MEDIATION OF PROSTACYCLIN SYNTHESIS IN THE RAT PENIS BY MUSCARINIC RECEPTORS

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Erection of the penis is a vascular phenomenon involving an increase in blood inflow relative to outflow. Since prostacyclin (PGI_2) has been ascribed roles in modulating vascular tone (as a vasodilator) and haemostasis (as an inhibitor of platelet aggregation), it may be that this prostanoid is involved in penile vasodilation, as well as protection against thrombosis during erection. PGI_2 synthesis by the rat penis was therefore investigated, using an in vitro model similar to that described for the rat aorta (Jeremy et al, 1985). As penile erection is primarily under parasympathetic nervous control and it has been suggested that vasoactive intestinal polypeptide (VIP) may also be involved (Ottesen et al,1984), the effect of methacholine and VIP on PGI_2 synthesis by the rat penis was studied. The effects of nicotine, noradrenaline, serotonin, histamine, dopamine and ATP were also investigated.

Penises were obtained from Sprague Dawley rats. All incubations were carried out in Dulbecco's Minimum Essential Medium (MEM) containing 25 mmol/l $NaHCO_3$, pregassed to pH 7.4 with 95/5:O_2/CO_2. Penises were cut into discs and pooled in MEM. Since cutting causes an initial surge of PGI_2 release, tissue was preincubated in MEM for 3 h, until PGI_2 release had reached a stable basal output. Penile discs were placed in incubation tubes containing 1 ml MEM and drug, in octuplicate for each drug dose, and incubated for 30 min at 37 °C. Control incubations, without drugs, were included. Following incubation, an aliquot of the supernatant was taken for estimation of 6-oxo-$PGF_{1\alpha}$ by specific radioimmunoassay (Jeremy et al, 1985).

Of the agonists investigated, only methacholine stimulated penile PGI_2 in a dose-dependent manner ($ID_{50}=10^{-6}$ mol/l), whereas nicotine, noradrenaline, serotonin, histamine, dopamine and ATP were without effect. Preincubation of the penile discs with ethylenetetraacetic acid (EDTA: 10 mmol/l) completely abolished the stimulatory effect of methacholine on penile PGI_2 synthesis.

Atropine (from 10^{-9} to 10^{-4} mol/l) and verapamil (from 10^{-6} to 10^{-3} mol/l) were incubated with the penile discs for 30 min prior to the addition of methacholine (final concentration: 10^{-6} mol/l) and incubated and assessed for PGI_2 release, as described above.

Atropine and verapamil inhibited methacholine-stimulated penile PGI_2 synthesis in a dose-dependent manner (ID_{50} atropine = 10^{-7} mol/l; ID_{50} verapamil = 6×10^{-5} mol/l). The antagonist action of atropine was shown to be competitive.

It is concluded that, in the rat penis, PGI_2 synthesis is under muscarinic control and is calcium dependent. These findings may be relevant to the vasodilatory phenomena of penile erection and to antithrombotic protection during erection.

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EFFECT OF THE THROMBOXANE RECEPTOR BLOCKING DRUG AH23848 UPON THROMBOCYTOPENIA INDUCED BY HAEMODIALYSIS IN THE ANAESTHETISED DOG

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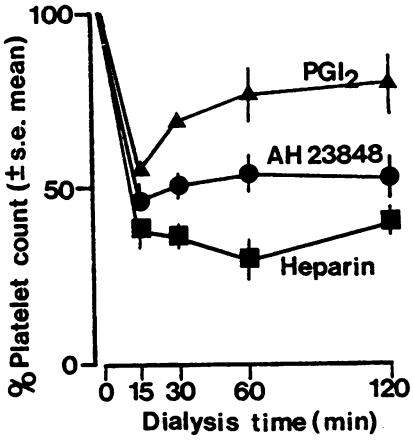
During haemodialysis platelets are activated through contact with the artificial surfaces of the dialyser (Lindsay et al., 1972). Prostacyclin (PGI_2) reduces the thrombocytopenia associated with haemodialysis in dog and man (Woods et al., 1978; Turney et al., 1980). Since platelet activation can cause the release of the potent platelet aggregatory substance thromboxane A_2 (TxA_2) we have examined the effect of the specific TxA_2-receptor blocking drug AH23848 (Humphrey & Lumley, 1984) upon the thrombocytopenia associated with haemodialysis in the anaesthetised dog.

Beagle dogs (12 - 15 kg) were anaesthetised with sodium barbitone (300 mg/kg i.p.). Access for dialysis was made via the left carotid artery and jugular vein and was carried out using a Travenol ($1m^2$) membrane unit perfused with dialysate (Renalyte S/136) at 200 ml/min and maintained at a temperature of 39 $\pm$ 1°C. Blood lines were primed with sterile saline (Steriflex). Three treatments (heparin alone, 300 IU/kg i.v. (n=6); heparin plus PGI_2, 200 ng/kg/min i.v. (n=4) and heparin plus AH23848, 75 µg/kg/min i.v. (n=5)) were investigated, drug administration being started 15 minutes prior to dialysis. Dialysis lasted for 2 hours over which time the dogs were given a constant i.v. infusion of Hartmanns solution (8 ml/min) and sodium pentobarbitone (0.9 mg/min). Platelets were counted (Haemalog 8/90 micro) in blood leaving the dialyser and platelet aggregation to collagen was performed in peripheral arterial blood ex vivo (Lumley & Humphrey, 1981).



With heparin alone platelet counts fell rapidly during the first 15 minutes of dialysis, fell further up to 60 min (64 $\pm$ 3%) and recovered slightly over the remaining 60 min (see fig). Compared with heparin alone, PGI_2 slightly, but significantly, reduced the initial rapid drop in platelet count; AH23848 was without effect. With PGI_2 platelet counts returned toward control values (18 $\pm$ 7% fall at 60 min) and stabilised for the final 60 min. With AH23848, after the initial drop, platelet count stabilised for the remainder of the experiment (47% $\pm$ 4% fall at 60 min). The total thrombocytopenia over the dialysis period (area above curves in fig) was significantly less with PGI_2 than with either of the other treatments and also significantly less with AH23848 than with heparin alone.

In summary the large fall in platelet count associated with haemodialysis in the dog was partially but significantly reduced by AH23848. Although the effect of AH23848 was less than that of PGI_2, profound inhibition of collagen-induced platelet aggregation ex vivo was observed following AH23848, indicating marked blockade of the platelet TxA_2-receptor. Thus it can be concluded that TxA_2 plays some role in the thrombocytopenia associated with haemodialysis in the anaesthetised dog but other mechanisms are also involved.

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AH 6809, A SELECTIVE ANTAGONIST AT THE HUMAN PLATELET DP-RECEPTOR?

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Prostaglandin receptors have recently been sub-classified and a nomenclature devised (Kennedy et al., 1982). AH 6809 has been reported to antagonise the actions of prostanoids at receptors of the EP_1 type in some smooth muscle preparations but has no activity at EP_2-, FP- or TP-receptors (Coleman et al., 1985). The present study describes the action of AH 6809 upon platelet TP-, IP- and DP-receptors.

Platelet aggregation was assessed in human whole blood (Lumley and Humphrey, 1981). Concentration-effect curves were determined for U46619-(a thromboxane A_2 mimetic and TP-receptor agonist, Coleman et al., 1981, Kennedy et al., 1982) or ADP-induced aggregation in the absence and presence of AH 6809 ($3x10^{-5}$ - $3x10^{-4}$ mol/l) and concentration-ratios (CR) calculated at the 50% response level. At the highest concentration of AH 6809 ($3x10^{-4}$ mol/l), a U46619 CR value (95% confidence limits) of 9.2 (7.8-10.7, n=4) was obtained. This concentration of AH 6809 was without effect upon ADP-induced aggregation (CR = 1.0 (0.7-1.4), n=4).

The effect of a single concentration of AH 6809 ($3x10^{-4}$ mol/l) was also investigated upon the anti-aggregatory effects of PGD_2, the hydantoin analogue BW 245C (Town et al., 1983) and PGI_2 in whole blood. All of the anti-aggregatory agents produced a rightward displacement of the ADP curve. The effects of PGD_2 and BW 245C but not that of PGI_2 were antagonised by AH 6809. This effect was analysed in more detail against PGD_2. Increasing concentrations of PGD_2 displaced ADP concentration effect curves progressively to the right. In the presence of a single concentration of AH 6809 higher concentrations of PGD_2 were required to produce a comparable effect against ADP. Four experiments at each of three concentrations of AH 6809 ($3x10^{-5}$, $1x10^{-4}$ or $3x10^{-4}$ mol/l) were performed. From each experiment the ratio of the concentrations of PGD_2 (PGD_2 CR) required to produce equal effects against ADP in the presence and absence of AH 6809 were determined. AH 6809 produced a concentration-related antagonism of the anti-aggregatory effects of PGD_2. PGD_2 CR values of 6.6 (5.1-8.4), 19.9 (13.6-29.3) and 35.5 (24.9-50.6) were obtained at $3x10^{-5}$, $1x10^{-4}$ and $3x10^{-4}$ mol/l AH 6809 respectively. In contrast, AH 6809 ($3x10^{-4}$ mol/l) did not antagonise the anti-aggregatory effects of PGI_2, rather, a small potentiation was observed (PGI_2 CR = 0.6 (0.4-0.9) n=4).

The potency of AH 6809 against the anti-aggregatory activity of PGD_2 and the aggregatory activity of U46619 was analysed by the method of Arunlakshana and Schild (1959). pA_2 values (slopes of the regression) of 5.35 (0.89) against PGD_2 and 4.44 (0.97) against U46619 were obtained. It is therefore concluded that AH 6809 blocks both the DP- and TP-receptors in human platelets. The activity of AH 6809 as a DP-receptor antagonist is further substantiated by its ability to antagonise the anti-aggregatory activity of BW 245C, a compound which has been shown to act at the platelet DP-receptor (Town et al., 1983). Thus, whilst AH 6809 is weak it appears to display sufficient selectivity at the platelet DP-receptor to be useful in classifying the action of anti-aggregatory prostanoids on human platelets.

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SELECTIVE ACTIONS OF SUBSTANCE P 6-11 ON TACHYKININ 'E' RECEPTORS

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Distinct differences between populations of receptors occupied by Substance P (SP) and other tachykinins have been demonstrated by numerous groups. One particular classification has designated receptors that are more sensitive to eledoisin (ELE) and kassinin (KASS) than to physalaemin (PHYS) and SP as 'E' and those showing similar sensitivities to all four agonists as 'P' (Lee et al. 1982). Later reports of differential antagonist affinities for these receptors have supported these findings (Growcott and Tarpey, 1983; Hunter and Maggio, 1984).

Preliminary evidence has been presented by Briggs et al (1984) that the C-terminal hexapeptide of SP (SP 6-11) could act preferentially at 'E'-type receptors causing motorneurone depolarization in the rat spinal cord (RSC). We now provide evidence of selective actions of SP 6-11 in other systems supporting these earlier findings.

Interactions with the agonist effects of SP, SP 6-11 and ELE by [D-Pro^2, D-Trp^{7,9}]-SP (PTT-SP) atropine and phenoxybenzamine have been examined in the guinea-pig urinary bladder (GPB), RSC and guinea-pig ileum (GPI).

PTT-SP (1-50μM, 15 min) antagonised both SP 6-11 and ELE to the same extent in both GPB (pA_2 5.50 ± 0.13, n=10 and 5.40 ± 0.05, n=4 respectively) and RSC (% change in response -67 ± 7, n=4 and -52 ± 7, n=4 respectively), whereas no antagonism of SP was seen in either preparation. Atropine pretreatment (1μM, 30 min) of the GPI caused identical rightward shifts in the SP 6-11 and ELE dose-response curves without any significant effects on responses to SP (ED_{50} dose ratios 4.2 ± 1.3, n=6, 4.3 ± 1.1, n=8, and 1.6 ± 0.1, n=6 respectively). In the GPB, phenoxybenzamine (20μM, 30 min) produced reductions in responses to SP 6-11 and ELE without altering responses produced by SP (log ED_{50} dose ratios 1.54 ± 0.26, n=9, 0.71 ± 0.07, n=6 and 0.21 ± 0.10, n=9 respectively) supporting our previous demonstration that phenoxybenzamine pretreatment of the GPB causes selective inactivation of 'E' receptor sites (Growcott et al. 1983).

The evidence presented suggests that in GPB, RSC and GPI, ELE and SP 6-11 act at similar sites distinct from those activated by SP. We conclude that SP 6-11 is a selective 'E' receptor agonist.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF NEUROPEPTIDE Y IN SINGLE NERVE FIBRES IN FRESH HUMAN HEART

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The thirty six amino acid peptide, neuropeptide Y, although only recently isolated is widely present throughout the peripheral nervous system (Gu et al 1983). In particular it has been demonstrated in large amounts in human heart post mortem (Gu et al 1983) and reduces myocardial perfusion in the isolated perfused rabbit heart (Allen et al 1983). In this report we demonstrate the presence of neuropeptide Y in nerve fibres in fresh human hearts. Recipient hearts were dissected fresh at the time of cardiac transplantation. Whole mount preparations were studied using a peroxidase antiperoxidase immunohistochemical technique (Costa et al 1980), modified for the localization of neuropeptide Y.

Neuropeptide Y containing nerves were demonstrated with high resolution in the sympathetic fibres running in the tunica media of large epicardial coronary arteries. In addition neuropeptide Y fibres were seen running along the intimal-medial border of small endomyocardial vessels. Neuropeptide Y fibres and terminals were also demonstrated directly impinging upon myocardium. Control experiments using peroxidase antiperoxidase antibody in the absence of NPY antisera produced no peroxidase staining at all.

These results strengthen the suggestion that neuropeptide Y is an important cardiac neuropeptide and in particular may be an important mediator of coronary artery tone.

