg) was effective in this model and inhibited significantly the cell infiltration (32%) and exudate formation (45%).

The results indicate at least partial involvement of chemotactic lipoxygenase products in the RPAR (but not in the DTHR), possible released from infiltrated PMNs following phagocytosis of immune complexes. These findings illustrate the potential value of lipoxygenase inhibitors in inflammatory disorders which are characteristic of Arthus-type reactions, notably, rheumatoid arthritis.

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PROSTAGLANDIN D_2 RELEASE BY GUINEA-PIG SKIN DURING IN VITRO ANAPHYLAXIS

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Prostaglandin D_2 (PGD_2) is the major cyclo-oxygenase product of rat and human mast cells after immunological challenge (Lewis et al, 1981). Flower et al (1976) have reported that PGD_2 produces vasodilation and erythema when injected intradermally in human skin. The release of PGD_2 in skin immediate hypersensitivity reactions may contribute to the inflammation produced.

We used the in vitro model of immediate cutaneous hypersensitivity in the guinea-pig (Yeoh et al, 1972) to study the release of PGD_2 and prostaglandin E_2 (PGE_2) in relation to histamine release. The abdominal skin from an egg albumen sensitised guinea-pig was cut into approximately 0.5-1.0 mm thick slices. Aliquots of the washed skin slices, weighing 0.7-0.8 g, were incubated in Tyrode solution at 37°C with or without egg albumen antigen. The supernatants were removed and prostaglandins extracted using octadecylsilyl silica (Powell, 1982). The extracts were subjected to straight phase high performance liquid chromatography and fractions with the same retention times as tritiated PGD_2 and PGE_2 were collected. PGE_2 was assayed by gas chromatography-mass spectrometry (GC-MS) as reported by Barr et al, 1982. PGD_2 was determined by radioimmunoassay using commercially available rabbit anti-PGD_2 antisera. After incubation the skin samples were freeze-dried to obtain dry weights. The histamine content of the skin samples was determined, after boiling in Tyrode solution, by bioassay on the guinea-pig ileum. Histamine release was expressed as the percentage reduction of the histamine content during incubation compared to control samples.

There was parallelism between the release of PGD_2 and histamine over a wide range of antigen concentration (0.1-1000 μg/ml). Maximal release of PGD_2 was achieved at around 10 μg/ml. The time course of release was similar for PGD_2 and histamine, reaching a plateau by about 10 min. In seven experiments significant ($p=0.004$) PGD_2 release was produced following incubation with 50 μg/ml of antigen for 10 min compared to unchallenged controls (358 $\pm$ 69 and 41 $\pm$ 13 ng/g dry weight of skin, respectively; mean $\pm$ s.e.m.). Antigen challenge had no effect on the release of PGE_2. Indomethacin (14 μM) completely inhibited the antigen-induced release of PGD_2. Confirmation of the identity of PGD_2, released during antigen challenge, was obtained by scanning GC-MS.

This report shows the enhanced generation of PGD_2 in guinea-pig skin during in vitro anaphylaxis and suggests that PGD_2 may play a role in such reactions in vivo.

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THE EFFECTS OF A SYNTHETIC PROSTANOID EPO92 ON INTRAVASCULAR PLATELET AGGREGATION AND BRONCHOCONSTRICTION

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Thromboxane A_2 is a potent aggregating agent and vasoconstrictor. It has been suggested that it is involved in the development of a variety of pathological conditions including thrombosis and angina. Jones et al (1983) have shown the effects of a competitive thromboxane receptor antagonist on platelet aggregation in vitro and in vivo. In this communication the in vivo effects of a more potent related compound (±)5-*endo*-(6'-carboxyhex-2'Z-enyl)-6-*exo*-{1"-[N-(phenylthiocarbamoyl)-hydrazono]-ethyl}-*bicyclo*[2,2,1] heptane, EPO92, will be described.

Intravascular platelet aggregation was measured in rats and guinea-pigs using the continuous platelet counting method (Smith & Freuler, 1973). The animals were anaesthetised and the trachea and a jugular vein cannulated. A double cannula was inserted into a carotid artery to facilitate the withdrawal of blood into the Technicon Autocounter. 1% EDTA solution was used to anticoagulate the blood as it left the artery. As the guinea-pigs had to be artificially ventilated, a transducer was inserted into the system to measure lung inflation pressure. To measure the oral activity of EPO92, the rats were gavaged with the compound or vehicle at Time 0, after 30 min or 90 min the rats were anaesthetised and after a priming dose of collagen, further doses of collagen were given at 15 min intervals, 1 to 2.5 h and 2 to 3.5 h after oral administration. Collagen 40 µg/kg i.v. and 11.9-epoxymethano-PGH_2, U46619, 0.35 µg/kg i.v. were used as agonists.

		Fall in Platelet Count % inhibition		Lung inflation pressure % inhibition	
Species	EPO92 mg/kg i.v.	11.9-Epoxy-methano-PGH_2	Collagen	11.9-Epoxy-methano-PGH_2	Collagen
Rat	2.5	-	25.1±5.35(7)	-	-
	5	-	36.7±6.8 (7)	-	-
Guinea-pig	0.25	72.2±1.71(4)	46.1±3.38(6)	88.3±2.02(7)	97.6±1.84(5)
	1	80.3±5.04(4)	66.8±10.95(4)	100(4)	100(4)

EPO92 produced a dose-dependent partial inhibition of collagen-induced aggregation in both rats and guinea-pigs. The compound was more than ten times as active in guinea-pigs than in rats. EPO92 produced nearly 100% inhibition of the platelet response to 11.9-epoxymethano-PGH_2 in guinea pigs. The increase in lung inflation pressure after collagen and 11.9 epoxymethano-PGH_2 was completely abolished by 1 mg/kg EPO92. In the rat oral study, the group of rats treated with 10 mg/kg EPO92 gave significantly lower responses to 40 µg/kg collagen (eg after 120 min, 17.3± 1.86% fall in platelet count) than did the control group (eg after 120 min, 31.8± 4.08% fall in platelet count). These results indicate that EPO92 is active at a dose below 1 mg/kg i.v. in the guinea-pig and that in rats it has significant activity after oral administration. It inhibited the response of platelets and bronchial smooth muscle to endogenous TxA_2 released by collagen. It also inhibited responses to a synthetic TxA_2 agonist confirming the in vitro observations that EPO92 is a thromboxane receptor antagonist (Armstrong et al, 1983).

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A COMPARATIVE STUDY OF LTD_4 ACTIONS ON GUINEA-PIG AIRWAYS USING THE TISSUE BATH AND CASCADE TEST SYSTEMS

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The activity of LTD_4 on guinea-pig airways alone or in the presence of FPL 55712 (a competitive LTD_4 antagonist) or indomethacin varies according to whether the tissue bath (TB) or cascade superfusion (CS) test system is used. Differences in the sources of LTD_4 and guinea-pigs used in these studies are variables which might be responsible for the discrepancies. Hedqvist & Dahlen (1983) suggested that such discrepancies occurred with LTD_4 because the superfusion system only allows indirect contractant mechanisms to occur.

We have studied the action of one sample of LTD_4 (supplied by T.S. Abram, Chemistry Dept., Miles Labs.) alone and in the presence of FPL 55712 or indomethacin, using the guinea-pig trachea and lung strip in both the TB and CS systems. Guinea-pig zig zag chains and lung strips were either suspended in 10ml tissue baths containing Tyrodes at 37°C aerated with 5% CO_2 in O_2 or continually superfused in baths with Tyrodes (5ml/min.) under the same conditions. Responses were monitored using a Harvard isotonic transducer with a load of 250-500mg for the trachea and 1g for the lung strip.

LTD_4 was compared with histamine throughout these studies. Histamine was equipotent on both tissues using both test systems. In contrast LTD_4 was significantly more potent on the guinea-pig lung strip. However, this greater potency varied with the test system, being some 54 fold for the TB but only 15 fold for the CS. LTD_4 was 2187x and 304x more potent than histamine in the TB and CS respectively. In contrast using the guinea-pig trachea LTD_4 was only 30x and 65x more potent than histamine in the TB and CS respectively.

The slow onset and long duration of action of the leukotriene response was comparable in both systems as was the development of tachyphylaxis with repeated identical doses.

FPL55712 was either inactive or potentiated higher doses of LTD_4 on the lung strip in both test systems. In contrast FPL produced a dose related rightward shift of the complete LTD_4 dose/response curve on the guinea-pig trachea for both test systems. Using Schild analysis the pA_2 and slope values for FPL were 6.98 ± 0.12, -1.67 ± 0.25 and 7.4 ± 0.39, -0.83 ± 0.18 for the SB and CS respectively.

In the presence of indomethacin (1µg/ml) the LTD_4 dose/response curve on the guinea-pig lung strip was shifted to the right but to differing amounts in both systems. The greatest shift occurred in the CS where the ED_{50} decreased by 105x whereas the decrease was only 19x for the TB. Although indomethacin reduced the inherent tone of the guinea-pig trachea in both systems no significant change in the ED_{50}/EC_{50} for LTD_4 occurred.

This study demonstrated that LTD_4 had significant myotropic activity in both test systems. Similarly the action of FPL55712 was comparable in both systems in that it only antagonised LTD_4 on the guinea-pig trachea. Significantly greater indirect activity was seen for LTD_4 on the lung strip using the cascade system but this was only 20-30% of its total activity over the whole dose response curve.

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LEUKOTRIENE-INDUCED INCREASE IN TXA_2 GENERATION BY GUINEA-PIG SMALL AIRWAYS: MECHANISM OF ACTION

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Previous studies have demonstrated that guinea-pig and human isolated airway preparations spontaneously produce cyclooxygenase products (Orehek et al 1973, Cuthbert and Gardiner, 1983). Exogenous leukotrienes (LT's) are known to increase this spontaneous production of prostanoids and on some tissues such prostanoids form a significant component of the leukotrienes smooth muscle activity. Consequently an understanding of the mechanism by which Lt's increase prostanoid levels is vital to the development of antagonists of these newly discovered pathophysiological agents. We have examined three possible mechanisms by which Lt's may be increasing cyclooxygenase products. Firstly, as a non-specific consequence of the LT-induced smooth muscle contraction, secondly following activitation of a specific membrane receptor responsible for smooth muscle contractior. or thirdly as a direct effect on the cyclooxygenase enzyme.

The guinea-pig isolated lung strip preparation was used throughout our studies. Smooth muscle responses and changes in cyclooxygenase product levels (using radioimmunoassay) were both monitored by the methods described in our previous studies (Cuthbert and Gardiner, 1983).

LTD_4,(10^{-7}M),K+(10^{-2}M) and histamine (10^{-5}M) were separately added to the preparations and were shown to induce significant contractant responses. However, LTD_4 was the only agonist which induced a significant increase in the level of a cyclooxygenase product. TXB_2 generation was increased from a basal value of 28±7 to 51±10 pg/mg tissue.

LTD_4 was retested at two concentrations 10^{-9} and 10^{-7}M and was shown to produce both dose related contractions and increases in TXB_2 production. In an attempt to determine whether the latter effects were inter related, FPL 55712 ($1 \mu g ml^{-1}$) an antagonist of leukotriene action on guinea-pig ileum and trachea was added to the tissue 15 min. prior to LTD_4. Unfortunately, however, FPL failed to antagonise the LTD_4 induced contraction of the tissue. It did, however, significantly decrease the rise in TXB_2 induced by the lower concentration of LTD_4. FPL 55712 had no effect on cyclooxygenase product levels spontaneously generated by the tissue.

Tachyphylaxis was induced to the LTD_4 (10^{-7}M) contractant response by frequent repeated challenges. The basal spontaneously generated endogenous levels of TXB_2 and other cyclooxygenase products continued high throughout the experiment suggesting that no depletion of free arachidonic acid was occurring. However, a concomitant tachyphylaxis occurred to the LTD_4-induced rise in TXB_2 levels.

Taken together these results eliminate the possibility that LTD_4-induced changes in the cyclooxygenase levels of this tissue are simply a secondary response to smooth muscle contraction. The concomitant dose related rise in TXB_2 levels with smooth muscle contraction and the concomitant tachyphylaxis to both effects without reduction in spontaneous prostanoid generation suggests that LT receptor(s) are responsible for both smooth muscle activity and changes in TXB_2 levels. Whether they are the same receptor(s) or not is uncertain.

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MEASUREMENT OF MOUSE PLATELET ACTIVITY IN VIVO: DOSE-RELATED RESPONSES TO COLLAGEN, ADP, THROMBIN, 5-HT AND U46619

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Measurement of platelet responsiveness in vitro is relatively simple, but the results may bear little relevance to events in vivo, especially with respect to the effects of inhibitors. Published methods of assessing platelet responsiveness in vivo have employed i.v. administration of aggregating agents and either measured death rate (Nishizawa et al, 1972; Silver et al, 1974) or, at sub-lethal doses, reduction of circulating platelet count in anaesthetized animals (methods discussed by Smith, 1981). Thrombocytopenia is certainly a preferable and more reliable reflection of platelet activation than death rate, but techniques so far described involve relatively elaborate surgical preparation. Here we outline a simple means of measuring fall in platelet count in mice in response to a range of platelet aggregating agents.

