THE RELATIONSHIP BETWEEN ANALGESIA INDUCED BY, AND PLASMA CONCENTRATION OF, BUPRENORPHINE IN THE SHEEP

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The antinociceptive activity of buprenorphine (6µg/kg I.V.) in the conscious unrestrained sheep was measured using a ramped thermal stimulus to the pinna of the ear to detect threshold (Livingston et al 1984). Blood samples were taken at intervals following the injection and plasma buprenorphine levels were measured by a modification of a previously reported radioimmunoassay (Bartlett et al 1980). The thermal tests indicated that at the dose level used, buprenorphine was a potent antinociceptive agent. Onset of analgesia varied from 5 to 45 minutes after injection and lasted from 90 to 300 minutes. Buprenorphine at the dose of 6µg/kg produced behavioural changes in the sheep, best described as agitation, which often caused difficulties in estimating the analgesic activity. Estimation of drug plasma levels showed that following I.V. administration the concentration fell in a triexponential manner. Correlation of plasma levels and antinociceptive effects indicated that plasma concentrations of 500-700 pg/ml produced antinociception in all animals, however effects could be occasionally seen in individuals with plasma levels as low as 130 pg/ml. In the animals with delayed onset of analgesia it is interesting to note that analgesic effects were not seen although plasma levels were as high as 34 ng/ml, the reasons for this effect are not apparent at present.

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STUDIES ON THE ANTINOCICEPTIVE PROPERTIES OF MORPHINE AND U50,488H IN ADRENAL DEMEDULLATED RATS

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Several chemically diverse compounds that are agonists at kappa opioid receptors increase urination after s.c. injections in normally hydrated rats; e.g. trans-3, 4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide (U50,488H) (Leander, 1983). Agonists at the μ (Leander, 1983) or δ (Cowan and Khunawat, 1986) opioid receptor do not share this property. Recently, it was observed that bilateral adrenal demedullation results in decreased urine output to kappa agonists in rats tested 3-7 days after surgery (Blackburn et al, 1985). The finding that kappa-induced diuresis is dependent upon an intact adrenal medulla prompted the present studies in which antinociception, normally associated with k and μ agonists in the rat hypertonic saline test (Collier and Schneider, 1979), was quantitated and compared in demedullated, sham-operated and intact (control) animals. Male Alderley Park rats (80-100g) were anaesthetized with Saffan (12mg/kg i.v.) and underwent either bilateral adrenal ennucleation or a sham-operation. Seven days later, groups of these rats (n=8-12) and control animals (n=12) were pretreated s.c. with saline, morphine HCl, (0.3, 1 and 3 mg/kg) or U50,488H (3,10,20mg/kg). Thirty minutes later, four rats were placed in an open tub (45cm long, 20cm wide; 19cm high) and injected i.p. with 4% (w/v) sodium chloride solution (1ml/100g). An animal was scored as a responder if at least one abdominal stretch occurred within 20s of injection. ED_{50}'s, 95% conf. limits and Chi-square values were obtained using the computer programs of Tallarida and Murray, (1981). The results are summarised in Table 1.

Table 1 Median effective antinociceptive doses of test agents.

Pretreatment	Morphine ED_{50} (mg/kg)	U50,488H ED_{50} (mg/kg)
Control	0.96 (0.40-2.26)	6.52 (3.63-11.68)
Sham-operated	0.88 (0.40-1.93)	13.38 (6.16-29.05)
Demedullated	1.09 (0.49-2.39)	6.30 (3.90-10.18)

The ED_{50} values obtained for morphine did not differ significantly ($p>0.05$) from one another. The same conclusion applies to the ED_{50} values for U50,488H. The top dose of U50,488H (20mg/kg) was particularly effective against visceral pain in demedullated (as opposed to sham-operated) rats; nevertheless, statistical significance at this dose was not achieved. A higher dose (30mg/kg) caused behavioural depression. It is known that demedullation does not markedly influence the antinociceptive potency of morphine in rat tail flick (Miller et al, 1955) and flinch-jump (Bodnar et al, 1982) tests. The results are therefore consistent with these reports. It may be concluded that whereas k induced diuresis is abolished in adrenal demedullated rats, k-induced antinociception is unaffected, at least in relation to visceral pain.

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DIFFERENTIAL EFFECTS OF ANTIPSYCHOTIC AND ANTIDEPRESSANT DRUGS ON HUMAN PLATELET AGGREGATION

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Several antipsychotics and antidepressant agents have been reported to inhibit cyclic AMP (cAMP) phosphodiesterase (PDE); an effect that would increase the intracellular concentration of cAMP. Since both adenosine and cAMP have been shown to inhibit platelet aggregation, it is concievable that these drugs may exert a similar effect, in platelets, and block platelet aggregation. It is possible therefore, that drugs that increase cAMP levels may diminish the risk of platelet aggregation as shown for prostacyclin, the most potent anti-aggregatory agent yet described. These observations prompted us to test the effects of various anti-psychotic agents on platelet aggregation induced by adenosine-5'-diphosphate (ADP), adrenaline (ADR) or arachidonic acid (AA).

Platelet aggregation was monitored turbidmetrically on a dual-channel Chrono-Log aggregometer. All aggregations were carried out at 37°C with platelet-rich plasma (PRP) having platelet count of 2-2.5 x 10^8/ml. Platelet aggregability was screened with ADP (2.2 uM), ADR (200 uM) or AA (1.7 mM) and all drugs were tested at 3-4 concentrations in triplicate. The mean values ± S.E.M. for inhibiting platelet aggregation by 50% (IC50) for various drugs are given in Table I.

Table I

Drug	Aggregating Agent		
	Adenosine-5'Diphosphate	Adrenaline	Arachidonic Acid
	IC50 (uM) ± S.E.M.		
Chlorpromazine	91.3 ± 9	125 ± 15	N.I.
Fluphenazine	144 ± 3	97 ± 4	N.I.
Haloperidol	95 ± 2.5	93 ± 7	101 ± 4
Promethazine	139 ± 19	144 ± 6	N.I.
Thioridazine	53 ± 9	70 ± 23	45 ± 10
Trifluoperazine	129 ± 11	84 ± 4.5	N.I.
Amitryptaline	53 ± 10	64 ± 10	370 ± 10
Protryptaline	6.7 ± 0.4	19 ± 4	N.I.
Normiphensine	60 ± 20	280 ± 3	N.I.

N.I. ; Not Inhibited. Statistical significance was assessed by students-t-test.

Among the various drugs investigated, thioridazine, haloperidol and amitryptaline appear to posses a strong inhibiting effect on platelet aggregation induced by various physiologically important aggregating agents (P 0.001). On the contrary, chlorpromazine, fluphenazine, normiphensine, promethazine, protryptaline and trifluoperazine specifically inhibited aggregation induced by ADP and ADR and had no effect on AA-induced aggregation. Such a differential effect of antipsychotic and antidepressant drugs towards platelet aggregation has not been reported before. Since ADP, ADR or AA induce platelet aggregation via different mechanisms, it is not unreasonable to postulate that these drugs inhibit platelet activation by one or more mechanisms.

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EFFECTS OF IONS AND GTP ON PLATELET α_2-ADRENOCEPTOR AGONIST AND ANTAGONIST BINDING.

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We have reported that the α2 adrenoreceptor agonist ligand ^{3}H-UK-14,304 labels approximately 60% of the total (labelled with ^{3}H-Yohimbine) platelet α2 adrenoreceptor population and this represents binding predominantly to a high affinity site of the receptor (Gibson et al, 1986).

Manganese ions increase the specific binding of ^{3}H-UK-14,304 to brain cortical membranes (Loftus et al, 1984). We now compare the effects of Mn^{2+}, Mg^{2+}, Na^+ (chloride salts) and GTP on the binding of this ligand and of the α2 adrenoreceptor antagonist ^{3}H-Yohimbine to previously frozen human platelet membranes.

Mn^{2+} up to 1mM increased ^{3}H-UK-14,304 (1.4nM) specific binding (maximally to 171±12% (n=10) of the control binding (50mM Tris buffer) at 0.1mM). Concentrations greater than 1mM progressively reduced binding, such that at 10mM binding was 51±1% of control. Mn^{2+} caused only a reduction in ^{3}H-Yohimbine (3.7nM) binding, to 51±5% at 10mM.

Mg^{2+} up to 1mM had no significant effect on the binding of either ligand but reduced the binding of both ligands at higher concentrations (at 10mM to 78±6% for ^{3}H-Yohimbine and 65±8% for ^{3}H-UK-14,304).

Na^+ (10-240mM) significantly increased the specific binding of ^{3}H-Yohimbine (to 166±37% at 30mM) but progressively reduced ^{3}H-UK-14-304 binding (to 45±10% at 30mM).

GTP up to 250μM had little effect on ^{3}H-Yohimbine binding but reduced ^{3}H-UK-14,304 binding to 45±2% at 1μM and to 12±5% of control at 250μM.

Saturation plots of ^{3}H-Yohimbine (0.25-14nM) in the presence of 0.1 mM Mn^{2+}, 1μM GTP or 30mM Na^+ did not significantly alter the number of binding sites compared to buffer alone. A slight reduction in K_D occurred with 30mM NaCl.

Unlabelled yohimbine displaced ^{3}H-Yohimbine (3nM) from a single population of sites under all the above conditions, with the same range of K_Ds as obtained from the saturation experiments.

Saturation plots of ^{3}H-UK-14,304 (0.15-7.2nM) in buffer alone, 1μM GTP or 30mM Na^+ showed a marked reduction in the percentage specific binding compared to those in the presence of 0.1mM Mn^{2+}, hence reducing the reliability of the estimates and preventing meaningful comparisons.

The displacement of ^{3}H-Yohimbine by unlabelled UK-14,304 under the above conditions was best fitted to a two site model, as reported for other agonists (Lenox et al, 1985). Mn^{2+} increased the proportion of high affinity sites (from 22% in buffer alone to 37%). GTP increased the K_D of both high and low affinity sites without changing their proportion compared to Mn^{2+} (K_D1 from 3.7 to 20nM; K_D2 from 144 to 314nM). Na^+ increased the K_D of both components and reduced the proportion of the high affinity binding sites to 21%.

These results further indicate the differential effects of ions on the binding of agonist and antagonist ligands at the platelet 2 adrenoreceptor.

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THE RAT ISOLATED STOMACH FUNDUS STRIP, A MODEL FOR 5-HT$_{1C}$ RECEPTORS

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In rat isolated stomach strips serotonin (5HT) and its derivatives induce contraction in nanomolar concentrations (Vane, 1957). 5HT-Antagonists like cyproheptadine, metitepin or methysergide inhibit this response in micromolar concentrations. A parallel with the definition of 5HT1 receptors by Peroutka & Snyder (1979) thus becomes obvious. It is however known that 5HT1 receptors can be subdivided into (at least) three subtypes, designated 5HT1A, 5HT1B and 5HT1C (Palacios et al,1984). It was the aim of the present study to determine which subtype is involved in the 5HT-induced contraction of the rat stomach.

Fundus strips of 2 cm length and 2 mm width were cut parallel to the greater curvature. Strips were mounted in Krebs' solution containing 50 µM pargyline at a resting tension of 1 g. After a 1.5 hr equilibration period the strips were challenged with 1 µM carbachol. After washout a cumulative concentration-response curve was established for the tryptamine analogue under investigation. 25 derivatives were tested and the agonistic potency characterised by pD_2-values, the efficacy expressed as % of the carbachol contraction.

For many analogues the concentration-response curve ranged over 5 log-units and sometimes was clearly biphasic. The curves were fitted by means of a non-linear regression analysis for an interaction with 1 or 2 independent receptors (Table 1).

Table 1 pD_2-values, means +/- SEM, (n) = number of experiments, relative efficac.

5HT	7.97 ± 0.09 (22)	53%	6.24 ± 0.07 (22)	19%
Tryptamine	7.20 ± 0.08 (11)	30%	5.11 ± 0.08 (12)	26%
5-Carboxamidotryptamine	6.90 ± 0.07 (6)	80%	-.--	
RU 24969	6.80 ± 0.15 (6)	8%	3.74 ± 0.07 (9)	64%

Since RU 24969 displayed a biphasic response with the high affinity contraction of low efficacy (8%), this compound was applied as antagonist to separate between the high and/or low affinity responses of the other 24 agonists. In a concentration (10 µM) just at the inflection point between the high and low affinity contraction RU 24969 inhibited the high affinity responses of all compounds with a biphasic concentration-response curve as well as the total responses of all compounds with a monophasic curve.