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THE RESPONSE OF RAT UTERINE TISSUE TO OXYTOCIN AND PROSTAGLANDIN $F_{2\alpha}$ IN THE PRESENCE OF CALCIUM CHANNEL ANTAGONISTS

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Oxytocin and prostaglandin F_2-alpha (PGF_2-alpha) are potent compounds in the induction of uterine contractions but their exact mechanism of action is unknown. Carsten and Miller (1977) have shown that both oxytocin and PGF_2-alpha facilitate the release of calcium from uterine microsomal vesicles, however, oxytocin also releases calcium from the intrinsic calcium pool. It is the purpose of this study to examine the responses to oxytocin and PGF_2-alpha on rat uterine tissue in the presence of three calcium channel antagonists which may show selectivity for different mechanisms of calcium blockade (Jarvis & Triggle, 1983).

Virgin female rats (Sprague-Dawley CD) (260-310g) were pre-treated with stilboestrol 1 mg kg^{-1} s.c. 18h prior to experiment. The uterine horns were removed and bisected to provide strips approximately 2 cm long. The strips were superfused (flow rate 2 ml min^{-1}) with either normal Krebs' solution or high potassium Krebs' solution (100 mM K^+) at 37°C gassed with 95% O_2 5% CO_2. Tension changes were analysed as area of the response using a response integration programme (Hewlett-Packard, HP-86) and were expressed as a percentage of the maximum response. Group size = 6.

Oxytocin and PGF_2-alpha produced dose-dependent responses in both normal and depolarising Krebs' solutions. Verapamil (2.0×10^{-7}M and 2.0×10^{-6}M) produced a concentration dependant inhibition of responses to oxytocin in both physiological solutions. The lower concentration of verapamil failed to inhibit the responses to PGF_2-alpha in normal Krebs' but in depolarising Krebs' both concentrations inhibited the PGF_2-alpha responses. Diltiazem (2.2×10^{-7}M and 2.2×10^{-6}M) produced a significant ($P<0.001$) inhibition of oxytocin responses in normal Krebs' but failed to inhibit these responses in high potassium Krebs' solution. The responses to PGF_2-alpha were significantly inhibited by the higher dose of diltiazem only in both normal and depolarising Krebs'. The lower dose of diltiazem significantly potentiated ($P<0.01$) the response to PGF_2-alpha in high potassium Krebs' solution. Bepridil (2.0×10^{-7}M and 2.0×10^{-6}M) produced a concentration-dependent inhibition of the responses to oxytocin in both types of Krebs' solution. The responses to PGF_2-alpha were also inhibited in normal Krebs' but not in depolarising Krebs' solution.

It would appear from these results that oxytocin produces uterine contractions by increasing the influx of extracellular calcium through potential dependant channels as well as being able to mobilize intracellular calcium. PGF_2-alpha appears not to have a major action through potential dependant channels but may act via a receptor operated channel as well as being able to release intracellular calcium.

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CHOLECYSTOKININ RECEPTOR ACTIVATION IN THE PANCREAS: EVIDENCE FOR AN INVOLVEMENT OF PROTEIN KINASE C

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The molecular events associated with receptor activation for a number of peptides remains unresolved. Recent interest has focussed on the involvement of the phosphatidylinositol (PI) cycle as a potential molecular mechanism underlying receptor activation. An integral part of the PI cycle is the Ca^{2+} and phospholipid-dependent enzyme, protein kinase C, which is activated on stimulation of the PI cycle. The aim of this study was to investigate whether or not protein kinase C is involved in cholecystokinin (CCK) receptor activation, utilising a variety of protein kinase C inhibitors and activators. CCK-stimulated pancreatic amylase release was used as the model system.

Pancreatic acinar cells were prepared by mild collagenase digestion using a modified procedure of Hahne et al (1983). Amylase release was determined by the Phadebas test (Ceska et al, 1969) and represented as the % of total amylase released. Acinar cells were incubated with CCK 26-33 (sulphated) in 100% O_2 in the presence or absence of chlorpromazine, polymyxin B and trifluoperazine - agents shown to block protein kinase C activation (Kuo et al, 1984). In addition, cells were incubated with a phorbol ester, phorbol 12-myristate, 13-acetate (TPA) - a potent activator of protein kinase C - or its inactive analogue 4-0-methyl TPA. CCK stimulated amylase release in a biphasic manner with a peak at 5 x 10^{-10}M CCK 26-33, ie, 15.5 $\pm$ 2.3% (30 minute incubation) and 28.1 $\pm$ 2.2% (60 minute incubation). TPA stimulated amylase release in a dose-dependent manner. At a concentration of 10^{-6}M TPA the amount of amylase released, ie, 27.9 $\pm$ 1.6% (60 minute incubation) was comparable to the peak concentration of amylase observed with CCK 26-33. 4-0-methyl TPA was 5-fold less active at 10^{-6}M than TPA itself. The ability of various inhibitors of protein kinase C to block CCK-stimulated amylase release is tabulated below:

Agent		% Total Amylase Released ($\pm$ S.E.M.)
CCK 26-33 alone (5 x 10^{-10}M)		11.8 $\pm$ 1.9
CCK 26-33 + chlorpromazine	10^{-4}M	1.8 $\pm$ 1.1
(5 x 10^{-10}M)	10^{-5}M	6.0 $\pm$ 2.1
CCK 26-33 + trifluoperazine	10^{-4}M	4.0 $\pm$ 1.2
(5 x 10^{-10}M)	10^{-5}M	8.9 $\pm$ 2.8
CCK 26-33 + polymyxin B	10^{-4}M	11.0 $\pm$ 0.9
(5 x 10^{-10}M)	10^{-5}M	7.7 $\pm$ 0.7

The present results provide evidence that CCK-receptor activation in the pancreas involves the enzyme complex, protein kinase C.

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DEVELOPMENT OF PROENKEPHALIN PRODUCTS IN RAT STRIATUM AND THE INHIBITORY EFFECTS OF LEAD EXPOSURE

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Certain developmental abnormalities have been associated with environmental exposure to lead. Our previous studies have indicated that the development of the endogenous opioid system is extremely sensitive to toxic effects of lead exposure (Kitchen et al., 1984; Winder et al., 1984). In connection with this, we report the ontogeny of proenkephalin products in the rat striatum and the effects of low level lead on this ontogeny.

Lead was administered in the maternal drinking water from conception to weaning at 100, 300 and 1000 ppm (Winder et al., 1984). Measurement of proenkephalin products, blood lead and body weight were made at 10, 21 and 30 days. Blood lead was determined by atomic absorption spectrophotometry. Proenkephalin products, [Met] enkephalin, [Leu] enkephalin, [Met] enkephalyl-Arg^{6}-Phe^{7} and [Met] enkephalyl-Arg^{6}-Gly^{7}-Leu^{8} were extracted by Sep-Pak/HPLC chromatography and assayed using a modified mouse vas deferens bioassay (Bailey and Kitchen, 1984).

At 10 days, the levels of the four proenkephalin products were similar, with values ranging from 1452 ± 224 pmol/g for [Met]enkephalin to 1090 ± 167 pmol/g for [Met] enkephalyl-Arg^{6}-Phe^{7}. The levels of [Leu]enkephalin and the extended [Met] enkephalins remained constant between 10 and 30 days. In contrast, the levels of [Met] enkephalin increased 2.3 fold between 10 and 21 days and returned towards their 10-day value by 30 days.

Lead caused a dose-related depression of all four proenkephalin products. The effect was most marked at 10 days where at the highest lead dose, levels were < 10% of control values. In four out of six samples at 300 ppm, and all samples at 1000 ppm the extended [Met] enkephalins could not be detected. At 21 days, lead prevented the elevation of [Met] enkephalin observed in control-treated rats. There was some recovery from the earlier depressant effects of lead by 30 days especially for the extended [Met] enkephalins. Peak blood lead levels were below 70 µg/100 ml in all lead-dosed groups. The toxicity of lead may be manifested on the processing enzymes.

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THE NATURE OF SUBSTANCE P RECEPTORS IN THE RAT GASTRIC CORPUS STRIP

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Field stimulation of non-adrenergic, non-cholinergic nerves of the rat gastric corpus strip produces an inhibitory response which is followed by a rebound contraction (Hunt et al (1978). Substance P (SP) or a related tachykinin may be responsible for the rebound contraction (Hunt et al. 1983). We have explored this possibility further and also attempted to discover whether a specific receptor type mediates the contractile effects of tachykinins in the rat gastric corpus strip.

Rat isolated gastric corpus strips were prepared as described by Hunt et al.(1978). In experiments using either the SP antagonist D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11} - SP or high doses of the tachykinins ($>10\,\mu M$) a 2.0ml isolated organ bath was used. Atropine ($10^{-6}M$), mepyramine ($5\times10^{-6}M$) and indomethacin ($10^{-5}M$) were present during experiments with the tachykinins, whereas only atropine ($10^{-6}M$) was present when the SP antagonist was tested. Guinea-pig isolated ileum preparations were set up as described by Kitchen (1984).

Incubation of gastric corpus strips with $2\,\mu M$ or $5\,\mu M$ concentrations of the SP antagonist for 20 min reduced the response of the preparations both to an applied 40 n M dose of SP [90.1 ± 23.3% s.e.m. (N=4) and 89.3 ± 17.6% s.e.m. (N=6) of controls] and to electrical field stimulation (pulse width 2ms, frequency 7Hz, voltage 140v) [80.9 ± 8.3% s.e.m. (N=4) and 92.3 ± 18.0% s.e.m. (N=4) of controls]. The antagonist also showed agonist activity in this tissue. In addition, 20 min incubation with the antagonist was effective in reducing the contraction of the guinea-pig isolated ileum produced by 10nM SP to about 10% of controls (N=2). The tachykinins SP, physalaemin, eledoisin and kassinin were added to the organ bath cumulatively. The four tachykinins produced parallel $\log_{10}$ dose - response curves. From the relative positions of these curves, it would appear that the order of potency is kassinin $\gg$ eledoisin $>$ physalaemin = SP.

The minimal effect of the SP antagonist on responses of the gastric corpus strip to either SP or electrical field stimulation suggests that this antagonist is unable to block the particular receptors involved in mediating responses to SP. A similar lack of effect of the antagonist on responses to SP has been reported for the hamster urinary bladder (Rosell, et al. 1983). The sites at which the SP-antagonist has been shown to be effective have been characterized as both SP-P and SP-E receptors (Watson, 1983). The lack of effect of the antagonist upon the gastric corpus and the hamster urinary bladder implies that the receptors in these tissues are predominantly not of these types. Furthermore, as kassinin is considerably more potent than other tachykinins in the gastric corpus, in contrast to the hamster bladder, perhaps an SP-K designation may be more appropriate (Buck, et al. 1984).

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ON THE ROLE OF α_2-ADRENOCEPTOR AGENTS IN THE MORPHINE ABSTINENCE SYNDROME

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There is considerable evidence that α_2-adrenoceptor agonists are effective in reducing the intensity of withdrawal signs occurring during narcotic abstinence (Charney, et al., 1982). There is however, little or no information either on the relative contribution of central and peripheral α_2-adrenoceptors to this abstinence syndrome or whether it is exacerbated by α_2-adrenoceptor antagonists. The present study examines the effects of the selective α_2-antagonist idazoxan (Doxey et al., 1983) on precipitated narcotic abstinence in the rat. The selective α_2-adrenoceptor agonist para-amino-clonidine (PAC), which has been shown to have severely restricted access to central sites (Roach, et al., 1984), was also examined and the results compared to those obtained with clonidine. Rats (male, Sprague-Dawley, 170 - 180 g, Olac) were made physically dependent to morphine by a process of continuous intraperitoneal infusion (McCarthy et al., 1982). Animals (n=8/10) were prepared with indwelling intraperitoneal cannulae through which morphine was infused for a period of 48h at a dose-rate of 100 mg/kg/24h. Narcotic abstinence was then induced by means of intravenous challenge with the narcotic antagonist naloxone. Abstinence signs were assessed as previously reported (McCarthy et al., 1982). The effects of drugs acting at α_2-adrenoceptors were studied by combining them with the naloxone challenge dose. Saline acted as the control throughout. The results indicate that naloxone (2 - 200 µg/kg,i.v.) produced a dose related increase in the intensity of narcotic abstinence signs. When clonidine (50 µg/kg, i.v.) was dosed with naloxone, the severity of the withdrawal syndrome was greatly reduced as measured by the behavioural signs. Except at the highest dose of naloxone tested (200 µg/kg,i.v.), the amount of weight loss experienced by rats challenged with clonidine/naloxone was greater than any other treatment group. These effects of clonidine were antagonised by idazoxan (100 µg/kg, i.v.). Neither PAC (2 µg/kg,i.v.) nor idazoxan (100 µg/kg,i.v.) had any effect on the nature or intensity of the narcotic withdrawal signs and body-weight loss precipitated by naloxone (2-200 µg/kg,i.v.). In the present studies both the effectiveness of clonidine and the inactivity of PAC argue against the existence of a significant peripheral contribution to the suppressant effects of α_2-adrenoceptor agonists on precipitated abstinence. The effects of clonidine were antagonised by idazoxan. Previous studies have demonstrated that precipitated abstinence is associated with increases in locus coeruleus firing rate (Aghajanian and Van der Maelen , 1982) and noradrenaline turnover (Attila and Ahtee, 1984). Although idazoxan itself induces similar acute changes in the naive rat (Freedman and Aghajanian, 1984.,Walter et al 1984), in the present studies it failed to potentiate the precipitating effects of naloxone. In this context it is interesting to note that lesioning of the dorsal noradrenergic bundle had no effect on either opiate withdrawal or its suppression by clonidine (Britton et al., 1984).

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DIRECT DEPENDENCE STUDIES IN RATS WITH AGENTS SELECTIVE FOR DIFFERENT SUBTYPES OF OPIOID RECEPTOR

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Our objective was to identify, describe and compare naloxone-induced abstinence syndromes in rats associated with the central infusion of agents that are claimed to be selective agonists at mu, delta and kappa opioid receptors. The following compounds were infused (at 1 μℓ/h for 70 h) to the caudal region of the Sylvian aqueduct of male Sprague Dawley rats (180-200 g) from a s.c. implanted osmotic minipump (Alzet 2001) (Wei, 1981): [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAGO, Peninsula - *mu* selective), [D-Pen^2,D-Pen^5]enkephalin (DPDPE - *delta*), dynorphin A (Peninsula - *kappa*), U-50,488H (VonVoigtlander et al, 1983 - *kappa*) and water. For each compound, the amount infused was 24.2 times the ED 50 value (i.c.v. nmol) in rat hot plate (Galligan et al, 1984) or paw pressure (Hayes et al, 1983) tests. This is equivalent to 49 nmol/70 h of morphine sulphate (Chang et al, 1983).