Collagen (Hormon-Chemie, Munich) was diluted in isotonic (2.17% w/v) glycine. Thrombin, 5-hydroxytryptamine (5-HT) and adenosine diphosphate (ADP) were dissolved in 154 mM NaCl. Solutions of sodium arachidonate and the thromboxane receptor agonist, U46619, were as previously described (Nunn, 1981; Nunn and Chamberlain, 1983). Male mice (20-25 g) were fasted overnight and warmed under lamps for 10-15 min in groups of 10-12. The tip (5-10 mm) of each tail was cut off with a scalpel blade and a micropipette (3 µl) immediately filled by inserting one end into the bubble of blood. The blood sample was transferred to a diluent reservoir (Clay Adams) and platelet count determined using an Ultra-Flo 100 Whole Blood Platelet Counter (Clay Adams). Aggregating agents (5 ml/Kg) were injected rapidly into a tail vein and, for all agents except collagen, the mouse immediately (within 6 s) placed in a box of CO_2. In the case of collagen, peak responses were obtained by allowing an interval of 30 s between injection and placing in CO_2. When respiration ceased (30-45 s), a second 3 µl blood sample was collected from the inferior vena cava snipped cleanly open near the renal veins. The platelet count in the post-injection sample was expressed as a per cent of that in the pre-injection sample for each mouse, and was 97 ± 6%(8), 100 ± 2%(12) and 102 ± 4%(12) (mean ± s.e. mean, (n)) in three control experiments in which only 154 mM NaCl was injected. Responses to each dose of aggregating agent were obtained in 6-12 mice and averaged. All agents induced dose-related reductions in platelet count, the maximum fall being 70-80% for non-lethal doses of collagen and thrombin, but only 40-50% for other agonists. The doses of each agent that typically gave a 50% maximal response were as follows: collagen (300 µg/Kg), thrombin (0.2 nmol (16 Units)/Kg), ADP (20 nmol/Kg), U46619 (40 nmol/Kg), 5-HT (70 nmol/Kg) and arachidonate (15 µmol/Kg).

This technique has so far proved useful in a number of studies: for example, it has allowed examination in vivo of the mechanism of action of collagen and the mechanism by which β-lactam antibiotics reduce platelet responsiveness to ADP. It has also been used for comparing in vivo antagonists at 5-HT and thromboxane A_2 receptors, and for the rapid screening of potential anti-thrombotic compounds.

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THE EFFECT OF SOME NEUROPEPTIDES ON TONE OF THE MOUSE ANOCOCCYGEUS MUSCLE

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Ultrastructurally, the non-adrenergic, non-cholinergic (NANC) inhibitory nerves of the anococcygeus muscle resemble the p-type fibres of the gastro-intestinal tract, suggesting that the NANC transmitter may be a peptide (Gibbins & Haller, 1979). Indeed, it has been proposed that vasoactive intestinal polypeptide (VIP) might be involved in NANC relaxations of the mouse anococcygeus (Gibson & Tucker, 1982). However, recently it has been shown that field stimulation-induced relaxations of this tissue are complex and that VIP is unlikely to be the sole mediator of NANC responses (Gibson & Yu, 1983). In the present study, therefore, we have investigated the effects of a wider range of neuropeptides on tone of the mouse anococcygeus.

Male mice anococcygeus muscles (LACA strain ; 25-35g) were set up for recording isometric tension responses as described previously (Gibson & Wedmore, 1981). To prevent effects due to release of noradrenaline from sympathetic nerves, each tissue was preincubated with guanethidine (30 μM ; 15 min) and the Krebs solution contained phentolamine (1 μM).

The following peptides neither contracted the resting muscle nor relaxed carbachol (4 μM)-induced tone : corticotrophin releasing factor ; luteinising hormone releasing hormone ; met-enkephalin ; and glucagon (all up to 10 μM).

Oxytocin (0.1-2 mU/ml) and Arg^8-vasopressin (0.4-100 mU/ml) produced slow, sustained contractions which reached equilibrium after 3-4 min. Substance P (0.5-8 μM) produced a variable response, contracting some preparations (3), but having no effect on others (2). When present, the contraction consisted of a rapid initial phase, during the first 30 s, followed by a slower, sustained contraction. Both thyrotrophin releasing hormone (TRH ; 4-100 μM) and neurotensin (2-100 μM) caused contractions which peaked within 2 min but which were poorly sustained ; the tone returned to baseline after 5 min, without washing out the peptide. At this time, contractions of the tissue to either peptide were reduced greatly (i.e., cross-desensitisation), although responses to carbachol (4 μM) were unaltered.

Three peptides were found to relax carbachol-induced tone. VIP (0.05-1 μM) caused powerful, rapid relaxations which were fully developed within 2 min. Somatostatin (10-80 μM) and its analogue urotensin II (0.07-6 μM), a dodecapeptide from the urophysis of the fish Gillichthys mirabilis (Pearson et al., 1980), produced weak, slowly developing relaxations, which required 5 min to reach equilibrium.

The main points from this study are : 1) the mouse anococcygeus contracts to low concentrations of oxytocin and Arg^8-vasopressin, one of the few smooth muscles from male mammals to respond to the former peptide ; 2) TRH and neurotensin appear to cause contraction via a common, distinct pathway ; 3) of the peptides studied, only VIP produced rapid, powerful relaxations in low concentrations, consistent with the proposal that it might play a role in NANC transmission in this tissue.

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THE EFFECT OF ACID PERFUSION AND VIP INFUSION ON THE NEUTRALISING CAPACITY OF THE DUODENUM OF THE ANAESTHETISED RAT

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Winship and Robinson have reported that, in man, the proximal duodenum forms the principal site of neutralisation of hydrochloric acid following instillation just distal to the pylorus. Only small amounts of acid remained to enter the distal duodenum such that the neutralising ability of this region of gut was seldom functional. In the rat cysteamine-induced ulcer model of Selye and Szabo ulcers only occur in the proximal duodenum. We wished to determine if there was any difference in neutralising capacity of the rat proximal and distal duodenum. The method, based on that of Ohe et al., was as follows:

Male Wistar rats [180-250g] were anaesthetised with urethane. A multibore perfusion catheter was passed via the stomach into the proximal duodenum and the pylorus and duodenum were ligated to form a 1cm pouch. For distal segment studies a 1cm length of transverse duodenum was cannulated. Biliary and pancreatic secretions were excluded from both pouches. Perfusion commenced with 0.9% sodium chloride solution (saline), pH 6.5 for 30 minutes at 0.1ml min^{-1} and outflow pH was recorded continuously. The perfusing medium was then changed successively for saline adjusted to pH 4, 3.5, 3 or 2.5 (with HCl) for 40 minutes each. Saline, pH 6.5, was then perfused for a further 30 minutes and the descending pH series repeated. This procedure gave 2 input pH (IpH)/output pH (OpH) curves, called 1st and 2nd pass. The effects of vasoactive intestinal polypeptide (VIP), given at a dose of 1µg $kg^{-1}h^{-1}$ as a 0.01ml min^{-1} infusion via a jugular vein, on the OpH was determined.

Proximal segment OpH values were significantly higher ($p<0.05$; 't' test) than distal segment OpH values at all IpH levels on the 1st and 2nd pass. Proximal segment 2nd pass OpH values were significantly higher ($p<0.05$; paired 't' test) than 1st pass OpH values at IpH 4, 3.5 and 3. Distal segment 1st and 2nd pass OpH values did not differ significantly. VIP significantly increased ($p<0.05$; 't' test) proximal segment 1st pass OpH values compared to controls at IpH 3 and 2.5 and also 2nd pass OpH values compared to controls at IpH 3.5, 3 and 2.5 ($p<0.02$; 't' test). VIP did not significantly alter distal segment 1st or 2nd pass OpH values compared to controls.

These data indicate that: i) the rat proximal duodenum may have a greater capacity to neutralise instilled acid compared to the distal duodenum; ii) acid perfusion of the proximal but not the distal duodenum can enhance the neutralising capacity to subsequent acid perfusion; iii) VIP can stimulate neutralising capacity in the proximal but not in the distal duodenum.

In the rat, as in man, the proximal duodenum contains large numbers of Brunner's glands. These glands which produce alkaline secretion and mucus, are only sparsely present in the distal duodenum. In our experiments acid is probably neutralised by the combination of Brunner's gland secretion, HCO_3^- secretion by the duodenal mucosa, and by back diffusion of H^+ ions. It is possible that this difference in neutralising capacity between the segments may, at least in part be due to Brunner's glands activity. Kirkegaard et al., have shown that VIP depletes granules in the Brunner's glands of the rat. Our data demonstrates that VIP increases neutralising capacity and suggests that Brunner's glands may play a cytoprotective role in the rat duodenum.

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PENETRATION OF α-MELANOCYTE-STIMULATING HORMONE THROUGH THE BLOOD-BRAIN BARRIER IN THE RAT

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The ability of alpha melanocyte-stimulating hormone (MSH) to cross the blood-brain barrier (BBB) of the rat was assessed by comparison of the rate of appearance of the peptide in cerebro-spinal fluid (CSF) following intravenous injection of 30nmol synthetic MSH with the rate of appearance of a simultaneously administered dose of 0.185MBq inulin(^{14}C)carboxylic acid. Pentobarbitone anaesthetized Wistar rats (380-520g) were prepared with carotid, jugular and tracheal cannulae. Artificial CSF was injected at 63ul/min into the right lateral ventricle and subsequently collected from a second cannula implanted into the cisterna magna. The concentration of MSH and inulin in plasma samples and in the CSF perfusate were monitored in six rats before and up to 45 min after injection, and in six control inulin injected animals by a specific radioimmunoassay and by liquid scintillation counting respectively.

Concentrations of endogenous MSH measured in CSF perfusates and in plasma of control rats remained relatively constant during the experiments such that peptide concentrations measured in animals injected with synthetic MSH could be corrected by subtraction of preinjection levels for analysis. Plasma level data for MSH fitted a two compartment open model where the concentration C at time t was described by the equation:-

$$C_t = Ae^{-at} + Be^{-bt}$$

Inulin plasma data were adequately described by a two compartment closed model where the rate constant b=0. Unknown parameters were estimated by non-linear least-squares minimization with weighting equal to the reciprocal of the drug level squared. CSF concentration data were fitted to a one compartment model with an input from plasma at rate j and a clearance at rate k. The equation describing the CSF concentration C at time t was:-

$$C_t = \frac{Aje^{-at}}{k-a} + \frac{Bje^{-bt}}{k-b} - \left[\frac{Aj}{k-a} + \frac{Bj}{k-b}\right] e^{-kt}$$

A,a,B and b were set at the values estimated from the plasma data for each animal and the unknowns j and k estimated by the weighted minimization procedure used previously. Differences between parameters were detected by paired Student t-tests.

The rate constant j for the entry of MSH into the CSF compartment was 0.00087±0.00034min-1 (±s.e.mean) which was not significantly different (P=0.18) from that for inulin of 0.00055±0.00027min-1. The rate constants k for clearance from the CSF compartment differed significantly (P<0.002), that for MSH of 1.30±0.09min-1 being greater than that for inulin 0.22±0.09min-1 due to the assumed central metabolism of the peptide. As MSH penetrated into CSF at a rate comparable to inulin, it is concluded that the BBB of the rat is impermeable to MSH. Entry to the brain will be via the areas where the BBB is absent; the preoptic recess, the area postrema and, notably for a pituitary hormone, the median eminence region.

THE EFFECT OF ANGIOTENSIN II AT THE CHICK BIVENTER CERVICIS NERVE-MUSCLE PREPARATION

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It has been reported that the polypeptide angiotensin II has a number of actions associated with excitable membranes. For example, angiotensin II may affect the renal and vascular smooth muscle fibres (Munday, Parsons & Poat, 1972; Ferrario et al., 1972). In addition, angiotensin II may facilitate synaptic transmission at the vertebrate sympathetic ganglia (Wallis, Williams & Wali, 1978). The present communication describes the effect of angiotensin II on impulse transmission and muscle contractility in the isolated chick biventer cervicis nerve-muscle preparation.

Angiotensin (1-1000 ng.ml^{-1}) increased the amplitudes of the twitch contractions elicited at 0.2 Hz with 5V and 0.5 ms pulse duration, by 23-31% of the control twitch tension (0.8±0.2 g, n=6). Angiotensin (100 ng.ml^{-1}) sometimes produced small contractures in the chick biventer cervicis muscle (upto 0.3 g tension). However, at higher concentrations, the peptide produced no contractures.

Repetitive nerve stimulation at 10-50 Hz produced tetanic contractions which were slightly potentiated by angiotensin (50 ng.ml^{-1}), by 13-17% (n=6, $p < 0.01$).

Angiotensin (50 ng.ml^{-1}) also potentiated the contractures produced by acetylcholine (1-1000 μg.ml^{-1}) and tetraethylammonium (0.5-5.0 mg.ml^{-1}) in the isolated chick muscle. A mean (±SEM) maximum contracture of 3.5±0.4 g and 0.8±0.1 g was produced by acetylcholine (600 μg.ml^{-1}) and tetraethylammonium (2.0 mg.ml^{-1}) respectively (n=6). The mean (±SEM) EC50s were 450±10 μg.ml^{-1}) and 1.5±0.02 mg.ml^{-1}) respectively. Angiotensin increased the maximum contractures by 18±2.0% and 12±1.0% respectively.

