IN VITRO EFFECTS OF A D-HOMO-CORTICOSTEROID ON HUMAN LYMPHOCYTE AND HUMAN EMBRYONIC LUNG FIBROBLAST PROLIFERATION

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Corticosteroids have been shown to inhibit a number of cellular functions, including DNA replication, mitosis and collagen synthesis (Fauci, 1979). Inhibition of mitosis has been demonstrated in cultured fibroblasts and in mitogen stimulated lymphocyte cultures. This paper describes some effects of Ro 12-7024, a novel D-homo-corticosteroid.

The study compared the effects on cell proliferation of the corticosteroid Ro 12-7024 (11B-Hydroxy-3,20-dioxo-D-homopregna-1,4-dien-17a-yl butyrate), with those of six reference steroids : betamethasone -17-propionate, dexamethasone, cortisol, prednisolone, clobetasol-17-propionate and 19-nortestosterone. The comparisons were made in two in-vitro cell culture systems. (1) ^{3}H-thymidine incorporation by phytohaemagglutinin P stimulated human peripheral blood lymphocytes was measured after challenge with 0.1, 1.0, 10 and 20 µg/ml of each steroid. (2) ^{3}H-thymidine incorporation by logarithmically growing human embryonic lung fibroblasts was measured after challenge with 0.1, 10, 20 and 40 µg/ml of each drug. The results indicated that all the investigated steroids produced a dose related inhibition of cell proliferation in both systems. The reference steroids inhibited cell proliferation to different degrees, compared to Ro 12-7024, over the range of doses studied. These differences allowed the establishment of an approximate rank order of inhibitory effectiveness shown in Figure 1.

Figure 1. In vitro inhibitory potency of Ro 12-7024

Ro 12-7024 < Clobetasol, nortestosterone.

Ro 12-7024 = Betamethasone, prednisolone

Ro 12-7024 > Cortisol, dexamethasone.

In the lymphocyte culture system significant inhibition of mitogen induced blastogenesis was observed at varying points in the PHA-P concentration range used.

The results obtained in this fibroblast system compare well with those of Ponec et al, 1977 and 1979 whose work in similar systems indicated the rank order of inhibitory effectiveness to be Clobetasol > Betamethasone > Cortisol.

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THE ACTION OF SELECTED ANTIMICROBIAL AGENTS ON CERTAIN FUNCTIONS OF HUMAN LEUCOCYTES

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During infections and acute inflammation there is a rapid accumulation, at the infected sites, of leucocytes which inactivate the organisms. The efficiency of arresting and killing the invading organisms, primarily depends on the functional status of neutrophils and lymphocytes. The functional integrity of these cells is of crucial importance in host resistance to infection so that functional defects are likely to have serious consequences (Kay et al, 1976).

This study set out to investigate the in-vitro effect of some antibacterial agents on quantitative neutrophil iodination and on lymphocyte transformation to Phytohaemagglutinin (PHA) stimulation. This investigation stems from earlier findings that human neutrophils and lymphocytes take up certain sulphonamides and trimethoprims in-vitro (Climax et al, 1981, 1982, 1983). The antimicrobial agents investigated were sulphadiazine (SDZ), sulphamerazine (SMZ), sulphanilamide (SNM), sulphamethoxazole (SMX), trimethoprim (TMP), brodimoprim (BMP), co-trimoxazole (CTX) and ceftriazone (CFS). Purified populations of neutrophils and lymphocytes from peripheral blood of seven healthy volunteers were studied.

Quantitative neutrophil iodination was performed with neutrophils that were pre-incubated with 10ug/ml of each of the investigated drugs, at 37°C for 60 minutes. Statistical analysis of the data revealed that SMX, SNM, TMP, BMP and CTX significantly increased ($p<0.05$ - $p<0.005$) quantitative neutrophil iodination. However, SMZ, SDZ and CFS produced no changes in the neutrophils' iodination capacity ($p>0.1$).

In the lymphocyte transformation study, 0.2µg of each of the investigated drugs was incubated with 10^7 cells in culture. The incorporation of ^3H-thymidine during transformation was measured. With the exception of CTX, none of the antimicrobial agents significantly modified lymphocyte transformation. The lymphocyte response to TMP and SMX approached significance ($p<0.1$). However, a highly significant ($p<0.005$) increase in lymphocyte transformation was produced by CTX, possibly due to synergism between the two components, SMX and TMP.

These observations suggest that some antimicrobial agents may enhance leucocyte function and thereby complement the already compromised status of leucocytes in infectious disease.

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DETECTION AND ISOLATION OF A STEROID-INDUCED ANTIPHOSPHOLIPASE PROTEIN OF HIGH MOLECULAR WEIGHT

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The glucocorticoids prevent the generation of eicosanoids by causing the synthesis and release of proteins which inhibit phospholipase A_2 activity. Hitherto several species have been described including macrocortin (mol. wt. ≃15 k; Blackwell et al. 1980), lipomodulin (mol. wt. ≃ 40 k; Hirata et al. 1981) and renocortin (mol. wt. ≃ 15k, 30k; Cloix et al. 1983). Because of similarities in the biological activity, immunological cross-reactivity and molecular weights we have suggested that macrocortin is a biologically active fragment of lipomodulin (Hirata et al. 1983). We now present data showing that when stimulated by glucocorticoids rat peritoneal macrophages also synthesise and release an anti-phospholipase protein with a molecular weight of ≃125 k.

To generate the protein, rats were injected with dexamethasone (0.2 mg/kg s.c.) and killed 1 h later by exposure to carbon dioxide. Peritoneal macrophages were recovered by lavage and either the cells themselves or the lavage fluid used as a source of the protein. When subjected to gel permeation chromatography on Sephacryl 300 S, antiphospholipase activity was associated with proteins having elution characteristics suggesting mol. wts. of 16K and 45K as we had previously observed, but in addition there was a prominent peak of biological activity with an apparent mol. wt. of 170K. This material was further purified by DEAE cellulose chromatography (approximately 20 fold purification) and finally subjected to phospholipase A_2 affinity chromatography. The latter step gave a purification of approximately 30 fold, and when the resulting material was subjected to SDS polyacrylamide gel electrophoresis under non-reducing conditions a major band was seen on the stained gel at 125k. Under reducing conditions this band disappeared and was replaced by major bands at 70 and 55-58k. Immune blotting, or immunoprecipitation of iodinated macrocortin preparations with the anti-macrocortin monoclonal antibody RM23 also revealed a strongly antigenic band of the same molecular weight whose immunogenicity disappeared after reduction. The 125k material did not react with other monoclonal antibodies of the same (2b) subclass as RM23, and did not itself contain any immunoglobulin, as judged by the absence of binding of iodinated rabbit anti-mouse immunoglobulin.

The partially purified (ex affinity column) material strongly inhibited the hydrolysis of labelled phospholipids in heat killed E. coli by the porcine pancreatic phospholipase A_2, with 50% reduction in the initial rate of reaction being given by approximately 100 ng protein of the partially purified fraction. Pre-incubation of the protein with a 1:75 dilution of the antibody RM23 at 4°C for only 30 min reduced the anti-phospholipase activity by >50%.

Many biologically active proteins are synthesised in cells as high molecular weight precursors. It is possible that the protein described here is a precursor for macrocortin but as yet we have no definitive evidence for this relationship.

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PROSTACYCLIN INHIBITS LEUKOTRIENE-INDUCED CONTRACTIONS OF AIRWAY SMOOTH MUSCLE

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The release of slow reacting substance of anaphylaxis (SRS-A) has been extensively studied using the technique of superfusing a series of bioassay tissues in cascade with the effluent from isolated perfused lungs. In these conditions, antigen-challenge of lungs from sensitized animals results in the slow contraction of strips of airway smooth muscle bathed in lung effluent (Piper and Vane, 1969). When lungs are treated with aspirin-like drugs, which selectively inhibit prostaglandin synthesis, the slow reacting responses of the tissues are augmented (Walker, 1973). We now know that SRS-A is a mixture of leukotrienes (LTs) and that LTC_4 and LTD_4 are potent inducers of slow contractile responses on isolated smooth muscle (Morris et al., 1980). In this paper we have investigated the interaction between prostaglandins and leukotrienes.

The tracheas and pulmonary arteries of anaesthetised male guinea pigs (Dunken-Hartley; 350-450g) were cannulated and the lungs removed, inflated and perfused with Krebs solution at 5 ml min^{-1}. Lung effluents were superfused over preparations of guinea pig tracheal smooth muscle or guinea pig lung parenchymal strips (Sirois et al., 1981). Responses of the tissues to bolus injections of LTC_4, LTD_4 or acetyl choline (ACh) into the superfusing medium were detected by auxotonic transducers (Paton, 1957). Tissue responses were compared before and after the infusion of prostaglandins or indomethacin into the superfusing fluid.

Guinea pig isolated tracheal strips (GPT) and lung parenchymal strips (GPP) contracted in a dose-dependent fashion to LTC_4 (10-300 ng), LTD_4 (10-250 ng) or ACh (5-80 µg). Approximately 50% of parenchymal preparations exhibited tachyphylaxis to the leukotrienes but not to ACh. Tachyphylaxis to LTD_4 was more marked than to LTC_4. Tissues superfused with lung effluent were markedly less sensitive to leukotrienes than tissues superfused with Krebs, requiring more than four times the dose of LTC_4 or LTD_4 to produce a response, while the sensitivity to ACh was unchanged. When tissues were superfused with lung effluent which had been collected and incubated at 37°C for 15 min the sensitivity to LTC_4 and LTD_4 was not affected. Similarly, tissues superfused with effluent from lungs perfused with Krebs containing indomethacin (0.5 µg ml^{-1}) also retained the sensitivity to leukotrienes. Infusions of prostacyclin (0.1-5 ng ml^{-1}) caused a dose-dependent reduction in responses of GPT and GPP strips to LTC_4 and LTD_4 without reducing ACh responses. Contractions of the GPT to leukotrienes were reduced by 50% at a concentration of 0.8 ng ml^{-1} prostacyclin added to the superfusing Krebs solution. PGE_2 (10 ng ml^{-1}), $PGF_{2\alpha}$ (10 ng ml^{-1}) or 6-keto-$PGF_{1\alpha}$ (20 ng ml^{-1}) had no effect on tissue responses to LTC_4 or LTD_4.

These results indicate that prostacyclin inhibits leukotriene-induced contraction of airway smooth muscle. It is possible that prostacyclin production in the lung modulates airway responses and inhibition of prostacyclin by aspirin-like drugs may increase airway reactivity. Our results also indicate that caution should be exercised when the effluent of organs is analysed by bioassay since interactions such as we describe here could lead to misinterpretation of results.

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AIRWAY HYPERREACTIVITY IN VIVO, INDUCED BY NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN GUINEA-PIGS

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Airway hyperreactivity is a cardinal feature of bronchial asthma, and is also observed during and following an acute bronchial infection (Empey et al., 1976). Although its aetiology is uncertain, results from in vitro studies have suggested that alterations in local arachidonic acid metabolism may be involved since non-steroidal anti-inflammatory drugs (NSAID) consistently increase responses of guinea-pig isolated trachea to histamine and other spasmogens (Orehek et al., 1975). However, attempts to induce hyperreactivity by NSAID administration to guinea-pigs in vivo have generally proved unsuccessful (Brink et al., 1976; Rossoni et al., 1980).

The current study was undertaken in an attempt to explain this discrepancy between in vitro and in vivo results. The method was a modification of that described by Herxheimer (1952). Male Dunkin-Hartley guinea-pigs (300-400g) were exposed individually for ten minutes to Wright's nebuliser aerosols of histamine, at a concentration (100 µg/ml in phosphate buffered saline) that produced threshold responses in control animals. Symptoms of respiratory distress during histamine exposure were assessed on an eight-point scale by observing and timing abdominal panting, cyanosis, spasms and collapse. In each experiment, treatments were allocated to groups of six animals using a randomised block design and symptoms scored "blind" by an independent observer. Hyperreactivity was defined as an increase in mean symptom score of between 2.5 and 3.5 times control, this being significant by Mann and Whitney U-test.

As in previous investigations, no enhancement of histamine-induced responses was observed when NSAID were administered i.p. to healthy guinea-pigs. However, alteration of the experimental conditions allowed the demonstration of pronounced and statistically significant hyperreactivity in three related models. Firstly, histamine-induced responses were increased in a dose-dependent manner, relating to cyclo-oxygenase inhibitor potencies, when NSAID were administered by aerosol to healthy guinea-pigs (Downs et al, 1980). Secondly, hyperreactivity was induced when either indomethacin (0.3-30 mg/kg) or aspirin (10-30 mg/kg) were administered i.p. 60 minutes before histamine aerosol to guinea-pigs suffering from a sub-clinical respiratory adenovirus infection. Enhancement was again dose-dependent, and the potency ratio between indomethacin and aspirin (1:0.1 respectively) favoured cyclo-oxygenase inhibition as the mechanism of action. Thirdly, i.p. NSAID (indomethacin 10 mg/kg; meclofenamic acid 30 mg/kg; aspirin 300 mg/kg) increased histamine-induced responses in guinea-pigs exposed to dilute hydrochloric acid (0.1 M) aerosols for 1s./g. body weight immediately before histamine challenge.

Thus, systemically-administered NSAID increase the reactivity of guinea-pig airways to histamine only if a predisposing local irritation (acid aerosol) or inflammation (adenovirus infection) is present. Since NSAID aerosols produce airway hyperreactivity in healthy animals, local irritation or inflammation probably increases lung vascular permeability so allowing systemically-administerd compounds to penetrate to their site of action, presumably airway smooth muscle.

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DOES TRIMETHOQUINOL INHIBIT THROMBOXANE A_2-INDUCED HUMAN PLATELET ACTIVATION BY BLOCKING A SPECIFIC TRANSDUCTION MECHANISM?

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Platelet activation induced by prostaglandin (PG)G_2, PGH_2, thromboxane (Tx)A_2 and by stable TxA_2-mimetics (e.g. U44069) is a receptor mediated process (MacIntyre, 1981). The mechanisms whereby agonist (TxA_2)-receptor combination are translated into platelet activation are not fully understood but may involve the degradation of phosphatidylinositol (PI) or polyphosphoinositides and consequent increase in $[Ca^{2+}]i$ (Armstrong et al., 1983a).

Several compounds including prostaglandin analogues and the tetrahydroisoquinoline, Trimethoquinol (TMQ) have been reported to selectively inhibit TxA_2-induced human platelet activation (MacIntyre, 1981). The effects of TMQ are stereospecific (R(+)-TMQ > S(-)-TMQ) and are assumed to be due to TxA_2 receptor antagonism (Mayo et al., 1981). However, unlike prostaglandin analogues (e.g. EP 045), R(+)-TMQ does not combine with the TxA_2 receptor on human platelets as assessed by lack of displacement by R(+)-TMQ of the radioligand, [^{3}H]-U44069, from its high affinity binding site (Armstrong et al., 1983b). In the present study we compared the ability of R(+)-TMQ and S(-)-TMQ to block the biochemical (PI degradation and Ca^{2+} flux) and biological (platelet aggregation) sequelae of TxA_2 receptor combination.

All studies were performed using intact human platelets and U44069 as agonist. Platelet aggregation was monitored photometrically and PI turnover, assessed as phosphatidic acid (PA) formation, was monitored using platelets pre-labelled with [^{32}P]-PO_4 (MacIntyre and Pollock, 1983). Platelet $[Ca^{2+}]i$ was measured using the fluorescent calcium indicator dye, Quin 2 (Tsien et al., 1982).

Addition of U44069 to human platelets resulted in platelet aggregation, accumulation of [^{32}P]-PA (3-4 fold above basal at U44069 = 330nM) and elevation of platelet $Ca^{2+}i$ (from a basal value of 62±13nM to 265±29nM at U44069 = 100nM; mean ± S.D. n = 6). R(+)-TMQ (0.3-30μM) produced a concentration dependent inhibition of U44069-induced platelet aggregation, [^{32}P]-PA formation and Ca^{2+} flux, and was at least 10 fold more potent than S(-)-TMQ.

These results indicate that, in common with the prostaglandin analogue EP 045, R(+)-TMQ > S(-)-TMQ inhibit the biochemical and biological events that follow the interaction of TxA_2 with its receptor on human platelets. As R(+)-TMQ does not inhibit the agonist-receptor interaction per se, the observed selective inhibition of TxA_2-induced platelet activation by TMQ may be mediated by blockade of a specific transduction process that links receptor occupancy to cellular response.

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ARACHIDONIC ACID AND HISTAMINE RELEASE FROM RAT MAST CELLS

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There is accumulating evidence that arachidonic acid metabolism may be necessary for the release of mediators from rat mast cells stimulated by a variety of secretagogues such as antigen, antiIgE, dextran, concanavalin A, compound 48/80 or the calcium ionophore A23187 (Goth, 1978; Sullivan and Parker, 1979) and from human basophils stimulated by antigen (Peters et al, 1981). Thus, in rat mast cells arachidonic acid metabolism is markedly enhanced during the release of mediators and since 5,8,11,14 eicosatetraynoic acid (ETYA), but not aspirin or indomethacin inhibits mediator release, it is likely that lipoxygenase, but not cyclooxygenase products of arachidonic acid are involved. Moreover, in basophils, the lipoxygenase product 5-hydroperoxyeicosatetraenoic acid (5-HPETE) enhances histamine release and reverses the inhibition of release caused by hormones and other agonists which act via the adenylcyclase system, whilst anti-inflammatory steroids, which impair the availability of arachidonic acid by interfering with the activity of phospholipase A_2 (Blackwell et al, 1980), have recently been shown to inhibit antigen induced histamine release from human basophils (Schlimer et al, 1981).

In the present experiments the effects of the anti-inflammatory steroid dexamethasone on histamine release from rat mast cells stimulated by dextran have been studied. In control experiments peritoneal cells obtained from Wistar rats (Tuck) by lavage with heparinised (10ug/ml) Tyrode solution and incubated (37°C, 15 min.) with dextran (5mg/ml) and phosphatidylserine (10ug/ml) released approximately 20% of their total histamine content. Pre-incubation of these cells with indomethacin (0.1 - 100uM) or aspirin (0.1 - 100uM) did not modify this release. However, preincubation of the cells with dexamethasone (100uM) produced a time-dependent inhibition of dextran induced histamine release (30 min. = 0%, 60 min. = 50% and 120 min. = 97% inhibition). Preincubation of the cells with the RNA and protein synthesis inhibitor cycloheximide (4 ug/ml) alongside dexamethasone antagonised this inhibitory effect, reducing it to 11% at 60 min. and 64% at 120 min.

In further experiments, in which preincubation of the cells for 90 min. with dexamethasone (100uM) or dexamethasone (100uM) and indomethacin (2.5uM) produced 84% inhibition of histamine release, the addition of 10uM arachidonic acid along with the secretagogue reversed this inhibitory effect to 8.5%.

These results show that dexamethasone inhibits dextran induced histamine release and suggest that this inhibition takes place via induction of protein synthesis: since this inhibition is overcome by arachidonic acid in the presence of indomethacin, these findings support the possibility that lipoxygenase products of arachidonate metabolism are associated with mediator release from rat mast cells.

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ULCEROGENESIS AND PROSTANOID INHIBITION IN THE CAT GASTRIC ANTRUM INDUCED BY PARENTERAL ASPIRIN BUT NOT SALICYLATE.

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Parenteral administration of aspirin during histamine-stimulated gastric acid secretion induces the acute formation of deep penetrating ulcers in the gastric antrum of the cat (Hansen et al. 1980). Ulceration of the antrum by intravenously infused aspirin can occur in the absence of changes in the indices of the gastric barrier seen with topical irritants (Bugat et al., 1976) and may result from the inhibition of gastric mucosal prostaglandin formation. We have now compared the ulcerogenic actions of sodium salicylate and aspirin in cat gastric antrum, following bolus intravenous injection, and have determined their effects on the synthesis of two major cyclo-oxygenase products by antral gastric mucosa.