Each rat lived in a Plexiglas observation box (26 cm long; 20 cm wide; 30 cm high). Signs of abstinence were monitored for 0.5 h (at 20 ± 0.5°C) before, and 0.5 h after, naloxone (3 mg/kg, s.c.) (Baldino et al, 1979). Three levels of abstinence were observed. (*a*) Negligible syndromes were precipitated by naloxone in rats on water, dynorphin A (114 nmol/70 h) or U-50,488H (2923 nmol). (*b*) Syndromes of low intensity were associated with DPDPE (859 nmol) (rears = 6.3±1.3, s.e. mean; body shakes = 4.0±1.0; head shakes = 7.3±1.3) and DAGO (0.034 nmol) (rears = 4.0±1.0; body shakes = 3.0±0.9; head shakes = 8.5±1.5). (c) A high intensity syndrome was obtained with DAGO (3.4 nmol) (jumps = 3.8±1.0; rears = 11.5±1.7; body shakes = 22.0±2.3; head shakes = 2.8±0.8; weight loss at 1 h = 3.3±0.9 g; digging - 75% of the rats; head scratching - 100%). There was never marked (rectal) hypothermia at +1 h. Excessive head scratching only occurred with the high dose DAGO abstinence syndrome; it was not observed during abstinence from morphine (49 nmol).

In vitro, ICI 174864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH; Aib is α-aminoisobutyric acid) is a selective antagonist at delta opioid receptors (Cotton et al, 1984). Does this compound precipitate abstinence in rats receiving an infusion of DPDPE (2062 nmol over 7 days) in the right lateral cerebral ventricle? When ICI 174864 (10 μg) was given into the left lateral cerebral ventricle at +7 days, the rats (n=5) displayed postural abnormalities, barrel rotation and hypothermia. This also occurred in rats receiving an infusion of water. When ICI 174864 (3 mg/kg) was injected s.c. in an additional 4 rats on DPDPE, the animals showed powerful abdominal writhes for the following 3-10 min. This behaviour did not appear when DPDPE (50 μg, i.c.v. at 0 h) and ICI 174864 (3 mg/kg, s.c. at +5 min) were given acutely to 4 rats; increased motor activity and immediate hyperthermia (2.6 ± 0.5 °C at 0.5 h) ensued. (These changes were caused by DPDPE rather than ICI 174864). Writhing may therefore be a sign of delta-mediated abstinence under our conditions. A cautious interpretation is necessary since ICI 174864 (3 mg/kg), by itself, elicited weak writhing in 2 out of 6 rats.

It is a pleasure to thank Dr. M.J. Rance (ICI, Macclesfield) for the ICI 174864 and Dr. R.A. Lahti (Upjohn, Kalamazoo) for the U-50,488H.

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BUPRENORPHINE: DEMONSTRATION OF MORPHINE-LIKE BUT NOT OF N-ALLYLNORMETAZOCINE-LIKE STIMULUS GENERALIZATION

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The use of narcotic drug discrimination tests has made a significant contribution to the classification of opioid-drug receptor interactions (Herling and Woods, 1981). Of particular value has been the use of the discriminative stimulus as a means of identifying N-allylnormetazocine - (NANM; SKF 10,047) and phencylidine-like effects (Holtzman, 1982, Shannon, 1983). The discriminative stimulus properties of morphine and ± NANM are considered to be mediated through mu-opiate receptors and (non-opiate) sigma receptors respectively (Quirion, 1981). Buprenorphine is an opioid partial agonist with a very high affinity for the mu opioid receptor subtype (Sadee et al., 1982). The discriminative stimulus properties of ± NANM and morphine have been utilised in the present study to investigate the degree of interaction displayed by buprenorphine (and a variety of other opioids) for the mu and sigma receptor subtypes. Discrimination training was performed using rats (male, Sprague Dawley, Cardiff University strain, approx. 300g) in standard 2-lever operant chambers, according to a fixed-ratio 10 schedule for food pellet reward. Using this procedure, two groups (n=8) of rats were trained to discriminate either morphine HCl (3 mg/kg,s.c.) or ± NANM (3 mg/kg,s.c.) from saline (1ml/kg,s.c.). Incorrect responses were not rewarded. An animal was considered to have discriminated the training drug from saline, when it had achieved 80% mean correct responding over the final ten consecutive days. Novel opioids were then tested for their ability to produce responding of a similar pattern to that of the training drug ('generalization'). The results of generalization tests were expressed in terms of the mean percentage responding on the drug-lever before ten responses were totalled on either the drug or the saline lever. The results of the morphine generalization tests indicated that whilst both buprenorphine and morphine generalized to morphine, no such phenomenon was observed for ± NANM, nalorphine, cyclazocine, ethylketocyclazocine or naltrexone. In the animals trained to discriminate ± NANM from saline cyclazocine produced ± NANM-like responding whilst buprenorphine failed to generalize to ± NANM. Ethylketocyclazocine, nalorphine, morphine and naltrexone also failed to generalize to ± NANM. It is widely considered that sigma receptors mediate dysphoria in a variety of species (Shannon, 1983). The lack of interaction between buprenorphine and sigma receptors demonstrated in the present study provides a pharmacological basis for the lack of dysphoric and hallucinatory reactions observed following buprenorphine administration in man (Harcus, et al.,1980).

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AN EXAMINATION OF THE THERMOREGULATORY EFFECTS OF MEPTAZINOL AND ITS QUATERNARIZED DERIVATIVE

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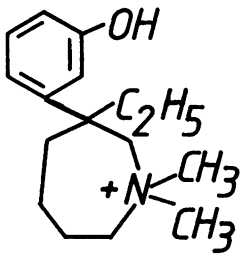
It is generally accepted that opioid agonists and antagonists can modify body temperature stereospecifically through interactions with opioid receptors (Cox et al 1979). Administration of morphine has been shown to produce biphasic changes in body temperature, low doses being hyperthermic and high doses hypothermic (Thornhill et al 1978). The present study examines the thermal changes evoked by the novel opioid meptazinol and its quaternary derivative N-methyl meptazinol (N-MM) in mice.



N-Methyl Meptazinol

Changes in body temperature following meptazinol and N-methyl meptazinol administration

Drug	Route	Dose Range	$\Delta^{o}C$
Meptazinol	s.c.	1 - 3mgkg^{-1}	↑
		20-80mglg^{-1}	↓
N-Methyl Meptazinol	s.c.	1 - 40mgkg^{-1}	-
Meptazinol	i.c.v.	2.5-5mcg mouse^{-1}	↑
		50-400mcg mouse^{-1}	↓
N-Methyl Meptazinol	i.c.v.	2.5-5mcg mouse^{-1}	↑
		10-40mcg mouse	↓

(- no change; ↑ hyperthermia; ↓ hypothermia)

These data demonstrate the biphasic dose-dependent effects of meptazinol on body temperature, where low doses were hyperthermic and higher doses produced long lasting hypothermia (90 mins). In addition these actions would appear to be predominantly mediated through central sites since N-MM produced a similar temperature profile only after i.c.v. administration.

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μ-OPIOID RECEPTORS: A ROLE IN THE CONTROL OF APPETITE IN NON-DEPRIVED RATS?

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The μ subtype of opioid receptor appears to play a complex role in the regulation of ingestive behaviour. The prototypic μ-agonist morphine increases food and water consumption in non-deprived animals whereas the opioid antagonist naloxone generally produces an opposite effect (for review see Morley et al. 1983). It has also been shown, however, that in deprived animals morphine can suppress the appetite (Sanger & McCarthy,1980), moreover, low doses of naloxone (0.001+0.01mg/kg i.p.) have been found to enhance intake of a 20% sucrose solution in water-deprived rats (Olson et al. 1981) and it has been suggested that a μ-anorectic system operates in these animals (Morley et al. 1984; Yim & Lowy,1984). The present investigation sets out to further probe a possible involvement of μ-receptors in the control of food and water intake in freely-feeding rats. Thus, the earlier work with morphine has been extended using the more highly-selective μ-agonist D-Ala^2,NMe-Phe^4,Gly-ol^5 -enkephalin (DAGO) alone and in combination with the long-acting opioid antagonist naltrexone. In addition, the appetitive effects of low doses of naltrexone have been examined using a similar rat feeding model.

Subjects were individually housed male Wistar rats (300-350g) which were allowed free access to powdered standard rat diet and tap water at all times. Experiments were carried out during the daylight period when the food and water intakes of control animals were minimal. Drugs were dissolved in sterile 0.9% NaCl. Naltrexone was administered intraperitoneally in a dose volume of 1ml/kg. DAGO was injected directly into the lateral ventricle through indwelling cannulae in a dose volume of 10μl/rat. Feeding jars and water bottles were weighed at the time of drug administration and after 1,2,3 and 4 hours enabling the calculation of mean cumulative intakes g/kg rat weight ± SEM. Statistical comparisons were made using the Mann Whitney 'U'-test.

Rats treated with DAGO (1+10μg i.c.v./rat) ate and drank significantly more than the control group over the test period. A lower dose of the peptide (0.1μg i.c.v./rat) did not have any appreciable effects on appetite. The increase in food and water intake induced by DAGO (1μg i.c.v./rat) was blocked by concurrent treatment with naltrexone (0.1mg/kg i.p.) thus confirming that its effects were being mediated via opioid receptors. The food and water consumption of animals injected with naltrexone alone (dose range 0.001-0.1mg/kg i.p.) did not significantly differ from that of the vehicle-treated controls during the 4 hours after drug administration.

The increase in food and water intake observed with DAGO and the lack of any intrinsic appetitive effects of low doses of naltrexone reported in this study suggest that in non-deprived rats at least, μ-receptors play a stimulatory as opposed to an inhibitory role in the control of ingestive behaviour. These findings emphasise the importance of factors such as the deprivation state of the animals and also diet in such behavioural experiments with the opioids.

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THE MECHANISM OF ENHANCEMENT OF RAT LIVER TRYPTOPHAN PYRROLASE ACTIVITY BY ACUTE MORPHINE ADMINISTRATION

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We have previously shown (Badawy & Evans, 1975a) that acute morphine administration to rats enhances the activity of liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11), which is a major determinant of the availability of tryptophan for various functions, including cerebral 5-hydroxytryptamine synthesis. Because morphine caused activation of the pyrrolase, the above authors suggested that the drug acts on this enzyme by either a substrate- or a cofactor-, but not a hormonal-type mechanism. A substrate-mediated activation is also more likely, in view of the recent finding (Evans et al., 1984) that morphine increases the availability of circulating free tryptophan, possibly by direct displacement from serum-protein-binding sites. It was therefore considered of interest to find out if tryptophan is involved in the activation of its pyrrolase by acute morphine administration.

Locally bred male Wistar rats (150-170g) were maintained on cube diet 41B (Oxoid, Basingstoke, Hants.) and water under standard conditions. Morphine sulphate was purchased from Macarthys Ltd. (Trecenydd Industrial Estate, Caerphilly, Mid Glam). Apart from some gifts (see below), all other chemicals were obtained from BDH or Sigma (both of Poole, Dorset). Morphine sulphate or an equal volume (2ml/kg) of 0.9% (w/v) NaCl (saline) was given i.p. Tryptophan pyrrolase activity was determined in fresh liver homogenates as described previously (Badawy & Evans, 1975b) either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2μM) haematin. The apoenzyme activity was obtained by difference. The extent of saturation of the enzyme with its cofactor haem was expressed as the percentage haem saturation (100 X holoenzyme activity/total enzyme activity). The half-life of the pyrrolase was determined by the cycloheximide method as described by Badawy & Evans (1975b).

Maximum activation of the pyrrolase occurred at 3hr after administration of a 40mg/kg dose of morphine sulphate. At this time interval, the drug increased the holoenzyme and total pyrrolase activities by 194% and 53% respectively and, therefore, elevated the percentage haem saturation by 93%. This morphine activation of the pyrrolase was not influenced by the opiate receptor antagonists naloxone, naltrexone, Mr-1452 and its inactive isomer Mr-1453, nor by the adrenoceptor-blocking agents ergotamine and propranolol. The above activation was also not influenced by the transcriptional inhibitor actinomycin D, but was prevented by the translational inhibitor cycloheximide. Combined administration of morphine plus cortisol gave an additive effect on pyrrolase activity, whereas that of morphine plus tryptophan did not. Morphine increased the half-life of the total pyrrolase from 2.24hr to 4.10hr.

It is suggested that acute morphine administration enhances rat liver tryptophan pyrrolase activity by a substrate-type mechanism involving activation and stabilization of the enzyme by tryptophan as a result of an increase in its availability to the liver secondarily to its displacement from serum-protein-binding sites.

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METABOLISM OF H_2-ANTAGONISTS BY A FLAVIN-CONTAINING MONOOXYGENASE

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Flavin-containing monooxygenase (FAD-monooxygenase) activity in human liver samples was first reported by Ziegler and Gold (1971). The structure of several H_2-antagonists is such that these might act as substrates for this enzyme.

FAD-monooxygenase activity was measured in fresh or stored (-15°C) guinea-pig liver microsomes by determination of the substrate-dependent increase in oxygen consumption, measured polarographically at 37°C with a Clark electrode. The reaction media contained 0.1M Tricine buffer pH 8.4, 0.5mM NADPH, microsomal protein (0.7 - 1.5 mg/ml) and 3mM n-octylamine, a compound known to inhibit cytochrome P-450 and to activate the FAD-monooxygenase (Ziegler, 1980). Methimazole (1mM) was used as a standard for measuring FAD-monooxygenase activity, since at this concentration it is not metabolised by the P-450 system. H_2-antagonists were used in the concentrations as indicated in Table 1.

All the H_2-antagonists studied appeared to be metabolised, at least in part, by the FAD-monooxygenase, since in the presence of n-octylamine there was still substrate-dependent oxygen consumption. Furthermore, in each case activity was lower at pH 7.4, in agreement with the reported pH optimum of 8.4 for the FAD-monooxygenase (Ziegler, 1980).