The potentiation of the contractile responses by angiotensin was unaffected by hexamethonium (600 μg.ml^{-1}), atropine (300 μg.ml^{-1}) or by isoprenaline (10 μg.ml^{-1}), but the twitch tension was reduced by tubocurarine (10 μg.ml^{-1}) and abolished by lignocaine (500 μg.ml^{-1}).

When the preparation was incubated in radioactive choline (^{3}H-methylcholine, bath concentration of 0.1 μCi.ml^{-1}), for 2 h and then angiotensin II was added, the uptake of tritiated choline was increased by 28±2.0% of the control preparations (1380±67 counts min^{-1}, CPM) (n=8).

Since the contractrature produced by angiotensin may indicate possible membrane depolarization, it may be that angiotensin II affects sodium permeability in the chick muscle. The potentiation by angiotensin of the twitch (and tetanic) contractions in the chick muscle may be explained if the peptide acted either by prolonging the duration of the terminal action potential, or by promoting Ca^{2+} entry and release within the preparation.

Experiments, using intracellular recording and voltage-clamp techniques, are needed to investigate further the mode(s) of action of angiotensin at the vertebrate neuromuscular junction.

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THE EFFECTS OF VASOPRESSIN ON APPETITE-MOTIVATED LEVER-PRESSING BEHAVIOUR IN RATS

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Many studies have looked at the effects of the neuropeptide vasopressin on passive avoidance behaviour (see Gash and Thomas, 1983) but little attempt has been made to assess the effects of the drug on operant conditioned tasks. The present study investigated the effects of arginine vasopressin (AVP) on the behaviour of rats trained in a variable interval (VI) operant reward paradigm.

Adult male Wistar rats (n = 8, wt 280-350g) were trained to press a lever for food reward on a VI 0.5 schedule. The rats were given AVP (0.0625, 0.125, 0.25, 0.5 and 1.0 IU/Kg) s.c. in random order, and the number of lever presses recorded at 10 min intervals for 1 hr. Saline control sessions were recorded on the day prior to each drug session. The scores from each drug session were compared with the corresponding scores for the preceding control day. Only one drug session was conducted per week, and each rat was tested at the same time of day.

AVP (0.0625 - 1.0 IU/Kg) caused a dose-related depression in the rate at which the rats pressed the lever. The most pronounced depression occurred in the first 30 min after administration. Thereafter the response returned slowly to near control values. This effect was most apparent at the high dose (i.e. 0.5 and 1.0 IU/Kg). Analysis of variance showed that AVP caused significant decreases ($p < 0.05$) in lever pressing rates at dose levels 0.5 - 1.0 IU/Kg.

An outstanding feature of these experiments was the observation that the highest dose of AVP (1.0 IU/Kg) could completely abolish lever pressing behaviour about 5-10 min after administration. The rats adopted an immobile crouched or prostrate posture with their heads turned away from the lever and the food chamber and refused to press the lever for up to 40 min. This effect was however only seen during an animal's 1st or 2nd experience of the drug. Lever pressing was not abolished if it was a rat's 3rd, 4th or 5th experience, regardless of the concentrations of the previous doses of AVP. Thus, for example, 10 to 20 min after administration AVP completely abolished lever responding in one rat that had received AVP for the 2nd time, but only caused an 11.5% decrease in lever responding in another rat that had received 4 previous injections of AVP. This observation is in agreement with another study (Ebenezer, 1983) in which it was shown that rats chronically treated with AVP develop a tolerance to the behavioural depressant effects of the drug.

The results of this study have shown that AVP (0.0625 - 1.0 IU/Kg) causes a dose-related depression in an appetitively motivated conditioned task in rats. These results are consistent with other behavioural studies which show that AVP reduces spontaneous activity in rats (Ebenezer, 1983). Interestingly, preliminary results from our laboratory have shown that AVP can cause a slight activation of the ECoG in both urethane anaesthetised and conscious rats. Thus it seems as if the behavioural depressant effects of AVP are not reflected by changes in the ECoG. It is not possible at present to speculate on the mechanisms involved in the behavioural depressant effects of AVP.

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A COMPARISON OF DIRECT AND INDIRECT ACTIONS OF SUBSTANCE P AND OTHER TACHYKININS IN GUINEA-PIG ILEUM LONGITUDINAL MUSCLE

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The neuronal distribution of the tachykinin substance P (SP) has been studied in the guinea-pig ileum by immunohistochemical techniques (Nilsson et al, 1975; Schultzberg et al, 1980), and other work has shown the majority of these nerves to be intrinsic to the enteric nervous system (Franco et al, 1979), although other evidence especially with the neurotoxin capsaicin suggests some release from sensory neurones. Although some nerve-released SP may directly excite smooth muscle (Holtzer & Lembeck, 1980) there is also considerable evidence of an indirect action on the enteric nervous system (eg, Katayama & North, 1978) and interactions with several other putative neurotransmitters have been described (eg, Monier & Kitabagi,1980; Featherstone & Morton, this meeting). When applied to the guinea-pig ileum preparation SP may act directly to produce an immediate response, and more slowly to give a contraction via release of acetylcholine (ACh)(Holtzer & Lembeck, 1980; Yau & Youther, 1982).

The experiments described here were designed to compare the potency of a number of tachykinins in evoking the immediate or direct response with that for release of ACh measured, in the presence of hemicholinium-3, as the efflux of [^{3}H] -ACh from stores in the myenteric plexus previously labelled with [^{3}H] -choline, with simultaneous measurement of isotonic contractions of the longitudinal muscle. The immediate contractions of longitudinal muscle strips were measured using conventional organ bath techniques with isotonic recording and a 20s contact time.

Tachykinin	Immediate Response	Evoked Release of [^{3}H]ACh
SP	1	1
Eledoisin	1.4	26.5
Kassinin	0.6	2.6
Physalaemin	1.9	0.4

The table shows the relative potencies of the tachykinins studied on these two responses, and it can be seen that two quite distinct orders of potency emerge. It is of interest that the order of potency for the immediate response, and the EC_{50} for SP (appx 3.2nM) were little altered in the presence of atropine (1μM) ruling out any important cholinergic component. This order of potency corresponds most closely to the 'P' subtype of receptor (Lee et al, 1982) whereas that for the [^{3}H] -ACh release is markedly of the 'E' type in view of the relatively high potency of eledoisin. It should be noted that the release process was Ca-dependent and unaffected by hyoscine (1μM), hexamethonium (0.5mM), guanethidine (10μM) and naloxone (1μM).

We conclude that the receptors for the pre- and postsynaptic actions of SP differ and this may well be of physiological significance. It will be of interest to see how many other presynaptic SP receptors prove to be of the eledoisin selective type especially in view of the possible existence of other endogenous mammalian tachykinins (Kimura et al, 1983).

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USE OF SUBSTANCE P ANTAGONISTS IN THE INVESTIGATION OF NON-ADRENERGIC NON-CHOLINERGIC NERVE MEDIATORS IN SMOOTH MUSCLE PREPARATIONS

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Substance P (SP) immunoreactive nerve fibres have been shown to have a wide distribution in various smooth muscle preparations (eg, Schultzberg et al, 1980) though the presence of other tachykinins should not be discounted. Release of neuronal SP has been inferred from desensitisation studies (eg, Franco et al, 1979), and actual release demonstrated in the guinea-pig ileum by immunoassay of perfusate (eg, Gintzler et al, 1983; Holzer, 1983).

Hence, SP antagonists have a potentially important role in analysis of non-adrenergic non-cholinergic (NANC) neurotransmission in smooth muscle preparations. We report here on the use of two such antagonists [D-Arg^{1},D-Pro^{2},D-Trp^{7,9},Leu^{11}]SP (SPA 1) and [D-Pro^{4},D-Trp^{7,9,10}]SP$_{4-11}$ (SPA2) in the guinea-pig ileum longitudinal muscle strip (GPI), guinea-pig taenia caeci (GPTC) and guinea-pig urinary bladder strip (GPB). In all experiments the preparations were suspended in Krebs solution at 30°C containing atropine (10^{-6}M) and guanethidine (5×10^{-6}M), in matched silanised 1 ml baths, with electrical stimulation via stainless steel wires attached to the ends of the preparations, and isometric recording of muscle contractions. Stimulation parameters were: GPI, 30Hz, 1 ms width, 20s trains; GPTC, 30 Hz, 1 ms width, 10s trains; GPB, 1 pulse per minute, 0.1 ms, 3 pulse trains (all at supramaximal voltage).

In experiments with the GPI comparing the reduction in responses to applied SP relative to reduction in NANC nerve-mediated responses, the two SP antagonists, in the concentration range 3×10^{-6}M - 10^{-5}M, were effective in reducing responses to both. In parallel studies (Bailey et al, 1983, and in press) these antagonists appeared to show some selectivity for the 'E' receptor subtype postulated by Lee et al (1982), but nevertheless, other tachykinins are antagonised. These antagonists show some stimulant or partial agonist action at higher concentrations which makes it difficult to achieve total blockade of responses.

CONCENTRATION SPA1 (M)	% REDUCTION RESPONSE (± S.E.(n))	
	SP	NANC nerve stimulation
3×10^{-6}	51.8 ± 4.6 (15)	24.6 ± 3.7 (15)
1×10^{-5}	55.8 ± 9.1 (10)	45.5 ± 4.3 (10)

In the GPTC the balance of receptor subtypes seemed rather labile, as judged from relative potency studies, but essentially similar results were obtained as with the GPI.

In contrast, with GPB the importance of testing concurrent exogenous tachykinin responses was at once apparent. SPA2 had little or no effect on NANC nerve stimulation or on responses to any of the tachykinins tested. We have shown elsewhere (Bailey et al, 1983, in press) that the tachykinin receptor type in the bladder differs from that in the other two preparations in its lack of susceptibility to SP antagonists, even at high concentrations. Hence, in preparations of this type lack of blockade by SP antagonists cannot be taken as evidence against a possible use of SP in NANC nerve mediation.

In conclusion, the SP antagonists proved capable of reducing responses to NANC nerve stimulation only in those preparations where applied tachykinins were also antagonised, but total blockade proved difficult in view of non-specific actions of the agents.

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INTERACTION OF ANGIOTENSIN II WITH SUBSTANCE P IN THE GUINEA-PIG ILEUM

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Whilst investigating the selectivity of the substance P antagonist [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] SP it was found that this compound reduced the contractile effects of angiotensin II (Ang II) on the guinea-pig ileum longitudinal muscle strip in vitro.

In the concentration range of antagonist used (1-10 μM) the dose-ratios determined for substance P (SP) and ANG II were similar in magnitude. However, for histamine, carbachol and bradykinin tested under similar conditions, no marked antagonism was apparent. Consequently experiments were designed to investigate whether this phenomenon resulted from non-selectivity of the SP antagonist or some true interaction of SP and Ang II receptor-mediated effects.

In cross-desensitisation experiments (see table) SP was able to markedly reduce responses to Ang II, but not vice versa. Also at 10 μM another SP antagonist [D-Pro^{4},D-Trp^{7,9,10}] SP_{4-11} reduced responses of Ang II in addition to those of SP, though the angiotensin antagonist [Sar^{1},Ile^{5},Ala^{8}]Ang II at a concentration (1 μM) that markedly reduced the response to Ang II had little or no effect on SP responses.

The possible involvement of nerve-mediated actions in this phenomenon was investigated with tetrodotoxin (TTX) and these results (see table) support such a mechanism. In further experiments pretreatment of the preparation with 1 μM TTX consistently reduced the effect of SP desensitisation on control Ang II responses.

TREATMENT	PERCENT REDUCTION IN RESPONSE	
	SUBSTANCE P	ANGIOTENSIN II
Substance P desensitisation	77.9 ± 11.2(8)***	81.2 ± 7.9(11)***
Angiotensin II desensitisation	10.3 ± 8.6(8)N.S.	82.9 ± 7.5(11)***
10 μM [D-Pro^{4},D-Trp^{7,9,10}] SP_{4-11}	78.9 ± 8.9(9)***	40.2 ± 4.5(10)***
1 μM [Sar^{1},Ile^{5},Ala^{8}] Angiotensin II	8.2 ± 4.1(5)N.S.	43.8 ± 8.6 (6)**
1 μM Tetrodotoxin	5.5 ± 5.5(6)N.S.	40.0 ± 9.5 (8)**

Reduction in control submaximal responses as mean ± sem. Significance of difference from control levels **$p < 0.01$, ***$p < 0.001$.

The present observations with cross-desensitisation seem at variance with certain earlier findings (Franco et al, 1979; Hutchison & Dockray, 1981), but our conclusions regarding such an interaction are supported by the data provided by the peptide antagonists and TTX. A possible complication is that both SP and Ang II have been shown to release ACh in the guinea-pig ileum (Godfraind et al, 1966; Yau & Youther, 1982) but, for the experiments reported here, atropine (1 μM) was present throughout to eliminate this possibility.

