BIPHASIC INOTROPIC RESPONSE TO HISTAMINE IN GUINEA-PIG RIGHT VENTRICULAR MUSCLE.

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Histamine produces a positive inotropic response in guinea-pig right ventricular strips which is depressed by cimetidine (Shigenobu et al. 1979). The poorly selective H_1 agonist, 2-pyridylethylamine, generates a qualitatively similar effect but only at high does (Verma and McNeill,1979) and that possibly by evoking catecholomine release. In left ventricular papillary muscle the increased tension produced by histamine is completely abolished competitively by cimetidine or metiamide and in the presence of H_2 receptor antagonists histamine generates a negative inotropic response which can be blocked by mepyramine (Wilson 1980). An attempt to clarify the differential role of H_1 and H_2 receptors in inotropic responses in right ventricular strips is reported.

Strips of male Dunkin-Hartley guinea-pig right ventricular muscle (14 x 2 mm) cut parallel to the orientation of the fibres were suspended vertically in Krebs-bicarbonate buffer at pH 7.4, gassed with 5% CO_2 in O_2, and held at 25.0 ± 1°C. The tissues were electrically paced via a platinum electrode at the apical end of the strip, with square wave pulses of 5.0 ms duration, 0.5 H_3, using a voltage 50% above threshold (generally between 0.4 - 0.8 V). Isometric tension was recorded via a suture at the basal end. Responses to single log unit sequential increases in histamine concentration were monitored first alone and then in the presence of antagonist.

In the right ventricular strip at 25°C, histamine produced dose-dependent increases in force of contraction between 10^{-8} and 10^{-5}M with EC_{50}ca. 3.6 x 10^{-7}M. Interestingly however, the nature of the positive inotropic response was also dependent on concentration. Below 10^{-6}M histamine caused monophasic responses, but above this concentration biphasic positive responses were produced in all preparations (n=5). Mepyramine (10^{-7}M) had little effect on these responses but cimetidine (10^{-4}M) completely abolished the monophasic responses to the lower doses of histamine and converted the responses to higher doses to biphasic responses comprising an initial negative phase followed by a positive phase. Mepyramine (10^{-7}M) abolished the negative phase of the inotropic response in the presence of cimetidine at all but the highest (10^{-3}M) histamine concentrations. All positive responses to histamine at concentrations up to 10^{-5}M observed in the presence of cimetidine were also completely abolished by the further addition of mepyramine. These results suggest that the inotropic response to histamine in the right ventricular strip may involve 3 components: an H_2-receptor mediated positive phase, responsible for the whole of the response to low doses of histamine and predominant in responses to higher doses of histamine; an H_1-receptor mediated negative phase, unmasked only in the presence of an H_2-receptor antagonist; and an H_1-receptor mediated positive phase, which contributes little to the overall positive response mediated by H_2-receptors.

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CONTRACTILITY OF GRANULATION TISSUE TO OXIDISING AGENTS AND FERROCHROMIUM METAL-FUME COMPONENTS.

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It has been shown that strips of granulation tissue respond to certain pharmacological agents by contracting (Majno et al., 1971). This contractile activity has been attributed to the development of myofibroblast cells: contractile fibroblasts, (Gabbiani & Montandon, 1977). Deposition of particulate material, from welding-fumes in skeletal muscle or lung tissue, has been shown to provoke fibroblast proliferation and collagen fibrosis (Hicks et al., 1983). These effects are believed to be due to the irritancy and fibrogenicity of fume or flux constituents, particularly hexavalent chromium, silica and manganese dioxide. The possibility that myofibroblasts may also develop has been investigated by attempting to demonstrate contractility to the unusual and characteristic range of pharmacological agents capable of such activity, e.g. mepyramine, 5-hydroxytryptamine. In case fume components, especially those in an oxidised state, might also provoke such contraction, chromates and other oxidising agents have been investigated.

Granuloma pouches were induced in male rats (CSE strain, 250-300g) by the dorsal subcutaneous injection of 20 ml of air, followed by injections of croton oil (0.1 ml in 1.0 ml corn oil) or welding fume particles (stainless steel, containing iron, chromium, nickel, manganese and silicon as oxides) 50 mg in 0.5 ml corn oil. Welding fume particles were generated and collected by the method of Hewitt et al. (1978). At 10 to 22 days after treatments, animals were sacrificed and granulation tissue dissected from around the sac or fume deposit. 2 cm strips were cut, weighing 0.12 ± 0.02g, and superfused with Krebs solution (37°C, 95% O_2, 5% CO_2) at a rate of 2 ml min^{-1}. An initial tension of 1g was applied and the tissue allowed to equilibrate for 1 hour. Contractions were measured by transducer, when agonists were added to superfusate.

Croton oil-induced granulation tissue strips responded with dose-related, reversible contractions to potassium dichromate and chromium trioxide (1-8 mg doses) as well as the known effects of mepyramine (1-8 mg). No contractile effects were produced by other fume components as soluble salts (e.g. nickel chloride), nor by a solution of chromic chloride. As the positive effect of dichromate or chromium trioxide suggested an association with oxidising activity, hydrogen peroxide was tested; in a concentration range of 0.8M to 8.8M it was found to produce reversible, dose-related contractions. Granulation tissue strips from welding-fume particle deposits were reactive to both mepyramine and hydrogen peroxide but less to potassium dichromate or chromium trioxide. Reactivity was less than croton oil-induced tissue and, unlike the latter, did not increase with time. Some contractile activity is therefore present in granulation tissue influenced by fibrogenic metal fume components but is weak, either because fewer myofibroblast are induced or because their growth is inhibited by toxic components. One type of myofibroblast contractile reactivity would appear to be associated with oxidative activity.

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PRO-INFLAMMATORY EFFECTS OF CYCLIC KININ ANALOGUES.

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The cyclic analogues of bradykinin (BK) Σ-cyclo (Lys^1, Gly^6)-BK (cyclo-BK) has been reported to be less active than BK at increasing vascular permeability in guinea pig and rabbit skin while being more potent than BK in rat skin and paw oedema (Whalley, 1985). In this study, we have compared the relative potency of BK and two cyclic analogues, cyclo-BK and cyclo-kallidin (cyclo-KD) at inducing oedema, hyperalgesia and, as a measure of blood flow, increased temperature in rat paws.

Groups of 6 female Alderley Park strain rats (approx. 60g) were given subplantar injections of kinin solutions in saline (right paw) and saline alone (left paw). Paw volume and nociceptive pressure threshold were measured pre- and 5 to 60 min post-injection as previously described (Haworth and Carey, 1985). Paw temperature was recorded using a digital thermometer connected to a thermocouple placed on the dorsal surface of the paw. Results were expressed relative to the control paw at each time point.

BK induced dose-dependent oedema which was maximal at 5-20 min post-injection and constant thereafter. The dose required to increase paw volume by 15% at 20 min (ED_{15}) was 1×10^{-9} mole. Cyclo-BK and cyclo-KD caused similar oedema but were more potent (ED_{15}, 2×10^{-11} mole and 4×10^{-11} mole respectively). BK induced dose-dependent hyperalgesia which was maximum at 5 min but had disappeared by 10-20 min. The dose required to reduce pressure threshold by 25% at 5 min (ED_{25}) was 1×10^{-8} mole. Cyclo-BK and cyclo-KD did not induce hyperalgesia at the highest concentration tested. (1×10^{-9} mole and 1×10^{-8} mole respectively). Paw temperature increased to a peak at 5-10 min after BK injection and thereafter declined to baseline. This effect was dose related and the dose which elevated paw temperature by 10% at 5 min (ED_{10}) was 6×10^{-9} mole. Cyclo-BK and cyclo-KD similarly increased paw temperature but were more potent (ED_{10} 3×10^{-10} mole and 1×10^{-9} mole respectively).

The relative lack of hyperalgesic activity of the cyclic kinin analogues compared with BK raises the possibility of differences between kinin receptors mediating permeability and blood flow changes and those involved with nociception in this model.

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ANTAGONISM OF THE ALGESIC ACTION OF BRADYKININ ON THE HUMAN BLISTER BASE.

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Kinins have been shown to reproduce the basic symptoms of inflammation in several species the algesic action in man of this group of peptides being first described by Keele et al. (see Keele & Armstrong, 1964). This study investigates, using the human blister base, the algesic action of bradykinin (BK) and compares it with the selective B_1 agonist des-Arg^9-BK (Regoli & Barabé, 1980) and the apparently selective hypotensive Σ-cyclo-(Lys^1-Gly^6)-BK (Cyclo-BK) and Σ-cyclo-Kallidin (Cyclo-KD) (Chipens et al., 1981). The effect of the B_1 + B_2 receptor antagonist B3824 (Vavrek & Stewart, 1985) and the relatively selective B_2 antagonist, S2441 (Whalley et al., 1984) on BK induced algesia was also investigated.

The blister base technique described by Keele et al. (see Keele & Armstrong, 1964) refined by Foster and Weston (1986) was used. Seven healthy male volunteers were used in the study. Eight blisters were raised overnight on the shaven dorsal surface of the forearm by applying a lipid based ointment containing 0.2% cantharidin. The following morning the blister was deroofed and cleaned with a physiological salt solution. The blister bases were maintained overnight as described by Foster & Weston (1986).

One or two drops ($\sim$ 0.05 ml each) of a drug in solution were applied to a blister base by means of a Pasteur pipette and the subject scored any pain produced. Pain intensity was assessed on an arbitary scale based on the following subjective reference standards 0=no pain; 2=detectable pain; 4=mild pain; 6=moderate pain; 8=severe pain; 10=very severe pain (as defined by a KCl 400 mmol/ℓ experience during training). A continuous visual readout of scores was achieved using a modified single channel pen recorder (Smiths) operated by the subject. After a preliminary training session using concentrations of KCl(6.25-400 mmol/ℓ) dose-response curves were produced to each kinin and the effect of B3824 and S2441 on BK investigated over the 2-day period. At least 4h was allowed between drug applications to any one blister base.

BK produced a dose-related characteristic slow onset, rate of rise and fall of response. From the agonist studies, taking 10 as the maximum score, the mean pD_2 values (-log of the concentration giving a score=5) for each kinin were BK, 6.55; Cyclo-BK, 3.45; Cyclo-KD, 3.35; des-Arg^9-BK, 2.2; (n=at least 4). Both B3824 (10^{-4}M) and S2441 (10^{-3}M) produced parallel rightward significant ($p < 0.05$) shifts of the dose response curves to BK, the mean pD_2 values for BK in the presence of each antagonist being 5.14 and 5.55 respectively. KCl responses were unaffected by B3824 or S2441.

The relative lack of algesic activity of des-Arg^9-BK and the antagonism of BK by both B3824 and S2441 provide evidence for a B_2 receptor mediating the algesic action of BK. The low potency of the cyclic kinin analogues compared to BK may indicate differences in kinin receptors mediating pain and those involved in blood flow changes (Chipens et al., 1981).

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THE DEXTRAN REACTION AND IgE IN THE RAT

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Parenteral injection of the glucose polymer dextran into rats produces an anaphylactoid reaction of oedema and hyperaemia in specific shock organs with progressive hypotension, haemoconcentration and hypothermia, mediated by release of histamine and 5 hydroxytyptamine from tissue mast cells. In vitro studies have suggested, on the one hand, that dextran may interact with glucose receptors on the mast cell membrane (Moodley et al 1982; Leoutsakos et al 1984), and on the other, that the polymer may act via combination with cell-fixed IgE (Hanahoe 1984). The experiments reported here were undertaken to study the effects of IgE on dextran induced histamine release using three in vivo models of the dextran anaphylactoid reaction.

Female Wistar rats from three populations were used: (1) High Titre IgE animals (150 - 300 g, Tuck, Rayleigh, Essex) sensitised to the nematode N.braziliensis (5000 larvae subcutaneously on days 1 and 30) and used within four months of sensitisation: (2) Control animals (150 - 200 g, Tuck): (3) Low Titre IgE animals (150 - 180 g, Olac, Oxfordshire) barrier maintained and isolator reared Category **** rats used within 24 hr of delivery. The sensitivity of these animals to dextran (Pharmacia T70) was assessed by measuring the haematocrit 20 min after intravenous administration (dose-range 15 mg Kg^{-1} to 300 mg Kg^{-1}), the foot volume 30 min after the subcutaneous injection of dextran into the left hind paw (0.1 mg to 10 mg) and the extravasation of blue dye following intravenous injection of Evans blue and intradermal administration of dextran into shaved dorsal skin (0.03 mg to 30 mg) 30 min previously.

The results showed that in each of these experiments the High Titre IgE rats developed a significantly greater reaction ($p < 0.05$), whilst the Low Titre IgE rats developed a significantly reduced ($p < 0.05$) reaction to dextran compared with the Control animals. These data indicate therefore, a relationship between the level of cell bound IgE and dextran induced histamine release in the three populations of rat studied and support the hypothesis that IgE plays a role in the dextran response. However, Category **** isolator reared rats, kept in a sterile environment and exposed to very limited antigenic stimulus, and which therefore display very low levels of immunoglobulin, developed an anaphylactoid response, albeit reduced compared to controls, on injection of dextran. This observation suggests that whilst the dextran reaction may involve cell-fixed IgE, there is also a likelihood that the glucose polymer may additionally interact with a non IgE receptor on the rat mast cell surface.

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EFFECT OF COTTON BRACT EXTRACT ON GUINEA PIG PERFUSED LUNG.

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Byssinosis is an occupational respiratory disease found in certain textile workers exposed to cotton dust. Although this condition has been well described, its aetiology and pathogenesis remain uncertain. Recently, it has been proposed that arachidonic acid metabolites may play a role in the bronchoconstriction occurring after inhalation of cotton dust (Mundie and Ainsworth, 1985). As the major component of cotton dust is the bract of the cotton boll (Cooke, 1979) a study of the effect of an aqueous extract of cotton bract upon perfused lung was undertaken. The airways of isolated lungs of adult female guinea pigs were perfused with Krebs-bicarbonate saline at 32°C via the trachea and the perfusion rate was 5 ml/min. Bract extract and various pharmacological agents were injected into the perfusion fluid and bronchoconstriction was assessed by measuring changes in perfusion pressure.