The metabolism of cimetidine and ranitidine by pig liver FAD-monooxygenase has recently been reported by Oldham and Chenery (1984). Our results would indicate however, that the metabolism of cimetidine and ranitidine, and metiamide also, involves both FAD-monooxygenase and cytochrome P-450 systems. With these H_2-antagonists, omission of n-octylamine increased oxygen consumption at pH 8.4, in contrast to the findings with methimazole whose metabolism under these conditions is known to involve only the FAD-monooxygenase. Oxmetidine and SK & F 93479 behaved like methimazole, indicating that their metabolism involves primarily FAD-monooxygenase activity.

We gratefully acknowledge the gift of ranitidine from Glaxo Group Research Ltd. and of the other antagonists from Smith Kline and French Laboratories.

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Table 1 EXTENT OF METABOLISM (as reflected by substrate-dependent O_2 consumption) OF A SERIES OF H_2-ANTAGONISTS BY A GUINEA-PIG LIVER FAD-MONOOXYGENASE

SUBSTRATE	SUBSTRATE-DEPENDENT OXYGEN CONSUMPTION (n moles O_2/min/mg microsomal protein)		
	Media, pH 8.4	Media, pH 8.4 minus n-octylamine	Media, pH 7.4
METHIMAZOLE (1mM)	10.4 ± 2.5	5.1 ± 1.3	5.7 ± 2.0
METIAMIDE (0.275mM)	3.2 ± 0.6	5.3 ± 1.4	2.2 ± 0.5
CIMETIDINE (4.9 mM)	2.4 ± 0.7	3.4 ± 0.4	1.4 ± 0.3
RANITIDINE (8.5 mM)	4.2 ± 1.3	5.5 ± 0.5	2.0 ± 0.9
OXMETIDINE (1mM)	6.9 ± 1.8	2.4 ± 0.7	3.6 ± 1.0
SK & F 93479 (5.3 mM)	7.5 ± 1.1	4.9 ± 0.8	3.4 ± 0.9

ABSORPTION FROM LIPOSOMES ADMINISTERED TO THE RAT LUNG

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The control of drug release from intravenously (i.v.) administered liposomes has been shown to be influenced by factors such as size, surface charge and lipid composition. The absorption of liposome encapsulated solutes following pulmonary administration has not been fully characterised. This communication reports the effect of liposome encapsulation in neutral and negative vesicles on the pulmonary absorption of 6-carboxyfluorescein (CF).

CF (94 mg ml^{-1}) was incorporated into neutral (dimyristoylphosphatidylcholine (DMPC)/cholesterol,1:1) and negative (DMPC/cholesterol/dicetylphosphate, 1:1:0.2) liposomes. After size reduction and purification the neutral and negative vesicles were 113±2nm and 139±4nm respectively, and each contained <5% of free dye. Groups of 5 male Wistar rats (200-250g), anaesthetised with pentobarbitone, received 0.1ml of liposome preparation (34mg lipid kg^{-1}) or free dye (2mg kg^{-1}) as a liquid intratracheal instillation. Serial blood samples were taken from a cannulated carotid artery and assayed for free and encapsulated CF using fluorimetry.

The absorption of CF from either a solution or liposomal preparations appeared to be rapid with peak concentrations attained within 60 min after dosing. However post-peak concentrations of CF declined with a half-life much longer than observed following i.v. dosing, indicating a prolonged absorption. The post-peak half-life of CF administered as a solution was 78(63-98)min (with 95% confidence interval in parantheses). Encapsulation in negative vesicles resulted in post-peak half-life of 123(99-153)min which was significantly different ($p<0.01$) from that observed after administration of the solution. Encapsulation in neutral liposomes produced a much larger increase in half-life to 281(183-425)min which was significantly different ($p<0.001$) from those observed from administration of either the solution or encapsulation in negative liposomes. These differences in post-peak half-life indicate changes in CF absorption kinetics and this is supported by the observed changes in mean absorption time (MAT). The absorption of CF from solution was characterised by a MAT of 108(83-141)min and a fraction available of 112(90-139)%. Encapsulation of CF in negatively charged vesicles resulted in a significant decrease ($p<0.001$) in the fraction available to 33(24-45)% and a small but insignificant increase in MAT to 137 (104-179)min. Encapsulation in neutral vesicles similarly resulted in a significantly decreased ($p<0.001$) fraction available to 16(9-28)%. However CF absorption was much slower from neutral vesicles with a MAT of approximately 415 min. This latter parameter could not be accurately determined due to the greatly prolonged absorption. The release rates of CF from the liposome preparations in vivo were calculated to be in excess of 10-fold greater than those seen in vitro. Kirby et al. (1980) reported that i.v. administered negatively charged liposomes released solutes more rapidly then neutral liposomes. Our results indicate that this also occurs within the pulmonary environment. We could not detect any encapsulated CF in blood and hence intact liposomes would not appear to be absorbed from the rat lung.

In conclusion, liposome encapsulation can markedly influence pulmonary absorption and solute release within the lungs. Furthermore, solute release in vivo is dependent upon liposomal lipid composition.

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THE TREATMENT OF EXPERIMENTAL CYANIDE POISONING

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In the United Kingdom the most widely-used specific treatment for cyanide poisoning is dicobalt edetate (Kelocyanor, Laboratories Laroche-Navarron, Puteaux, France) introduced by Paulet (1958). This antidote is reputedly successful but sometimes gives rise to unpredictable reactions (Bryson, 1978). It acts by forming a complex with cyanide, cyanide and Kelocyanor reciprocally neutralizing one another's toxicity (Paulet, 1961). 4-Dimethylaminophenol (DMAP) has been successfully used in the treatment of experimental cyanide poisoning (Lörcher and Weger, 1970) and in its prophylaxis (Marrs et al, 1983). DMAP acts by generating a therapeutic methaemoglobinaemia, the methaemoglobin reacting with cyanide to form cyanmethaemoglobin inside the red cell. It is recommended that DMAP treatment be followed by intravenous sodium thiosulphate solution to hasten the detoxification of cyanide by conversion to thiocyanate. The main factors which would appear to govern the choice between Kelocyanor and DMAP are their relative toxicities and relative efficacies: the present study is directed towards the latter.

After cannulation of the cephalic vein of one forelimb beagle bitches were given 30 mg kg^{-1} of potassium cyanide by gavage. When respiration had ceased for about 20 s, four of these bitches were treated with an intravenous dose of 6 ml Kelocyanor solution (90 mg dicobalt edetate, 1.2 g glucose in 6 ml water) followed by a further 6 ml about 5 min later. Seven bitches were treated with 5 mg kg^{-1} DMAP intravenously which produces a peak methaemoglobin of about 50%. This was followed by 10 ml 50% sodium thiosulphate about 5 min later. 10 ml blood samples were taken 5, 15 and 45 min after poisoning for estimation of blood and plasma cyanide by the method of Epstein (1947) and Feldstein and Klendshoj (1954). Only one out of four animals treated with Kelocyanor survived the study, whilst all seven treated with DMAP did so. This difference in survival is statistically significant ($p = 0.048$, Fisher's exact test). Those treated with DMAP showed plasma cyanide concentrations of 0.29 ± 0.26, 0.17 ± 0.23 and 0.26 ± 0.32 mg l^{-1} ($\bar{x}$ ± SD), 5, 15 and 45 min after poisoning. Corresponding whole blood cyanide levels were 22.1 ± 16.3, 57.2 ± 26.6 and 56.3 ± 27.8 mg l^{-1} showing that the cyanide was mainly in the red cells. Plasma cyanide levels after Kelocyanor treatment were 0.34 ± 0.20 and 0.64 ± 0.10 mg l^{-1} at 5 and 15 min. Corresponding whole blood cyanide levels were 1.7 ± 0.49 and 2.7 ± 0.20 mg l^{-1}. It is concluded that under the conditions of this experiment DMAP was a superior treatment to Kelocyanor.

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INDUCTION OF METHAEMOGLOBIN BY AMINOGLUTETHIMIDE IN THE MOUSE

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Aminoglutethimide is an aromatic amine that inhibits aromatase and is used in the treatment of advanced breast cancer in oophorectomized and post-menopausal women (Harris et al. 1983). As aniline derivatives may produce methaemoglobin (Kiese, 1966), it was of interest to determine the activity of aminoglutethimide in this respect, although methaemoglobinaemia has not been reported as a blood dyscrasia with this drug. Dapsone, a known methaemoglobin inducer (Cucinell et al. 1972) was employed as a positive control.

The drugs were suspended in 0.5% (w/v) sodium carboxymethylcellulose for oral administration to fasting female albino mice (25-30g) in groups of 5. Blood was collected by cardiac puncture under ether anaesthesia and methaemoglobin was determined by the method of Fairbanks (1976). After a dose of 500mg/kg aminoglutethimide, methaemoglobin was found in the blood reaching a maximum concentration (8.2±1.1%) at 45 min. However, within 90 min, levels had returned to baseline values. An equimolar dose of dapsone (534mg/kg) produced a maximum effect of 10.1±1.5% within 30 min and values returned slowly to baseline over 240 min. The mean ED_5 values were 420 and 95mg/kg for aminoglutethimide and dapsone respectively when determined 45 min after dosing groups of mice with various doses of the two drugs.

For many aromatic amines, including dapsone, it is thought that their N-hydroxy metabolites may be the active methaemoglobin-inducing agents (Cucinell et al.1972). With this in mind, heparinized mouse blood was incubated at 37°C for 45 min in the presence of either aminoglutethimide or dapsone each, at a final concentration of 2µg/ml, but no methaemoglobin formation was detected. However, when the incubation was repeated in the presence of the 10,000 x g supernatant of mouse liver homogenate 8.0±1.3% and 6.3±0.9% conversion to methaemoglobin occurred in 45 min for aminoglutethimide and dapsone respectively. This suggests that metabolic activation of aminoglutethimide at this concentration (within the levels achieved from therapeutic doses in man) is necessary for methaemoglobin induction. The in vivo activity of this drug in mice may be related to its metabolism to an N-hydroxy product which has been described as an autoinduced metabolite in man (Jarman et al. 1983) and recently in the mouse (Jarman, personal communication).

The present experiments suggest that in the mouse aminoglutethimide has a similar order of methaemoglobin-inducing activity to that of dapsone. We have found no evidence of raised methaemoglobin levels in the blood of two normal human subjects following a single 500mg oral dose of aminoglutethimide. However, the relevance of the methaemoglobin-inducing activity of aminoglutethimide in the human situation should be established for chronic dosing.

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TISSUE DISTRIBUTION OF [^{14}C]-PENTOBARBITONE IN RATS WITH EXPERIMENTAL CHRONIC RENAL FAILURE

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The hypnotic effect of barbiturates is significantly prolonged in renal failure (Leber et al. 1978). Information on the tissue distribution of these drugs is scarce in spite of some limited evidence for an altered pharmacokinetic profile (Reidenberg et al. 1976; Ghonheim and Pandya, 1975). We have investigated the in vivo tissue distribution of (^{14}C) pentobarbitone in experimental chronic renal failure.

The 5/6th nephrectomy described by McCance and Morrison (1956) was used to induce renal failure in male Wistar rats (180g). The animals were matched with pair-fed sham-operated controls. Injection of (^{14}C) pentobarbitone 10mg.kg^{-1}(6μCi.kg^{-1}) into the jugular vein was made under ether anaesthesia. Animals in groups of 3 (nephrectomized and pair-fed sham-operated controls) were sacrificed at 14 and 28 days after surgery, 1 hour after injection when tissue samples of liver, brain, kidney and plasma were collected. The concentration of total radioactivity (cpm.g^{-1} tissue) was obtained following standard tissue digestion and liquid scintillation counting techniques. Unchanged (^{14}C) pentobarbitone (μg.g^{-1} tissue) was assayed by modifications to the method of Kunztman et al. 1967. Results were analysed statistically by the Mann Whitney 'U' test.

Plasma urea concentrations were significantly ($P<0.05$) raised in nephrectomized rats (101±10mg.100ml^{-1}) when compared to control animals (55±2.3mg.100ml^{-1}). Tissue total radioactivity concentration was significantly ($P<0.05$) raised in the nephrectomized groups (at 14 days) when compared to their respective controls. Furthermore, a significantly ($P<0.05$) higher concentration of unchanged (^{14}C) pentobarbitone was found in the liver of these nephrectomized rats (Table 1), and there was a similar trend for the other tissues. Results from renal tissue were difficult to compare in view of the drastic reduction in renal mass in nephrectomized groups.

Table 1. Tissue distribution of (^{14}C) pentobarbitone 10mg.kg^{-1}(6μCi.kg^{-1}) i.v. 1h post-injection in male Wistar rats 14 days after 5/6ths nephrectomy

	Tissue radioactivity cpm.10^{3}.g^{-1}		(^{14}C) Pentobarbitone μg.g^{-1} tissue	
	N	C	N	C
LIVER	81.3 ± 12.9*	31.2 ± 2.1	308 ± 22*	221 ± 9
BRAIN	21.4 ± 6.5	21.3 ± 6.5	160 ± 14	149 ±12
PLASMA	29.1 ± 2.6*	6.5 ± 0.1	123 ± 25	103 ±13
KIDNEY	49.4 ± 6.4	59.7 ±17.7	301 ± 47*	154 ±28

Values are means ± s.e.m. *$p<0.05$ N = Nephrectomized C = Control

Results at 28 days were qualitatively similar to those at 14 days. The findings suggest that in the 5/6th nephrectomized rat the lowered clearance of radioactivity may be due, not only to a decreased renal elimination of labelled metabolites, but also to a decreased metabolic clearance of pentobarbitone.

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LITHIUM DISTRIBUTION IN A THREE COMPARTMENT SYSTEM OF GUINEA-PIG GASTROINTESTINAL TRACT: EFFECTS OF DRUGS

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Lithium is used in the prophylactic treatment of manic-depressive psychoses. However, there is controversy surrounding the so called "slow release formulations" of lithium (Birch 1982). In an attempt to resolve this issue, absorption characteristics *in vitro* of lithium in a multicompartment system (Birch *et al* 1983) and a three compartment model (Karim *et al* 1984) have been previously reported. We now report the effects of drugs on unidirectional fluxes in the absorptive (mucosal (M) to serosal (S)) and secretory (S to M) directions together with tissue uptake from both sides.

