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THE EFFECT OF PROBENECID ON THE DISPOSITION AND IMMUNOGENICITY OF BENZYLPENICILLIN IN THE RAT

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Probenecid has been used in conjunction with penicillin in therapy, because it increases the plasma concentrations of penicillin in man by competing with the active tubular secretion of penicillin (Overbosch et al., 1988). Penicillin allergy is thought to involve formation of penicilloyl-protein conjugates, which can then act as immunogens. Significant alterations in the degree of drug-protein conjugation in vivo, which could occur following increased plasma concentrations of benzylpenicillin (BP), may affect immunogenicity. We have therefore, investigated the effect of probenecid administration on the irreversible binding of BP to rat plasma proteins in vivo and in vitro, and on the immunogenicity of BP in the rat.

Probenecid (140 μmol/kg) was given as an i.v. bolus, immediately followed by infusion of probenecid (210 μmol/kg/h) to anaesthetised male Wistar rats. [^{3}H]BP (27 μmol/kg; 20 μCi), was administered i.v. 1 h after commencement of probenecid infusion, and serial blood samples were taken. [^{3}H]BP irreversibly bound to plasma proteins was measured by equilibrium dialysis. The plasma concentration of free BP and polar-acetone extractable metabolites were measured by liquid scintillation counting after separation by h.p.l.c (Kitteringham et al, 1987). The reaction between[^{3}H]BP (42 nmol;0.35 μCi)and rat plasma proteins (0.25 ml) in vitro was similarly investigated. The effect of i.p. probenecid (70 and 175 μmol/kg) on the immunogenicity of BP (2.7 mmol/kg) was assessed after chronic administration (4 days at 4 week intervals). Probenecid was administered 1 h before and 3 h after BP administration. IgG anti-benzylpenicilloyl (BPO) activity was determined by enzyme-linked immunosorbent assay (Christie et al, 1987).

Probenecid decreased the clearance of BP, indicated by the AUC (0-3 h) for total plasma radioactivity (mean; 152 nmol/ml.h) and free BP (mean; 65 nmol/ml.h), compared with control (mean; 57 nmol/ml.h and 8 nmol/ml.h respectively). Despite this there was no significant increase in irreversible binding at 3 h, which represented <0.05% dose/ml. Probenecid produced a concentration-dependent inhibition in the irreversible binding of [^{3}H]BP to rat plasma proteins in vitro, 5.6 ± 3.0% (n=10) after 3 h in the absence of probenecid and 1.8 ± 0.3% (n=8) in the presence of 0.5 mM probenecid. Serum IgG antibodies directed against BPO were not detected in any blood samples from any group.

These studies show that although probenecid administration caused an increase in the plasma concentration of BP, there was no corresponding increase in the irreversibe binding of BP, which can be attributed to probenecid inhibiting BP binding, as detected in vitro. Accordingly, there was no effect on the immunogenicity of BP in the rat. Therefore, these findings and previous observations on the effect of impurities in BP (Christie et al, 1987), indicate that qualitative rather than quantitative changes in disposition are more important determinants of the immunogenicity of the drug.

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INFLUENCE OF DONOR AGE ON THE MAINTENANCE OF CYTOCHROME P_{450}-RELATED ENZYME ACTIVITIES OF RAT HEPATOCYTES IN CULTURE

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It is recognized that cytochrome P_{450}-related enzyme activities decline rapidly upon placing rat hepatocytes in culture, and that dexamethasone can attenuate this loss of activity for some of the isozymic forms of P_{450} (Warren & Fry, 1988). It is also recognised that the P_{450} isozyme profile changes with age, particularly with the onset of sexual maturity (Gram et al., 1969). Accordingly, we have measured the activity and maintenance in culture of a number of P_{450}-related enzyme activities in hepatocytes isolated from 4 week (immature) and 8 week (sexually mature) male Wistar rats. The activities measured were the high affinity (substrate concentration of 10 or 20 μM) and total activity (500 μM) forms of 7-methoxy- and 7-ethoxy-O-dealkylases (7-MCOD, 7-ECOD).

Hepatocytes were isolated by lobe perfusion, and cultured for 24 hours in William's E medium containing 10% (v/v) foetal calf serum, 10^{-6}M dexamethasone and 5mM nicotinamide. Enzyme activities were measured in the freshly-isolated and cultured cells as described previously (Warren & Fry, 1988). The results are presented in the Table, and represent the mean ± s.e.mean (fresh-cell activities) or mean and range (maintenance values) for 6 animals. Statistical analysis was performed by Student's t-test (fresh-cell values) or Wilcoxon rank sum test (maintenance).

Table 1 Influence of donor age on activity and maintenance in culture of enzyme activities

Enzyme Activity	Fresh-cell value[1]		Maintenance[2]	
	4wk	8wk	4wk	8wk
7-MCOD (20μM)	17±2	10±1^{+}	42 (19-59)	89^{+} (57-146)
7-MCOD (500μM)	85±7	155±10^{++}	54 (44-75)	47^{+} (34-54)
7-ECOD (10μM)	18±2	11±2	74 (56-94)	140^{+} (89-220)
7-ECOD (500μM)	233±17	199±25	44 (35-58)	103^{+} (71-156)

[1] pmol/min/mg protein [2] percent of fresh-cell value
Significantly different from 4-week value at $^{+}P<0.01$; $^{++}P<0.001$

High-affinity 7-MCOD activity was significantly reduced at 8 weeks, whereas the total activity was significantly increased. 7-ECOD activities showed no variation with age. Maintenance of three of the four activities was significantly increased when 8 week donor rats were used; maintenance of the total 7-MCOD activity was not altered by using 8 week donor rats.

These results suggest that P_{450}-related enzyme activities of rat hepatocytes in culture are maintained better when older animals are used as donors, but that some selectivity in effect is still apparent. It is possible that the improved maintenance using hepatocytes from older rats is associated with the reported decreased turnover of P_{450} (Levin et al., 1975).

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CORRELATION OF CYTOTOXICITY IN VITRO OF ANTI-TUMOUR COMPOUNDS WITH ACUTE TOXICITY

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The LD_{50} values for a number of (potential) anti-tumour compounds, determined in NCI strain mice (i.p. administration), have recently been tabulated (Quinn & Milne, 1986). We have determined the in vitro cytotoxicity of eight of these compounds, which encompass a wide range of LD_{50} values, to assess the possible usefulness of in vitro cytotoxicity measurements for prediction of acute toxicity.

Cytotoxicity was determined with the Chinese hamster V79 cell line as described previously, using reduction in cell protein as the index of toxicity (Garle et al., 1987). The results (expressed as ID_{50} values) are presented in the Table, and represent the mean ± s.e.mean of 3-6 separate experiments. The LD_{50} values are taken from Quinn & Milne (1986).

Table 1 Cytotoxicity and lethality of eight (potential) anti-tumour agents

Agent	ID_{50} (μg/ml)	LD_{50} (mg/kg)
Actinomycin D	0.0028±0.0010	1.4
Colchicine	0.015±0.001	5.0
Methotrexate	0.038±0.002	87
Thioguanine	0.21±0.01	140
Melphalan	0.60±0.03	22
6-Mercaptopurine	3.2 ±0.6	523
Chloramphenicol	76 ±21	1077
Cyclophosphamide	>1500	372

The order of cytotoxicity agreed reasonably well with that for acute toxicity, with the exceptions of melphalan and cyclophosphamide (CPA). The anomaly with CPA can be ascribed to the absence of a liver metabolism component in the cytotoxicity assay for activation for CPA to its toxic species (Horner et al., 1985). The reasons underlying the anomalous results with melphalan are at present unclear. When the CPA data was omitted for the reason given above, the ranking of the LD_{50} values for the 7 remaining compounds correlated with the ranking of the ID_{50} values (Kendall's rank coefficient = 0.810, $P<0.02$).

It is concluded that measurement of cytotoxicity in vitro may be used for prediction of acute toxicity of potential anti-tumour compounds, but that assessment of the role of metabolism in cytotoxicity must also be made.

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ORAL ADMINISTRATION OF A NEW FORMULATION OF VITAMIN K_1 TO RABBITS

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Treatment of anticoagulant overdose involves administration of vitamin K_1, which is given by injection because of poor and variable oral bioavailability (Hart et al. 1984; Park et al. 1984). However, intravenous administration of the formulation of vitamin K_1 in current use (PE-K) can be problematic, due to the presence of polyethoxylated castor oil as a stabiliser (Rich & Drage 1982). A mixed micelle formulation of vitamin K_1 has now been produced for intravenous use; and in theory, the presence of bile salts might increase absorption of the vitamin from the gut. We have therefore investigated the pharmacokinetics and procoagulant activity of the mixed-micellar preparation of vitamin K_1 (MM-K) in male, New Zealand White rabbits.

Doses of 10 mg/kg MM-K (n=5) or PE-K (n=5) were given by gastric gavage, or via the left marginal ear vein for the measurement of bioavailability. Blood samples were taken from the right marginal ear vein at 0,1,2,3,5,7 and 12 h after dosing. A further 8 rabbits were given the potent and long-acting coumarin, brodifacoum (10 mg/kg i.p.) 24 h before administration of MM-K (n=4) or PE-K (n=4). Blood samples were collected as described above, and an additional 0.9 ml of blood was collected into trisodium citrate (10% by volume) for the measurement of prothrombin complex activity (PCA).

Oral administration of MM-K, alone, caused a significant ($p<0.01$) increase in the plasma concentrations of vitamin K_1. Maximum plasma concentrations of vitamin K_1 (450 ng/ml, range 133-824 ng/ml) were achieved later, and were significantly ($p<0.05$) greater than those seen after administration of the existing formulation of the vitamin (PE-K; 260 ng/ml, range 198-390 ng/ml). AUC after MM-K (4.6 ug/ml/h, range 2.1-6.3 ug/ml/h) was also significantly ($p< 0.05$) greater than after PE-K (1.6 ug/ml/h, range 1.0-2.1 ug/ml/h). However, the bioavailability of vitamin K_1 after MM-K was poor (9.4%), and there was considerable intra-individual variability between the concentrations of vitamin K_1 measured. Both preparations of vitamin K_1 stimulated clotting factor synthesis in rabbits anticoagulated with brodifacoum. Maximum stimulation of clotting factor synthesis after MM-K (PCA 87%, range 44-124%) was seen later (t max 12 h) than after PE-K (PCA 82%, range 47-125%; t max 5h), but there was no difference between the maximum effects of the two preparations on clotting factor synthesis. Moreover, there was considerable intra-individual variability between the two groups of rabbits.

It is concluded that the incorporation of K_1 into bile salt micelles does not improve the bioavailability or procoagulant effect to an extent likely to be of clinical benefit after oral administration. The value of MM-K in clinical use will result from its lower toxicity after intravenous administration.

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LOW CELLULAR LITHIUM CONCENTRATIONS: A CONSEQUENCE OF PARACELLULAR TRANSPORT IN ACUTE STUDIES?

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The mode of action of lithium in the prophylaxis of affective disorders is unknown: many areas have been explored (Birch, 1988). The intestinal transport of lithium in rodents has been shown to be a passive process, possibly utilising a paracellular route through the tight junctions (Birch et al, 1985a, 1985b). We now report further investigations of lithium transport and tissue uptake in guinea pig jejunal mucosa.

Isolated guinea pig jejunal mucosa were prepared as previously described (Birch et al, 1985a, 1985b) using a method based on that of Lauterbach (1977). Krebs Tris buffer (pH 7.4) was present in both compartments of the flux chambers. Mucosal-side buffer contained lithium ranging in concentration from 5 to 30 mM, (^{14}C) labelled D-glucose (14 mM), and (^{3}H) polyethylene glycol (PEG) 900 (0.4 mM) as an extracellular marker. Serosal-side buffer was PEG and lithium free. Transport and tissue uptake of lithium were measured, after 45 min incubation at 37°C, using atomic absorption spectroscopy. (^{14}C) D-glucose and (^{3}H) PEG 900 were determined by liquid scintillation counting. Q_{10} was determined after temperature reduction to 27°C (Hofer, 1981).

Lithium transport in the mucosal to serosal direction was linear over the concentration range 5 to 30 mM at 37°C with no evidence of saturation. 2,4 dinitrophenol had no effect on transepithelial transport of 20 mM lithium but significantly reduced glucose transport ($P<0.01$). 10° C temperature reduction significantly reduced both lithium ($P<0.05$) and glucose transport ($P<0.01$). Q_{10} for lithium transport was 1.53, and for glucose 2.47.

The concentration of lithium associated with the tissue was lower than transported lithium (mean ratio 0.477, s.e.mean 0.031). Luminal buffer lithium concentrations and apparent tissue uptake showed a strongly positive and highly significant correlation ($r=+0.925$, $P<0.001$). The lithium content of the extracellular fluid associated with the tissue was calculated and was shown to be similar to the experimentally measured tissue lithium content ($r=+0.947$, $n=15$, $P=<0.001$, regression slope 1.03).

We infer that lithium transport is primarily a passive process and that most of this lithium resides in the extracellular fluids. This supports preliminary evidence of low cellular uptake of lithium by other cell types (Thomas et al, 1988; Partridge et al, 1988). However, a minor active component of lithium transport, which may be transcellular, cannot be excluded.

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THYROID STIMULATING HORMONE (TSH) ACTIVATES Na^+K^+-ATPASE AND IODIDE ACCUMULATION IN THE RAT FRTL-5 THYROID CELL LINE

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A number of ion-pumps and channels are located in the plasma membrane of thyroid epithelial cells. For some, their function and interrelationships remain to be precisely clarified in relation to control of growth and function (thyroid hormone synthesis) of the thyroid gland. For example, there is a K^+,H^+-ATPase (Saccomani et al, 1979) a voltage-dependent Na^+-channel, and a Na^+,H^+-antiporter (Marcocci et al, 1987) which appears to be located at the apical membrane and may be involved in TSH-regulated cell growth. Much more, however, is known about the thyroidal Na^+K^+-ATPase (Gerard et al, 1985). It is localised at the basolateral membrane, is activated by TSH and generates Na^+ gradients necessary for iodide transport. We have now further characterized this pump in rat thyroid FRTL-5 cells in vitro, and determined the time-course of and correlation between TSH-stimulated iodide accumulation and Na,K^+-ATPase activity, since this is still unclear.