Therefore, there seems good evidence that Ang II exerts part of its action by releasing SP from the enteric nervous system and in this respect resembles some other neuropeptides such as neurotensin and cholecystokinin (Hutchinson & Dockray, 1981; Monier & Kitabgi, 1980).

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INHIBITION OF DOPAMINE UPTAKE IN RAT STRIATAL SLICES BY PHENYLETHYLAMINE DERIVATIVES

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The presence of at least two saturable systems for the uptake of dopamine in rat striatal tissue has been demonstrated (Mireylees, 1975). The evidence suggesting a high affinity mechanism Km 1.19μM, Vmax 0.16 nmol/g wet wt/min and a low affinity mechanism Km 24.4 μM, Vmax 19.9 nmol/g wet wt/min. The high affinity uptake system has characteristics similar to those of peripheral neuronal uptake (Mireylees, 1975, Horn, 1973). However little is know of the characteristics of the low affinity uptake system. In order to study this system the effect on uptake of a variety of phenylethylamine based compounds was investigated. The accumulation of dopamine in striatal slices was also studied in the presence of benztropine and desipramine.

Uptake of (3H)-dopamine was studied using slices (0.5 mm thick) of striatum from the brain of male Sprague-Dawley rats (175-250g). The slices were preincubated for 5 min at 37 ^{o}C in Krebs' solution containing the compound under investigation. Drug concentrations in the range 10^{-3}M - 10^{-8}M were used. An aliquot of the incubation solution containing (3H)-DA was added (final concentrations 100 nCi/ml). At a total dopamine concentration of 100 μM the low affinity uptake system is the major system of the total uptake mechanism whereas at a dopamine concentration of 0.1 μM the high affinity system predominates. Incubation was for two min. Slices were removed by filtration, washed with 2ml of ice cold Krebs' solution, blotted, weighed, digested with Soluene-350 and the radioactivity measured by liquid scintillation.

The results indicate that complete inhibition of catecholamine uptake was not possible, the fraction of the total uptake which cannot be inhibited is taken to be due to diffusion or non-specific binding. The IC50 values were determined relative to the maximum inhibitable uptake obtained experimentally; 60% at 100 μM dopamine and 90% at 0.1 μM dopamine. The calculation of IC50 values was performed using the GLIM program on a DEC 20 computer.

At a dopamine concentration of 100 μM the rank order of potency of inhibition of these compounds was: desipramine > adrenaline = metaraminol =tyramine = benztropine > noradrenaline =6-hydroxydopamine >isoprenaline =octopamine > hordenine, whereas at a dopamine concentration of 0.1 μM the rank order was: desipramine= benztropine> hordenine =metaraminol= noradrenaline= tyramine> isoprenaline= octopamine >adrenaline> 6-hydroxydopamine.

These results suggest that the low affinity site shows the characteristics of specific binding. The low affinity system exhibits a pattern of inhibition different from that found for the high affinity system.

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USE OF IMMOBILISED LECTINS FOR PARTIAL PURIFICATION OF D_2 DOPAMINE RECEPTORS

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A necessary stage in the understanding of D_2 dopamine receptors is the purification of the receptor protein. Partial purification of detergent-solubilised receptors may be obtained by either gel filtration or sucrose density gradient centrifugation but more selective techniques are required in order to achieve greater purification. Accordingly we have investigated the interaction of solubilised D_2 dopamine receptors with immobilised lectins. For these studies D_2 dopamine receptors were solubilised from bovine caudate nucleus using cholate (0.2%)/sodium chloride (1M) and assayed using [^{3}H]spiperone binding.

Solubilised D_2 receptors did not adsorb well to a column of concanavalin A sepharose but good adsorption was observed to a wheat germ agglutinin (WGA)-agarose column. About 40% of the applied solubilised D_2 dopamine receptors were retained by the WGA-agarose and they could be eluted quantitatively using N-acetylglucosamine (0.1M) resulting in an approximately seven fold enrichment of receptors. No elution was obtained using other sugars indicating that the elution with N-acetylglucosamine was a specific one. The ligand-binding properties of the solubilised receptors were largely unchanged after this purification step.

The D_2 dopamine receptor is therefore a glycoprotein that can be partially purified using lectin affinity chromatography.

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CHARACTERISATION OF THE D_2 DOPAMINE RECEPTOR OF BOVINE ANTERIOR PITUITARY

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D_2 dopamine receptors have been studied in membrane preparations of bovine anterior pituitary by [^{3}H]spiperone binding. Specific [^{3}H]spiperone binding (B_{max} 99 fmol/mg protein, K_d 85pM, defined using 3.3μM (+)-butaclamol) was displaced by a series of drugs. The K_i values indicated a typical D_2 dopamine receptor pharmacological profile ((+)-butaclamol (13nM), (-)-butaclamol (1520nM), domperidone (6nM), sulpiride (360nM), apomorphine (70nM), dopamine (1670nM)). No serotonin S_2 receptor component of [^{3}H]spiperone binding was detected.

Whereas antagonist displacements gave pseudo-Hill coefficients close to one and were insensitive to GTP, agonist displacements gave pseudo-Hill coefficients less than one and agonist affinities were reduced by GTP. Agonist displacement curves both in the absence and presence of GTP could best be described in terms of two sites with different affinities. The effect of GTP was to alter both the affinities and relative proportions of the two sites.

The effects of two potentially irreversible antagonists have been investigated. Phenoxybenzamine inhibited [^{3}H]spiperone binding to membranes and extensive washing failed to alter this inhibition indicating it to be irreversible. When membranes were treated with α-flupenthixyl chloride (FPC) followed by extensive washing [^{3}H]spiperone binding was inhibited. Similar inhibition was observed, however, with the parent substance, α-flupenthixol (α-FPT) although this was not a non-specific effect as similar experiments with β-FPT showed no inhibition. It seems likely, therefore, that FPC is not an irreversible antagonist at D_2 receptors. Rather, owing to its high lipophilicity it is adsorbed on to membranes and interferes in subsequent binding assays.

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$D\text{-}_1$ AND $D\text{-}_2$ DOPAMINE AGONIST-ANTAGONIST ACTION IN THE NUCLEUS ACCUMBENS TO MODIFY MOUSE SPONTANEOUS CLIMBING BEHAVIOUR

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The possibility that dopamine receptors may be subclassified as linked (D-1) or non-linked (D-2) to adenylate cyclase (Kebabian & Calne, 1979) has received much attention, but behavioural analysis of the hypothesis has proven difficult, not least because a selective D-1 antagonist has not been available. However, such a compound has now been defined by receptor binding studies, SCH23390 (Hyttel, 1983; Waddington, personal communication). In the present study this compound is used with (-)sulpiride (selective D-2 antagonist), SK&F38393 and LY141865 (selective D-1 and D-2 agonists respectively, Lehmann et al, 1983) to determine whether these biochemically defined selectivities can be extrapolated to a behavioural situation in the mouse, spontaneous climbing. Intracerebral administration was used to avoid problems of blood-brain barrier penetration. Thus, female albino B.K.W mice were stereotaxically implanted with chronically indwelling guide cannulae (Bradbury et al, 1983) for intra-accumbens (ACB) injection (Costall et al, 1983). Spontaneous climbing was measured in 'Mouse Climbing Monitors' (Costall et al, 1982).

Both (-)sulpiride and SCH23390 were potent to dose-dependently inhibit spontaneous climbing on intra-ACB injection (ED_{50} values of 11 and 4.6 ng/ACB respectively). Low doses of SK&F38393 and LY141865 dose-dependently decreased spontaneous climbing behaviour (ED_{50} values of 35 and 530 ng/ACB respectively). Increased doses of SK&F38393 (>1 μg) but not of LY141865 (up to 5 μg) enhanced spontaneous climbing. Intra-ACB (-)sulpiride (1 ng 15 min pretreatment, causing no behavioural change per se) antagonised the motor inhibitory actions of intra-ACB SK&F38393 and LY141865 (0.1 and 1.0 μg respectively, near-maximal doses). In contrast, intra-ACB SCH23390 (0.2 ng 15 min pretreatment, having minimal effect on climbing per se) failed to modify the motor inhibitory effects of the dopamine agonists. The enhanced climbing caused by SK&F38393 (4 μg) was antagonised by both (-)sulpiride (10 ng) and SCH23390 (2 ng)(15 min pretreatments). Neither the motor inhibitory or facilitatory effects of the dopamine agonists were modified by prazosin or yohimbine.

Hence, the present studies show both the D-2 antagonist (-)sulpiride and the D-1 antagonist SCH23390 to potently inhibit normal dopamine function in the limbic system. However, that (-)sulpiride antagonised the motor inhibitory effects of both the D-1 agonist SK&F38393 and the D-2 agonist LY141865, and that both (-)sulpiride and SCH23390 antagonised the motor facilitatory effects of SK&F38393 would not support an interpretation in terms of distinct and independently operating D-1 and D-2 receptors. The failure of SCH23390 to antagonise the motor inhibitory effects of SK&F38393 and LY141865 is supportive of a selective action on postsynaptic dopamine receptors (Iorio et al, 1981) and is being further assessed.

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THE HYPOTHALAMUS AS A SITE OF ACTION FOR METOCLOPRAMIDE AND CLEBOPRIDE TO FACILITATE GASTRIC EMPTYING

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The actions of metoclopramide and clebopride to facilitate gastric emptying may involve an enhancement of smooth muscle contraction processes in the gastro-intestinal system (see Harrington et al, 1983; Roberts, 1982). Additionally, the enhanced gastric emptying following the injection of metoclopramide and clebopride into the ventricular system may reflect an action at an unspecified central site (Costall et al, 1983). We report here that the hypothalamus may be a relevant site of action.

The studies used starved Dunkin-Hartley guinea pigs, 500 ± 50 g, having chronically indwelling intracerebral injection guide cannulae stereotaxically located at Ant. 9.0, Lat. ± 1.6 and 1.5 mm below the dura to allow bilateral injection (1 μl/hemisphere) into the lateral area of the hypothalamus 8 mm below the guide tips. Gastric emptying was measured using the non-invasive fluoroscopic method of Cox & Ennis (1980), emptying being determined from the number of barium sulphate spheroids remaining in the stomach after 0.5-4 h (Costall et al, 1983).

The injection of metoclopramide (50-500 ng) into the hypothalamus caused a dose-related and rapid increase in gastric emptying, the rate being increased by 30-60% at 1 h ($n = 5-6$, $p < 0.001$, Mann-Whitney U test). Clebopride was approximately five times more potent than metoclopramide. Metoclopramide methiodide, having one fifth the potency of metoclopramide as a local anaesthetic (Fozard, personal communication), was equipotent with metoclopramide to facilitate gastric emptying on intrahypothalamic injection whilst procainamide (1000 ng) was ineffective. Haloperidol and domperidone (1000 ng) were also ineffective to modify gastric emptying as was the injection of metoclopramide (500 ng) 1.0 mm anterior to the above coordinates. The ability of intrahypothalamic metoclopramide (500 ng) to facilitate gastric emptying was antagonised by intrahypothalamic atropine (200 ng).

Thus, metoclopramide and clebopride are shown to be approximately one thousand-fold more potent to facilitate gastric emptying on intrahypothalamic injection than on injection into the ventricular system. This action does not involve a local anaesthetic effect and does not reflect a classical dopamine receptor blockade. It is suggested that the benzamide action in the hypothalamus to facilitate gastric emptying involves an enhanced cholinergic activity. The specificity of this action and its precise topography in the hypothalamus are presently being established.

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EFFECTS OF PIMOZIDE ON OPERANT PERFORMANCE IN VARIABLE-INTERVAL SCHEDULES: FAILURE TO OBTAIN SUPPORT FOR THE ANHEDONIA HYPOTHESIS

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According to the 'anhedonia' hypothesis of the mode of action of neuroleptics, these drugs exert their suppressant effects on operant behaviour by reducing the effectiveness of (i.e., by 'devaluing') reinforcing stimuli, and not by impairing the organism's capacity to respond (Wise, 1982). In the present experiment we tested a prediction derived from the anhedonia hypothesis using a new behavioural test system based on Herrnstein's (1970) quantitative formulation of steady-state operant behaviour.

The rate of responding (R) in variable-interval schedules of positive reinforcement is a hyperbolic function of the reinforcement frequency (r):

$$R = R_{max} \cdot r / (K_H + r) \qquad (1)$$

where R_{max} and K_H are constants expressing the theoretical maximum response rate and the reinforcement frequency needed to obtain the half-maximum response rate (Herrnstein, 1970). The value of R_{max} is sensitive to variables which impair the organism's capacity to respond (e.g., Bradshaw et al, 1983a), whereas the value of K_H is sensitive to variables which 'devalue' the reinforcer (e.g., Bradshaw et al, 1983b). It follows from Equation 1 that a drug which elevates K_H will have a more profound suppressant effect on performance maintained by low reinforcement frequencies than on performance maintained by high reinforcement frequencies, whereas a drug which reduces the value of R_{max} will have suppressant effects which are independent of reinforcement frequency. The present experiment examined whether the neuroleptic pimozide belongs to the former category, as is implied by the anhedonia hypothesis.