Non steroidal anti-inflammatory drugs (NSAID) such as indomethacin and piroxicam were reported to increase plasma lithium levels by suppressing renal clearance of lithium (Reimann *et al* 1983 and Stockley (1983). Amiloride, a potassium sparing diuretic has been reported to cause lithium retention (Jefferson 1980) and is known to affect magnesium absorption in the kidney (Devane *et al* 1983). However, any contribution that might be made by alteration by these drugs of gastrointestinal absorption of lithium to the raised levels of serum lithium was not considered.

Guinea pig isolated mucosae were prepared as described by Lauterbach (1977) and lithium determined by atomic absorption spectroscopy (Birch and Jenner, 1973).

Absorption of lithium in the presence of indomethacin and piroxicam was unaffected. More importantly, the cellular levels of lithium were also unaffected. In addition the movement of lithium in the S to M direction remained unaltered in the presence of these drugs on the serosal side. Amiloride was unable to alter either the absorption of lithium across the small intestine or permeation in the secretory direction. Cellular concentration was also unaffected.

We conclude that neither NSAID, indomethacin and piroxicam, nor amiloride produced effects on gastrointestinal absorption and tissue uptake of lithium. There appears to be no gastrointestinal contribution to the raised levels of lithium which have been reported during concomittant treatment with these drugs.

We are grateful to Merck Sharp and Dohme Ltd for the gift of amiloride.

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THE FORMATION AND ELIMINATION OF DINITROPHENYL-PROTEIN CONJUGATES IN VIVO IN THE RAT AND RABBIT

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It has been proposed that drug allergies may be due to the conversion of endogenous proteins into immunogenic protein conjugates, with either the drug itself or a reactive metabolite acting as a hapten. Antibodies raised against such protein-hapten conjugates can lead to the formation of immune complexes, and it is the deposition of these complexes that results in tissue lesions that typify a particular drug hypersensitivity reaction. The aim of this study was twofold: first, to show that protein-hapten conjugates can be formed in vivo after administration of a model chemically reactive metabolite, dinitrofluorobenzene (DNFB) (Kitteringham et al., 1985), to rats. Secondly, to investigate the immunogenicity of dinitrophenyl conjugates of endogenous serum proteins synthesised in vitro with different epitope densities (dinitrophenyl groups/protein molecule) (Kitteringham et al., 1985).

Male Wistar rats, anaesthetized with urethane, received [^{3}H]-DNFB (10 μCi; 5 μg, 5mg or 25mg/kg) in polyethylene glycol/saline (50/50, v/v) via the jugular vein. Blood samples were removed at regular intervals and after 3h the animals were killed and the major organs were removed. The covalent binding to protein of radiolabelled material was measured in plasma and tissue homogenates. [^{3}H]-DNFB (15 μCi; 5 or 25 mg/kg) was also given i.v. to conscious rats and urine collections were made every 24h for 4 days. The identification and quantification of the major radiolabelled urinary metabolites was accomplished using h.p.l.c., u.v. spectoscopy, mass spectrometry and n.m.r spectrometry.

Radioactivity rapidly disappeared from the plasma of anaesthetized rats. After 3h only 0.7 ± 0.03% dose/ml plasma (mean ± SD) remained, but of that 56 ± 14% was covalently bound to proteins. Tissue radioactivity was greatest in the kidney (1.3 ± 0.3%/g), but only 17 ± 5% was covalently bound. In conscious rats the principal urinary radiolabelled metabolite of [^{3}H]-DNFB was dinitrophenyl mercapturate (32-64% of radioactivity in 24h), however N^{2}-acetyl-N^{6}-dinitrophenyl-lysine was also detected and this compound is the principal metabolite of dinitrophenylated albumin in rats (Kitteringham et al., 1985). These data indicate that DNFB is mainly eliminated as low-molecular-weight metabolites, but that covalent binding to plasma proteins leads to small amounts of circulating protein-hapten conjugates.

To investigate the immunogenicity of dinitrophenyl-protein conjugates, male New Zealand White rabbits were injected i.v. with dinitrophenyl conjugates of either their own rabbit serum protein (RSP) (50mg/kg in 1ml/kg saline) (epitope densities 0.5,15 and 30) or bovine serum albumin (BSA) (epitope density 0.5) at the same dose. Rabbits given the BSA conjugate exhibited a precipitous decline in plasma radioactivity between days 7 and 10, which is due to formation of immune complexes (Wilson and Dixon, 1971). However no such phenomenon was observed with the RSP conjugates.

In conclusion, administration of DNFB to rats results in the formation of covalently bound conjugates in plasma. Conjugates, formed in vitro from rabbit serum, did not appear to elicit an immune response when injected back into the same animal.

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COVALENT BINDING OF PROPRANOLOL TO RAT AND HUMAN LIVER MICROSOMES: RELATION TO INHIBITION OF LIGNOCAINE METABOLISM

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Previous studies have shown that liver microsomes from rats pre-treated in vivo with propranolol (P) had a decreased ability to metabolise lignocaine (L) despite the absence of significant residues of unchanged P (Al-Asady et al, 1982). Using ^{3}H-P, Schneck and Pritchard (1981) presented evidence suggesting that a metabolic intermediate product of P binds covalently to the cytochrome P-450 system and causes autoinhibition of P metabolism. We have used ^{14}C-P to confirm this binding in both rat and human liver microsomes and to investigate its relationship to inhibition of L metabolism.

Study 1: P was incubated with Wistar rat and human liver microsomes at 37°C and pH 7.4, alone or together with L (4.27μM). Putative covalently bound material was measured after exhaustive solvent extraction. L and its metabolites were measured by hplc. In rat microsomal incubates (P: 50μM containing 0.146μCi) covalently bound material increased from 0.38 ± 0.04s.d. nmol.mg protein^{-1} after 6min to 1.03 ± 0.08s.d. nmol.mg protein^{-1} at 30min (4 livers), at which time it represent-ed 4% of the dose. In human microsomal incubates (P: 100μM containing 0.5uCi) 0.031 ± 0.004s.d. nmol. mg protein^{-1} was bound after 60min (3 livers). This represented 0.15% of the dose. Exclusion of metabolic cofactors lowered the binding by 96% in rat and by 85% in human liver microsomes. Addition of glutathi-one (1mM) decreased binding by 70% (rat) and 73% (human) but had no effect on the inhibition of L metabolism by P.

Study 2: Four rats received a daily dose of P (100 mg.kg^{-1}, 26uCi.kg^{-1}) by intra-gastric intubation for 5 days and were killed at various times after the last dose. The decline in the amount of covalently bound material in liver microsomes from 0.33 nmol.mg protein^{-1} at 18h to 0.28 nmol.mg protein^{-1} at 24h and 0.16 nmol. mg protein^{-1} at 48h paralleled the decrease in inhibition of L metabolism (6min incubation: L loss 7.88 ± 0.10s.d., 9.38 ± 0.08s.d. and 16.60 ± 3.86s.d. umol.mg protein^{-1}.min^{-1} at 18, 24, 48h, respectively). The appearance of the hydroxy-product of L increased with time while that of the N-dealkylation product decreased.

These data indicate that a covalently bound product of P metabolism may contribute to the inhibition of L metabolism by P pre-treatment but not when L and P are coincubated with liver microsomes.

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MEASURING THE DISSOCIATION CONSTANT OF A PARTIAL AGONIST FROM ITS INTERACTION WITH A FULL AGONIST

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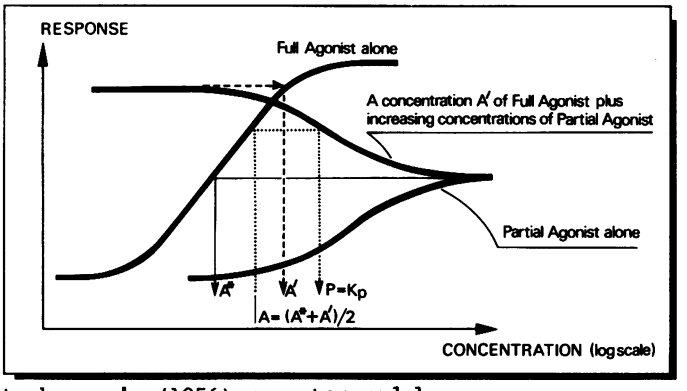
Methods have been described to estimate the equilibrium constant of a partial agonist from its log concentration response curve (Furchgott & Bursztyn, 1967; Roberts, 1984) or from the interaction between a single concentration of the partial with increasing concentrations of a full agonist (Stephenson, 1956; Kaumann & Blinks, 1980). Alternatively the reverse situation can be used as shown in the diagram below:-



According to Stephenson's (1956) receptor model:

$$e_A \cdot \frac{A}{K_A} = e_A \cdot \frac{A'}{K_A}\left(\frac{K_P}{P + K_P}\right) + e_P\left(\frac{P}{P + K_P}\right) \qquad (1)$$

where A is the concentration of full agonist alone that produces the same response as a concentration A' plus P of the partial agonist; e_A , e_P , K_A, K_P are the efficacies and dissociation constants of the full and partial agonist respectively.

But if A* is the concentration of full agonist alone that produces a response equal to the maximum of the partial, from Stephenson's (1956) model, then:

$$e_A \cdot \frac{A^*}{K_A} = e_P \text{ , and so } A^* = \frac{e_P}{e_A} \cdot K_A \qquad (2)$$

From this equation and rearranging (1) then:

$$K_P = P \cdot (A-A^*)/(A' - A) \qquad (3)$$

Thus when $A = (A^* + A')/2$, $(A - A^*) = (A' - A)$, $K_P = P$

K_P can thus be obtained from the log concentration response curves either 'by-eye' as shown in the diagram, or using suitable 'curve-fit' procedures.

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ON-LINE ANALYSIS OF CARDIOVASCULAR DATA USING A MINI-COMPUTER SYSTEM

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The analysis of multiple channels of data from physiological and pharmacological cardiovascular experiments by manual measurement is tedious and time-consuming. A commercially available computer system for analysing this type of data 'on-line' has been described (Algate et al, 1983), however the original system could not analyse electrocardiographic (ecg) data, nor accept data from more than one animal at a time. Recently, the software of this system has been upgraded by CED Ltd., the manufacturers, for Wellcome Research Laboratories, to include ecg analysis and to allow input from upto four animals at one time (with the total number of analogue inputs limited to eight).

We regarded it as essential, before using the system routinely, to validate the on-line analysis by comparing the computer-generated data with that measured manually from paper traces (Gould Brush Recorder System). Accordingly, the cardiovascular data from anaesthetised dogs and cats, and from chronically instrumented conscious dogs, were compared. The signals analysed included systemic arterial pressure, left ventricular pressure, ecg, blood flows (using electromagnetic flow meters), cardiac dimensions and segment lengths (using sonomicrometry) and also respiration excursion and volume. The computer can also generate a wide range of parameters that are calculated from these signals, and these too have been compared. The correlations between computer- and manually-measured data have proved totally acceptable, and will be displayed at the meeting of the Society. Two computer systems have now been implemented for routine use in our department.

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FURTHER STUDIES ON THE ACTIONS OF MDL 72222 ON RESPONSES TO 5-HT IN RABBIT NODOSE (NG) AND SUPERIOR CERVICAL (SGG) GANGLIA

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5-hydroxytryptamine (5-HT) has a potent depolarizing action on the cell bodies of vagal afferents in NG which is blocked by tropine 3,5,-dichlorobenzoate (MDL 72222) (Azami et al,1984a,b). Our earlier experiments indicated that, after equilibration with the antagonist for 1 hr, concentrations up to 100 nM caused a rightward shift of the dose-response (DR) curve with no change in slope. Higher concentrations induced some degree of further, but insurmountable blockade, and there appeared to be an MDL 72222-resistant component of the response. We now report that further blockade of very slow onset occurs when the antagonist superfuses the tissue for several hours, which causes a reduction in slope and depression of the maximum of the DR curve. No clear MDL 72222-resistant component can now be identified.

Membrane potential change in a population of neurones was measured using the sucrose-gap technique. NG or SCG were prepared as described by Azami et al (1984b). They were continuously superfused with Krebs solution at 19-20°C. Reproducible depolarisations were obtained by injecting into the superfusion stream to the ganglion amounts of 5-HT ranging from 5-80 nmol (0.05-0.4ml of a solution in Krebs).

In agreement with our previous results equilibration of the tissue with MDL 72222 (10 and 100 nM) for 1 hr produced a parallel rightward shift of the DR curve in NG; the dose-ratios measured at the ED50 were 2.8 and 4.0, respectively. Longer exposures (3-4 hr) to 100nM shifted the DR curve to the right with a reduction of slope and of the maximum, an effect also seen on exposure to 1uM MDL 72222 both for 1 hr and 3-4 hr. Dose-ratios were measured at the ED25 in these experiments and were: 100nM, 3-4 hr, 6.0; 1uM, 1 hr, 4.8; 1uM, 3-4 hr, 59.6. Control DR curves repeated at these time intervals showed a small, parallel rightward shift, dose-ratios at ED50 being 1.4 (1 hr) and 2.1 (3-4 hr). On the SCG 10 and 100 nM MDL 72222 produced a rightward shift from the control curve after 1 hr exposure; the dose-ratios measured at the ED50 were 1.8 and 8.3, respectively.

Metoclopramide and ketanserin were also tested as antagonists of the 5-HT response of NG. Metoclopramide (100 nM and 1 uM) was an effective competitive antagonist; dose-ratios measured at the ED50 after equilibration for 1 hr were 6.5 and 13.9, respectively. Ketanserin (10 and 100 nM), on the other hand, caused no shift in the DR curves relative to controls.

Thus, while 1 hr exposures to MDL 72222 can produce competitive blockade, more prolonged exposure produces a further and insurmountable blockade similar to that reported for antagonism of 5-HT responses of rat vagus nerve (Fortune & Ireland, 1984).