FRTL-5 thyroid cells (obtained by permission of L, Kohn, NIH, USA) were passaged using trypsin-collagenase and plated at a density of 2 x 10^5 cells/ml in 24 x 0.5 ml tissue culture dishes in a Coon's Modified Ham's F 12 medium with hormone supplements plus TSH (6H medium - Brown et al, 1985) for 3 days. After 7 days in a TSH-free medium (5H) the medium in each well was replaced by a one containing different TSH concentrations (0-500 µU/ml). For periods up to 48 hours (0-24 hours TSH followed by 24 hours in 5H medium) the cells were then assessed for their ability to accumulate ^{125}Iodide by the method described previously (Brown et al, 1985). Na^+K^+-ATPase activity was determined by measuring ouabain-sensitive ^{86}Rubidium (^{86}Rb) uptake against total uptake. Culture medium was removed and replaced with 500 µl Hanks Balanced Salt Solution (HBSS) ± ouabain (5 mM) for 30 mins, and then incubated with 1 µCi/ml ^{86}Rb for 5 mins. The medium was then aspirated and the cell layer washed once with ice-cold HBSS. Cells were dissolved in 0.1 M NaOH for approx 20 hrs and ß emission measured.

Exposure to TSH for 27 hours activated ^{125}Iodide transport but not ouabain-sensitive ^{86}Rb transport in the thyroid cells in vitro, whereas after 36 hrs and above there were significant increases in both transporters (up to 300-400% increases). This was similar to results obtained in propylthiouracil-treated rats in vivo where activation of thyroidal Na^+,K^+-ATPase activity in thyroid membranes occurred around 24-48 hrs following rises in serum TSH. These data confirm the presence of a ouabain-sensitive Na^+,K^+-ATPase in rat FRTL-5 cells which appears to be activated several hours after iodide-accumulation by physiological TSH concentrations. The data suggest stimulated synthesis of new transporter proteins by TSH and contradict early work where TSH was reported to rapidly activate the Na^+-pump in thyroid cell membranes (Turkington, 1962).

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INCREASE IN BLOOD-BRAIN BARRIER PERMEABILITY DUE TO AMITRIPTYLINE IS ACCOMPANIED BY AUGMENTED PINOCYTOSIS IN CEREBRAL CAPILLARIES

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Catecholamines were previously observed to modify pinocytotic activity in dog vessels (Azevedo et al.,1984). Since it is known that both stimulation of Locus Coeruleus (Raichle et al.,1975) and administration of amitriptyline (Preskorn et al., 1982) increase the permeability of blood brain barrier, the hypothesis of an effect of catecholamines on the pinocytosis of cerebral capillaries and consequently on blood brain barrier permeability was considered.

To evaluate pinocytosis three different methods were used: direct visualization and quantification of pinocytotic vesicles in capillary endothelial cells at the electron microscope; transport of horseradish peroxidase (HRP) with visualization in histochemical ultrastructural preparations; and brain extraction rate of ^{131}I-human albumin after i.v. injection.

Adult Wistar rats were anaesthetized (sodium pentobarbital, 40 mg/kg, i.p.) and amitriptyline (34 mg/kg, i.p.) or the same volume of saline injected. Under artificial ventilation, the abdominal aorta was clamped, the ascending aorta catheterized and perfused with Karnowsky fixative for 30 min (beginning 10 min after amitriptyline or saline injection). Fragments of the right parietal cortex were collected and processed for ultrastructural study. When used, HRP was injected (75 mg i.v.) 20 min before amitriptyline or saline. In this case the tissue samples were histochemically processed for ultrastructural visualization of HRP.

To calculate brain ^{131}I-albumin extraction rate, 30 μCi ^{131}I-albumin or the same volume of saline were injected i.v. in adult anaesthetized Wistar rats (see above). Thirty minutes later blood samples were obtained, the animals sacrificed and parietal cortex fragments collected. Radioactivity was measured in both blood and cerebral tissue samples and the extraction rate of ^{131}I-albumin calculated according to the formula: $\frac{\text{cpm/mg brain}}{\text{cpm/mg blood}} \times 100$.

In control preparations pinocytotic activity in capillary endothelial cells was scarce: 1.39 vesicles/μm^2. Amitriptyline significantly increased the density of pinocytotic vesicles in capillary endothelial cells, to 5.55 vesicles/μm^2 ($n=129$; $p<0.001$).

In amitriptyline and HRP injected rats, many pinocytotic vesicles were dark stained, the reaction product being also detected beyond the capillaries in brain cell lysosomes; in the animals injected only with HRP these aspects only occurred in a small degree. Amitriptyline did not induce other changes in capillary endothelial cells, namely separation of intercellular junctions. The brain ^{131}I-albumin extraction rate in control animals was 1.16 ± 0.05. Amitriptyline significantly increased that value by 20% ($n=12$; $p < 0.05$).

We conclude that amitriptyline induced increase in the blood-brain barrier permeability appears to be ascribable to enhanced pinocytosis.

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POLYMYXIN B DOES NOT MODIFY THE EFFECT OF ADENOSINE BUT PREVENTS THE EFFECT OF PHORBOL ESTERS AT THE FROG NEUROMUSCULAR JUNCTION

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It has been shown that the activator of protein kinase C, 4β-phorbol, 12,13-diacetate (Castagna et al., 1982) attenuates the inhibitory action of adenosine on neuromuscular transmission at the frog neuromuscular junction (Sebastião & Ribeiro, 1988). It seemed therefore of interest to know whether an inhibitor of protein kinase C, polymyxin B (Kuo et al., 1983) would modify the inhibitory effect of adenosine on neuromuscular transmission.

The experiments were carried out at room temperature (22 - 25°C) on the isolated nerve-sartorius muscle of the frog. The nerve was stimulated at a constant rate (0.5 Hz) with supramaximal rectangular pulses of 20 μs duration. Intracellular techniques for recording evoked endplate potentials (e.p.ps) were conventional (e.g. Ribeiro & Sebastião, 1987). The normal bathing solution (pH 7.0) contained (mM): NaCl 117, KCl 2.5, NaH_2PO_4 1, Na_2HPO_4 1, $CaCl_2$ 1.8, $MgCl_2$ 1.2. Muscle action potentials and twitches in response to nerve stimulation were prevented by increasing the Mg^{2+} concentration (9.0 - 12.5 mM) in the bath. The effects of adenosine or phorbol esters in the absence and in the presence of polymyxin B were compared in the same endplates.

Polymyxin B (0.5 - 2.5 μg/ml ≈ 0.5 - 2.5 μM) reversibly decreased in a concentration-dependent manner (8 - 31%) the amplitude of e.p.ps without changing the membrane resting potential of the muscle fibres (n=6). In these concentrations polymyxin B did not modify in an appreciable manner the inhibitory effect of adenosine (1 - 30 μM) on the amplitude of e.p.ps; for example, adenosine (3 μM) decreased e.p.ps amplitude by 25 ± 8% (n=6) in the absence of polymyxin B, and by 27 ± 9% (n=6) in the presence of polymyxin B (1 μg/ml). Polymyxin B (1 μg/ml) almost abolished the enhancement in neuromuscular transmission caused by 4β-phorbol,12,13-diacetate (100 nM), which increased e.p.ps amplitude by 23 ± 3% (n=2) in the absence of polymyxin B, and by only 4 ± 4% (n=2) in the presence of polymyxin B (1 μg/ml).

These results suggest that the attenuation caused by phorbol esters in the action of adenosine on neuromuscular transmission might be a consequence of an indirect interaction between the cascade process operated by the adenosine receptor and protein kinase C, rather than a direct interaction of adenosine with the mechanisms leading to modification of protein kinase C activity.

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PURINE RECEPTORS IN RAT ISOLATED SUPERIOR CERVICAL GANGLIA

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Adenosine (AD) is known to hyperpolarize (Brown, Caulfield & Kirby 1979) and inhibit calcium dependent potentials recorded intracellularly from postganglionic neurones (Henon & McAfee 1983) of isolated rat superior cervical ganglia (SCG) but no receptor subtype has been assigned. We have shown that AD antagonised the postganglionic response to (±) muscarine (Connolly & Stone 1988) and now extend these studies to include the effects of AD analogues.

D.C. potential changes were recorded from the internal carotid nerves of desheathed SCG in vitro by a modification the method of Bowery & Tulett (1975), employing a grease-gap. Each ganglion (n values refer to the number of animals used) was superfused (2-2.5ml/min) with medium at 25°C, pre-equilibrated with 5% CO_2 in O_2 , containing (mM) NaCl 125, KCl 5, KH_2PO_4 1, $CaCl_2$ 2.5, $MgSO_4$ 1, $NaHCO_3$ 25 and D-glucose 10. Muscarine (100nM) was applied via the superfusate for 1 min, or 1 min from the start of perfusion with adenosine or analogues for 5 min, for 1 min. The response size in AD was calculated as a percentage of the control responses.

The response to muscarine was reduced in a concentration-dependent manner by AD and its analogues. The A1 and A2 receptor ligands, N6-(Phenylisopropyl) adenosine, R(-) isomer [R(-)PIA] and N-ethyl-carboxamidoadenosine [NECA], respectively, were equally potent. S(+)PIA the diastereomer of R(-)PIA was less potent than R(-)PIA with a R/S ratio of about 7. The IC50 calculated from half the maximum inhibition of muscarine recorded, gives values for NECA, or R(-)PIA, S(+)PIA and AD of 45, 45, 300, 1200nM respectively (n of 3 to 9).

The ability to hyperpolarise the ganglion paralleled the ability to reduce the muscarinic depolarization, ie., order of potency being NECA = R(-)PIA > S(+)PIA > AD.

By using a series of adenosine analogues we hoped to classify the purine receptor/s of the rat SCG. The equipotency of R(-)PIA & NECA is suggestive of the presence of A2 receptors, alone or together with A1 receptors and the less than ten fold difference in the R/S PIA potency ratio, supports this view.

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ADENOSINE ANTAGONISES DEPOLARIZATION TO MUSCARINE BUT NOT GABA ON RAT SUPERIOR CERVICAL GANGLIA

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Adenosine (AD) and it's nucleotides are implicated as neuromodulators of synaptic transmiss in a variety of tissues, enhancing or depressing neurotransmitter function (Stone 1981).

Henon & McAfee (1983) found three calcium dependent potentials produced by preganglionic electrical stimulation of isolated rat superior cervical ganglia (SCG) which could be inhibited by AD. More recently Cox & Walker (1985) have shown that AD inhibits the depolarization of Helix parietal ganglia by exogenous acetylcholine, via inhibition of a calcium component of the response. Given these findings we have investigated the interaction of AD with the depolarization produced by exogenous compounds on the rat SCG.

Ganglia from urethane anaesthetised male Wistar rats were excised, desheathed and placed in a three compartment bath, with the ganglion body in the central chamber and the pre- and post-ganglionic trunks protruded through greased slots into the two outer chambers. Each ganglion (n values refers to the number of animals used) was continuously superfused with medium at 25°C, pre-equilibrated with 5% CO_2 in O_2 , containing (mM) NaCl 125, KCl 5, KH_2PO_4 1, $CaCl_2$ 2.5, $MgSO_4$ 1, $NaHCO_3$ 25 and D-glucose 10. Ganglionic potentials were recorded differentially between the earthed central chamber and the internal carotid nerve, via Ag/AgCl electrodes. Muscarine or GABA was applied via the superfusate for 1 min, or 1 min from the start of perfusion with adenosine (5 mins) for 1 min. The response size in AD was calculated as a percentage of the control responses.

Muscarine at 30nm (186 ± 21μV, n=16) and 100nM (501 ± 35, n=25) gave similar depolarisations to 3μM (258 ± 54, n=11) and 10μM GABA (578 ± 104μV, n=9) respectively. Various combinations of agonist and AD concentrations were tested. AD at up to 100μM depressed depolarisations to muscarine by 24.7% ± 2.7, n=8, whereas at 100μM AD, the GABA responses (10μM) were not reduced (response size 104 ± 4 n=7).

Both the hyperpolarization by adenosine and the antagonism of the muscarinic depolarization were concentration-dependent and abolished by the AD antagonist, 8-para-sulphophenyltheophylline (10μM, 10 min before AD).

We conclude that adenosine can selectively modulate the postganglionic depolarization to muscarine but not GABA.

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CONSTANT CURRENT BIPOLAR STIMULATION IN TISSUE BATHS

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We report the development of a new device which combines and modifies the twin positive unipolar outputs of a standard Grass Instruments S88 electrical stimulator so as to deliver symmetrical rectangular bipolar pulses of pre-determined constant current over a range of resistances such as are encountered during electrical stimulation of isolated tissues. Such pulses are easily quantified, irrespective of their frequency or amplitude (unlike harmonic or more complex stimuli) and (unlike unipolar or unbalanced pulses) minimise damaging or confounding electrolytic effects (Orrego, 1979). The tendency of current to decline with time (Palmer, et al, 1986) and our own observation (Jamieson & Selbie, 1988) of variations in tissue bath resistance (approximately 200-600 ohms), principally due to bubbled gases, support the use of a constant current source. We have stimulated, in single 3 ml tissue baths, neurogenic contractions in guinea pig jejunal myenteric plexus and have induced tetrodotoxin-resistant contractions in human basilar and uterine artery preparations, employing 45-60 mA. The device is easily modified and has wide potential application. Without augmentation of the S88's limited output voltage (maximum 150V) the present version of the device delivers a predefined maximum constant current stimulus (I_C) in the range 0-100mA, independent of load (tissue bath) resistance (RL) in the range 0-1000 Ohm. Minimum voltage required to reach I_C depends on RL but current is independent of voltage once I_C has been achieved (Figure 1).

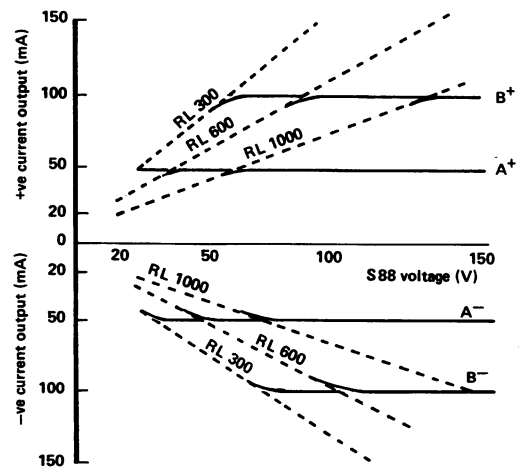

Figure 1

Relationship between Grass S88 output voltage and current across load resistances (RL) of 0-1000 Ohms. A and B reflect settings of the device's variable internal resistances producing I_C 50 and 100mA. + and - represent +ve and -ve components of the bipolar pulse

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INHIBITION OF NERVE CONDUCTION IN FROG ISOLATED SCIATIC NERVE-GASTROCNEMIUS MUSCLE BY ALTERNATION MAGNETIC FIELD

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Electromagnetic stimulation of the nervous tissue has been extensively studied by many workers (Kolin,Brill & Broberg,1959; Oberg,1973; Polson,Barker & Freeston, 1982). So far, the stimulatory effects of electromagnetic field have been reported and there is no literature reference on the inhibitory effect.