Fifteen female albino Wistar rats maintained at 80% of their free-feeding body weights were trained to press levers in conventional operant conditioning chambers, using 0.05 ml 0.60 M sucrose as the reinforcer. In Phase I, performance was maintained under a variable-interval 10-sec schedule. Five groups of three rats were tested, respectively, 10 minutes, one hour, two hours, three hours and four hours after intraperitoneal injections of pimozide dissolved in 0.1 M tartaric acid. Each rat received four injections, at three-day intervals, of each of the following doses of pimozide: 0.125, 0.250, 0.330 and 0.500 mg/kg. In Phase II, performance was maintained under a variable-interval 100-sec schedule, and the entire procedure was repeated.

Pimozide exerted a suppressant effect on response rates. The maximum effect was attained two hours after the injection, and was maintained for at least a further two hours. A two-factor analysis of variance (dose of pimozide, schedule of reinforcement), with repeated measures on both factors, revealed a significant effect of dose ($P < 0.001$), but there was no tendency for pimozide to exert a greater effect on performance maintained under variable-interval 100-sec than on performance maintained under variable-interval 10-sec ($P > 0.1$).

These results do not provide any evidence that the suppressant effect of pimozide on variable-interval performance is due to a 'devaluation' of the reinforcer. Indeed, the results are compatible with the hypothesis that the effects of pimozide reflect an impairment of the capacity to respond.

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RELATIONSHIP OF SPONTANEOUS OROFACIAL DYSKINESIA TO DOPAMINERGIC FUNCTION IN AGED RATS

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Orofacial movements in patients receiving long-term neuroleptic treatment are commonly termed 'tardive' dyskinesia, reflecting their presumed late onset as a side effect of such medication. While older patients may be more likely to develop the syndrome, some elderly subjects show similar dyskinesia without prior exposure to neuroleptics (Kane & Smith, 1982; Waddington, 1983). We have investigated possibly similar phenomena in aged rats.

Male Sprague-Dawley rats of 20-22 months were studied in parallel with matched 2-4 month counterparts. They were inspected individually for spontaneous mouth movements and rated as shown below. To assess dopaminergic function, behavioural responses to 0.125-8.0 mg/kg s.c. apomorphine were rated by a behavioural check list and a stereotypy scale (Molloy & Waddington, 1983). Striatal tissue was later assayed for ^{3}H-spiperone binding (O'Boyle & Waddington, 1983).

The number of animals with orofacial movements, predominantly of the jaw and similar to vacuous chewing, at each level of severity, are given in the Table.

	NONE	MINIMAL	MILD	MODERATE	SEVERE
Young (n = 16)	11	5	0	0	0
Aged (n = 15)	2	2	7	3	1

More aged than young animals showed orofacial movements of mild or greater severity ($P < 0.01$), but no evidence of spontaneous dopaminergic hyperfunction. Sniffing and locomotor responses to low apomorphine doses (0.125-0.5 mg/kg) were potentiated in aged animals, especially in the late phase of drug action ($P < 0.05$ @ 50 mins), and may represent a pharmacokinetic effect. At higher doses (2.0-8.0 mg/kg) no potentiation was noted, only a trend towards reduced licking responses in aged animals. The density of striatal D_2 dopamine receptors was reduced by 30% in aged animals: B_{max}, 20.8 $\pm$ 0.9 v/s 14.5 $\pm$ 1.5 pmol/gm, $P < 0.05$; K_d, 0.11 $\pm$ 0.01 v/s 0.13 $\pm$ 0.02 nM.

In aged animals, spontaneous orofacial dyskinesia occurred in the absence of either behavioural or neurochemical evidence for dopaminergic hyperfunction. In some aged individuals the effect was similar to that seen, using similar techniques, in other younger animals 2.5 months after initiating depot neuroleptic treatment (Crow et al, 1981). These data further support our propositions (Crow et al, 1981; Waddington et al, 1983; Waddington, 1983) that (i) 'tardive' dyskinesia, especially in elderly populations, may be contaminated by factors unrelated to neuroleptic treatment, and (ii) the role of dopaminergic hyperfunction in mediating these effects may have been overemphasised.

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EFFECTS OF THE TWO ENANTIOMERS OF 3-PPP ON DOPAMINE RELEASE MONITORED IN VIVO AND ON DOPAMINE SYNTHESIS MEASURED IN VITRO

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The recently synthesized dopamine-receptor ligands (+) and (-) 3-PPP (3-(3-hydroxyphenyl)-N-n-propylpiperidine) offer the possibility of studying the effects of selective activation of subclasses of dopamine receptors (Hjorth et al., 1983). We looked at the effects of the two enantiomers on dopamine synthesis in rat striatal synaptosomes, using the tritium release method. Both isomers produced a dose-dependent depression of synthesis, (+) 3-PPP being the more effective (range studied: 1-100 μM). This confirms that both (+) and (-) 3-PPP act as agonists at the pre-synaptic dopamine autoreceptor.

We also monitored changes in extracellular homovanillic acid in the striatum as an index of dopamine release after intraperitoneal administration of the two drugs; for this we used microprocessor-controlled linear sweep voltammetry with carbon paste electrodes implanted chronically in unrestrained rats (O'Neill et al., 1983). This revealed clear differences between the effects of the two isomers. (+) 3-PPP produced a dose-dependent depression of dopamine release (doses were 1, 5, 10 and 25 mg/kg), an effect similar to that of apomorphine. (-) 3-PPP, on the other hand, depressed dopamine release at 1 and 5 mg/kg but caused enhanced release at 25 mg/kg.

These results confirm previous findings using different techniques which show that (-) 3-PPP acts as a pre-synaptic agonist at low concentrations and as a post-synaptic antagonist at higher doses, while (+) 3-PPP acts as a dopamine agonist at all concentrations.

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FPL 60278 (DOPEXAMINE): ACTIONS AT PRE AND POSTJUNCTIONAL DOPAMINE RECEPTORS AND AT α- AND β- ADRENOCEPTORS

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Dopexamine is a novel dopamine (DA) analogue developed for the treatment of acute heart failure (Brown et al,1984). We have examined its interactions with peripheral DA receptors and α- and β-adrenoceptors in several well-known assay systems.

Falls in renal vascular resistance (RVR) to DA, dopexamine and salbutamol (SAL) were compared by i.a. injection to pentobarbitone anaesthetised dogs (Goldberg et al, 1978; Brown et al, 1983), treated with phenoxybenzamine (10 mg/kg, i.v.) to block α-adrenoceptors and haloperidol (HP,50μg/kg, i.v., hourly) to block DA_2 receptors. Mean (n=5-8) doses (mol/kg, i.a.) producing a 20% fall in RVR were DA, 1.07×10^{-8}; SAL, 0.39×10^{-8} and dopexamine, 0.91×10^{-8}. Responses to SAL were selectively antagonised (approx. 70-fold shift) by the β_2-adrenoceptor antagonist ICI 118551 (3.3μg/kg. min^{-1}, i.a.; Bilski et al, 1981) and those to DA by simultaneous administration of HP (100μg/kg, i.a.) indicating the latter effect to be mediated by vascular DA_1-receptors. Dose response curves for dopexamine exhibited only a small (approx. 3-fold) rightward shift in the presence of ICI 118551 but were shifted further (approx. 5-fold, comparable to DA) by added simultaneous injection of HP (100μg/kg, i.a.), indicating a response mediated in part by β_2- and largely by DA_1-receptor stimulation.

Rabbit isolated ear artery (REA) preparations (Brown & O'Connor, 1981) were used to assess prejunctional DA_2-receptor mediated inhibition of sympathetic neurogenic vasoconstriction sensitive to blockade by metoclopramide (MET,2.5×10^{-6}M). Dopexamine had approximately 1/6 potency of DA and unlike DA was only slightly antagonised by MET. Molar IC50s (n=10-19) under control conditions (and in the presence of MET) were: DA,1.9×10^{-7} (2.57×10^{-6}) and dopexamine, 1.14×10^{-6} (2.8×10^{-6}), suggesting the possibility of weak DA_2-receptor stimulation.

β-adrenoceptor mediated effects were examined in guinea-pig isolated spontaneously beating atria (β_1) and isolated spirally cut tracheal (β_2) preparations contracted with carbachol(2×10^{-7}M). In atria, dopexamine showed weak activity, the maximum rate increase at 3×10^{-5}M being only 10% of the maximum response to isoprenaline (ISO). Concentrations $>10^{-4}$M depressed atrial rate. EC50s(M) for DA, SAL & ISO were 8.2×10^{-5}, 7.2×10^{-7} and 9.3×10^{-9} respectively. In tracheal spirals (n=4-34) in the presence of β_1-blockade (atenolol,4×10^{-6}M), dopexamine was a full agonist (IC50,4.9×10^{-5}M) and although less potent than SAL (3.1×10^{-6}M) or ISO (3.6×10^{-7}M) was more potent than DA (>3×10^{-4}M).

Interactions with postjunctional α_1- and α_2- adrenoceptors were investigated in dog isolated saphenous vein strips, (Langer & Shepperson, 1981). Both DA(EC50, 9.5×10^{-6}M, n=6) and noradrenaline (EC50, 7.5×10^{-8}M,n=9) were potent agonists but dopexamine was devoid of activity up to a concentration of 10^{-4}M(n=6).

Dopexamine is therefore a potent agonist at postjunctional DA_1-receptors and has some β_2-adrenoceptor and prejunctional DA_2-receptor agonist properties. Its overall pharmacological profile differs considerably from that of DA and in some circumstances may prove to be clinically advantageous.

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BEHAVIOURAL EVIDENCE FOR MESOLIMBIC DOPAMINE RECEPTOR SUPER-SENSITIVITY FOLLOWING LONG TERM DIAZEPAM TREATMENT

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It is now well established that the behavioural responses produced by dopamine-like agonists are enhanced following long term neuroleptic treatment (Muller & Seeman, 1978). However it is also becoming apparent that a number of drugs which are not dopamine antagonists may also modify dopamine mediated behaviours following long term dosing. For example, Spyraki & Fibiger (1981) found that chronic treatment with the tricyclic antidepressant desipramine selectively enhanced behaviours mediated via the mesolimbic dopamine system. We now report that 22 day treatment with diazepam selectively enhances the behavioural responses produced by an intra-accumbens injection of dopamine.

Male Wistar rats (Charles River) weighing 130-150g at the start of the study were dosed orally for 22 days with either diazepam 10mg/kg, haloperidol 3mg/kg, or vehicle. On termination of dosing (Day 23) the animals were implanted with guide cannulae for direct injections into either the nucleus accumbens (A + 9.4,L ± 1.6,V 0) or caudate nucleus (A + 8.0,L ± 3.0,V + 1.0; Pellegrino & Cushman, 1967) using standard stereotaxic techniques. Five days later (Day 28) locomotor activity was measured following bilateral dopamine injections (2.5 µg) into the nucleus accumbens. Animals with caudate cannulae received 10 µg of dopamine bilaterally and were scored for stereotyped behaviour. All the animals received nialamide 50 mg/kg i.p. 2 hrs prior to the dopamine injection.

Both treatments produced a significant enhancement of the dopamine induced locomotor activity in the nucleus accumbens group (Mean activity ± S.E.M. 80min. after receiving the dopamine; vehicle group 19±9, diazepam group 83±21 $p<0.01$, haloperidol group 300±71 $p<0.001$). The enhancement observed in the diazepam group was not as marked and was of shorter duration than the effect seen in the haloperidol group. Chronic haloperidol also significantly enhanced the stereotyped behaviour produced by the intracaudate injection of dopamine, whilst diazepam had no effect (mean score ± S.E.M. 90 min. after the dopamine injection; vehicle group 1.6 ± 0.3, diazepam group 1.7 ± 0.2, haloperidol group 2.6 ± 0.3 $p<0.05$). These results demonstrate that 22 day treatment with diazepam produces a selective behavioural supersensitivity in mesolimbic regions. The clinical significance of this enhancement requires further investigation.

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NOMIFENSINE INDUCES AN EXAGGERATED STEREOTPYED RESPONSE IN RATS RECEIVING CONTINUOUS HALPERIDOL INTAKE FOR 20 MONTHS

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Chronic continuous neuroleptic administration to rats causes striatal dopamine receptor supersensitivity characterised by increased dopamine receptor density and enhanced striatal acetylcholine content (Murugaiah et al,1982). Rats treated in this manner show an exaggerated stereotyped response to high doses of apomorphine (0.5-1.0 mg/kg) but may also display apparent inhibition of stereotypy induced by a low dose of apomorphine (0.125 mg/kg) (Clow et al,1979). One interpretation of this latter effect would be maintained functional blockade of cerebral dopamine receptors after chronic neuroleptic treatment (Waddington et al,1981). We now compare the stereotyped response to nomifensine and desipramine with that produced by apomorphine in rats treated for 20 months with haloperidol.