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THE HAEMODYNAMIC PROFILE AND MECHANISM OF THE HYPOTENSIVE ACTION OF 5-CARBOXAMIDOTRYPTAMINE IN CONSCIOUS DOGS

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5-Carboxamidotryptamine (5-CT) is a potent agonist at 5-HT receptors mediating relaxation of vascular smooth muscle in vitro (Feniuk et al., 1984) and in causing inhibition of sympathetic neurotransmission (Feniuk et al., 1981; Engel et al., 1983). The present study was undertaken to determine the haemodynamic effects of 5-CT in conscious normotensive dogs.

Male beagle dogs weighing 10-15 kg were prepared for measurement of arterial blood pressure from an indwelling catheter in the common carotid artery and ascending aortic blood flow with a cuffed electromagnetic flow probe. Heart rate was measured by counting the number of blood pressure pulses during one minute periods. Doses of 5-CT were added cumulatively from 0.001-100 µg/kg administered into the cephalic vein.

In untreated animals, 5-CT had little effect on diastolic blood pressure and cardiac output in doses up to 0.1 µg/kg i.v. However, larger doses caused a lowering of diastolic blood pressure (maximum decrease = 30 $\pm$ 8 mmHg, mean $\pm$ s.e. mean from 5 dogs) which was accompanied by an increase in heart rate and cardiac output. The hypotensive effects of low doses of 5-CT (0.1-3 µg/kg i.v.) were transient (<15 mins), not always dose-related, and accompanied by increases in heart rate and cardiac output of progressively increasing magnitude and duration. Higher doses of 5-CT (10-100 µg/kg i.v.) produced sustained and dose-related decreases in diastolic blood pressure but little further change in heart rate or cardiac output. The hypotensive action of 5-CT was attributable to falls in total peripheral resistance.

In further experiments, the same animals were pretreated with mecamylamine (10mg/kg i.v.) and atropine (0.2 mg/kg i.v.) which increased heart rate from 66 $\pm$ 4 beats/min to 117 $\pm$ 5 beats/min (mean $\pm$ s.e.m. n=5). This tachycardia was due to a withdrawal of vagal tone and resulted in diastolic blood pressure increasing from 80 $\pm$ 5 mmHg to 96 $\pm$ 5 mmHg (see Drew et al., 1983). In these animals, 5-CT (0.1-100 µg/kg i.v.) produced even greater and more sustained decreases in arterial blood pressure (maximum change = 60 $\pm$ 7 mmHg) and total peripheral resistance. There was little effect of 5-CT on heart rate or cardiac output after ganglion blockade. Despite a higher resting heart rate following ganglion blockade, isoprenaline (10-300 ng/kg i.v.) produced further increases in heart rate and cardiac output.

It is concluded that in conscious dogs, 5-CT reduces total peripheral resistance which induces a reflexly mediated tachycardia and increase in cardiac output. When the latter increases no further, hypotension then results. The hypotensive action of 5-CT appears to be predominantly mediated through a direct vasodilator action since it is still present in animals after ganglion blockade.

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311P

5,7-DIHYDROXYTRYPTAMINE LESIONING INCREASES BOTH 5-HT_2-MEDIATED BEHAVIOUR AND CORTICAL 5-HT_2 RECEPTOR NUMBER IN MICE

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5-Hydroxytryptamine- (5-HT) mediated head-twitches in mice and 'wet-dog' shakes in rats are probably specifically mediated by 5-HT_2 receptors (see Green & Heal, 1985). Lesioning rat brain with 5,7-dihydroxytryptamine (5,7-DHT) enhances 'wet-dog' shake behaviour (see Green & Heal, 1985) but ligand binding studies have not shown an increase in 5-HT_2 receptor number (e.g. Leysen et al, 1983).

We have now studied the effects of 5,7-DHT lesioning on both 5-HT_2-mediated head-twitch and 5-HT_2 receptor number in the same mice. 5,7-DHT (50 µg salt in 2 µl ice-cold saline containing ascorbic acid 0.4 mg/ml) was injected intracerebroventricularly into desipramine (5 mg/kg) pretreated conscious mice using the stereotaxic apparatus described by Heal (1984). Sham-lesioned controls received 2 µl ascorbate solution. After 2 weeks head-twitches were determined in the 6min following injection of 5-methoxy-N,N-dimethyltryptamine (2 mg/kg). One week later, 5-HT_2 binding in frontal cortex was measured with [^{3}H]-ketanserin using a modification of the method of Leysen et al (1982). Scatchard analyses and 'single-point' binding were performed on pooled tissues. 5-HT concentrations were measured by HPLC with electrochemical detection. All results are expressed as mean ± S.D.

5,7-DHT lesioning markedly elevated both the head-twitch response and cortical 5-HT_2 receptor number (see below).

	5-HT_2 receptor binding		Head-twitch
	K_D	B_{max}	
Sham-lesion	0.89 ± 0.11 (4)	276 ± 16 (4)	10.1 ± 1.1 (7)
Lesion	0.99 ± 0.17 (3)	353 ± 11 (3)**	34.8 ± 11.0 (18)**

**$p < 0.01$. K_D :nM. B_{max}:fmol/mg protein.

Mean % 5-HT depletion was 70 ± 8 (16) in cortex and 63 ± 8 (17) in mid/hindbrain. Mice lesioned with lower doses of 5,7-DHT (5-20 µg) displayed no head-twitch enhancement (sham-lesion: 10.3 ± 2.1, n = 6; lesion: 9.0 ± 4.5, n = 8) and no increase in 5-HT_2 receptor binding at a [^{3}H]-ketanserin concentration of 2.1 nM (sham-lesion: 216 ± 48, n = 4; lesion: 225 ± 58, n = 4; fmol/mg protein). Mean % 5-HT depletion was 32 ± 17 in cortex and 41 ± 10 in mid/hindbrain.

We have thus shown that where 5-HT depletion is sufficient to enhance 5-HT_2-mediated behaviour, there is also an increase in 5-HT_2 receptor number. Furthermore, when lesioning failed to enhance the behaviour there was no increase in 5-HT_2 receptors. Although these two events may be associated, head-twitch responses are probably mediated via the hindbrain and brainstem (see Green & Heal, 1985) whereas binding was performed in frontal cortex. The failure of previous studies to show increased cortical 5-HT_2 receptor number following 5,7-DHT lesioning may be due to physiological differences between mice and rats or, possibly, the degree of 5-HT_2 depletion obtained.

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EVIDENCE FOR THE PRESENCE OF 5-HT_2 RECEPTORS ON THE RAT FUNDIC STRIP PREPARATION

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Gaddum and Picarelli (1957) classified 5-hydroxytryptamine (5-HT) receptors in the gut into those located in the myenteric plexus, termed 'M' and those located on the smooth muscle termed 'D'. On the basis of radiolabelled ligand binding studies in the mammalian CNS, Peroutka and Snyder (1979) put forward an alternative classification of 5-HT receptors: 5-HT_1 and 5-HT_2. In this report we have investigated the ability of the 5-HT_2 receptor antagonists mianserin and ketanserin to inhibit 5-HT induced contraction of the isolated rat fundic strip preparation (Vane, 1957).

Fundic strips were incubated at 35°C in Ringer-Locke solution containing 1μM atropine in order to eliminate the possibility of 5-HT contractile effects caused via activation of 5-HT 'M' receptors. Dose response curves were constructed for 5-HT in the presence and absence of mianserin (10^{-9}M - 10^{-7}M), ketanserin (10^{-9}M - 10^{-7}M) and N,N-dimethyltryptamine (N,N-DMT, 10^{-6}M). In each case, the tissue was allowed to equilibrate with the antagonist for 30 min prior to retesting the effects of 5-HT. ED_{50} values (mean ± S.E.) were compared and pD_2' estimated. Use of fundic strips not exposed to 5-HT antagonists showed that, when administered at 5 min intervals, the response to standard doses of 5-HT became constant after 3 - 5 additions and remained stable for at least 3h.

Mianserin (10^{-9}M) increased the ED_{50} values for 5-HT from 3.8 ± 0.3 X 10^{-8}M to 1.4 ± 0.2 X 10^{-7}M (n= 8; significantly different with $P < 0.025$; $pD_2' = 8.09 \pm 0.09$) but the antagonism was insurmountable with only 85% of the 5-HT maximum being attained. Similarly, ketanserin (10^{-9}M) increased the ED_{50} for 5-HT from 5.7 ± 0.8 X 10^{-8}M to 2.0 ± 0.3 X 10^{-7}M (n= 7; significantly different with $P < 0.025$; $pD_2' = 8.03 \pm 0.1$) as well as reducing the maximum response. Higher concentrations of both mianserin and ketanserin caused progressive decreases in the maxima of 5-HT dose response curves which is characteristic of insurmountable antagonism. By comparison, N,N-DMT (10^{-6}M) was a far less potent antagonist than either mianserin or ketanserin. Thus, the ED_{50} increased from 3.0 ± 0.4 X 10^{-8}M to 1.6 ± 0.2 X 10^{-7}M (n= 12; significantly different with $P < 0.01$). Finally, RU24969, the highly potent and selective agonist 5-HT_1 agonist (Hunt et al, 1981) had no contractile effect on the fundic strip at concentrations ranging from 10^{-8}M - 10^{-4}M.

The data suggests that the 5-HT 'D' receptor located on the rat fundic strip has the properties that would be expected of a 5-HT_2 receptor.

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313P

IN VITRO AND IN VIVO ACTIVITY OF A NOVEL MELATONIN ANALOGUE

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In order to assess the physiological role of the mammalian pineal gland, it would be advantageous to be able to specifically antagonise the effects of the pineal hormone, melatonin (MT). We report here preliminary findings of a novel analogue of MT, N-methyl-[5-methoxyindole]-propionamide, the reversed amide (RA) of melatonin which was synthesised by a method to be reported elsewhere.

In vitro studies demonstrated that RA interacts with antibodies raised specifically against MT. In the radioimmunoassay of Ho & Smith (1982), tritiated-MT was equilibrated with the antibody in the presence of either MT or RA. After precipitation with ammonium sulphate and separation, the percentage bindings of labelled-MT at different concentrations of MT and RA were as follows

Concentration of MT or RA (pg/250ul)		5	10	20	40	80	200
Percentage binding	- MT	60.9	60.0	51.0	43.6	21.4	9.9
	- RA	64.5	63.4	62.2	60.4	46.1	38.8

Intra-assay variation was 11%. Thus, although the antibody does not cross-react with many related indoles (Ho & Smith, 1982), RA cross-reacted with the antibody at approximately three times the concentration of MT, 50% binding being achieved with 25pg/tube of MT or with 74pg/tube of RA.

The in vivo agonist potencies of RA and MT were assayed on adult Xenopus laevis (3-10g body wt) equilibrated to a constantly illuminated black background. Groups of 12 toads were placed into a range of concentrations in water of RA or MT and the state of expansion of the dermal melanophores of the hind limb foot webs noted by microscopic examination after 30 min using the scale of Hogben & Slome (1931). Drugs were first dissolved in ethanol, the final concentration of which was 1 or 2%. RA produced a paling response at concentrations above 10^{-6} M. The dose-response curve was parallel to that of MT and the relative potency of RA was 0.000074 (95% confidence limits 0.000059, 0.000092).

The antagonist potency of RA against MT was assessed either by adding the drugs simultaneously or with RA added 30 min before MT. In the former case, a concentration of 10^{-6} M RA failed to cause antagonism, the relative potency of MT in the presence of RA being 1.27 (95% limits 0.97,1.65). When added 30 min before MT, RA produced parallel shifts to the right in the MT dose-response curve. The potency of MT after 10^{-7} M RA was 0.23 (95% limits 0.18, 0.29) and after 10^{-6} M RA 0.099 (95% limits 0.075, 0.131). A Schild plot gave a pA2 value of 8.23 for the antagonism but the slope the line differed from unity being 0.43.

In conclusion, the novel analogue RA was recognised in vitro by an antiserum raised against MT, whilst in vivo, on toad skin melanophores, it had very weak agonist potency compared with MT. When applied before exposing toads to MT, it significantly antagonised the effects of the pineal hormone, though the shallow Schild plot indicated that this was not true competitive antagonism. Thus, RA is a possible candidate for the specific antagonism of MT in biological systems.

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SEROTONIN RECEPTOR SUBTYPES IN RAT BASAL FOREBRAIN AND THEIR ROLE IN NUCLEUS ACCUMBENS-EVOKED ACTIVITY

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Dopamine agonists injected into the nucleus accumbens (NA) of rat brain cause locomotor stimulation. Many NA efferents terminate in subpallidal regions, especially in the ventral pallidum (VP, Williams et al, 1977). Some NA efferents to VP and the substantia innominata (SI) may be GABAergic, and modulation of GABA function in these subpallidal areas affects NA-evoked hyperactivity (Walaas & Fonnum, 1979; Patel & Slater, 1983). As part of an investigation of transmitters in the basal forebrain, we have measured serotonin (5-HT) receptors in VP and SI and examined the effects of 5-HT agonists in VP on NA-evoked hyperactivity.

Female Sprague Dawley rats (180 g) were fitted with guide cannulae for bilateral injections into NA (A 9.7, H -1.3, L 1.3) and VP (A 7.4, H -2.1, L 1.5). ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene, 10 μg in 1 μl) in NA caused locomotor hyperactivity. 5-HT compounds were injected into VP after 1 h and activity (photobeam crossings) was recorded for 5 h. 5-HT receptor subtypes were measured autoradiographically in 15 μm brain sections (Patel & Slater, 1984). The ligands were 2 nM [^{3}H]5-HT (5-HT$_1$ sites) and 2 nM [^{3}H]ketanserin (5-HT$_2$ sites). High concentrations of forebrain 5-HT$_1$ receptors were visualized in the globus pallidus, lateral septum, the VP and the SI. All of these areas contained low levels of 5-HT$_2$ receptors.

ADTN-induced locomotion reached a maximum at 4 h with a mean score (counts/h) of 2234 ± 53 (n=8). 5-HT in the VP reduced the hyperactivity - the doses given and the counts/h were: 0.5 μg, 1989 ± 251; 1 μg, 1087 ± 98; 2 μg, 799 ± 67 (n=4-6). 5-Methoxy-N,N-dimethyltryptamine in VP also reduced the activity: 1 μg, 372 ± 99; 2 μg, 245 ± 91 (n=5). The 5-HT agonists had no effects on rat locomotor activity when injected into VP in the absence of ADTN in NA.