Histamine dihydrochloride (160 μg $kg^{-1}h^{-1}$) in a dose stimulating near-maximal rates of gastric acid secretion, was infused via a catheter in a foreleg vein for 1 h prior to bolus injection of aspirin or salicylate and throughout the subsequent 4 h in the unanaesthetised cat. The area of lesions in the cat gastric antrum were then assessed (Hansen et al., 1980) and the generation of both 6-oxo-$PGF_{1\alpha}$ and PGE_2 from washed strips (0.3-0.5 g) of antral mucosal tissue following 1 min vortex-incubation in 50 mM Tris buffer (pH 7.4 at 22°C) (Whittle, 1981) was determined by radioimmunoassay (Salmon, 1978). The plasma and mucosal-tissue levels of total salicylate were also determined using HPLC techniques.

Intravenous bolus injection of aspirin (40 mg kg^{-1}; 0.2 mmole kg^{-1}) significantly ($P < 0.05$) increased the area of antral ulceration during the 4 h histamine infusion from 1 ± 0.8 mm^2 (n=4) to 91 ± 35 mm^2 (n=5) whereas sodium salicyalte (35 mg kg^{-1} i.v.; 0.2 mmole kg^{-1}) caused no substantial increase in lesion area (12 ± 6 mm^2, n=5; $P > 0.05$). Sodium salicylate caused no significant inhibition in the ex vivo generation of either 6-oxo-$PGF_{1\alpha}$ or PGE_2, whereas aspirin induced 92 ± 3% and 97 ± 1% inhibition of generation of these prostanoids respectively. There was no significant difference between the total salicylate levels in plasma 4 h following aspirin or sodium salicylate administration (113 ± 27 and 85 ± 14 μg ml^{-1} respectively) or between the levels determined in the antral mucosal tissue (25 ± 6 and 16 ± 2 μg g^{-1}).

These findings confirm the previous observations in the rat that sodium salicylate, unlike aspirin, fails to inhibit gastric mucosal cyclo-oxygenase (Whittle et al., 1980). Furthermore, despite comparable plasma and mucosal tissue levels of total salicylate, intravenous bolus injection of aspirin but not sodium salicylate induces substantial ulcer formation in the cat gastric antrum during histamine-induced acid secretion. These observations support the premise that cyclo-oxygenase inhibition is an important mechanism underlying gastric damage induced by aspirin when administered parenterally.

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$PGF_{2\alpha}$ IS NOT A TXA_2 RECEPTOR ANTAGONIST ON HUMAN PLATELETS

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It has recently been suggested that $PGF_{2\alpha}$ is a platelet TXA_2 receptor antagonist (Hung et al, 1982; 1983). However the inability of $PGF_{2\alpha}$ (>28 μM) to displace the binding of a tritiated TXA_2 mimetic to washed human platelets suggested that an alternative mode of action for $PGF_{2\alpha}$ must be sought (Armstrong et al, 1983).

We have found that $PGF_{2\alpha}$ (8 μM) inhibits aggregation of human platelets induced by the TXA_2 mimetic 11,9-epoxymethano PGH_2 (100%), platelet activating factor (PAF) (72.2%) and thrombin (65.5%), but not adenosine diphosphate (ADP) (n = 4 donors). Unlike the specific TXA_2 receptor antagonist EP 045 (Jones et al, 1983), $PGF_{2\alpha}$ inhibits primary aggregation induced by PAF or thrombin in both platelets desensitised to 11,9-epoxymethano PGH_2 and in platelets from aspirin-treated donors. Furthermore, $PGF_{2\alpha}$ inhibition of thrombin is still evident in platelets desensitised to PAF.

$PGF_{2\alpha}$ (20 μM) can raise platelet cGMP levels 1.88 ± 0.13 fold (n = 16, 4 donors). 8-Bromo cGMP can mimic the effects of increased platelet cGMP levels (Pareti et al, 1978) and inhibits primary aggregation to 11,9-epoxymethano PGH_2, thrombin and PAF, but unlike $PGF_{2\alpha}$ inhibits ADP to a similar extent. In addition, the guanylate cyclase inhibitor N-methyl hydroxylamine (Deguchi et al, 1978) did not inhibit the effect of $PGF_{2\alpha}$ at 0.04 mM and at 0.4 mM itself inhibited aggregation and acted additively with $PGF_{2\alpha}$. The increase in cGMP seen with $PGF_{2\alpha}$ is unlikely to account for its inhibitory actions.

$PGF_{2\alpha}$ (20 μM) also increases basal cAMP levels 1.74 ± 0.07 fold (n = 16, 4 donors) and this increase is inhibited to a greater extent by ADP (31% at 5×10^{-7} M) than by thrombin (15% at 0.5 u/ml), PAF (11.3% at 75 nM) or 11,9-epoxymethano PGH_2 (5.9% at 1.5 μM). This is consistent with ADP directly inhibiting the enzyme adenyl cyclase (Mellwig & Jakobs, 1980). The ability of aggregating agents to inhibit the increase in cAMP induced by $PGF_{2\alpha}$ correlates inversely with their sensitivity to inhibition by $PGF_{2\alpha}$. The suggestion that the small increase in cAMP is sufficient to account for the effect of $PGF_{2\alpha}$ is supported by the ability of SQ 22536 (100 μM) (Harris et al, 1979) to inhibit the action of $PGF_{2\alpha}$. Also the concentration of PGE_1 required to match the inhibition of PAF by $PGF_{2\alpha}$ is 2 nM; - at 5 nM PGE_1 increases basal cAMP levels 1.36 ± 0.06 fold (n = 16, 4 donors) and has a minimal inhibitory effect on ADP. It is of interest that in some donors a slight potentiation of the ADP wave was seen with $PGF_{2\alpha}$, similar to that reported for PGE_2 (Shio et al, 1972).

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SULPHASALAZINE AND THE INHIBITION OF THROMBOXANE SYNTHESIS IN HUMAN COLONIC MUCOSA

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The mode of action of sulphazalazine (SASP), used in the treatment of ulcerative colitis is unknown. It is unlikely to be due to overall inhibition of the increased colonic synthesis of prostanoids seen in this disease since more potent cyclo-oxygenase inhibitors have failed to be of therapeutic benefit (Rampton and Sladen, 1981). We have previously characterised the main cyclo-oxygenase and lipoxygenase products of [^{14}C]-arachidonic acid (AA) metabolism formed by homogenates of human colonic mucosa (Hawkey, et al., 1982) and now report on the effects of SASP and its cleavage products 5-amino salicylic acid (5-ASA) and sulphapyridine (SP) on their synthesis.

Normal colonic mucosa obtained from specimens resected for carcinoma was homogenised in Tris buffer (50 mM; pH 7.4) to give a 50 mg ml^{-1} w/v suspension. Aliquots (2 ml) were preincubated (20 min, 0^{o}C) with the compounds (at 5 x 10^{-5}M and 10^{-3}M) prior to incubation (30 mins, 37^{o}C) with [^{14}C]-AA (840 ng, 57.6 µCi mmol^{-1}). After addition of ethanol (1.5 ml at 0^{o}C) and acidification to pH 3.5 with formic acid, unmetabolized [^{14}C]-AA and its products were extracted into chloroform and separated by two T.L.C. systems which allowed resolution of both cyclo-oxygenase and lipoxygenase products (Hawkey, et al., 1982).

The major radioactive bands separated by T.L.C. were characterised as PGE_2, TXB_2, $PGF_{2\alpha}$ and 11-, 12-, 15-HETE (which run together) by co-chromatography with authentic standards and previously identified ^{14}C-AA metabolites of rabbit polymorphonuclear leukocytes and human platelets. The 11-,12-, 15-HETE band was further characterised using HPLC and contained predominantly 12- and 15-HETE (Hawkey, et al., 1982).

5-ASA (10^{-3}M) inhibited the formation of PGE_2 (50 ± 7% inhibition, n=9, P < 0.001) but was without significant effect on the other metabolites of [^{14}C]-AA. SASP inhibited TXB_2 formation at both concentrations used (by 64 ± 18% at 10^{-3}M, n=9, P < 0.01; and by 47 ± 11% at 5 x 10^{-5}M, n=7, P < 0.01). Although SASP (10^{-3}M) had no significant effect on PGE_2 formation, and caused only a small inhibition of the minor product 6-oxo-$PGF_{1\alpha}$ (23 ± 6% inhibition; n=9; P < 0.01), there was a significant enhancement of $PGF_{2\alpha}$ formation (85 ± 24% increase; n=9; P < 0.01). The formation of TXB_2 was also significantly inhibited by 10^{-3}M SP (42 ± 11% inhibition; n=9; P < 0.01) while there was an enhanced formation of PGE_2 (72 ± 17% increase; n=9; P < 0.01).

Formation of the major lipoxygenase products separated, which co-chromatographed with 11-, 12-, 15-HETE was inhibited by SASP (39 ± 12% inhibition; n=7; P < 0.05) at 10^{-3}M, but was unaffected by either 5-ASA or SP.

In this study, both SASP and SP inhibited TXB_2 formation while increasing the synthesis of either $PGF_{2\alpha}$ or PGE_2 respectively. SASP at a high concentration also inhibited the formation of 11-, 12-, 15-HETE. Thus, although 5-ASA has been identified as the major therapeutic moeity of SASP (Azad Kahn, et al., 1977), our present observations emphasise that both unmetabolised SASP and SP may also contribute to the actions of SASP in ulcerative colitis but may act by different mechanisms.

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DOES IMMUNOLOGICAL SENSITIZATION AFFECT THE ACTIVITY OF GUINEA-PIG LUNG ADENYLATE CYCLASE?

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Extrinsic or allergic bronchial asthma is caused by an immediate-type hypersensitivity reaction to allergens, and is mediated primarily by IgE antibodies (Ishizaka, 1976). Recently, Andersson (1980) demonstrated that suitably sensitized guinea-pigs are able to produce IgG and IgE antibodies. The purpose of this study was to examine whether immunological sensitization affected the activation of lung adenylate cyclase.

Guinea-pigs were sensitized to evoke (I) IgG antibodies only, or (II) IgG and IgE antibodies. Control animals were sham injected with adjuvant. After an appropriate sensitization period qualitative assessment of sensitization was examined in vitro on the isolated guinea pig ileum, and serum was analysed for IgG and IgE antibodies by passive cutaneous anaphylaxis (PCA) according to the method of Watanabe and Ovary (1977). For the adenylate cyclase activity measurements,the lungs were perfused with ice-cold saline (0.9%), homogenised and a 1600g (x10min) fraction prepared. Adenylate cyclase activity was determined according to a modified method of Albano et al (1973).

Experiments on the isolated guinea-pig ileum confirmed that ovalbumin injected animals were sensitized. Subsequently PCA identified the presence of IgG antibodies (I) and IgG and IgE antibodies (II), in the sera of the sensitized guinea-pigs. The sera from control animals failed to demonstrate the presence of either antibody. Basal adenylate cyclase activity of control and IgG sensitized lungs showed no difference (Table 1). However a highly significant difference was observed in the basal adenylate cyclase activity of lung homogenate membranes prepared from IgG and IgE sensitized animals. Incubation with 5mM sodium fluoride stimulated the enzyme in all groups, and the accumulation of cAMP was increased approximately two-fold.

Table 1. Adenylate cyclase activity of guinea-pig lung 1600g membranes

	Control	Sensitization I	Sensitization II
	p moles cAMP formed/min/mg protein		
Basal	239 ± 34 (4)	220 ± 35 (3)	496 ± 23 (3)*
5mM Naf	401 ± 57 (4)	397 ± 67 (3)	1097 ± 136 (3)

Values represent mean ± s.e. of triplicate measurements in (N) experiments
Student's t-test *p < 0.01.

The results suggest that the sensitization procedure resulting in the formation of IgG and IgE antibodies, appears to affect the adenylate cyclase complex in such a manner as to markedly increase the basal activation of the enzyme. We are at present engaged in elucidating the precise molecular component of the cyclase which has been affected by the sensitization process.

Alison Gadd is a NAPP Laboratories Ltd. (Cambridge Science Park) Research Student.

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THE EFFECT OF HISTAMINE H_1- AND H_2-RECEPTOR ANTAGONISTS ON HISTAMINE-STIMULATED cAMP ACCUMULATION IN CHICK CEREBRAL CORTEX SLICES

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Histamine H_1- and H_2-receptors mediate histamine-stimulated cAMP accumulation in guinea-pig brain slices (Palacios et al., 1978). Previous studies have suggested that histamine-stimulated cAMP accumulation in chick cerebral hemisphere slices is mediated only by H_2-receptors (Nahorski et al., 1977). The aim of the present study is to characterise more fully the nature of receptors mediating the stimulation in chick brain, using a range of histamine receptor agonists and antagonists.

The method of Nahorski et al. (1977) was used. Slices (0.25 x 0.25 mm) were pre-incubated for 1 hour at 37 °C in Krebs bicarbonate buffer containing 10 mM glucose gassed to pH 7.4 with O_2:CO_2 (95:5). Slices (0.16 - 0.22 mg protein) were tranferred to fresh buffer and incubated for 10 min with agonists and antagonists as appropriate. Incubations were terminated by boiling and cAMP in the supernatant assayed as described by Brown et al. (1971). Dose response curves were fitted to the logistic equation using an iterative curve-fitting procedure (ALLFIT) developed by De Lean et al. (1978).

Histamine elicited a large increase in cAMP from basal levels of 63 ± 11 to a maximal stimulation by 100 μM HA of 566 ± 66 pmol/mg protein, EC_{50} 3.0 ± 0.5 μM. Dose ratios derived from the dextral shift of histamine dose-response curves were used to construct Schild plots for cimetidine (pA_2 7.70 ± 0.23, corresponding to a K_B value of 0.02 μM) and metiamide (pA_2 6.91 ± 0.25, K_B 0.12 μM). Plot slopes did not differ significantly from unity.

Inhibition by a range of concentrations of several antagonists against a fixed concentration of histamine (100 μM) was also investigated. SK&F 93479 (atrial K_B value 0.017 μM; Blakemore et al., 1981) and mepyramine exhibited apparent affinities (K_i values 0.014 ± 0.004 and 6.4 ± 1.8 μM respectively) in agreement with their H_2-receptor K_B values. However metiamide (K_i 0.17 ± 0.07 μM) and particularly cimetidine (K_i 0.016 ± 0.004 μM) were again more potent in inhibiting cAMP accumulation than would be expected from their affinities at the H_2-receptor (atrial H_2-receptor K_B values 0.92 and 0.79 μM respectively).

The specific H_2-receptor agonists impromidine and dimaprit elicited no agonist activity. However, impromidine (but not dimaprit) was a potent antagonist (K_i 0.05 ± 0.001 μM). The anomalously high affinity of the H_2-receptor antagonists cimetidine, and to a lesser extent, metiamide in this system require an explanation: studies utilising a wider range of histamine agonists and antagonists are in progress.

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THE PROBLEM OF INSURMOUNTABLE ANTAGONISM IN 5-HYDROXYTRYPTAMINE RECEPTOR CLASSIFICATION

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The operational classification of 5-hydroxytryptamine (5-HT) receptors has been complicated by the discovery that many specific antagonists do not show clearly-defined competitive pharmacodynamics on all tissues. For example, in canine coronary artery segments methysergide behaved as a competitive antagonist (Brazenor & Angus, 1981) whereas trazodone was an insurmountable antagonist of 5-HT responses. Lysergic acid diethylamide (LSD) also generally behaves as an insurmountable antagonist of 5-HT responses in isolated but intact tissues (Müller-Schweinitzer & Weidmann, 1978) thus raising the possibility that 5-HT and LSD are not acting at the same site. Nevertheless, LSD has been used extensively in radioligand binding studies (Peroutka & Snyder, 1979) to identify and classify 5-HT receptors. As no rigorous functional characterization of LSD as a 5-HT antagonist appears to have been published, we have compared its activity with trazodone and methysergide on rabbit aortic segments *in vitro*. This tissue was chosen for the analytical convenience that low concentrations of 5-HT (ED_{50} = 6.99 ± 0.05 M, mean ± S.E.) produced sustained, relatively fade-free, contractions.

Changes in isometric force produced by 5-HT were measured in preparations pretreated with the selective irreversible α-adrenoceptor antagonist benextramine (3×10^{-6} M for 30 min). "Treated" preparations were equilibrated with a single concentration of antagonist drug for 60 min before obtaining a cumulative concentration-response curve to 5-HT. Logistic functions were fitted to each set of concentration-response data and an iterative least squares procedure was used to fit the model of simple competition and thus derive estimates of pK_B and the parameter n (equivalent to the slope in a Schild plot).

Methysergide (10^{-8} - 10^{-6} M) showed competitive antagonism to 5-HT (pK_B = 8.25 ± 0.05, n = 1.00 ± 0.03), consistent with reported results (Apperley *et al*, 1976). Competitive 5-HT antagonism was also seen with trazodone (pK_B = 7.22 ± 0.02, n = 0.99 ± 0.06). Although 5-HT antagonism has been reported for this compound (Brazenor and Angus, 1982) a quantitative analysis of its functional interactions with the 5-HT receptor has not been published before. Concomitant equilibration of tissues with methysergide (1.5×10^{-7} M) and trazodone (5×10^{-7} M) displaced the 5-HT concentration-response curves, consistent with the relationship $CR_{1,2} = CR_1 + CR_2 - 1$ predicted for two antagonists competing for the same receptor, where $CR_{1,2}$ is the displacement produced by the antagonists concomitantly and CR_1 and CR_2 are the displacements produced by each antagonist alone (Paton and Rang, 1965). Apparently methysergide and trazodone compete with each other and with 5-HT for a common site.

LSD (3×10^{-10} - 3×10^{-8} M) produced a progressive decrease in the maxima of 5-HT concentration-response curves characteristic of insurmountable antagonism. LSD alone also showed agonist activity, 14% of the 5-HT maximum. Concomitant equilibration of LSD (3×10^{-9} M) and either methysergide (10^{-8} M) or trazodone (10^{-5} M) not only inhibited the agonist activity of LSD but also prevented the attenuating effects of LSD on the 5-HT curves. Therefore both the agonist and antagonist effects of LSD appear to be the result of an affinity for the same receptor site with which methysergide, trazodone and 5-HT interact.

The mechanism of the insurmountable, syntopic, antagonism produced by LSD remains to be elucidated.

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EFFECTS OF KETANSERIN AND METHYSERGIDE ON THE CARDIOVASCULAR SYSTEM OF THE CAT

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Ketanserin is a 5-HT_2 antagonist with affinity for α_1 adrenoceptors (Leysen et al, 1981). It lowers blood pressure in both normotensive and spontaneously hypertensive rats (Van Nueten et al, 1981) and in hypertensive man (De Cree et al, 1981). The mechanism by which ketanserin produces its hypotensive action has been attributed to blockade of either 5-HT_2 receptors (Van Nueten et al, 1981) or α_1 adrenoceptors (Fozard, 1982). The following experiments were carried out to investigate further the mechanisms by which ketanserin causes its hypotensive action by comparing it with methysergide which has also been reported to lower blood pressure (Antanaccio & Taylor, 1977) and to bind to 5-HT_2 receptors (Leysen et al, 1981).

Cats were anaesthetised with α-chloralose (70 mg kg^{-1} i.v.) and pentobarbitone sodium (12 mg). Simultaneous recordings were made of brachial arterial pressure (BP), heart rate (HR), femoral arterial flow (from which conductance (FAC) is derived) and preganglionic sympathetic nerve activity (PSNA) as previously described (Ramage, 1982). In all experiments an initial infusion of vehicle (0.04M lactic acid) was given for 20 min before administration of test solutions.

Table 1 Effects of 30 min infusions of test solutions

TEST SOLUTIONS	ΔBP mm Hg	ΔHR Beats min^{-1}	ΔFAC ($\times 10^{-3}$) ml mm Hg^{-1} min^{-1}	ΔPSNA %
Vehicle (1.5 ml)	-2±1	-1±2	+2±3	0±9
Ketanserin (0.25 mg kg^{-1})	-13±2***	-8±4	+42±7**	-39±8*
Ketanserin (0.5 mg kg^{-1})	-20±5**	-17±4**	+45±9**	-58±3***
Methysergide (1.0 mg kg^{-1})	-27±11*	-17±6*	-17±8	+8±23

*p < 0.05 **p < 0.01 ***p < 0.001 results as mean (n=5) ± s.e.