Male Wistar rats (205±14 g at the start of the experiment) received haloperidol (1.4-1.6 mg/kg/day) dissolved in their daily drinking water continuously for 20 months. Control rats maintained alongside drug-treated animals received distilled water alone. At the end of this period, and while drug administration continued, administration of a high dose of apomorphine hydrochloride (1.0 mg/kg sc 15 min previously) to haloperidol-treated rats induced an exaggerated stereotyped response (Table 1). In contrast, administration of a low dose of apomorphine hydrochloride (0.125 mg/kg sc) produced a reduced stereotyped response compared to that observed in age-matched control rats. Blockade of dopamine reuptake following administration of nomifensine (3.12-25 mg/kg 30 min previously) to control rats caused a dose-related increase in stereotyped behaviour. In rats receiving chronic haloperidol treatment, stereotypy induced by high doses of nomifensine (9.37-25 mg/kg) was exaggerated compared to control animals (Table 1). However, the stereotyped response to low doses of nomifensine (3.12-6.25 mg/kg) were not different from control values. Blockade of noradrenaline reuptake after treatment with desipramine (1.62-25 mg/kg 30 min previously) did not induce stereotypy in any animals (Table 1).

Table 1 Stereotypy induced by apomorphine, nomifensine or desipramine in animals treated for 20 months with haloperidol (1.4-1.6 mg/kg) or age-matched control rats

Drug treatment	Dose (mg/kg ip)	Mean stereotypy score (± 1 SEM) Control	Haloperidol
Apomorphine	1.0	3.33 ± 0.33	4.17 ± 0.17*
Nomifensine	25	2.17 ± 0.17	3.17 ± 0.31*
Desipramine	25	0 ± 0	0 ± 0

$p < 0.05$ compared to control rats, Mann-Whitney U test. n = 6

The lack of inhibition of low dose stereotypy induced by elevation of brain dopamine content using nomifensine suggests that enduring blockade of stereotyped behaviour does not persist following chronic neuroleptic treatment. The apparent effect of apomorphine may relate to a disruption of the stereotypy scoring system as previously suggested (Boyce et al,1983).

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INTERACTION OF INDOLEAMINE ANTAGONISTS WITH $5HT_1$ RECEPTORS IN GUINEA PIG BRAINSTEM

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5-Hydroxytryptamine (5HT) agonist-induced myoclonus in guinea-pigs appears to involve a 5HT-1 receptor in the brainstem (Jenner et al,1983). This contrasts with the accepted correlation between indoleamine induced behaviours and action at 5HT-2 receptors (Peroutka et al,1981; Leysen,1981). We now investigate the ability of indoleamine antagonists to displace ^{3}H-5HT from guinea pig brainstem preparations in relation to their reported efficacy at 5HT-1 and 5HT-2 receptors.

Brainstem (minus cerebellum) preparations from female guinea pigs (250-500 g) were used for ligand binding assays. This tissue preparation specifically binds ^{3}H-5HT to both high and low affinity sites but specific ^{3}H-spiperone binding is low and cannot be consistently detected, indicating a prevalence of 5HT-1 binding sites (Jenner et al,1983; Peroutka & Snyder,1981). The capacity of indoleamine antagonists (8 concentrations; 10^{-9} M to 10^{-4} M) to displace specific ^{3}H-5HT (4 nM) binding (as defined using 10^{-5} M 5HT) from brainstem homogenates was examined.

The indoleamine antagonists differed widely in their ability to displace specific ^{3}H-5HT binding (Table 1). Methergoline and methysergide were most potent while cinanserin was only weakly active. Comparison of the order of potency of antagonists in displacing ^{3}H-5HT with that for action at 5HT-1 and 5HT-2 receptors in forebrain preparations showed the potency of ^{3}H-5HT displacement from guinea pig brainstem by indoleamine antagonists to closely parallel their ability in the rat to displace ^{3}H-5HT from 5HT-1 receptors, but not ^{3}H-spiperone from 5HT-2 receptors. In contrast, the ability of indoleamine antagonists to inhibit myoclonus induced by administration of 5-hydroxytryptophan (75 mg/kg sc) to carbidopa (25 mg/kg 60 min previously)-treated animals showed a differing order of potency. Methergoline (5 mg/kg ip) and cyproheptadine (10 mg/kg) were most potent. Mianserin (10 mg/kg) was less active and methysergide (10 mg/kg) and cyproheptadine only weakly active. The order of potency did not correlate with activity at 5HT-1 or 5HT-2 receptors.

Table 1 The ability of indoleamine antagonists to displace ^{3}H-ligands from brain 5HT-1 and 5HT-2 receptors and to inhibit 5HTP-induced myoclonus

Indoleamine antagonist	IC_{50} (nM) ^{3}H-5HT (5HT-1) a	b	c	^{3}H-spiperone (5HT-2) b	c	% reduction 5HTP myoclonus score
Methergoline	7.1± 1.5	9.9	25	2.1	4	65
Methysergide	8.9± 2.5	150	126	3.1	35	15
Mianserin	499 ± 99	82	1259	4.7	40	48
Cyproheptadine	607 ± 501	1110	794	2.4	20	92
Cinanserin	3377 ± 870	1800	3981	15	126	12

a:this study; guinea pig brainstem (^{3}H-5HT). b: Peroutka et al,1981; rat cortex (^{3}H-5HT and ^{3}H-spiperone). c: Leysen,1981;rat hippocampus (^{3}H-5HT) and frontal cortex (^{3}H-spiperone)

The data supports the involvement of a 5HT-1 receptor in guinea pig brainstem mediating myoclonus. The lack of correlation between in vivo antagonism of 5HTP induced myoclonus and in vitro displacement of ^{3}H-5HT binding may reflect pharmacokinetic effects on drug action.

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RECEPTOR OCCUPATION OF STRIATAL D_1-RECEPTORS MEASURED USING (^{3}H)-PIFLUTIXOL BINDING TO INHIBIT ADENYLATE CYCLASE ACTIVITY

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Specific binding of ^{3}H-piflutixol (in the presence of sulpiride) to striatal membranes may identify the D-1 dopamine receptor linked to adenylate cyclase (AC) (Hyttel,1981). We now study occuption of D-1 receptors by investigating the kinetics of ^{3}H-piflutixol binding and, in the same samples, measuring the activity of dopamine stimulated AC.

Pooled rat striatal tissue was homogenised in 20 volumes of 2 mM Tris maleate,2 mM EGTA pH 7.4 homogenising buffer at 4°C and centrifuged at 45,000 x g for 15 min. The resulting pellet was resuspended in 200 volumes of homogenising buffer. Aliquots (100 ul) of tissue suspension were incubated for 20 min at 30°C with ^{3}H-piflutixol (0-4.0 nM) in the presence of 3 x 10^{-5} M sulpiride. Samples were placed on ice and 750 ul of ice-cold 80 mM Tris-maleate, 2 mM $MgSO_4$, 10 mM theophylline, 0.2 mM EGTA, pH 7.4 buffer containing 0, 10 or 100 uM dopamine added. ATP (500 uM final concentration) and GTP (25 uM final concentration) were added and after 2 min samples were incubated for 2 min at 30°C. Some samples were then placed in boiling water for 2.5 min and assayed for cyclic AMP content (Brown et al,1972). Remaining samples were immediately filtered over Whatman GF/C filters and washed twice with ice-cold 50 mM Tris HCl buffer, pH 7.4, to determine ^{3}H-piflutixol binding. Specific binding was defined in the presence and absence of 10^{-6} M cis-flupenthixol.

Specific binding of ^{3}H-piflutixol over a range of concentrations was analysed using Scatchard analysis and computer curve fitting. This was compared with the inhibition of dopamine stimulated AC activity produced by addition of ^{3}H-piflutixol. The fraction of receptors occupied at each concentration of ^{3}H-piflutixol was plotted against the degree of inhibition of dopamine stimulated AC produced by ^{3}H-piflutixol. Using 100 uM dopamine to stimulate AC a good correlation exists between the inhibition of dopamine stimulated AC activity and the specific binding of ^{3}H-piflutixol (Table 1). However, when 10 uM dopamine was used to stimulate AC activity only 75% of ^{3}H-piflutixol binding sites had to be occupied to totally inhibit enzyme activity.

Table 1 Receptor occupation and inhibition of AC

Dopamine concentration used to stimulate AC	% receptor occupation giving no inhibition of AC (± 1 SEM)	% receptor occupation giving 100% inhibition of AC (± 1 SEM)	'r'
10 uM	0 (9)	75.1 (4)	0.962
100 uM	2.9 (10)	100.5 (6)	0.964

We conclude that a close relationship exists between the inhibition of dopamine stimulation of AC activity and the specific binding of ^{3}H-piflutixol to D-1 sites in rat striatum.

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THE ACTION OF Y-AMINOBUTYRIC ACID (GABA) ANALOGUES ON IDENTIFIED CENTRAL NEURONES OF HELIX ASPERSA

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GABA can excite, inhibit or have no effect on central neurones of the snail, Helix aspersa. The inhibitory response is associated with an increase in chloride conductance while the excitatory response is mainly associated with an increase in sodium conductance. Walker et al (1975) have shown that the excitatory and inhibitory responses are mediated via receptors that have different structural requirements for GABAergic activity. For activation of the excitatory receptor, the preferred conformation for GABA is extended while for the inhibitory receptor, it is partly folded. In the present study a number of muscimol analogues have been tested on both GABA receptors to obtain further information on the structural requirements of these receptors.

Intracellular microelectrode recordings were made from identified neurones in the isolated suboesophageal ganglia of Helix. The ganglia were prepared as described by Walker (1968) and conventional electrophysiological recording techniques used. GABA and the putative agonists were applied to the preparation in 0.4ml Helix Ringer via a glass pipette. From the resulting responses, equipotent molar ratio (epmr) values were calculated such that where the analogue under test was more potent than GABA, then the value obtained was less than one. For GABA excitation, cells 15, 16 and 17 in the visceral ganglion were used while cell 5 in the left parietal ganglion was used for the inhibitory response.

Table 1 Epmr values for GABA agonists on both excitatory (D) and inhibitory (H) receptors.

	D response	H response
GABA	1.0	1.0
Muscimol	0.0013 ± 0.0004	0.45 ± 0.11
Dihydromuscimol	0.0072 ± 0.0008	0.50 ± 0.10
Homomuscimol	0.43 ± 0.05	>100
Thiomuscimol	2.10 ± 0.16	---
Isomuscimol	>500	---
Piperidine-4-carboxylic acid	13.4 ± 1.4	>100
Piperidine-4-sulphonic acid	Antagonist	>100

Table 1 shows the epmr values obtained, the excitatory and inhibitory receptors having similar sensitivity to GABA and each epmr is the mean of 4-7 experiments. Muscimol is 750 times more potent than GABA on the excitatory receptor but only twice as potent on the inhibitory receptor. Piperidine-4-sulphonic acid is a potent and specific antagonist of the GABA excitatory response but has no agonist activity at this site. It is a very weak agonist at the inhibitory receptor site.

These results confirm that the structural requirements for the inhibitory and excitatory actions of GABA on Helix neurones are different. The most potent compound so far tested is muscimol which is more potent than GABA on both receptors. No compound has yet been found which is more potent on the inhibitory receptor than on the excitatory receptor.

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A SYSTEMATIC STUDY OF PARAMETERS DEFINING THE RESPONSE OF A NEURONE (THE LEECH LEYDIG CELL) TO EXOGENOUSLY-APPLIED AMINES

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The Leydig cells are a pair of large, spontaneously active interneurones on the ventrolateral sides of each leech segmental ganglion (Keyser et al, 1982). Since the leech is a segmental worm, the segmental ganglion is a repeating unit of the nervous system. Leech ganglia were set up for intracellular recording as described by Sunderland et al (1979). Activity of the cells persisted in 20mM Mg^{2+} Ringer, which removes synaptic input. Action potentials (APs) had an amplitude of 50-70mV and a duration of 15-20ms.

Initial screening of various amines, all of which hyperpolarised the cell, indicated a higher sensitivity to octopamine (OA) than to its analogues eg. tyramine, dopamine, noradrenaline, phenylethanolamine. OA was 10-100 times more potent than any other amine tested. However, when attempts were made to quantify the response, it proved difficult to obtain reliable dose-response curves. It was clear that factors other than dose were influencing the response and appreciable desensitization was also noted. Hence it was necessary to standardize the experimental procedure. Experiments were carried out using a single dose on each ganglion: it is possible to obtain 15-16 separate ganglia from one leech, thus reducing variation between individuals. Each preparation was allowed to settle to a steady firing level for 20 mins and, if necessary, adjusted to an initial AP frequency of approximately 1Hz using intracellular current injection via an amplifier bridge circuit. The drug was then applied for a period of 2 min during which level of membrane potential and change in AP frequency were monitored every 5s. Actual dose and ganglion number (ie. position of segmental ganglion in the leech) were randomised.

A neurophysiological response is multidimensional. Response to a drug, for instance, can be expressed in terms of the changes in membrane potential, amplitude, duration and frequency of APs as well as the time after its application. A multivariate statistical procedure, canonical correlation (Harris, 1975), was thus undertaken. The variation in a two-dimensional response (observed AP frequency and membrane voltage after drug treatment) was summarised in a set of independent variables (initial frequency, initial voltage, dose of drug applied, ganglion number). A canonical variate was obtained which accounted for 88% of the variation in the response set. This variate described the change in observed voltage ($r=0.99$) and was highly correlated with the dose of OA applied ($r=-0.93$); it should serve as a basis of a dose-response curve for future studies.