The bract extract (equivalent to 10-100 mg dried cotton bract) induced a reversible bronchoconstriction with a time-course similar to that elicited by either cotton dust extract (10-100 mg) or the calcium ionophore A-23187. Compared with responses elicited by histamine and carbachol, the onset was slower and the duration longer with the bract. The response to the bract extract was repeatable and dose-dependent and it was unaffected by atropine (0.34 µg/ml), mepyramine (0.34 µg/ml), nordihydroguaiaretic acid (3 µg/ml), FPL55712 (1 µg/ml), diethylcarbamazine (4 mg/ml) and imidazole (197 µg/ml). However, bract-induced bronchoconstriction was reduced by about 50% in the presence of either indomethacin (6 µg/ml) or methysergide (0.1 µg/ml). A combination of both these agents abolished the response of the lung to the bract extract. These results indicate that the bract-induced bronchoconstriction is unlikely to be mediated by release of arachidonate products of the lipoxygenase pathway. As the bract extract employed did not contain any 5-HT, the methysergide-sensitive component of the bronchoconstrictor effect of bract suggests the presence of an unidentified 5-HT receptor agonist and this confirms the findings of Russell et al. (1982). The inhibitory effect of indomethacin on the methysergide-insensitive response of lung suggests that this bronchoactivity may be mediated by the release of arachidonic acid metabolites of the cyclo-oxygenase pathway. This constrasts with the bronchoconstriction induced by cotton dust extract in this preparation which appears to be mediated by the release of a leukotriene-like metabolite of arachidonic acid (El-Mahdy and Nicholls, 1986).

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CROTON OIL INDUCED MYOFIBROBLASTS AND THEIR SENSITIVITY TO 5-HYDROXYTRYPTAMINE - AN ALTERNATIVE PROPOSAL.

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Croton oil induced granulation tissue is well documented as containing myofibroblasts (Gabbiani et al 1972). This "tissue" responds to a range of pharmacological agents with either a contractile or relaxatory response which is usually ascribed to the myofibroblasts present in the tissue. One such agent is 5HT which causes myofibroblast contraction (Gabbiani et al, 1972; Ryan et al, 1974; Garcia-Valdecasas et al, 1981) and is said to be a 'classical' property of such cells. However, the effect is reported to be both irreversible (response >2hr) and tachyphylactic and clearly unlike the effect which 5HT produces on smooth muscle, the cell type with which the myofibroblast is usually compared. In an earlier study (Illingworth and Naylor, 1982) these unusual effects of 5HT could not be reproduced and this study was undertaken to determine if the reported 'classical' effects of 5HT on granulation tissue were correct.

Female rats (250-300g, Bradford strain) were used in three groups (a) untreated controls (n=8), (b) solvent treated (n=8), and (c) Selye's technique (1953) (n=8) per time interval. Animals were killed 12-33 days after injection and from group c strips of the granuloma pouch or from group a and b fundic strips were arranged for superfusion in (a) Krebs solution (37°C) at 2 ml min-1 of (b) calcium free Krebs. An initial tension of 1g was applied and tissues were equilibrated for 1 hour. Agonists tested were KCl, acetylcholine, $BaCl_2$,5HT and mepyramine.

Barium and potassium ions were ineffective at any of the doses used (1-8mg) on granulation tissue but caused a dose dependent, reversible contraction on fundic strip. 5HT produced dose dependent, fully reversible and repeatable responses on both tissues. In contrast mepyramine produced a sustained response on granulation tissue but only a brief effect on the fundic strip, although in both cases the tension developed was significantly less than with the 5HT. Calcium free Krebs significantly ($P<0.001$) reduced the effects of 5HT on fundic strip but not on granulation tissue. The sensitivity to 5HT was dependent on the age of the pouch. Pouch sensitivity was found to be maximal at 21-22 days. Consequently although croton oil induced myofibroblasts showed a sensitivity to 5HT it was clearly different to that widely accepted as the 'classical' property of such cells. The proposal is made that the 5HT sensitivity in these experiments is perhaps more representative of its activity in vivo rather than in the earlier experiments which used 'one dose' techniques, poor apparatus, multi-drug exposures, an unusual choice of physiological fluid for rat tissue, and limited sample numbers and repeats. In addition the usually quoted comparison with smooth muscle cells is seriously questioned by (a) the finding that the 5HT response on myofibroblasts does not depend on extracellular calcium ions, (b) the response to mepyramine and (c) the lack of effect with barium or potassium ions and acetylcholine. Further experiments are in progress to characterise the nature of the 5HT receptors involved.

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THE ROLE OF CALCIUM IONS AND cAMP IN THE CONTRACTILITY OF MYOFIBROBLASTS IN THE RAT TESTICULAR CAPSULE.

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Papaverine has been shown to relax granulation tissue (Majno et al., 1971; Baker et al, 1981; Garcia-Valdecasas et al., 1981) due to an action on the myofibroblasts present in this tissue. Since it has been suggested that these cells contract in a similar way to smooth muscle it was of interest to determine if it was the modifications to the calcium ion movements and/or the effect on cAMP that was responsible for the inhibitory effects produced by papaverine.

Myofibroblast contractility was studied using the rat testicular capsule as previously described (Lal and Naylor, 1985). A minor modification was the use of capsular strips rather than whole preparations to aid reproducibility and rapidity of responses. Mepyramine (10-80μg) was used as the agonist (Lal and Naylor, 1986) and its response was studied in the absence and presence of: sodium dantrolene, verapamil, nifedipine (all 1-100μM), theophylline, ICI 63197 and papaverine (all 10-100μM). Dose response curves were constructed for each antagonist, only one antagonist being used on each preparation. Six tissues were used for each concentration studied.

Group	Drug	Effect
	Papaverine	Inhibitory(see text)
Ca^{2+}(intracellular blocker)	Na dantrolene	Inhibitory*(100μM P<0.05)
Ca^{2+}(channel blockers)	Verapamil	No effect
	Nifedipine	Inhibitory*(100μM P<0.05)
Phosphodiesterase	Theophylline	No effect
inhibitors	ICI 63197	No effect

*Inhibitory = an effect on both the maximal change in tension and the duration of the response to mepyramine.

Papaverine was found to inhibit the responses of testicular capsule to mepyramine in a concentration dependent manner. At the highest concentration used (100μM) extensive washing did not reverse its effect, suggesting the similarity of myofibroblasts in rat testicular capsule to those found in granulation tissue. The results with Na dantrolene and nifedipine suggest that the response to mepyramine is dependent on both intracellular and ion channel calcium movements. In contrast, the lack of effect of verapamil, previously shown to relax granulation tissue taken from the Selye's pouch model (Garcia-Valdecasas et al., 1981) and the lack of effect produced by both theophylline and ICI 63197, both inhibitors of phosphodiesterase, suggests that the role of extracellular calcium ions and cAMP in the contractile processes is not identical in croton oil induced cells and those found in the testicular capsule.

The results indicate that myofibroblast contractility has clear differences to that normally found in smooth muscle cells but the concentrations necessary to evoke an effect, the lack of effect with ICI 63197 and theophylline and verapamil suggest that the often quoted similarities of myofibroblasts with smooth muscle cells is not clearly shown by testicular myofibroblasts. The effects of ions other than calcium are currently under investigation.

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SOME OBSERVATIONS ON THE CHEMOTHERAPY OF OSTEOARTHRITIS IN STR/ORT MICE.

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The STR/ORT strain of mouse spontaneously develops osteoarthritis (OA), predominantly in the knee joints, which is more severe in males and in which there is a positive relationship between severe arthropathy and both patella subluxation and calcification of the medial collateral ligament (Walton, 1977). We have used this strain to evaluate a range of compounds reported as effective in other models of degenerative joint disease.

Male mice at about 10 weeks of age were anaesthetised (pentobarbitone sodium, 80 $mg.kg^{-1}$ i.p.) and both hind limbs laterally X-rayed at X10 magnification by microfocal radiography (Cashin et al, 1980) using intensifying screens (Trimax 8) to minimise radiation exposure. Mice showing bilateral calcification of the quadriceps tendons were selected for test and randomly allocated to treatment groups. The remainder were subjected to the same procedure at two weekly intervals on up to four occasions. This approach was adopted in an attempt to ensure that the development of the syndrome had reached the same stage in each mouse at the start of dosing. Test compounds were administered three times weekly for twelve weeks to groups of 10 mice at which time the mice were killed, the hind limbs were removed from the hip joint and fixed in neutral buffered formalin. Lateral and anterior-posterior microfocal radiographs were taken as above, except non-screen film (3MR2) was used. The hind limbs were then decalcified with EDTA and embedded in paraffin wax. Serial coronal sections were cut at 8µM and every fourth section stained with alcian blue, haemotoxylin and eosin. The sections were scored for patella displacement and erosion of the medial tibia and femoral condyles using a qualitative scoring system based on that of Walton (1977). Erosions were scored on a scale in which 1 represented loss of cartilage staining, progressing to 8 which represented complete cartilage loss and extensive eburnation. Erosions were scored radiologically by selecting radiographs from control groups showing low, medium and high histological erosion scores, allocating scores of 2, 4 and 6 respectively to these and then comparing test radiographs with these standards. Patella displacement and ligamentous calcification were both scored on 1-3 scales.

Drugs failing to show significant reductions in erosion scored histologically or radiologically were tamoxifen (1 $mg.kg^{-1}$ p.o.), tribenoside (100 and 500 $mg.kg^{-1}$ p.o.), diacetylrhein (100 $mg.kg^{-1}$ p.o.), etretinate (5 and 10 $mg.kg^{-1}$ p.o.), tenoxicam (3 $mg.kg^{-1}$ p.o.), dexamethasone (0.1 $mg.kg^{-1}$ s.c.) and Arteparon (100 $mg.kg^{-1}$ s.c.). Calcitriol (0.1 and 0.3 $\mu g.kg^{-1}$ p.o.) did however significantly (U-test) reduce both histological and radiological changes indicating that an impairment of calcium homeostasis may contribute to the development of the disease. No significant drug effects on patella subluxation or ligamentous calcification were seen. These results indicate that OA in STR/ORT mice is relatively insensitive to therapeutic intervention, a view supported by Maier and Wilhelmi (1984) who consider the C57 black mouse more suitable since the disease develops more slowly.

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PROPRANOLOL PLASMA CONCENTRATIONS AND PROTEIN BINDING IN RATS WITH ADJUVANT-INDUCED INFLAMMATION.

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The effect of adjuvant-induced inflammation in rats on the relationship between area under the propranolol plasma concentration versus time curve (AUC) and plasma protein binding (PPB) was investigated.

On day 1, the hind foot-pads of rats were injected using 15 µl of either H37Ra, Freunds complete adjuvant (FCA), Freunds incomplete adjuvant (FIA) or saline. On day 5, propranolol was administered either orally (p.o.) (2 mg) or intraperitoneally (i.p.) (0.25 mg) to sub-groups of these adjuvant and saline treated rats. Inflammation was assessed by measuring swelling of the hind foot-pads and ankle joints. After p.o. dosing with propranolol, animals were sacrificed at 10, 20, 40, 60, 90, 120 and 180 min and following i.p. dosing at 10, 20, 30, 60 and 120 min and the blood was collected from each animal. Propranolol PPB was determined as described by Vlahos et al (1982) and propranolol was assayed by the method of Shand et al (1970). The binding parameters were assessed by the method of Horth et al (1986).

Significant correlations were observed between foot-pad swelling and PPB ($r = 0.39$, $p < 0.001$) and between ankle swelling and PPB ($r = 0.405$, $p < 0.01$). Greater AUC values and PPB values were shown in adjuvant treated rats compared with controls. The AUC values for p.o. administration in the H37Ra treated rats were 8 times that in the saline treated rats whereas for i.p. administration, the AUC values for the H37Ra rats were 1.3 times that of the saline treated rats. The results appeared to indicate the importance of gut wall metabolism in the presystemic loss of the drug. There was a significant difference in the binding to high affinity sites (42.9% vs 31.1%, $p < 0.001$) but not in low affinity sites (48.6% vs 50.9%) when comparing H37Ra treated rats with saline treated rats. The higher PPB in H37Ra treated rats was associated with a higher Ns (16.7×10^{-7} M) compared with saline controls (3.3×10^{-7} M) rather than the difference in Ks (3.4×10^{5} M^{-1} for H37Ra and 9.4×10^{5} M^{-1} for saline). The binding parameters for the low affinity sites were not implicated.

Although there was a significant association between propranolol PPB and inflammation, more evidence is required before it can be established that the increased protein binding due to inflammation is directly responsible for the increased plasma propranolol levels.

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EFFECTS OF RETINOIDS IN AN IMMUNE MODEL OF INFLAMMATION IN MICE.

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The anti-inflammatory effects of retinoids such as etretinate, differs from both immunosuppressive agents and non-steroidal anti-inflammatory drugs (NSAIDs) (Bradshaw et al, 1985). In the delayed type hypersensitivity response to methylated bovine serum albumin (MBSA) in mice, immunosuppressive agents are active when given at the time of sensitisation whilst NSAIDs are only active when given at the time of antigen challenge (Cashin et al, 1979). Etretinate is active during the sensitisation phase but its reported delayed onset of activity in vivo makes its mechanism of action difficult to classify (Bradshaw et al, 1985). The effect of the retinoids has now been further examined in this model.

Groups of mice (MF1♂ 20-25g) were sensitised on day 0 by i.d. injection of equal volumes of 0.5% w/v MBSA and Freunds Complete Adjuvant. On day 8 they were challenged by subplantar injection of 0.02 ml 1% w/v MBSA in one hind paw and an equal volume of sterile saline in the other. 24 h later paw volumes were measured by H_2O displacement plethysmography and the oedema expressed as the % increase in volume of the challenged paw compared to the control paw. Plasma serum amyloid P levels (SAP) were measured by a method of crossed electrophoresis in samples obtained by cardiac puncture. Drugs were administered (5 ml kg^{-1} p.o.) on the days indicated in the table below.

Retinoid	Dosing schedule days	n	% paw swelling
Control[1]	-	49	107.1 ± 2.12
All-trans retinoic acid 30 mg kg^{-1} p.o.	-7 ⟶ -3	8	67.1 ± 6.4*
	0 ⟶ 4	10	70.3 ± 4.4*
Etretinate 30 mg kg^{-1} p.o.	-7 ⟶ -3	10	86.9 ± 13.5
	0 ⟶ 4	10	64.5 ± 9.5*
	7 ⟶ 9	9	99.3 ± 8.6
	1 ⟶ 9	10	45.7 ± 9.3**

[1] Control values for each dosing schedule were not significantly different (101-111.9%) and have been combined. **$p<0.01$; *$p<0.02$ Student's 't'-test.