A 30 min infusion of ketanserin (higher dose) or methysergide caused a significant (p < 0.05) fall in BP and HR compared with the vehicle. The lower dose of ketanserin caused a significant fall only in BP over this period. Methysergide differed from ketanserin in that it failed to cause a significant rise in FAC and a decrease in PSNA (Table 1). Further, methysergide caused over the first 10 min a significant decrease in FAC and an increase in PSNA.

These results show that there are differences in the mode of hypotensive action between ketanserin and methysergide. The differences may be explained by the partial agonist action of methysergide on 5-HT receptors (Apperley et al, 1980) or the ability of ketanserin to block α_1 adrenoceptors. Evidence to support the latter mechanism comes from the observation that prazosin in the above experimental system causes a fall in BP along with a reduction in PSNA, HR and increase in FAC (Ramage, 1982).

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STIMULATION BY PROPRANOLOL OF (^{86}Rb) FLUXES IN RAT THYMOCYTES

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β-Adrenoceptor antagonists may interfere with potassium homeostasis. Rosa et al (1980) demonstrated that propranolol affected the ability of human subjects to handle an acute KCl load. The Medical Research Council Working Party on Mild to Moderate Hypertension (1981) have reported that serum potassium increased significantly in patients on propranolol treatment. Rat thymocytes are a useful model for investigation of cation composition and transport rates (Jones et al 1981). We previously reported that propranolol increased ^{86}Rb (used as a analogue of K) influx in rat thymocytes (Murphy & Ryan, 1982). We have now extended these investigations.

Thymus glands were removed from Male Wistar rats (100-150 g) and thymocytes were isolated at 4°C by mincing, aspiration and separation through gauze. For influx experiments, cells (1 X 10^8/ml) were incubated at 37°C in medium 199 containing 20% foetal calf serum. ^{86}Rb was added at time zero and triplicate samples (200 µl) were removed at time intervals up to 90 min. For efflux experiments, cells (1 X 10^8/ml) were incubated in medium containing ^{86}Rb at 37°C for three hours. Cells were then washed twice by centrifugation (300 g). Cells were resuspended in radioactive-free medium at 37°C and triplicate samples (200 µl) were removed at time intervals up to 90 min. After sampling, cells were rapidly separated from medium by centrifugation through di-n-butylphthalate. Flux rates were calculated by the procedure of Segel & Lichtman (1976) using our own estimates of cell potassium and water.

Total control influx rate was 7.79 ± 0.36 f.mol $cell^{-1}$ h^{-1}. Ouabain (1 mM) reduced this rate to 2.41 ± 0.06 f.mol $cell^{-1}$ h^{-1}. (±)-Propranolol (50 µM) significantly increased the total influx rate ($p<0.001$). The stimulation of ^{86}Rb influx by (±)-propranolol was also demonstrated in the presence of ouabain (500 µM). Nadolol (50 µM), a β-adrenoceptor antagonist with no reported membrane-stabilizing activity, did not increase ^{86}Rb influx rates. (+)-Propranolol, an isomer which is inactive at β-adrenoceptors, significantly increased the influx rate ($p<0.001$). (±)-Propranolol (50 µM) did not stimulate influx rate in experiments carried out in calcium-free medium. Control efflux rates were 8.04 ± 0.48 f.mol $cell^{-1}$ h^{-1}. (±)-Propranolol (50 µM) significantly increased the efflux rate ($p<0.001$).

In conclusion, the effect of propranolol on ^{86}Rb fluxes can be demonstrated in the presence of ouabain and is not directly related to β-adrenoceptor antagonism but rather to a membrane action which is calcium dependent.

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VERAPAMIL AND BEPRIDIL ON POTASSIUM-EVOKED CONTRACTURES OF GUINEA-PIG ILEUM

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Depolarising solutions of potassium when applied to guinea-pig ileum, result in a biphasic mechanical response. Initially the tissue undergoes a rapid, transient spike contraction (phasic component) which is maintained, often at a lower level (Weiss et al, 1961). Both components of the response are dependent on extracellular calcium ions. However, it has been suggested that each represents a distinct potential-operated calcium channel. (Hurwitz et al, 1982). In the present study, the effects of two calcium antagonists, verapamil and bepridil, were studied on these two components of the response.

Segments of whole guinea-pig ileum were bathed in Kreb's solution in an isolated organ bath at 37°C, gassed with 95%O_2, 5% CO_2. Responses were evoked by the addition of KCl solution, sufficient to give a bath concentration of 40mM, and recorded isotonically. After reproducable control responses were obtained, verapamil (10^{-8} - 10^{-6}M) or bepridil (10^{-7} - 10^{-5}M) were added to the bathing medium and the tissues allowed to equilibrate for 15-20 mins. The tissues were then again subjected to K^+ depolarisation.

Each calcium antagonist reduced the magnitude of both phasic and tonic components of the response (fig. 1). Bepridil was unselective and inhibited both parts of the response equally ($P>0.05$, Mann-Whitney U Test). Verapamil, however, inhibited the tonic component to a significantly ($P<0.05$) greater extent than the phasic component (fig. 1). There was more than a six-fold difference in inhibition between the phasic and tonic components at 10^{-7}M verapamil. These experiments indicate a difference in action between verapamil and bepridil and underline the need for care when using calcium antagonists as investigative tools to study calcium activation mechanisms in smooth muscle.

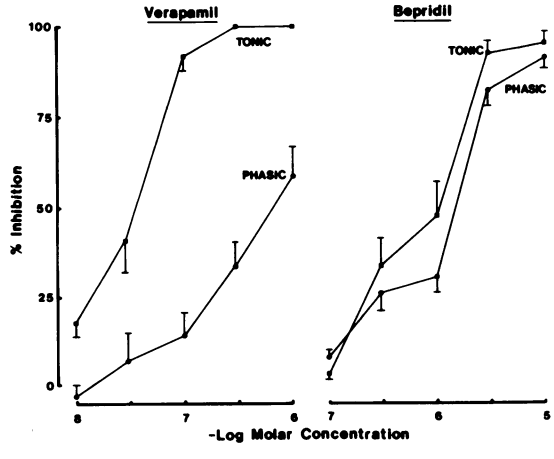

Fig. 1

Inhibition of K^+ evoked contractures of guinea-pig ileum by verapamil (10^{-8}- 10^{-6}M) and bepridil (10^{-7}- 10^{-5}M). Each point represents the mean value from 8 preparations ± SEM

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INHIBITION OF CALCIUM-DEPENDENT RESPONSES BY BW755C

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Leukotrienes and hydroxy acids are derived from arachidonic acid by the action of 5' lipoxygenase. The activity of this enzyme is not readily detected in normal tissues but addition of the divalent cation ionophore A23187 has proved a potent stimulus to the formation of 5' lipoxygenase products in a number of systems. The effects of A23187 are considerably reduced in the absence of extra-cellular calcium, suggesting that 5' lipoxygenase is calcium-dependent. The phenyl-pyrazoline BW755C is a potent inhibitor of lipoxygenase (Higgs et al., 1979) and, in common with known calcium channel blockers such as the dihydropyridines, BW755C has a dihydro-aromatic structure. We have now investigated the effects of BW755C on calcium-dependent responses using preparations of isolated smooth muscle.

Contractions of the rat isolated stomach strip caused by 5-hydroxytryptamine (5-HT) are dependent upon extra-cellular calcium whereas responses to acetyl choline (ACh) are not (Weinstock and Weiss, 1979). In this study, strips of smooth muscle from the rat stomach fundus or guinea pig trachea were superfused at 5 ml min^{-1} with Krebs or Tyrode solution. In some experiments tissues were superfused with calcium-poor medium from which calcium chloride had been omitted. Tissue contractions following bolus injections (0.05-0.2 ml) of 5-HT, ACh, histamine, prostaglandin (PG)E_2 or calcium chloride were detected by auxotonic transducers (Paton, 1957). Dose-response curves were repeated in the presence and absence of different concentrations of indomethacin, verapamil or BW755C.

Rat stomach strips contracted in a dose-dependent fashion to 5-HT (1-500 ng) and PGE_2 (1-500 ng). Verapamil (2×10^{-7} to 10^{-5}M) or BW755C (4×10^{-6} to 4×10^{-4}M) inhibited 5-HT contractions but did not reduce PGE_2 responses; verapamil was approximately 100 times more potent than BW755C in this property. The effects of BW755C but not verapamil were rapidly reversible. In calcium-poor medium rat stomach strips contracted to ACh (10-5000 ng) and calcium chloride (0.5-500 µmoles) but higher doses of 5-HT (20-1000 µg) were required to induce contractions. BW755C caused a concentration-related, non-parallel shift to the right of the dose response curve to calcium chloride with a depression of the maximum response (IC_{50} = 3.4×10^{-5}M). BW755C selectively inhibited calcium chloride-induced contractions, was less effective against responses to ACh (IC_{50} = 2.6×10^{-4}M) and did not reduce the residual calcium-independent contractions caused by 5-HT.

Indomethacin augments histamine-induced contractions of the guinea-pig trachea and this enhancement is reversed by BW755C (Adcock and Garland, 1980). In the present study, indomethacin (2.8×10^{-6}M) increased maximal contractions of the trachea to histamine by approximately 50%. Verapamil (4.4×10^{-7}M) or BW755C (8.7×10^{-5}M) did not affect histamine responses in the absence of indomethacin but reversed the augmentation of these responses in the presence of indomethacin.

These results indicate that BW755C is a weak inhibitor of responses of the rat stomach strip which are dependent on extra-cellular calcium. The effects of BW755C on calcium may be related to inhibition of lipoxygenase by this compound and it is possible, therefore, that a lipoxygenase product acts as a calcium ionophore. The interaction between indomethacin and calcium-dependent responses is being further investigated.

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CARDIOVASCULAR PROFILE OF FELODIPINE, A NEW Ca-ANTAGONIST, IN CONSCIOUS RENAL HYPERTENSIVE RABBITS: A COMPARISON WITH HYDRALAZINE

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The possible application of Ca-antagonists in anti-hypertensive therapy is based upon the decrease in total peripheral resistance induced by these agents (Vanhoutte, 1982). However, little information is available on the relative effects of these drugs on different vascular beds. We used the radioactive microsphere technique to study the systemic and regional haemodynamic effects of felodipine, 10 minutes after the i.v. administration of the drug in conscious rabbits with bilateral cellophane perinephritis hypertension (Bolt & Saxena, 1983). To facilitate the interpretation of the results, a comparison was made with hydralazine.

Felodipine (10, 30 and 100 nmol/kg, n=8) caused a dose-dependent decrease in mean blood pressure (MBP) (-5±2%, -21±4%, -40±3%) and total peripheral resistance (TPR) (-8±12%, -34±7%, -57±3%), which was accompanied by a reflex increase in heart rate (HR) (+21±3%, +31±4%, +57±3%) and cardiac output (CO) (+13±10%, +28±13%, +43±7%). After the i.v. administration of hydralazine (1.5, 5 and 15 µmol/kg, n=10) the changes in HR (+16±4%, +28±5%, +29±6%), CO (+39±8%, +33±13%, +31±10%) and TPR (-23±4%, -27±6%, -37±5%) preceded the hypotensive effect of the drug; MBP decreased only after the 2nd and 3rd dose of hydralazine by -9±3% and -21±4% respectively. Figure 1 shows that after felodipine a rather uniform increase in regional vascular conductance was measured, the changes being most pronounced in the heart, brain, muscles and intestines. With hydralazine large increases in vascular conductance were observed in the heart, brain and kidneys, while a remarkable reduction was measured in the skin, stomach and small intestine, probably due to α-receptor stimulation, as a result of the reflex increase in circulating catecholamines.

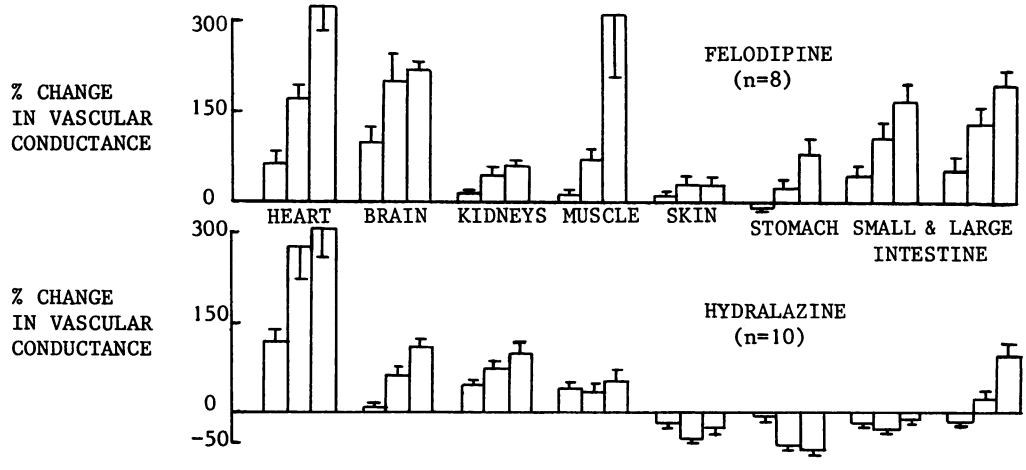

Figure 1 Percentage change in regional vascular conductance (ml.min^{-1}/mmHg) after felodipine (10, 30 and 100 nmol/kg) and hydralazine (1.5, 5 and 15 µmol/kg).

In conclusion, felodipine is an effective vasodilator. When compared with hydralazine, the reflex mediated changes in systemic and regional haemodynamic variables, opposing the hypotensive effect of the drug, were less after felodipine.

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THE EFFECTS OF CALCIUM ANTAGONISTS ON THE NEUROMUSCULAR BLOCKING ACTIONS OF VECURONIUM BROMIDE IN ANAESTHETIZED CATS

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Because the major mechanism of action of calcium antagonists involves inhibition of depolarisation-induced influx of calcium, it is generally expected that these drugs would exert little effect on excitation-contraction coupling in skeletal muscle. However, it has been recently shown that the calcium antagonist, verapamil, both inhibits neuromuscular transmission in anaesthetised dogs (Lawson et al. 1983) and potentiates the effects of neuromuscular blocking agents on isolated rat diaphragm (Bikhazi et al. 1982).

We have therefore studied in chloralose-anaesthetised cats, the effects of the calcium antagonists, nifedipine (0.05 mg/kg), verapamil (0.5 and 1.0 mg/kg) and bepridil (2 and 5 mg/kg) on sciatic nerve induced twitches of the tibialis anterior muscle (0.2 ms duration, 0.1 Hz, twice max. voltage) and on the neuromuscular blocking effects produced by cumulative administration (at 1 h intervals) of the non-depolarising agent, vecuronium bromide (Marshall, I.G. et al. 1980). The doses of the calcium antagonists chosen produced similar, dose-dependent but transient (<15 min) decreases in blood pressure of between 17 and 66 mmHg and (in the case of bepridil and verapamil) more sustained (30-40 min) decreases in heart rate of between 12 - 30 beats/min. In contrast none of the calcium antagonists significantly affected twitches of the tibialis muscle although small increases were occasionally seen immediately after injection of nifedipine, verapamil or bepridil. However, all three calcium antagonists significantly potentiated the neuromuscular blocking effects of vecuronium (assessed as computerized regression line EC_{50} values; Table 1.). Recovery time(i.e.25-75%) from the vecuronium-induced block was not modified by the calcium antagonists.

Table 1

			Neuromuscular block EC_{50} (μg/kg)		
Drug	Dose(mg/kg)	n	Pre-drug	5' post-drug	75' post-drug
Controls	-	9	33±5	32±5	35±4
Verapamil	0.5	4	51±4	40±7 *	52±5
Nifedipine	0.05	3	36±3	28±2 *	33±1
Bepridil	5.0	4	47±6	27±2 *	35±6

* $p < 0.05$ Significantly different from pre-drug.

In conclusion, in intravenous doses which cause calcium antagonist effects on vascular and cardiac muscle, nifedipine, verapamil and bepridil did not affect indirectly-elicited twitches of a peripheral voluntary muscle. However, their ability to potentiate the effects of a non-depolarising neuromuscular blocker may be relevant to anaesthetic practice.

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EFFECTS OF THREE Ca^{2+}-ENTRY BLOCKERS ON CONTRACTION, NORADRENALINE RELEASE AND α-ADRENOCEPTOR BINDING IN THE FEMALE RABBIT URETHRA

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Organic "Ca^{2+}-entry blockers", such as nifedipine, verapamil and diltiazem, are powerful relaxants of vascular smooth muscle (Fleckenstein, 1977). However, depolarization-induced transmitter release in vertebrate neurons appears to be relatively resistant to these drugs (Hagiwara & Byerly, 1981). In order to further elucidate the actions of Ca^{2+}-entry blockers on smooth muscle and nerve tissue, the effects of nifedipine, verapamil and diltiazem on noradrenaline (NA)—induced contraction and electrically evoked ^{3}H-NA release were investigated in the female rabbit urethra. Possible influence of the Ca^{2+}-entry blockers on urethral α-adrenoceptors was also examined using radioligand binding technique.

Urethral ring preparations (≈ 2 mm wide) were suspended in Krebs solution (1.5 mM Ca^{2+}), containing 10 μM cocaine and 0.3 μM propranolol, and contractions elicited by 1 μM NA (≈ EC_{50}) were measured "isometrically". Adrenergic transmitter release was studied on preparations preincubated with ^{3}H-NA. The tissues were stimulated with alternating square pulses (0.8 ms), delivered at a frequency of 10 Hz for periods of 1 min. Binding was measured on a crude membrane preparation of the bladder base and urethra using ^{3}H-dihydro-α-ergocryptine (^{3}H-DHE) as a marker for α-adrenoceptors.

The Ca^{2+}-entry blockers inhibited the contractile response to NA in the following order of potency: nifedipine (EC_{50} = 20 nM) > diltiazem (EC_{50} = 0.4 μM) ≈ verapamil (EC_{50} = 0.5 μM). The maximum inhibitions produced by nifedipine (55 ± 5, n = 7) and diltiazem (60 ± 3, n = 6) were significantly smaller ($p < 0.001$) than that produced by verapamil (96 ± 3, n = 9). The electrically evoked fractional efflux of ^{3}H, on the other hand, was unaffected by nifedipine in the concentration range 3 nM to 3 μM, whereas it was significantly decreased by 1 and 10 μM diltiazem (19 and 24 %) and increased by 0.1 and 1 μM verapamil (8 and 29 %). Only verapamil inhibited specific ^{3}H-DHE binding (K_i = 0.1 μM), and the inhibition appeared to be of the competitive type. Since the effects of verapamil on NA-induced contraction and adrenergic transmitter release occurred in roughly the same concentration interval as that inhibiting ^{3}H-DHE binding, it is suggested that blockade of pre- and post-junctional α-adrenoceptors contributed to the verapamil-induced inhibition of contraction and enhancement of transmitter release. The ability of verapamil to interact with urethral α-adrenoceptors may explain why this drug, in contrast to nifedipine and diltiazem, managed to abolish the contractile response to NA.

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α -ADRENOCEPTORS IN THE IN SITU BLOOD PERFUSED SUPERIOR MESENTERIC ARTERIAL BED OF THE RAT

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α-adrenoceptors mediating contraction of vascular smooth muscle have been shown to include the α_2 as well as the α_1-subtype (McGrath, 1982). The relative importance of these different sub-types of receptor in different vascular beds, however, has not been extensively studied. Using an *in vitro* preparation (which does not contain the smaller resistance vessels) it has recently been shown that the mesenteric arterial bed of the rat does not appear to possess any postjunctional vasoconstrictor α_2-adrenoceptors (Fiotakis & Pipili, 1983). It is, therefore, of interest to establish the presence or absence of such receptors in the intact mesenteric arterial bed of the rat *in vivo*.

Under pentobarbitone anaesthesia (60 mg kg^{-1}) male Wistar rats (250-350g) were prepared for the *in situ* blood perfusion of the superior mesenteric arterial bed essentially as described by Jackson & Campbell (1980) except that a trapped air system, to reduce pressure fluctuations, and a heat exchanger, to reheat blood to body temperature, were included in the extracorporeal circuit and the sympathetic nerves were left intact.