A second variate associated variation in the membrane potential to the ganglion number ($r=-0.98$); higher levels of response being associated with the more anterior ganglia. However, there is a 13% chance that this variate is describing random error. If the sensitivity of the cell to OA varies throughout the length of the animal, there must be a further complexity to the organisation of the nervous system that is, as yet, not fully understood. The specificity of the OA response suggests that this chemical may play a natural role in the leech nervous system.

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THE EFFECT OF β-ADRENOCEPTOR AGONISTS AND ANTAGONISTS ON HEAD-TWITCH IN MALE MICE

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Previous studies have shown that β_2-adrenoceptor agonists potentiate the quipazine hyperactivity syndrome mediated by 5-hydroxytryptamine (5-HT) receptors. The effect of antagonists was however not clearcut (Cowan et al, 1982). Since β_2-adrenoceptor agonists also potentiate the L-5-hydroxytryptophan (L-5-HTP) head-twitch (Ortmann et al, 1981), we have investigated the interactions of β_1- and β_2-adrenoceptor selective agonists and antagonists on this model.

Male TO mice (18-30g) received the agonists salbutamol or dobutamine s.c. simultaneously with carbidopa (9mg/kg s.c.) 15 min before L-5-HTP (200mg/kg i.p.) Head twitches were counted for 5 minutes starting 20 minutes after L-5-HTP. Each mouse was observed in parallel with a control animal (saline/L-5-HTP/carbidopa).

Potentiation of the head-twitch syndrome was caused by both the β_1- and β_2- adreno ceptor agonists (ED200 i.e. dose to double the control response:- dobutamine 1.53 (1.36-1.74) mg/kg;salbutamol 0.12(0.06-0.24) mg/kg). The legitimacy of the β_1/β_2-adrenoceptor subclassification has recently been questioned (Leclerc et al, 1981); however the effect of antagonists did provide some evidence that separate receptors may be involved (Table 1). The antagonists were injected s.c. immediately before salbutamol or dobutamine (except for practolol which was administered icv [Brittain and Handley, 1967]); parallel control groups received agonist only (control groups for practolol also received saline icv.). None of the selective antagonists affected the L-5-HTP response when given alone. The ability of butoxamine to prevent the effect of salbutamol while leaving dobutamine potentiation unaffected suggests that the former effect was caused by an action at β_2-adrenoceptors while the latter effect was not. However both the β_1-adrenoceptor antagonists metoprolol and practolol, prevented the respose to both agonists. This raises questions about the selectivity of the antagonists at the doses used; although practolol does have a relatively high selectivity it is still only 9 times more potent at β_1-adrenoceptors (Leclerc et al,1981).

Table 1 Interaction of β_1- and β_2-adrenoceptor ligands on head-twitch counts following L-5-HTP/carbidopa (mean ± s.e.mean).

	AGONIST					
	dobutamine (2.5mg/kg)		salbutamol (0.25mg/kg)		saline	
ANTAGONIST	alone	+antagonist	alone	+antagonist	alone	+antagonist
metoprolol (2.5 mg/kg)	43.0±7.3	13.9±1.9**	38.1±6.4	17.9±5.2**	28.0±12.2	14.0±3.8
practolol (10 ug icv)	34.0±7.0	10.9±2.9**	84.9±7.3	34.9±12.2***	13.6±3.1	12.6±3.9
butoxamine (10 mg/kg)	32.7±7.7	39.4±7.0	44.4±2.5	9.0±2.6***	17.0±0.7	17.4±1.4

(n/group=7) * p<0.05 ** p<0.01, *** p<0.001 paired t-test

These results suggest the possibility that both β_1- and β_2-adrenoceptors may be involved in modulation of the 5-HT mediated head-twitch response. The complex pattern of antagonist action may indicate problems of selectivity or alternatively may reflect the neuronal organisation of the receptors involved.

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EFFECTS ON PUNISHED RESPONDING OF DRUGS ACTING AT α_2- ADRENOCEPTORS

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Clonidine may possess anxiolytic activity (Hoehn-Saric et al, 1981; Uhde et al, 1981) and yohimbine and piperoxane cause anxiety and panic attacks (Holmberg & Gershon, 1961; Goldenberg et el, 1947). The effects of α_2-adrenoceptor ligands on operant conflict (Geller & Seifter, 1960), a widely accepted test for anxiolytics, have therefore been examined.

Two groups of male Lister hooded rats were trained on a 52 min variable interval (VI 2 min) schedule in which 3 min periods of continuous reinforcement with contingent footshock (CRFs) were interposed every 10 min. Drugs were injected ip at weekly intervals and effects compared (Wilcoxon matched pairs-signed ranks) with the preceding (saline control) day. Group 1 rats (footshock titrated to produce >75% suppression) were already benzodiazepine and clonidine experienced at the time of these experiments. Group 2 rats (titrated to <25% suppression) were yohimbine experienced.

Table 1 shows that both clonidine and azepexole increased responding during CRFs although effects were poorly dose related. Clonidine reduced VI responding at all doses. Yohimbine and idazoxan produced further suppression of CRFs responding. Only the highest dose of yohimbine significantly reduced responding during VI.

Table 1 Total lever presses/rat during CRFs (Punished) periods.

Group 1 (n=5)	mean ± sem		Group 2 (n=6)		
drug/dose (mg/kg)	saline	drug	drug/dose (mg/kg)	saline	drug
clonidine 0.0125	3.0±1.0	11.6±1.7*	yohimbine 1.25	18.5±5.4	8.2±3.4*
clonidine 0.025	3.0±1.2	9.6±1.7*	yohimbine 2.5	22.7±4.9	10.3±3.3*
clonidine 0.05	3.0±1.2	10.4±2.9*	yohimbine 5.0	21.5±5.2	2.3±0.9*
clonidine 0.075	2.6±1.0	3.6±2.0	idazoxan 0.125	33.5±5.0	22.5±4.6*
azepexole 1.0	6.8±1.5	18.0±1.8*	idazoxan 0.25	38.2± 3.0	11.0±3.8*
azepexole 2.0	4.8±1.3	12.6±1.3*	(* p<0.05)		

The Geller-Seifter conflict paradigm as used here is open to the possibility of rate-dependency effects. However, the results demonstrate that these α_2-adrenoceptor agonists are capable of having anxiolytic- and the antagonists 'anxiogenic'-like effects on punished responding. They also reinforce the likelihood of the involvement of noradrenergic systems in anxiety.

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SELECTIVE BLOCKADE BY MDL 72222 OF THE DEPOLARIZING ACTION OF 5-HYDROXYTRYPTAMINE ON VAGAL PRIMARY AFFERENTS

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5-Hydroxytryptamine (5-HT) has a potent depolarizing action on the vagal afferents whose cell bodies lie in the nodose ganglion. This action is antagonized by quipazine (Wallis et al, 1982). MDL 72222 (1αH, 3α, 5αH-tropan-3-yl-3,5-dichlorobenzoate) is the most potent of a series of benzoic acid esters of tropine in blocking the excitatory action of 5-HT on sympathetic nerve terminals (Fozard & Gittos, 1983). Since the compound is also a potent antagonist of the Bezold-Jarisch reflex elicited by 5-HT acting on vagal afferents (Fozard, 1983), its blocking activity has been investigated on vagal cell bodies.

Membrane potential change in a population of neurones was measured using the sucrose gap technique. Nodose ganglia were rapidly removed from rabbits (2-2.5 kg) killed by air embolism and desheathed before mounting in the apparatus described previously (Wallis et al., 1982). They were continuously superfused with Krebs solution at 19 - 20°C. Reproducible depolarizations were obtained by injecting into the superfusion stream to the ganglion amounts of 5-HT ranging from 5 - 80 nmol (0.05-0.4 ml of a solution in Krebs). Comparison was made with depolarizations to GABA (40 nmol), dimethylphenylpiperazinium, DMPP (80 nmol) and noradrenaline (1 µmol).

With 5-HT, repeated dose-response curves could be obtained from the tissue providing supramaximal test amounts were avoided. The ED_{50} determined from 16 ganglia was approximately 14 nmol ; 80 nmol gave maximal or near maximal responses. MDL 72222 was a potent antagonist of 5-HT responses, the threshold for antagonism being 5-10 nM and blockade being surmountable. Antagonism was concentration-related although not in a manner consistent with simple competitive antagonism. At a concentration of 100 nM, which caused a rightward shift in the dose-response curve to 5-HT with no evidence of a change in slope or a reduction in the maximum, the 5-HT ED_{50} was shifted to 76 nmol. Blockade of 5-HT by MDL 72222 was selective. At 100 nM, MDL 72222 had no significant effects on submaximal responses to GABA or noradrenaline ; at 1 µM a small effect on DMPP responses was observed (33% reduction) but GABA responses were unaffected.

Thus, MDL 72222 is a potent and selective antagonist of depolarization induced by 5-HT in the cell bodies of vagal primary afferents. Blockade, is, however, partial in that even high concentrations of MDL 72222 fail to abolish the response to 5-HT. The reason for this remains to be established although it may simply reflect activation by 5-HT of two distinct excitatory neuronal receptors: one, the equivalent to that on sympathetic neurones, being potently antagonized by MDL 72222 ; the other, analogous to that on the cholinergic nerves of the guinea-pig ileum, being unaffected by the drug (Fozard, 1983).

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THE EFFECTS OF CLONIDINE AND XYLAZINE ON TAIL FLICK LATENCY DURING NALOXONE INDUCED WITHDRAWAL IN MORPHINE TOLERANT RATS

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There have been various reports that the α_2 adrenoceptor agonist clonidine can reduce opiate withdrawal effects in both man (Gold, Redmond & Kleber, 1979) and laboratory animals (Dwoskin, Neal & Sparber, 1983). In this study we have compared the effects of xylazine, another α_2 agonist, with those of clonidine on the naloxone induced hyperalgesia in morphine dependent rats. Adult male Wistar rats, body weight 300-450g, were injected intraperitoneally with morphine hydrochloride solution, 7.5 mg/kg, on a 3 days on, 2 days off, 2 days on dosage regime to induce tolerance. On the eighth day the animals were injected intraperitoneally with naloxone hydrochloride solution, 5 mg/kg, to induce withdrawal symptoms. Analgesia was measured by using the tail flick latency in a water bath at 55°C as described by Sewell & Spencer (1976). Readings were taken before the injection of drugs on the eighth day and at 15, 45 and 60 minutes after. The change in tail flick latency relative to the pretreatment value was calculated. Preliminary investigations had indicated that doses of 0.2 mg/kg clonidine and 20 mg/kg xylazine were approximately equipotent in raising tail flick latency in naive rats and so the effects of these doses of the α_2 agonists on the naloxone induced hyperalgesia were measured. In addition further experiments were done to measure the effects of an α_2 antagonist RS 21361 (Syntex), 10 mg/kg, on the responses to the α_2 agonists during withdrawal.

Naloxone produced a decrease in tail flick latency of 1.20 ± 0.23 sec ($n = 10$) at 15 mins after injection, a decrease of 1.01 ± 0.31 sec at 45 mins and the effect had worn off by 60 mins with a slight increase of 0.39 ± 0.28 sec.

Clonidine produced a clear reversal of the effects of naloxone with an increase in latency of 1.06 ± 0.21 sec at 15 min ($n = 12$) an increase of 3.39 ± 0.86 sec at 45 min and an increase of 3.38 ± 0.46 sec at 60 min. Xylazine on the other hand had little effect on the decrease in latency at 15 min with a value of 1.26 ± 0.35 sec ($n = 12$), at 45 min there was an increase in latency of 0.88 ± 0.47 sec and a similar value of 0.90 ± 0.56 sec at 60 min. It is interesting to note that in naive rats clonidine gave increased latencies of 1.73 ± 0.51, 2.67 ± 0.61, and 1.40 ± 0.48 sec ($n = 6$) at the three time points whilst xylazine gave increased values of 1.07 ± 0.36, 2.41 ± 0.33, and 2.20 ± 0.62 sec ($n = 18$).

If the α_2 adrenoceptor antagonist RS 21361 was given 10 mins before the clonidine or xylazine it exerted a clear effect in both groups of experiments. In the clonidine treated group the increased tail flick latency at 15 mins was reduced to 0.08 ± 0.38 sec ($n = 12$), at 45 min the increase was reduced to 2.26 ± 0.48 sec and at 60 min to 2.05 ± 0.43 sec. In the xylazine group however the decrease in latency at 15 min was abolished with a slight increase in latency of 0.26 ± 0.46 sec ($n = 11$), at 45 min the increase was enlarged to 2.75 ± 0.52 sec and similarly at 60 min to 2.62 ± 0.32 sec.

Thus, in summary, it would appear that clonidine is more effective than xylazine at reversing naloxone induced hyperalgesia in tolerant rats, and that this effect with clonidine is reversible by α_2 antagonists, whereas the effects of xylazine do not appear to be susceptible to α_2 antagonist reversal in the same way.