Receptor binding assays for the selective determination of affinity to 5HT1A, 5HT-1B, 5HT1C and 5HT2 binding sites were carried out as described in detail by Hoyer et al (1985). pD_2-values of contractile responses susceptible to antagonism by RU 24969, were compared with pK_D-values for the above mentioned 5HT receptors. A highly significant correlation was calculated between pK_D-values for the 5HT1C binding site and the pD_2-values for contraction ($r = 0.885$, $n = 25$, $P = 0.0001$). The correlation coefficients for the 5HT1A, 5HT1B and 5HT2 binding site amounted to 0.085, 0.529 and 0.558, respectively. These data strongly suggest that in the rat stomach fundus preparation one of the components of the contractile response to 5HT is elicited by activation of 5HT1C receptors. The receptor mediating the low affinity contractile response has not been characterised yet. It should be realised that the assessment of antagonists using a non-selective agonist, may be complicated by this second contractile effect.

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RU24969 AND 8-OH-DPAT ANTAGONISE 5-HT INDUCED CONTRACTION OF THE RAT COLON AND ILEUM.

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RU24969, a highly potent and selective 5-HT_1 agonist (Hunt et al, 1981), has been shown to antagonise 5-HT induced contraction of the rat fundic strip without showing any agonist properties (Osborne, 1985). 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a centrally active 5-HT agonist (Hjorth et al, 1982), which shows selectivity for the 5-HT_1 recognition site (Middlemiss & Fozard, 1983), has been shown to have an agonist action in the guinea pig ileum (Fozard & Kilbinger, 1986). In this report we demonstrate that both RU24969 and 8-OH-DPAT antagonise atropine-sensitive 5-HT induced contraction of the isolated rat colon and ileum preparations.

Rat colon and ileum sections were incubated at 35°C in Tyrode's solution and dose response curves constructed for 5-HT and ACh in the presence and absence of RU24969 (1μM), 8-OH-DPAT (1μM), ketanserin (1μM) and methysergide (1μM). In each case, the tissues were allowed to equilibrate with the antagonist for 30 min prior to retesting the effects of 5-HT and ACh and ED_{50} or ED_{25} values (mean ± S.E. ; n = 5 - 7) determined.

RU24969 (0.01 - 1μM) and 8-OH-DPAT (0.01 - 1μM) showed no contractile activity on either tissue. However, RU24969 (1μM) proved to be a competitive antagonist of 5-HT in both tissues significantly increasing ED_{50} values (ileum: 1.1 ± 0.2 X 10^{-6}M to 9.8 ± 0.6 X 10^{-6}M, $P<0.01$; colon: 1 ± 0.2 X 10^{-7}M to 9 ± 0.4 X10^{-7}M, $P<0.01$). Similarly, 8-OH-DPAT (1μM) increased the ED_{50} value for 5-HT on the colon from 1.3 ± 0.1 X 10^{-7}M to 4.6 ± 0.5 X 10^{-7}M ($P<0.05$) as well as reducing the maximum response by 35%. This non competitive effect of 8-OH-DPAT (1μM) was even more marked on the ileum where the 5-HT maximum was reduced by 56% and the ED_{25} value increased from 1.3 ± 0.1 X 10^{-6}M to 7 ± 0.2 X 10^{-6} M ($P<0.025$). Neither RU24969 nor 8-OH-DPAT effected ACh induced contraction of the colon. However, RU24969 (1μM) caused a significant increase in the response of the ileum to ACh with the latter's ED_{50} value being reduced from 1.5 ± 0.2 X 10^{-7}M to 5.2 ± 0.6 X 10^{-8}M ($P<0.05$). 8-OH-DPAT (1μM) caused a similar but much smaller effect which was not significant.

Methysergide (1μM) significantly reduced 5-HT contractile effects in both tissues in a non competitive manner with the maximum response being reduced by 60%. Conversely, ketanserin (1μM) had no significant effect on 5-HT induced contraction, which was abolished by atropine (1μM), suggesting that there was a very low population of 5-HT_2 receptors in both tissues. These results suggest that RU24969 and 8-OH-DPAT may be antagonists of neuronal receptors in the rat gut, an observation which is at variance with observations of agonist activity by 8-OH-DPAT (Fozard & Kilbinger, 1986) in the guinea pig ileum, suggesting that there may be differences in 5-HT receptors between these two species.

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DIFFERENCES IN THE RELAXANT EFFECTS OF 5-HT, 5-CT AND 8-OH-DPAT ON RAT AORTA AND GUINEA PIG ILEUM

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5-Hydroxytryptamine (5-HT) has been reported to induce relaxations of the guinea-pig ileum (Feniuk et al, 1983) and of several vascular tissues, including the cat saphenous vein (Feniuk et al, 1983). These actions have been described as being mediated through '5-HT1-like' receptors (Feniuk et al, 1985). The contractile effects of 5-HT on the rat aorta have been reported to be mediated through 5-HT2 receptors (Cohen et al, 1981). However, preliminary experiments have shown that high concentrations of 5-HT also induce relaxation. The aim of the study was to compare the relaxant effects of 5-HT and 5-HT1 'selective' agonists on the isolated rat aorta and guinea-pig ileum.

Rings of rat aorta were placed in Krebs solution containing panuramine (0.1 μM), to optimise sensitivity to 5-HT, and ketanserin (1 μM), to block 5-HT2 mediated-contractions. These were precontracted to approx. 1.0 g tension, using phenylephrine (10 μM). Guinea-pig ilea were placed in Tyrode solution containing atropine (1 μM) and precontracted using histamine (3 μM) as described by Feniuk et al (1983). All agonists were added cumulatively and all experiments were conducted at 37°C, pH 7.4.

Concentration-dependent relaxations (0.3 - 3 mM) were observed in the rat aorta with 5-HT, 5-carboxamidotryptamine (5-CT), and 8-hydroxy-dipropylaminotetralin (8-OH-DPAT); at 1 mM each agonist relaxed induced tone by 46%, 44%, and 43% respectively (mean, n = 4). These relaxations were similar in both intact and endothelial-denuded rings and were unaffected by the presence of methysergide (1 μM), pindolol (10 μM), indomethacin (3 μM) or imiloxan (1 μM). The ileum was also relaxed by all three agonists (EC_{50} = 3.14, 0.172, and 3.37 μM respectively). Methysergide antagonised the relaxant effect of 5-HT (pA_2 = 6.85, slope = 0.86, (n = 4) and 5-CT, but not that of 8-OH-DPAT .

In summary, relaxations were observed in the rat aorta to 5-HT, 5-CT, and 8-OH-DPAT at concentrations 1000 fold greater than those observed in the ileum, indicating a non-specific action of all the agonists in this tissue. In agreement with previous reports (Feniuk et al, 1983; Feniuk et al, 1984) 5-HT- and 5-CT-induced relaxations of the ileum were probably mediated by '5-HT1-like' receptor stimulation. However, in contrast to the relaxant effects of 5-HT and 5-CT, 8-OH-DPAT induces relaxations of the guinea-pig ileum which are not mediated by '5-HT1-like' receptors. This action may resemble the relaxant effects observed with 5-HT, 5-CT and 8-OH-DPAT in the rat aorta. Thus, 8-OH-DPAT may exert effects in isolated tissues which are not related to its affinity for 5-HT1 receptors.

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THE 5-HT AUTORECEPTOR IN THE RAT HYPOTHALAMUS RESEMBLES A $5HT_{1B}$ RECEPTOR

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The central 5-HT autoreceptor has been classified as a 5-HT_1 rather than a 5-HT_2 receptor (Martin and Sanders-Bush, 1982) but the 5-HT_1 receptor itself appears to encompass 3 subtypes: 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} (Pazos et al, 1985). The present study was designed to extend our earlier observations (Ennis and Cox, 1982) and to characterise further the 5-HT autoreceptor in the rat hypothalamus.

Slices of rat hypothalamus (0.25 x 0.25 x 2.0 mm) were preloaded with $|^3H|$-5HT (10^{-7}M, specific activity 14Ci $mmol^{-1}$) and superfused at a rate of 0.4 ml min^{-1} with Krebs-Henseleit solution containing chlorimipramine (10^{-6}M). Two 4 min pulses of Krebs soulution containing 25 mM K^+ were administered 68 (S_1) and 92 (S_2) min after the start of the superfusion. Drugs were added to the superfusing medium immediately after the first pulse. The results were expressed as the S_2/S_1 ratio.

The selective 5-HT_{1A}, agonist 8-hydroxy-2-(di-n-propylamino) tetralin (Middlemiss and Fozard, 1983) had no significant effect on the K^+-evoked release of 5-HT, producing only 10% inhibition at 10^{-6}M (n=4). In contrast, the selective 5-HT_{1B} receptor agonist RU 24969 (Goodwin and Green, 1985) produced a concentration-dependent inhibition (max. 69±2% at 10^{-6}M) of 5-HT release with a pD_2 value of 7.2±0.09 (n=6). The concentration-effect curve to RU 24969 was shifted to the right in a parallel manner in the presence of (-) propranolol which has been reported to be an antagonist at the $5HT_{1B}$ receptor (Middlemiss, 1984). The pA_2 value was 8.4±0.19 (n=4). The inhibition of 5-HT release produced by RU 24969 was not antagonised by either spiperone (10^{-7}M) or mianserin (10^{-7}M) which have been reported to be selective for the 5-HT_{1A} and 5-HT_{1C} sites respectively. In the presence of methiothepin (10^{-7}M), an agent previously shown to be an antagonist at the 5-HT autoreceptor (Ennis and Cox, 1982), the inhibition produced by RU 24969 (10^{-7}M) was reduced from 44% to 26% ($p < 0.05$).

These results suggest that the 5-HT autoreceptor in the rat hypothalamus can be classified as a $5HT_{1B}$ receptor and is therefore similar to the 5HT autoreceptor in the frontal cortex which has also been suggested to be of the $5HT_{1B}$ type (Middlemiss, 1984).

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FURTHER CHARACTERISATION OF THE DISCRIMINATIVE STIMULUS INDUCED BY 8-HYDROXY-2-(DI-N-PROPYLAMINO) TETRALIN IN RATS

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8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) has both high affinity and selectivity for the 5-HT_{1A} subtype of the 5-HT_1 recognition site in rat brain cortex (Middlemiss & Fozard, 1983). Previous work has shown that 8-OH-DPAT can produce a discriminative stimulus in rats and evidence consistent with its being mediated by the putative 5-HT_{1A} receptor has been obtained (Cunningham & Callaghan, 1985). At similar doses 8-OH-DPAT also lowers blood pressure and heart rate and again, pharmacological analysis suggests the putative 5-HT_{1A} receptor is involved; however a catecholaminergic mechanism is also implicated since α_2-adrenoceptor blocking agents proved effective antagonists of the cardiovascular reponse to 8-OH-DPAT (Fozard & McDermott, 1985). The primary objective of the present work was to explore the possibility that the 8-OH-DPAT discriminative stimulus involved α_2-adrenoceptors. In addition we have sought an explanation for why neither 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) nor RU 24969, both of which have high affinity for the 5-HT_{1A} recognition site, (Gozlan et al., 1983), generalized to the 8-OH-DPAT cue.

40 male Sprague-Dawley rats were trained to discriminate 8-OH-DPAT (50 µg/kg injected subcutaneously (s.c.) 30 min before testing) from saline, using a two lever FR10 food-reinforced schedule, according to the method of Colpaert et al. (1976).

The α_2-adrenoceptor agonist clonidine, at doses (3-60 µg/kg) which did not cause overt sedation, did not generalise to the 8-OH-DPAT cue when injected s.c. 30 min prior to testing. The α_2-adrenoceptor antagonists, idazoxan and WY 26392 (0.1-1.0 mg/kg), injected s.c. 30 min prior to 8-OH-DPAT, did not block the capacity of the rats to discriminate the cue.

5-MeODMT (0.125-0.5 mg/kg s.c.) generalised only partially and RU 24969 (0.125-0.5 mg/kg s.c.) not at all to the 8-OH-DPAT cue. When tested at the same doses 30 min following ketanserin (1.25 mg/kg), a selective 5-HT_2 receptor antagonist which per se had no effect on the cue, 5-MeODMT generalised completely to 8-OH-DPAT. In contrast, the response to RU 24969 was unaffected by prior treatment with ketanserin.