In the present investigation, the effect of intermittent electromagnetic stimulation,on nerve conduction in frog isolated sciatic nerve-gastrocnemius muscle preparation,was studied.

The nerve trunk was placed,longitudinally,inside an induction coil (a 100 turn induction copper coil,0.5 mm diameter and 0.6 Ω). The coil was wound on a P.V.C. tube (internal diameter of 2 mm). The nerve trunk was moistened with Ringer's solution and the gastrocnemius muscle laid outside the coil,in an organ bath containing 80 ml of Ringer's solution,at room temperature. The nerve trunk was stimulated,repetitively at 0.5 Hz with 0.25-0.6 V (supramaximal) and 1 ms pulse duration. The induction coil was connected to a d.c. source,1.5-4.0 V,via a make-and-break switch. The induced current was obtained at various frequencies (1-100 min^{-1}) and durations (20-120 s). The effect of this induced current on nerve conduction was assessed by analysing the amplitude of the twitch tension,of the gastrocnemius muscle, recorded isometrically.

In the control experiments,i.e. with no induced current,repetitive electrical nerve stimulation,produced twitch contractions (3.7±0.21 g tension,mean±s.e.,n=10) in the frog isolated gastrocnemius muscle. When a current, from a d.c. source of 1.5 V,at frequency of 100^{-1}, was passed through the coil,i.e. by operating via the make-and-break switch,the twitch contractions were completely blocked in 42± 5 minutes. The inhibition was frequency-dependent,as well as voltage-dependent. Complete inhibition occurred at 1.5 V and above (e.g 4 V). At 4 V, there was an immediate initial contraction (5.2±0.4 g tension,n=6),followed by a complete inhibition of the contractions.

Recovery from electromagnetic inhibition of the nerve conduction was easily reversible,after the cessation of current induction, recovery was usually achieved within 1-2 min of removal of the induced current.

In conclusion, electromagnetic induction inhibited impulse conduction,in frog isolated sciatic nerve,leading to a block of twitch contraction. The mechanism of this inhibition was not further analysed,but it is possible that the induced current interferes with the ionic mechanisms associated with the propagation of the nerve action potential.

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EFFECTS OF VERAPAMIL ON RESPONSES PRODUCED BY NORADRENALINE, ACETYLCHOLINE AND POTASSIUM CHLORIDE IN RAT SEMINAL VESICLE IN VITRO

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The autonomic innervation of the smooth muscle of the seminal vesicle of the rat, is primarily innervated by adrenergic neurones, but also by some cholinergic and peptidergic neurones (Meldrum & Burnstock, 1985; Fedan, Besse, Carpenter & Teague, 1977; Moss, Crowe & Burnstock, 1987). Thus, neurotransmitters, such as noradrenaline (NA), and acetylcholine (ACh), will produce marked contractions, comparable to those produced by potassium chloride (KCL), in the rat isolated seminal vesicle. In the present investigation, the effect of verapamil, an organic calcium antagonist, or calcium entry blocker, on the contractile responses produced by NA, ACh, carbachol (CCh) and KCl, was studied, in isolated seminal vesicle of the rat, to see to how and to what extent does verapamil inhibit these responses produced by different mechanisms.

Seminal vesicles, from adult male Sprague-Dawley rats, weighing 200-300 g, were used. The preparation was cut spirally (2-3 cm long and 2 mm wide), and it was set up in an organ bath, containing 20 ml of Krebs-Henseleit solution, maintained at 38±2°C and bubbled with 5% CO_2 in O_2. The contractile responses produced by the action of neurotransmitters and by other depolarizing agents, were recorded isometrically.

Noradrenaline (0.78-156.8 µM), ACh (8.8-880 µM), CCh (2.75-275 µM) and KCl (1.34-134 mM) produced concentration-dependent contractions in the smooth muscle of the rat isolated seminal vesicle. The mean EC_{50} values (i.e. concentration to produce 50% maximum contraction) are shown in Table 1. In the presence of verapamil (1-10 uM), which on its own did not alter the tone and contractility of the rat seminal vesicle, the responses were significantly reduced ($P < 0.05$ & $P < 0.001$) (means± s.e., n=6).

Table 1. Contractile responses produced by noradrenaline, acetylcholine, carbachol and potassium chloride, in the rat isolated seminal vesicle, in the absence and presence of verapamil (means±s.e., n=6).

	Contraction (g) (Max.)	EC_{50} value (µM/ mM) (C)	EC_{50} value in Verap. (µM/mM) (V)	Ratio of EC_{50} (V/C)	P <
NA	2.5±0.1	31±1.8	85±13.1	2.7:1.0	0.01
ACh	2.1±0.2	88±14.5	167±16.2	1.9:1.0	0.05
CCh	1.0±0.1	55±2.1	95±12.5	1.7:1.0	0.05
KCl	3.2±0.4	19±1.5	58±1.3	3.1:1.0	0.001

Verapamil (1 µM), significantly reduced the contractions produced by neurotransmitters and by the depolarizing agents, greater reductions occurred in the KCl, NA compared to ACh and CCh. These results suggest the selectivity of the action of verapamil in inhibiting these different responses, and the results also suggest the multiple innervations (adrenergic/cholinergic) present in the rat isolated seminal vesicle.

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INFLUENCE OF NEURONAL RE-UPTAKE ON THE RELEASE OF [^{3}H]-NORADRENALINE (^{3}H-NA) EVOKED BY ELECTRICAL STIMULATION (ST)

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Using dog saphenous vein, it was shown that 3H-NA is only partially released by ST(i.e. about 50% of the total amine accumulated, TA, was the "bound fraction" BF, Brandão et al., 1985). Since in these experiments cocaine(C) was present during ST, it was not possible to know the influence of neuronal re-uptake on the release. Thus, it was decided to continue this investigation in order to study: 1)the fate of neuronal re-uptake of 3H-NA (whether incorporated in the BF or in the releasable compartment); 2) if the kinetic characteristics of release are dependent on the experimental conditions (continuous or intermitent train of stimuli). The strips were loaded with 3H-NA (0.23µmol), under inhibition of MAO, COMT and extraneuronal uptake. The strips were then continuously perifused during 200 min. From the 100th min up to 200 min, the strips were stimulated. Two types of ST were used: a) continuous with pulses of 2ms of duration and frequency of 2 Hz; b) intermitent, at 10 Hz (2 min in each period of 10 min of perifusion) and at 20 Hz (2 min in each period of 20 min of perifusion); thus the total number of pulses was the same in all experiments. The ST were made in the absence (control conditions) and in the presence of C (10 µmol/l). The results are shown in the table as arithmetic mean±SEM for Releasable Compartment and geometric mean for t/2.

Characteristics of release of 3H-NA evoked by ST

Control	n	Releasable Compartment in % of TA	t/2 (min) of efflux
2Hz	7	39.5±3.0 a)	135.1 g)
10Hz	5	45.6±4.5 b)	197.5 h)
20Hz	7	33.6±2.6 c)	98.7 i)
Cocaine			
2Hz	7	52.1±3.2 d)	119.3 j)
10Hz	7	53.9±3.0 e)	130.0 k)
20Hz	6	40.0±4.8 f)	115.9 l)

The results were statistically significant between: a and d $P<0.02$; b and c; e and f; g and h; h and i; h and k $P<0.05$

Conclusion - The fate of 3H-NA depends on the experimental conditions: 1) For intermitent ST at 20Hz, there appears to be no reuptake of 3H-NA; 2) On the contrary, for continuous ST at 2Hz and intermitent ST at 10Hz, an important fraction of 3H-NA is taken up by the adrenergical terminals, to be incorporated in the BF in the case of continuous ST and in the releasable compartment in the case of intermitent ST. For intermitent ST at 20 Hz we assume a "fading effect", since the releasable compartment is smaller than that found for 10Hz(with or without cocaine).

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INFLUENCE OF INHIBITION OF EXTRANEURONAL UPTAKE AND O-METHYLATION ON THE SENSITIVITY OF SYMPATHOMIMETIC AMINES

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In vascular tissue and for a given dose, the concentration of noradrenaline and adrenaline available for their alpha-adrenoceptor-mediated effects is mainly governed by uptake into sympathetic nerve endings, while O-methylation is the main factor determining the concentration of those agonists available for the beta-adrenoceptor-mediated effects (Guimarães, 1982). On the other hand, it has been suggested that $beta_1$-adrenoceptors respond primarily to neurotransmitter and are therefore innervated, while $beta_2$-adrenoceptors are non-innervated and responsive to circulating catecholamines (Bryan et al, 1981; Broadley et al, 1984). More recently, it has been shown that O-methylating activity is functionally more effective in modulating $beta_2$-adrenoceptor-mediated responses than responses mediated by $beta_1$-adrenoceptors (Proença et al, 1988).

In the present study, the influence of inhibition of extraneuronal uptake and of inhibition of catechol-O-methyl transferase on the sensitivity of the isolated guinea-pig trachea to some sympathomimetic amines was compared.

After having been removed and cleaned of excess tissue, the tracheae were spirally cut, each strip was then approximately halved and each half suspended in a 10 ml organ bath. The preparations were contracted with 0.3 $\mu mol.l^{-1}$ carbachol to allow determination of concentration-response curves for the relaxation caused by sympathomimetic amines. The enhancement caused by the inhibition of extraneuronal uptake (by hydrocortisone) or by the inhibition of COMT (by U-0521; dihydroxy-2-methyl propiophenone) of the relaxing action of the amines was determined by the leftward displacement of the concentration-response curves at the EC50 level.

The results obtained are shown in the Table 1:

Table 1. Influence of U-0521 (60 $\mu mol.l^{-1}$) or hydrocortisone (200 $\mu mol.l^{-1}$) on the sensitivity of guinea-pig tracheal muscle to sympathomimetic amines. The tracheal muscle was previously contracted by 0.3 $\mu mol.l^{-1}$ carbachol in the presence of 12 $\mu mol.l^{-1}$ cocaine to inhibit neuronal uptake

Amine	ED50 (control)	F_1	ED50 (control)	F_2
(-)-Isoprenaline	10.5 nmol.l^{-1} (8.5-13.0)	3.9 (2.5.-5.0)	9.1 nmol.l^{-1} (7.5-11.0)	1.9* (1.7-2.1)
(-)-Adrenaline	36.3 nmol.l^{-1} (28.2-46.9)	2.2 (1.9-2.5)	39.1 nmol.l^{-1} (26.4-58.2)	1.8* (1.7-2.1)
Dopamine	30.1 $\mu mol.l^{-1}$ (21.7-41.6)	2.2 (1.7-2.7)	25.4 $\mu mol.l^{-1}$ (20.1-32.1)	1.5* (1.3-1.7)
(±)-Dobutmine	2.3 $\mu mol.l^{-1}$ (1.7-3.1)	3.9 (3.6-4.2)	2.3 $\mu mol.l^{-1}$ (1.8- 2.9)	1.4* (1.3-1.5)

F_1=ratio ED50 control/ED50 after U-0521

F_2=ratio ED50 control/ED50 after hydrocortisone

The values represent geometric means and confidence limits

*significantly different from the corresponding F_1 value

These results show that the sensitivity of the tracheal muscle to these amines is more markedly enhanced by the inhibition of COMT (by U-0521) than by the inhibition of extraneuronal uptake (by hydrocortisone).

The high degree of supersensitivity caused by U-0521 is compatible with the hypothesis that COMT is situated in the membrane of the extraneuronal cells.

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ERGOMETRINE DISPLACEMENT OF [^{3}H]-5-HYDROXYTRYPTAMINE BINDING IN RAT BRAIN AND MYOMETRIUM

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Functional studies suggest that ergometrine in rat uterus is both a spasmogen (EC50 approximately 30 nM), whose action involves receptors with characteristics of 5-HT$_2$ receptors, and a selective non-competitive antagonist at 5-HT receptors (1 μM produced a 230-fold antagonism of 5-HT) (Hollingsworth et al., 1988). The aim of this study was to determine if these actions of ergometrine involved a direct interaction with 5-HT receptors in myometrium by assessment of the ability of ergometrine to displace [^{3}H]-5-HT. Displacement of [^{3}H]-5-HT from brain was used for comparison.

Whole brain (excluding cerebellum and pons-medulla) or endometrium-free uterus from non-pregnant rats was homogenised in 0.05 M Tris-HCl and centrifuged at 35,000 RCF. The pellet was resuspended in 0.05 M Tris-HCl and incubated with [^{3}H]-5-HT (3.4 nM; 537 GBq/mmol), in the presence of pargyline (1 μM), for 30 min at 37°C in the absence or presence of various concentrations of non-radioactive 5-HT or ergometrine. Bound and free [^{3}H]-5-HT were separated by vacuum filtration using Whatman GF/C filters. Each assay was performed in triplicate. Non-specific binding was defined as the amount of [^{3}H]-5-HT not displaced by 10 μM 5-HT.

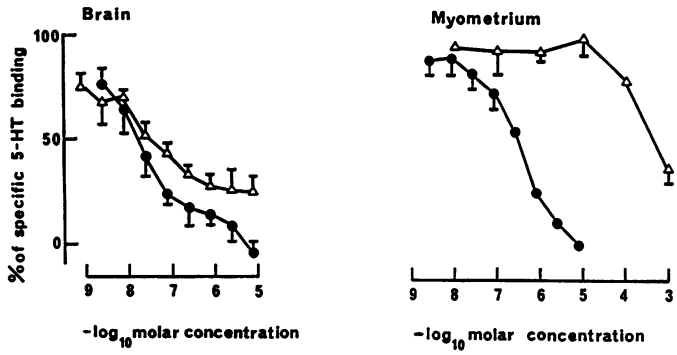

Figure 1 Displacement of specific [^{3}H]-5HT binding in brain and myometrium by 5-HT (● —— ●) and ergometrine (△——△). Values are means ± s.e.m.