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INHIBITION WITH ADENOSINE DERIVATIVES OF OPIATE WITHDRAWAL EFFECTS

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That adenosine suppressed the withdrawal contracture of normorphine-dependent guinea-pig ileum in vitro (Collier & Tucker, 1983) led us to test whether two stabler adenosine derivatives, likely to act on the same receptor, would also inhibit the normorphine withdrawal contracture of opiate-dependent ileum and withdrawal effects in morphine-dependent mice.

Tested acutely on pieces of ileum stimulated with supramaximal electrical pulses at 0.5 ms and 0.1 Hz, 2-chloroadenosine (2-CA) and N^6-cyclohexyladenosine (CHA) gave IC50 values ($\pm$ s.e. mean) respectively of 54 $\pm$ 7 nM and and 72 $\pm$ 8 nM. In normorphine-dependent ilea, 100 nM 2-CA reduced by 75.3% the withdrawal contracture precipitated with 300 nM naloxone ($P<0.01$). CHA, 100 nM, given 2-3 min after precipitation of withdrawal with the same dose of naloxone, largely suppressed the contracture.

Mice were made dependent by implanting subcutaneously one 35 mg pellet of morphine. Three days later, 25 min after administration of test drug or saline in coded solution, withdrawal was precipitated with naloxone, 1mg/kg i.p. After naloxone, for 15, 20 and 30 min respectively, withdrawal jumping was counted, weight-loss recorded and the incidence of diarrhoea noted. 2-CA at 0.2 - 3.125 mg/kg i.p. significantly ($P<0.01$) inhibited jumping, weight-loss and diarrhoea in a dose-related manner. CHA at 0.5 - 3.125 mg/kg i.p. was likewise significantly ($P<0.01$) effective against all three withdrawal signs. Doses of chlordiazepoxide, which had comparable sedative effects to 2-CA, did not suppress withdrawal signs.

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CHRONIC HYDROCORTISONE INDUCES CENTRAL SEROTONERGIC SUPERSENSITIVITY WITHOUT AFFECTING A BEHAVIOURAL PARADIGM FOR DEPRESSION

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Corticosteroids have been reported to change mental state, including the induction of depression (Carpenter & Bunney, 1971). After chronic hydrocortisone guinea pigs display a subsensitive response to L-5-hydroxytryptophan(5HTP)-induced myoclonic jerking (Nausieda et al, 1982), and decreased 5-hydroxytryptamine (5HT) function may be partly implicated in clinical depression. Thus we have examined the effects of chronic hydrocortisone administration both on the response of mice in a putative test model of depression (Porsolt test; Porsolt et al, 1977) and in a model of cerebral 5HT receptor function (5HTP-induced head twitch; Corne et al, 1963).

Male CD1 mice (15-17g; Charles River; n = 20 per group) were chronically administered (p.o., b.i.d.) either hydrocortisone 21-hemisuccinate (10mg/kg) or deionised water (control group) for 2 or 4 weeks. Six hours after the final dose, the mobility time of mice was measured during a 4 min period in the Porsolt test. A further group of mice was administered 5HTP (200 mg/kg i.p. plus carbidopa 25 mg/kg i.p. 1h prior to 5HTP) 6h after the final dose of hydrocortisone or water, and the number of head twitches was counted during a 2 min period starting 30 min after 5HTP treatment.

Chronic hydrocortisone administration for 4 weeks attenuated weight gain in mice and also reduced adrenal gland weight (control group = 1.85 ± 0.14mg; hydrocortisone group = 1.25 ± 0.09 mg; $P<0.001$).

After 2 and 4 weeks of hydrocortisone administration, mice respectively displayed a small decrease (27% ; n.s.) and a small increase (21% ; n.s.) in immobility time compared to the response of control mice in the Porsolt test. Following both pretreatment periods, however, hydrocortisone-treated mice were markedly supersensitive to 5HTP administration displaying 164% ($P<0.001$; 2 weeks) to 365% ($P<0.001$; 4 weeks) more head twitches than control mice, and additionally exhibiting gross tremor and hind limb abduction.

Chronic hydrocortisone administration therefore evokes supersensitivity in the mouse brain stem 5HT receptors which mediate head twitch responses without altering behaviour in the Porsolt test. If supersensitive 5HT receptors are also induced in other brain areas it may indicate either that alterations in cerebral 5HT receptor sensitivity are not related to the response in the Porsolt test, or that the test is relatively weak in detecting drug-induced changes in brain 5HT function (Porsolt, 1981).

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EFFECT OF 5-HYDROXYTRYPTAMINE ON POTASSIUM-EVOKED RELEASE OF GLUTAMATE FROM RAT CEREBELLAR SLICES

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Anatomical studies have revealed the existence of serotonergic projections from the raphe nuclei to the cerebellar cortex (Bobillier et al 1976) which terminate in both the molecular and granule cell layers, with the latter projection appearing to synapse on granule cell dendrites (Chan-Palay 1977). Evidence suggests that the excitatory amino-acid glutamate is the transmitter of the granule cells (Sandoval & Cotman, 1978). In this study the effect of 5-hydroxytryptamine (5-HT) on K^+-evoked release of glutamate from cerebellar slices was investigated.

Parasaggital slices (250 μm) were superfused (0.5 ml/minute) with artificial CSF oxygenated with $95\%O_2/5\%CO_2$ at 37^oC. Initial experiments showed that the release of glutamate stabilized at a basal level within 40 minutes. Three, two minute aliquots of superfusate were collected between 40-46 minutes and then the slices were superfused for 4 minutes with CSF containing varying concentrations of K^+. In the experiments investigating the effect of 5-HT on glutamate release 35 mM K^+ was used. Varying concentrations of 5-HT were added to the stock CSF and the slices were superfused with this solution throughout. Two minute aliquots of superfusate were collected and freeze-dried prior to analysis by HPLC. The analytical method involved pre-column derivatization with O-pthaldialdehyde, followed by separation on a C_{18} reversed-phase chromatography column with fluorescence detection.

The K^+-evoked release of endogenous glutamate was found to be concentration and calcium dependent (Table I). Maximal release of glutamate was found to occur at 35 mM. The addition of 5-HT to the superfusion medium for the duration of the experiment produced a concentration-dependent inhibition of the K^+-evoked release of glutamate down to a concentration of 10^{-12} 5-HT (Table I).

TABLE I Effect of 5-HT on K^+-evoked release of glutamate (Mean ± SEM)

K^+(mM)	5-HT (M)	Glutamate release* (pmoles/mg of tissue)	n	% Inhibition
20	-	8.54 ± 3.03	6	-
30	-	34.54 ± 9.68	8	-
35	-	192.41 ± 31.60	7	-
35(Ca^{++}Free)	-	9.19 ± 3.40	8	-
40	-	191.19 ± 28.43	7	-
50	-	179.97 ± 47.38	8	-
35	10^{-5}	6.59 ± 2.46[a]	8	96.58
35	10^{-7}	52.08 ± 17.31[a]	8	72.93
35	10^{-8}	60.23 ± 13.16[a]	11	68.70
35	10^{-12}	60.27 ± 14.26[b]	6	68.70
35	10^{-14}	169.11 ± 63.96	7	12.11

*The evoked release of glutamate is expressed as the total increase in release above the pre-stimulation level. a - $p < 0.001$; b - $p < 0.01$ Students t-test

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THE REACTION OF RAT BRAIN CHOLINE ACETYLTRANSFERASE (ChAT) WITH ETHYLCHOLINE MUSTARD AZIRIDINIUM (ECMA) AND PHENOXYBENZAMINE (PB)

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Dixon plots suggested that choline mustard aziridinium (CMA) and ECMA caused competitive inhibition of soluble ChAT from rat brain (Ki 1.8 and 5.1 mM respectively, Rylett & Colhoun, 1979; Mantione et al, 1983). However 5 μM-CMA also caused time-dependent inhibition of intrasynaptosomal ChAT, interpreted as irreversible inhibition by alkylation (Rylett & Colhoun, 1980). We therefore characterised the inhibition of ChAT by ECMA and PB in synaptosome (P_2) and solubilized preparations.

Choline uptake was measured in slices (0.1 x 0.1 x 1 mm) of cerebral cortex pre-incubated (10 mg tissue) in 2 ml Tris-Krebs (118 mM Na^+, 37°C, 5 min), incubated with ECMA or PB (5 min) then with ^{3}H-choline (1 μM, 1 μCi, 4 min), filtered (GF/C discs) and corrected for uptake at 0°C. IC_{50} ECMA approx. 2 μM, PB approx. 30 μM. P_2 preparations or homogenised samples (glass-teflon, 5000 rpm, 0.5% Triton X-100, 1 h, 0°C) incubated with ECMA or PB (10 mg original tissue, 200 μl Na^+ Tris-Krebs, 1 h, 37°C) were assayed for ChAT (Fonnum, 1975), P_2 samples were washed (5% v/v x 3, Na^+ Tris-Krebs) and solubilized before assay.

30 μM ECMA inhibited P_2-ChAT by 50%, solubilized ChAT was less sensitive at low doses (300 μM ECMA, 50% inhibition) but 1 mM ECMA caused 81-83% inhibition in both preparations. Similar results were obtained with preparations from guinea pig cortex. 300 μM PB inhibited P_2 ChAT by 50%, 200 μM PB inhibited solubilized ChAT by 50%. Inhibitions by ECMA and PB (equipotent doses) had approximately the same time course in soluble and P_2 preparations. Hemicholinium (HC-3), at doses completely inhibiting specific uptake of choline, protected P_2 ChAT against 30 μM ECMA (table 1), but not against 300 μM PB. HC-3 did not protect the solubilized enzyme against 300 μM ECMA or 300 μM PB.

Table 1: HC-3 protection of ChAT activity‡ in synaptosomes incubated with ECMA.

HC-3 (μM)	control	ECMA (30 μM)
0	1.22 (0.07)	0.56 (0.03)**
0.4	1.31 (0.05)	0.70 (0.02)*
4	1.35 (0.03)	1.03 (0.05)
40	1.25 (0.08)	1.17 (0.04)

‡ μmol/hr/g tissue; mean values of quadruplicate determinations, in parentheses s.e. mean; ** $p < 0.01$, * $p < 0.05$ (2 tailed t-tests against controls).

Thus solublized as well as intrasynaptosomal (P_2) ChAT exhibits time dependent inhibition by ECMA and PB. That ECMA is no more effective against than is PB suggests non-specific alkylations. HC-3 sensitive uptake potentiates the inhibition of P_2 ChAT by ECMA, suggesting carrier-mediated concentration (10-fold) of ECMA within ChAT containing synaptosomes rather than alkylation by a non-specific contaminant. PB inhibits the carrier without apparent transport.

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CALCIUM IONS REGULATE EXCITATORY AMINO ACID RECEPTORS LABELLED WITH DL-(^{3}H)-2-AMINO-4-PHOSPHONOBUTYRATE

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DL-^{3}H-2-amino-4-phosphonobutyrate (APB) labels a population of synaptic membrane binding sites (Butcher et al.,1983a,b), which appears to correspond with the predominant type labelled by L-^{3}H-glutamate (Fagg et al., 1982). APB binding is almost totally dependent on the presence of Cl^- (EC_{50}=0.6mM), while the further addition of Ca^{2+} produces a two-fold enhancement in binding. In this study, we have investigated the regulation of APB binding by Ca^{2+}, and suggest possible mechanisms involved.

Whole rat brain synaptic membranes were prepared as described previously (Sharif & Roberts, 1980) and binding assays were performed in 50mM HEPES-KOH buffer (pH 7.4) at 37°C, at a final DL-^{3}H-APB concentration of 30nM. Binding assays were terminated by centrifugation, and non-specific binding was defined as that not inhibited by the inclusion of 1mM L-glutamate.

Specific DL-^{3}H-APB binding (in the presence of 2.5mM $CaCl_2$) was biphasic with respect to time. An initial equilibrium value of approximately 240 fmol bound mg^{-1} protein was attained within 10 min. Binding then increased, reaching a new equilibrium value of 600 fmol mg^{-1} protein after 50-60 min incubation. The K_D was unaltered throughout the incubation. Comparison of the pharmacological characteristics of binding during 10 and 60 min incubations, revealed no significant differences; this suggests that the "new" binding sites were identical to those detected with shorter incubations. The effect of Ca^{2+} in enhancing binding appeared to be irreversible, since binding was not reduced following the addition and then, subsequent removal of this ion.

Calcium ions induce the breakdown of certain membrane-associated proteins, by activation of calpain I (Siman et al., 1983); we have therefore investigated the effects of a number of protease inhibitors on $CaCl_2$ -dependent DL-^{3}H-APB binding. Benzethonium chloride (0.1mM) was without effect, while PMSF (0.1mM) reduced binding by approximately one third. PCMB (0.1mM), which alkylates thiol groups, virtually abolished binding. Leupeptin however, which inhibits the activity of calcium-sensitive neutral thiol-proteinases, reduced binding in the presence of Ca^{2+} to the level observed with Cl^- alone. These findings are consistent with the report of the uncovering of glutamate receptors by calcium, and its prevention by leupeptin (Baudry et al., 1983) in the rat hippocampal slice preparation.

Such mechanisms as outlined here may play a fundamental role for the rapid up-regulation of receptors.

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