These data render it unlikely that the 8-OH-DPAT cue passes through an α_2-adrenoceptor and thus clearly differentiate this mechanism from that involved in the cardiovascular response (Fozard & McDermott, 1985). The data also suggest that 5-MeODMT does not generalize completely to 8-OH-DPAT because concomitant activation of 5-HT_2 receptors provides a disruptive stimulus. A similar explanation does not, however, apply in the case of RU 24969 and the reason why this nominally selective putative 5-HT_1 receptor agonist does not generalize to 8-OH-DPAT remains to be established.

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THE EFFECTS OF 5-HYDROXYTRYPTAMINE ON GUINEA PIG STOMACH MUSCLE IN THE PRESENCE OF ATROPINE

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There is considerable evidence to suggest that 5-hydroxytryptamine (5-HT) affects muscle contractions within the gastrointestinal tract via modification of neurotransmitter, particularly acetylcholine, release (see review by Gershon 1982). An analysis of the potential postsynaptic actions of 5-HT on muscle cells may be obscured by the pre-synaptic effects and, in the present study, 5-HT action on guinea-pig gastric circular muscle was investigated after the inhibition of cholinergic function.

Male Dunkin-Hartley guinea-pigs (450-550g) were killed by cervical dislocation and the stomachs removed. Gastric body circular muscle strips (one from each animal, 20 mm long and 5 mm wide, with the mucosal layer removed) were placed in tissue baths containing oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution at 37°C. 1g tension was applied to the tissues which were allowed to equilibrate for 45 min before being subject to drug treatments. At least 6 tissues were used for each treatment and the significance of differences between treatments determined using the Mann-Whitney U test.

In the presence of atropine (5×10^{-8}M), 5-HT (10^{-7}-4×10^{-6}M) caused either a concentration-related relaxation in approximately 80% of the tissues or, in the remainder, an initial contraction at low concentrations (2×10^{-7}-4×10^{-7}M) reverting to relaxation at higher concentrations (8×10^{-7}-8×10^{-6}M). Data obtained from the latter tissues is not considered further. The 5-HT antagonists mesulergine, methiothepin, methysergide (10^{-6}-10^{-5}M) and metoclopramide (10^{-5}-5×10^{-5}M) antagonised the relaxation responses to 5-HT causing 4 to 5 fold shifts in the dose response curves ($n = 8$, $P<0.05$). In some tissues, approximately 20%, metoclopramide (10^{-5}M) and methiothepin (10^{-6}M) reversed the relaxations to contractions. Ketanserin (8×10^{-8}-2×10^{-6}M) and ICS 205-930 (10^{-9}-10^{-7}M) failed to antagonise the 5-HT-induced relaxations except at high concentrations (8×10^{-6}M and 10^{-6}/10^{-5}M respectively). Tetrodotoxin (10^{-7}M), prazosin, propranolol, yohimbine and haloperidol (5×10^{-7}M) failed to modify the relaxation responses. At the concentrations indicated above, none of the antagonists modified muscle tension in their own right.

The 5-HT agonist 2-CH_3 5-HT (3×10^{-6}-10^{-4}M) caused a concentration-related biphasic response of relaxation followed by contraction whereas 8-OHDPAT (2×10^{-6}-10^{-4}M) caused only contraction. S(+)-α-CH_3 5-HT (3×10^{-6}-2×10^{-5}M) and 5-hydroxytryptophan (3×10^{-5}-1.5×10^{-4}M) were ineffective.

It is concluded that in the absence of cholinergic function relaxation is the dominant response of the gastric circular muscle preparation to 5-HT. Attempts to characterise the receptor type using a series of 5-HT antagonists proved inconclusive since many agents were inhibitory but were effective at high and perhaps non-specific concentrations. An inconsistent action of 5-HT was to cause contractions, more reliably shown using 2-CH_3.5HT and 8-OHDPAT, where again the receptor type remains to be established.

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THE ACTIONS OF METOCLOPRAMIDE AND ICS 205-930 ON THE GUINEA PIG ILEUM ARE DEPENDENT ON THE TYPE OF PREPARATION USED

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Metoclopramide and ICS 205-930 have been shown to enhance field stimulation (FS) induced contractions of the guinea-pig ileum (see review by Kilbinger & Weihrauch 1982; Bradbury et al, 1985). In this study we investigate the actions of these two compounds to modify FS-induced responses in three different ileum preparations.

Male Dunkin-Hartley guinea-pigs (450-550g) were killed by cervical dislocation and the gastrointestinal tract removed. Ileum segments (2 cm long) were obtained 30 cm from the pyloric sphincter and prepared as intact ileum, mucosal stripped ileum or as the longitudinal muscle myenteric plexus preparations (LMMP). Preparations were placed in tissue baths containing oxygenated (95% O_2; 5% CO_2) Krebs-Henseleit solution at 37°C. 1g tension was applied to the tissues which were allowed to equilibrate for 45 min before being subject to electrical stimulation (stainless steel wire electrodes were 6mm apart, 35 volts, 0.2 ms pulse width, 0.1-10Hz) or drug treatments. The significance of differences between treatments was determined using the Mann-Whitney U test. In all experiments n = 6-8.

FS (0.1-10Hz) of the intact ileum, mucosal stripped ileum and LMMP preparation for 30s at 5 min intervals caused contraction responses which were repeatable at least 6 times over a 4h period and which were abolished by atropine ($5x10^{-8}M$) and tetrodotoxin ($10^{-7}M$). Typical changes in gram tension at 1 Hz were 2.8±0.2, 3.2±0.15 and 2.3±0.2 for the intact ileum, mucosal stripped ileum and the LMMP preparation respectively. Similar levels of spontaneous activity were observed with all 3 preparations. ICS 205-930 (10^{-10}-$10^{-7}M$) and metoclopramide (10^{-7}-$10^{-5}M$) caused concentration-related enhancements, particularly at lower frequencies (0.1-0.5Hz, to 150-300% of control values, $P<0.01$) of FS-induced contractions of the intact ileum preparation. Only metoclopramide ($10^{-5}M$) caused a marked potentiation of spontaneous activity of the intact ileum preparation.

In the mucosal stripped ileum both ICS 205-930 (10^{-10}-$10^{-6}M$) and metoclopramide (10^{-8}-$10^{-5}M$) failed to significantly modify the FS-induced contractions. High concentrations of ICS 205-930 (10^{-7}-$10^{-5}M$) caused concentration-related reductions (to 30% of control values, $P<0.01$) of the FS-induced contractions of the LMMP preparation whereas metoclopramide (10^{-7}-$10^{-5}M$) failed to consistently modify the FS-induced contractions. Methysergide (10^{-7}-$10^{-5}M$) failed to modify the contractions in any of the three preparations.

It is concluded, firstly, that the mucosal layer of the ileum is important for the actions of ICS 205-930 and metoclopramide to facilitate the contractions caused by FS. Secondly, in the absence of the mucosal layer in the LMMP preparation ICS 205-930 is revealed as having a potential to reduce FS-contractions. This may indicate the presence of two 5-HT receptor types moderating FS-induced contractions since ICS 205-930 and metoclopramide have been considered to act as neuronal 5-HT antagonists within the gastrointestinal tract (see Buchheit et al, 1985).

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COMPARISON OF THE EFFECT OF BRL 24924, METOCLOPRAMIDE AND DOMPERIDONE ON CISPLATIN-INDUCED EMESIS IN THE FERRET.

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It has been previously reported that metoclopramide HCl (Mcp), a gut motility stimulant with dopamine antagonist properties (Pinder et al, 1976), inhibits cisplatin induced emesis in the ferret (Florczyk et al, 1982). Using this model, the effects of Mcp have been compared to those of domperidone (Domp), a predominantly peripheral dopamine antagonist (Laduron & Leysen, 1979) and BRL 24924, a novel benzamide which stimulates gut motility in the absence of dopamine antagonism (Cooper et al, 1986) and which may also be a potent 5-hydroxytryptamine (5-HT) M-receptor antagonist (Dunbar et al, 1986).

Male ferrets, 1.3-1.8kg, with a chronic indwelling venous catheter were used. For each series of experiments, a dose of cisplatin (Neoplatin) just sufficient to give a consistent maximal emetic response was established. The doses of Mcp and Domp used inhibit apomorphine-induced emesis (dopamine antagonism); the doses of BRL 24924 had no such effect (unpublished). Each drug was administered 30 min pre- and 45 min post-cisplatin. Time to onset of the first emetic episode (latency period) and the total number of emetic episodes were observed for up to 4h post cisplatin injection. BRL 24924 and Mcp were of the same order of potency in increasing the latency period and reducing the number of emetic episodes in response to cisplatin. Domp was ineffective on the number of emetic episodes but reduced the latency period at the dose used (Table 1).

Table 1: Effect of BRL 24924, Mcp and Domp on Cisplatin-induced Emesis

Treatment mg kg^{-1} i.v.		Group Size	No. of animals completely protected	Group mean ± s.e. of mean No. of emetic episodes	Latency period (min)
Cisplatin 10					
vehicle	x 2	10	0	15.7 ± 1.5	72 ± 3
BRL 24924	0.65 x 2	6	1	6.2 ± 2.5*	>139**
	1.25 x 2	6	4	2.8 ± 2.1*	>197**
	2.5 x 2	6	3	2.8 ± 1.5**	>169*
Cisplatin 7.1					
vehicle	x 2	10	0	18.4 ± 2.4	85 ± 5
Mcp	0.65 x 2	6	1	4.7 ± 2.2*	>139
	1.25 x 2	6	2	4.2 ± 2.1*	>161**
	2.5 x 2	6	5	1.8 ± 1.8**	>218**
Domp	1.0 x 2	5	0	18.4 ± 5.4	58 ± 14*

Comparison with vehicle dosed animals *P<0.05; **P<0.01 (Mann-Whitney)
Latency period taken as 240 min (total observation period) if animal completely protected from vomiting. Drugs as parts free base.

The results with Domp and BRL 24924 suggest that dopamine antagonism is not essential for the inhibition of cisplatin emesis in the ferret. The efficacy of Mcp and BRL 24924 may therefore involve other properties, such as 5-HT M-receptor antagonism and/or effects on gut motility. If this is applicable to man, the absence of dopamine antagonism and related side effects may afford a therapeutic advantage to BRL 24924 in the treatment of cisplatin-induced emesis.

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A MODEL OF NAUSEA AND EMESIS IN THE COMMON MARMOSET

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Nausea and emesis associated directly with the administration of, or in anticipation of, cancer chemotherapy cause the patient serious psychological and medical discomfort (Borison & McCarthy, 1983). Emesis induced by dopamine agonists and chemotherapeutic agents in the dog and ferret has been used to detect anti-emetic activity, although the induction of nausea in such species, 'the conscious perception of discomfort', has not been reported. In the present study we investigate the use of the marmoset for the detection of agents having anti-emetic and anti-nauseant potential.

Common marmosets (Callithrix jacchus, male, 350-400g) were injected s.c. with the dopamine agonist 2-di-n-propylamino-5,6-dihydroxytretralin (tetralin), vehicle or antagonist (i.p. 45 min pretreatment) (in a volume of 1 ml/kg body weight) and were placed individually in the observation cage (760 cm high, 500 cm wide and 600 cm deep with a clear perspex front). Behavioural changes were assessed by remote video recording. The time taken for emesis to occur was noted, emesis being recorded as present or absent. 'Nausea', clearly a subjective experience, was assessed by allocating a point to each of the following behaviours: alteration in facial expression involving increased blinking and eye closure, retraction of the ears, mouthing movements including salivation and tongue protrusion, nose rubbing on the perch, a hunched body posture, drooped head and/or neck stretching and slowness of movement. Thus, each marmoset could be allocated a maximum 'nausea score' of 7. There were 4 animals in each experimental group to provide a total nausea score of 28 for each treatment group.