Specific binding of [^{3}H]-5-HT was 56.4 ± 4.8 % (brain, n = 4) and 64.3 ± 4.8 % (myometrium, n = 8, mean ± s.e.m.) of total binding. 5-HT produced concentration-dependent displacement of [^{3}H]-5-HT with a pIC50 of 7.81 ± 0.26 for brain and 6.64 ± 0.11 for myometrium (Figure 1). Ergometrine partially displaced [^{3}H]-5-HT in brain, the maximum displacement being 75.0 ± 7.0 %, with a pIC50 of 7.35 ± 0.29 (n = 5). Ergometrine produced significant displacement of [^{3}H]-5-HT in myometrium only at 1 mM (n = 5).

These results suggest that ergometrine can interact with at least one type of 5-HT binding site in rat brain. Although there are specific binding sites for 5-HT in the rat myometrium, ergometrine does not interact at functionally important concentrations with these sites. These binding studies indicate that the agonist and antagonist actions of ergometrine in rat uterus arise from its initial interaction with sites other than those for 5-HT.

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INVOLVEMENT OF EXTRACELLULAR CALCIUM IN AGONIST-INDUCED CONTRACTION OF GUINEA-PIG DETRUSOR

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Acetylcholine and ATP are established neurotransmitters in the guinea-pig detrusor and both can produce contraction of isolated strips of urinary bladder (Burnstock et al., 1978). Electrophysiological evidence suggests that ATP stimulates P_2-purine receptors to induce depolarisation and smooth muscle contraction (Fujii, 1987). Muscarinic receptor stimulation in many tissues leads to the hydrolysis of inositol phospholipids to produce inositol-1,4,5-trisphosphate which can release calcium from intracellular stores (Michell, 1987). This study was undertaken to determine the dependence of the contractile responses to purine- and muscarinic-receptor stimulation on extracellular calcium in guinea-pig bladder.

5 x 1mm strips of guinea-pig detrusor were prepared and mounted in standard organ baths. Contractile responses to carbachol, ATP and histamine were measured isometrically in a Krebs bicarbonate buffer containing calcium (2.5mM) and in a calcium-free Krebs medium. Agonist-induced accumulation of [^{3}H]-inositol phosphates was measured in a suspension of detrusor smooth muscle segments (six bladders per experiment), prelabelled with [^{3}H]-inositol, as described by Hall & Hill (1988).

Carbachol (EC_{50} = 9 $\pm$ 1 μM, n=5) and histamine (EC_{50} = 18 $\pm$ 6 μM, n=5) produced dose-dependent contractions of detrusor strips. ATP gave a much smaller response: 1mM ATP produced a response which was 34 $\pm$ 7% (n=5) of the maximum produced by carbachol (0.1mM). 10μM α,β,methylene ATP, a stable analogue of ATP, similarly elicited a response which was 47 $\pm$ 4% (n=3) of the maximal carbachol response. When the Krebs medium was changed for a calcium-free medium the response to ATP (1mM) was reduced to 7 $\pm$ 4% (n=5) of the original response after 10 min. In contrast, the response to carbachol (0.5 and 100μM) was much better maintained in calcium-free Krebs medium (50 $\pm$ 12% and 79 $\pm$ 8% of the original responses were obtained after 10 min with 0.5 and 100 μM respectively, n=5). Histamine (10μM) also produced a substantial response in the absence of calcium (72 $\pm$ 13% after 10 min in calcium-free medium, n=5). Carbachol (100 μM) and histamine (100 μM) produced 12 $\pm$ 3 (EC_{50} = 4μM) and 4 $\pm$ 1 fold increases in [^{3}H]-inositol phosphate accumulation over basal levels (n=3 in each case). These responses were abolished by atropine (0.1μM) and mepyramine (0.1μM) respectively. Neither ATP (1mM) nor α,β,methylene ATP (10μM) elicited such a response (n=3).

The results of this study show that the contractile response to ATP is more dependent on extracellular calcium than the contractile responses to carbachol and histamine, which are also accompanied by a stimulation of inositol phospholipid hydrolysis. These data suggest that purine receptor stimulation induces an influx of extracellular calcium while the contractile responses to muscarinic and histamine H_1-receptor stimulation can mobilise intacellular calcium via the production of inositol-1,4,5-trisphosphate.

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GANGLIOSIDE TREATMENT ATTENUATES IMPAIRED NERVE REGENERATION IN STREPTOZOTOCIN-DIABETIC RATS

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As part of our interest in the aetiology of diabetic neuropathy we designed the present study to examine the rate of regeneration of crushed nerve axons in diabetic rats and to determine whether treatment with gangliosides might offer some protection against any defect found.

Rats were made diabetic with streptozotocin (50 mg/kg i.p.) in normal saline, age-matched controls received saline alone. In Experiment 1 nerves were crushed, to promote regeneration, three weeks later and ganglioside treatment (10 mg/kg/day i.p. mixed bovine brain gangliosides in normal saline: Fidia, Abano Terme, Italy) begun on the day before crushing the nerve. In Experiment 2 ganglioside treatment began after 4 weeks of diabetes and nerves were crushed 7 days later. Sciatic nerves were crushed at a mid-femoral site under recovery halothane anaesthesia. After 3 or 7 days the rats were again anaesthetised, the sciatic nerve exposed and pinched with fine forceps, beginning 30 mm distal to the crush and moving proximally by 1 mm steps. The furthest point of regenerated sensory axons was noted when the pinch elicited fasciculations of the back muscles. This regeneration distance (mm $\pm$ standard deviation) was measured relative to the crush.

In Experiment 1 untreated controls showed regeneration both 3 days (6.0 ± 1.0; n=5) and 7 days (21.4 ± 0.8; n=4) after crush; ganglioside treatment of non-diabetic rats did not affect the distances (6.0 ± 0.3, n=7 and 19.0 ± 1.4, n=5). The untreated diabetic rats showed some impairment (regeneration distances of 3.3 ± 0.7, n=6 at 3 days and 16.0 ± 1.3, n=4 at 7 days). Ganglioside treatment attenuated the deficit slightly (3 days - 4.6 ± 0.8, n=6 and 7 days - 17.8 ± 0.6, n=5). In Experiment 2 the longer duration of diabetes was studied with 7 days regeneration only. Again untreated controls and ganglioside-treated non-diabetic rats gave similar regeneration distances (respectively 21.4 ± 1.8, n=7 and 20.4 ± 1.6, n=7). In untreated diabetic rats the deficit was more marked (10.0 ± 0.5, n=6; $p<0.001$ vs. controls) and was significantly attenuated in the ganglioside-treated diabetic group (18.7 ± 1.2, n=4; $p<0.001$ vs. untreated diabetics).

These findings show a reduced rate of regeneration of sciatic sensory fibres from a nerve crush in diabetic rats, an effect markedly attenuated by ganglioside treatment.

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TACHYKININ RECEPTOR SUBTYPES IN THE IRIS SPHINCTER PUPILLAE OF THE RABBIT AND SHEEP

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Neurokinin receptors have been previously characterised by a number of workers in the rabbit iris sphincter using naturally occurring tachykinins. Utilising the more selective synthetic ligands now available, we have re-examined these receptors in the rabbit and report, for the first time, on those in the sheep.

Relative activities were compared for two series of agonists; first, the naturally occurring peptides neurokinin A (NKA), neurokinin B (NKB), substance P (SP), physalaemin (PHY), kassinin (KAS) and eledoisin (ELE) and, second, the synthetic tachykinin agonists, SP methyl ester (SPOMe), $[Glp^6,L\text{-}Pro^9]\text{-}SP_{(6\text{-}11)}$ (Septide), $[Glp^6,D\text{-}Pro^9]\text{-}SP_{(6\text{-}11)}$(D-isomer), GlpPhePhe(R)Gly[ANC-2]LeuMetNH$_2$ (WPC) and Succ-$[Asp^6,Me\text{-}Phe^8]\text{-}SP_{(6\text{-}11)}$ (Senktide). We also have estimated affinity constants for two putative tachykinin antagonists using selected agonists. The tissues were suspended in Krebs' solution containing ibuprofen (1μM), mepyramine (1μM), hexamethonium (1μM) and guanethidine (5μM). Cumulative dose-response curves were constructed to the six tachykinin agonists within a series using a 6x6 Latin square design, with regular dosing of carbachol as an internal control, estimating responses as a percentage of the maximal to carbachol for each tissue. The potencies for the naturally occurring peptides were similar (within a 10-fold range) in both the rabbit and the sheep iris sphincter, and in the order PHY>SP>ELE>KAS>NKA>NKB, which indicates that there is a predominant NK_1 receptor. In the rabbit preparation the rank order of potency for the synthetic tachykinins was septide>SPOMe>SP>WPC>senktide>D-isomer which again indicates the predominant presence of NK_1 receptors. The ratio of activities of the L:D-Pro^9 isomers is thought to be a valuable criterion of receptor subtype (Iversen *et al*, 1987) and the estimate here (c.12) strongly indicates the NK_1 subtype, though the NK_3 selective ligand senktide has appreciable activity. For **antagonist studies**, matched tissues were used, where the test preparation was treated with 10μM of the antagonist 5 minutes prior to application of the tachykinin agonists. The analogue $[D\text{-}Arg^1,D\text{-}Pro^2,D\text{-}Trp^{7,9},Leu^{11}]\text{-}SP_{(1\text{-}11)}$ though essentially inactive against SP, PHY and NKB, was active against the other analogues giving pA_2 estimates in the range 5.2-6.2. Similar results were obtained with $[D\text{-}Pro_4,D\text{-}Trp^{7,9,10}]SP_{(4\text{-}11)}$.

Taken together, the findings with the tachykinin agonist relative activities provide strong evidence of a predominant NK_1 receptor population in the rabbit and the sheep iris sphincter pupillae. On the other hand, the antagonists showed quite marked agonist selectivity which, if interpreted in terms of their higher affinity for one site in a mixed population, would indicate a degree of receptor heterogeneity; though we have noted similar behaviour in other tissue types where there were few other indications of mixed subtypes (eg, Bailey *et al*, 1986), so these data should be treated with caution. However, there are further observations by ourselves and others in iris preparations regarding agonist specific actions (including differing kinetics, selective desensitisation and phenoxybenzamine sensitivity) that may be readily explained in terms of multiple receptor subtypes. Taken overall, we conclude there is good evidence of a predominant NK_1 receptor population in the iris of the rabbit and sheep, but that contributions from other subtypes cannot be excluded.

We thank Drs LL Iversen & BJ Williams of MSD Research Laboratories for generous gifts of tachykinins. (WPC=L-363,851).

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MUSCARINIC BINDING SITE SUBTYPE IN RAT BRAINSTEM AND ILEUM MUSCLE

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We have found that a series of five antagonists can distinguish two classes of non-M_1 muscarinic receptors in rat tissues (Lazareno & Roberts, this meeting). The receptors studied functionally in isolated ileum and atrium preparations appear, respectively, to be similar to the binding sites labelled by ^{3}H-N-methylscopolamine (^{3}H-NMS) in the submandibular gland and heart. We have also used these antagonists to study the binding sites in rat brainstem and ileum and as shown below these preparations each appear to contain a mixture of non-M_1 sites.

Tissues were obtained from 2-4 month old male Lister hooded rats. The ileum muscle preparation contained longitudinal and circular muscle and myenteric plexus. The brainstem preparation included the colliculi, but not the cerebella. Washed membranes were incubated with ^{3}H-NMS and antagonist in 20mM Na Hepes/100mM NaCl/0.5mM EDTA buffer, pH 7.4, at 23°C for 90 min. The membranes were collected with centrifugation or filtration. Each experiment used one type of tissue and consisted, in duplicate, of a 22-point saturation curve and 13- or 16-point inhibition curves for four or five antagonists against 0.2-0.3nM ^{3}H-NMS. The curves from each experiment were analysed simultaneously with the program LIGAND (Munson & Rodbard, 1980). A two-site fit was always necessary. ^{3}H-NMS was constrained to be non-selective for the subtypes within the brainstem (K_D =175±24pM, n=7) and within the ileum (K_D = 287±49pM, n=6). The major subtype (84±2% of total sites in brainstem, 69±9% in ileum) was designated R1. The potencies (pKi) of the drugs at the two sites in each tissue and correlations with potencies found in heart and submandibular gland binding (Lazareno & Roberts, this meeting) are shown in Table 1 (mean ± sem, n≥3).

Table 1 (a) Antagonist pK_i at muscarinic receptor subtypes in brainstem and ileum and (b) Correlation coefficients with values found in heart and gland

		Brainstem		Ileum	
		R_1	R_2	R_1	R_2
(a)	4-DAMP	8.4±0.1	9.2±0.1	8.1±0.2	8.8±0.1
	Hexahydro-siladifenidol	7.1±0.1	8.2±0.1	6.6±0.2	7.7±0.3
	AF-DX 116	7.4±0.1	6.3±0.1	7.5±0.2	6.4±0.2
	Himbacine	8.3±0.0	6.6±0.1	8.6±0.1	7.3±0.2
	Methoctramine	8.4±0.1	6.4±0.1	8.4±0.1	6.8±0.1
(b)	Heart	0.97	-0.02	0.95	0.29
	Gland	0.07	1.00	-0.28	0.96

In conclusion, a series of selective antagonists has revealed heterogeneity in the muscarinic binding sites in rat brainstem and ileum muscle. The major binding component (70-80% of total sites) has a similar pharmacology to that found in heart, while the minor component appears similar to the sites found in submandibular gland. The relationship between these sites and functional responses of these preparations to muscarinic agonists is as yet unclear.

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MEPTAZINOL POTENTIATES NON-ADRENERGIC NON-CHOLINERGIC RESPONSES OF MOUSE VAS DEFERENS TO ELECTRICAL STIMULATION

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Many opioid agonists inhibit responses to electrical stimulation of sympathetic nerves. However, in mouse isolated vas deferens (T.O. strain; 30-35 g; NaCl 128, KCl 5.63, $CaCl_2$ 2.16, NaH_2PO_4 1.19, $NaHCO_3$ 25, glucose 11.1, sucrose 13.1 mM; gassed with 5% CO_2 in oxygen) twitch responses induced by field stimulation (40 volts, 2 msec, 0.1 Hz; isotonic recording; load 150 mg) were potentiated by meptazinol (10 - 300 μM), up to 3-fold at the highest concentration.

As we have shown previously (Duchesne et al, 1984) this effect was still seen in the presence of naloxone (20 nM; added to the physiological saline for all further experiments) and was easily reversed by washing. The potentiation produced by meptazinol was reproducible and was still apparant when experiments were performed in the presence of physostigmine (0.1 μM), atropine (0.1 μM), yohimbine (1 μM), propranolol (1 μM) or prazosin (1 μM). None of these drugs applied alone affected the size of the twitch response.