All-trans retinoic acid inhibited the inflammation to a similar extent whether dosed during (d0-4) or before sensitisation (d-7⟶-3). Etretinate was similarly active although the inhibition following predosing was not significant. It had no effect when given at the time of antigen challenge (d7-9). The inhibition of paw swelling by etretinate was paralleled by a decrease in SAP levels (control SAP 166 µg ml^{-1} (n=4); etretinate group 78 µg ml^{-1} (n=5). In contrast neither NSAIDs not immunosuppressive agents affect SAP levels (Ball et al, this meeting). In an extension to these experiments mice were rechallenged with MBSA on d17 and paw swelling measured 24 h later. The second increase in paw volume (58.3% ± 6.8, n=4) was again reduced (46%) in mice previously treated with etretinate on d0-4 (30 mg kg^{-1} p.o.).

These results suggest that the retinoids exert an inhibitory effect in this model by modulating the process of sensitisation to MBSA. Since IL-1 is an inducer of SAP, the effect of the retinoids on this acute phase protein warrant further study.

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SERUM AMYLOID P LEVELS IN A DELAYED TYPE HYPERSENSITIVITY REACTION IN MICE

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Measurement of acute phase reactants (APRs) has been used clinically in the assessment of inflammatory reactions and drug efficacy (McConkey et al, 1973). Serum amyloid P is a major APR in mice, homologous with C reactive protein in man. We have now measured SAP levels in a delayed hypersensitivity reaction in mice to investigate the correlation between inflammation and SAP and the effect of drugs on both parameters.

Groups of mice MF1 ♂ 20-25 g) were sensitised on day 0 by i.d. injection of equal volumes of 0.5% methylated bovine serum albumin (MBSA) and Freunds Complete Adjuvant. On day 8 they were challenged by subplantar injection of 0.02 ml 1% w/v MBSA in one hind paw and an equal volume of sterile saline in the other. 24h later paw volumes were measured by H_2O displacement plethysmography and oedema expressed as the % increase in volume of the challenged paw compared to the control paw. Drugs were administered (5 $ml.kg^{-1}$ p.o.) on either days 0-4 (sensitisation) or days 7-9 (elicitation) (Cashin et al, 1979). Blood samples were taken by cardiac puncture and SAP measured by rocket electrophoresis.

SAP levels in naive mice were 25 μgml^{-1} ± 3.8 (n=8) but 24h after challenge of sensitised mice with MBSA, there was a 4-5 fold rise in SAP. This was accompanied by a marked swelling in the challenged paw ranging from 90-110% increase. Experiments to compare the effect of a NSAID, an immunosuppressive agent and two retinoids, showed a similar efficacy in reducing the paw oedema but varied effects on SAP.

Drug	Dose $mgkg^{-1}$	Treatment period	n	SAP μgml^{-1}	n	% paw swelling
Control	-	7-9	10	102±17.6	10	111.5±11.9
Indomethacin	2	7-9	10	124±14.4	10	64.3±12.7[1]
Control	-	0-4	10	114±23.3	10	89.2±7.9
Azathioprine	100	0-4	10	86.9±8	10	44.6±10.7[3]
Control	-	0-4	4	152±23.6	8	91.1±12
Ro 04-3780	100	0-4	4	72±19[1]	8	63.3±7.7
Ro 13-7410	0.01	0-4	4	66±21[1]	8	41±8.2[3]

All values are mean ± sem [1]$p<0.05$; [2]$p<0.02$; [3]$p<0.01$ Student's 't'-test

Both indomethacin and azathioprine reduced oedema without significantly affecting SAP levels whilst the inhibition by 13-cis retinoic acid (Ro 04-3780) and the arotinoid (Ro 13-7410) was paralleled by a decrease in SAP. Thus there was no consistent correlation between degree of inflammation and SAP levels.

These results suggest that the activity of the retinoids in this model differs both from that of the NSAIDs and conventional immunosuppressives. It has been reported recently that injection of rIL-1 in mice results in a marked increase in SAP (Westmacott et al, 1986) and it is interesting to speculate that in the present experiments IL-1 mediated events, reflected by the changes in SAP, may be modulated by the retinoids.

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COMPARATIVE EFFECTS OF TENOXICAM ON TYPE II COLLAGEN-INDUCED ARTHRITIS IN THE RAT.

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Tenoxicam is a new non-steroidal anti-inflammatory drug (NSAID) of the oxicam family which has potent analgesic and anti-inflammatory activity and a long plasma elimination half-life in man, allowing once-daily dosing. Its activity has been demonstrated in a number of biological systems (Bradshaw et al, 1984) and as an extension of this work tenoxicam has now been compared with several NSAID and the steroid dexamethasone for its effects on collagen-induced arthritis in the rat, a model which in many respects resembles human rheumatoid arthritis (Trentham, 1982).

Arthritis was induced in female Alderley Park strain 1 rats (weight range 150-180 g), by the intradermal injection of a type II collagen/Freund's incomplete adjuvant emulsion. Following the onset of hind-paw inflammation the animals were placed in groups, matched according to paw volume and body weight and the compounds were administered once daily (p.o.) for 15 days. The doses of the compounds used were: tenoxicam, 0.3, 1.0 and 3.0 $mgkg^{-1}$; piroxicam 1.0 and 3.0 $mgkg^{-1}$; naproxen 10 $mgkg^{-1}$; indomethacin 1.0 $mgkg^{-1}$ and dexamethasone 0.5 $mgkg^{-1}$ for 5 days and 0.25 $mgkg^{-1}$ thereafter. Hind paw volumes were taken at intervals throughout the course of the arthritis using water displacement plethysmography. On termination of the experiment blood was taken for the determination of plasma acute phase reactants (APR), using a COBAS BIO centrifugal analyser, and serum anti-collagen antibody, using a haemagglutination technique. Bone changes in hind paws were assessed using X-radiography.

All compounds except naproxen caused a statistically significant (Student's 't' test) reduction in the inflammatory response judged in terms of hind paw volume. Radiographic deterioration in the tarsus region, assessed using an arbitrary scoring system (0=no effect to 6=severe deterioration) was reduced to a statistically significant degree (Mann-Whitney 'U' test) for all the NSAID except naproxen. Actual mean radiographic scores at the 1.0 $mgkg^{-1}$ dose level were tenoxicam 1.7; piroxicam 1.9 and indomethacin 2.0 compared with 3.7 for the arthritic control group. Dexamethasone was also effective, producing a mean radiographic score of 1.6 in treated animals compared with 3.4 in a corresponding control group.

Of the APR measured, caeruloplasmin, fibrinogen, seromucoid and haptoglobin were increased in arthritic animals compared with normals whereas albumin and iron levels were decreased. In general, none of the NSAID had any significant effect on APR levels while dexamethasone restored all APR, except haptoglobin, towards or beyond normal levels. Body weight gain was greater than arthritic controls in all groups treated with NSAID but was adversely affected by dexamethasone.

In conclusion, tenoxicam is effective in reducing the inflammation and bone changes which occur during collagen-induced arthritis. In this respect it is similar to other NSAID and its potency is approximately equivalent to that of piroxicam and indomethacin. The NSAID differ from the steroid dexamethasone in their lack of effect on APR levels but the significance of this is not yet entirely clear.

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ROLE OF INTERLEUKIN-1 IN THE EFFECT OF RETINOIDS ON 'NON-RESPONDER' COLLAGEN ARTHRITIC RATS

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Certain retinoids have been shown to exacerbate collagen arthritis in the rat (Trentham and Brinckerhoff, 1982; Bradshaw et al, 1985). Recently, retinoic acid (RA) has been shown to stimulate the release of interleukin-1 (IL-1) from both murine and human mononuclear cells in vitro (Trechsel, 1985), suggesting increased IL-1 production as one possible mechanism for this exacerbatory effect. Following sensitisation of rats with collagen most animals respond with hind paw swelling by day 15 after injection, but there is a percentage of animals which do not respond. In the present work we have examined the effects of RA and an arotinoid ethyl sulphone (Ro 15-1570), a compound which does not induce IL-1 production in mononuclear cells (Trechsel, personal communication), on these non-responder (NR) rats.

Female Alderley Park strain 1 rats (weight range 150-180g) were sensitised by the intradermal injection of a type II collagen/Freund's incomplete adjuvant emulsion. Animals not responding with hind paw swelling by day 15 following sensitisation were selected for experimentation. These NR rats received RA (30 $mgkg^{-1}day^{-1}$), Ro 15-1570 (5 $mgkg^{-1}day^{-1}$) or the arachis oil vehicle, all administered orally. Hind paw inflammation was assessed visually and by measurement of hind paw volume by water displacement plethysmography. X-radiography was used to determine any bone changes occurring following treatment and anti-collagen antibody was determined by a haemagglutination technique.

Of 15 NR rats receiving RA for 4 days 12 responded with hind paw swelling whereas there was no response in a group of 14 vehicle-treated animals. X-radiography showed no evidence of bone changes in animals responding to RA even when they were left untreated for a further 11 days. There was no difference in anti-collagen antibody levels in rats responding to RA compared with controls. RA did not induce hind paw inflammation in non-sensitised rats.

Ro 15-1570 failed to induce hind paw inflammation in NR rats after 4 days of treatment at a dose which produced signs of hypervitaminosis A, indicating that its lack of effect was not due to poor bioavailability. Mean paw volumes for the treated and control groups were 1.56 ± 0.05 ml and 1.59 ± 0.06 ml respectively. Subsequent challenge of this control group with RA for 3 days produced a response in all animals which was detected as a significant increase in paw volume to 1.94 ± 0.09 ml ($p<0.01$, Student's 't'-test, compared with controls). When the group previously treated with Ro 15-1570 was similarly given RA a response was elicited in only 50% of the animals.

These results indicate that RA not only exacerbates an existing collagen arthritis in rats, as previously reported, but induces arthritis in otherwise non-responding rats. Ro 15-1570 does not share this property and in fact appears to inhibit the response to RA. These results are consistent with the hypothesis that retinoids induce/exacerbate arthritis in collagen-sensitised rats by a mechanism involving an increase in IL-1 production. However, in preliminary experiments in which up to 20,000 Units of human recombinant IL-1 were administered i.p. to rats which had responded to collagen sensitisation, a slight inhibition of the arthritis was detected. It is possible that this reflects a difference between the local and systemic effect of IL-1 but further work is required to clarify these findings.

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PERIPHERAL ANTITUSSIVE ACTION OF CODEINE AND MORPHINE IN THE GUINEA-PIG.

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The antitussive effects of opiates are considered generally to be central in origin, although some peripherally mediated actions on the cough reflex have been reported (Yanaura et al., 1981) . Quaternary salts with their high polarity and consequent restricted access to the CNS have been used to investigate peripheral actions and, using these pharmacological tools, peripherally mediated gastrointestinal and antinociceptive effects of opiates have been demonstrated (Smith & Clark, 1982). In the present study the antitussive action of codeine and morphine in the guinea-pig have been investigated with the quarternary opiate antagonist, N-methylnalorphine. To ensure that the quaternary salt used was essentially excluded from the CNS, the distribution of N-[^{14}C-methyl]-methylnalorphine iodide was investigated. In various species, minimal radioactivity appeared in the brain at any time and, of that observed, a large part was accounted for by intravascular material.

Cough was induced by exposure of conscious male albino guinea-pigs to vapour generated from a nebulized aqueous solution of citric acid (Boura et al., 1971). Prior treatment of the guinea-pig with codeine produced a dose-related inhibition of cough response; subcutaneous ED_{50} being 1.9mg/kg. The corresponding ED_{50} for morphine was 3.1mg/kg s.c. The antitussive effects of opiates were antagonised significantly by N-methylnalophine 3.0mg/kg s.c. and by nalorphine 3.0mg/kg s.c. The antinociceptive effect of codeine in guinea-pigs (Collier et al., 1961) in a toe-pinch test (ED_{50} 18.0mg/kg), however, was unaffected by pretreatment with N-methyl nalorphine (3mg/kg s.c.). Similarly the antinociceptive action of morphine (ED_{50} 2.3mg/kg s.c.) was also unaffected by N-methylnalorphine. In contrast, nalorphine (30mg/kg s.c.) significantly antagonised the antinociceptive effects of both codeine and morphine.

In the guinea-pig models used, therefore, the antitussive, but not the antinociceptive, effects of codeine and morphine may be considered to be mediated peripherally.

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ADENOSINE AND REBOUND CONTRACTIONS OF THE RAT GASTRIC CORPUS TO NON-ADRENERGIC NON-CHOLINERGIC NERVE STIMULATION.

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Neural stimulation releases adenosine in both the guinea pig stomach (Burnstock et al.1970) and intestine (Hayashi et al. 1978). Furthermore adenosine inhibits the release of acetylcholine and noradrenaline from autonomic nerves (Vizi 1979). The possibility that adenosine can modulate non-adrenergic, non-cholinergic (NANC) nerve transmission has been investigated using the rat gastric corpus muscle in which field stimulation of NANC nerves produces a relaxation followed by a rebound contraction (Hunt et al. 1978).

The rat gastric corpus muscle was prepared and field stimulation carried out as described by Hunt et al. (1981). Adenosine (10^{-6} - 5 x 10^{-4}M) induced a dose dependent reversible inhibition of the rebound contraction without affecting either the relaxation or the tone of the tissue. Contractile responses to carbachol (10^{-6}M) or direct electrical stimulation of the muscle (supramaximal voltage, pulse width 5ms, frequency 10 HZ for 10 sec every 100 sec in the presence of tetrodotoxin (10^{-6}M))were not inhibited by adenosine (10^{-4}M). Theophylline (10^{-5}- 5 x 10^{-5}M), a P1 purinergic receptor antagonist (Burnstock 1978), did not affect either the relaxation or the rebound contraction to field stimulation but it did antagonise the adenosine induced suppression of the rebound contraction in a non-competitive manner. At a higher dose theophylline (10^{-4}M) inhibited the rebound contraction without affecting the inhibitory response. The inhibitors of either adenosine uptake, dipyridamole (10^{-7}- 5 x 10^{-4}M), dilazep (10^{-7} - 5 x 10^{-4}M) or adenosine deaminase, erythro -9- (-2- hydroxy -3- nonyl) adenine (EHNA 10^{-7} - 5 x 10^{-4}M) independently caused dose dependent inhibition of the rebound contraction without affecting the inhibitory response. Moreover either dipyridamole (10^{-6}M), dilazep (10^{-6}M) or EHNA (10^{-6}M) increased the pD_2 for adenosine's inhibition of the rebound contraction from 5.5 to 6.7, 6.5 and 7.4 respectively. Adenosine deaminase (0.22 units cm^{-3}) significantly potentiated the rebound contraction to NANC nerve stimulation and decreased the adenosine (10^{-7}- 10^{-3}M) induced inhibition of the rebound contraction but did not affect the relaxation to field stimulation.