2.5 µg/kg of the tetralin compound was selected as the minimum dose to cause nausea and emesis in all animals, the nausea developing within 1-4 min, the emesis within 5-10 min. Minimally effective doses of haloperidol and metoclopramide to abolish the tetralin (2.5 µg/kg) - induced emesis, but without any significant reductions in the nausea scores (28 and 26 respectively), were 0.013 mg/kg and 0.1 mg/kg respectively. Increasing the dose of metoclopramide to 5.0 mg/kg failed to reduce nausea (score 24, $P>0.05$) whilst a 4-fold increase in the dose of haloperidol, to 0.05 mg/kg, lead to a reduction in the nausea score (to 14, $P<0.01$). Animals treated with the high dose of metoclopramide appeared sedated, although animals remained alert following haloperidol treatment. In contrast to the data obtained using haloperidol and metoclopramide, the lowest anti-emetic dose of sulpiride (0.02 mg/kg) also reduced nausea (to score 19, $P<0.05$). As the dose of sulpiride was increased the anti-emetic action was maintained and the anti-nauseant action increased (nausea score reduced to 9 at 0.31 mg/kg sulpiride, $P<0.001$) with nausea completely abolished at 2.5 mg/kg sulpiride.

Thus, in the common marmoset 2-di-n-propylamino-5,6-dihydroxytetralin is shown to be potent to cause a syndrome of apparent discomfort, reasoned to be analogous to nausea in man, followed by emesis. Both the nausea and emesis are reproducible. That emesis can be antagonised by neuroleptic agents independently of nausea supports the hypothesis that different systems may mediate the two effects (Borison & McCarthy, 1983). The relevance of nausea and emesis responding of the marmoset for studies on the mechanisms controlling these two phenomena, both drug-induced and anticipatory, is presently being assessed.

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THE EFFECT OF CLONIDINE UPON EXOGENOUS AND ENDOGENOUS NORADRENALINE RELEASE FROM SUPERFUSED RAT CORTICAL TISSUE

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The use of radiolabelled transmitters in release experiments is widespread and has allowed the modulation of transmitter release to be studied in a large number of brain regions (Chesselet, 1984). In particular the release of ^{3}H-noradrenaline from rat cortical tissue, and its inhibition by α_2-adrenoceptor agonists, has been extensively investigated (see Heepe and Starke, 1985). The significance of such release, however, may be in doubt since it is thought that newly synthesised, as opposed to stored, transmitter is preferentially released and is of major importance in the maintenance of release under continual stimulation (De Belleroche, 1976). For this reason the release of both endogenous and exogenous, radiolabelled noradrenaline from rat cortical tissue, and the effect of clonidine upon this release, was studied and compared. Rat cortical cubes (250 μm) were incubated for 20 minutes at 37°C in gassed Krebs bicarbonate buffer (KBB) of the following (mM) composition : (NaCl 136 ; glucose 10 ; KCl 5 ; $CaCl_2$ 2 ; KH_2PO_4 1.25 ; $MgSO_4$ 1.2 ; $NaHCO_3$ 0.25 ; ascorbate 0.2 ; tyrosine 0.05 ; pargyline 0.01). In some experiments ^{3}H-noradrenaline (100 nM) was included. Subsequently 20 chambers, each containing approximately 100 μl packed tissue, were superfused at 0.5 ml min^{-1} for 20 minutes (endogenous) or 60 minutes (exogenous) after which time 2 pairs of 10 minute aliquots were collected separated by a period of 10 minutes. Of each pair the first aliquot was an estimate of basal release ; the second encompased the release evoked by a 4 minute pulse of KBB containing 25 mM KCl which commenced at the start of the second aliquot. An S_2/S_1 ratio was derived from the 2 stimulations : the effect of clonidine (10^{-10} - 10^{-6} M) was assessed by adding the drug immediately following the first stimulation until the completion of the experiment. Evoked tritium was measured by liquid scintillation spectrometry. ^{3}H-noradrenaline represented approximately 73 % of the potassium evoked tritium ; subsequent experiments assumed a constant proportion of ^{3}H-noradrenaline. Endogenous noradrenaline was assayed by HPLC with electrochemical detection. The data were expressed as % inhibition of release and were analysed using computerised non-linear regression (Allfit) (De Lean et al., 1978).

Clonidine inhibited ^{3}H-noradrenaline and noradrenaline release in a concentration-dependent manner with IC_{50} values (nM) of 11.1 (n=5) and 4.9 (n=3) respectively with slopes of 0.63 and 0.85. Clonidine (10^{-6} M) maximally inhibited ^{3}H-noradrenaline and noradrenaline release by 58 % and 56 % respectively.

No differences, therefore, were found between the clonidine-mediated inhibition of ^{3}H-noradrenaline and endogenous noradrenaline release from superfused rat cortical tissue.

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THE EFFECT OF MAPROTILINE ON DIETHYLPROPION-INDUCED ANOREXIA IN THE RAT.

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Anorexia nervosa is a disease most prevalent in adolescent females. It is characterised by a marked reduction in energy intake and hence in weight. Due to a strong psychological factor in the aetiology of the disorder, it has proved difficult to find an animal model of anorexia nervosa. Potentially useful drugs in this disorder may be investigated by studying their effects in animals with drug-induced anorexia. Using this method it has been shown that the antidepressant viloxazine reverses the anorectic effects of fenfluramine (Pleece et al 1980). Fenfluramine is known to exert its anorectic action by increasing 5HT release in the CNS thus causing 5HT depletion (Costa et al 1971). It was suggested that viloxazine reversed the effect of fenfluramine by preventing its access to intraneuronal 5HT stores (Pleece et al 1980). In this study the anorectic agent diethylpropion was used. It is a centrally acting anorectic drug but unlike fenfluramine, it exerts its action on central noradrenergic neurones, causing release and preventing reuptake (Borsini et al 1979). Having demonstrated the anorectic activity of diethylpropion the effects of the antidepressant drugs viloxazine and maprotiline on the resulting anorexia were investigated.

Female Wistar rats 200g $\pm$ 10g were individually housed and used in groups of six. Water was freely available but access to food was only possible for 2h in 24 (11.00h - 13.00h). Each animal received 30g standard laboratory pellet diet and the remainder was removed after 2h and weighed. All drugs were dissolved in 0.9% saline and administered i.p. Unless otherwise stated, diethylpropion was given 30min before feeding and the antidepressants 60 min prior to feeding. Appropriate control injections of 0.9% saline were used.

Diethylpropion (6mg/kg and 9mg/kg) caused a significant decrease in food intake ($P < 0.01$ and $P < 0.001$: unpaired Students t-test).

When diethylpropion was preceded by viloxazine (5mg/kg and 10mg/kg) no change in the anorectic activity of diethylpropion was observed. However, when diethylpropion injection was preceded by maprotiline (5mg/kg, 7.5mg/kg and 10mg/kg) there was a dose-dependent reversal of the anorectic effects. If the order of the diethylpropion and maprotiline injections was reversed, there was no antagonism of the anorexia.

Maprotiline has potent noradrenaline uptake blocking activity (Delina-Stula 1972). Maprotiline has been shown in this study to block the anorectic effects of diethylpropion, a drug known to deplete central neurones of noradrenaline. A possible mechanism by which maprotiline may block this effect is by preventing diethylpropion entering the noradrenergic neurones and therefore preventing the depletion of noradrenaline stores.

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NON-μ, NON-δ OPIATE BINDING SITE DISTRIBUTION IN RAT BRAIN

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While the binding properties and anatomical distribution of μ and δ opiate binding sites in rat brain are reasonably well characterised, the situation is not so clear in the case of the κ binding sites. In particular, there is some controversy concerning the properties of the binding sites labelled by benzomorphans and oripavines under conditions where binding to μ and δ receptors has been blocked. It has been suggested that these sites represent 'benzomorphan' receptors (Chang et al, 1981), or κ receptors (Gillan & Kosterlitz, 1982). Equally, little information is available concerning the anatomical distribution of these sites. We have therefore used ^{3}H-bremazocine and ^{3}H-diprenorphine (in the presence of saturating concentrations of D-Ala^2 MePhe^{4}Glyol^{5}enkephalin (DAGO) and D-Ala^{2}D-Leu^{5}enkephalin (DADL) to block μ and δ receptor binding respectively) to localise putative κ-opiate binding sites by autoradiography using slide-mounted sections of rat brain.

Coronal sections (20μm) were incubated with either (a) 1 nM ^{3}H-bremazocine in the presence of 1 μM DAGO and 1 μM DADL, or (b) 2.5 nM ^{3}H-diprenorphine in the presence of 1 μM DAGO and 1 μM DADL. After 5 1 minute rinses in ice-cold tris buffer, slides were apposed to LKB Ultrofilm for periods of 12 - 20 weeks to permit autoradiographic visualisation of binding sites. In biochemical experiments, sections were wiped from the slides, and the amount of bound radioactivity assessed by liquid scintillation counting. Non-specific binding was defined using (a) 5 μM U50488H or (b) 1 μM diprenorphine.

Under these conditions, the binding of ^{3}H-bremazocine was displaceable by a series of κ-selective ligands. The binding sites showed a unique distribution, with levels highest in the claustrum, medial preoptic area, medial amygdaloid nucleus and superior colliculus. However, ^{3}H-diprenorphine labelled, in addition, another class of binding site which was not displaceable using the κ-selective drug U50488H. These latter sites showed a highly discrete distribution, with notable binding only in the globus pallidus, substantia nigra pars reticulata, interpeduncular nucleus and locus coeruleus.

We conclude that the sites labelled by ^{3}H-bremazocine represent κ-receptors, while the identity of the additional site labelled by ^{3}H-diprenorphine remains unclear.

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CALCIUM ENTRY BLOCKERS AS ANTICONVULSANT DRUGS IN DBA/2 MICE

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Compounds acting as selective antagonists of Ca^{++} influx possess anticonvulsant properties in animal models of experimental epilepsy (Wauquier et al., 1985; De Sarro et al., 1984).

In the present study six calcium entry blockers belonging to different classes were tested in an experimental model of epilepsy (sound induced seizures in DBA/2 mice) to assess whether these drugs were able to attenuate or suppress seizures. During exposure to auditory stimulation the incidence and timing of successive seizure phases (wild running, clonus, tonus and respiratory arrest) were recorded as previously described (Anlezark et al., 1976; De Sarro et al., 1984). All drugs were given i.p. 45 min before test, at 3-6 doses up to a maximum of 168 µmol/kg. ED50 was calculated according to Litchfield and Wilcoxon (1949).

Table. ED50-values (± 95% confidence limits) in µmol/kg of calcium entry blockers in DBA/2 mice.

	WILD RUNNING	CLONUS	TONUS
Flunarizine	37 (26-54)	21 (14-32)	7 (5-10)
Nifedipine	94 (64-140)	39 (22-68)	21 (12-36)
Nimodipine	71 (49-103)	51 (36-72)	29 (18-47)
Diltiazem	115 (85-156)	63 (32-124)	53 (37-74)
Nitrendipine	N.A.	84 (53-134)	46 (27-79)
Verapamil	N.A.		

N.A.: inactive at dose levels studied

The present results confirm an anticonvulsant effect of some calcium entry blockers and show that there are substantial differences among different classes. Flunarizine is the most potent and verapamil the least potent. Calcium antagonists of the dihidropyridine class show an intermediate potency. The reasons for these differences are not known. They possibly involve actions on different Ca^{++} conductances, different penetration of the blood brain-barrier and different actions on vascular resistance.

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(3-(^{125}I)-IODOTYROSYL^{3}) NEUROTENSIN BINDING IN POST-MORTEM BRAIN FROM ALZHEIMER-TYPE DEMENTIA PATIENTS AND CONTROLS

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Neurotensin (NT) is a putative neurotransmitter peptide which has a wide distribution throughout the brain. Specific high affinity binding sites for NT ligands have been described in several species, including man (Uhl, 1982). NT-like immunoreactivity has been reported to be unchanged in post-mortem brain from patients with Alzheimer-type dementia (ATD), (Yates et al., 1985). However, there have been no reports of investigations of high affinity binding sites for NT in this disease.

In the present study, NT binding sites were assessed by a method similar to that of Mazella et al, (1983). Brain samples from controls, and histologically confirmed ATD patients were matched for age, sex, time at 4°C prior to autopsy, and storage time over liquid nitrogen. Binding was evaluated in four brain regions using 0.05 nM (3-(^{125}I)-iodotyrosyl^{3}) neurotensin (^{125}I-NT), and defining specific binding as that displaced by 1 μM NT. The results are presented in table 1.