Desmethylimipramine (DMI; 3, 30 and 300 nM) reduced the size of the twitch to 66±4, 38±6 and 22±5% of the initial twitch respectively (mean±s.e.mean; n=4). In the presence of 30 nM DMI meptazinol still produced potentiation of the twitch though the magnitude of the effect was smaller than that produced by meptazinol alone. Cocaine (1 μM) also reduced the twitch size when applied alone and in the presence of cocaine the potentiation produced by meptazinol was not reduced. Incubation (15 min) with $\alpha\beta$ methyleneATP (1 μM) abolished the twitch completely in each of 4 experiments.

Vasa deferentia previously incubated with ^{3}H-noradrenaline for 45 min were washed every 2 min for 60 minutes. Electrical stimulation (400 mA, 2 msec, 2.5 Hz for 90 sec every 14 min) was applied through parallel electrodes. The effluent from the tissue was counted for tritium and fractional resting and evoked overflows were determined and expressed as a ratio to the value in a control period of stimulation in each tissue (Sx/S2 ratios). Meptazinol did not affect this measure of resting or evoked overflow at 10, 30 or 100 μM ($P>0.05$; Student's t-test) but at 300 μM resting overflow was increased (0.62±0.02 and 0.74±0.02) and evoked overflow was reduced (0.83±0.08 and 0.61±0.08) significantly ($P<0.05$; control and treated values respectively; mean±s.e.mean; n=6).

It is concluded that the potentiation produced by meptazinol does not involve cholinergic or noradrenergic effects but may be mediated by an action through the non-cholinergic, non-adrenergic transmitter (see Blakeley et al, 1988) thought to be present in this tissue.

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THE EFFECT OF EXTRACELLULAR CALCIUM AND D600 ON THE IONOPHORE A23187 AND ACETYLCHOLINE DOSE-EFFECT CURVES

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The ability of A23187 (Ionoph.) to carry calcium and to mimic drugs in the generation of calcium dependent effects has been reported in the gastro-intestinal smooth muscle(Triggle et al ,1975; Mandreck & Golenhofen,1977).It seemed interesting to quantitatively analise and compare the effects of changing the extracelular calcium ([Ca2+]o) and of a calcium antagonist, methoxyverapamil, (D600), on the dose-effect curves of acetylcholine and A23187 in the longitudinal muscle of the guinea-pig ileum(Rang,1964).

A magnesium free Krebs solution at 37ºC gassed with 95%O2+5%CO2 (mM): NaCl 113;KCl 4.7; NaHCO3 24.76; K2HPO4 1.19 and glucose 11.56,was used.The [Ca2+]o were 2.55; 1.275; 0.638 and 0.319 mM for acetylcholine and 1.275; 0.638 and 0.319 for A23187.For acetylcholine in [Ca2+]o=2.55 mM the D600 concentrations were 12.5; 25; 50 and 100 uM. For A23187 in [Ca2+]o=1.275 mM the D600 concentrations were 0.01 and 0.02 uM. The muscle contractions were isometrically recorded with a mechano-electric transducer. The parameters of the dose- effect curves (Ym and ED50%) were calculated using a nonlinear regression analysis.The maximal responses are given as a ratio to the maximal effect obtained in the control conditions (Ym norm.).The results are given as mean±s.e.mean.

[Ca2+]o mM	Ach.Ym norm.	Ach.ED50% nM	Ionoph.Ym norm.	Ionoph.ED50% nM
2.55	1 (12)	353±94(12)		
1.275	0.81±0.09(7)	485±107(7)	1 (16)	246±57(16)
0.638	0.64±0.07(7)**	1072±297(9)*	0.55±0.08(10)*	186±69(12)
0.319	0.48±0.08(7)**	1039±299(9)*	0.38±0.07(11)*	186±47(11)

[D600] uM	Ach.Ym norm.	Ach.ED50% nM	Ionoph.Ym norm.	Ionoph.ED50% M
Control	1(10)	152 ± 40(10)	1 (10)	152.13±29.52(10)
0.01			0.65±0.09(7)**	79.42±15.82(7)
0.02			0.31±0.06(10)**	118.58±31.78(8)
12.5	0.70±0.13(5)*	171±21(5)		
25	0.67±0.13(6)*	275±75(7)		
50	0.52±0.06(8)**	372±106(8)		
100	0.39±0.11(4)**	662±219(4)*		

* P<0.05
** P<0.001

From these results we may see that the decrease in [Ca2+]o induces a shift of the acetylcholine dose-effect curves with simultaneous decrease of the maximal response and an increase of the ED50%, while the A23187 curves show only a decrease of the maximal response. The blockade of calcium channels only decreases the maximal response with only a small increase in the ED50% both in acetylcoline and A23187. These results suggest that the response to acetylcholine depends more on the mobilization of cellular calcium than that of the A231187. The A23187 response does not seem to depend exclusively from the amounts of calcium transported by the Ionophore.

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ACTIONS OF CALCIUM CHANNEL ANTAGONISTS ON SYNAPTIC TRANSMISSION IN RAT PARASYMPATHETIC GANGLIA

synaptic transmission, calcium channel antagonists, parasympathetic ganglia.

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The aim of this study was to investigate the action of calcium channel antagonists on neurotransmitter release in mammalian parasympathetic ganglia. Several classes of calcium channels (N,T and L) have been identified on the basis of biophysical and pharmacological criteria in mammalian central and spinal neurones (Fox et al 1987). The significance of these channel types in transmitter release is unclear, however, pharmacological evidence indicates that the dihydropyridine sensitive L-type calcium channels are not involved in neurotransmitter release in rat sympathetic neurones (Hirning et al 1988), and extracellular recording from the perineural sheath of the mouse M. triangularis sterni preparation indicates that at least two types of channel may be involved (Penner and Dreyer 1986).

Excitatory post-synaptic potentials (EPPs) were recorded from the submandibular parasympathetic ganglia of newborn rats (10-20 days old), using intracellular recording techniques (electrodes 90-120 Mohm, 4 M K-acetate) and a suction electrode to deliver stimulus trains to the lingual nerve (15 stimuli at 0.1, 0.3, 1, 3, and 10 Hz, 22 C). Only evoked responses without voltage-dependent action potentials were analyzed (observed at membrane potentials negative to -70 mV), and EPP amplitudes were determined for the plateau responses during each train (5 - 15th response).The inorganic calcium channel antagonist, cadmium, reduced EPP amplitudes in a dose-dependent manner (Kd 74 μM, $P < 0.01$). In contrast, low concentrations of verapamil (0.1 - 30 μM), a phenylamine, had no significant effects upon EPSP amplitudes at any frequency examined. Diltiazem, a benzothiazepine, reduced EPSP amplitudes in a frequency- and dose-dependent manner (80% block at 30 μM and 10 Hz), whereas L-cis diltiazem was without effect. Amiloride, a potassium-sparing diuretic, also antagonized the amplitudes of evoked responses in a frequency- and dose-dependent manner (Kd's of 10 and 560 μM, $R^2 = 0.979$). It is not clear whether this effect of amiloride is specific for presynaptic calcium channels, but its potency in reducing evoked response amplitudes in this preparation is comparable to the suppression of T-type calcium currents in chick DRG neurones and mouse neuroblastoma (Tang et al 1988). Neither omega-conotoxin VIA (1 μM) nor nickel (300 μM), were effective in blocking synaptic transmission in these ganglia. The effects of these calcium channel antagonists are unlikely to be of post-synaptic origin, as miniature EPPs were still observed during these treatments.

In conclusion, various calcium channel antagonists have been shown to suppress synaptic transmission in rat parasympathetic ganglia. The differential effects of these probes indicate that either more than one type of calcium channel may be involved in transmitter release processes in this preparation, or that these presynaptic calcium channels possess different pharmacological sensitivities to those channel types described in neuronal cell bodies.

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α-ADRENOCEPTOR RESERVE AND THE RIGHTWARD SHIFT OF THE DOSE-RESPONSE CURVES CAUSED BY PHENOXYBENZAMINE

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In a recent study it was shown that in the dog saphenous vein, where both postsynaptic alpha$_1$-and alpha$_2$-adrenoceptors co-exist, at only one concentration of phenoxybenzamine (3 nmol.l^{-1}) was the concentration-response curve for noradrenaline shifted to the right without a reduction of the maximum effect. However, in other vascular tissues of the same species where only alpha$_1$-adrenoceptors exist postsynaptically (mesenteric and renal arteries), phenoxybenzamine never caused any rightward shift of the noradrenaline concentration-response curve without depressing the maximum effect. Furthermore, in none of the tissues did phenoxybenzamine, at any concentration, shift the concentration-response curve for phenylephrine to the right without depressing its maximum (Guimarães & Paiva, 1987). It was concluded that according to the original definition of "spare receptors" - "receptors in excess of those required to produce a maximal response" (Clark, 1933) there was no "receptor reserve" in the dog vascular tissues for sympathomimetic agonists.

To widen the basis supporting this conclusion similar experiments were performed using the canine cephalic vein, another vascular tissue where both alpha$_1$- and alpha$_2$-adrenoceptors exist postsynaptically (Shoji et al, 1982). The effect of three different concentrations of phenoxybenzamine (1,3 and 9 nmol.l^{-1}) on the concentration-response curves to phenylephrine (a selective alpha$_1$-adrenoceptor agonist) and to noradrenaline and adrenaline (mixed alpha$_1$- and alpha$_2$-adrenoceptor agonists) was compared in this tissue.

Both cephalic veins of the dogs were spirally cut as described by Guimarães & Osswald (1969) and then cut in 6-8 strips of about 2x20 mm. Each of these strips was used to determine two concentration-response curves for only one agonist: one before and the other after phenoxybenzamine. After the first concentration-response curve had been determined phenoxybenzamine was added to the bath and left in contact with the tissue for 30 min and was then washed out before starting the determination of the second concentration-response curve. 1 nmol.l^{-1} phenoxybenzamine caused very small but significant rightward parallel displacements of the concentration-response curves for noradrenaline and adrenaline (0.5 and 0.6 log units, respectively; n=5) but caused rightward non-parallel shift of that for phenylephrine. Both 3 and 9 nmol.l^{-1} phenoxybenzamine caused non-parallel displacements of the concentration-response curves for the three amines and the reduction of the maximum effects observed were larger after 9 than after 3 nmol.l^{-1} phenoxybenzamine; on the other hand, for one same concentration of phenoxybenzamine the depression of the maximum effect of phenylephrine was larger than that observed for noradrenaline and adrenaline. These results confirm that there is no "receptor reserve" in the dog vascular tissues for sympathomimetic amines.

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α_2-ADRENOCEPTOR-MEDIATED EFFECTS OF SELECTIVE α_1-ADRENOCEPTOR AGONISTS, AFTER PHENOXYBENZAMINE IN VIVO

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Recently it was proposed that the differential antagonism by calcium entry blockers of the responses mediated by vascular alpha$_1$-and alpha$_2$-adrenoceptors might be related to differences in receptor reserves between the postsynaptic vascular alpha-adrenoceptor subtypes rather than to specific blockade of the processes set in motion by activation of the receptor subtype (Hamilton et al. 1983; Ruffolo et al. 1984). This hypothesis was proposed on the basis that, after elimination of spare receptors by phenoxybenzamine, the pressor response to alpha$_1$- and alpha$_2$-adrenoceptor agonists became equally sensitive to antagonism by calcium entry blockers. It was assumed that after phenoxybenzamine, responses to the selective alpha$_1$-adrenoceptor agonist cirazoline were due to activation of the remaining alpha$_1$-adrenoceptors. This hypothesis was questioned on the basis that after removal of alpha$_1$-adrenoceptor reserve by phenoxybenzamine, the responses to selective alpha$_1$-adrenoceptor agonists were predominantly alpha$_2$-adrenoceptor-mediated (Guimarães et al. 1987). However, the results of these authors involved contractile responses of the dog saphenous vein *in vitro* to phenylephrine and methoxamine, while those of Ruffolo et al. (1984) involved pressor responses to cirazoline in the pithed rat. The present study was undertaken to re-assess which alpha-adrenoceptor subtype is involved in the pressor responses to cirazoline after phenoxybenzamine in the pithed rat.

The influence of prazosin (0.1 mg/kg) and of yohimbine (1 mg/kg) on the dose-response curves to cirazoline in the pithed rat was compared after different doses of phenoxybenzamine. After 0.05 mg/kg phenoxybenzamine, prazosin caused a displacement of the dose-response curve for cirazoline to the right which was much larger than that caused by yohimbine (2.0 vs. 0.4 log units); after 0.3 mg/kg phenoxybenzamine, prazosin and yohimbine caused about equal displacements of that dose-response curve (0.8 vs. 0.9 log units); after 1 mg/kg phenoxybenzamine, yohimbine caused a marked displacement while prazosin was without effect (1.2 vs. 0.0 log units).

It is concluded that in the pithed rat after phenoxybenzamine the pressor response to the selective alpha$_1$-adrenoceptor agonist cirazoline is predominantly if not totally alpha$_2$-adrenoceptor-mediated. This can explain why, after phenoxybenzamine, the selective alpha$_1$- and alpha$_2$-adrenoceptor agonists are equally antagonized by calcium entry blockers.

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ADDITIONAL STUDIES ON THE PHARMACOLOGICAL ACTIONS OF GR50360A, AN α_2-ADRENOCEPTOR ANTAGONIST

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Blockade of α_2-adrenoceptors in the CNS is known to enhance stimulation-evoked release of noradrenaline (Farnebo and Hamberger, 1971) and this is the rationale for the suggestion that α_2-adrenoceptor antagonists may be useful as antidepressants (Langer, 1978). We have reported some of the pharmacological properties of a novel α_2-adrenoceptor antagonist, GR50360A (Halliday et al., this meeting). This paper describes additional studies to demonstrate the selectivity of action of GR50360A in vitro and its potency and duration of action as an α_2-adrenoceptor antagonist in vivo.

The α_2-adrenoceptor antagonist properties of GR50360A were determined in vitro using the guinea-pig field stimulated ileum (Drew, 1978). GR50360A caused parallel rightwards shifts of UK-14304 (Cambridge, 1981) dose-response curves with no change in the max. response (pK_B = 7.89±0.07, slope = 1.04). In using other functional receptor preparations the specificity of action of GR50360A was good with little or no affinity for muscarinic acetylcholine (rat ileum, heart and cerebral cortex), histamine H_1 (guinea-pig ileum) and H_2 (guinea-pig atria), dopamine D_2 (rat striatum), 5-HT_2 (rabbit aorta), β_1 (rat atria) and β_2 (guinea-pig trachea) receptors at concentrations up to 1000 times the α_2-adrenoceptor pK_B value. There was weak affinity for 5-HT_1 binding sites (pIC_{50} of 5.9 at 5-HT_{1A}, pK_i of 5.5 at 5-HT_{1B}).