These results are consistent with the view that both endogenous and exogenous adenosine can inhibit the rebound contraction by reducing the release of the NANC excitatory transmitter by a presynaptic mechanism. The inability of theophylline to potentiate the rebound contraction in this preparation contrasts with the observations of Bauer et al (1982) and Bartho et al (1985) who observed potentiation of NANC excitatory responses in the guinea-pig colon and ileum respectively. In our experiments the P1 antagonist activity of theophylline against endogenous adenosine may have been masked by inhibition of phosphodiesterase at higher doses.

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THE AGONIST ACTION OF AH 23848 AT GUINEA-PIG VASCULAR AND AIRWAY SMOOTH MUSCLE TP-RECEPTORS IN VIVO

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Several thromboxane receptor (TP-receptor) blocking drugs have been described which contract vascular smooth muscle but when added to platelets have no direct agonist activity (e.g. carbocyclic thromboxane A_2 (CTA_2) Lefer et al., 1980). AH 23848 is a potent specific TP-receptor blocking drug that is devoid of direct or pro-aggregatory effects on platelets but does produce small transient contractions of human isolated arteries and dog saphenous veins (Brittain et al., 1985). AH 23848 has also been reported to increase blood pressure in anaesthetised animals (Lumley, 1986). This activity is particularly marked in the guinea-pig and the present study examines further the nature of this effect.

Male guinea-pigs (250-500g) were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.) and prepared for recording blood pressure (BP) and tracheal inflation pressure (TIP). AH 23848 (0.003 - 1.0 mg/kg) was administered as single i.v. bolus injections. Each animal received one dose of drug which was preceded by a cumulative dose-response curve to the TxA_2 mimetic, U-46619. AH 23848 produced a transient (peak 15-30s, duration <120s) dose-related vasopressor response. At the highest dose tested (1 mg/kg) the mean (± s.e. mean) increase in diastolic BP was 32 ± 1 mmHg (n=6). At this dose AH 23848 also produced an increase in TIP of 23 ± 4% (n=6). Vehicle produced no significant effect. Compared with U-46619, AH 23848 was some 40 times less potent as a vasoconstrictor and >1000 times less potent as a bronchconstrictor in this animal model. In parallel experiments using the same dose range, the compound was without effect upon the circulating platelet count. Cumulative i.v. bolus administration of AH 23848 (0.01 - 1.0 mg/kg) resulted in increases in both diastolic BP and TIP, although the peak responses after 1 mg/kg were smaller (18 ± 1 mmHg, 10 ± 2% respectively, n=6) than following the equivalent single dose, indicating that some desensitization had occurred. This was examined further by administering three consecutive i.v. bolus doses of AH 23848 (1 mg/kg) to the same animal at 15 min intervals. The response to the first dose of AH 23848 in naive animals was comparable to earlier experiments. However responses to subsequent doses were absent. In contrast, with repeated administration of U-46619 no such tachyphylaxis was seen. Pre-treatment of conscious animals with an oral dose of AH 23848 (1 mg/kg) or i.v. bolus administration (5 mg/kg) of the TP-receptor blocking drug BM 13.177 (Patscheke and Stegmeier, 1984) one hour or 15 minutes respectively prior to a subsequent i.v. bolus dose of AH 23848 also resulted in the marked reduction or abolition of the agonist responses.

The present results therefore suggest that AH 23848 exhibits some agonist activity, albeit transient, at vascular and airways smooth muscle TP-receptors in the guinea-pig. It appears to be devoid of such activity at the platelet TP-receptor where it acts solely as an antagonist. Similar profiles of action with compounds such as CTA_2 have led to the suggestion of differences between the platelet and vascular smooth muscle TP-receptors (Lefer et. al., 1980). Such a profile could, however, be equally explained by partial agonism and differences in receptor density or stimulus-response coupling in the two systems.

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EFFECT OF VERAPAMIL ON UPTAKE OF CALCIUM INTO VASCULAR AND TRACHEAL SMOOTH MUSCLE OF THE RAT.

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Verapamil, an organic calcium antagonist, is used in the treatment of cardiovascular disorders,e.g. in cardiac dysrythmias (Singh,Ellrodt & Peter,1978; Reves, Kissin,lell & Tosone,1982). It is known that verapamil blocks influx of calcium through voltage-dependent slow " calcium" channels in cardiac and smooth muscle membrane (Fleckenstein,1977; Bou,Llenas & Massingham,1983). In the present investigation,we have used a biochemical technique to measure and quantify uptake of calcium into rat muscle,in the presence and absence of verapamil,to see if verapamil produce a differential effect on uptake of calcium in these different tissues of the same species.

Tissues, heart (2g), aorta (100 mg), saphenous vein (100 mg),and trachea (100 mg), were incubated, for 10 min, in separate organ baths,containing 25 ml of Krebs-Henseleit solution maintained at 38± 2°C and bubbled with 5% CO_2 in O_2, in the presence and absence of verapamil (28 µM).

Uptake of calcium was measured by means of a Kone Micrlyte Analyser (Kone Corporation Instruments Division, SF- 0.2321, ESP0032, Finland),which is an ion-selective electrode that can directly measure the ionic activity of calcium (Ca^{2+}) in a biological or body fluid (Robertson,1976; Ladenson,1977). The ionix activity of calcium is converted into ionic concentration (mM) using the formula: $I = \frac{1}{2} Z^2 x. C x$, where I is the ionic strength, Z is the charge of ionic species x, and C is the ionic concentration of x. With this technique, only the ionized calcium concentration is measured and this can be related to the total calcium concentration of about 2.5 mM present in the control Krebs solution (Elliott & Wali,1983).

In the control experiments,uptake of calcium (ionized calcium) varied from preparation to preparation-uptake by the heart muscle being,on average,20-30 times less than in the blood vessels and in the tracheal smooth muscle (Table 1). Verapamil (28 µM) reduced the uptake of calcium in all these tissues by 30-60% of the control values-greater reductions occurred in the cardiac and aortic muscle. Thus,in the presence of verapamil (28 µM) ,there was a differential reduction in the amount of calcium taken up by the different tissues-indicating tissue selectivity in the action of verapamil within the same animal species.

Table 1. Effect of verapamil on uptake of calcium in rat cardiac and smooth muscle

Tissue	Control $ng.mg^{-1}.min^{-1}$ mean±S.E.	In Verapamil $ng.mg^{-1}.min^{-1}$ mean±S.E.	% Reduction mean±S.E.	P <	n
Heart	68± 4	30± 3	56± 5%	0.001	6
Aorta	1320± 120	605± 22	54± 2%	0.001	6
Saph. Vein	1595± 280	1155± 93	28± 1%	0.001	6
Trachea	2035± 410	1375± 135	32± 5%	0.001	6

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NORADRENALINE RESPONSES OF VASCULAR MUSCLE FROM RATS PRETREATED WITH ETHINYLOESTRADIOL OR NORETHISTERONE

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Pretreatment of rats with 17B-oestradiol has been shown to induce a leftward shift in the dose response curves (DRC) for the contraction of mesenteric arteries to noradrenaline (NA) (Altura,1976, Colucci et al, 1982). The aim of the present study was to determine the effect of pretreating female rats with either the oestrogenic agent, ethinyloestradiol (EO) or the progestogenic agent, norethisterone acetate (NAc) upon contractile responses of the aortic strip and portal vein to NA.

Female Wistar rats (200g-300g) were given s.c. EO (800 g/kg) or NAc (70 mg/kg) in polyethylene glycol 300 (PEG) for 9 days. Controls were given PEG alone. The first injection was made at dioestrus. Rats were killed by cervical dislocation 24h after the last injection. Helical strips of rat aorta and longitudinal preparations of portal vein were suspended in Krebs' solution at 37°C, gassed with 5% CO_2 in O_2 under a resting tension of 9.8mN (aorta) or 4.9 mN (portal vein). Non-cummulative DRC to NA were determined; contractions of the aorta were recorded isometrically as the initial peak tension at 20s and the sustained peak tension, contractions of the portal vein were recorded as the integral of isometric tension. There was a significant, parallel leftward shift of the DRC for both components of the response to NA in aortae from rats pretreated with EO compared with PEG. The EC50 for the response at 20s was decreased from 9.5±2.5 nM (n=6) to 1.1±2.8 nM (n=7) and for the peak response from 2.2±0.9 nM (n=6) to 0.2±0.05 nM (n=7) (both $P<0.05$). The maxima of both the initial (2.7+0.2 mN, n=7) and the peak (5.8+0.6 mN, n=7) components of the contraction of the aorta to NA after EO pretreatment were significantly greater ($P<0.05$) than after PEG pretreatment (1.9+0.2 and 3.9+0.5 mN respectively, n=6). The maximum contraction of the portal vein to NA was unaltered by EO pretreatment but the EC50 was significantly less than that in preparations from PEG pretreated rats, 2.0+0.9 mN and 7.8+2.4 mN respectively (n=6). Pretreatment with NAc caused no significant change in the contractions of the aorta or portal vein to NA.

The leftward shift of the DRC for NA on aorta and portal vein from rats pretreated with EO is similar to the effect of 17B-oestradiol upon responses of mesenteric arteries to NA [vide supra] and is consistent with the suggestion that oestrogenic agents increase agonist affinity of vascular alpha-adrenoceptors (Colucci et al, 1982). Since high concentrations of NAc had no effect upon the response of either preparation to NA and since responses of mesenteric artery to NA are unaffected by testosterone pretreatment (Colucci et al, 1982) it is possible that this action is only observed with oestrogenic steroids. The reason for the increase in maximum response of the aorta following EO pretreatment is unknown.

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DIBUTYRYLcAMP, FORSKOLIN AND MDL 12,330A MODULATE DOPAMINE INHIBITION AND A DOPAMINERGIC IPSP IN IDENTIFIED HELIX NEURONES.

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It has previously been reported (Cox & Walker 1986) that dibutyrylcAMP (dbcAMP) enhances the dopamine induced inhibition of an identified neurone of Helix aspersa. In this present study, two compounds which modify the activity of adenylate cyclase were tested, forskolin which enhances and MDL 12,330A which depresses adenylate cyclase activity (Seamon et al 1981; Silinsky 1984). In addition, the effects of dbcAMP, forskolin and MDL 12,330A on an evoked inhibitory postsynaptic potential (IPSP) in an identified neurone were examined.

Microelectrode recordings were made from cells F-5/6 in the isolated suboesophageal ganglionic mass of Helix aspersa. Cells were voltage clamped at membrane potential using a Dagan 8100 single electrode clamp. A single stimulus to the intestinal nerve, 2-3 V and 2.0 msec duration, elicited an antidromic spike followed by an IPSP in cell F-1 (Kerkut et al 1975). Drugs, apart from forskolin which was made up initially in ethanol, were made up in Helix saline and added directly to the bath while dopamine was applied ionophoretically from a second electrode containing 0.5 M dopamine, pH 4.5. Experiments were repeated at least five times and mean $\pm$ sem values are given.

The dopamine response was enhanced by 0.1 µM bath applied forskolin. Typically the dopamine current was increased from control values of 0.78 nA to 3.6 nA. The % enhancement was 283 $\pm$ 61.7. The adenylate cyclase inhibitor, MDL 12,330A, 1.2 µM, depressed the dopamine response where in a typical experiment, the control dopamine current decreased from 1.65 nA to 0.46 nA. The % depression was 73.1 $\pm$ 12.0. The IPSP recorded from F-1 was antagonised by ergometrine, 1.2 µM, suggesting it was dopamine mediated (Walker et al 1968; Kerkut et al 1969). Bath addition of 1 µM dbcAMP enhanced the IPSP in a time dependent manner. The peak enhancement was obtained 35 minutes after addition of the dbcAMP. Typically the IPSP was increased from 4.0 mV to 7.5 mV with a % enhancement of 119 $\pm$ 25.5. Forskolin, 1 µM, also potentiated the IPSP, increasing the control IPSP typically from 4.5 mV to 8.25 mV with a % enhancement of 85.9 $\pm$ 14.4. MDL 12,330A, 1.2 µM, reduced the size of the IPSP typically from 6.0 mV to 3.75 mV with % depression of 35.8 $\pm$ 4.38. The maximum depression was obtained 25 minutes after addition of the MDL 12,330A.

This study shows that dbcAMP and compounds which alter the activity of adenylate cyclase affect both the dopamine induced current and a dopaminergic IPSP in identified neurones of the snail, Helix aspersa. In this respect the Helix dopamine inhibitory receptor resembles the D-1 receptor proposed by Kebabian and Calne (1979) for mammalian central dopamine receptors.

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THE ACTION OF AMIDANTEL AND ITS DEACYLATED DERIVATIVES ON ACETYLCHOLINE RECEPTORS OF CENTRAL NEURONES OF HELIX ASPERSA.

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Amidantel (BAY d 8815) has been described as a promising representative of a new class of p-aminophenylamidine anthelmintic drugs (Wollweber et al 1979). This compound is effective against nematodes, filariae and cestodes in rodents (Wollweber et al 1979). Amidantel is deacylated in vivo to the corresponding free amino (BAY d 9216), which also exhibits anthelmintic activity (Tomlinson et al 1985). It has been reported by these authors that effects of amidantel and deacylated amidantel may be antagonised by the nicotinic antagonists, d-tubocurarine and gallamine. In the present study the effects of amidantel and its deacylated derivative were tested for activity at acetylcholine (ACh) receptors on Helix neurones which can be excited ('D' cells) or inhibited ('H' cells) by ACh.

Intracellular recordings were made from identified cells in the suboesophageal ganglia of the snail, Helix aspersa. Cell membrane activity was amplified using conventional electrophysiological methods and displayed as a permanent record on a Watanabe recorder. Compounds were dissolved in Helix saline and applied directly to the preparation. The relative potency ratios were calculated from partial dose-response curves such that if the compound under test was less active than ACh then its potency ratio was greater than one. In the ion substitution experiments, chloride was replaced by acetate while sodium was replaced by Tris. Experiments were repeated on at least four separate preparations and mean $\pm$ sem values are given.