Table 1 ^{125}I-NT binding in controls and ATD patients

	CONTROL	ATD
Frontal Cortex	0.28 ± 0.05 (7)	0.32 ± 0.18 (7)
Temporal Cortex	0.24 ± 0.04 (8)	0.12 ± 0.02 (8) *
Amygdala	0.40 ± 0.07 (8)	0.13 ± 0.03 (6) **
Caudate Nucleus	0.16 ± 0.03 (8)	0.16 ± 0.04 (8)

(Values are fmol/mg protein; mean ± s.e. mean (n)) *$p < 0.025$ **$p < 0.01$

Binding of ^{125}I-NT was decreased by 68% ($p < 0.01$) in the amygdala, and by 49% ($p < 0.025$) in the temporal cortex of ATD patients compared with control values.

The localization of this decrease in NT binding is consistent with the neuropathological changes in ATD, and suggests that there is a loss of cells with NT binding sites in this disease. Further investigations of NT mechanisms might provide information relating to the disease process of ATD.

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PALMITYL CARNITINE, A LIPID METABOLITE PRODUCED IN ISCHAEMIA, ACTIVATES Ca^{++} CHANNELS IN SMOOTH MUSCLE

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Inhibition of fatty acid oxidation by ischaemia causes marked accumulation of acyl carnitine derivatives in the cytosol. These amphiphilic derivatives accumulate in cell membranes where they have detergent properties and affect channel gating; palmityl carnitine has been reported to increase Ca^{++} current in the heart by a surface charge effect (Inoue & Pappano, 1983). We have investigated the effects of palmityl carnitine in smooth muscle and on (^{3}H)nitrendipine and (^{3}H)diltiazem binding to rat cortical membranes.

Taenia preparations from the guinea-pig caecum (Spedding, 1985) were set up in 10ml organ baths in K^+(40mM)-Tyrode solution and isotonic contractions in response to Ca^{++} recorded. (^{3}H)nitrendipine and (^{3}H)diltiazem binding assays were performed, using rat cortical membranes, with minor modifications of previously described methods (Ehlert et al., 1982 and Schoemaker & Langer,1985, respectively). Palmityl carnitine (10µM -1mM) and Bay K 8644 (1nM - 1µM) increased established Ca^{++}-induced contractions, whereas carnitine (10 - 300µM) was inactive and palmitic acid (0.1 - 1mM) relaxed Ca^{++}-induced contractions. Palmityl carnitine (0.1mM) and Bay K 8644 (1µM) shifted Ca^{++} concentration-response curves to the left; the shifts were additive.

The Ca^{++} channel activator, Bay K 8644, has characteristic interactions with the various classes of calcium-antagonists, in that the inhibitory effects of dihydropyridines were competitively antagonized, the effects of verapamil and diltiazem were non-competitively reduced, but the inhibitory effects of class III calcium antagonists (e.g., flunarizine) were unaffected (Spedding, 1985). Similar effects were observed with palmityl carnitine. Thus, the pA_2 of nifedipine as an antagonist of Ca^{++}-induced contractions was reduced from 9.73±0.20 (mean ± SEM) by palmityl carnitine (9.35±0.20, 0.03mM; 8.91±0.10, 0.1mM; 8.31±0.11, 0.3mM), the inhibitory effects of verapamil and diltiazem were reversed by palmityl carnitine (0.1mM), but the effects of flunarizine were unaffected. The effects of the detergents, Triton X-100, Tween 80, saponin and sodium dodecyl hydrogen sulphate did not resemble those of palmityl carnitine.

Whereas carnitine (10-300µM) was inactive, palmityl carnitine and palmitic acid reduced (^{3}H)nitrendipine binding to rat cortical membrane preparations with IC_{50} values of 120±1µM and 170±3µM, respectively. Palmityl carnitine displaced (^{3}H)diltiazem binding with an IC_{50} of 120±5µM and the displacement was temperature-independent.

These findings show that endogenously occurring lipid metabolites can directly affect Ca^{++} channels and may be expected to modulate channel function in certain conditions.

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INVESTIGATION OF THE IRREGULAR EFFECT OF RAUWOLCINE ON ADRENERGIC RESPONSES OF THE MESENTERIC ARTERIAL BED OF THE RAT

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Rauwolcine, an a_2-selective antagonist (Timmermans, Kwa et al, 1979) has been reported to increase responses to noradrenaline (NA) and phenylephrine (PE) in the mesenteric arterial bed of the rat (Fiotakis & Pipili, 1983). This irregular effect was further investigated by looking at the involvement of β-adrenoceptors and of prostaglandin I_2 (PGI_2) and thromboxane A_2 (TXA_2). The mesenteric arterial bed of the rat was prepared as already reported (Pipili & Poyser, 1981). Noradrenaline and PE were given as bolus injections and dose-response curves were constructed. Rauwolcine and propranolol were continually present in the perfusion fluid when their effect was studied. The stable metabolites 6-Keto-PGF_{1a} and TXB_2 of PGI_2 and TXA_2 respectively were measured in the perfusion fluid by R.I.A. according to the methods of Pipili and Poyser (1981) and Levine, Morgan et al (1981).

In the presence of the β-antagonist propranolol (10^{-6}M), rauwolcine no longer potentiates responses to NA but reduces them at all concentrations used (10^{-8}-10^{-6}M) with a PA_2 of 6.6±0.11, n=12. Control responses to NA were not significantly altered by propranolol. Responses to PE were potentiated by 10^{-8}M and reduced by 10^{-6}M rauwolcine in the presence of propranolol. The potentiation, however, is not significant compared to time controls of PE (PE responses increase with time -NA responses do not vary considerably). The reduction in responses is significant with a calculated PA_2 of 6.3±0.42, n=12. Noradrenaline causes a significant increase in the release of both 6-keto-PGF_{1a} and TXB_2 ($p<0.01$, n=6). Rauwolcine (10^{-8}-10^{-6}M) does not appreciably affect the increase in 6-keto-PGF_{1a} release but lowers its basal production compared to time controls. The increase in TXB_2 release is not affected by 10^{-8}M and it appears somewhat reduced by 10^{-7} and 10^{-6}M rauwolcine. The baseline remains unaltered.

The fact that propranolol modifies the effect of rauwolcine on NA responses in the rat mesenteric arterial bed indicates that stimulation of β-adrenoceptos may be necessary for this potentiation to occur. The reduction in NA and PE caused by rauwolcine is at least partly due to a_1-antagonism as suggested by the PA_2 values. (PA_2 values for rauwolcine against a_2-adrenergic responses are reported around 7.0, Timmermans & VanZwieten, 1980). The alterations in the pattern of PGI_2 and TXA_2 release by rauwolcine cannot be at present directly related to its irregular effect and require further experiments.

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BRL 24924: A NEW AND POTENT GASTRIC MOTILITY STIMULANT.

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Side chain modification of metoclopramide can lead to retention of gastric motility stimulant activity in the apparent absence of dopamine blocking activity e.g. BRL 20627 (McClelland et al, 1983). A considerably more potent novel benzamide, BRL 24924 [(±)-(endo)-4-amino-5-chloro-2-methoxy-N-(1-azabicyclo[3.3.1]non-4-yl) benzamide HCl] is now compared to metoclopramide HCl (Mcp).

Drug effects in rat on gastric motility (changes in mean amplitude of intragastric pressure waves; IGP) and emptying of a liquid test meal (5ml, 7mg ml^{-1} citric acid), were determined as described (McClelland et al, 1983). BRL 24924 (0.05-0.5 mg kg^{-1} s.c.) or Mcp (0.25-10mg kg^{-1} s.c.) increased the mean amplitude of IGP with no effect on baseline pressure. Above these dose ranges, stimulation was reduced with both drugs. ED_{50} values (significant increase [$P<0.05$; 't' test] in mean amplitudes in 50% of animals) over the increasing part of the dose response curve showed BRL 24924 to be 8 times more potent than Mcp (Table 1).

Compared to vehicle dosed animals, BRL 24924 (0.04-1mg kg^{-1} s.c.) significantly ($P<0.05$; 't' test; n=5 or 6) increased gastric emptying of the liquid test meal by 30-43%. Considerably higher doses of Mcp (5 and 10mg kg^{-1} s.c.) were required to obtain a similar effect. Based on lowest doses that significantly increase gastric emptying, BRL 24924 is about 100 times more potent than Mcp in this test.

To obtain an index of dopamine antagonist activity, the drugs were assessed for their ability to increase immunoreactive plasma prolactin levels in the rat (Carlson et al, 1977) and to antagonise apomorphine-induced climbing by mice (Protais et al, 1976). Mcp (0.25-10mg kg^{-1} s.c.) dose-dependently and significantly ($P<0.05$; 't' test) elevated plasma prolactin levels in the non-stressed rat and antagonised apomorphine-induced climbing by the mouse (0.5-2mg kg^{-1} s.c.). In contrast, BRL 24924 had no significant effect on prolactin levels at doses of 0.1-20mg kg^{-1} s.c. and was 60 times less potent than Mcp in antagonising apomorphine-induced climbing (Table 1). The low potency of BRL 24924 in these tests is consistent with its low affinity for dopamine receptor binding sites in vitro ($IC_{50} > 10^{-5}M$ against [^{3}H]-spiroperidol binding to rat striatal membranes).

Table 1: Comparison of BRL 24924 and Mcp. (Doses in mg kg^{-1} s.c; free base).

Drug	Stimulation of gastric motility ED_{50}[1]	Increase in gastric emptying lowest active dose	Central dopamine antagonism ED_{50}[1]	Prolactin release ED_{500}[2]
BRL 24924	0.12(0.06-0.25)	0.04	48(28-80)	> 20
Mcp	1.0(0.3-3.7)	5	0.8(0.6-1.10)	0.10

[1] ED_{50} with 95% confidence limits (Litchfield & Wilcoxon, 1949); n=10. [2] ED_{500} value - dose which increased mean plasma prolactin to 500% of vehicle control; n=6.

It can be concluded that BRL 24924 is considerably more potent than Mcp as a gastric motility stimulant in the rat. If this is applicable to man, BRL 24924 may also have therapeutic advantages in the treatment of gut motility disorders with the absence of side effects associated with dopamine antagonism.

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THE CONTRIBUTION OF CHANGES IN RECEPTOR NUMBER AND AFFINITY TO VARIATION IN VASCULAR RESPONSIVENESS TO NORADRENALINE

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The arterial tree of the rabbit is not homogeneous in its α-adrenoceptor responsiveness to noradrenaline (NA). In the rabbit the NA ED_{50} of the aorta is approximately 10^{-7} M and there is considerable receptor reserve. At specific locations along many of its primary branches, variable changes in sensitivity occur abruptly, often by 1-2 orders of magnitude. There may be an associated increase or decrease in receptor reserve, but in these vessels NA still elicits a maximum tissue response. As these arteries successively branch, in some regional vascular beds there are further changes: for example, in some smaller arteries the responses that can be elicited by NA are considerably less than the capacity of the artery to contract to other agonists.

We have measured the affinity (K_A) for NA in the aorta and a number of its major branches that show these differences in NA ED_{50} using the method of Furchgott and Bursztyn, (1967), and also receptor reserve defined as antilog [-log ED_{50} - pK_A]. In 12 arteries, the thoracic and abdominal aorta, basilar, ear, external, internal, and common iliac, ovarian, large and small pulmonary, renal and superior mesenteric arteries, where NA elicited a contraction equal to maximum tissue contraction, sensitivity defined as -log ED_{50} could be positively correlated with pK_A (r=0.70). Receptor reserve varied from 100 for the ear artery to 1.1 for the small pulmonary artery. In the basilar and a small pulmonary artery where NA maxima were 57 and 35% of tissue maximum respectively the relationship between receptor occupation and response was estimated. There was no receptor reserve for NA and maximum contraction was achieved only with all receptors occupied. We interpret this as indicating that contraction in these vessels is limited by receptor number.

We conclude that in the larger arteries of the rabbit where NA causes a full contraction, affinity of the agonist for its receptors seems to dominate the response of the vascular smooth muscle to NA. In some smaller arteries when response to NA is less than maximum, receptor number seems to limit the capacity of the muscle to respond to this physiologically important amine. These two influences vary independently.

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EFFECTS OF BAY K 8644 AND NIFEDIPINE ON ISOLATED PERFUSED RAT TAIL ARTERY UNDER DIFFERENT OXYGEN TENSIONS

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An earlier observation on rat anococcygeus smooth muscle (McGrath 1982) suggested that the susceptibility to the calcium entry blocker (CEB), nifedipine of contractions to noradrenaline (NA) was less at low PO_2. We have now investigated this further on isolated perfused rat tail artery, whose contractions to NA and potassium chloride (KCl) have been demonstrated to be attenuated by nifedipine and facilitated by BAY K 8644 (Su, et al. 1984) under the high PO_2 customarily employed in pharmacological experiments.