Intra-arterial injections of noradrenaline (50-5000 ng) into the extracorporal circuit at constant flow (2 ml min^{-1}) produced dose related increases in perfusion pressure with a maximal pressor of response of 189 ± 10 mm Hg (n=10). Phentolamine, 100 μg kg^{-1} i.v., produced a parallel shift to the right of the noradrenaline log dose-response curve whereas propranolol (0.5 mg kg^{-1} i.v.) potentiated the response to higher doses of noradrenaline indicating that this pressor response is mediated via α-adrenoceptors. Prazosin, a selective α_1 antagonist (10 μg kg^{-1} i.v.) also produced a parallel shift of the curve to the right which was further shifted by increasing the dose of prazosin to 50 μg kg^{-1}. Intra-arterial xylazine (a relatively selective α_2 agonist), at doses up to 2000 times greater than that of noradrenaline required to increase perfusion pressure by 50 mm Hg, produced only small, inconsistent pressor responses of less than 10 mm Hg. However, these doses consistently produced systemic hypotension and bradycardia. Also, separate experiments in pithed rats showed xylazine to be approximately 750 times less potent than noradrenaline as a pressor agent. Clonidine, a less selective α_2 agonist, produced similar effects with maximal pressor responses of approximately 10 mm Hg being obtained with 50 μg. Repetition of the high doses of xylazine and clonidine elicited either a much smaller or no response and the responses to noradrenaline after high doses of xylazine and clonidine were markedly attenuated.

It is concluded that the superior mesenteric arterial bed of the rat *in vivo* contains postjunctional α_1-adrenoceptors which mediate vasoconstriction but does not contain a significant population of postjunctional vasoconstrictor α_2-adrenoceptors. Xylazine and clonidine appear to act as partial α_1 agonists, as reported for other α_2 agonists on central neurones (Bradshaw et al. 1981) and aortic strip (Ruffolo & Waddell 1982). Alternatively as proposed by Fiotakis & Pipili (1983), this vascular bed may contain non-pressor postjunctional α_2 receptors, activation of which may inhibit the function of the α_1 receptors.

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NORADRENALINE CONCENTRATION AT PREJUNCTIONAL α_2-ADRENOCEPTORS AFTER SINGLE PULSE STIMULATION

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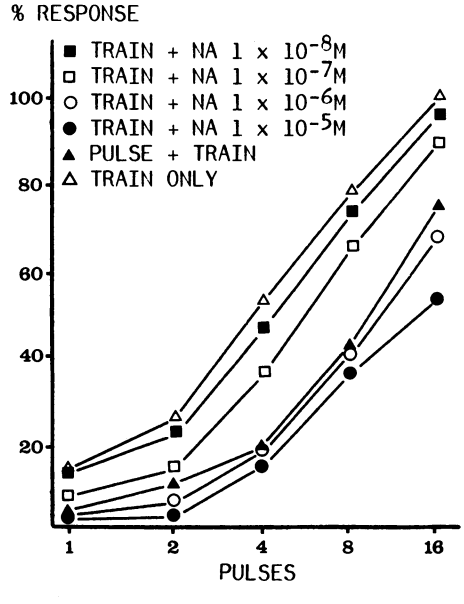
Direct measurement of noradrenaline (NA) concentration at the sympathetic neuroeffector junction resulting from nerve stimulation is difficult. Indirect methods rely heavily on the use of radiolabels and electon microscope derived estimates of vesicles, varicosities and junction volume (Falkow & Haggendal, 1970).

Prostatic portions of vas deferens from mature rats were set up as previously described (French & Scott 1981). Cocaine 1 x 10^{-5} M, and EDTA 2.7 x 10^{-5}M were added to the Ringer to inhibit uptake$_1$ and to retard NA oxidation respectively. Prazosin 5 x 10^{-8}M was also added to prevent α_1-receptor mediated facilitation of the nonadrenergic-noncholinergic response (French & Scott, 1982). Tissues were stimulated by (a): a single pulse 1 ms, 300 mA, followed, 2 or 8 s later by a train of 1,2,4,8 or 16 pulses at 500 Hz, 0.3 ms, 300 mA: (b): a train of 1 - 16 pulses only. Protocol (b) was repeated in the presence of NA 1 x 10^{-8} M - 1 x 10^{-5} M. All responses were expressed as a percentage of the response to a 16-pulse train in the absence of NA.
Stimulation with trains alone resulted in a pulse-related twitch response. When trains were preceded by a single pulse, the twitch-response curve was displaced to the right in a parallel manner. The degree of displacement was inversely related to the interval between pulse and train. NA also produced a dose-related parallel diplacement of the pulse-response curve (see fig.). The responses of this preparation to trains are due to the synchronised action of increasing amounts of released transmitter. These conditions of high-frequency stimulation preclude any element of negative feedback. (French & Scott, unpub data). However, when a train is preceded by a single pulse there is a pulse-interval dependent inhibitory effect on the train response. The effect is exacerbated by inhibitors of uptake$_1$ and is reduced by α_2-adrenoceptor antagonists, thus reflecting classical negative feedback characteristics. The inhibitory effect is mimicked by exogenous NA and it may be expected that comparable degrees of inhibition should reflect comparable degrees of prejunctional α_2-adrenoceptor activation, thus suggesting comparable concentrations of NA at the neuroeffector junction. The data from this series of experiments indicates that under these conditions, the concentration of NA at the α_2-receptor 8s and 2s after a single stimulus is aproximately 1 x 10^{-7}M and 1 x 10^{-6} M respectively. These estimates are comparable with those of other workers and are based on fewer variables and unknowns, the only assumption being the classical equal stimuli produce equal responses.



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NO EVIDENCE FOR DIFFERENCES BETWEEN PRE- AND POSTSYNAPTIC α-ADRENOCEPTORS OF THE SAME SUBTYPE

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In the pithed rat, pressor responses are mediated by both $alpha_1$- and $alpha_2$-adrenoceptors (Docherty & McGrath, 1980) and, in addition to the predominant $alpha_2$ population, $alpha_1$-adrenoceptors are present on sympathetic nerves; these, like the $alpha_2$-receptors, mediate inhibition of neurotransmitter release (Docherty, 1983). Since it has been suggested that presynaptic and postsynaptic receptors of the same subtype may differ (Kobinger & Pichler, 1980; Hicks, 1981), this work was carried out to examine whether sufficient evidence exists to suggest that presynaptic $alpha_1$ and $alpha_2$ differ from postsynaptic $alpha_1$- and $alpha_2$-adrenoceptors.

In pithed rats, presynaptic alpha-receptor agonism was assessed in terms of the dose of agonist producing 50% inhibition of the cardioacceleration to a single stimulus pulse (ID_{50}), and postsynaptic alpha-agonism as the dose of agonist producing a rise in diastolic blood pressure which was 50% of the maximum obtainable to that agonist (ED_{50}). Potency at $alpha_1$-receptors was assessed in the presence of yohimbine (1 mg/kg) and potency at $alpha_2$ receptors in the presence of prazosin (1 mg/kg). Potency of antagonists was assessed as the shift produced in the agonist ID_{50} or ED_{50}.

The order of agonist potency was the same at pre- and postsynaptic $alpha_2$-receptors: Clonidine > xylazine > cirazoline > amidephrine. At $alpha_1$-receptors cirazoline was approximately five times more potent than amidephrine both pre- and postsynaptically.

The antagonists yohimbine (1 mg/kg), phentolamine (1 mg/kg) and phenoxybenzamine (10 mg/kg) were all markedly more potent at antagonising the pre- than postsynaptic effects of the $alpha_2$-agonist xylazine, producing shifts in the presynaptic ID_{50} of 63, 102 & 66 as compared to shifts in the postsynaptic ED_{50} of 5, 21 & 8, respectively. Prazosin (1 mg/kg) had no effect on either pre- or postsynaptic potency of xylazine.

The antagonists prazosin (1 mg/kg), phentolamine (1 mg/kg) and phenoxybenzamine (1 mg/kg) produced greater shifts in the post- than presynaptic potency of the $alpha_1$-agonists cirazoline and amidephrine: prazosin (1 mg/kg) produced shifts in the presynaptic ID_{50} and postsynaptic ED_{50} of amidephrine by 8 & 200, respectively. However, in low doses prazosin was approximately equipotent pre- and postsynaptically: prazosin (10 μg/kg) produced shifts in the presynaptic ID_{50} and postsynaptic ED_{50} of 3.1 and 4.8 respectively.

In conclusion, whereas relative agonist and antagonist potencies showed no difference between pre- and postsynaptic alpha-adrenoceptors of either subtype, the absolute potencies of antagonists differed: $alpha_1$-antagonists were more potent postsynaptically and $alpha_2$-antagonists were more potent presynaptically. However, these differences may be due to the experimental conditions and different receptor distributions rather than differences within receptor subtypes.

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CARDIAC β-ADRENOCEPTOR CHARACTERISTICS AFTER CHRONIC TREATMENT WITH β-ADRENOCEPTOR ANTAGONISTS

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Abrupt cessation of chronic propranolol treatment to patients has been associated with the precipitation of angina, cardiac arrhythmias and myocardial infarction, an effect known as the "propranolol withdrawal syndrome" (Shand & Wood, 1978). A possible explanation for this phenomenon is an elevation in sensitivity (Nattel, Rangno & Van Loon, 1979) and an increase in receptor number (Glaubiger & Lefkowitz, 1977). We have therefore examined β-adrenoceptor characteristics pharmacologically and by radioligand binding following propranolol withdrawal.

Propranolol ($3.6 mgkg^{-1}day^{-1}$) was administered to guinea-pigs (330g) by means of "Alzet" osmotic minipumps implanted subcutaneously for 14 days. This dosage was shown to produce adequate β-adrenoceptor blockage by implanting minipumps and after 3 days measuring the blood pressure and heart rate responses to isoprenaline under urethane anaesthesia. The ED_{50} values for the positive chronotropic response ($71.0(33.3-150.4) ngkg^{-1}$) and vasodepressor response ($443.5(209.0-950.4) ngkg^{-1}$) were significantly ($P<0.05$) larger than the control values obtained in animals implanted with empty minipumps ($13.3(1.3-128.8) ngkg^{-1}$ and 108.7 (26.8-449.0) $ngkg^{-1}$ respectively).

To examine the sensitivity after withdrawal of propranolol, the minipumps were removed after 14 days. 48hr later the animals were killed and isolated atria set up. Control experiments were performed identically but with empty minipumps. Tissue sensitivity was determined by constructing cumulative dose-response curves to isoprenaline, orciprenaline and salbutamol. The left atrial tension responses were not altered by propranolol pretreatment, the EC_{50} values in pretreated and control tissues respectively were 25.0 (5.7-109.9)nM and 25.6 (8.3-76.1)nM for isoprenaline and 6.9 (2.1-22.5)μM and 7.14 (2.7-18.9)μM for orciprenaline. Salbutamol was a partial agonist, the maximum responses being 17.0 ± 3.2 and 15.8 ± 3.8% of the isoprenaline maximum. The right atrial chronotropic responses however exhibited supersensitivity after propranolol withdrawal. The EC_{50} values for isoprenaline (8.0(4.6-13.8)) and orciprenaline (2.4(1.14-4.0)nM) were significantly ($P<0.05$) smaller than the values from control animals (34.7 (25.8-46.6)nM and 23.1 (11.0-47.6)nM). The maximum response to salbutamol (45.8 ± 3.4%) was also significantly ($P<0.05$) greater than the control value (31.3 ± 6.4%).

(^3H) Dihydroalprenolol binding was examined in ventricular membranes prepared from the same animals used for the pharmacological analysis. The dissociation constants (K_D) and maximum number of binding sites (B_M) for (^3H)DHA binding to ventricular membranes were similar in control (2.33 ± 0.56nM; 123.3 ± 14.0 fmoles mg^{-1} protein) and propranolol-treated animals (1.83 ± 0.72nM; 114.5 ± 9.6 fmoles mg^{-1} protein). The pharmacological data would suggest that a rebound increase in sensitivity occurs for cardiac chronotropic responses after two weeks of pretreatment. This is not supported by the left atrial responses and ventricular binding, where longer periods of treatment may be required for the development of supersensitivity.

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DIFFERENCES IN SPECIFICITY OF RESERPINE-INDUCED SUPERSENSITIVITY AT CARDIAC β-ADRENOCEPTORS AND AORTIC α-ADRENOCEPTORS

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The mechanism by which chronic reserpine pretreatment induces supersensitivity to catecholamines is as yet unclear. Although a non-specific mechanism has been suggested (Fleming et al. 1973), Broadley and Lumley (1977) find supersensitivity to be β-adrenoceptor specific in guinea-pig cardiac tissue. Since a large proportion of the observations supporting a non-specific mechanism of action have been made in tissues where the response is mediated via α-adrenoceptors (Fleming et al. 1973), the present study examines the possibility of supersensitivity being induced by different mechanisms at α- and β-adrenoceptors.

Left atria, papillary muscle, aortic spiral tension and right atrial rate were recorded with the tissues suspended in Krebs-bicarbonate Ringer, gassed with 5% CO_2 in oxygen at 38°C. Left atria and papillary muscles were paced at 2Hz with a threshold voltage + 50% and 5ms pulse width. Cardiac tissues were incubated throughout with phentolamine (5×10^{-6}M) and metanephrine (10^{-5}M) to inhibit α-adrenoceptors and extraneuronal uptake respectively. Aortic spirals were incubated with propranolol (10^{-6}M), cocaine (10^{-5}M) and metanephrine (10^{-5}M) to inhibit β-adrenoceptors, neuronal and extraneuronal uptake respectively. Treated animals were given reserpine i.p. for 3 days ($5mgkg^{-1}$ 72h, $3mgkg^{-1}$ 48h & 24h) prior to sacrifice. Cumulative dose response curves were constructed for isoprenaline in cardiac tissue and noradrenaline in aortic spirals, followed by cumulative curves to calcium with the tissue bathed in either normal Ringer or calcium-free Ringer. Geometric mean EC_{50} values were calculated.

Left atria, right atria and papillary muscles showed significant supersensitivity ($P<0.05$) for the positive inotropic and chronotropic effects of isoprenaline after reserpine pretreatment, the mean EC_{50} values ($n>5$) falling from $1.4x10^{-8}$M, $1.1x10^{-8}$M and $1.1x10^{-8}$M, to $3.0x10^{-9}$M, $4.1x10^{-9}$M and $4.4x10^{-9}$M. Mean control EC_{50} values ($n>5$) of calcium for left atria ($1.4x10^{-3}$M), right atria ($1.4x10^{-3}$M) and papillary muscles ($4.4x10^{-3}$) in normal Ringer and $2.6x10^{-3}$M, $1.9x10^{-3}$M and $2.8x10^{-3}$M in calcium-free Ringer were not significantly different ($P>0.05$) from mean EC_{50} values for left atria, right atria and papillary muscle of $2.6x10^{-3}$M, $1.8x10^{-3}$M and $3.9x10^{-3}$M in normal Ringer and $2.3x10^{-3}$M, $1.8x10^{-3}$M and $2.7x10^{-3}$M in calcium-free Ringer after pretreatment, showing reserpine has no effect on calcium sensitivity of cardiac tissue.

Reserpine pretreatment induced supersensitivity to noradrenaline in the aortic spiral indicated by a significant fall in the mean EC_{50} ($n=10$) from $3.0x10^{-6}$M to $4.4x10^{-7}$M ($P<0.05$). In normal Krebs solution calcium failed to elicit a response. However, in calcium-free solution, unlike cardiac tissue reserpine pretreatment produced an increase in sensitivity to calcium with mean EC_{50} ($n=4$) values falling from $3.9x10^{-3}$M to $2.2x10^{-4}$M ($P>0.05$).

It therefore appears that reserpine induced supersensitivity is a specific phenomenon at cardiac β-adrenoceptors while at the α-adrenoceptors of the aorta the mechanism appears to be more nonspecific. This may reflect the more intimate coupling to external calcium of α-adrenoceptor mediated responses (Exton, 1982).

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FACILITATORY PRESYNAPTIC β-ADRENOCEPTORS IN HUMAN ATRIA

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Activation of presynaptic β-adrenoceptors located on sympathetic nerve terminals leads to facilitation of noradrenaline release (Adler-Graschinsky & Langer, 1975). These receptors have been identified in a number of animal tissues and also in human veins (Callanan et al., 1982). The present experiments were undertaken to investigate such receptors in human atrial tissue and to make a preliminary assessment of their physiological relevance.

Paired atrial strips, 1 cm in length, were taken from patients undergoing open heart surgery and incubated with 12 μCi(^{3}H)-adrenaline (s. act. 10-18 Ci. m.mol^{-1}). Tissues were suspended between stainless steel electrodes in an organ bath, superfused with gassed Locke's solution and subjected to three periods of electrical stimulation (S_1-S_3) applied at 18 min. intervals (2Hz, 0.2 ms, 50V). Drugs were added 12 min. before S_3.

The addition of 100 nM isoprenaline caused a significant increase in evoked release (Table 1) which was abolished by 10 μM butoxamine, a selective β_2-adrenoceptor antagonist. Simultaneous recordings of muscle contractility showed that the isoprenaline-induced tachycardia was unaffected by butoxamine. When butoxamine was administered alone there was a significant fall in evoked release of tritium.

It can be inferred from these results that in human atria, presynaptic β-adrenoceptors exist, which can be activated by neuronally released adrenaline to facilitate further transmitter release. In complementary experiments with rat atria, the butoxamine-mediated effect was only seen when tissues were preloaded with (^{3}H)-adrenaline or (^{3}H)-noradrenaline/adrenaline mixtures. Under physiological conditions it is conceivable that this adrenaline could be supplied via raised circulating levels following enhanced adrenal medullary secretion.

Table 1. Effects of different treatments on evoked release of tritium from human atria.

Treatment	S_3/S_1	n
Control	0.78 ± 0.04	4
isoprenaline (100 nM)	1.16 ± 0.03**	3
isoprenaline (100 nM) + butoxamine (10 μM)	0.73 ± 0.05$^+$	3
butoxamine (10 μM)	0.51 ± 0.06*	4

significantly different from control: *p<0.05, **p<0.01
$^+$significantly different from isoprenaline control: p<0.01.

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THE EFFECT OF PINACIDIL ON EXPERIMENTAL CARDIAC ARRHYTHMIAS IN DOGS

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Pinacidil has been shown to lower blood pressure in animals and man chiefly by a direct vasodilator effect (Arrigoni-Martelli et al, 1980). It has a haemodynamic profile similar to that seen with other vasodilators acting on precapillary resistance vessels (Kardel et al, 1981; Carlsen et al, 1981). We have studied the effect of pinacidil on experimental arrhythmias in dogs.

In anaesthetised dogs cardio-toxic doses of ouabain produced a ventricular tachycardia which was stable for at least 100 min. In 7 dogs in which ouabain (mean dose - 56.4 ± 6.4 μg/Kg) produced a stable arrhythmia, pinacidil was administered by increasing bolus injections of 0.25, 0.5, 1.0, 2.0, 4.0 mg/Kg every 5 min (n=4) and by infusions of 0.02 (n=1), 0.08 (n=1)and 0.16 (n=1) mg/Kg/min for 50 min. Pinacidil did not produce a return to sinus rhythm but did reduce blood pressure.

Two stage ligation of the anterior descending branch of the left coronary artery in dogs produces a ventricular arrhythmia which is present for up to 48 hours after ligation (Allen et al, 1977). Observations were made with measurement of arterial blood pressure and the ECG in 9 conscious dogs 24 hours after ligation of the coronary artery when 8.0 ± 2.1% of the ventricular beats were of sinus origin.