Amidantel and its deacylated derivative were tested on both 'H' and 'D' cells and their potencies compared to that of ACh. Amidantel possessed weak ACh-like activity but was more than 100 times less potent than ACh on both cell types. Deacylated amidantel produced a similar depolarization and increase in conductance to that of ACh but was less potent with an equieffective molar ratio (EMR) of 26.0 $\pm$ 1.0. On 'H' cells, this compound produced a similar hyperpolarization and conductance increase to ACh with an EMR of 76.28 $\pm$ 3.25. In ion substitution experiments both deacylated amidantel and ACh excitatory responses were sodium dependent while for inhibitory responses, both compounds enhanced chloride conductance and in low chloride saline, the inhibitory responses were reduced or reversed. d-Tubocurarine, 50μM, reversibly antagonised both the excitatory and inhibitory ACh responses. The same concentration of d-tubocurarine reversibly antagonised the responses of deacylated amidantel on both 'H' and 'D' cells.

This study shows that amidantel and its deacylated derivative appear to act on the ACh receptors of Helix aspersa central neurones. The ionic mechanisms associated with the responses of deacylated amidantel appear to be similar to those for ACh. d-Tubocurarine was also found to reversibly antagonise the response to ACh and deacylated amidantel on both 'H' and 'D' cells. The present study supports the conclusion of Tomlinson et al (1985) that the primary mode of action of these compounds is at the level of the ACh receptor.

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EFFECTS OF ANTAGONISTS ON 5-HT AND OCTOPAMINE RECEPTORS IN THE FOREGUT OF SCHISTOCERCA GREGARIA.

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The foregut of the locust Schistocerca gregaria is innervated by nerves originating in the corpora cardiaca where 5-hydroxytryptamine (5-HT; Klemm, 1972) and octopamine (OA; Evans, 1978) are known to be present. Recently, Banner et al. (1986) demonstrated that the isolated foregut of Schistocerca gregaria was contracted by proctolin and relaxed by OA and 5-HT. They went on to suggest that, as 5-HT-inducted relaxation was blocked by mianserin and mimicked by MK212, the effects of 5-HT were mediated by a 5-HT_2-like receptor. The aim of this study was to further characterize receptors involved in foregut motility by comparing the effects of a range of antagonists.

Isolated foreguts (oesophagus to proventriculus) of Schistocerca gregaria were incubated in Clarke Insect Ringer at room temperature (18 $\pm$ 2°C) for 20 min, and using a 6 min cycle with 2 washes, dose response curves were constructed for 5-HT and OA. The general tonus of the tissue was maintained using alternate doses of proctolin (10^{-7}M) and either 5-HT or OA. The effects of mianserin (10^{-8}M - 5 X 10^{-5}M), ketanserin (10^{-8}M - 5 X 10^{-5}M) and ICS 205-930 (10^{-5}M) on the responses to 5-HT and OA were investigated, the tissue being incubated with the antagonist for 20 min prior to retesting the effects of these agonists.

5-HT (ED_{50}:4 $\pm$ 0.6 X 10^{-7}M; n = 18) and OA (ED_{50}:1.6 $\pm$ 0.2 X 10^{-6}M; n = 9) caused dose dependent relaxation of the foregut although the maximum response to OA was only 45% of that caused by 5-HT. Mianserin (10^{-8}M - 5 X 10^{-5}M) was found to be a competitive antagonist of 5-HT (pA_2 = 6.3; slope = 0.81; n = 8) and a non competitive antagonist of OA causing an approximate reduction of 50% in the maximum response to OA at a concentration of 10^{-6}M. By comparison, ketanserin had no effect on OA-induced relaxation but was a competitive antagonist of 5-HT (pA_2 = 5.65; slope = 1.11; n = 6) while ICS 205-930 had no effect on responses to 5-HT or OA.

The antagonistic effect of ketanserin on 5-HT-induced relaxation of the locust foregut and the lack of effect of the potent 'M' receptor antagonist ICS 205-930 (Richardson et al., 1985) adds further support to the presence of a 5-HT_2-like receptor in this tissue. The failure of ketanserin to antagonize OA-induced relaxation and the different kinetics of antagonism exhibited by mianserin against OA and 5-HT suggests that effects of these amines are mediated through different receptors. This is as would be expected with innervation by both 5-HT and OA containing neurons.

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RAPID SCREENING OF ALGAL CULTURES FOR ENZYME INHIBITORS WITH POTENTIAL PHARMACOLOGICAL ACTIVITY.

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Screening culture and organic solvent extracts of microorganisms, especially Streptomycetes, has resulted in the identification of a wide spectrum of compounds with biological activity, including antibiotics, antifungal agents and a range of enzyme inhibitors with pharmacological activities (Demain 1983, Schindler 1980.) However, such large scale screening programmes for pharmacologically active compounds have not yet been applied to algae. Some reports on antibacterial and antifungal activities from algae exist, but reports of enzyme inhibitors from algae are rare. We have therefore developed a range of rapid through-put assays for screening for the presence of enzyme inhibitors in culture filtrates and organic solvent extracts of both freshwater and marine algae. In excess of 500 samples can be screened in a normal working day with most assays, the majority of which are simple colourimetric microtitre plate assays. All assays have been optimised for the detection of inhibitors. Although developed specifically for screening algal cultures, these assays can also be used to screen bacterial or fungal cultures, plant extracts, animal tissue extracts, etc.

To date rapid assays for inhibitors of the following sixteen enzymes have been developed; Lipoxygenase, β-lactamase, ornithine decarboxylase, acetyl cholinesterase, collagenase, elastase, adenosine deaminase, α-amylase, α-glucosidase, β-galactosidase, papain, chymotrypsin, trypsin, leucine aminopeptidase, carboxypeptidase A and alkaline phosphatase. The usefulness of such assays has been demonstrated in an initial screen of 300 marine and freshwater algal cultures, where a total of 76 enzyme inhibitors were detected. (A total of 71 antibacterial effects and 25 antifungal effects were also detected from these cultures.) These results indicate that algae may well have considerable potential as a source of novel pharmacologically active compounds.

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THE LACK OF EFFECT OF SODIUM L-3,3,5-TRIIODOTHYRONINE ON LEAD II ECG SIGNALS IN CONSCIOUS CATS.

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Thyrotoxicosis causes cardiac disorders and ECG abnormalities in animals and man (Peterson et al 1982). This study was designed to investigate ECG changes in animals subjected to sustained, high plasma concentrations of Sodium L-3,3,5-triiodothyronine (L-T_3). Titanium electrodes, sited to facilitate the recording of lead II ECG signals, were implanted subcutaneously in eleven male cats, under halothane in N_2O/O_2 anaesthesia. The effects of chronic (fourteen day) oral administration of L-T_3, 0.25 mg/kg/day and vehicle alone (alkaline saline) were assessed daily on ECG parameters, Table 1. Body weight was recorded daily. Plasma T_3 concentrations were measured (using a Amerlex T_3 RIA kit) in both groups before, and at times during the treatment period. Significant differences between treatment groups were determined by analysis of variance. After a training period, of 6 weeks during which ECG signals were monitored, three cats were discarded due to inherent abnormalities, one with ectopics, one with predominant S-waves and the third with unacceptable day to day variation. The remaining cats were allocated to two groups of four.

TABLE 1
A comparison between published Lead II ECG values and those recorded in this study during the week before dosing

Parameter	Published Values Tilley L.P. (1985)	This Study Values represent the mean ± s.e.m. (n=8).
Mean: (beats/min)	197	239 ± 2.8
Rhythm	Normal sinus rhythm	Normal sinus rhythm
P-wave (mv)	0.2 Max.	0.13 ± 0.006
P-R interval (sec)	0.05-0.09	0.085 ± 0.001
QRS complex (sec)	0.04 Max.	0.046 ± 0.001
Height of R-wave (mv)	0.9 Max.	0.87 ± 0.033
S-T segment	No marked depression or elevation.	No marked depression or elevation.
T-wave (mv)	0.3 Max. Can be +ve or -ve	0.26 ± 0.009 All +ve.
Q-T interval (sec)	0.07-0.2	0.18 ± 0.002

No statistically significant changes in ECG patterns or heart rate were seen during or following treatment with L-T_3 or vehicle. However, a marked though not statistically significant increase in R-wave height was recorded in the group that received L-T_3. Pre-dose R-wave values were reestablished during the second week after the end of dosing. Plasma T_3 concentrations were less than 0.5 ng/ml at all times in the vehicle dosed cats, but reached 3.9 ± 0.5 and 29.7 ± 3.8 ng/kg, 3 and 24 hrs respectively, after dosing with L-T_3. There was also a statistically significant reduction in body weight in the L-T_3 dosed group. This study has shown that in animals with increased plasma thyroid hormone concentrations for 14 days, sufficient to cause a significant reduction in body weight, there were no effects on heart rate and minimal changes to ECG signals.

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THYROXINE ALTERS PRE-AND POST-SYNAPTIC SENSITIVITY IN THE MOUSE VAS DEFERENS.

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Thyroid hormones influence the sensitivity of sympathetically-innervated tissues to agonists (Gibson, 1981). For example, hyperthyroidism increases and hypothyroidism decreases the sensitivity of the heart to β-adrenoceptor agonists. Ligand binding studies support these results and indicate that hyperthyroidism increases the number of β-adrenoceptors and decreases the number of α-adrenoceptors, whilst hypothyroidism has the opposite effects (Kunos, 1977). Little attention has been focussed on effects of thyroid hormones on pre-synaptic receptors despite the important role of α_2-adrenoceptors in regulating transmitter release at the sympathetic neuro-effector junction (Gillespie, 1980). This study investigated the effects of thyroxine on the sensitivity of pre-synaptic α_2-adrenoceptors and opiate receptors and on the sensitivity of post-synaptic α_1-adrenoceptors and muscarinic cholinoceptors to agonists in the mouse vas deferens.

Adult male T.O. mice (25-30 g) were pretreated with L-thyroxine (T_4) (16 days, 20 mg kg^{-1} day^{-1} orally in drinking water). The effectiveness of this treatment was monitored by measuring free serum T_4 levels radiochemically by competitive binding analysis. Vasa were inserted in silver ring electrodes in organ baths containing Krebs' solution (37°C), gassed with 95% O_2/5% CO_2 and were field stimulated (10 pulses of 0.5 ms duration, 20 Hz, supramaximal voltage at 100 s intervals). Contractions of vasa to field stimulation or agonists were recorded isometrically.

T_4 pretreatment raised serum free T_4 levels from 0.9 ± 0.2 pmol l^{-1} in controls to 360 ± 30 pmol l^{-1} (mean ± s.e. mean, n = 8, P<0.001). This change was accompanied by complex changes in the sensitivity of the vas to agonists acting at both pre- and post-synaptic sites. T_4 pretreatment reduced the maximum percentage inhibition produced by clonidine from 87.2 ± 2 in controls to 46.8 ± 3.2 and the maximum percentage inhibition produced by morphine from 67.6 ± 1.2 in controls to 57.2 ± 4 (mean ± s.e. mean, n = 8. P<0.001). In addition, postsynaptic sensitivity changes also occurred. The maximum contractile responses (g) to noradrenaline and carbachol were reduced respectively from 0.49 ± 0.1 to 0.33 ± 0.05 and from 0.53 ± 0.05 to 0.3 ± 0.05 (mean ± s.e. mean, n = 8, 0.05>P>0.01). Moreover, the dose response curve for carbachol was displaced to the right by T_4 pretreatment. These results show that thyroxine influences both pre- and post-synaptic sensitivity in the mouse vas deferens.

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SK&F L-94901 - A NOVEL SELECTIVE THYROMIMETIC WITH POTENT HYPOCHOLESTEROLAEMIC ACTIVITY.

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In man thyroid hormones are known to reduce plasma cholesterol probably by effects mediated via thyroid hormone receptors in the liver. However, their potential utility as hypocholesterolaemic agents has not been realised therapeutically because of direct stimulation of thyroid hormone receptors in the heart, leading to serious cardiac complications[1]. Consequently a programme was instituted to design selectively acting drugs with hepatic thyromimetic activity but lacking direct cardiac effects. Additionally, to be clinically useful, a selective thyromimetic should only minimally affect the thyroid/pituitary axis or whole body oxygen consumption (WBOC) at therapeutic doses. Administration of thyromimetics to rats is known to cause specific and dose-dependent increases in the activity of the enzyme, mitochondrial glycerol-3-phosphate dehydrogenase (GPDH) in the heart and liver[2], and this effect has been used as a primary screen for novel compounds. In all the experiments described below rats, either euthyroid or rendered hypothyroid by pretreatment with methimazole, were given 7, daily, consecutive doses of thyromimetics, and measurements made 24 h after the final dose.

SK&F L-94901 is the most potent, selective thyromimetic yet synthesised. After intramuscular administration to hypothyroid rats its ED_{50} in the liver on GPDH activity was 36×10^{-9} moles $kg^{-1}d^{-1}$. By contrast, in the heart doses up to 10^{-6} moles $kg^{-1}d^{-1}$, had no effect. The ED_{50} for T_3 under these conditions was 46×10^{-9} moles $kg^{-1}d^{-1}$ and 58×10^{-9} moles $kg^{-1}d^{-1}$ in heart and liver respectively. The rate of beating of atria isolated from these rats was also determined. The ED_{50} for stimulation by T_3 was 4×10^{-8} moles $kg^{-1}d^{-1}$, but again doses of SK&F L-94901 up to 10^{-6} moles $kg^{-1}d^{-1}$ had no effect. In euthyroid rats after oral administration, SK&F L-94901 and T_3 were similar in activity as hepatic thyromimetics (ED_{50} 46×10^{-9} and 120×10^{-9} moles $kg^{-1}d^{-1}$ respectively) whereas SK&F L-94901 had no effect on cardiac GPDH at doses up to 1.8×10^{-5} nmoles $kg^{-1}d^{-1}$ (ED_{50} for T_3 106×10^{-9} moles $kg^{-1}d^{-1}$).

SK&F L-94901 given orally to euthyroid rats had no effect on circulating plasma T_3 concentrations at doses up to 1.8×10^{-6} moles $kg^{-1}d^{-1}$. By contrast plasma T_4 concentrations were affected in a dose-dependent manner: 1.8×10^{-7} moles $kg^{-1}d^{-1}$ causing a drop of 50%. When given orally to euthyroid rats T_3 increased WBOC by a maximum of 150% of the pretreatment value: SK&F L-94901 caused a maximum increase of 32% at 1.8×10^{-5} moles $kg^{-1}d^{-1}$.