The proximal segment of male rat tail artery (Wistar, 300-350g) was perfused at 2-3 ml/min. and the perfusion pressure was recorded. In the first set of experiments, $[Ca^{2+}]$ response curves were constructed by starting in a nominally zero saline using a sub-maximal [NA] of 3μM. In other experiments $[Ca^{2+}]_{free}$ was buffered with EGTA and nitriloacetic acid (NTA) (2.5mM each) and contractions induced by NA (3μM) and KCl (100mM). In each case the solution was bubbled with different gas mixtures of 95% O_2 and 5% CO_2; 16% O_2, 5% CO_2 and 79% N_2 and 4% O_2, 5% CO_2 & 91% N_2.

NA: - At 95% O_2 there was a small shift to the left with BAY K 8644 and a shift to the right with nifedipine. As the PO_2 decreased, the control curve shifted to the right but the curves in BAY K 8644 or nifedipine hardly changed. We interpret this as indicating that the nifedipine-sensitive channels play a steadily decreasing role as PO_2 decreases but that they can be re-activated by BAY K 8644. This has implications for the effects of these drugs under physiological conditions or in hypoxia. In hypoxia, nifedipine would not be likely to reverse NA-induced vasoconstriction whereas BAY K 8644 might intensify constriction. It is now of interest to determine whether the interaction is competitive or not.

KCl: - Since the clearest demonstration of facilitation of contraction of smooth muscle by BAY K 8644 is usually against KCl-induced depolarisation (Schramm & Towart, 1983) we repeated these experiments with KCl 100mM. At 95% O_2 we demonstrated a small facilitation of contraction with BAY K 8644 and an almost complete blockade with nifedipine. At lower PO_2 sensitivity to KCl declined slightly and nifedipine blockade remained. However, we now could not facilitate responses with BAY K 8644. On the contrary it reduced responses. This was unexpected and contrasts with the effects against NA. This appears to have considerable consequences for the interpretation of the mode of action of BAY K 8644 under physiological conditions.

As a general conclusion, we suggest that oxygen may exert sufficient influence on Ca^{2+} channels; that effects of drugs modifying Ca^{2+} channel function may be qualitatively and quantitatively altered by excessive oxygenation *in vitro* and that the action of such drugs in life may be more fruitfully explored under physiological conditions with particular regard to oxygen.

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EFFECTS OF BAY K 8644 ON BLOOD PRESSURE AND HEART RATE IN ANAESTHETISED RATS

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Bay K 8644 is a dihydropyridine which activates Ca^{2+} channels in both cardiac and smooth muscle and is active in a similar dose range to nifedipine (Schramm et al 1983). In order to examine the cardiac and vascular effects of this compound in the same animal we have determined the effects of Bay K 8644 on blood pressure and heart rate in two groups of rats with i) reflexes intact ii) reflexes abolished by ganglion blockade. Rats were anaesthetised with Inactin (100 mg/kg^{-1}, i.p.). A carotid artery and left jugular vein were cannulated for measurement of blood pressure and heart rate and administration of drugs respectively. Heart rate was derived from the blood pressure signal. Dose response curves to Bay K 8644 were carried out in two groups of four rats. One group of animals received pentolinium 20 mg/kg i.p. 15 min before Bay K 8644. In a further group of rats responses to Bay K 8644 were examined at 15 intervals in the absence (n=4) and presence (n=4) of Nitrendipine (0.1 $mg.kg^{-1}$).

Administration of Bay K 8644 (1-30 µg/kg) induced a dose dependant increase in diastolic (DBP) and pulse pressure and a bradycardia.

	Dose Bay K 8644 $\mu g.kg^{-1}$			
	1	3	10	30
Δ Systolic BP (mmHg)	30±6	38±8	61±13	63±10
Δ Diastolic BP (mmHg)	19±6	26±5	48±13	44±11
Δ HR ($bts.min^{-1}$)	-48±14	-105±27	-68±27	-113±34

Pretreatment with pentolinium abolished the reflex response to carotid artery occlusion, potentiated the pressor responses and attenuated the bradycardia so that administration of Bay K 8644 at a dose of 30 $\mu g.kg^{-1}$ now increased systolic and diastolic blood pressure by 109±4 mmHg and 74±2 mmHg respectively and induced bradycardia of 51±10 $bts.min^{-1}$. In the ganglion blocked rat Bay K 8644 increased diastolic blood pressure by 15±4 and 35±5 mmHg at doses of 1 and 3 $\mu g.kg^{-1}$ respectively. Pulse pressure was increased only at 10 and 30 $\mu gm.kg.^{-1}$ doses of Bay K 8644. Methoxamine (1-300$\mu g.kg^{-1}$) increased systolic and diastolic pressure with no change in pulse pressure.

In the ganglion blocked rat reproducible pressor responses every 15 min were produced by administration of Bay K 8644 at 2 $\mu gm.kg^{-1}$. Administration of Nitrendipine (0.1 $mg.kg^{-1}$) after two Bay K 8644 responses completely blocked the pressor response 15 min later. This dose of Nitrendipine produced a small decrease in blood pressure which had returned to control values before the administration of Bay K 8644. After 60 min the Bay K 8644 pressor response had returned to control values.

These results suggest that in the anaesthetised ganglion blocked rat, low doses of Bay K 8644 increase peripheral resistance and higher doses are required to induce increased pulse pressure which is indicative of increased cardiac contraction. The ganglion blocked animal is a useful preparation in which to study the cardiac and vascular actions of Bay K 8644 and antagonism of these effects by drugs.

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NITRENDIPINE INHIBITS THE UPTAKE OF CALCIUM INTO RAT HEART MITOCHONDRIA

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During ischemia the capacity of mitochondria to generate ATP is reduced, resulting from the accummulation of intracellular calcium (Ca^{2+}), following the failure of the ATP-dependent mechanisms responsible for maintaining cellular Ca^{2+} homeostasis (Nayler, 1981). Recent reports have indicated that the prophylactic use of Ca^{2+} antagonists protects against Ca^{2+}-induced mitochondrial damage (Leblondel et al, 1984). In this study the ability of nitrendipine to alter mitochondrial Ca^{2+}-uptake is reported.

Tightly coupled heart mitochondria were isolated from female Wistar rats by a modification of the method of Vercesi et al(1978). Ca^{2+} uptake was followed with a Corning Ca^{2+}-specific electrode coupled to a Pentracourt PM 10pH-meter and a BBC recorder (Model SE120) according to the method of McNamee et al (1985). Protein was determined by the method of Gornall et al (1949).

Using a Ca^{2+}-specific electrode nitrendipine (25-100μM) was found to cause a concentration dependent inhibition of Ca^{2+} influx into isolated rat heart mitochondria, resulting in an IC_{50} value of 54.8 ± 4.3μM (n=4). Under control conditions, the rate of influx was 286.1 ± 24.5nmol Ca^{2+} min^{-1} mg of $protein^{-1}$ (n=4); in the presence of nitrendipine (100μM) the rate decreased to 51.8 ± 2.8 nmol Ca^{2+} min^{-1} mg of $protein^{-1}$ (n=4). Similar findings were obtained when either verapamil (IC_{50} = 19.5 ± 2.0μM, n=4) or diltiazem (IC_{50} = 93.8 ± 8.3μM, n=4) replaced nitrendipine. Over the same concentration range, nitrendipine (25-100μM) also reduced total Ca^{2+} uptake into mitochondria, with the amount taken up being reduced from 220.7 ± 20.8 to 41.4 ± 6.9nmol Ca^{2+} mg of $protein^{-1}$ (n=4); resulting in an IC_{50} value of 85.7 ± 2.9μM (n=4). Qualitatively similar results were obtained for verapamil (IC_{50} 14.8 ± 1.1μM, n=4) and diltiazem (IC_{50} = 82.5 ± 7.8μM, n=4).

The findings presented here indicate that concentrations of nitrendipine and the other antagonists producing changes in Ca^{2+}-influx were significantly ($p < 0.05$) lower than those known to modify mitochondrial ATP synthesis (Vaghy et al, 1981) suggesting that the ability of nitrendipine to modify Ca^{2+} transport is not dependent on its ability to uncouple oxidation from phosphorylation.

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MODULATION OF DEPOLARIZATION STIMULATED $^{45}CA^{2+}$ UPTAKE IN CULTURED NEURONAL CELLS BY CALCIUM AGONISTS AND ANTAGONISTS

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Calcium influx through voltage sensitive calcium channels represents an important mechanism in regulating the intracellular Ca^{2+} concentration in many tissues (Reuter, 1983). Drugs which have the ability to block Ca^{2+} flow through these channels are widely used in the treatment of angina and hypertension (McCallister, 1982).. The most potent of these compounds are the dihydropyridines. Studies of the binding of radiolabelled dihydropyridines suggest that these compounds bind to sites with characteristics indicative of an interaction at the calcium channel in a number of tissues, including brain (Ehlert et al., 1982). However, although these drugs are potent inhibitors of Ca^{2+} channels in cardiac and smooth muscle, they are virtually inactive in neuronal preparations (Miller and Freedman, 1984). To further investigate the basis for this apparent absence of pharmacological effects of dihydropyridines in neuronal tissue, we have studied the effect of dihydropyridine Ca^{2+} agonists and antagonists on $^{45}Ca^{2+}$ uptake into cultured neuronal cells.

Primary cultures of neurons dissociated from foetal rat brains were prepared as described by Hefti et al. (1985). Cells were grown in culture for 6-10 days in the presence of 10 μM arabinose C to obtain pure neuronal cultures. Hefti et al (1985) have demonstrated that under these conditions all cells in these cultures were labelled with tetanus toxin, a specific neuronal marker, and <1% of the cells were positively stained for glial fibrillary acid protein.

Under resting conditions an initial rapid uptake of $^{45}Ca^{2+}$ into the neurons was followed by a slower phase. Depolarization of the cells with 50 mM KCl led to an increase in the initial rate of $^{45}Ca^{2+}$ uptake and to a 50% increase in the maximal accumulation. The dihydropyridine calcium agonist Bay K 8644 enhanced uptake in the presence of 50 mM KCl but not in resting conditions. K^+ stimulated $^{45}Ca^{2+}$ uptake was concentration dependent and was insensitive to TTX (1 μM) indicating that Na^+ channels are not involved in this response. The dihydropyridine calcium agonists Bay K 8644 and (+)-S-202-791 enhanced K^+ stimulated $^{45}Ca^{2+}$ uptake in a concentration dependent manner with EC_{50} values of 21 nM and 67 nM respectively. The dihydropyridine calcium antagonists PN 200-110 and (-)-R-202-791 had little or no effect on K^+ stimulated $^{45}Ca^{2+}$ uptake, but inhibited that stimulated by Bay K 8644 (1 μM) with IC_{50} values of 20 nM and 130 nM respectively.

The data suggests that voltage sensitive calcium channels of these neurons are sensitive to dihydropyridines and thus that binding sites for these compounds have a functional role in these neuronal cultures.

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EFFECTS OF EXTRACELLULAR Ca^{2+} REMOVAL AND NITRENDIPINE ON THE FIELD-STIMULATED AND K^+-CONTRACTED HUMAN COLON

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Calcium antagonists have actions on many vascular and non-vascular smooth muscles but tissue susceptibility is very variable (Schwartz & Triggle, 1984).

They act predominantly at voltage-activated Ca^{2+} channels but possibly also at other stages of activation which depend upon Ca^{2+}. As far as we can ascertain there is only one report of their action on the field-stimulated human colon in vitro (Zar & Gooptu, 1983).

We have investigated the effect of the dihydropyridine Ca^{2+} antagonist nitrendipine and the dependence upon extracellular Ca^{2+} of K^+ and field stimulation(FS)-induced contractility of longitudinal strips of human colon smooth muscle in Krebs solution at 37°C.