In 4 dogs pinacidil was administered by increasing bolus injections of 0.25,0.5, 1.0 mg/Kg every 5 min. Pinacidil increased the number of ventricular beats of sinus origin to 54.2 ± 14.7, 62.0 ± 13.9 and 63.2 ± 16.8% of the total ventricular rate and reduced the mean blood pressure by 30.4 ± 7.3, 47.5 ± 13.3 and 64.9 ± 11.7 mmHg after the 0.25, 0.5 and 1.0 mg/Kg doses of pinacidil respectively. In all dogs pinacidil increased the incidence of ventricular beats of sinus origin to at least 50% of the total ventricular rate. The 2.0 mg/Kg dose produced agitation and excitement in the dogs. In 5 dogs pinacidil was administered by increasing infusions of 3.1, 6.2, 12.5, 25, 50 μg/Kg/min etc every 5 min until at least 50% of the ventricular beats were of sinus origin. In 4 of 5 dogs an infusion rate of 50 μg/Kg/min or less for 5 min and in 1 of 5 dogs an infusion rate of 200 μg/Kg/min for 5 min produced a rhythm in which at least 50% of the beats were of sinus origin. A mean cumulative dose of 0.82 ± 0.3 mg/Kg pinacidil produced a rhythm in which 77.2 ± 8.9% of the beats were of sinus origin. This was accompanied by a 35.0 ± 10.0 mmHg decrease in mean blood pressure. The high incidence of sinus beats and the decrease in blood pressure were maintained for at least 25 minutes.

In conclusion pinacidil produces a marked anti-arrhythmic effect on the arrhythmia produced by coronary artery ligation at doses which produce a significant reduction in blood pressure. The exact mechanism of action of pinacidil on this arrhythmia is unknown.

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COMPARISONS BETWEEN THE MONOAMINE OXIDASE ACTIVITIES ASSOCIATED WITH MITOCHONDRIA AND MICROSOMES IN RAT LIVER

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Although the majority of liver monoamine oxidase activity is associated with the mitochondria a small proportion has been found to be associated with the microsomal fraction. On the basis of differences in the recoveries of the enzyme activities in these two fractions after administration of irreversible inhibitors, it has been suggested that the activity associated with the microsomal fraction may represent a precursor of the mitochondrial enzyme (Erwin & Simon, 1969).

In this study we have compared the specificities and inhibitor-sensitivities of the enzyme activities associated with these two subcellular fractions. Mitochondria and microsomes were prepared by standard differential centrifugation procedures and also by using the Ca^{++}-precipitation method Schenkman & Cinti (1978). The specific activities towards 5-hydroxytryptamine (a substrate for the A form) and 2-phenethylamine (a substrate for the B form at low concentrations) are compared in Table 1 which also shows the ratios of activities of the two forms determined in this way.

Table 1 Activities of monoamine oxidase in the microsomal and mitochondrial fractions towards 5-hydroxytryptamine (5-HT) and 2-phenethylamine (PEA).

Fraction	Specific Activity ($pmol.min^{-1}.mg^{-1}$)		A/B
	5-HT (A)	PEA (B)	
Mitochondrial	1622 ± 173	1937 ± 378	0.86 ± 0.12
Microsomal	796 ± 102	996 ± 126	0.93 ± 0.12

Results are mean values ± S.E. Mean of 10 separate triplicate determinations

Preincubation of the enzyme preparations with different concentrations of clorgyline and 1-deprenyl confirmed that, in both fractions, the activity towards 5-hydroxytryptamine was due to the clorgyline-sensitive, deprenyl insensitive A-form of the enzyme and that the activity towards 2-phenethylamine was due to the deprenyl-sensitive, clorgyline-insensitive B-form of the enzyme. Inhibition of the activity towards tyramine, a substrate for both forms of the enzyme, by clorgyline and deprenyl was consistent with the ratios of the activities of the two forms shown in Table 1. However, although incubation of the mitochondrial fraction for 30 min., at 37°C, with 10^{-8} M deprenyl gave substantial inhibition of the B-form, an incubation time of 60 min. was required to obtain a similar effect with the microsomal fraction.

Although it is not possible to exclude the possibility of some contamination of the microsomal fraction with mitochondrial fragments the procedures used in this work were designed to minimise this. The results show no significant differences between the specificities of the monoamine oxidase activities associated with the microsomal and mitochondrial fractions and suggest that, if the former does indeed represent a precursor of the latter, there is no significant alteration in these properties during the process of transfer to the mitochondrial outer membrane.

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EFFECTS OF QUINACRINE ON MONOAMINE OXIDASE ACTIVITIES IN SOME TISSUES OF THE RAT

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Quinacrine (mepacrine), a drug used for many years in the treatment of malaria has long been known to be an inhibitor of many enzymes with flavin prosthetic groups (Wright and Sabine, 1944; Bovarnick et al, 1946). Indeed inhibition by quinacrine has been taken as proof of the importance of flavoprotein in a particular enzyme reaction (Hemker & Hülsmann, 1960). If this were true, quinacrine might provide a convenient and simple test to determine the relative importance of FAD-dependent amine oxidases such as monoamine oxidase (MAO) and polyamine amine oxidase and carbonyl reagent sensitive amine oxidases such as clorgyline-resistant (semicarbazide-sensitive) amine oxidase (CRAO) in the deamination of various monoamines in tissue homogenates where several amine oxidase activities may be present.

For such a test to be useful quinacrine must be either an irreversible or tight binding inhibitor of all relevant FAD-dependent enzymes and of no others. In addition its interaction with enzyme must approach completion within a convenient time to limit the effect of other possible effects on the enzymes being tested.

To examine the usefulness of quinacrine, homogenates, in 1mM potassium phosphate buffer; pH 7.8, were made from heart, liver and interscapular brown fat of male Wistar rats. The homogenates after centrifugation at 600g for 10 min to remove nuclei and cell debris were used immediately or within a week after storage at -20°C. MAO and CRAO activities were measured radiochemically with ^{3}H-5-hydroxytryptamine (5-HT) and ^{3}H-tyramine as substrates for MAO-A in liver and heart respectively, and ^{14}C-benzylamine as a substrate for MAO-B in liver and CRAO in brown fat.

In the heart with tyramine as substrate for MAO-A, quinacrine (10µM-1mM) without preincubation before addition of substrate, appeared to be competitive from double reciprocal and S/v vs S plots. Quinacrine also appeared competitive with 5-HT as substrate in heart and liver. Dixon plots of the tyramine data also indicated a competitive interaction and gave a K_i for quinacrine against MAO-A of 200µM. With benzylamine as substrate for MAO-B in liver, quinacrine was apparently competitive, with a K_i of 470µM against this form of the enzyme.

After 1h preincubation with homogenates of heart tissue before addition of tyramine the inhibition appeared mixed in nature especially at high concentrations of quinacrine. Similar effects were seen with 5-HT in liver. Dixon plots were curved and approached a maximum value. This suggests that even when MAO-A has bound all the quinacrine that it can, it is still able to deaminate tyramine although at a reduced rate . With MAO-B inhibition remained predominantly competitive. Quinacrine (1µM-1mM) even when preincubated for 90 min had no effect on the deamination of benzylamine by brown fat CRAO.

These results are consistent with at least two kinetic models of interaction between MAO-A and quinacrine; one involves the formation of ternary complexes between enzyme substrate and inhibitor and the other assumes the presence of two inhibitor binding sites (Segal, 1975). The results do not show the presence of a major irreversible inhibitory component on either MAO-A or -B, which is important in distinguishing flavin-dependent amine oxidases from others with similar activities.

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EFFECTS OF MONOAMINE OXIDASE INHIBITOR PRETREATMENT ON THE FATE OF INTRADUODENALLY INSTILLED (^{14}C) - β - PHENETHYLAMINE

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β-Phenethylamine (PEA) can precipitate migraine and is present in cheese, red wine and chocolate (Sandler et al. 1974). The fate of (^{14}C)-PEA instilled intraduodenally (i.d.) was examined in chloralosed cats, serial portal venous (PV) and cranial mesenteric arterial (CMA) blood samples being removed and chromatographed (Modified method of Tacker et al 1970) for scintillation counting.

Principal (^{14}C)-compounds recovered from PV and CMA blood were phenylacetic acid (PAA) and PEA, the proportion of PEA increasing progressively with larger dose. PEA and PAA accounted for 88% of total radioactivity, an amount unaltered by pretreating cats with (-)-deprenyl or mebanazine. PEA in CMA blood was 21% to 40% of that in PV blood, while corresponding values for PAA were 64 to 74%. Concentration of PEA in PV blood peaked 5 to 10 min after instillation, indicating rapid absorption. The presence of PEA in CMA blood, meant it survived passage through the liver, heart and lungs. Despite its lipid solubility, (^{14}C)-PEA was not absorbed from the stomach.

PEA is a preferred substrate for MAO B (Yang & Neff 1973) but PEA in PV or CMA blood was not significantly elevated nor PAA so reduced, following (-)-deprenyl i.d. 90 min prior to PEA instillation (Table 1), or after clorgyline. Arterial concentrations of PEA were significantly elevated by pretreatment with mebanazine, or clorgyline together with deprenyl; PAA concentrations were reduced. The nictitating membrane did not contract in control cats given PEA but did so in cats given PEA 8.5μmol/kg i.d. after mebanazine or clorgyline with deprenyl, half-maximum contraction occurring at mean PEA arterial concentrations of about 2nmol/ml. Control liver, lung, gut and kidney mean concentrations of PEA ranged from 0.4-1.0nmol/g (PEA 1.7μmol/kg) to 9.8-13.1nmol/kg (PEA 17μmol/kg), indicating much PEA eluded deamination; PEA concentrations exceeded those of PAA, except in the kidney where the total for PEA and PAA was 5-fold that in other tissues. Tissue concentrations of PEA were significantly increased by mebanazine or clorgyline combined with deprenyl.

Table 1. (^{14}C)-β-Phenethylamine (PEA) and phenylacetic acid (PAA) in portal venous (PV) blood and cranial mesenteric arterial (CMA) blood for control cats and those pretreated with MAO inhibitors

		1.7μmol/kg + 5μCi			8.5μmol/kg + 5μCi			17μmol/kg + 5μCi		
		PEA	PAA	n	PEA	PAA	n	PEA	PAA	n
		pmol/ml			pmol/ml			pmol/ml		
Controls	PV	295	1262	4	2549*	5341**	6	5903*	6970	4
	CMA	113	866		709***	3921		1256	4485	
Deprenyl (4.5μmol/kg)	PV	400	620	4	1921	3765	3			
	CMA	121	260*		562	4216				
Clorgyline (24.5μmol/kg)	PV				1939	2332	3			
	CMA				807	3309				
Deprenyl (4.5μmol/kg) +	PV	975**	848	4	7825*	2593*	5			
Clorgyline (24.5μmol/kg	CMA	370**	629		2685***	2013				
Mebanazine (40μmol/kg)	PV				6546*	2070*	5			
	CMA				3376***	2131				

Values are mean results of serial determinations (12 per experiment) in n experiments.
*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

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DRUG INTERACTIONS WITH THE CNS NUCLEOSIDE TRANSPORT SYSTEM

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Several classes of drugs have been proposed to act via a potentiation of adenosine action resulting from inhibition of nucleoside transport (Phillis & Wu, 1982). Study of the nucleoside transport system (NTS) of erythrocytes and cultured cells (Paterson et al, 1981), and more recently guinea pig (Hammond & Clanachan, 1982) and rat (Marangos et al, 1982) CNS membranes has been facilitated with the use of nitrobenzylthioinosine (NBMPR), a potent nucleoside transport inhibitor. NBMPR binds tightly, but reversibly, to functional transporters and inhibition of transport is related to site occupancy (Cass et al, 1974). Inhibition constants (K_i values) of several agents for inhibition of the binding of NBMPR reflect accurately the ability of these agents to inhibit nucleoside transport (Hammond et al, 1983). We have now estimated inhibition constants of a variety of compounds for this transport inhibitory site in CNS membranes through inhibition of the site-specific binding of [G-^{3}H]NBMPR.

In guinea pig cortex, several recognized nucleoside transport inhibitors, e.g. dipyridamole, dilazep and hexobendine, were potent, competitive inhibitors of the binding of NBMPR. Metabolism by cholinesterases reduced the apparent potency of dilazep and hexobendine as inclusion of physostigmine significantly increased their activity as inhibitors of the binding of NBMPR. Purine and pyrimidine nucleosides were also competitive inhibitors of the binding of NBMPR with K_i values (100 µM to 10 mM) which were similar to their Michaelis-Menten constants (K_m) for translocation by the NTS. Various benzodiazepines inhibited the site-specific binding of NBMPR but they had low affinity (K_i values > 5 µM) relative to the recognized transport inhibitors. Similarly, β-carboline derivatives and phenothiazines were poor inhibitors of the binding of NBMPR.

Interestingly, the NBMPR binding sites in rat cortex, which displayed similar affinities for benzodiazepines as the sites in guinea pig cortex, had about a 37 fold lower affinity for dipyridamole; K_i values of 404 and 11 nM were obtained for dipyridamole in rat and guinea pig cortical membranes, respectively.

The finding that recognized inhibitors of the NTS were potent inhibitors of the binding of NBMPR in guinea pig CNS membranes supports the proposal that NBMPR binds to transport inhibitory elements of functional nucleoside transporters in these membranes. Competitive inhibition of the binding of NBMPR by nucleosides indicates that NBMPR may bind at or near the permeant site of the NTS. Although it has been proposed that inhibition of the NTS by benzodiazepines and phenothiazines contributes to their therapeutic activities (Phillis & Wu, 1982), the present finding that these agents have low affinity for NBMPR sites suggests that they are poor inhibitors of the NTS. Rat membranes contain a different form of NTS which can be identified by its significantly lower affinity for dipyridamole.

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THE EFFECT OF p-CHLOROPHENYLALANINE ON SUBSENSITIVITY OF RAT INTESTINE TO 5-HYDROXYTRYPTAMINE DURING A NEMATODE INFECTION

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During infection of rats with Nippostrongylus brasiliensis, isolated small intestine exhibits (a) non-specific increases in maximum response to acetylcholine and 5HT, and (b)specific subsensitivity to 5HT reflected as a decrease in pD_2 (Farmer 1981). Prolonged exposure of rat intestine to 5HT renders this tissue unresponsive to this amine (Gillan & Pollock, 1980). Since intestinal 5HT levels are elevated during infection (Murray et al, 1971) the present study set out to examine the effect of treating infected rats with p-chlorophenylalanine (PCPA), which depletes tissue 5HT (Koe & Weissman, 1966), on intestinal sensitivity to 5HT. It was also interesting to determine the effect of PCPA on immune expulsion of the parasites, a process in which 5HT has been widely implicated (Boreham & Wright, 1976).

Male PVG rats (150-200g) were infected with 5000 N.brasiliensis larvae and injected on day 3 post-infection with PCPA (200 $mgkg^{-1}$ i.p.). On days, 5,7,9,11 and 13 these animals received 100 $mgkg^{-1}$ PCPA. Uninfected rats also received the drug and groups of uninfected and infected rats received 0.9% saline i.p. on corresponding days. Rats were killed on day 14 of the experiment and 4-5 cm segments of small intestine excised. Tissues were bisected, half being weighed and frozen in liquid N_2 prior to estimation of 5HT content, and half suspended in oxygenated Krebs' solution (37°C). Responses to 5HT were recorded isometrically, dose-response curves obtained and pD_2 values compared by Duncan's multiple range test. Intestinal 5HT was assayed fluorometrically (Curzon et al, 1981).

In uninfected rats PCPA depleted intestinal 5HT by 75% and caused supersensitivity to 5HT (Table 1). N.brasiliensis infection doubled intestinal 5HT content and rendered the gut subsensitive to 5HT. In infected rats PCPA treatment not only prevented the increase in intestinal 5HT but also inhibited the development of intestinal subsensitivity to 5HT. It therefore seems likely that specific subsensitivity of gut to 5HT during infection is due to elevated levels of this substance. From Table 1 it can be seen that PCPA did not affect the number of worms remaining in the intestine at day 14. This casts doubt on the hypothesis that 5HT plays a role in parasite expulsion (Murray et al, 1971).

Table 1: Effect of p-chlorophenylalanine on sensitivity to, and levels of 5HT in isolated intestine of N.brasiliensis-infected rats.

	Group	pD_2	Amount of 5HT in gut ($ug\ g^{-1}$)	Worms present
A	Control	7.45 ± 0.03^{a}	2.73 ± 0.21^{ab}	-
B	PCPA treated	7.82 ± 0.04^{ab}	0.73 ± 0.27^{a}	-
C	Infected	6.70 ± 0.10^{ab}	4.74 ± 0.54^{ab}	130 ± 36
D	Infected + PCPA treated	7.13 ± 0.11^{b}	0.49 ± 0.13^{b}	142 ± 58

Values expressed as mean ± s.e. mean of at least 4 observations. Within columns values bearing the same superscript are significantly different at the 95% level at least.

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CERTAIN ANTICONVULSANTS INHIBIT FOLATE ACCUMULATION INTO RAT BRAIN IN VIVO

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Long term therapy with some anti-epileptic drugs e.g. phenytoin and phenobarbitone, is known to reduce CSF folate concentrations in at least 50% of patients (Reynolds, 1972). The mechanism of action is unknown but it has been suggested by Reynolds that the anticonvulsants may inhibit folate transport into the CNS. However, animal studies using isolated preparations of choroid plexus, known to possess a carrier mediated uptake system, which transports reduced folates, the biologically active form, from blood to CSF, have revealed an inconsistent effect of phenobarbitone and no effect of phenytoin (Taguchi et al, 1977, Chen et al, 1975). A preliminary, limited study on the accumulation of folates into rat brain in vivo suggested that phenobarbitone and valproate, but not phenytoin and carbamazepine, inhibited accumulation (Smith et al, 1981). We have now undertaken a more detailed study on the effect of several anticonvulsants, given acutely at "therapeutic doses", on the accumulation of folate by rat brain in vivo following the peripheral administration of folic acid (FA).

Anticonvulsant drugs, at ED_{95} values obtained in the maximal electroshock or leptazol (70 mg kg^{-1}, i.v.) tests, or saline, were administered to rats (Wistar, male, 200 to 250g) 1h before FA at 10 mg kg^{-1} (i.p.), a dose which significantly ($p < 0.001$) elevated brain folate concentrations (mean brain concentration ± s.e. in saline and FA treated rats were 665 ± 16 ng g^{-1}, n=38, and 1250 ± 18 ng g^{-1}, n=41, respectively). One hour later, the rats were killed and their brain folate concentrations determined by radioassay using β-lactoglobulin as the binding protein (Rothenberg et al, 1972, and Tigner et al, 1979). A significant ($p < 0.001$) reduction in folate accumulation was induced by phenytoin (59 mg kg^{-1} p.o., n=16), phenobarbitone (22 mg kg^{-1} p.o., n=16), valproate (1480 mg kg^{-1} p.o., n=13) and troxidone (575 mg kg^{-1} p.o., n=14). In contrast, carbamazepine (30.5 mg kg^{-1} p.o., n=16), diazepam (22 mg kg^{-1} p.o., n=17) and ethosuximide (300 mg kg^{-1} s.c., n=15) had no significant effect. None of the drugs tested when given alone had any significant effect on endogenous brain folate concentrations. In an attempt to characterise the active component of folate accumulation by rat brain, probenecid, an inhibitor of organic acid transport, was administered at 200 mg kg^{-1} i.p. (n=16), a dose known to inhibit homovanillic acid transport from rat brain (Bartholini, 1977): folate accumulation was significantly inhibited by 33% ($p < 0.001$).

Our studies have shown that rat brain folate concentrations are significantly increased following the peripheral administration of FA. The increase is due, at least in part, to an active transport system as evidenced by the effect of probenecid. The mechanism(s) by which certain anticonvulsants inhibit this uptake are, as yet, unknown, but may include inhibition of the CNS folate transport system and/or reduced conversion of FA to the reduced forms required for transport.

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THE EFFECTS OF FREE RADICAL SCAVENGERS ON REPERFUSION ARRHYTHMIAS IN THE ISOLATED RAT HEART

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Reperfusion of the ischaemic myocardium induces severe arrhythmias in most species studied and this type of arrhythmia may be a cause of sudden death in man. The immediate causes of these arrhythmias are not known but one possibility is that they are caused by free radicals generated during the reoxygenation which occurs following reperfusion. Free radicals affect myocardial calcium movements (Hess et al 1981), and lipid metabolism (Meerson et al 1982) and both of these factors have been implicated in arrhythmogenesis. For these reasons we have studied the effects of the free radical scavengers glutathione, mannitol, catalase and superoxide dismutase (S.O.D.) on reperfusion arrhythmias in the Langendorff perfused coronary ligated rat heart.