In rats made hypercholesterolaemic by feeding a diet rich in cholesterol, SK&F L-94901 was equipotent with T_3 in reducing plasma cholesterol levels. In hypothyroid rats, given a dose of SK&F L-94901 of 2×10^{-9} moles $kg^{-1}d^{-1}$ orally for seven days, plasma cholesterol was 236±56 (6) mg dl^{-1} compared to a control value of 438±108 (6) mg dl^{-1}.

In conclusion SK&F L-94901 is a highly potent hepatic thyromimetic and hypocholesterolaemic agent but with minimal effects on the heart, circulating thyroid hormones or WBOC, and could thus be of value in treating hypercholesterolaemia.

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CIRAZOLINE AND UK-14,304 REVERSE ISCHAEMIC DEPRESSION OF SKELETAL MUSCLE CONTRACTIONS.

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An in vivo rat model has been developed to study the action of vaso-active drugs on skeletal muscle function compromised by partial ischaemia. Male Wistar rats (180-250g) were anaesthetised with Inactin (60-120mg/kg i.p.). The tendon of the right or left extensor digitorum longus muscle (EDL) was exposed in the foot, sectioned and the proximal end connected via a cotton thread to an isometric transducer. The leg was firmly clamped at the ankle and knee and a resting tension of 5.0g applied to the EDL. The sciatic nerve was exposed, sectioned and bipolar platinum ring electrodes were placed around the distal cut end. Supramaximal (5V;100μs) stimuli were applied to the nerve at a frequency of 0.1Hz. Every 5 min a burst of impulses of 5Hz was given for 30 s; the mean twitch height of three 5Hz stimulation periods was taken as the control twitch height. Blood pressure (BP) and heart rate (HR) were measured from a polythylene cannula inserted into the right carotid artery. One jugular vein was cannulated for the infusion of drugs. Animals were given heparin (1000u/kg;i.p.).

Partial ischaemia was produced by manually occluding the femoral artery by pulling a cotton tie placed around it. The artery was left occluded for 45 s in every min for a total of 15 min. Three periods of ischaemia were performed in each experiment, (each involving three bursts of 5Hz stimulation) a control ischaemia (I_1), a drug infused ischaemic period (I_2) and a post infusion ischaemia (I_3). At the end of the third 5Hz stimulation period during I_1 the twitch height had fallen to 36.8 ± 2.7% (n=38) of controls. Both the α_1-agonist cirazoline (CIR) and the α_2-agonist UK-14,304 (UK) (dose range 33 to 165μg/kg/min for both) caused a significant ($P<0.05$) dose independent reversal of the effect of ischaemia. The figures quoted below are for 82.5μg/kg/min UK and 33μg/kg/min CIR. At the end of the third 5Hz stimulation period the twitch height (during I_2) in the presence of CIR was 62.4 ± 6.8% (n=6) and in the presence of UK was 62.3 ± 3.9% (n=6) of control values. Following cessation of drug infusion, the ischaemic twitch height (I_3) returned to the level seen before drug infusion with UK (ie. I_1). However after CIR the post-ischaemic twitch height (I_3), was significantly ($P<0.05$) smaller than the I_1 twitch height, and was only 22 ± 5% (n=14) of the control twitch height. Cirazoline produced dose dependent increases in both mean BP and HR; 33μg/kg/min led to an increase of 10.0 ± 5.2mmHg and 5.0 ± 3.9 b.p.m. respectively whereas 165μg/kg/min resulted in an increase of 70.5 ± 6.9mmHg and 40.0 ± 5.86 b.p.m. respectively. UK had no significant effect on BP or HR with any dose used. Infusion of salbutamol (SAL) (β_2-agonist) 33μg/kg/min caused a significant ($p<0.05$) exacerbation of the ischaemic depression of skeletal muscle function. After the third 5Hz stimulation period the twitch height, (I_2) was only 14.1 ± 4.2% (n=11) of controls. Following cessation of SAL infusion the ischaemic twitch height (I_3) did not recover to I_1 levels. SAL infusion was accompanied by a decrease in mean BP of 18.6 ± 6.7mmHg (n=7) and an increase in HR of 80.8 ± 17.1 b.p.m. (n=6).

The reason for the reversal of ischaemia-induced depression of skeletal muscle function by UK is not known, changes in perfusion pressure with SAL and CIR may have contributed to the effects observed on skeletal muscle function with these agents.

A-L Humphreys is an S.E.R.C. (CASE) student
UK-14,304 was a gift from Pfizer

AMINOGLUTETHIMIDE POTENTIATES THE ANTI-NOCICEPTIVE ACTIVITY OF MEPTAZINOL IN MICE

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Aminoglutethimide (AG) is a non-steroidal agent used in the therapy of oestrogen-dependent breast cancers in post-menopausal women. As it is a competetive inhibitor of several cytochrome P-450 dependent enzymes (Daly et al 1986), the possibility of drug interactions with AG at this level exists. The present study was designed to determine the effect of AG on the anti-nociceptive activity of meptazinol (M) in mice using conventional anti-nociceptive tests.

The abdominal constriction test (Koster et al 1959) involved injecting acetic acid (3%v/v, 0.1ml/10g) intraperitoneally (i.p.) and counting the total number of abdominal constrictions elicited in the 30 minute period following injection of the irritant. A dose-response curve to M, given orally as a suspension (in 0.1%w/v carboxymethylcellulose/0.1%v/v Tween 80, 0.1ml/10g) 45 minutes before the i.p. irritant, was established to select an effective sub-maximal dose of M (20mg/kg) that could be given in combination with AG. The dose of AG chosen (10mg/kg) did not exhibit marked anti-nociceptive activity per se (< 2% change of control response); however, higher doses (25-100mg/kg) had a more pronounced protective effect. The drug combination (AG 10mg/kg + M 20mg/kg) was also tested using the mouse tail immersion test at 48°C (Sewell and Spencer 1976). Nociceptive sensitivity was monitored for 120 minutes. The results (table) show that AG markedly potentiates the anti-nociceptive activity of M in both tests.

Table: Anti-nociceptive activity (% ± S.E.M.) of M ± AG

TEST	ANTI-NOCICEPTIVE ACTIVITY (% ± S.E.M.)* -AG	+AG	Statistical Analysis used
Abdominal Constriction (n = 16)	33.5±1.6	55.1±3.8	Linear model $P < 0.005$
Tail Immersion (n = 20)	75.2±7.4	136.8±9.5	Mann-Whitney-U-Test $P < 0.0001$

Doses used, M 20mg/kg, AG 10mg/kg; * Expressed as % vehicle-treated animals

Since M is the active species, potentiation of its protective effect may indicate a decrease in its metabolism. When M was co-administered with SKF-525A (β-diethylaminoethyldiphenylpropyl acetate 10mg/kg i.p.)the increase in anti-nociceptive activity was similar to that obtained with the same dose of AG. There is a large species variation in the metabolism of M and it is not unlikely that the mouse eliminates a greater percentage by oxidation than man (R.A. Franklin, personal communication). Taken together with the fact that AG even at this low dose inhibits microsomal enzyme systems, these findings would seem to indicate that the interaction between M and AG is probably pharmacokinetic in nature.

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TOLERANCE TO THE CNS EFFECTS OF AMINOGLUTETHIMIDE IN MICE.

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Aminoglutethimide (AG) is an established treatment for advanced oestrogen-dependent breast cancer in postmenopausal women. Commonly occurring side effects such as ataxia, dizziness and lethargy (Harris 1985) are most likely related to CNS-depressant effects of the drug which was originally employed as an anti-epileptic agent. These effects are self-limiting and decrease within 2-6 weeks of commencing therapy (Santen et al., 1982). The present study examines the development of tolerance in mice to some CNS effects of the drug.

Male albino mice (20-22g) were employed for both tests selected for CNS activity (rota rod performance, Henauer et al., 1984; antileptazol activity, Swinyard et al., 1952). AG (50 mg/kg suspended in 0.75%w/v carboxymethylcellulose) or vehicle was administered orally to mice daily for 14 days. On day 16, groups (n = 10) of the chronically-dosed mice received either vehicle or one of several doses of AG orally. One hour later, when the drug had achieved its max.plasma level, the animals were evaluated in either of the two tests.

In both vehicle- and AG-pretreated mice, there was a well-defined log dose - response relationship for the antileptazol activity of AG ($r = 0.99$ and 0.98 respectively) over the dose range 10-70 mg/kg. The dose-response curve for the chronically dosed AG group was significantly ($P < 0.01$) shifted to the right (log potency ratio = 0.125). Rota rod performance was also linearly related to log dose of AG (range 50-300 mg/kg) in both vehicle- and AG-pretreated mice ($r = -0.92$ and -0.98 respectively). The curve for the AG-pretreated animals was significantly ($P < 0.05$) shifted to the right (log potency ratio = 0.275). These results indicate that a modest but significant degree of tolerance to the CNS depressant effects of AG occurs when it is administered chronically to mice. The mechanism for this phenomenon has not yet been established. However, in similarly pretreated animals not subjected to pharmacological evaluation, the 1 h mean plasma level of AG following a 50 mg/kg challenge dose of the drug was significantly ($P < 0.05$) lower in the AG-pretreated than in the vehicle-pretreated mice (14.3±0.4 and 17.0±0.3 µg/ml respectively). This suggests that some degree of dispostional tolerance may be involved.

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STUDIES ON THE δ-OPIOID RECEPTOR MEDIATED INHIBITION OF Isc IN THE ISOLATED GUINEA-PIG ILEAL MUCOSAL PREPARATION.

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Recent evidence suggests the antidiarrhoeal activity of opioids can be attributed to an antisecretory as well as an antipropulsive action. Experiments to study the opioid receptor mediating inhibition of anion secretion in isolated guinea-pig ileal mucosa (gpIm) suggests that it is of the delta subtype (Vinayek et al., 1983), although the mechanism involved is not known. The present study was designed to examine further this opioid receptor-mediated effect.

Isolated ileal mucosal preparations from male Dunkin-Hartley guinea-pigs were mounted in Ussing chambers and short-circuit current (Isc) recorded (Kachur et al., 1980). The serosal surface was bathed in Krebs-Ringer maintained at 37°C containing the following (mM concentration) : glucose, 11.1; NaCl, 117; KCL, 4.7; $NaHCO_3$, 24.8; $MgSO_4$, 1.2; KH_2PO_4 1.2; $CaCl_2$, 0.63; and gassed with 5% CO_2 in O_2 (pH 7.4). The mucosal surface was bathed in the same medium except mannitol replaced glucose. Opioids were added to the serosal side and cumulative dose-response curves constructed.

Table 1	IC_{50}(nM)	Emax(↓in Isc μA cm^{-2})	ICI174864 pA_2	Slope	n
DADLE	4±1	44±3	7.4 (7.1-8.0)	1.2±0.1	4
DSLET	8±3	49±3	not determined	-	
DPDPE	15±3	59±6	8.0 (7.4-9.4)	1.1±0.1	4
FK33824	600±150	49±3	7.6 (7.3-9.8)	1.1±0.2	4
DAGO	3310±1800	52±6	7.9 (7.6-8.2)*	-	6

*Single concentration pA_2 value.

Table 1 shows that the rank order of potency for inhibiting Isc was DADLE>DSLET>DPDPE>FK33824>DAGO; all produced similar maximal effects. U50488 and fentanyl were inactive at 10^{-5}M. The delta-selective opioid antagonist, ICI174864, antagonised the decreases in Isc. The pA_2 values determined from Schild analysis were consistent with an interaction at delta receptors.

Pre-treatment of the serosal side with the irreversible opioid antagonist ß-funaltrexamine (ß-FNA; 10^{-6}M for 30 min), followed by extensive washout, shifted the dose-response curves for DADLE and DPDPE to the right and reduced the maximal response, confirming previous evidence (Hayes et al., 1985) that ß-FNA has delta- as well as mu-receptor antagonist properties. Agonist affinities were calculated using the method of irreversible antagonism and were as follows : DADLE, 12±7 nM (n=4); DPDPE, 26±8 nM (n=4).

In rabbit ileal mucosa lowering [Ca^{2+}] has been shown to increase the inhibitory effect of opioids on Isc (McKay et al., 1981). However, in the present study, there was no difference in the potency of the opioid peptides DADLE, DSLET, FK33824 and DPDPE in either 0.63 mM or 2.5 mM Ca^{2+}. The lower [Ca^{2+}] was used routinely as the mucosal preparations were more stable under these conditions. Interestingly TTX (30nM) reduced Isc (↓ 51±11 μA cm^{-2}) suggesting that a stimulatory endogenous tone exists in the gpIm; DADLE had no effect in the presence of TTX.

The results confirm the role of delta receptors in inhibiting anion secretion in gpIm and suggest that this occurs by a calcium-independent mechanism.

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DOES FEVERFEW EXTRACT EXHIBIT PHOSPHOLIPASE A_2 INHIBITORY ACTIVITY IN VIVO?

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Since the Middle Ages, feverfew (Tanacetum parthenium) has been used to relieve fever and a variety of conditions such as asthma, arthritis and migraine. Recent studies in vitro with human platelets have suggested that feverfew extract may possess phospholipase A_2 (PLA_2) inhibitory activity (Collier et al., 1980; Makheja and Bailey, 1982). We were therefore interested to see whether PLA_2 inhibitory activity of feverfew extract could be demonstrated in vivo. In the anaesthetised guinea-pig, collagen- and arachidonic acid (AA)-induced bronchoconstriction is mediated by thromboxane (Tx)A_2 (Vargaftig et al., 1979). Bronchoconstriction to both collagen and AA can be inhibited by cyclo-oxygenase inhibitors such as aspirin and indomethacin or thromboxane (TP) receptor blocking drugs such as AH 23848 (Lumley and Humphrey, 1985). In contrast to exogenously administered AA, collagen-induced TxA_2 generation is believed to require activation of PLA_2 to release endogenous AA from membrane phospholipids prior to its subsequent metabolism . Thus a compound with PLA_2 inhibitory activity should antagonise bronchoconstriction produced by collagen but not that produced by AA.