Longitudinal strips (2mm x 15mm) of normal colon muscle with mucosa removed were set up in Krebs solution (NaCl 118.6; KCl 4.75; $NaHCO_3$ 25; KH_2PO_4 1.19; $MgSO_4.7H_2O$ 1.19; $CaCl_2$ 2.57; glucose 11.54mM) oxygenated with 95% O_2/5% CO_2. They were stimulated with additional 40-80mM K^+ with a contact time of 20min added every 30-60min, or with trains of pulses 10-30Hz for 10-20sec and 0.5ms pulse width delivered from parallel platinum electrodes every 4min.

K^+ 40-80mM caused a slow contraction exhibiting a more steeply rising phase over a period of about 5min (phasic response) and then a relatively maintained plateau (tonic phase) measured at 20min. When consistent responses were obtained Ca^{2+}-free EGTA Krebs was introduced with frequent washings (10 strips from 4 patients). After 40-255min tonic responses were reduced by 61% (range 7.3-82) but abolition of response was not achieved. Responses to K^+ were still obtained in 4 strips kept for 18h at 4°C in Ca^{2+}-free Krebs with frequent washings before setting up in Ca^{2+}-free EGTA Krebs at 37°C.

The contractility seen with FS was usually that of a post-stimulation response such that increased tension developed after switching off the stimulus. Tetrodotoxin 10^{-6}M abolished this response. The response was also abolished on introducing Ca^{2+}-free EGTA Krebs in all cases within 20min and reintroduction of normal Krebs restored responses to or above normal (N=11).

Addition of nitrendipine (2×10^{-6}M) for 20min did not significantly affect KCl-induced contractions in 13 of 17 strips tested while in the remainder the average reduction was by 46 ± 15% (mean ± SD) of the original tonic response.

Nitrendipine (10^{-6}M-10^{-5}M) did not significantly affect FS contractions in 6 out of 17 strips tested. In the remaining 11 the average reduction was 70 ± 23.8%. The average time to 50% reduction was 5.3 ± 2.8min.

It appears that K^+-induced but not FS-induced contractility of the isolated longitudinal colon has only partial dependence upon extracellular Ca^{2+}. The types of Ca^{2+} channels activated by K^+ are also less susceptible to nitrendipine than those activated by FS.

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A COMPARISON OF THE H_2-RECEPTOR ANTAGONIST AND ANTISECRETORY EFFECTS OF FAMOTIDINE AND RANITIDINE

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Famotidine has been claimed to be an unsurmountable histamine H_2-receptor antagonist with a longer duration of antisecretory action than ranitidine (Pendleton et al, 1983). However other workers found famotidine to be, like ranitidine, a competitive H_2-antagonist (Black et al, 1985) and to have a similar duration of action (Buyniski et al, 1984). We have compared the H_2-blocking activities of famotidine and ranitidine against histamine-induced tachycardia in the guinea pig isolated right atrium and histamine-induced acid secretion in the rat isolated gastric mucosa. In addition the antisecretory potencies and durations of action of the two drugs were studied in the conscious Heidenhain pouch dog.

Famotidine, like ranitidine, caused parallel displacements of histamine concentration-response curves on both isolated atrium and mucosa. The pA_2 values (95% confidence limits) on atrium and mucosa respectively were: famotidine 8.06 (7.86-8.35) and 7.50 (7.26-7.92), ranitidine 7.20 (7.01-7.45) and 6.62 (6.36-7.04). The maximum response of the mucosa to histamine was unaffected by famotidine (1μmol/l) or ranitidine (10μmol/l) while the maximum response of the atrium was slightly depressed (by 22±3%) by famotidine (0.3μmol/l) but not by ranitidine at 3μmol/l. Schild plot slopes on atrium and mucosa respectively were: famotidine 1.12 (0.90-1.33) and 1.26 (0.90-1.64), ranitidine 0.99 (0.83-1.16) and 1.18 (0.87-1.50), none of these values being significantly different from unity.

In the Heidenhain pouch dog (n=4) famotidine was more potent than ranitidine as an inhibitor of histamine-induced gastric acid secretion. Antisecretory ED_{50} values (μg/kg; 95% confidence limits) were: intravenously famotidine 5(3-8), ranitidine 29(11-78) and orally famotidine 20(12-33), ranitidine 110(60-200). However, when equieffective oral dose levels of famotidine and ranitidine were compared there was no difference between the duration of action of the two drugs (Table 1).

Table 1 Inhibition of gastric secretion in the dog.

Drug	Oral dose mg/kg	Mean (± s.e.) % inhibition of secretion at 1h	2h	4h	6h	8h	12h
Ranitidine	0.30	59 ± 15	81 ± 7	55 ± 12	54 ± 11	39 ± 7	16 ± 9
Famotidine	0.06	52 ± 19	87 ± 6	75 ± 7	52 ± 8	49 ± 10	21 ± 4

In conclusion, famotidine's profile of action as an H_2-receptor antagonist resembles that of the competitive antagonist ranitidine rather than the unsurmountable antagonist loxtidine (Brittain et al, 1985). Although famotidine is more potent than ranitidine, the two drugs have a similar duration of antisecretory action when administered orally.

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SULPHASALAZINE INHIBITS PLATELET AGGREGATION, AND THROMBOXANE FORMATION IN HUMAN PLATELETS AND COLON

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Sulphasalazine (SASP), inhibits the formation of radiolabelled thromboxane B_2 (TXB_2) by human colonic mucosa (Hawkey et al, 1985) and human platelets (Stenson & Lobos, 1983) from ^{14}C-arachidonic acid. We have now compared the activity of SASP with the thromboxane synthase inhibitor, 1-benzyl imidazole (BZI) on human platelet aggregation and the concurrent formation of TXB_2, and on the formation from endogenous substrate of TXB_2, prostaglandin (PG)E_2 and 6-keto-$PG_{1\alpha}$ by homogenates of human colonic mucosa.

Human platelet-rich-plasma (PRP) was incubated in a aggregometer (37°C, stirred at 900 rpm) and platelet aggregation induced by submaximal concentrations of collagen (0.4 - 1 µg/ml) . The drugs under study were pre-incubated (1 min) with PRP before adding collagen. Following aggregation (4 min), indomethacin (10 µg/ml) was added and the PRP frozen (-20°C) for later radioimmunoassay (RIA) of TXB_2.

Normal colonic mucosa, from resections for carcinoma (stored at -20°C) was homogenized (60 secs, Ultraturex) in tris buffer (50 mM, pH 7.4) to give a 25 mg/ml suspension. Aliquots (1ml) alone or with compounds under study were incubated for 30 min (37°C), at which time BW755C (100 µg/ml) was added. Following centrifugation (10,000g for 1 min), the supernatant was stored (-20°C) for later specific RIA for TXB_2, 6-keto-$PGF_{1\alpha}$ and PGE_2.

BZI (1-500 µM) produced a dose-related inhibition of platelet aggregation (IC_{50} 22 µM), which was accompanied by inhibition of platelet TXB_2 formation (IC_{50} 6 µM). Similarly, SASP (0.4 - 4 mM) produced a dose-related inhibition of platelet aggregation (IC_{50} 2 mM) and inhibition of platelet TXB_2 formation (IC_{50} 4 mM).

Homogenates of human colonic mucosa formed: 6-keto-$PGF_{1\alpha}$ (688 ± 234 ng/g of tissue, m ± sem, n=8), TXB_2 (224 ± 42 ng/g) and PGE_2 (218 ± 69 ng/g). BZI (0.4 - 730 µM) inhibited the formation of TXB_2 in a dose-related manner (IC_{50} 0.4 µM) but did not affect the synthesis of PGE_2 or 6-keto-$PGF_{1\alpha}$. SASP (IC_{50} 30 µM), which at higher concentrations (100 - 1000 µM) was accompanied by a significant ($p < 0.05$) increase in PGE_2 and 6-keto-$PGF_{1\alpha}$ formation. The SASP product, 5-aminosalicylic acid, (1-1000 µM) was without effect on the arachidonate metabolites measured.

At high concentrations, SASP inhibited the aggregation of human platelets induced by collagen, but was some 100-fold less potent than the thromboxane synthase inhibitor, BZI. Both SASP and BZI inhibited the formation of TXB_2 by human platelets and homogenates of human colonic mucosa from endogenous substrate, confirming previous studies using ^{14}C-AA conversion. SASP was a more potent inhibitor of TXB_2 formation in human colon than in human platelets stimulated by collagen. This may reflect the bioavailability of SASP in the broken-cell preparation of human colon compared to that in the stimulated intact-platelet suspensions.

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IS 9,11-EPOXYMETHANO PGH_2 (U44069) A PARTIAL AGONIST AT THE HUMAN PLATELET THROMBOXANE A_2 RECEPTOR?

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Thromboxane (Tx)A_2 mimetics (e.g. U44069, U46619) are routinely used to investigate the effects and mechanism(s) of action of the natural agonist (TxA_2) on platelets (MacIntyre, 1981; Pollock et al, 1984) and other TxA_2-sensitive preparations (Jones et al, 1982). EP 171 is the most potent TxA_2-mimetic reported to date (Jones et al, 1985). In the present study we compared the effectiveness of EP 171 and U44069, alone and in combination, on human platelet functional responses and on the transduction processes, namely phosphoinositide hydrolysis and consequent elevation of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$), that link TxA_2-receptor occupancy to platelet activation.

All studies were performed using freshly-isolated human platelets. Platelet aggregation was monitored photometrically and ATP secretion by luciferin-luciferase luminescence. $[Ca^{2+}]i$ was measured by using Quin 2 and phosphoinositide hydrolysis was monitored as $[^{32}P]$-phosphatidate (-PtdA) formation in platelets pre-labelled with $[^{32}P]$-PO_4 (Pollock et al, 1984).

U44069 (10nM - 3μM) and EP 171 (1 - 100nM) elicited, in a concentration-dependent manner, platelet aggregation, ATP secretion, elevation of $[Ca^{2+}]i$ and $[^{32}P]$-PtdA formation. The maximal extent of aggregation and of elevation of $[Ca^{2+}]i$ (~400nM above the basal level of 90 ± 3nM) induced by EP 171 and U44069 were similar. However, the maximum extent of ATP secretion (expressed as a % of that induced by Thrombin, 50nM) and of $[^{32}P]$-PtdA formation (expressed as fold stimulation of basal) elicited by EP 171, respectively 70 ± 12% and 8.0 ± 0.2 fold, exceeded those elicited by U44069, respectively 33 ± 12% and 3.6 ± 0.3 fold (results are mean values ± S.E., n = 3-7). Moreover, the effects of EP 171 (10 - 100nM) on ATP secretion and $[^{32}P]$-PtdA formation were attenuated by simultaneous addition of U44069 (3μM). The latter effect of U44069 was shared by 9,11-ethano-PGH_2 (30μM).

Thus, besides being more potent than U44069, EP 171 is also more effective than U44069 in inducing platelet ATP secretion and phosphoinositide hydrolysis. Furthermore, co-addition of U44069 mirrored the effects of co-addition of 9,11-ethano-PGH_2, a known partial agonist at the platelet TxA_2 receptor (Jones & Wilson, 1980), in suppressing the effects of EP 171. These observations might best be explained were EP 171 to act as a full agonist and U44069 to act as a partial agonist at TxA_2 receptors on human platelets.

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LEUKOTRIENE-INDUCED RELAXATION OF DOG MESENTERIC ARTERY *IN VITRO* IS ENDOTHELIAL CELL DEPENDENT

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Despite having potent pressor actions *in vivo*, few isolated blood vessels have been reported to contract in response to leukotrienes (Kito et al., 1981; Berkowitz, et al., 1984). Endothelial cells (EC) can modify blood vessel function by releasing a relaxant factor (Furchgott, 1984) and we have now investigated whether EC modify leukotriene (LT)-induced effects on the isolated mesenteric artery preparation.

Rings of dog superior mesenteric artery (approximately 5mm i.d.) were mounted for isometric recording (Edvinsson et al., 1974) and allowed to equilibrate for 2-3 hours in Krebs solution at 37°C. Resting tension was adjusted to 2g. Active tension was induced with an EC_{50} concentration of phenylephrine (PE; 1-5 x 10^{-6}M) and relaxation in response to acetylcholine (Ach) or LTC_4, LTD_4, LTE_4 and LTF_4 was investigated. The leukotriene antagonist, FPL55712 was preincubated with the tissues for 15 minutes before addition of LTC_4 or LTD_4. Where used, indomethacin was present throughout the time course of the experiment.