In the mouse (CRH male, 20-26g) clonidine (0.15mg/kg i.p.) caused a fall in oesophageal temperature of 2°C at 30 minutes. This hypothermic response was halved by 0.68±0.10 and 0.84±0.09mg/kg respectively of GR50360A and idazoxan given orally 60 minutes before clonidine. In another study in the mouse, clonidine (0.05mg/kg s.c.) prevented the abdominal constriction response to phenylbenzoquinone (0.02%w/v i.p.). This antinociceptive action of clonidine was reduced in mice pretreated orally 60 minutes earlier with the α_2-adrenoceptor antagonists. The following ED_{50} (95% conf. lim.) values were obtained: GR50360A 0.75 (0.54-1.07)mg/kg, idazoxan 0.65 (0.40-1.03)mg/kg or yohimbine 1.10 (0.28-1.94)mg/kg.

In conscious beagles a 30 minute oral pretreatment with GR50360A produced a dose-related and persistent (>5h) antagonism of the bradycardia and sedation resulting from a submaximal dose of UK-14304 (0.05mg/kg s.c.). The doses of GR50360A inhibiting by 50 per cent the maximal UK-14304-induced bradycardia and sedation were 1.9±0.6 and 2.5±0.1mg/kg respectively.

In summary, GR50360A is highly selective for α_2-adrenoceptors and causes long-lasting inhibition of the gross effects of selective α_2-adrenoceptor agonists in rodents and dogs. Of these effects, sedation is known to be mediated by α_2-adrenoceptors in the CNS (Drew et al., 1979) indicating that GR50360A readily penetrates the blood brain barrier.

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EFFECTS OF THE SELECTIVE DOPAMINE DA_1 RECEPTOR AGONIST FENOLDOPAM ON RENAL FUNCTION IN THE ANAESTHETISED DOG

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In man, the selective DA_1 receptor agonist fenoldopam (SK&F 82526) has been reported to cause diuresis and natriuresis (Harvey et al., 1986; Glück et al., 1987). We have examined the effects of fenoldopam, on renal function in pentobarbitone anaesthetised dogs.

Artificially respired dogs were infused with sodium bicarbonate (0.05 mmol/kg/min) to prevent metabolic acidosis. Aortic blood pressure (BP) and left renal artery blood flow (RBF) were measured and both ureters cannulated for urine collection. Glomerular filtration rate (GFR) was calculated from creatinine clearance. Saline (30 ml/kg i.v.) was infused over 2 h followed by a maintenance infusion (0.1 ml/kg/min i.v.). In control animals, there was little variation (< 10%) in the measured parameters. In contrast, intrarenal infusion of fenoldopam caused statistically significant ($P<0.05$) decreases in renal vascular resistance (RVR) and increases in urine flow (U_V) compared to the control group, at doses (0.01-0.3 µg/kg/min) that had little effect on BP and GFR (Table 1). Although sodium excretion ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) also tended to increase, the changes did not achieve statistical significance. Higher doses of fenoldopam (1.0-10 µg/kg/min) reduced BP, U_V, $U_{Na}V$ and FE_{Na} (results not shown).

TABLE 1: Effects of intrarenally infused fenoldopam

SK&F 82526 µg/kg/min	Diastolic BP	RVR	U_V	$U_{Na}V$	FE_{Na}	GFR
0.01	-1±1	-9±1	12±4	-1±10	8±11	-9±4
0.03	-3±2	-20±2	22±6	29±14	17±17	12±9
0.1	-6±3	-31±2	38±10	20±13	21±12	-2±9
0.3	-10±3	-38±2	45±15	15±7	7±10	7±7

Values in the table are the percentage change in the group mean ± s.e.m. (n=6) from pre-fenoldopam basal measurements. Each dose of fenoldopam was infused sequentially for a period of 10 min.

In separate dogs (n=6) SCH 23390 (30 µg/kg i.v.), a selective DA_1 receptor antagonist (Hilditch et al., 1984), caused a small significant, ($P = 0.03$) increase in U_V (6.21 ± 1.1 to 7.36 ± 1.4 ml/10 min); other parameters remained unchanged. Fenoldopam dose-response curves (in which the response to each dose was expressed as a percent change from post-SCH 23390 values) on BP, RVR and U_V were displaced about 5, 14 and 14 - fold respectively to the right of those obtained in antagonist-free dogs. Jose et al., (1987) have demonstrated a more marked effect of fenoldopam on sodium excretion in dogs than we observed. This may reflect differences in the experimental conditions. However, like Jose et al., (1987) this study demonstrates that fenoldopam causes renal vasodilatation and diuresis in anaesthetised dogs and additionally, suggests that these effects are mediated via DA_1 receptors.

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PERIPHERAL EFFECTS OF SK&F 101468-A ARE REVERSED BY DOMPERIDONE

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SK&F 101468-A is a potent, selective, dopamine DA_2 agonist with activity in the periphery as a hypotensive agent (Eden, Owen, Parker and Wright. 1988). This activity, however, is subject to rapid onset of tolerance (Eden, Harvey, Owen and Parker. 1988). A possible side effect of sympatholytic activity such as that shown by this compound, is orthostasis. This study describes experiments to investigate this possibility and the potential to reverse any such effects with a peripheral dopamine antagonist.

Cats were anaesthetised with α-chloralose, and blood pressure and heart rate measured from a femoral artery. The right brachial vein was cannulated for the administration of the drug or anaesthetic. A haemodynamic event mimicking the effects of orthostatic hypotension was simulated using a Swan-Ganz catheter (4F) inserted into the vena-cava, via a femoral vein, to the level of the renal veins. The balloon at the catheter tip was inflated, for a period of 6 minutes with 0.35 ml air, to partially occlude the vena-cava and thus diminish venous return. The procedure was repeated at 20 minute intervals. Cardiovascular responses to this manoeuvre were measured before and after SK&F 101468-A, 500 $\mu g.kg^{-1}$ i.v. and following subsequent administration of domperidone, 500 $\mu g.kg^{-1}$ i.v. Data was analysed by ANOVA using animals, treatments and occlusions as factors. The S.E.M. are common for each group.

Table 1. Comparison of hypotensive episodes, in response to a reduction in venous return, before and after drug treatments. (n=4)
Values represent change in mmHg over 6 min during and 14 min post occlusion.

Treatment	Mean AUC during occlusion	Difference in AUC (+ 95% C.I.)	Mean AUC following occlusion	Difference in AUC (+ 95% C.I.)
Control	-63		8	
		Control Vs 101468		Control Vs 101468
SK&F 101468	-106	-43 (-92 to 6)	129	121* (22 to 220
		101468 Vs domperidone		101468 Vs domperidone
Domperidone	8	113* (65 to 162)	30	-99 (-195 to 0)
(S.E.M.)	(13.9)		(28.4)	

* AUCs were statistically significantly different from its control ($P<0.05$) when 95% confidence intervals do not overlap zero.

Resting blood pressure and heart rate were reduced significantly, and the hypotensive response to the cardiovascular intervention approximately doubled (Table 1), after dosing with SK&F 101468-A. There was also a statistically significant reactive hyperaemia. The tachycardia resulting from the occlusion was halved (AUC from 101 to 43 beats per 6 min). These effects were completely reversed by domperidone. These results confirm the acute cardiovascular effects of SK&F 101468-A and that any peripheral side effects can be readily reversed by domperidone.

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PHENYLEPHRINE - IS IT A SELECTIVE α_1-ADRENOCEPTOR AGONIST?

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α-adrenoceptors may be subdivided into α-1 and α-2 subtypes. It is now recognised that both subtypes may mediate vasoconstriction, at least in some blood vessels. We recently reported the existence of a population of α-2 adrenoceptors in isolated resistance arteries taken from human omentum and subcutaneous tissue (Nielsen et al., 1987). In this study we have examined the effect of phenylephrine (PE), a selective α-1 adrenoceptor agonist on isolated human resistance arteries

Omental (n=15) and subcutaneous (n=8) arteries (median internal diameter = 281 and 211μm respectively) were obtained from tissues removed during surgery. Arteries were mounted in a myograph (Mulvany & Halpern 1977) and isometric tension measured. The myograph contained 10ml physiological saline (PSS) at 37°C and aerated with 95% O_2 and 5% CO_2. Following equilibration, arteries were set to an internal circumference equal to 0.9 L100, where L100 is the internal circumference corresponding to a passive wall tension which would be produced by 100mmHg distending pressure. Tissues were allowed to equilibrate for a further 30mins and vessel viability was assessed by exposing arteries to a sequence of PSS containing 125mM K (KPSS), noradrenaline 10μM (NA) and KPSS containing NA. Arteries not generating force equivalent to 90mmHg in response to KPSS were not used for studies. Concentration response data were generated by cumulative addition of agonist. EC_{50} data are expressed as geometric means (95% confidence limits) and maximum tensions (max) as means ± s.e.mean. Antagonist contact time was 20 mins.

Omental and subcutaneous arteries contracted in response to KPSS (max = 3.1 ± 0.3 and 2.3 ± 0.8N/m respectively) and NA (max = 1.5 ± 0.2 and 1.8 ± 0.6N/m respectively). PE (100nM - 100μM) induced a concentration dependent contraction in arteries from both sites (EC_{50} = 9.4 (4.5 - 19.6) and 2,2 (0.8 - 6.1)μM, max = 1.2 ± 0.6 and 1.4 ± 0.3N/m respectively). Responses to PE were antagonised by both doxazosin (10nM - 1μM), a selective α-1 adrenoceptor antagonist and yohimbine (10nM - 1μM), a selective α-2 adrenoceptor antagonist.

In conclusion, these results suggest that PE acts as a non-selective adrenoceptor agonist in isolated human resistance arteries. Alabaster and colleagues (1985) have reported that PE acts as an α-2 adrenoceptor agonist in rabbit saphenous vein. Possibly, our findings reflect a large α-2 adrenoceptor population in these human resistance arteries. In addition, these results suggest some caution in interpreting the action of PE as solely due to α-1 adrenoceptor activation in the absence of other supporting evidence.

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ACTION OF B-FUNALTREXAMINE AT μ-OPIOID BINDING SITES IN GUINEA-PIG ILEUM

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B-Funaltrexamine (B-FNA) is an irreversible μ-opioid receptor antagonist (Ward et ai., 1982). Pre-incubation of myenteric plexus longitudinal muscle (MPLM) strips of the guinea-pig ileum with B-FNA decreases the potency of μ-agonists on this tissue (Corbett et al., 1985). In confirmation of this ligand-binding assays in brain homogenates show an irreversible blockade of μ-opioid binding sites (Ward et al., 1980; Corbett et al., 1985). However in homogenates of MPLM irreversible binding of B-FNA to the μ-binding site could not be demonstrated (Corbett et al., 1985). In order to explain this apparent anomaly we have investigated the action of B-FNA on μ-opioid binding, as defined by the selective agonist [^{3}H][D-Ala^2, MePhe^4, Gly-ol^5]enkephalin ([^{3}H]DAGO) in MPLM preparations under various conditions.

Strips of guinea-pig ileum MPLM, or guinea-pig brain-cerebellum, were homogenised (Corbett et al., 1985), then treated with B-FNA for 60 min at 37°C in Tris-HCl buffer (pH 7.4) or Tris-HCl containing NaCl (100mM) and the GTP analogue GppNHp (50μM). After washing four-times with Tris buffer the binding of [^{3}H]DAGO (4nM) was studied at 25°C for 40 min. Non-specific binding was defined using naloxone (10μM). In a further set of experiments 2cm strips of MPLM were treated with B-FNA for 60 min at 37°C in Krebs solution buffered with HEPES (25mM, pH 7.4). The treated whole tissues were washed extensively, then homogenised and the binding of [^{3}H]DAGO studied as above.

The control binding of [^{3}H]DAGO to homogenates of MPLM (7.1±0.7 fmols/mg protein) was not altered by treatment with B-FNA (1μM) (8.8±1.4 fmols/mg), in accordance with previous reports (Corbett et al., 1985). In contrast B-FNA (0.1μM) under similar conditions reduced [^{3}H]DAGO binding in homogenates of guinea-pig brain by 48.2±8.5%. However after treatment of intact strips of MPLM with B-FNA (1μM) in Krebs-HEPES the binding of [^{3}H]DAGO in subsequently prepared homogenates was reduced from a control value of 9.7±2.2 fmols/mg to 4.4±1.5 fmols/mg. A reduction in μ-binding in MPLM could also be demonstrated when B-FNA (1μM) treatment of homogenates was done in the presence of NaCl and GppNHp. In this case control binding was lowered from 7.9±1.9 fmols/mg to 3.9±0.9 fmols/mg, an inhibition of 51.2±3.4%.

The results suggest that the reported shifts in μ-agonist potency on the guinea-pig MPLM preparation can be explained on the basis of an irreversible interference with μ-ligand binding. It is of interest that in MPLM, unlike brain tissue, the irreversible action of B-FNA is seen only in whole tissue, or in the presence of NaCl and GppNHp. NaCl and GppNHp will promote the formation of a low agonist affinity state of the μ-receptor. This conformation of the μ-receptor is sensitive to B-FNA alkylation and would seem therefore to be the form present under physiological conditions in whole MPLM tissue. This supports the idea that this is the physiologically relevant form of the μ-opioid receptor (Carroll et al., 1988).

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EFFECTS OF SELECTIVE OPIOID RECEPTOR BLOCKADE ON HYPOTHALAMO-PITUITARY GONADAL FUNCTION IN THE MALE RAT

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Endogenous opioid peptides are believed to contribute to the complex physiological mechanisms which control the activity of the hypothalamo-pituitary-gonadal axis (Kalra & Kalra, 1984). Their effects appear to be mediated by specific receptors in the hypothalamus, which influence the secretion of luteinizing hormone releasing hormone (LHRH). In order to investigate their mode of action further and to characterize the relevant receptors we have studied the effects of selective antagonists of mu-(naloxone), delta-(ICI 174864) and kappa-(MR2266) opioid receptors on the secretion of luteinizing hormone (LH) and LHRH in the rat.