Pentobarbitone (60mg/kg i.p.) anaesthetised guinea-pigs (male 300-500g, Porcellus) were surgically prepared for recording tracheal inflation pressure (TIP), blood pressure (BP) and heart rate (HR). Feverfew extract was prepared by homogenising 1g of chopped fresh leaves in 2.5 ml distilled water for 2-3 minutes. The homogenate was centrifuged at 10,000g for 5 minutes and the resulting supernatant (pH 5.2 ± 0.2, mean ± s.e. mean, n=18) stored on ice until used. Following a 20 minute stabilisation period feverfew extract or pH adjusted vehicle was administered to the animals. The extract (1 ml i.v.) produced a small but reversible bronchoconstriction (25 ± 5% increase in TIP) as well as a transient reduction in diastolic BP (6 ± 5 mmHg) and reduction in HR (20 ± 4 beats/min). Fifteen minutes after administration of the extract, 5-HT (3 μg/kg i.v.) or U-46619 (1 μg/kg i.v.) was administered to the animals. A further 15 minutes later collagen (0.1 mg/kg i.v.) or AA (1 mg/kg i.v.) was administered. Feverfew extract significantly inhibited collagen-induced bronchoconstriction (115 ± 22 versus 36 ± 5% increase in TIP, n=9, 69% inhibition $P<0.01$) whereas that induced by AA was not affected (298 ± 18 versus 297 ± 17% increase in TIP, n=9). The bronchoconstriction produced by agents which induce this effect independently of TxA_2 formation (e.g. 5-HT and U-46619) were not significantly inhibited.

These data are consistent with feverfew extract possessing PLA_2 inhibitory activity in vivo. Whether this activity is responsible for the claimed efficacy of feverfew in diseases such as migraine (Johnson et al., 1985) remains to be determined.

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pH DEPENDENT STIMULATION BY FREE CALCIUM OF PROSTACYCLIN SYNTHESIS BY RAT AORTIC RINGS.

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Calcium influences prostacyclin (PGI_2) synthesis, which is stimulated by calcium ionophore (A23187) (Weksler et al, 1978), and inhibited by 8(N,N-diethylamino)-octyl-3,4,5-trimethoxybenzoate (TMB-8) (Brotherton & Hoak 1982), an antagonist of calcium release from intracellular stores. We now report that extracellular free calcium stimulates PGI_2 synthesis by rat aortic rings at physiological pH even in the absence of calcium ionophore. However, stimulation is much reduced at or below pH 7, explaining earlier negative findings (Ritter et al, 1982). Matched groups of fresh aortic rings were prepared from male Sprague-Dawley rats (Ritter et al, 1982) and incubated (60 min, 37°C) in balanced salt solutions with varying concentrations of calcium chloride, and buffered with HEPES. PGI_2 was measured by radioimmunoassay of its stable hydrolysis product, 6-oxo-prostaglandin (PG) $F_{1\alpha}$ (Orchard et al, 1982). Stimulation was sought by comparison by paired t test of 6-oxo-$PGF_{1\alpha}$ production (ng/mg) by rings from each rat in the presence or absence of calcium, and considered significant (*) when $P < 0.05$. Values are given as means ± SE mean. In one group of experiments (n = 6), rings were incubated at pH 6.5, 7.0, 7.4, and 8.0 with or without calcium (20 mM). 6-Oxo-$PGF_{1\alpha}$ production (ng/mg) in the absence/ presence of calcium was 2.2 ± 0.1/3.7 ± 0.1; 2.0 ± 0.4/4.3 ± 1.1; 3.9 ± 0.9/11.1 ± 2.2*; 6.8 ± 0.9/27.7 ± 4.2* at the respective pH values. In contrast, A23187 (5×10^{-6} M) caused similar stimulation of 6-oxo-$PGF_{1\alpha}$ production at pH 7 and pH 8 (calcium = 2.5 mM), n = 3: 22.1 ± 3.8 (no ionophore)/40.8 ± 3.9 (with ionophore) at pH 7, and 25.6 ± 2.6 (no ionophore)/41.2 ± 4.8 (with ionophore) at pH 8. In experiments at pH 8, calcium concentration was varied in the absence of A23187 (n = 7): 6-oxo-$PGF_{1\alpha}$ production was 8.1 ± 1.9; 10.4 ± 2.0*; 11.0 ± 2.7*; 15.0 ± 2.3*; 24.6 ± 4.7* and 35.4 ± 4.9* at calcium concentrations of 0, 0.5, 2.5, 5.0, 10.0 and 20.0 mM respectively. Stimulation by 20 mM calcium at pH 8 was abolished by a molar excess of sodium citrate (n = 3) or sodium oxalate (n = 3). TMB-8 (2×10^{-4} M) inhibited basal and calcium (20 mM) stimulated 6-oxo-$PGF_{1\alpha}$ synthesis similarly at pH 8 (47.8 ± 8.2% and 46.8 ± 4.0% respectively). Nifedipine (10^{-4} - 10^{-7} M) did not inhibit basal 6-oxo-$PGF_{1\alpha}$ synthesis, but did inhibit calcium (20 mM) stimulated synthesis (pH 8), by 25.1 ± 5.3% (10^{-7} M) - 75.8 ± 6.6% (10^{-4} M), n = 6. A structurally related dihydropyridine, BAY K 8644, that activates calcium channels, did not enhance basal or calcium stimulated 6-oxo-$PGF_{1\alpha}$ synthesis, at 10^{-5} or 10^{-4} M. Verapamil (10^{-4} M) and diltiazem (10^{-4} M) did not inhibit basal or calcium stimulated 6-oxo-$PGF_{1\alpha}$ synthesis. We conclude that there is an interaction between extracellular H^+ and free calcium on PGI_2 synthesis by rat aorta. Since effects of pH and calcium concentration occur over the physiological range, this may represent an important control mechanism.

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A NOVEL CYCLOHEXANETRIONE DERIVATIVE WHICH STIMULATES THE PRODUCTION OF PROSTAGLANDINS BY RAT POLYMORPHONUCLEAR LEUCOCYTES (PMN).

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A novel series of cyclohexanetrione derivatives has been identified which stimulate prostaglandin synthesis in whole cells. The action of one of these compounds, Ro 31-0521, 3,3,5,5-tetraallyl-2-hydroxy-N-(5-methyl-3-isoxazolyl)-4,6-dioxo-1-cyclohexane-1-carboxamide, has been studied on phagocytosing PMN to try to ascertain its mode of action.

Rat peritoneal PMN, induced by casein, were harvested and suspended at $3x10^7$ cells/ml in Gey's Balanced Salt Solution (GBSS). This suspension (400µl) was added to a preincubated suspension of complement coated yeast cells, $6x10^8$ particles, in a solution of test compound (plus ferricytochrome C & catalase for assay of $O_2^{\bullet}$) in 1.6ml GBSS. Phagocytosis was allowed to proceed at 37°C for 30 min and was terminated by centrifugation. The supernatant fluid was assayed for inflammatory mediators. Products of the arachidonic acid pathway were extracted from acidified solution into ethyl acetate and assayed by bioassay on rat stomach strip (PGE_2) or by radioimmuno assay. The lysosomal enzyme ß-glucuronidase was assayed using phenolphthalein-ß-D-glucuronide as substrate. Release of $O_2^{\bullet}$ during phagocytosis was estimated by the reduction of ferricytochrome C measured photometrically at 555nm.

Table 1 Effect of Ro 31-0521 on mediator release from PMN

CONCENTRATION of Ro 31-0521	% STIMULATION OF MEDIATOR RELEASE					
	PGE_2 BIOASSAY	PGE_2 RIA	TXB_2	PGI_2 as 6-keto-$PGF_{1\alpha}$	ß-GLUC	$O_2^{\bullet}$
10^{-4}M	230		>240	500	6	0
10^{-5}M	130	120			-8	-10
10^{-6}M	110	85			-12	-4

PGE_2 values are the means of at least 9 separate experiments.
Others 2 or 3 experiments.

Ro 31-0521 apparently stimulates the whole of the cycloxygenase pathway. However, separate experiments showed it was without effect on PGE_2 synthesis from arachidonic acid by isolated "prostaglandin synthetase" from ovine seminal vesicles or lung homogenates. Thus the effect is unlikely to be due to direct action on enzymes of this pathway post arachidonate release. However, lysosomal enzyme & superoxide release were not significantly effected. Thus the action is not one of general cell stimulation.

Compounds which can raise tissue prostaglandin levels may have therapeutic value in the treatment of gastric ulcers (Pomarelli et al, 1980) or for the induction of labour.

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ACTION OF INHIBITORS OF Na^+/H^+ EXCHANGE ON CALCIUM STIMULATED PROSTACYCLIN SYNTHESIS BY RAT AORTIC RINGS.

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Free calcium stimulates prostacyclin (PGI_2) synthesis by rat aortic rings at pH 7.4 and greater, but not below pH 7 (Frazer et al, 1986). Several enzymes of the eicosanoid cascade are calcium-dependent and it is possible that the effect of extracellular H^+ operates indirectly by an action on intracellular H^+. One mechanism implicated in the control of intracellular H^+ is Na^+/H^+ exchange (Johnson et al, 1976), and inhibitors of Na^+/H^+ exchange block stimulus provoked arachidonic acid release in human platelets (Sweatt et al, 1985). The object of this study was to determine the effect of inhibitors of Na^+/H^+ exchange on PGI_2 synthesis by aortic rings. These were freshly prepared from male Sprague-Dawley rats and allotted to 4 matched groups per animal (Ritter et al, 1982). Each group of aortic rings was incubated at 37°C for 60 min in balanced salt solution (NaCl 120 mM; KCl 4 mM; glucose 5 mM; HEPES 25 mM: pH 8). Groups of rings were incubated with and without calcium chloride (20 mM) in the presence and absence of an inhibitor of Na^+/H^+ exchange. PGI_2 synthesis was determined by radioimmunoassay of its stable hydrolysis product, 6-oxo-prostaglandin (PG) $F_{1\alpha}$ (Orchard et al, 1982). Results are given as means ± SEM; inhibition was assessed by the paired t test and considered significant when $P < 0.05$. Two 5-alkylamino substituted analogues of amiloride which are more potent inhibitors of Na^+/H^+ exchange than is amiloride itself (Zhuang et al, 1984) were tested. 3-Amino-5-dimethylamino-6-chloro-N-(diaminomethylene) pyrazinecarboxamide (A1), 4×10^{-5} M, did not affect basal PGI_2 synthesis (6-oxo-$PGF_{1\alpha}$ production 23.9 ± 4.7 ng/mg and 24.9 ± 4.3 ng/mg in the absence and presence of A1 respectively). At the same concentration, A1 inhibited significantly calcium stimulated 6-oxo-$PGF_{1\alpha}$ synthesis, by 38.1 ± 8.4% (n = 8). 3 Amino-5-ethyl, propyl, amino-6-chloro-N-(diaminomethylene) pyrazinecarboxamide (A7), 10^{-5} M, also did not affect basal PGI_2 synthesis (6-oxo-$PGF_{1\alpha}$ production 21.0 ± 3.8 ng/mg and 18.1 ± 4.3 ng/mg in absence and presence of A7 respectively). This concentration of A7 likewise inhibited significantly calcium stimulated 6-oxo-$PGF_{1\alpha}$ synthesis, by 45.7 ± 11.0 % (n = 8). Higher concentrations of A1 or A7 (4×10^{-4} M and 10^{-4} M respectively), increased basal PGI_2 synthesis, perhaps because of a non-specific toxic action (Zhuang et al, 1984). We conclude that inhibitors of Na^+/H^+ exchange inhibit calcium stimulated PGI_2 synthesis in rat aortic rings, perhaps by an action on intracellular pH. Alternatively, a direct action on calcium entry or calcium/sodium exchange has not yet been excluded.

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EFFECTS OF PHENTOLAMINE ON THE RELEASE OF [^{3}H]-(-)- AND ENDOGENOUS -NORADRENALINE BY DIRECT ELECTRICAL STIMULATION OF VASA DEFERENTIA.

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Most of the evidence in favour of α-adrenoceptor mediated feedback was obtained in tissues labelled with tritiated noradrenaline (Starke, 1977; Langer, 1981). Results reported by Hughes (1973) using a fluorimetric assay and Moura et al (1984) using HPLC-EC demonstrate that recently incorporated amines ([^{3}H]-noradrenaline or adrenaline) do not mix homogeneously with the tissue stores of noradrenaline and are preferentially released by electrical stimulation. We have looked for differential effects of α-adrenoceptor blockade by phentolamine on endogenous and [^{3}H]-noradrenaline simultaneously released by electrical stimulation of guinea-pig vas deferens preloaded with the radioactive amine.

The preparations (n=8) were incubated with [^{3}H]-(-)-noradrenaline (specific activity 23.1 Ci mmol^{-1}) in a concentration of 2.3 μmol l^{-1} during 1 h. Hydrocortisone (40 μmol l^{-1}) was present in the Krebs solution fluid throughout the experiment. The vasa deferentia were previously submitted to a 30 min preincubation with 1 mmol l^{-1} pargyline. The tissues were placed in perfusion chambers and washed out for 110 min; subsequently the fluid was continuously collected in samples of 30 min. Two periods of electrical stimulation (S1 and S2) with platinum electrodes directly applied to the ends of the preparation were performed at 170 min and 260 min (1 Hz, 2 msec, 100 V, 25 min). Cocaine 7.5 μmol l^{-1} was introduced 30 min before S1. Phentolamine in a concentration of 3 μmol l^{-1} was added to the medium 30 min before S2.

The basal and stimulation-induced efflux were analysed by scintillation counting and HPLC-EC. Results are summarized in the table.

	Tissue (μg/g)	Fractional release (x10^{-5}) S1	S2	S2/S1
Tritiated	1.47 (±0.15)	13.64 (9.71 19.16)	25.93 (21.54 31.21)	1.90 (1.44 1.52)
Endogenous	28.04 (±1.42)	4.63 (3.38 6.33)	8.55 (5.92 12.35)	1.85 (1.42 2.40)

Results are expressed as arithmetic mean ± S.E. or geometric mean and 95% confidence limits (in brackets).

The release of endogenous noradrenaline expressed as a fraction of the tissue amine content was much lower than the fractional release of [^{3}H]-(-)-noradrenaline. However, the increase of [^{3}H]-(-)-noradrenaline overflow by phentolamine was the same as that observed for endogenous noradrenaline.

These results show that the release of the endogenous transmitter and that of the recently incorporated amine are equally governed by α-adrenoceptor negative feedback. The fractional release of [^{3}H]-(-)-noradrenaline was higher than that of endogenous noradrenaline, indicating that the incorporated amine is accumulated predominantly in the releasable pool. This can be due to a more peripheral location of the releasable pool, thus more readily accessible to amines present in the surrounding medium.