Hearts from male Wistar rats were perfused at 10ml/min and 37^{o} with a solution composed of (mM) 118NaCl; 1.2$MgCl_2$; 1.2$CaCl_2$; 25$NaHCO_3$; 1.2KH_2PO_4: 2.0KCl and 11.5 glucose. The main left coronary artery was occluded and 10 mins later the occlusion was released. Epicardial E.C.G's were recorded over the following 3 mins. The incidence, onset and duration of ventricular fibrillation (V.F.); ventricular tachycardia (V.T.); and the number of premature ventricular contractions were recorded. The effects of glutathione, S.O.D. catalase and mannitol on these reperfusion arrhythmias are shown in Table 1.

Table 1

	n	VF % Incid.	VT % Incid.	PVC's
Control	21	90	86	142±32
Glutathione 10^{-5}M	9	67	78	257±115
10^{-4}M	9	22*	78	142±38
10^{-3}M	9	0*	56	121±38
S.O.D. 5 units/ml	9	56	78	136±38
10 units/ml	9	33*	56	57±20
20 units/ml	9	22*	56	61±17
Catalase 100 units/ml	9	56	67	102±29
300 units/ml	9	56	78	82±14
Mannitol 20mM	9	56	89	64±13
S.O.D. 10 units/ml) +Catalase 100 units/ml) +Mannitol 20mM)	9	0*	22*	20±10*

*$p<0.05$ compared with controls

These results show that reperfusion arrhythmias in the isolated rat heart can be prevented by free radical scavengers, this suggests that free radicals may be important mediators of this type of arrhythmia.

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INFLUENCE OF ISOPRENALINE AND PROPRANOLOL ON BINDING OF DHA AND PROPRANOLOL IN RAT HEART

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The beta-adrenoceptors of rat ventricular myocardium are responsive to drug-induced adrenergic stimulation, or blockade, through alterations in receptor density. While treatment of rats with an agonist such as isoprenaline caused a down regulation of receptor number (B_{max} reduced) as indicated by binding of ^{3}H-DHA (Tse et al., 1979), administration of propranolol has been variously reported to increase (Glaubiger and Lefkowitz, 1977) or to leave unchanged (Baker and Potter, 1980) the binding of ^{3}H-DHA to ventricular membranes. We have investigated the effects in rats of administered isoprenaline or propranolol on binding characteristics of both ^{3}H-DHA and of ^{3}H-propranolol. The effects of thyroid hormone and of clonidine on myocardial receptors for both ligands have been compared with those of the adrenergic drugs.

All experiments were performed using male Wistars ranging from 150 - 220 g weight. At conclusion of treatment ventricles were rapidly removed after sacrifice and a membrane preparation made according to the method of Alexander et al. (1975) with minor modifications. The density of beta-receptors was assayed using cold propranolol as competitor, and the same competitor was employed for ^{3}H-propranolol binding. Thyroxine, propranolol and clonidine were given by the intraperitoneal route, while isoprenaline was administered subcutaneously. **All drugs were withdrawn for 24 hours prior to sacrifice.**

Thyroxine at 800 μg/kg twice daily for 5 days increased the ventricular B_{max} for DHA, leaving the affinity for the ligand unchanged. The binding of ^{3}H-propranolol was not significantly altered by thyroxine. Isoprenaline administered at dosage 10 mg/kg for 10 days caused a reduction in DHA binding density but this agent also did not affect the binding of labelled propranolol. Propranolol at 20 mg/kg daily in divided dose for 7 days caused a significant increase in density of sites for DHA, without altering affinity for that ligand. The binding of ^{3}H-propranolol itself was not affected by propranolol treatment. Clonidine given at 0.15 mg Kg^{-1} **daily for 3 or 21 days did not alter the** binding of either ligand.

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ACTIONS OF BENZODIAZEPINE ANTAGONISTS ON THE RAT SUPERIOR CERVICAL GANGLION

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It has been reported that benzodiazepines potentiate the responses of the rat superior cervical ganglion to GABA when these responses were partially antagonised by bicuculline (Bowery and Dray, 1978) and we have previously shown that this effect is antagonised by the benzodiazepine Ro 15-1788 (Nutt et al., 1982). The present studies show that the effects of these drugs on the responses of this tissue GABA can be seen in the absence of bicuculline. In addition, the inverse agonists methyl- and ethyl-β-carboline-3-carboxylate decreased GABA responses and these effects were also blocked by Ro 15-1788, as are their in vivo actions (Nutt et al., 1982). No consistent difference was seen with the effects of these drugs with bicuculline, with the exception of high doses of Ro 15-1788 which increased the effects of GABA only when bicuculline was present.

The method of Brown and Marsh (1974) was used for recording extracellular potential changes from the isolated perfused ganglion. Doses of GABA were applied at 15 min intervals and the amplitudes of the responses were expressed as percentages of the first (steady) control response on each preparation (mean ± S.E.M.). The doses of GABA were 9.7 μM, giving half-maximal responses, and 38.8 μM which, with 27.7 μM bicuculline, gave responses of similar amplitude (absolute amplitude range 400 μV - 1.2 mV).

Controls 9.7 μM GABA	Chlordiazepoxide 14.9 μM	CDP + Ro 15-1788, 3.34 μM
(7) 108 ± 5, 97 ± 7	144 ± 9, 144 ± 11 *	102 ± 10, 87 ± 9
Controls 38.8 μM GABA (+ bicuculline)		
(5) 106 ± 3, 97 ± 4	141 ± 4, 152 ± 12 *	91 ± 10, 91 ± 10
Controls 9.7 μM GABA	βCCM 219 nM	βCCM + Ro 15-1788, 3.34 μM
(7) 109 ± 4, 100 ± 7	76 ± 6, 70 ± 7 *	129 ± 15, 117 ± 12
Controls 38.8 μM GABA (+ bicuculline)		
(5) 93 ± 3, 97 ± 3	40 ± 8, 43 ± 10 *	90 ± 11, 87 ± 8
Controls 9.7 μM GABA	βCCE 1 μM	βCCE + Ro 15-1788, 3.34 μM
(6) 98 ± 8, 94 ± 5	73 ± 10, 77 ± 12 *	101 ± 11, 90 ± 8
Controls 38.8 μM GABA (+ bicuculline)		
(6) 92 ± 2, 96 ± 6	81 ± 4, 74 ± 3 *	127 ± 14, 113 ± 12
Controls 9.7 μM GABA	Ro 15-1788, 3.34 μM	Ro 15-1788, 334 μM
(6) 101 ± 3, 101 ± 3	99 ± 2, 93 ± 3	85 ± 5, 87 ± 5 *
Controls 38.8 μM GABA (+ bicuculline)		
(6) 101 ± 2, 97 ± 2	99 ± 5, 95 ± 4	119 ± 5, 120 ± 5 *

( ) = No. of preparations, * $P < 0.05$, compared with controls (analysis of variance).

These results show that the interaction between the drugs in their effects on GABA responses parallel those interactions see in vivo, even though the ganglion is not thought to contain neurones which normally use GABA as a transmitter.

We are grateful to - Hoffman-La-Roche for gifts of chlordiazepoxide and Ro 15-1788, and to Glaxo for βCCE and βCCM.

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ACTION OF MULTIVALENT CATIONS ON THE CAFFEINE CONTRACTURE OF RAT SOLEUS MUSCLE

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Experiments performed on intact and subcellular fractions of skeletal muscle have led to the hypothesis that caffeine acts intracellularly, releasing calcium from the terminal cisternae and blocking the reuptake of calcium by the longitudinal sarcoplasmic reticulum (Weber & Herz 1968). However, the site of action of caffeine may also be explained in terms of an extracellular site of action (Luttgau & Oetliker 1968). This study investigated the site of action of caffeine in mammalian skeletal muscle.

The in vitro application of 12 mM caffeine in Ringer's solution (containing 2.52 mM Ca and 0.8 mM Mg) to the soleus muscle of 50 g rats at 37°C caused biphasic contractures. The first phase which averaged 1.36 ± 0.13 g and had a rise time of 30.75 ± 4.31 sec was followed by a small drop in tension and then a second phase. The second phase attained a peak tension of 3.50 ± 0.26 g after 22.10 ± 1.95 min and slowly declined (10% decline over 15 min).

Equilibration of the muscle for 2 or 10 min in 10^{-9}M Ca (no added Ca, 1 mM EGTA, 0.8 mM Mg) prior to the addition of 12 mM caffeine in 10^{-9}M Ca increased the amplitude of the first phase to 2.48 ± 0.32 and 1.79 ± 0.14 g respectively. The rise time declined to 11.50 ± 0.96 and 17.25 ± 2.53 sec respectively. However the second phase was usually abolished so that the contracture was transient with a half decay time of less than 1 min. Equilibration for longer than 10 min in 10^{-9}M Ca reduced the first phase and after 2 hr equilibration in 10^{-9}M Ca, the caffeine contracture was abolished completely.

Equilibration of the muscle in 1 mM La for 10 min prior to the addition of caffeine abolished the caffeine contracture. Addition of caffeine in 0.5 mM La Ringer solution after prior equilibration for 2 or 10 min in 0.5 mM La increased the amplitude of the first phase to 1.46 ± 0.43 and 2.43 ± 0.21 g respectively. The second phase tension was increased to 3.84 ± 0.66 and 5.31 ± 0.90 after 2 and 10 minutes equilibration in 0.5 mM La and the time to peak tension was reduced by over 40%. The potentiated first phase observed after equilibration for 2 min in 10^{-9}M Ca was reduced 31% and the time to attain first phase tension was increased 148% by the addition of 0.5 mM La. La prolonged the half decay time of the contracture by over 400%.

Raising external K from the control value of 4.6 mM to between 10 and 40 mM, one minute prior to the addition of caffeine potentiated the amplitude of the first and second phases of the contracture. The largest potentiation occurred with 10 K, when first and second phases of tension were increased 174% and 59% respectively. Concentrations of K above 60 mM reduced the amplitude of first and second phase tnesion and in 180 mM K these reductions were greatest being 10% and 41% respectivley.

These findings indicate that caffeine acts at an external site in mammalian skeletal muscle. The changes produced in contractures by altering cation concentrations in the bathing solution indicate that caffeine acts by moving the potassium activation curve to more negative potentials and the inactivation curve to more positive potentials.

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CHARACTERISATION OF CATECHOLAMINES BY HIGH SPEED CYCLIC VOLTAMMETRY

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High speed cyclic voltammetry at carbonfibre microelectrodes has been used to quantify ionophoresis of catecholamines and 5-HT (Armstrong-James et al 1981, Kruk et al 1980), and detection and measurement of ascorbic acid in vivo (Kruk & Stamford 1983). In all these experiments, signal capture has been achieved using a digital storage oscilloscope with 8-bit resolution, and while satisfactory for the applications above, it was not possible to distinguish catecholamines from each other on the basis of electroxidatiion and reduction peaks. An oscilloscope with 12-bit resolution, and facilities for digital subtraction of waveforms, overcomes this difficulty.

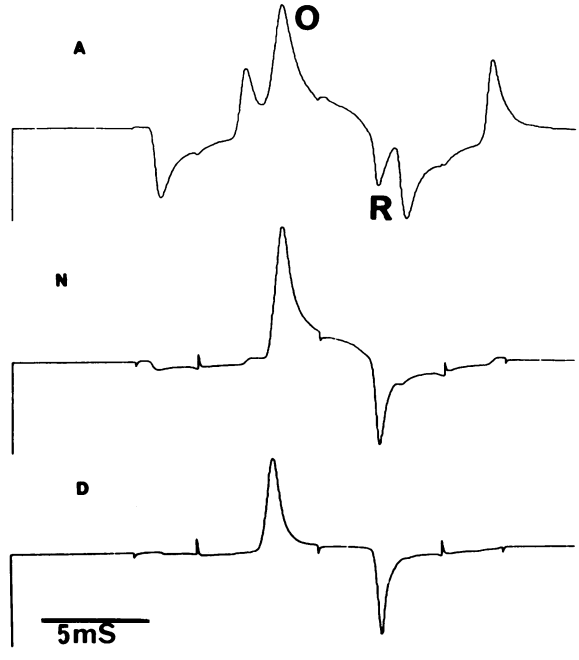

Figure 1. Oxidation 'O' and reduction 'R' peaks of dopamine (D), noradrenaline (N), and adrenaline (A) after subtraction of background signal. A 1.5 cycle linear voltage ramp 900 mV about 0.0V at 81.5 Hz was used as the driving voltage, and applied 1/s. Principle oxidation peaks are at 706 mV(D), 765 mV(N), 818 mV(A). Principle reduction peaks are at 207 mV(D), 266 mV(N), 372 mV(A). Secondary oxidation and reduction peaks are seen with adrenaline. In the concentration range 5.10^{-6} M to 1.10^{-4} M, within electrode variation in peak position is less than 2%. Between electrode variation in peak position can be greater than 10%, thus emphasising the need to calibrate each electrode.

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OPIATES AT ANALGESIC DOSES SUPPRESS OXYTOCIN RELEASE IN THE RAT

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Oxytocin is released during labour and is thought to promote maximal uterine contractions to accelerate expulsion of the foetus during the second stage. The same hormone is also known to be indispensable for milk ejection during nursing. We have shown that oxytocin release is blocked by morphine and endogenous opioid peptides when given by intracerebroventricular injection and can be potentiated by naloxone (Clarke et al., 1979; Wright et al., 1982). Since an opiate, usually pethidine, is often given during labour we felt it was important to know whether opiates at analgesic doses interfere with oxytocin release. We have therefore compared the effects of morphine, pethidine and pentazocine.

Lactating Wistar rats which had been separated from their pups overnight were used in these experiments. On days 7-10 of lactation the rats were divided into two groups. In the first group the intraperitoneal dose of each opiate required to produce analgesia was determined using the tail-flick test. The analgesic ED_{50} was taken as the dose that in 50% of the animals caused a 100% increase in response latency from baseline, for 3 or more consecutive (5 min) readings. The second group of animals was used to determine the effect of opiates on the release of oxytocin during suckling. The rats were anaesthetised with urethane ($1.2g\ kg^{-1}$, i.p.) and the hungry pups placed on the nipples 3 hours later. Pulsatile release of oxytocin, sufficient to cause milk ejection which could be detected by the characteristic behaviour of the pups (Lincoln et al., 1973), usually began within 20 min and continued for several hours at regular (approximately 5 min) intervals. The ED_{50} for suppression of oxytocin release was taken as the intraperitoneal dose which suppressed 3 or more consecutive milk ejections in 50% of the animals.

The ED_{50} for analgesia and suppression of oxytocin release was the same for pentazocine (analgesic ED_{50} : $15mg.kg^{-1}$; oxytocin suppression ED_{50} : $15mg.kg^{-1}$) and similar for pethidine (analgesic ED_{50} : $10mg.kg^{-1}$; oxytocin suppression ED_{50} : $11mg.kg^{-1}$). In contrast subanalgesic doses of morphine blocked the release of oxytocin, the oxytocin suppression ED_{50} was $0.67mg.kg^{-1}$ whereas the analgesic ED_{50} was $5mg.kg^{-1}$. A dose of $1mg.kg^{-1}$ of morphine inhibited oxytocin release in 7/9 (77.8%) experiments but was analgesic in only 1/7 (14.3%) experiments.

Opiates are often administered to alleviate pain in labour. Our experiments clearly show that in the rat pentazocine, pethidine and morphine markedly suppressed oxytocin release at doses sufficient to cause analgesia; the order of potency for both effects were identical. Morphine, unlike pethidine and pentazocine, even blocked oxytocin release at subanalgesic doses. As suppression of oxytocin release during labour may be detrimental, the use of an analgesic that suppresses oxytocin release may be inappropriate.

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EFFECTS OF CANNABIS AND THC ON SOCIAL BEHAVIOUR IN MICE

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Cannabis and its chief psychoactive constituent, delta-9-tetrahydrocannabinol (THC), are known to exert complex neurochemical changes affecting cholinergic, serotonergic and catecholaminergic systems and also to influence hypothalamo-pituitary functions (Chakravarty et al, 1982; Dalterio et al, 1982; Miczek & Dixit, 1980). A variety of behavioural correlates to the neuro-chemical actions of the cannabinoids have been described although relatively few studies have analysed effects upon social behaviour (Mackintosh et al, 1977; Miczek & Dixit, 1980; Sieber et al, 1982).

In this study acute effects of cannabis and THC on social interactions between mice were examined by ethological procedures as described by Mackintosh et al (1977). Male CFW mice aged 10-12 weeks were caged in pairs for 1 week prior to experiments and on the day of treatment, 1 male from each pair received an i.p. injection of physiological saline containing 4% Tween 80 while the other was injected with cannabis or THC suspended within 4% Tween-saline. Cannabis (containing 3.6% THC, 2.5% cannabidiol and 0.6% cannabichromene) was given at dose levels of 4, 50 and 100 mg kg^{-1} and THC at a level of 5 mg kg^{-1} prior to encounters with unfamiliar Tween-saline injected male partners. Cannabis also was given at 12.5 and 25 mg kg^{-1} prior to encounters with untreated control female partners while Tween-saline injected males encountered a group of control females. At 45 minutes after injection, behaviour shown by the mice in a neutral cage was recorded by 2 observers for a 10 minute period using a check list of 53 behavioural elements (Mackintosh et al, 1977).

A common pattern of behavioural change followed the treatments with cannabis and THC. Immobility, (consisting of the elements 'Flop' and 'Sit'), and the Flight elements 'Flag, Evade, Retreat and Flee' were increased in frequency after injections of THC and cannabis (50 and 100 mg kg^{-1} in male-male encounters and 25 mg kg^{-1} in male-female encounters) whereas 'Scan', 'Wash and Self-Groom' and some elements of Social Investigation and Sexual Behaviour were reduced. In particular 'Mounts and Attempted Mounts' were reduced in cannabis-treated males encountering female partners (12.5 and 25 mg kg^{-1}) and in THC treated males encountering male partners. It is possible that the known inhibitory actions of THC on serum androgen levels (Chakravarty et al, 1982) could underlie the decrease in sexual behaviour observed in these experiments. Analysis of the response sequence of cannabis-treated mice encountering male partners showed the drug to significantly increase Flight in response to Aggression.

On a dose-response basis, the enhancement of Immobility and reduction of Scanning produced by cannabis were approximately of the level to be expected from its THC content whereas the Flight response after cannabis treatment was less than expected in relation to its content of THC. It is possible that cannabidiol or other constituents of the cannabis might be inhibiting some of the actions of the THC within the cannabis sample (Zuardi et al, 1982).

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SODIUM VALPROATE: EFFECTS ON DEVELOPMENT AND SOCIAL BEHAVIOUR IN THE OFFSPRING OF MICE

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Many drugs and other agents can adversely influence brain development and subsequent behaviour when children or young animals are exposed to them during prenatal or early postnatal life. Anticonvulsant drugs in general are within the group of compounds sharing such undesirable actions. The newer anticonvulsant, sodium valproate, may not be any safer in this respect since some workers have found it to be both a physical teratogen (Kao et al, 1981) and also to adversely influence brain development and subsequent learning ability (Diaz and Shields, 1978). Despite the clinical relevance, effects on social behaviour are rarely analysed in the preclinical evaluation of drugs. We have examined effects sodium valproate produces on social interactions as well as on physical development in mice when these had been exposed to the drug in utero and throughout postnatal life.

Sodium valproate was given in drinking fluid (600 mg/l) during pregnancy and lactation to outbred mice and to their offspring after weaning. A corresponding group of controls received tap water to drink and a further treated group were given sodium valproate from the time of weaning using the same dose level. No detectable effect of the drug was observed on breeding performance, maternal behaviour or physical development of pups up to the time of weaning. The average daily intake of valproate ranged from 160-180 mg/kg body weight, depending on age and sex.

After weaning, mice were housed in groups of 5-7 animals of the same sex prior to observations of their behaviour. Behaviour of each animal was examined for a 10 min period when mice had been placed in a neutral cage during the early phase of the dark period of their 24 hour light-dark cycle. Ethological procedures as described by Mackintosh et al (1977) were used to obtain an objective record of the acts and postures shown by each mouse when encountering an unfamiliar animal of the same sex and treatment group. Behaviour shown by each animal in the diad was recorded by two observers.