These findings suggest that the very high concentration of 5-HT$_1$ receptors in the ventral pallidum can modulate NA-evoked locomotor activity. The innervation and function of these receptors have to be established.

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315P

[^{3}H]-8-OH-DPAT DOES NOT BIND TO THE 5-HT AUTORECEPTOR IN RAT STRIATUM

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8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) is a central 5-HT receptor agonist which has been reported to decrease 5-HT turnover, an effect which could reflect agonist activity at the 5-HT autoreceptor. A study of the effects of 8-OH-DPAT on the 5-HT autoreceptor in vitro has however failed to detect any agonist properties in the frontal cortex, striatum, hippocampus or piriform cortex of the rat brain (Middlemiss, 1984). In contrast, [^{3}H] 8-OH-DPAT appears to bind to a presynaptic site in rat striatal membranes and this has been suggested to be the 5-HT autoreceptor (Gozlan et al., 1983). One observation which argues against this interpretation is the relative lack of activity of the 5-HT agonist 5-methoxytryptamine (5-MeOT) in displacing [^{3}H] 8-OH-DPAT from its recognition site in striatal membranes (Gozlan et al., 1983) since, at least in the cortex, 5-MeOT is a potent 5-HT autoreceptor agonist (Engel et al., 1983). The present study was carried out to compare the potency of 5-MeOT as a 5-HT autoreceptor agonist with its activity to displace [^{3}H] 8-OH-DPAT from its recognition site in several areas of the rat brain.

The effect of 5-MeOT as an agonist at the 5-HT autoreceptor was assessed using inhibition of continuous 25 mM K^+ evoked release of preloaded [^{3}H] 5-HT from rat brain slices (Engel et al., 1983 ; Middlemiss, 1984). Receptor binding studies with [^{3}H] 8-OH-DPAT (116 Ci/mmole, CEA, France) were carried out in 50 mM Tris/HCl buffer as described by Gozlan et al. (1983).

5-MeOT was a potent agonist at the 5-HT autoreceptor in the striatum, frontal cortex and hippocampus (Table 1) with no significant difference in potency being evident in the three brain areas studied. In contrast, a marked regional variation was found in the ability of 5-MeOT to displace [^{3}H] 8-OH-DPAT from its recognition site. Thus 5-MeOT was only weakly active in displacing [^{3}H] 8-OH-DPAT from its binding site in the striatum, but was more potent in the frontal cortex and hippocampus. 8-OH-DPAT itself was equipotent in these latter two areas but was somewhat less active in the striatum as has previously been shown by Gozlan et al. (1983).

Table 1 Potency of 5-MeOT and 8-OH-DPAT in displacing [^{3}H] 8-OH-DPAT from its recognition site and the potency of 5-MeOT as an agonist at the 5-HT autoreceptor in various areas of the rat brain.

Brain Area	[^{3}H] 8-OH-DPAT recognition site (pIC_{50})		5-HT autoreceptor (pEC_{30})
	5-MeOT	8-OH-DPAT	5-MeOT
Striatum	5.00±0.03	7.47±0.16	6.81±0.11
Frontal Cortex	6.03±0.05	8.35±0.03	6.81±0.17
Hippocampus	6.64±0.15	8.17±0.28	7.02±0.13

These results, together with those from previous studies which failed to detect agonist activity at the 5-HT autoreceptor in these brain areas for 8-OH-DPAT itself (Middlemiss, 1984), prompts the conclusion that [^{3}H] 8-OH-DPAT does not bind to the 5-HT autoreceptor in the rat striatum.

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PHARMACOLOGICAL COMPARISON BETWEEN 5-HYDROXYTRYPTAMINE (5-HT) AND 5-CARBOXAMIDO-TRYPTAMINE (5-CT)

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In contrast to the effects of 5-HT mediated by M or 5-HT_2 receptors, the functional correlates of 5-HT_1 receptors are less well recognised. Recently, 5-CT was shown to stimulate 'atypical' 5-HT receptors, probably of the 5-HT_1-type (Feniuk *et al.*, 1981, 1984; Engel *et al.*, 1983; Saxena & Verdouw, 1985), since it selectively binds with 5-HT_1 recognition sites (Engel *et al.*, 1983). In this investigation, we have compared some cardiovascular and smooth muscle effects of 5-HT and 5-CT with a view to study the functional selectivity of 5-CT.

Heart rate, and arterial and urinary bladder pressures (rats), and bronchial resistance (guinea-pigs) in pentobarbital-anaesthetized animals, and isometric contractions of guinea-pig isolated ileum, suspended in a Tyrode solution, were recorded on a Grass polygraph.

In normal rats 5-HT caused bradycardia and a triphasic blood pressure response. The initial bradycardia and hypotensive response to 5-HT, being mediated by M receptors on afferent neurons, was abolished by mecamylamine (10 mg/kg, i.v.). After mecamylamine, in 12 rats 5-HT (6.25, 12.5, 25, 50, 100 and 200 µg/kg, i.v.) caused tachycardia (5±2, 6±2, 10±3, 13±3, 25±4 and 33±4 beats/min), and hypertension (19±3, 32±3, 42±3, 54±4, 69±4 and 76±5 mmHg), followed by hypotension (18±3, 20±4, 25±4, 17±2, 16±3 and 10±3 mmHg). The tachycardia and hypertension, but not hypotension, were antagonised by 0.5 mg/kg, i.v. of cyproheptadine or ketanserin. 5-CT did not affect heart rate, but caused already with a 6.25 µg/kg, i.v. dose a long-lasting hypotension, which in 6 ganglion blocked rats amounted to 44±2 mmHg. High doses of 5-CT produced a small, cyproheptadine-susceptible, hypertensive response (10 mmHg after 200 µg/kg, i.v.). In 5 rats we observed that methysergide (>2.5 mg/kg, i.v.) antagonized the hypotensive effect (31±7, 38±9 and 43±8 mmHg) caused by 5-HT (3.12, 6.25 and 12.5 µg/kg, i.v.). Similarly, peak hypotensive effect in rats (n=6-8 each) injected with 5-CT (0.5, 2 and 8 µg/kg, i.v.) was less in methysergide (5 mg/kg, i.v.) treated rats (7±2, 7±2 and 6±2 mmHg) than in saline treated rats (28±6, 52±4 and 51±3 mmHg). On the urinary bladder and bronchi, which were contracted by 5-HT (threshold dose, µg/kg: 1.56 i.a. for bladder, and 12.5 i.v. for bronchi) via 5-HT_2 and, in the case of bladder, also via M receptors. 5-CT (up to 25-50 µg/kg) was without any effect. The isolated ileum was slightly relaxed by low concentration (5×10^{-6} M) of 5-CT, probably via 5-HT_1 receptors (Feniuk *et al.*, 1984), and contracted by higher concentrations. However, the contractile effect of 5-HT, exerted via M and 5-HT_2 receptors, was much more than that of 5-CT.

Our results show that 5-CT possesses no or little activity on M or 5-HT_2 receptors. The hypotensive effects of both 5-HT and 5-CT are mediated by 5-HT_1 receptors, since they were unaffected by cyproheptadine or ketanserin, but antagonised by methysergide, which has appreciable affinity for 5-HT_1 recognition sites (Peroutka & Snyder, 1979). Therefore, 5-CT not only has a binding, but also a functional specificity for 5-HT_1 receptors. This compound seems to be a useful tool for investigating 5-HT_1 receptor mediated functional responses.

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ISOMETRIC MEASUREMENT OF CAT TRACHEALIS MUSCLE TENSION IN SITU

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The physiological and pharmacological properties of airway smooth muscle have been investigated primarily in vitro. However, by definition, such studies cannot be entirely predictive of the equivalent properties of this tissue in vivo. We have developed a method in cats which allows quantitative measurement of isometric tension in trachealis muscle in situ. This method also allows simultaneous estimation of intrathoracic airway calibre and is based on that originally described by Brown et al (1980) using dogs.

Male mongrel cats (2-3kg) were anaesthetised initially with halothane (5%) and thereafter with chloralose (60-80 mg/kg i.v.). Each animal was placed on its back and ventilated mechanically with 27 strokes/min of 15ml of laboratory air/kg body wt through a low cervical tracheostomy. The remainder of the cervical trachea was exposed up to the larynx. A segment approximately 2 cm in length and containing 8 cartilage rings was isolated by caudal and cranial transections taking care not to damage underlying structures. The isolated segment was longitudinally bisected along its anterior margin and the cut edges sutured to 2 aluminium strips. These were connected by stainless steel wire to a metal rod on one side of the cats neck and to a force-displacement transducer (Grass FTO3) on the other. The transducer and metal rod were each mounted on a rack and pinion assembly so that moving them either medially or laterally altered the load applied across the the preparation, which was usually fixed at 10 grams (equating to a resting tension of 5-6 g/cm longitudinal length). Pulmonary inflation pressure, an index of intrathoracic airway calibre (Dixon and Brodie 1903), was measured via a lateral port in the tracheostomy circuit with a pressure transducer (Elcomatic EM750). Catheters were placed in both femoral veins for administration of test substances, and in a femoral artery for measurement of blood pressure/heart rate.

Bolus injections of 5-HT (0.1 - 30 µg/kg i.v., n=4-9) provoked transient dose-related increases in trachealis resting tension of up to 3.3 ± 0.5g/cm and concomitant increases in pulmonary inflation pressure of up to 15.6 ± 2.3 cm H_2O. Acetylcholine (0.1 - 30 µg/kg i.v., n=3-4) also provoked dose-related increases in both resting tension of the tracheal segment and pulmonary inflation pressure, of up to 1.0 ± 0.2g/cm and 6.8 ± 2.1 cm H_2O respectively. Histamine (0.1 -30 µg/kg i.v., n=3-4) provoked a small initial increase in resting tension of up to 0.6 ± 0.2 g/cm quickly followed by a fall of similar magnitude, the time course of which paralleled the decline of the accompanying rise in inflation pressure (up to 6.0 ± 2.0 cm H_2O). The relaxant effect of histamine in the tracheal segment was antagonised by (±)propranolol (2 mg/kg i.v. + infusion of 20 µg/kg/min i.v.). At the same time the duration ($t^1/2$) of intrathoracic airway constriction induced by histamine was extended, consistent with the blockade of an endogenous adrenergic homeostatic bronchodilator reflex mechanism (McCulloch et al 1967; Blaber and Fryer 1985).

These preliminary results indicate that measurement of isometric tension in cat trachealis in situ may be a useful model in which to investigate quantitatively airway pharmacology in vivo as an alternative to that previously described in the dog (Brown et al, 1980).

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COMPARISON OF THE α_2-ADRENOCEPTORS WHICH MODULATE NORADRENALINE RELEASE IN RABBITS AND RAT OCCIPITAL CORTEX

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It is well established that α_2-adrenoceptors modulate the release of noradrenaline (NA) from slices of occipital cortex of both the rat (Taube et al, 1977) and the rabbit (Reichenbacher et al, 1982). The results of the present study, however, suggest that the α_2-adrenoceptors in the rabbit brain are not identical to those previously described in the rat brain (Ennis, 1983).

The potassium (30mM)-evoked release of tritium from slices of rabbit occipital cortex preloaded with $|^3H|$-NA was measured using a superfusion system as described previously (Ennis, 1983).

Clonidine produced a concentration-related inhibition of K^+-evoked tritium release with an IC_{50} of 38.9 ± 1.2nM and a maximum inhibition of 70% (n=15). Methoxamine (10^{-6}M) had no significant effect on K^+-evoked tritium release.

The potency of α-adrenoceptor antagonists to reverse the effect of clonidine was investigated using an Arunlakshana-Schild analysis. The pA_2 values are shown in Table 1 together with those previously obtained using slices of rat occipital cortex, (Ennis, 1983).

Table 1: pA_2 values for the antagonism of clonidine in slices of rabbit and rat occipital cortex (n=3)

	Rabbit	Rat
Yohimbine	7.80 ± 0.08	7.69 ± 0.02
Idazoxan	7.25 ± 0.05*	8.24 ± 0.12
Wy 26703	6.59 ± 0.07*	8.23 ± 0.06
Wy 26392	6.64 ± 0.06*	8.16 ± 0.22
WB 4101	< 6.00	< 6.5

*$p < 0.01$ pA_2 value significantly different from that in the rat.

The order of potency for the antagonists in the rabbit cortex was: yohimbine > idazoxan > Wy 26703 > Wy 26392 > WB4101 and that in the rat: Wy 26703 = Wy 26392 = idazoxan > yohimbine > WB4101. The lack of effect of WB4101 and methoxamine suggests that the adrenoceptors on the noradrenergic nerve terminals in the rabbit occipital cortex are not of the α_1-subtype. Yohimbine did not discriminate between the receptors in the two species whereas the benzodioxan, idazoxan was 10 times less potent and the benzoquinolizines, Wy 26703 and Wy 26392 (Lattimer et al) 40 times less potent at the α_2-adrenoceptor in the rabbit than the rat brain. The results of the present study support a previous suggestion (Reichenbacher et al, 1982) that the α_2-adrenoceptors in rabbit and rat cortex may have slightly different properties and suggest that idazoxan, Wy 26703 and Wy 26392 are more potent at the type of α_2-adrenoceptor found in the rat than the rabbit occipital cortex.

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THE REGULATION OF GASTRIC ACID SECRETION IN THE RAT IN VIVO; A ROLE FOR α_2-ADRENOCEPTORS?

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In conscious rats fitted with cannulated gastric fistulae clonidine (CLO), acting as an agonist at the α_2-adrenoceptor, inhibits gastric acid (H^+) secretion (Jennewein, 1977). In the present study we investigated the effects of CLO, para-aminoclonidine (PAC) a potent α_2-agonist that does not penetrate readily into the CNS (Cavero et al, 1980), UK14304 a potent, selective α_2-agonist which enters the CNS (Cambridge, 1981) and WHR1370A a weak but selective α_2-agonist which is reported not to pass easily into the brain (Taylor et al, 1980) on basal H^+ secretion in the rat in-vivo.