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PHARMACOLOGICAL ACTION OF HORDENINE (N,N-DIMETHYLTYRAMINE) ON THE RAT VAS DEFERENS.

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Tyramine and its N-methylated derivatives, N-methyl tyramine and N,N-dimethyl tyramine (hordenine) occur in foods (Barwell & Blunden, 1981). Both tyramine and N-methyl tyramine are indirectly acting sympathomimetic amines with similar potency upon the isolated rat vas deferens (Patil et al., 1967). The pharmacological actions of hordenine do not appear to have been determined. We have investigated the effects of hordenine upon the isolated rat vas deferens, as part of a study to assess the pharmacological significance of dietary hordenine.

Vasa deferentia from Sprague-Dawley rats (200-300g) were used in Mg^{2+}-free Tyrodes bubbled with 95% O_2 : 5% CO_2. Some rats received chronic treatment with guanethidine (injected i.p. 25 mg/kg per day 5 days for 6 weeks) in order to destroy adrenergic nerves (Burnstock et al., 1971).

Exogenous noradrenaline and tyramine produced concentration-dependent contractions of untreated vasa. Half-maximal contraction was produced by 3 μM noradrenaline and 20 μM tyramine. In contrast, hordenine at up to 150 μM produced no measurable contraction. However, hordenine (25 μM) did affect responses to noradrenaline and tyramine. Responses to sub-maximal doses of noradrenaline were potentiated whilst responses to tyramine were inhibited. In denervated vasa neither tyramine nor hordenine produced contractions and hordenine (25 μM) had no effect upon responses to noradrenaline. Table 1 illustrates these effects of hordenine upon responses to noradrenaline (3 μM, untreated vasa: 0.1 μM, treated vasa) and tyramine (20 μM, untreated vasa).

Table 1

	% Change of Responses to	
	noradrenaline	tyramine
Hordenine (25 μM)	+94.6 ± 2.1*	-75.2 ± 1.9*
Denervation + Hordenine (25 μM)	-2.8 ± 2.4	-

mean ± S.E.M., n = 4, + increase, - decrease, t-test: *p <0.001

The results indicate that hordenine is not an indirectly acting sympathomimetic amine, in contrast to tyramine and N-methyl tyramine. However, it does exhibit pharmacological activity on vasa. The inhibition of responses to tyramine, which must be taken up presynaptically in order to produce contractions, indicates inhibition of neuronal amine uptake, as does the potentiation of responses to noradrenaline. This is supported by the observation that hordenine did not alter responses of denervated vasa to noradrenaline. Thus it appears that, if absorbed, dietary hordenine may interact with noradrenergic mechanisms and potentiate responses to noradrenaline.

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CALCITONIN IN ENDOTOXIN SHOCK.

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Naloxone raises blood pressure after endotoxin (Holaday et al, 1978) and after haemorrhage. Salmon calcitonin (sCT) has a pressor effect in rats rendered hypotensive by haemorrhage (Bates et al, 1983), but Quimby et al, 1985 have shown a depressor effect of sCT in baboons during toxic shock syndrome. We have therefore compared the effects of calcitonin with those of naloxone on blood pressure of anaesthetised rats subjected to endotoxin shock.

Sprague-Dawley rats (150-300g) were anaesthetised with i.p. sodium pentobarbital (60mg.kg^{-1}). The trachea, jugular vein, and carotid artery were cannulated for artificial ventilation, drug administration, and the measurement of blood pressure respectively. Rats were maintained on a ventilator (50 strokes/min) throughout the experiment, and anaesthesia was maintained by s.c. sodium pentobarbital as required. After a 20 min stabilisation period an i.v. infusion of E.coli lipopolysaccharide (0127:B8) was initiated (2mg.kg^{-1} over 2 hr at 0.03 ml/min). This produced a rapid fall in mean arterial pressure (M.A.P.), the maximum response (-47 ± 8mm Hg) occurring at 30 min. Twenty-five min after the start of the endotoxin infusion i.v. naloxone in 0.9% NaCl, sCT in 0.15M sodium phosphate buffer containing 0.1% BSA, or appropriate vehicles were administered. M.A.P. was monitored for a further 95 min. Statistical analysis was by analysis of variance followed by the students 't' test as appropriate.

Table 1 Increase in M.A.P. after drug treatment in rats subjected to endotoxin shock.

Treatment	ΔM.A.P. (mmHg) : post drug treatment ($\bar{x}$ ± S.E., *p< 0.05) n= 6.		
	15min	35min	75min
Endotoxin alone	7 ± 8	2 ± 10	4 ± 11
Endotoxin + naloxone (10mg.kg^{-1})	27 ± 6	31 ± 6 *	30 ± 7 *
Endotoxin + 0.9% NaCl	18 ± 4	9 ± 5	6 ± 7
Endotoxin + sCT (10U.kg^{-1})	18 ± 6	14 ± 7	11 ± 5
Endotoxin + sodium phosphate	29 ± 6	19 ± 4	16 ± 4

In the rats treated with endotoxin infusion and either vehicle there was a transient increase in M.A.P. during the first 15 min. These increases, however, were not significant. As shown in table 1, naloxone resulted in a significant increase in M.A.P. at 35 min. This pressor response was maintained for the duration of the experiment. In contrast, sCT had no significant effects on M.A.P. It was noted, however, that when compared with the corresponding control sCT tended towards a lower rather than a higher M.A.P.

We have shown that sCT had no significant effect on M.A.P. of rats treated with endotoxin.

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A DIFFERENTIAL EFFECT OF PRAZOSIN ON THE TWO PHASES OF EGTA-RESISTANT CONTRACTIONS OF RAT AORTA TO NORADRENALINE.

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We have previously described a selective reduction by prazosin (PZ) of the initial, transient EGTA-resistant contraction of rat aorta to noradrenaline (NA) (Downing et al, 1983), an effect not seen with corynanthine (COR). We have suggested that this effect of PZ may be related to its slow rate of dissociation from the adrenoceptors (Downing et al, 1985). Since Heaslip and Rahwan (1982) have shown that EGTA-resistant responses of rat aorta have two distinct phases, representing two distinct pools of calcium, we have reinvestigated the effect of prazosin on these two phases and compared it action with that of COR.

2-3mm circular preparations of thoracic aortae from male Wistar rats (180-200g) were suspended under a resting tension of 2g in Krebs' solution containing 2.5mM Ca^{++} and gassed with 5% CO_2 in O_2. Reproducible responses were obtained to $3x10^{-6}$M NA and the effects of a 30 min incubation with COR ($1x10^{-6}$M) or PZ ($1x10^{-9}$M) were determined. The effects of these antagonists were also studied on contractions to $3x10^{-6}$M NA following 5 min exposure to Ca^{++}-free Krebs' containing 0.5mM EGTA (EGTA-resistant responses). Contractions to $3x10^{-6}$M NA in the presence of Ca^{++} were biphasic and the initial fast component measured at the inflexion point was 38.1 ± 1.3% (n=8) of the maintained slow component. COR and PZ caused a similar small reduction of the slow component, 4.1 ± 2.4% and 5.9 ± 2.9% (n=8) respectively, but the reduction of the fast component was significantly greater following PZ than following COR (82.4 ± 4.2% and 39.7 ± 10.5% (n=8) respectively). The EGTA-resistant responses to $3x10^{-6}$M NA were also biphasic with an initial transient peak phase, measured at 15-20 seconds and a secondary sustained phase measured at 25 min. PZ caused a significantly greater ($p<0.05$) reduction of the peak EGTA-resistant response than did COR. No difference was seen between the two agents on the sustained phase of the EGTA-resistant contractions; both agents produced a small reduction of this phase which was not significant ($p>0.05$) (Table 1).

Table 1. Effect of PZ and COR upon responses to NA ($3x10^{-6}$M) in the presence and absence (EGTA-resistant response) of Ca^{++}. (* $p<0.05$) n=8.

	Slow response with Ca^{++}	Peak EGTA	Sustained EGTA
NA	100%	27.0 ± 0.9%	12.0 ± 1.5%
NA + PZ	94.1 ± 2.9%	8.8 ± 0.8%*	9.4 ± 1.0%
NA + COR	95.9 ± 2.4%	23.2 ± 1.4%	9.6 ± 1.1%

The selective reduction by PZ of the initial phase of contractions to a high concentration of NA (in the presence or absence of Ca^{++}) together with similar reductions by both agents of the slow and sustained phases supports the view that this effect is due to a slow rate of dissociation of PZ from the receptors and mitigates against a selective effect of PZ on calcium release per se. The corollory of this suggestion is that agonists with low rates of association would not be expected to produce an initial transient release of calcium but should be able to induce a later sustained release.

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PREJUNCTIONAL INHIBITORY ACTIONS OF α_1-ADRENOCEPTOR AGONISTS IN THE RAT.

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While inhibitory alpha-adrenoceptors on adrenergic nerve terminals were initially thought to be exclusively of the alpha-2 subtype, evidence has accumulated for a proportion of alpha-1 adrenoceptors prejunctionally in pithed rat heart, dog heart, guinea-pig atria and rat vas deferens (see Warnock et al., 1985 for references). However, de Jonge et al. (1986) have suggested that the cardio-inhibitory actions of alpha-1 adrenoceptor agonists in the pithed rat heart are due to post-junctional actions of the drug, since these authors found that alpha-1 adrenoceptor agonists inhibited the tachycardia to isoprenaline as well as that to nerve stimulation. The object of this study was to re-investigate the inhibitory actions of alpha-1 adrenoceptor agonists on the pithed rat heart.

Male Wistar rats were pithed by the method of Gillespie et al. (1970), and respired with 100 % O2. Animals were given atropine (0.6 mg kg-1) before beginning experiments. Cardiac effects of sympathetic stimulation via the pithing rod (supramaximal voltage, 0.5 ms pulses, single stimulus or 1 Hz 4 pulses), noradrenaline (NA)(1 µg kg-1) and isoprenaline (ISO)(3 ng kg-1) were examined before and after cirazoline (100 µg kg-1). This dose of cirazoline caused marked bronchoconstriction and oedema, and was occasionally fatal.

Control cardioaccelerator responses to 1 pulse, 1Hz 4 pulses, NA (1 µg kg-1) and ISO (3 ng kg-1) were 22.5 $\pm$ 6.8 min-1, 71.0 $\pm$ 3.1 min-1, 57.0 $\pm$ 9.3 min-1 and 40.2 $\pm$ 5.9 min-1 (n = 4 each). Cirazoline (100 µg kg-1) caused a tachycardia of 18.8 $\pm$ 4.3 min-1 (n=5), although the heart rate had partially recovered when agonist and stimulation responses were measured e.g. heart rate was elevated by 8.2 $\pm$ 5.6 min-1 (n=4) when responses to a single stimulus were obtained. Cirazoline significantly reduced the cardioacceleration to a single pulse and to 1 Hz 4 pulses to 12.3 $\pm$ 6.3 % of control and 51.9 $\pm$ 15.0 % of control, respectively, but did not significantly affect the tachycardia to NA (78.5 $\pm$ 5.2 %) or ISO (91.7 $\pm$ 18.3 %) as compared with the effects of saline. Yohimbine (1 mg kg-1) did not alter the inhibitory effects of cirazoline, but prazosin (1 mg kg-1) pretreatment prevented the cardio-inhibitory effects of cirazoline against the responses to nerve stimulation.

Hence, although cirazoline does produce a small tachycardia by alpha-1 adrenoceptor stimulation, this does not explain the inhibitory actions against stimulation evoked cardioacceleration, since responses to NA or ISO were unaffected. This inhibitory action of alpha-1 adrenoceptor agonists appears to be prejunctional in origin, and indeed a direct inhibition of the stimulation evoked release of NA has recently been demonstrated (Story et al., 1985).

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PREJUNCTIONAL INHIBITORY 5-HYDROXYTRYPTAMINE (5-HT) RECEPTORS IN THE RAT HEART AND VAS DEFERENS.

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Prejunctional inhibitory 5-HT receptors in rat brain cortex (Engel et al., 1986) and in rat vena cava (Göthert et al., 1986) have been reported to be similar to 5-HT-1B binding sites in terms of agonist and antagonist potencies. We have investigated inhibitory 5-HT receptors in pithed rat heart and rat isolated vas deferens.

Male Wistar rats were pithed by the method of Gillespie et al. (1970), and the pithing rod was used to stimulate the cardioaccelerator nerves with a single stimulus every 2 min (supramaximal voltage, 0.5 ms pulses). Epididymal portions of rat vas deferens, treated with nifedipine (10 μM) to eliminate postjunctional actions of 5-HT receptor agonists, were stimulated with a single electrical pulse (supramaximal voltage, 0.5 ms pulses) every 5 min. Effects of agonist drugs were assessed against stimulation evoked responses in the presence or absence of antagonist drugs.

In pithed rats, 5-carboxyamidotryptamine (5-CT) inhibited the stimulation-evoked cardioacceleration with a potency approximately 200 times greater than that of the selective 5-HT-1A receptor agonist 8OHDPAT (Middlemiss & Fozard, 1983). The 5-HT-1A selective antagonist spiroxatrine (0.3 mg kg-1) was ineffective at antagonising the inhibitory effects of 5-CT, as was yohimbine (1 mg kg-1) and cyproheptadine (1 mg kg-1). Cyanopindolol, a potent 5-HT-1B receptor antagonist, could not be examined since it caused a marked tachycardia in pithed rats.

In epididymal portions of rat vas deferens, 5-CT was approximately 5 times more potent than 8OHDPAT and 100 times more potent than 5-HT at reducing the stimulation evoked contraction. However, 5-CT behaved as a partial agonist, producing a maximum inhibition of the stimulation evoked contraction of only approximately 50%. Propranolol (1-10 μM), cyanopindolol (1 μM), cyproheptadine (1-10 μM), spiroxatrine (0.1 μM) and rauwolscine (0,3 μM) failed to alter the potency of 5-CT or 6OHDPAT.

In conclusion, our data are in agreement with previous findings in so far as we found no evidence in favour of a 5-HT-1A receptor involvement in the prejunctional inhibitory effects of 5-HT receptor agonists. However, the failure of cyanopindolol to antagonise the effects of 5-CT in rat vas deferens makes it difficult to identify the receptor with a 5-HT-1B binding site (see Engel et al., 1986). A 5-HT-1C site is also unlikely due to the failure of cyproheptadine to antagonise responses to 5-CT. The inhibitory prejunctional 5-HT receptors in rat heart and vas deferens do not seem to be identical with any 5-HT binding site.

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