At 5 weeks of age, treated male and female mice exposed to valproate throughout their life showed no significant differences in any form of behaviour from control animals. We have thus obtained no evidence that valproate at the dosage level employed, has produced any adverse effects. Administration of the drug for 1-2 weeks from the time of weaning, however, did cause some behaviour change, with females showing an increased mean frequency of social investigation (Treated 110 $\pm$ 32; Control 84 $\pm$ 25; $P < 0.05$) but no other behavioural difference from control animals.

These experiments thus indicate that valproate causes no significant interference with general development or behaviour at this dose level in the mouse although its short-term administration to juvenile animals can bring about some degree of behavioural damage via an as yet unknown mechanism.

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BEHAVIOURAL STIMULATION BY THE ATYPICAL DOPAMINE AGONIST SK & F 38393: STEREOSPECIFICITY AND NEUROLEPTIC SENSITIVITY

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In agreement with the view that D_2 receptors mediate most dopaminergic drug effects (Seeman, 1980), the putative D_1 agonist SK & F 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine) fails to induce stereotyped behaviour (Setler et al, 1978; Waddington et al, 1982). We have reported that this agonist (2.5-40.0 mg/kg s.c.) can induce behavioural stimulation in a non-stereotyped fashion when assessed using a novel rapid sampling behavioural check-list technique; grooming was particularly promoted (Molloy & Waddington, 1983). With the resolution of the enantiomers of this racemic compound, we have studied the stereospecificity and neuroleptic sensitivity of the actions of *R*- and *S*-SK & F 38393. Male Sprague-Dawley rats were used.

Animals were habituated to the test cage and injected s.c. with drug or vehicle. For rapid sampling by behavioural check list, each subject was observed for 5 second periods at 1 min intervals over 5 consecutive minutes and behaviours recorded for each period; they were then immediately assessed using a conventional stereotypy rating scale. This cycle repeated at 10 min intervals.

Using the behavioural check list technique, *R*- SK & F 38393 characteristically induced intense grooming, with sniffing, locomotion and rearing also being promoted. The effect of 20 mg/kg of the *R*-enantiomer was comparable with that produced by 40 mg/kg of the racemic compound; there was negligible activity in its *S*-antipode. Using the conventional stereotypy rating scale, only discontinuous behavioural stimulation was weakly noted; no typical stereotypy, such as that induced by apomorphine, was recorded.

The dopaminergic nature of these effects was investigated using the isomers of flupenthixol. Behavioural responses to *R*- SK & F 38393 were antagonised ($P < 0.05$) by 0.1-0.5 mg/kg cis(Z)-Flupenthixol given 30 min previously; 0.5 mg/kg trans (E)-Flupenthixol given similarly was without effect.

Behavioural stimulation induced by the putative D_1 dopaminergic agonist SK & F 38393 was stereospecific for the *R*-enantiomer. This response was itself stereospecifically antagonised by cis(Z)-Flupenthixol, indicating the likelihood of a dopaminergic mechanism being involved. Flupenthixol is a non-selective antagonist which cannot therefore distinguish any sub-type of receptor possibly involved in responsivity to SK & F 38393. However, we have found (O'Boyle & Waddington, this Meeting) *R*- SK & F 38393 to stereospecifically displace the binding of ^{3}H-piflutixol to D_1 dopaminergic sites, with little activity at the D_2 site labelled by ^{3}H-spiperone. The use of selective antagonists will aid clarifying the nature of these responses.

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BENZODIAZEPINE "ANTAGONIST" BLOCKS THE RELEASING EFFECT OF CHLORDIAZEPOXIDE ON A RAT CONFLICT TEST

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A conditioned suppression "conflict" test for rats has been developed. The test is sensitive to benzodiazepines and allows repeated drug treatments on a stable baseline, yet requires only 8 training sessions to establish. In the present paper we report specific blockade of the anxiolytic effects of chlordiazepoxide on this test by a benzodiazepine "antagonist" (Ro 15-1788), and of the similar effect of morphine by naloxone in the same test.

Rats (n=16) were first trained to lever-press on an FR1 schedule in a standard conditioning chamber. All animals then received a 20-minute conditioning session each day for eight days. Each session was partitioned into alternating 5-minute light (L) and 2.5-min dark (D) periods in a fixed LDLDL sequence. During both periods (L.D), every lever-press delivered a food pellet; in the dark periods only, a random 50% of lever-presses also elicited a footshock (0.6mA, 0.5s). All conditioning contingencies were controlled by BASIC programs running on a low-cost laboratory microcomputer. All drugs were administered i.p. in 0.9% saline.

Animals were drug-tested in extinction (shock off) sessions separated by 1 or 2 sessions of re-training. Results are expressed here as mean lever-pressing rates over the 5-minute dark (D) component of the session. The Wilcoxon test was used throughout for statistical comparisons. The main findings were:

(1) Selective release of lever-pressing in the 5-minute dark period by chlordiazepoxide (10 mg kg-1, i.p.) on first (25.9 +/- 2.4 s.e. presses) and final (29.4 +/- 3.7 s.e.) test days relative to saline control days (5.8 +/- 1.9 s.e., 4.6 +/- 1.6 s.e.; both effects significant at $p<0.005$), and blockade of this effect (3.1 +/- 1.3 s.e.; significant at $p<0.005$ compared with release effect) by the benzodiazepine "antagonist" Ro 15-1788 (10 mg kg-1 i.p.).

(2) Selective release of lever-pressings by morphine (3 mg kg-1) on two test days (21.8 +/- 4.0 s.e., 20.1 +/- 4.3 s.e.) relative to saline control (7.0 +/- 3.0 s.e.; either significant at $p<0.005$) and a partial blockade of this effect (11.2 +/- 3.4 s.e.; significant at $p<0.05$ compared with release effect) by naloxone (1 mg kg-1 i.p.).

In conclusion, we describe a relatively simple, rapid and reliable conditioned behavioural test for drugs with anxiolytic potential. The test is sensitive to at least two pharmacologically distinct classes of drugs (benzodiazepines and opiates) and to blockade of the effects of these drugs by their respective antagonists. An important feature of the test is that it is based upon a conditioned emotional response (CER), rather than upon immediate punishment as in the Geller-Seifter test. With this feature, the CER-based test described here may prove a useful addition to the test available for detecting drugs of different classes with anxiolytic properties.

DOTHIEPIN AND DESIPRAMINE INDUCE DIFFERENT CHANGES IN NORADRENERGIC AND SEROTONINERGIC BEHAVIOUR AFTER SUBCHRONIC TREATMENT

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Subchronic administration of the antidepressants dothiepin and desipramine to rats induces down-regulation of noradrenergic β-receptors and the receptor-coupled adenylate cyclase (Buckett & Thomas, 1982; Banerjee et al, 1977; Buckett & Diggory, 1982). Less is known either of behavioural correlates with these neurochemical findings or of the rate of recovery from such down-regulation. This communication presents evidence to show that the behavioural changes induced by dothiepin and desipramine differ both in degree and in temporal characteristics.

Male Sprague-Dawley (Charles River) rats (155 ± 10g body wt) were dosed orally twice daily with dothiepin HCl (30 mg/kg for 21 days), desipramine HCl (20mg/kg for 14 days) or 0.9% saline (control; 14 or 21 days). Animals were then tested at 2, 5, 9 and 12 days after cessation of treatment in (i) a noradrenergic (NA) behavioural model: behavioural hypoactivity induced by clonidine (0.1 mg/kg i.p.) (Drew et al, 1979) and (ii) models of 5-hydroxytryptamine(5HT)-mediated behaviour: "wet -dog" shaking induced by l-5-hydroxytryptophan (5HTP)(100mg/kg s.c. plus carbidopa 25 mg/kg i.p. 30 min previously)(Bedard & Pycock, 1977) and the syndrome induced by 5-methoxy-N,N-dimethyltryptamine (5MeODMT)(5 mg/kg i.p.)(Grahame-Smith, 1971). Dothiepin pretreatment produced, on the second day after the end of treatment, an attenuation of 50% (5HTP) and 40% (5MeODMT) in 5HT-mediated behaviours, whereas the NA-mediated clonidine response was identical to that of saline-treated rats. Normal responses were restored by the ninth (5HTP) and fifth (5MeODMT) day of withdrawal from dothiepin. In contrast, desipramine pretreatment led to total abolition of the clonidine response on the second and fifth days after termination of desipramine and it only returned to 49% of the control response by the twelfth withdrawal day. The 5HT-mediated behaviours were reduced by 41% (5HTP) and 30% (5MeODMT) on the second day of withdrawal, but were restored after five and nine days respectively.

Subchronic dothiepin administration therefore leads to a marked down-regulation of cerebral 5HT systems reversible in 5 to 9 days, whereas acute dothiepin pretreatment has little effect on 5HT-mediated behaviours. The inability of dothiepin to antagonise clonidine-induced hypoactivity is unexpected since dothiepin and all its major metabolites are more effective in vitro in inhibiting NA uptake than 5HT uptake. Under these conditions dothiepin thus appears to preferentially down-regulate 5HT mechanisms. This selectivity is in contrast to the effect of subchronic desipramine, a relatively selective inhibitor of NA uptake (Maître et al, 1979), which produced very marked changes in NA-mediated behaviour accompanied by readily reversible 5HT-mediated responses.

Thus it appears that antidepressants may down-regulate neurotransmitter systems preferentially and the offset of their effects may occur at various times after cessation of drug treatment.

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CHANGES IN BRAIN ACETYLCHOLINESTERASE ACTIVITY AFTER CHRONIC ETHANOL ADMINISTRATION AND WITHDRAWAL

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Ethanol can produce alterations in synaptic membrane composition and this may have consequences for normal brain neurotransmitter function (Littleton, 1983). Membrane-bound enzymes involved in neurotransmitter metabolism such as acetylcholinesterase (AChE), may be particularly susceptible to the effects of ethanol, although few studies have looked at this in detail (Karczmar, 1978). In this communication, the effects of chronic ethanol treatment and withdrawal on AChE activity in three cholinergic brain regions are presented.

Male Sprague Dawley rats (150-180g) were administered ethanol in their drinking water to give an approximate daily intake of 10g/kg for 28 days (Morinan, 1983). Some of these animals were withdrawn 7 days or 48h before the end of the experimental period. Brain regions were homogenized in 30mM sodium phosphate buffer/1% (v/v) Triton-X-100/pH 7.0 followed by centrifugation at 100,000g for 60 minutes. AChE activity was assayed in the supernatant using 1mM acetylthiocholine (or 0.1-1.0mM for kinetic analysis) as the substrate, and 10 µM ethopropazine to inhibit butyryl ChE (Ellman et al, 1961).

TABLE 1. Brain regional AChE after chronic ethanol administration and withdrawal

	BRAIN STEM	HIPPOCAMPUS	CORPUS STRIATUM
CON	11.7 ± 0.6 (10)	6.8 ± 0.3 (10)	28.0 ± 0.9 (6)
ALC	11.7 ± 0.8 (9)	7.5 ± 0.3 (9)	22.9 ± 1.4 (6)
48h	15.0 ± 1.7 (5)*	7.6 ± 0.5 (5)	25.7 ± 2.1 (6)
7dy	10.6 ± 0.6 (4)	7.1 ± 0.3 (4)	26.6 ± 1.5 (6)

Each value is the mean enzyme activity (µ mol product/mg protein/h) ± SEM (n)

* $P<0.05$ compared to CON, ALC and 7dy (one-way ANOVA + Tukey test).

Brain stem AChE activity was significantly increased after 48h withdrawal (Table 1) Analysis of the kinetics for striatal AChE showed that V_{max} was significantly lower in the ALC rats compared to the other groups:- (ALC) 23.7 ± 2.3, (48h) 31.9± 0.3, (7dy) 31.0 ± 2.6 and (CON) 30.7 ±0.5 µmol/mg/h (mean ± SEM for n = 3), but K_m was unaltered:- (ALC) 54 ± 3, (48h) 57 ± 3, (7dy) 52± 1 and (CON) 61 ± 6µM. At a concentration of 10mM in vitro, ethanol caused no inhibition of AChE, whereas the anticholinesterase physostigmine, had an IC_{50} value of 40nM.

These results suggest that chronic ethanol administration inhibits striatal AChE non-competitively, although this is reversible on subsequent withdrawal. It remains to be seen if this is also true after longer treatment periods, and whether there are implications for a cholinergic involvement in ethanol dependence.

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EFFECT OF CLONIDINE ADMINISTRATION ON ALCOHOL WITHDRAWAL: CHANGES IN MEMBRANE-BOUND ENZYMES

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The principal action of alcohol on the brain appears to be associated with changes in the composition of the neuronal membrane (Johnson et al., 1978). As some of the enzymes involved in neurotransmission are closely associated with such membranes, it is not surprising to find that their activities change following the chronic administration of this drug (Keane & Leonard, 1983). The aim of the present study was to see whether the changes in adenylate cyclase and calcium dependent ATP'ase that occur during alcohol withdrawal could be affected by the administration of clonidine.

Groups of Wistar rats (male 230 - 250g, N = 8 per group) were given a sweetened milk diet for a period of 15 days (Keane & Leonard, 1983). Alcohol was added to the milk solution at a concentration of 15%; the daily alcohol intake was 9-10g/kg/day. The control group was given a sweetened milk solution. After 15 days, alcohol was withdrawn. Clonidine (150 ug/kg i.p.) was then administered 16 and 8 hr. before decapitation to both the experimental and control animals. Adenylate cyclase (basal and noradrenaline stimulated) was determined in synaptosomal fractions by the method of Von Hungen (1977) and calcium ATP'ase by the method of Sulakhe et al. (1973).

The results are summarized in the Table. In the hippocampus, basal adenylate cyclase activity is unchanged following alcohol withdrawal while noradrenaline stimulated cyclase activity is reduced. In this brain area, and in the mid-brain, calcium dependent ATP'ase activity is unchanged (control value 7.2 $\pm$ 0.5 u mol Pi/mg. protein/hr.). By contrast, in the mid-brain regions changes occurred in both the basal and noradrenaline stimulated cyclase. Following alcohol withdrawal both activities are decreased; former effect was normalized by clonidine-pre-treatment. It may be concluded that the effect of clonidine is mediated indirectly via α_2 receptors.

TABLE 1. Effect of clonidine treatment on adenylate cyclase following alcohol withdrawal.
(P mol cAMP/mg protein $\pm$ S.E.N. N = 8).

	Control	Control+ Clonidine	Experimental	Experimental+ Drug
Mid-brain basal	2157.4+160.43	2231.65+144.1	1598.2+199.04**	2109.08+122.2
NA-Stimulated●	5427.6+153.4	4365.1+343.4	4372.4+191.2 **	4134.7 +254.7
Hippocampus	Control	Control+ Clonidine	Experimental	Experimental+ Clonidine
Basal	2711.76+218.9	2482.74+117.6	2645.79+267.95	2545.79+233.1
NA-Stimulated	3391.23+245.25	2228.7+135.5*	2536.18+182.7*	2238.2 +180.8*

* P 0.02 ** P 0.01 compared to control animals using Student's t-test (2-tailed. ● Noradrenaline (NA) concentration 10^{-4}M.

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THE EFFECT OF N-ACETYLHISTAMINE ON REGIONAL TRH LEVELS IN RAT BRAIN AND SPINAL CORD

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Thyrotrophin releasing hormone (TRH) is widely distributed in the CNS where evidence suggests TRH may act as a neurotransmitter (Yarbrough, 1979; Emson, 1979). Similarly, histamine may function as a neurotransmitter in the hypothalamus and other brain regions and has been shown to release TRH from hypothalamic tissue slices and synaptosomes acting via H_2 receptors (Joseph-Bravo et al, 1979; Bennett, 1981; Barraclough et al, 1983). Acetylhistamine treatment has been reported previously to be more effective than histidine loading to increase rat brain histamine (Endo, 1979) and in the present study regional changes in rat brain TRH and histamine have been determined following acetylhistamine.

Male Wistar rats were injected with 0.5 ml N-Acetylhistamine (800 mg/Kg i.p.) or vehicle saline. After 45 min the rats were killed and TRH measured by radioimmunoassay in various brain and spinal cord regions. Histamine was measured by the isotopic microassay of Taylor and Snyder (1972) in similar brain regions from separate groups of animals.

Table. Effect of N-Acetylhistamine on TRH in specific brain regions.

Treatment	Hypothalamus	Nucleus accumbens	Septum	Brain stem	Hippocampus
Saline control	913±104	327±45	596±87	71±12	18±3
N-acetyl-histamine	652^{+}±87	168*±31	576±73	63.5±9	6.5*±2.5

Results given as pgTRH/mg protein±SEM (n=6-8) *$p<0.02$ $^{+}p<0.05$

Acetylhistamine treatment caused a marked reduction in the TRH content of the nucleus accumbens and hippocampus with a smaller depletion in hypothalamus. The histamine content of these brain regions (pg/mg tissue; SEM (n=6)), nucleus accumbens (169±70), hippocampus (81±3.5) and hypothalamus (679±14) was increased by 83%, 20% and 2%, respectively, following acetylhistamine. In contrast, TRH in other brain regions, including the septum, striatum and brain stem, and in dorsal or ventral cervical and lumbar spinal cord showed no change.

The present results indicate possible functional relationships between histamine and TRH in the nucleus accumbens and hippocampus. It remains to be determined whether the changes in TRH content of these regions results from histamine-induced increased release and metabolism of peptide as shown previously in the hypothalamus, or alternatively, is due to decreased TRH synthesis.

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5-HYDROXYTRYPTAMINE, OPIOID PEPTIDES AND THE MODULATION OF BURSTING ACTIVITY IN RAT ARCUATE NEURONES IN VITRO

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Bursting activity in peptidergic neurones is advantageous for the release of their neurosecretory product. The arcuate nucleus, which is rich in peptidergic neurones, and in which some neurones display bursting activity (MacMillan, 1983), has several opioid peptide containing cell groups and also 5-hydroxytryptamine within nerve terminals. Since these substances are implicated in the release of several hypothalamic peptides we have examined the effects of an opioid peptide and 5-hydroxytryptamine on neurones displaying bursting activity in the arcuate nucleus in vitro.

Extracellular activity was recorded from 400μm thick coronal hypothalamic slices taken from lactating Wistar rats. D-ala-D-leu-enkephalin (DADLE), 5-hydroxytryptamine (5HT) or their respective antagonists, naloxone and methergoline were added to the perfusion medium at a concentration of 10μM.

The first group of neurones showed solely bursting activity. The burst characteristics were: 6 spikes (range 3-11), duration 62ms (range 23-211), and intraburst interspike intervals of 12ms (range 9-24). These neurones (n=16) were profoundly inhibited by DADLE; their mean frequency of discharge decreased from 4.1±1.4 to 0.0 spikes/sec. In contrast 5HT increased the firing rate of 8 bursting neurones from 4.3±1.6 to 6.4±1.8, which was due to an increase in burst generation.

The second group (n=19) of neurones studied showed bursts of activity superimposed on a background of irregular discharge. The burst characteristics were: 5 spikes (range 3-10), duration 58ms (range 27-291), and intraburst interspike intervals of 15ms (range 12-31), with 15% of the total activity occurring as bursts. When DADLE was applied to these neurones there was a reduction in the mean firing rate from 5.2±1.4 to 4.5±1.2 spikes/sec, due entirely to a suppression of the bursting component of the activity. The number of interspike intervals of less than 40ms was reduced from 16.0±7.4% to 4.8±3.3% ($P<0.01$). This group of neurones (n=12) was also excited by 5HT which caused an increase in bursting activity: this is reflected by the number of interspike intervals of less than 40ms which increased from 10.1±3.8% to 24.2±6.1% ($P<0.01$). The overall firing frequency increased from 5.4±1.7 spikes/sec to 6.5±1.3 spikes/sec. In 6/6 tests the effects of DADLE were reversed by the concurrent administration of naloxone, and bursting activity increased when naloxone was administered alone. In 4/4 tests the actions of 5HT were antagonised by the concurrent administration of methergoline, and bursting activity was either suppressed or abolished with methergoline alone.

These results show that 5HT can facilitate and an opioid peptide can suppress burst generation in some arcuate neurones. The presence of both 5HT and endogenous opioid peptides in the arcuate nucleus, and the observation that their antagonists administered alone affected inherent bursting activity, suggests that these agents might be responsible for the endogenous bursts displayed by these two sub-populations of arcuate neurones.