Gastric H^+ secretion was measured in conscious rats previously prepared, under full surgical anaesthesia, with cannulated gastric fistulae. Gastric juice was collected by gravity drainage and H^+ concentration ($[H^+]$; mM) determined by electrometric titration to pH 7.00 with 0.01M NaOH. The data have been recalculated as areas under the curves (AUC; mM 30 min^{-1}).

PAC (0.001-0.01mgkg^{-1},i.v.), CLO (0.005-0.03mgkg^{-1},i.v.), UK14304 (0.003-0.3mgkg^{-1},i.v.) and WHR1370A (0.03-0.3mgkg^{-1},i.v.) significantly reduced basal $[H^+]$ in a dose-dependent manner (Fig. 1); the rank order of potency being PAC > CLO $\geq$ UK14304 > WHR1370A. The inhibitory effects of UK14304 (0.03mgkg^{-1},i.v.) and WHR1370A (0.3mgkg^{-1},i.v.) on basal H^+ secretion were antagonized by idazoxan (0.3mgkg^{-1},i.v.) a selective α_2-antagonist (Chapleo et al, 1980). Idazoxan (0.01-10mgkg^{-1},i.v.) alone was without effect on basal $[H^+]$. The α_1-adrenoceptor agonist phenylephrine (0.3-3.0mgkg^{-1},i.v.) and the antagonist prazosin (0.1-1.0mgkg^{-1},i.v.) were also without effect on basal $[H^+]$ and prazosin (1.0mgkg^{-1},i.v.) did not affect the inhibitory effect of UK14304 (0.3mgkg^{-1},i.v.).

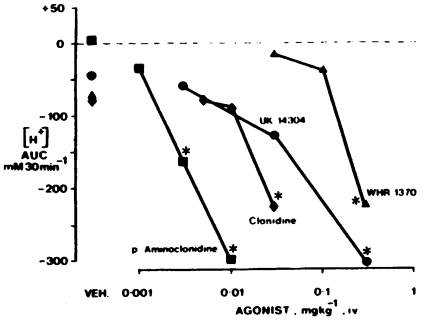

Fig. 1: Effect of α_2-adrenoceptor agonists on basal $[H^+]$. Each point is the mean (n=6), * $p<0.05$ (Dunnett's test), VEH = saline control.

The results suggest that α_2-adrenoceptors are involved in the regulation of gastric H^+ secretion in conscious rats.

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THE α-ADRENOCEPTOR MEDIATED RESPONSES OF THE GUINEA-PIG AORTA AND ILEUM: EFFECT OF TEMPERATURE

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Reducing the bath temperature of isolated tissues causes an increase in sensitivity of β_1- but not β_2-adrenoceptor mediated responses (Williams & Broadley,1982). This change in sensitivity is associated with an increase in agonist but not antagonist β_1-adrenoceptor affinity (Chess-Williams, Broadley & Duncan,1984). This study examines the effect of temperature on the α-adrenoceptor-mediated responses of the guinea-pig aorta (α_1) and ileum (α_2).

Tissues were set up in a Krebs-bicarbonate solution gassed with 5% CO_2 in oxygen and containing metanephrine (10μM) and propranolol (1μM). Aortic spirals were set up under 1g tension and isometric contractions recorded. Segments of distal ileum were transmurally stimulated at 0.1Hz (suprathreshold voltage, 5msec pulse-width) and isotonic contractions recorded. Cumulative concentration-response curves to methoxamine on aorta and clonidine on the stimulated ileum were obtained at either 38°C or 30°C.

Lowering the bath temperature from 38°C to 30°C reduced the maximum contractile response of the aorta from 0.58±0.20g to 0.13±0.04g but had no significant effect on tissue sensitivity to methoxamine. The EC_{50} values were 13.2 (2.4-73.1)μM and 15.0 (3.9-59.2)μM at 38 and 30°C respectively. Intestinal inhibitory responses to clonidine, however, were potentiated at the lower temperature. Clonidine produced a maximum 92% inhibition of the twitch response at both temperatures, but the EC_{50} value was significantly lower ($P<0.05$) at 30°C, 0.22 (0.11-0.47)μM compared with 3.5 (0.3-40.0)μM at 38°C.

Antagonist potency on the ileum was also investigated. Schild plots for the antagonism of clonidine responses by phentolamine yielded similar pA_2 values of 10.18±0.2 at 38°C and 9.92±0.15 at 30°C. The slopes of these plots at 0.9±0.2 (38°C) and 1.2±0.2 (30°C) were not significantly different ($P>0.05$) from unity.

It appears that an increase in agonist potency with hypothermia occurs for the α_2-adrenoceptor-mediated responses of the intestine but not for the α_1-adrenoceptor mediated responses of the aorta. The change in agonist sensitivity is not accompanied by a change of antagonist affinity for the α_2-adrenoceptor.

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ESTIMATES OF THE ANTAGONIST POTENCY OF WY 26392 AGAINST VARIOUS α_2-ADRENOCEPTOR AGONISTS IN THE PITHED RAT

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It has been reported that in vivo estimates of α_2 adrenoceptor antagonist potency depend upon both the agonist and test system employed. This finding led to the suggestion that differences in antagonist potencies may reflect differences in α_2 adrenoceptors. (Berridge et al, 1984). Agonist responses obtained in vivo are subject to a variety of influences thus making receptor classification potentially erroneous. We have investigated how different experimental conditions influence the estimation of antagonist potency at postjunctional α_2 adrenoceptors in vivo.

Female Sprague Dawley rats (230-270g) were pithed, bivagotomised and respired with room air. Diastolic blood pressure (DBP) was recorded from a carotid artery and drugs injected via a jugular vein or subcutaneously. Following pretreatment (15 min) with saline vehicle (1.0 $mlkg^{-1}$) or the selective α_2 adrenoceptor antagonist Wy 26392 (1.0 $mgkg^{-1}$, Paciorek et al, 1984) pressor dose response curves were constructed by cumulative administration of preferential α_2 adrenoceptor agonists (Timmermans and VanZwieten, 1982). ED_{50} values (agonist dose required to increase DBP by 50% of maximal response) were calculated and are presented as mean (s.e. mean), n=4-5. Dose ratios were calculated (ED_{50} Wy 26392 /ED_{50} saline vehicle) as an estimate of the α_2 antagonist potency. These experiments were performed following 3 differing pretreatments.

Experimental Conditions	Agonist	Agonist ED_{50} (μgkg^{-1}) Vehicle	Wy 26392	Dose Ratio
(1) No pretreatment	B-HT 933	91.9(12)	2088(399)	23
	UK-14,304	3.91(1.2)	74.4(17)	19
	Clonidine	2.26(0.6)	11.5(3.2)	5
	Guanabenz	13.6 (4.7)	64.6 (12)	5
	αMeNA	0.80(0.26)	52.3(5.7)	65
(2) Pretreatment with Prazosin 0.1 $mgkg^{-1}$ Propranolol 1.0 $mgkg^{-1}$ Cocaine 1.0 $mgkg^{-1}$	B-HT 933	123(14)	5625(1248)	46
	UK-14,304	7.6(1.9)	151(39)	20
	Clonidine	11.1(4.2)	116(18)	11
	Guanabenz	13.4(1.3)	154(25)	12
	αMeNA	1.18(0.18)	21.5(7.2)	18
(3) Pretreatment as (2) plus reserpine 5 $mgkg^{-1}$ s.c. 24hrs pre-dose	B-HT 933	70.0(3.4)	570(75)	8
	UK-14,304	2.2(0.3)	17.0(4.7)	7
	Clonidine	5.7(1.7)	65.0(20)	11
	Guanabenz	3.6(0.6)	46.5(10)	13
	αMeNA	0.36(0.16)	5.8(1.7)	16

α MeNA=α methylnoradrenaline. All shifts significant ($p<0.05$, t test)

This data shows that the potencies of this series of α_2 adrenoceptor agonists vary markedly and are altered following the pretreatments we have employed. The apparent potency of Wy 26392 depends on the agonist employed, but not its potency. Although there is a large variation in the apparent potency of Wy 26392 (13 fold variation in the dose ratio) in experiment (1), this is reduced to 4 fold in experiment (2), and to 2 fold in experiment (3). Thus large variations in antagonist potency should not be taken as evidence for receptor subdivision unless they occur after steps have been taken to eliminate other pharmacological actions of the agonists.

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EFFECT OF SELECTED α-ADRENOCEPTOR AGENTS ON RESPONSES OF RAT ISOLATED GASTRIC MUCOSA TO ELECTRICAL FIELD STIMULATION

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Clonidine inhibits gastric acid secretion in rats (Hoefke & Kobinger, 1966) and this action has been related to its α_2-adrenoceptor agonist activity. We have investigated the action of clonidine and several α-adrenoceptor agents on acid secretory responses elicited by electrical field stimulation of the rat isolated gastric mucosa preparation; field stimulation of gastric mucosa is believed to stimulate acid secretion through the mediation of postganglionic cholinergic nerves (Baird & Main, 1978).

Gastric mucosa preparations were maintained in Krebs solution according to the method described by Main & Pearce (1978) using male Sprague Dawley rats, 150-180g. Mucosae were superfused with unbuffered modified Krebs solution and the pH of the superfusate was monitored continuously. Acid secretory responses were elicited by electrical field stimulation (5v, 2.5Hz, 0.5ms for 15 min) using platinum electrodes placed above and below the mucosa. The preparation was equilibrated for 90 min after which the drug was added to the bath and field stimulation in the absence of drug. The responses were measured in terms of $\mu eq\ H^+cm^{-2}h^{-1}$ and corrected for basal output.

The α_2-adrenoceptor agonists clonidine, UK14304 and para-aminoclonidine reduced the responses of the mucosa to electrical stimulation (Table 1) and this effect was antagonised by idazoxan (10^{-5}M), a selective α_2-adrenoceptor antagonist; the antagonist alone did not affect the acid secretory responses. WHR1370A, an antisecretory drug with reported α_2-adrenoceptor agonist activity (DiJoseph et al., 1984) caused a non-significant reduction in the responses.

Table 1 The effect of clonidine and selected α-adrenoceptor agents on the responses of the rat isolated gastric mucosa preparation to electrical field stimulation

Drug	Responses, $\mu eq\ H^+\ cm^{-2}\ h^{-1}$
control	1.182 ± 0.119 (18)
clonidine (10^{-5}M)	0.321 ± 0.042 (6) ***
para-aminoclonidine (10^{-7}M)	0.257 ± 0.161 (6) ***
UK14304 (10^{-6}M)	0.433 ± 0.116 (6) ***
WHR1370A (10^{-4}M)	0.774 ± 0.293 (6)
phenylephrine (10^{-6}M)	1.117 ± 0.312 (6)

Values shown are means ± SEM (n), *** $p < 0.001$ (Student's t-test).

The results demonstrate that the acid secretory responses elicited by electrical field stimulation of the rat isolated gastric mucosa may be inhibited by α_2-adrenoceptor agonists which presumably act at α_2-adrenoceptors located on postganglionic cholinergic nerve terminals.

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CHANGES IN [^{3}H]-YOHIMBINE BINDING IN RABBITS ASSOCIATED WITH OESTROGEN/PROGESTERONE TREATMENT AND PREGNANCY

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Modulation of α_2-adrenoceptor number by oestrogens appears to be tissue and species specific. In the rabbit, oestrogen treatment increases the number of α_2-adrenoceptors in the uterus and bladder but decreases that in the platelet (Elliott et al, 1980). In women, platelet α_2-adrenoceptor number is increased when plasma oestrogen levels are high (Peters et al, 1979) and decrease following the rapid fall in oestrogen levels after parturition (Metz et al, 1983). This decrease in platelet α_2-adrenoceptors also corresponds with the appearance of maternity blues syndrome, suggesting that hormonally-induced changes in the platelet may reflect similar receptor changes within the brain. The objectives of the present study were to determine in the rabbit whether changes in platelet α_2-adrenoceptors following oestrogen treatment reflected similar changes within the brain and whether such changes also occurred after parturition.

Female New Zealand white rabbits (weight 3-5 kg) were used throughout. Oestrogen (ß-oestradiol benzoate, 150 mg/day) and progesterone (10 mg/day) were administered s.c. for 7 days and animals killed 24h after the last dose. Pregnant animals were tested either ante-partum (27-29 days pregnant, gestation period 31 days) or post-partum (7-8 days after parturition). Animals were bled by cardiac puncture under anaesthetic and the brain then isolated and dissected. Following homogenisation and slow centrifugation to remove cell debris, the brain membrane preparation was washed and finally resuspended in 25 mM glycylglycine (pH 7.5). Platelets were separated from whole blood by differential centrifugation, broken by sonication and membranes prepared in an identical buffer solution. α_2-Adrenoceptor binding was characterised using [^{3}H]-yohimbine (1.5-17 nM) incubated at 37°C for 30min. Non-specific binding was defined by 5 µM phentolamine.

Binding capacities (fmol/mg protein) for platelet and hypothalamic membrane preparations are shown below. Each figure represents the mean ± s.e.m. for 6-10 individual animals. There were no significant differences in binding affinity.

	Control	Oestrogen	Oestrogen + progesterone	Ante-partum	Ante-partum
Hypothalamus	423 ± 28	302 ± 36*	396 ± 58	372 ± 27	361 ± 47
Platelet	326 ± 8	238 ± 24**	260 ± 51	294 ± 53	284 ± 27

*$p < 0.05$ **$p < 0.01$ vs control. Unpaired t-test.

As in previous studies, platelet α_2-adrenoceptor capacity was significantly decreased following oestrogen treatment. This was accompanied by a similar, though proportionately smaller decrease in binding in the hypothalamus. Treatment with both oestrogen and progesterone resulted in a smaller decrease in receptor number than occurred with oestrogen alone. No significant changes in α_2-adrenoceptor number were observed post-partum relative to ante-partum.

These results suggest that significant changes in platelet α_2-adrenoceptor number following oestrogen treatment do reflect similar changes occurring within the hypothalamus. However, no changes in α_2-adrenoceptor number were observed in the peri-partum period, indicating the limitations of the rabbit as a model for man.

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