The serum concentrations of immunoreactive LH in male Sprague-Dawley rats (150g) were determined at various times after subcutaneous injection of one of the antagonists. Naloxone (5mg/kg) stimulated the release of LH. Its effects were maximal 20 minutes after injection when the serum LH concentration was increased from 0.45^{+} 0.07 ng/ml (n = 5) in vehicle (2.0 ml/kg) - treated controls to $1.42 \pm$ 0.05 ng/ml (n = 5). In contrast, neither ICI 174864 (1.5 mg/kg) nor MR2266 (1.5 and 3.0 mg/kg) influenced significantly (P > 0.2) the resting serum concentrations of LH. In vitro experiments were performed to investigate directly the effects of these antagonists on the release of immunoreactive LHRH. Hypothalami, removed from adult (200g) or immature (50g) Sprague-Dawley rats, were incubated as described previously (Buckingham & Hodges, 1977) at 37°C in oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid, containing glucose (0.2%) and BSA (0.25%). Addition to the incubation medium of naloxone (10^{-7}M and 10^{-6}M) significantly (P < 0.05, n = 5) increased, in a dose related manner, the release of LHRH from hypothalami obtained from adult rats. Similarly, hypothalami from pre-pubertal rats responded to naloxone (10^{-8}M - 10^{-6}M) treatment with significant (P < 0.05, n = 5) dose-related increases in LHRH release. In contrast, neither ICI 174864 (10^{-6}M) nor MR2266 (10^{-6}M) significantly (P > 0.2) affected the release of LHRH from hypothalami obtained from adult or pre-pubertal rats. The $alpha_1$-adrenoceptor antagonist, alfuzosin (10^{-6}M), was also ineffective in this respect. However, it abolished the secretory responses to naloxone (10^{-6}M) by hypothalami obtained from both adult and pre-pubertal rats.

The results suggest firstly that the tonic inhibitory effects of the opioid peptides on the release of LHRH are mediated via mu-receptors and secondly that the LHRH response to opioid-receptor blockade in immature and adult rats involves disinhibition of the noradrenergic neurones which control the secretion of the releasing hormone.

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PURINE MEDIATED RELAXATION AND CONTRACTION OF ISOLATED HUMAN RESISTANCE ARTERIES

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Purines have direct actions on blood vessels, producing vasodilatation and/or vasoconstriction. Both pre and post junctional mechanisms of action and a number of receptor subtypes for purines have now been identified (Williams, 1987). ATP may function as a cotransmitter in perivascular sympathetic nerves (Su, 1975). In the perfused rat mesentery adenosine and ATP both attenuate the pressor response to sympathetic nerve stimulation. In SHR this attenuation required doses 6 times those required in WKY rats (Kamikawa, 1980). We have determined the actions of the endogenous purines in isolated human resistance arteries.

Arteries (I.D. 100-700 μM) were dissected from omentum or subcutaneous fat removed at surgery and were mounted, in Krebs buffer, in a Mulvany myograph maintained at 37°C and gassed with 95% O_2/5% CO_2. The vessels were then normalised to a resting diameter 0.9 x L100 where L100 is the diameter producing the wall tension equivalent to that produced by 100 mm Hg distending pressure calculated by the Laplace relationship (Mulvany & Halpern, 1977). Arteries were then allowed to equilibrate for 1 hr. Viability was confirmed by demonstration of contractile responses to a depolarising K^+ solution (120 mM) and noradrenaline (10 μM). Arteries producing a tension equivalent to less than 90 mm Hg were discarded. Contractions are expressed as a percent of K^+ (120 mM) response and relaxations as a percent reduction of preconstricted tone.

When added cumulatively ATP (1 nM - 1 mM) alone produced a dose dependent contraction over the upper dosage range (1 μM - 1 mM), which required the presence of extracellular Ca^{++}, with an Emax of 55% (n = 25). This Emax was increased to 82% if a composite dose response curve was prepared by addition of single doses to separate preparations (n = 21, 3 each dose). The EC_{50}s, however, were unaltered by the different methodologies, being 72 μM and 74 μM for cumulative and composite respectively. Adenosine alone did not produce contraction. Following preconstriction with noradrenaline (1 μM) cumulative addition of ATP (1 nM - 300 nM) produced a dose dependent relaxation, Emax 83% and EC_{50} 52 nM (n = 20) which did not bear a linear relationship to acetylcholine relaxation. In some instances further cumulative addition of ATP (1 μM - 1 mM) reversed this relaxation. Adenosine (10 nM - 10 μM) also produced a dose dependent relaxation, Emax 87%, EC_{50} 6.2 μM (n = 10) following noradrenaline preconstriction. Neither ATP nor adenosine induced relaxations were significantly affected by indomethacin (10 μM) (n = 4). Adenosine, but not ATP, induced relaxations were antagonised non-competitively by the P1 purinoceptor antagonist, 8-phenyltheophylline (10 μM) (n = 6).

These findings demonstrate the presence of purinoceptors mediating both relaxation and contraction of human resistance arteries. Further assessment of their physiological role and sympathetic interactions is important to the understanding of vascular control mechanisms.

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EFFECT OF ELECTRICAL PRETREATMENT ON MICROCARBON FIBRE ELECTRODES: AN ELECTRON MICROSCOPIC AND VOLTAMMETRIC STUDY

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Electrically pretreated microcarbon fibre electrodes (CFE) allow, when combined with differential pulse voltammetry (DPV), direct monitoring in vitro and in vivo of dopamine and serotonin metabolites (Crespi et al., 1984). This technique has advantages over sampling techniques (e.g. intracerebral dialysis) in speed and frequency of measurement and in the small size (12 µm diam) of the biosensor, due to better localisation and less tissue trauma. However, it is still unclear why electrical pretreatment increases the sensitivity and selectivity of the CFE. In the present study, using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) in association with DPV, we have investigated the effect of electrical pretreatment on the structural characteristics of the CFE.

Observed with the SEM at two magnifications (3,176X and 15,176X), the surface of the untreated 12 µm CFE shows numerous irregular longitudinal grooves along the whole length of the fibre. DPV performed on the untreated CFE in phosphate buffer containing ascorbic acid (AA 500 µM), 3,4-dihydroxyphenylacetic acid (DOPAC 50 µM) and 5-hydroxyindoleacetic acid (5HIAA 20 µM), produces a wide oxidation wave with no separation between AA, DOPAC or 5HIAA. Electrical pretreatment performed on the 12 µm CFE (triangular wave from 0 to 3V, 15 sec; from 0 to 2.5V, 20 sec; from 0 to 1.5V, 30 sec at 70Hz; then D.C. + 1.5V 10 sec, DC -1V 10 sec) allows clear voltammetric separation of AA (Peak 1 at -50mV), DOPAC (Peak 2 at +90mV) and 5HIAA (Peak 3 at +280mV) in vitro. This treatment modifies the surface of the CFE; covering the tip with small crystals which are probably sulphonate-based and may be important for the increased sensitivity and selectivity of the electrode. The electrically pretreated CFE are more fragile and shatter more readily than untreated carbon fibres during cutting on the ultramicrotome for the subsequent TEM analysis. Furthermore, the TEM observations show loss of the uniform small dense discs (about 1.1 µm in diameter) observed before pretreatment. The discs probably correspond to the core of the untreated carbon fibres (Bennett & Johnson, 1978). The data suggests that electrical pretreatment markedly alters not only the surface but the entire structure of the carbon fibre resulting in an increased carbon surface available for interactions with electroactive compounds. It has been reported already that anodic treatments increase red-ox couples (Engstrom, 1982) and the presence of oxides on the surface of the carbon fibres (Sherwood, 1983). The present study indicates that these red-ox couples and oxides, which probably interact with electroactive compounds, could be increased also in the internal structure. In conclusion, the chemical and physical modifications observed may explain the high selectivity and sensitivity of the electrically pretreated CFE. At present the chemistry of the crystals observed after electrical pretreatment is being determined using X-ray microanalysis and scanning electron microscopy. Such studies will allow a more predictive approach to the development of specific electrical pretreatments for carbon fibre voltammetric electrodes and aid the production of electrodes with greater selectivity and sensitivity.

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IN VIVO MEASUREMENT OF BRAIN 5-HT RELEASE USING MICRODIALYSIS: CHANGES IN RELATION TO 5-HT-ERGIC NEURONAL ACTIVITY

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Intracerebral perfusion by microdialysis, is used to study the release of 5-HT from central neurones in the animal brain *in vivo*. However, given that the method samples 5-HT in the brain extracellular space, a proportion of the 5-HT measured may arise from non-neuronal sources, in particular the blood. The aim of the present study was to better determine the relationship between dialysate levels of 5-HT and both the integrity and electrical activity of central 5-HT neurones. Spontaneous output of 5-HT into dialysates of rat ventral hippocampus was measured in response to a) lesion of central 5-HT neurones using 5,7-dihydroxytryptamine (5,7-DHT), b) electrical stimulation of the dorsal raphé nucleus, which contains 5-HT neurones innervating ventral hippocampus, and c) local perfusion with tetrodotoxin, a sodium channel blocker which prevents neurone depolarization.

Non-treated rats (male Sprague Dawley, 260-300g), or rats which had 14 days earlier received i.c.v. injections of 5,7-DHT (150 µg in 15 µl saline), were anaesthetized with chloral hydrate and a dialysis probe was stereotaxically implanted into the ventral hippocampus. Probes were perfused (1 µl/min) with artificial CSF containing the 5-HT reuptake inhibitor citalopram (10^{-6}M) and dialysates were collected every 20 min for analysis of 5-HT using HPLC-EC (Sharp et al, 1988).

5-HT levels in hippocampal dialysates collected from both non-treated and 5,7-DHT-injected rats declined over the first 80-100 min post probe implantation but then became constant. The stabilized output, but not the initially falling amounts of 5-HT, was markedly reduced (-57%, $p<0.002$, n=8) in the 5,7-DHT-treated versus control group. 5,7-DHT decreased whole tissue hippocampal 5-HT by 70% in a separate group of rats. Following a 2h control period, electrical stimulation (cathodal monophasic pulse, 1 msec duration, 4v, 2-20 Hz) of the dorsal raphé nucleus for 20 min evoked a short-lasting, frequency-dependent release of 5-HT (2Hz +12%, 5Hz +78%, 10Hz +142%, 20Hz +327%, n=3 rats in each case). Addition of 1 µM tetrodotoxin to the perfusion medium for 60 min reduced 5-HT to 74% of control values.

From these finding we conclude that under our experimental conditions a large proportion of the constant, spontaneous output of endogenous 5-HT into rat hippocampal dialysates derives from central 5-HT neurones and changes in accordance with altered 5-HT neuronal activity.

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TRANYLCYPROMINE AND 5-HYDROXY-L-TRYPTOPHAN ANTAGONISE CISPLATIN-INDUCED EMESIS IN THE FERRET

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The ability of 5-HT_3 receptor antagonists to attenuate cisplatin induced emesis in the ferret and dog (Alphin et al. 1986; Costall et al, 1986, 1987; Miner et al, 1986) indicates that the emetic response may be mediated via a 5-hydroxytryptamine (5-HT) system. If this hypothesis is correct then agents acting to increase 5-HT function should exacerbate the response and in the present study we investigate the effects of tranylcypromine, fluoxetine and 5-hydroxy-L-tryptophan (5-HTP) to modify cisplatin induced emesis in the ferret.

Male ferrets (1.3 - 1.8 kg) were housed individually and presented with cat food at 12.00-13.00 h. At 13.30-14.30 h the ferrets were anaesthetised with fluothane (N_2O/O_2 carrier) and cisplatin (5.0 or 10.0 mg/kg) was injected into the jugular vein. 5-HTP (20.0 mg/kg i.p.) and fluoxetine (2.5 and 10.0 mg/kg i.p.) were administered immediately before and 30 min prior to the injection of cisplatin respectively; tranylcypromine (2.0 and 5.0 mg/kg i.p.) was administered as a 4h pretreatment. In each animal the time to onset of emesis, and the number of vomits, retches and emetic episodes were recorded over a 4h period.

Table 1. The effect of tranylcypromine (Tran.), fluoxetine (Flu.) and 5-HTP to modify cisplatin-induced emesis.

Treatment/ dose		n	Onset of emesis (min)	No. of vomits	No. of retches	No. of episodes
Cisplatin	5.0mg/kg	5	>240	0	0	0
+ Tran.	5.0mg/kg	5	>240	0	0	0
+ Flu.	2.5mg/kg	5	>240	0	0	0
Cisplatin	10 mg/kg	4	69±4	14±2	87±22	17±2
+ Tran.	2.0mg/kg	4	130±38*	4±3	31±27	4±3*
+ Tran.	5.0mg/kg	5	216±24*	0*	2±2*	1±1*
+ Flu.	2.5mg/kg	4	77±8	8±2	71±21	11±2
+ Flu.	10 mg/kg	4	75±8	8±3	65±18	11±3
+ 5-HTP	20 mg/kg	4	147±20*	6±2*	39±10	7±1*

Each value is the mean ±S.E.M. Significant delays in the onset of emesis or reductions in vomiting/retching behaviour are indicated as *$P<0.05$, (Mann-Whitney U-test).

The failure of the monoamine oxidase inhibitor tranylcypromine and the 5-HT reuptake inhibitor fluoxetine to precipitate an emetic response to a subthreshold dose of cisplatin (5.0 mg/kg) prompted the subsequent use of an emetic dose of cisplatin (10 mg/kg). However, tranylcypromine, fluoxetine and 5-HTP failed to enhance emesis and indeed tranylcypromine and 5-HTP significantly reduced the response to cisplatin; the reductions afforded by fluoxetine were not significant ($P>0.05$). It is concluded that 5-HT has a complex involvement in the control of emetic behaviour.

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EFFECTS OF THE 5-HT_3 RECEPTOR ANTAGONIST, GR38032F, ON THE SYNTHESIS AND METABOLISM OF 5-HT AND DOPAMINE IN RAT FOREBRAIN

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$5HT_3$ receptor antagonists reduce the hyperactivity response to dopamine (DA) infusions into the nucleus accumbens (Costall et al., 1987). GR38032F inhibits both the behavioural response and the increased DA metabolism resulting from injection of a neurokinin receptor agonist into the ventral tegmental area of the rat (Hagan et al., 1987). $5HT_3$ binding sites are present in cortical and limbic areas of rat forebrain (Kilpatrick et al., 1987). In order to investigate further the mechanism of action of GR38032F in modifying mesolimbic DA systems, we have examined its effects on the synthesis and metabolism of DA and 5HT in several areas of rat forebrain (male, random hooded strain, Glaxo) in (a) vehicle treated control animals, (b) animals pretreated with the aromatic amino acid decarboxylase inhibitor, NSD 1015 (100mg/kg i.p., 30min before death). The effects of GR38032F were also studied in animals in which forebrain DA turnover was increased by pretreatment with haloperidol (HAL, 0.1mg/kg s.c., 30min before death). In all cases, vehicle or GR38032F was administered 45min before death. DA, 5HT, their precursors and metabolites were determined by HPLC with electrochemical detection (Hagan et al., 1987).