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THE PHARMACOLOGY OF ADENOSINE AND DIPYRIDAMOLE ON EXCITATORY TRANSMISSION IN THE GUIEA-PIG OLFACTORY CORTEX

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Adenosine depresses the compound excitatory potential evoked from slices of guinea-pig olfactory cortex without any effect on membrane resistance or potential (Scholfield, 1978). However, with this tissue, cellular uptake of drugs can severely depress the potency of drugs (Galvan and Scholfield, 1978) so further experiments on adenosine action have been performed in the presence of the purine uptake inhibitor dipyridamole.

These experiments were performed on 600 µm thick surface slices of guinea-pig olfactory cortex maintained in an incubating bath through which Krebs solution flowed at 25°C (Scholfield, 1981). A pair of silver wires were placed across the lateral olfactory tract (LOT) for continuous orthodromic stimulation at 0.1 Hz. Compound evoked potentials were recorded from the pial surface of the slice over the pyriform area via a single saline-filled glass pipette and an indifferent electrode in the subjacent solution. Adenosine and its analogues were applied to the preparation via the bathing solution for 4 min periods. Other drugs were added 15 min before the purines.

Adenosine (0.1 - 100 µmol/l) alone depressed the amplitude of the compound monosynaptic e.p.s.p. by up to 80% in a dose-related manner. In the presence of dipyridamole (1 µmol/l), the potency of adenosine was increased: a shift in the dose-response curve to the left from 46±17 to 3.2±0.8 µmol/l (mean ± SEM concentration producing a 40% depression of the monosynaptic e.p.s.p. in 6 slices). Dipyridamole was also tested on the effect of the uptake resistant adenosine analogue, cyclohexyladenosine. Contrary to the potentiating effect of dipyridamole on adenosine, cyclohexyladenosine action was antagonised: the cyclohexyladenosine dose-depression curve was shifted to the right by dipyridamole from 0.39±0.08 to 1.63±0.83 µmol/l (mean±SEM concentration producing a 40% depression in 8 slices). Dipyridamole also antagonised another adenosine analogue, L-N^6-phenylisopropyladenosine. Addition of a low concentration of adenosine to replicate the depressant effect of 1 µmol/l dipyridamole alone had no effect on the action of cyclohexyladenosine.

Dipyridamole (1-100 µmol/l) alone depressed the compound e.p.s.p. in a manner similar to the purines. This effect was antagonised by 8-phenyltheophylline (3 µmol/l) suggesting that dipyridamole might be acting at the adenosine receptor.

These results indicated that adenosine potency is probably reduced by its own uptake. However, because of the two secondary actions of dipyridamole of adenosine receptor activation and blockade the usefulness of dipyridamole as a specific purine uptake inhibitor for brain is suspect.

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LOCUS OF $GABA_B$ SITES IN THE SPINAL CORD

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The anti-spasticity effect of baclofen is believed to reside in the spinal cord (Bein, 1972). In addition the analgesic activity of this drug may also result from an action within the cord (Cutting and Jordan, 1980). Since baclofen is a selective ligand for $GABA_B$ sites (Bowery et al. 1981: Hill and Bowery, 1981) and $GABA_B$ sites have been implicated in GABA mediated analgesia (Bartolini et al, 1981: Sawynok and LaBella, 1982) we have examined the location of these sites in rat spinal cord. A dry-mounting autoradiographic technique has been employed to visualise ^{3}H-GABA binding sites. Incubation conditions were the same as previously described for the detection of $GABA_A$ and $GABA_B$ sites in the cerebellum. 10 µm sections were incubated for 20 min at room temperature and then rinsed twice briefly in Tris solution. After allowing to air-dry, emulsion-coated coverslips (Ilford K5 emulsion) were placed in close apposition. The emulsion was developed after 14-21 days' exposure and the grains visualised under dark-field optics. $GABA_A$ and $GABA_B$ binding sites could be detected throughout the grey matter of the spinal cord. Results of the grain counts are summarised in Table 1.

Table 1 Distribution of $GABA_A$ and $GABA_B$ sites in spinal cord

Laminae	$GABA_A$	$GABA_B$	
I,II,III	14.7 + 1.2	50.1 + 7.8	Specific grains/
IV	11.0 + 3.8	23.3 + 6.2	1000 sq µm
V	11.9 + 3.2	10.1 + 1.9	
VI	10.3 + 2.0	10.4 + 1.6	
VII	11.7 + 2.3	9.1 + 2.3	
VIII	6.6 + 3.0	9.6 + 3.3	
IX	9.0 + 3.9	7.8 + 2.3	Data from 4 rats
X	12.5 + 2.0	13.3 + 4.2	3 areas/lamina/animal

$GABA_B$ sites appear to be concentrated in the substantia gelatinosa whereas $GABA_A$ sites show no such specificity. It is established that activation of $GABA_B$ sites on peripheral nerve terminals can reduce neurotransmitter release. If these sites are located presynaptically in the cord they may be of significance in the modulation of sensory transmitter output. The effect of neonatally administered capsaicin on $GABA_B$ site distribution has therefore been examined because this drug produces a selective degeneration of sensory afferents in the cord (Jancso et al, 1977). Capsaicin was injected according to the regimen of Faulkner and Growcott (1980). Four months after treatment the concentration of $GABA_B$ sites in the substantia gelatinosa was reduced by 40-50% when compared to control littermates. This suggests that a proportion of $GABA_B$ sites are present on sensory afferent terminals.

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EFFECT OF SOME TETRAHYDRONAPHTHALENES ON CNS DOPAMINE ACTIVITY

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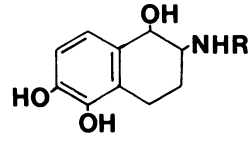
A series of 2-amino-1,5,6-trihydroxy-1,2,3,4-tetrahydronapthalene derivatives, synthesized by Itoh et al (1977), were tested for dopamine agonist activity.

The tests used were the rat striatal dopamine sensitive adenylate cyclase (Kebabian et al., 1972); locomotor activity following microinjection into the nucleus accumbens of conscious rats (Elkhawad and Woodruff, 1975) and the ability of the drugs to displace ^{3}H-sulpiride binding in striatal homogenates (Freedman et al., 1981). The results are shown in Table 1.

Table 1 The effect of Drugs on Dopamine Activity



Drug	R		Adenylate Cyclase EC_{50} (μM)	Locomotor Activity I	D(hrs)	Displacement of ^{3}H-sulpiride IC_{50} (μM)
Dopamine	-	-	4.68	Not tested		0.40
ADTN	-	-	3.50	high	18	0.016
T15520	H	trans	17.00	high	19	3.16
T15512	H	cis	inactive	low	3	> 10
T14893	CH_3	trans	18.00	medium	11	8.90
T14895	CH_3	cis	14.00	medium	11	-
T18771	cyclobutyl	trans	inactive	low	2	> 10
T14891	$C(CH_3)_3$	trans	inactive	low	2	> 10

EC_{50} is the dose required to produce 50% of the maximum stimulation (100 μM dopamine) of adenylate cyclase. IC_{50} is the dose displacing 50% of specifically-bound ^{3}H-sulpiride. I = intensity and D= duration of locomotor stimulation produced by the bilateral injection (85 nmol each side) of the drug into the nucleus accumbens.

On the dopamine sensitive adenylate cyclase and on the ^{3}H-sulpiride binding, T15520, T14893 and T14895 all showed activity, although they were less active than ADTN (Elkhawad and Woodruff, 1975). However T15520 was equipotent with ADTN in stimulating locomotor activity, suggesting that factors other than direct dopamine receptor stimulating activity contribute to potency in this test.

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INTERACTION OF THE ENANTIOMERS OF 3-PPP WITH POSTSYNAPTIC DOPAMINE-SENSITIVE ADENYLATE CYCLASE IN CARP RETINA

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It has been suggested that the phenylpropylpiperidine derivative 3-(3-hydroxyphenyl)-N-n-propylpiperidine (3PPP) may interact selectively with dopamine (DA) autoreceptors in the central nervous system (Hjorth et al., 1981). In support of this hypothesis, (±)-3PPP possesses no agonist activity at postsynaptic DA receptors associated with cyclic AMP production in carp retina (Watling and Williams, 1982). However, recent experiments have shown that (±)-3PPP can inhibit DA-stimulated cyclic AMP accumulation in this tissue (Watling et al., 1982). In the present study, the inhibitory effects of the enantiomers of 3PPP have been examined on the postsynaptic DA-sensitive adenylate cyclase of carp retina.

Cyclic AMP accumulation was measured in intact pieces of carp retina as previously described (Dowling and Watling, 1981). While 10 µM DA evoked an approximate 3-fold increase in cyclic AMP accumulation, from 258.2 ± 9.2 pmol cyclic AMP/mg protein (n=15) to 642 ± 59.2 pmol cyclic AMP/mg protein(n=18), neither (+)-3PPP nor (-)-3PPP stimulated cyclic AMP accumulation at concentrations up to 300 µM. However, when examined for its ability to antagonise 10 µM DA-stimulated cyclic AMP accumulation, (-)-3PPP induced a dose-dependent inhibition of the DA response, with 50% inhibition occuring at a concentration of approximately 20 µM. In marked contrast, (+)-3PPP did not antagonise 10 µM DA-stimulated cyclic AMP accumulation at concentrations up to 300 µM.

The inhibitory effects of (-)-3PPP were also examined on DA-stimulated adenylate cyclase activity in homogenates of carp retina using previously described techniques (Watling and Dowling, 1981). At concentrations up to 100 µM, (-)-3PPP induced a dose-dependent inhibition of 10 µM DA-stimulated adenylate cyclase activity, with 50% inhibition of the DA response again occuring at a concentration of approximately 20 µM. These data yielded an apparent Ki value for (-)-3PPP of 1.8 X 10^{-6}M. In experiments where the DA dose response curve was repeated in the presence of a single, fixed concentration of (-)-3PPP (30 µM), this enantiomer induced a parallel shift to the right of the DA dose response curve indicating that (-)-3PPP acts as a competitive inhibitor. From this series of experiments, the apparent Ki value for (-)-3PPP was calculated to be 0.64 X 10^{-6}M. As was the case in the intact retina, (+)-3PPP was without effect on 10 µM DA-stimulated adenylate cyclase activity at concentrations up to 300 µM.

These data confirm that the putative selective DA autoreceptor agonist 3PPP is a weak competitive antagonist at postsynaptic cyclase-linked DA receptors in carp retina. Furthermore, this antagonist activity would appear to reside in the (-)-enantiomer of this compound.

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A STUDY OF MESOLIMBIC DOPAMINE AUTORECEPTOR FUNCTION USING IN VIVO VOLTAMMETRY

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Evidence from electrophysiological studies suggest the presence of dopamine (DA) autoreceptors on the cell bodies and dendrites of ascending DA neurones in the substantia nigra (SN) and ventral tegmental area (VTA). Stimulation of the receptors with dopamine agonists inhibits the firing of the DA neurones whereas DA antagonists have an excitatory effect (Groves et al, 1975). Similarly, infusion of dopamine into the SN of rats decreases ^{3}H-DA release in the ipsilateral caudate nucleus (Cheramy et al, 1977) whilst haloperidol causes a small increase in release (Nieoullon et al, 1979). The present study has used in vivo voltammetry to monitor dopamine turnover in the n. accumbens of anaesthetized rats after infusion of DA or haloperidol into the ipsilateral VTA.

Male Sprague Dawley rats (280-340 g) were anaesthetised with chloral hydrate (600 mg/kg i.p.) and stereotaxically implanted with 23 gauge stainless steel guide cannulae 3 mm dorsal to the VTA and a carbon fibre microelectrode (Gonon et al, 1980) in the ipsilateral n. accumbens. Voltammograms were recorded every 4 mins and after a stabilisation period drugs were administered to the VTA via 31 gauge stainless steel injection cannulae.

Combining the techniques of in vivo voltammetry and intracerebral dialysis we have recently confirmed the suggestion (Gonon et al, 1980) that electrically pretreated carbon fibre microelectrodes used with differential pulse voltammetry are capable of closely monitoring levels of the DA metabolite dihydroxyphenylacetic acid (DOPAC) in vivo. In the present study the DOPAC signal in the n. accumbens increased after infusion of haloperidol (2.5 μg/0.5 μℓ) into the ipsilateral VTA to a maximum of 48%±10 S.E. above control levels 80 mins post-infusion (n=6). A higher dose of haloperidol (10 μg/2 μℓ) produced a similar maximum increase (48%±15 S.E.) 90 mins after infusion but this was preceded by a transient decrease (19%±5 S.E.) in DOPAC levels (n=5). Infusion of DA (100 μg/0.5 μℓ) produced a maximal decrease in the DOPAC signal of 36%±6 S.E. within 20 mins (n=5). However, this was followed by a large but variable (50-100%) increase above baseline levels. The initial decrease in DOPAC levels in the n. accumbens after infusion of DA into the ipsilateral VTA and the increase after haloperidol (2.5 μg) support evidence for autoreceptor control of the mesolimbic DA system. It is likely that the transient decrease in DOPAC levels produced by the higher dose of haloperidol is a result of a local anaesthetic effect (Groves et al, 1975) although it is also possible that it reflects an action on other transmitter receptors within the VTA. Whilst it is tempting to speculate on the mechanism responsible for the secondary increase in DOPAC levels after DA infusion into the VTA it must be realised that diffusion of infused DA to the n. accumbens may have occurred. This possibility is currently under investigation.

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A STUDY OF SOME BRAIN AREAS INVOLVED IN THE MYOCLONUS PRODUCED BY INTRASTRIATAL ADMINISTRATION OF PICROTOXIN

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Picrotoxin injected into the striatum of conscious rats causes myoclonic jerking of the contralateral forelimb (Slater & Dickinson,1982). We have investigated the brain areas involved in this motor response.

Female Sprague Dawley rats (180-190g) were anaesthetized and fitted with a vertical guide cannula for injection into one striatum (A 8.5, L 2.6, H -1.0;slightly modified from König & Klippel,1963). Picrotoxin (1 µg) caused sustained, regular myoclonic jerking of the opposite forelimb in the conscious animal. The latency, frequency and intensity (on a scale of 0-4; Slater & Dickinson,1982) were recorded (Table 1). The volume injected was 1 µl in all cases.

Rats received 500 µCi/kg i.v. of 2-fluoro-D-[5,6-^{3}H]-glucose (2-DG) 5 min after an injection of picrotoxin into the striatum. The rats (n=3) were killed 45 min later and frozen brain sections were exposed to tritium-sensitive film for 4 weeks. The autoradiographs showed a unilateral increase in 2-DG uptake by the frontal cerebral cortex on the injected side - the change was most pronounced in sections cut at the level A 9.2. 2-DG uptake by the ventro-lateral (VL) thalamus and subthalamic nucleus (STN) was increased on the injected side.

Table 1 Effects of brain lesions on myoclonus

	N	Latency (min)	Frequency (jerks/min)	Intensity (0 - 4)
Striatal picrotoxin				
Normal	12	3.5 ± 0.1	27.0 ± 1.4	3.5 ± 0.1
STN lesion	6	4.6 ± 0.8	14.8 ± 3.4*	1.9 ± 0.4*
VL thalamus lesion	6	4.9 ± 0.5	15.4 ± 1.3*	3.0 ± 0.1
Cortical picrotoxin				
Normal	12	17.3 ± 0.8	14.3 ± 0.1	2.2 ± 0.2
STN lesion	6	18.4 ± 2.0	14.7 ± 1.3	2.7 ± 0.2
VL thalamus lesion	6	17.3 ± 0.9	5.8 ± 0.7*	1.4 ± 0.2

* $P < 0.05$

Picrotoxin (2 µg) placed on one cortical surface (A 9.2, L 3.0) produced myoclonus after a long latency (Table 1). When placed above the striatal injection site, picrotoxin was less effective (frequency 11.9 ± 0.6/min; intensity 1.5 ± 0.2, n=6). A unilateral STN electrolesion reduced the myoclonus induced by intrastriatal picrotoxin without affecting the cortically-evoked response (Table 1). A lesion in the VL thalamus reduced the mean frequency of myoclonus evoked from both sites.

Myoclonus produced by intrastriatal picrotoxin probably involves cortical mechanisms. Our findings, especially with STN lesions, suggest that the striatum can initiate myoclonus independently of the cortex and lend little support to the proposal that myoclonus is caused by leakage of picrotoxin from the striatum to the cortex (Palfreyman et al, 1980).

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TETRAHYDRO-β-CARBOLINE (THBC), AN ANTAGONIST OF TRYPTAMINE-INDUCED CONTRACTIONS IN PERFUSED RAT TAIL ARTERY

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The tetrahydro β-carbolines (THBC's) are formed endogenously in mammals and have been linked with a neuromodulator function in brain and peripheral tissues particularly in relation to indoleamine neuronal pathways (see reviews Buckholtz, 1980 ; Airaksinen and Kari, 1981). Recently THBC has been shown to have a high affinity for ^{3}H-tryptamine but not ^{3}H-5HT binding sites (Cascio and Kellar, 1982) although it is not known whether this represents an agonist or antagonist interaction. We now report on the antagonist properties of THBC against tryptamine-induced contractile effects in the perfused rat tail artery. The results are discussed in terms of differences between 5HT and "tryptamine"-receptors in this preparation.

Male Sprague-Dawley rats (300 - 350 g) were used. Segments of proximal tail artery were removed under pentobarbital anaesthesia (60 mg/kg, i.p.), cannulated at both ends, and perfused and superfused with Krebs' bicarbonate. Propranolol (1 μM), indomethacin (2.5 μM) and pargyline (10 μM) were routinely present. Increases in perfusion pressure (mmHg) were measured in response to increasing molar concentrations of the agonists. One concentration of antagonist was employed in each preparation (30 min contact), n=4-7.

Tryptamine and 5HT contracted the rat tail artery. The EC_{50}'s (increase in perfusion pressure by 50% ± 95% confidence limits) were 1.52 (1.02 - 1.85) and 0.076 (0.061 - 0.094) μM respectively. Both the Emax (mmHg) for 5HT (142 [128 - 162]) and slope of the dose-response curve (91.7 [79.6 - 103.8]) were significantly different ($p<0.05$) from those obtained with tryptamine 108 [93 - 122] and 58.5 [51.1 - 65.9] respectively. The unsubstituted THBC did not act as an agonist over the concentration range 0.1 - 100 μM, but was a weak competitive antagonist of tryptamine induced responses (pA_2 5.4, slope 0.95, r=0.95). In contrast, THBC (30 μM) caused only a small rightward displacement of the 5HT dose-response curve (log DR 0.39 ± 0.05) which was not further increased at 100 μM. THBC (1, 10 and 100 μM) progressively antagonised responses to periarterial field stimulation (1 - 30 Hz, 0.3 ms, 40 V) and responses to phenylephrine, indicating α_1-adrenoceptor blocking properties of the compound. THBC did not, however, antagonise the responses induced by K^+ (40 mM).

Prazosin (100 nM) in combination with the α_2-receptor antagonist RX 781094 (100 nM) failed to antagonise the contractile responses to tryptamine. Dose-response curves to 5HT were progressively displaced to the right by methysergide (1 and 3 nM) with a reduction in the maximum effect at 30 nM (log DR = 2.52 ± 0.2). The "apparent pA_2" for methysergide was 9.3 with a slope of 1.4. In contrast to the results on 5HT, methysergide (30 nM) caused only a small rightward shift of the tryptamine dose-response curve with a depression of the maximum effect (log DR = 1.30 ± 0.04). This antagonism against tryptamine was not further increased by methysergide at 300 nM (log DR = 1.34 ± 0.26).

These results suggest that the responses to tryptamine on the smooth muscle of the rat tail artery are not mediated by classical 5HT receptors which apparently mediate the response to 5HT in this preparation. THBC is a weak competitive antagonist of tryptamine, but not of 5HT. It is possible that the affinity of THBC for the ^{3}H-tryptamine binding sites in the central nervous system represents an antagonist interaction. In addition our results provide evidence for a distinct "tryptamine" receptor in vascular smooth muscle, which mediates constriction.

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