Pretreatment with GR38032F (0.01–0.3mg/kg) had no statistically significant effect ($p>0.05$, Dunnett's t-test) on the accumulation of 5HTP or DOPA in the nucleus accumbens of rats treated with NSD 1015 (Table 1).

Table 1

Treatment (mg/kg)	(n)	5HTP accumulation (ng/mg/protein/hr)	DOPA accumulation mean ± S.E.M.
Vehicle	5	4.6±0.4	35.6±2.5
GR38032F (0.01)	5	4.6±0.4	34.2±1.2
(0.03)	4	4.1±0.1	30.1±1.7
(0.1)	4	4.7±0.3	32.9±1.4
(0.3)	3	5.1±0.4	32.1±1.6

GR38032F (0.1mg/kg) also had no statistically significant effect on the levels of DA, 5HT and their metabolites in the nucleus accumbens, olfactory tubercles, frontal cortex, entorhinal cortex, amygdala and striatum; nor on HAL-induced increases in DA metabolism, expressed as the ratio DOPAC/DA, in nucleus accumbens, amygdala or striatum. Nucleus accumbens (mean ± s.e. mean, n=5 per group): control, 0.133±0.002; GR38032F, 0.126±0.008; HAL, 0.370±0.012; GR38032F + HAL, 0.347±0.009 ($p>0.05$ vs HAL alone).

The results suggest that GR38032F, in common with its lack of effect on normal rat behaviour, does not affect normal rates of 5HT and DA metabolism in limbic areas of rat brain. In contrast to our previous findings, whereby GR38032F antagonised the increase in DA metabolism in animals where the mesolimbic DA system was selectively activated, it had no effect on HAL-induced increases in DA metabolism. The ability of GR38032F to antagonise increased mesolimbic DA metabolism may thus depend on the post-synaptic expression of increased dopaminergic activity, which is lacking in HAL treated animals.

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BRAIN 5-HT AND 5-HIAA CONCENTRATIONS IN DEPRESSED SUICIDES

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Suicidal behaviour in depressed patients, particularly when violent in nature, is associated with low CSF 5-hydroxyindoleacetic acid (5-HIAA) concentrations (Asberg et al, 1976). This finding has been interpreted as reflecting decreased central 5-HT turnover. Studies of post-mortem brain from suicide victims have generally found 5-HT or 5-HIAA concentrations to be lower in the hind brain but not in higher brain centres compared to controls (Stanley et al, 1986). We now report 5-HT and 5-HIAA concentrations in six brain regions from a group of depressed suicide victims and controls.

Deaths confirmed as suicide were subjected to retrospective diagnosis. A firm diagnosis of depression was established in 19 cases (14 M, 5 F, mean age ± sem 39±3 years). Thirteen of these subjects had not been prescribed psychoactive drugs recently and none were detected in blood taken at post-mortem (drug-free). Five subjects were receiving antidepressant drugs (alone or in combination with other drugs) prior to death (antidepressant-treated). Control subjects died suddenly from natural causes or accidents (14 M, 5 F, 40±3 years).

5-HT and 5-HIAA concentrations (ng/g tissue, mean±sem) were determined by reverse phase HPLC with electrochemical detection in frontal and temporal cortex, hippocampus, amygdala, caudate and putamen.

5-HT, 5-HIAA and 5-HIAA/5-HT ratio did not differ significantly between the total, drug-free and antidepressant-treated suicides and their matched controls in frontal and temporal cortex and caudate. 5-HIAA concentration was significantly higher in the amygdala of drug-free suicides (482±26) than controls (387±22), whereas 5-HT and 5-HIAA/5-HT were unaltered. Similarly 5-HIAA was higher in the hippocampus (282±22 v 223±11) but failed to reach statistical significance; again 5-HT and 5-HIAA/5-HT did not differ. No differences in 5-HT, 5-HIAA and 5-HIAA/5-HT were apparent in these two regions when the antidepressant-treated suicides were compared to controls. 5-HT concentration in putamen was significantly lower in the total (259±13) and antidepressant treated (246±24) suicides compared to controls (323±15). A reduction of similar magnitude was also apparent in drug-free suicides, but was not statistically significant. 5-HIAA concentration did not differ significantly in the putamen between the suicide and control groups, but the 5-HIAA/5-HT ratio was significantly higher in the total suicide and drug-free suicides compared to controls. 5-HT and 5-HIAA concentrations in drug-free suicides who died by non-violent means (224±20, 649±28 respectively, n=8) were significantly lower in the putamen, but not other brain areas, than those subjects who died by violent means (307±32, 927±94 respectively, n=5). Differences between suicides and controls could not be attributed to age, sex or post-mortem delay.

In contrast to the findings of reduced CSF 5-HIAA concentration in depressed patients who attempt suicide, we found no evidence of reduced 5-HT turnover in the brains of depressed patients who had died by suicide.

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MECHANISM OF THE ATTENUATION OF NALOXONE-INDUCED JUMPING BEHAVIOUR IN MORPHINE DEPENDENT MICE BY 5-HT_1 RECEPTOR AGONISTS

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A variety of evidence implicates 5-HT in the mechanism of the jumping behaviour associated with naloxone-precipitated morphine withdraval in rats (Cervo et al., 1981; Romandini et al., 1984). Recently, Dzoljic & Saxena (1987) have demonstrated that the 5-HT_1 receptor agonists, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and 5-methoxy-3-[1,2,3,6-tetrahydro-4-pyridinyl]-1-H-indole (RU 24969), inhibit most of the signs of morphine withdraval in the rat. The present study extends this work to the mouse and, by the use of other selective 5-HT_1 receptor agonists and various antagonists, attempts to determine the underlying mechanisms.

Female OF1-mice were rendered tolerant to, and dependent on, morphine by implantation of a pellet containing 75 mg morphine base in the dorsal subcutaneous tissue. After five days, abstinence signs were precipitated by an intraperitoneal injection of 1 mg/kg naloxone. The animals were placed individually in a cylindrical glass and the number of jumps was recorded for 20 min. Test drugs were injected subcutaneously 30 min before naloxone, except for 8-OH-DPAT, which was injected 5 min before naloxone. The antagonists were injected 40 min before naloxone. All treatment groups consisted of 10 animals.

8-OH-DPAT and RU 24969 attenuated the naloxone-precipitated jumping behaviour. After 8-OH-DPAT the dose reponse relationship followed a U-shaped curve with maximum effects (51 ± 10% of control) between 1 and 2 mg/kg. RU 24969 acted dose-dependently, being effective at the lowest dose of 0.25 mg/kg (38 ± 10% of control) with maximum effects at 2 mg/kg (6 ± 3% of control). The selective 5-HT_{1A} receptor ligands, buspirone (2-5 mg/kg), ipsapirone (10 mg/kg) and flesinoxan (0.5-3 mg/kg), also suppressed jumping. Depletion of 5-HT with para-chlorophenylalanine (2 x 150 mg/kg, 48 and 72 h before naloxone) had only minimal effects on jumping and did not alter the attenuating effects of 8-OH-DPAT and RU 24969.

The dose response curve of RU 24969 was displaced to the right by (-)pindolol (2 mg/kg). The same dose of (-)pindolol neither influenced the action of 8-OH-DPAT nor showed an effect per se. The actions of 8-OH-DPAT and buspirone, but not of RU 24969 were blocked by spiroperidol pretreatment (0.5 mg/kg). However, both haloperidol (0.1 mg/kg) and prazosin (0.1 mg/kg) also blocked 8-OH-DPAT without affecting RU 24969.

The present data show that drugs with selectivity for subtypes of the 5-HT_1 recognition site inhibit naloxone-induced jumping in the morphine-dependent mouse and the integrity of central 5-HT stores seems not to be a prerequisite for these actions. With respect to 8-OH-DPAT, no evidence has been obtained for an involvement of the 5-HT_{1A} receptor, although the response can be mimicked by other 5-HT_{1A} selective ligands. RU 24969 behaves quite differently from 8-OH-DPAT and here strong evidence implicates the 5-HT_{1B} receptor in the response. Since 5-HT_{1B} receptors have not been detected in the human brain (Hoyer et al., 1986), the wisdom of an approach to the therapy of opiate withdrawal based on 5-HT_1 receptor agonism, as suggested by Dzoljic & Saxena (1987), might be questioned.

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THE EFFECT OF GR38032F ON ALCOHOL CONSUMPTION IN THE MARMOSET

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It has been reported that both benzodiazepines (Cahn et al., 1983) and more recently the novel anxiolytic buspirone (Collins & Myers, 1987) can reduce alcohol intake in alcohol-habituated experimental animals. In this study, we have investigated the effect of the potent 5-HT_3 antagonist GR38032F (Butler et al., 1988), a potential anti-anxiety agent (Jones et al., 1988), on alcohol intake in the marmoset. Additionally, the effect of GR38032F on alcohol-induced withdrawal signs was also examined.

Eight female marmosets (Callithrix jacchus), housed singly, were gradually introduced to and maintained on 3% v/v alcohol (ethanol - ANALAR) in their drinking water. They were then withdrawn from alcohol and allowed water only to drink for 1 week. For the week following they were allowed a free choice of 3% v/v alcohol or water. During the whole experiment fluid consumption was measured daily and mean weekly fluid intakes calculated. The effect of twice daily dosing with either vehicle (water - 5ml/kg p.o.) or GR38032F (0.01mg/kg p.o.) on fluid intake over the withdrawal and choice phases was determined using a cross-over design. The effect of GR38032F (0.01mg/kg p.o. b.d.) on the withdrawal-induced anxiety as measured by aggressive postures and time spent at the cage front (see Jones et al., 1988) was also assessed during the withdrawal phase.

In the two stages of the cross-over designs, the baseline alcohol consumption was consistent (180.5±29.3ml/wk and 185.6±32.2ml/wk, respectively). In the withdrawal phase, where only water was offered, fluid consumption was reduced to 101.8±18.8ml/wk and 115.4±14.5ml/wk under vehicle and GR38032F treatment, respectively. In the subsequent choice phase, the total fluid intake under vehicle treatment was back to pretest levels (177.6±27.8ml/wk) of which 130.6±29.5ml was alcohol and 47.0±8.9ml was water. However, under GR38032F treatment, the amount of fluid intake was reduced to 128.9±17.5ml/wk with significantly ($P<0.05$ - Mann Whitney 'U') less alcohol (72.5±10.1ml) but a similar amount of water (56.4±14.4ml) being drunk.

In the withdrawal phase, the amount of aggressive posturing was significantly ($P<0.05$ Dunnett's 't') increased from 3.8±1.1 to 9.0±2.4 which was significantly ($P<0.05$ Dunnett's 't') attenuated by concomitant GR38032F treatment (2.8±0.4). The time spent at the cage front was also significantly reduced on alcohol withdrawal from 53.6±6.4s to 10.4±5.7s and this was attenuated by GR38032F treatment (21.0±6.0 s).

Thus GR38032F reduced alcohol intake after withdrawal and subsequent re-exposure, as well as reducing the anxiety related withdrawal behaviours. These results suggest a possible clinical use for this compound in controlling alcohol intake and withdrawal in alcohol-dependent individuals.

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CHARACTERISATION OF [^{3}H]-GR65630 BINDING TO 5-HT$_3$ RECEPTORS IN HOMOGENATES OF RAT AREA POSTREMA AND VAGUS NERVE

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The tritiated derivative of the potent and selective 5HT$_3$ antagonist, GR65630, has been shown to bind to a specific site in homogenates of rat entorhinal cortex that has been identified as representing the 5HT$_3$ receptor (Kilpatrick et al., 1987). Central 5HT$_3$ sites have also been labelled with ^{3}H-quipazine (Peroutka & Hamik, 1988) and ^{3}H-zacopride (Barnes et al., 1988). We have previously reported that the area postrema (AP) had the highest concentration of specific ^{3}H-GR65630 binding of 16 brain regions tested (Higgins et al., 1988). We now report on the characterisation of specific ^{3}H-GR65630 binding to AP homogenates. We also report on the characterisation of specific ^{3}H-GR65630 binding to the rat vagus nerve (RVN), a tissue where a 5HT$_3$ receptor mediated effect (depolarisation) can be measured (Ireland & Tyers, 1987).

Tissue homogenates were prepared and specific ^{3}H-GR65630 binding assayed as described previously (Kilpatrick et al., 1987) except that a final tissue dilution of 250 volumes was employed (0.05-0.07mg protein/assay tube). Saturation analysis revealed that the specific binding of ^{3}H-GR65630 (0.01-2nM) represented a single high affinity site in both AP and RVN homogenates (A.P. Kd 0.24±0.08nM, B_{max} 44.4±0.2fmol/mg protein; RVN Kd 0.50±0.026nM, B_{max} 89.1±4.8fmol/mg protein; means ± range; n=2). 5HT$_3$ agonists and antagonists inhibited ^{3}H-GR65630 (0.2nM) binding to AP and RVN homogenates (Table 1). Hill numbers were close to unity. These compounds inhibited up to 80% of total binding in AP homogenates and up to 70% of total binding in RVN homogenates. Although GR38032F inhibited up to 90% of total binding in both tissues, this additional inhibition is likely to represent a saturable low affinity site unrelated to 5HT$_3$ receptors (Kilpatrick et al., 1987).

Table 1 The inhibition constants of 5HT$_3$ agonists and antagonists to inhibit ^{3}H-GR65630 binding to AP and RVN homogenates

	RVN IC_{50} (nM)	AP IC_{50} (nM)	
Quipazine	1.1	0.87	
ICS 205-930	1.8	1.1	
BRL 43694	2.5	2.3	
GR38032F	1.2*	2.8*	
MDL 72222	30.2	27.9	
Metoclopramide	654	508	
5HT	102	131	* Ki
2-methyl-5HT	301	375	

The IC_{50} values for 5HT$_3$ antagonists to inhibit ^{3}H-GR65630 binding to AP and RVN homogenates correlated well and also with their affinities to inhibit ^{3}H-GR65630 binding to rat entorhinal cortex and 5HT-induced depolarisation of the RVN (see Kilpatrick et al., 1987). These observations provide further support for the suggestion that specific ^{3}H-GR65630 binding sites represent the 5HT$_3$ receptor.

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