Acetylcholine produced almost complete relaxation of PE-induced tone, with an EC_{50} of 1.93 ± 0.26 x 10^{-8}M (mean ± S.E.M., n=22). LTC_4 and LTD_4 also produced relaxation with a maximum effect equivalent to 50-60% reversal of the PE-induced tone, with an EC_{50} of 8.12±1.55x10^{-7}M (mean ± S.E.M., n=8) and 1.40 ± 0.27 x 10^{-7}M (mean ± S.E.M., n=8) for LTC_4 and LTD_4 respectively. Both LTE_4 and LTF_4 were inactive up to 10^{-5}M. Indomethacin ($3x10^{-6}$M) had no effect on either Ach or LT-induced relaxation. Similarly, FPL55712 (10^{-5}M) or LTE_4 (10^{-5}M) had no effect on LTC_4 or LTD_4 responses in this tissue. Histological examination demonstrated that both Ach and LT-induced relaxation was dependent on the presence of EC. In the absence of a functional EC layer (i.e. lack of Ach-induced relaxation) LTC_4 or LTD_4 did not induce contraction.

This work shows that there are no LT receptors mediating contraction of the isolated dog mesenteric artery, even in the absence of a functional EC. There are, however, LT receptors located on EC which mediate relaxation. The endothelial cell-dependent relaxation may have relevance to LT action in man, where vasodilation has been observed (Kaijser, 1982). The inactivity of FPL55712 suggests that caution should be exercised when using this compound to implicate a role for LT in pathology and physiology.

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RELEASE OF PROSTAGLANDIN D_2 (PGD_2) FROM HUMAN SKIN IN VIVO DURING CUTANEOUS ANAPHYLAXIS

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PGD_2 has been shown to be released following stimulation of human lung mast cells in vitro (Holgate et al,1984) and by human nasal mucosa in vivo following antigen challenge (Naclerio et al,1985). Attempts to demonstrate PGD_2 release from human skin in cold urticaria, where mast cell degranulation is probably involved, have been unsuccessful although PGD_2 and histamine are elevated in the venous blood draining the cold challenged arm (Heavey et al,1985).

We studied the release of PGD_2 and prostaglandin E (PGE) from the skin of 5 atopic subjects who gave positive skin reactions to Dermatophagoides pteronyssinus (Dp) antigen (Pharmacia). Cylindrical acrylic chambers were attached to three $1cm^2$ abraded areas of skin on the thigh (Dowd et al,1983). Each site was washed twice for 5 min with 2ml of sterile Tyrode solution. The chambers were then filled with 1ml Tyrode solution and at 5 min intervals a $200\mu\ell$ aliquot was removed for PG analysis by radioimmunoassay. Fresh Tyrode solution was added to each chamber to maintain the volume at 1ml. At 25 min after the initial washes Dp antigen was added to give final concentrations of 200 and 1000 Biological Units (BU)/ml in two chambers, with Tyrode solution alone in the third chamber as control. Further $200\mu\ell$ aliquots were taken at 5 min intervals up to 50 min.

The five subjects gave wheal and flare reactions which were usually antigen-dose related, with little reaction at the control site. The mean flare areas at 10 min after challenge for control, 200 BU/ml and 1000 BU/ml sites were 2.0 ± 2.0, 21.7 ± 7.2 and 26.3 ± 3.6 cm^2 respectively ($\pm$ SEM). Release of PGD_2 varied widely between the subjects with four out of five showing increased PGD_2 at antigen challenged sites relative to control. Mean values (with ranges, n=5) for the increase in PGD_2 in the 25 min post-challenge period were: control site 107 pg (56-256), 200 BU/ml antigen 524 pg (106-1283) and 1000 BU/ml antigen 998 pg (181-2791). PGE measured in the same samples was unaffected by antigen challenge. 3 control, non-atopic, subjects challenged using the above procedure with 1000 BU/ml of Dp antigen showed no observable reaction and gave a mean 112 pg (range 85-132) PGD_2 in the 25 min post-challenge period.

The time course of PGD_2 release was further studied in 2 experiments using neonatal human foreskin slices in vitro, passively sensitised with reaginic serum (20% for 90 min at 37°C). Aliquots for PGD_2 radioimmunoassay were removed from skin slice incubations with and without Dp antigen (1000 BU/ml) at intervals up to 20 min. PGD_2 release was evident at 2 min after challenge, reaching 50% of maximum at 4 and 6.5 min in the 2 experiments, with net production at 20 min of 918 and 870 pg PGD_2/mg dry weight of skin.

This study shows that there is stimulated synthesis of PGD_2 after induction of immediate allergic reactions in human skin in vivo and in vitro. PGD_2 released in such reactions could contribute either directly or in synergism with other mediators to the inflammatory reaction.

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DO LEUKOTRIENES MEDIATE HYPOXIC PULMONARY VASOCONSTRICTION (HPV) IN DOGS?

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The mechanism of HPV remains unknown, but recently leukotrienes (LTs) have been proposed as potential mediators, primarily because HPV is inhibited in sheep, piglets and rats by the LT receptor antagonists FPL 55712 and FPL 57231, and also in rats by diethylcarbamazine and piriprost, which inhibit LT synthesis (Ahmed & Oliver, 1983; Morganroth et al, 1984). This was investigated further in dogs.

Chloralose-anaesthetised dogs (3-4 per group) were ventilated artificially and instrumented to record pulmonary arterial pressure (Ppa), pulmonary arterial wedge pressure (Ppaw), cardiac output (CO, by thermodilution via Swan-Ganz catheter), systemic arterial pressure, heart rate, pulmonary mechanics (R_L & C_{dyn}), arterial blood pH, PO_2 and pCO_2. Pulmonary and systemic vascular resistances (PVR & SVR) were calculated. At intervals, hypoxia was induced by breathing 10% O_2 in N_2 for at least 10min. After two consistent vasoconstrictor responses, groups were treated i.v. as follows: (a) FPL 57231 at $1mg.kg^{-1}.min$ followed by $2mg.kg^{-1}.min$ each for 45 min. (b) phenidone $10mg.kg^{-1}$ followed by indomethacin $1mg.kg^{-1}$ and then FPL 57231 $2mg.kg^{-1}.min$. (c) FPL 57321 $2mg.kg^{-1}.min$ after cervical vagosympathectomy and adrenoceptor blockade (propranolol $0.25mg.kg^{-1}.h$ and phentolamine $1mg.kg^{-1}$ before each hypoxic challenge).

Hypoxic challenge produced severe hypoxaemia (Table 1) without changes in pCO_2, pH, R_L or C_{dyn}. HPV was inhibited by FPL 57231 at the higher dose only. Neither HPV nor the response to FPL 57231 was affected by phenidone, indomethacin or vagosympathectomy. In two dogs LTD_4 ($1-10\mu g.kg^{-1}$) was given directly into the pulmonary artery without effect.

Although FPL 57231 clearly inhibits HPV, it is considered unlikely that LTs are involved because of the high dose of FPL 57231 required, the ineffectiveness of phenidone at a dose considered sufficient to inhibit lipoxygenase, and the relative insensitivity of canine pulmonary vasculature to LTs. The mechanism of action of FPL 57231 in this model remains unknown.

Table 1. Peak changes induced by hypoxia (mean ± s.e.mean)
* $p<0.05$ compared with control.

	Control	FPL 57231 $1 mg.kg^{-1}.min$	FPL 57231 $2 mg.kg^{-1}.min$
pO_2 (mmHg)	-59.6 ± 2.6	-50.3 ± 2.9	-47.5 ± 2.5
CO ($l.min^{-1}$)	0.21 ± 0.13	0.37 ± 0.20	0.26 ± 0.14
Ppa (mmHg)	16.5 ± 1.7	15.0 ± 1.2	4.3 ± 0.6*
PVR ($mmHg.l^{-1}.min$)	9.6 ± 2.1	7.7 ± 1.7	0.8 ± 1.0*
SVR ($mmHg.l^{-1}.min$)	1.1 ± 7.2	8.4 ± 12.5	13.2 ± 17.1

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INHIBITION OF HYPOXIC PULMONARY VASOCONSTRICTION (HPV) IN RATS.

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The possible role of leukotrienes (LTs) in the HPV response is receiving increasing attention following experiments in rats, ferrets, sheep, piglets and dogs. In rats, HPV is inhibited by the LT receptor antagonist FPL 55712 and also by diethylcarbamazine and piriprost which inhibit leukotriene synthesis (Morganroth et al, 1984a). Furthermore LTC_4 has been detected in lung lavage fluid from rats undergoing HPV (Morganroth et al, 1984b). These observations have been extended in the present study using the LT receptor antagonist FPL 57231 and phenidone, a lipoxygenase inhibitor.

Male Wistar rats (200-300g) were anaesthetised with pentobarbitone (60mg kg^{-1} i.p.) and their lungs perfused in situ with blood as described by Emery et al (1981). The rate of perfusion was initially adjusted to achieve a mean pulmonary arterial pressure (Ppa) of 10-15mmHg. The lungs were ventilated with room air, resulting in a blood pO_2 >100mmHg. HPV was induced by ventilating with 5% CO_2 in N_2 (reducing pO_2 to approximately 35mmHg) until the increase in Ppa had stabilised, typically 5-10 min. The lungs were then ventilated with room air for a further 10 min before the next hypoxic challenge. After 3 consistent HPV responses, vehicle or test compound was added to the blood 4 min before the next hypoxic challenge. Further doses were added later in a cumulative fashion as necessary. HPV responses varied between animals, and so were expressed as a percentage of the mean of the 3 pretreatment responses.

Diethylcarbamazine citrate (DEC) at a single concentration of 2.5mM inhibited HPV by approximately 50%, as reported by Morganroth et al (1984a). However, this concentration of DEC induced a pressor response itself, equivalent to 18% of the control HPV response. FPL 57231 at 1mM significantly inhibited HPV; lower concentrations had little effect. This is approximately 10,000 times the concentration required to antagonise LTD_4 on guinea pig ileum. Phenidone at 2.5mM abolished HPV, but lower concentrations had no significant effect. In various intact cell systems phenidone has been shown to virtually abolish LT synthesis at concentrations below 100μM (eg. Boot et al, 1985).

These observations do not support the concept that LTs are involved in the production of HPV in rats.

Table 1. HPV responses (as % of mean pretreatment response). mean ± s.e. mean
n = 5-7 per group.
* p <0.05 compared with saline

saline	FPL 57231 (1mM)	saline	DEC (2.5mM)	saline	phenidone (2.5mM)
94.2 ± 14.6	36.8 ± 9.0*	99.7 ± 17.2	41.6 ± 6.8*	106.1 ± 19.8	0 ± 0*

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VENTRICULAR ARRHYTHMIAS INDUCED BY LOCAL INJECTIONS OF VASOCONSTRICTORS FOLLOWING CORONARY OCCLUSION

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Noradrenaline, thromboxane and the leukotrienes are all potent vasoconstrictors *in vivo* and studies with LTC_4 and LTD_4 have shown that these leukotrienes are capable of causing constriction when injected directly into the coronary circulation of dogs (Fiedler *et al.*, 1984). The aim of this study was to determine whether such vasoconstrictor agents induced ventricular arrhythmias when injected directly into the ischaemic myocardium.

Greyhounds of either sex were anaesthetised with chloralose and prepared for occlusion of the left anterior descending coronary artery (LAD). Catheters were placed in the femoral artery, pulmonary artery and left ventricular cavity for the measurement of systemic arterial pressure, pulmonary artery pressure and left ventricular pressure and dP/dt_{max} respectively. Immediately following occlusion of the LAD a catheter was placed in that coronary artery for measurement of peripheral coronary pressure (PCP; Marshall and Parratt, 1980). LTC_4, LTD_4, U46619 (the stable thromboxanemimetic) and noradrenaline were injected directly into the occluded artery commencing 30 min after the onset of ischaemia. The severity of arrhythmias that occurred following each drug dose was assessed using a semi-quantitative scoring system.

The rank order of potencies for increasing PCP was noradrenaline> U46619> LTC_4= LTD_4. Neither of the leukotrienes (1-20 µg) produced any other general haemodynamic responses. The noradrenaline responses (0.01-0.1 µg) were accompanied by a significant rise in systemic arterial pressure and an increase in myocardial contractility. U46619 (0.1-10 µg) did not cause any changes in systemic pressure but elicited a rise in pulmonary artery pressure. Noradrenaline, LTC_4 and U46619 all caused some arrhythmic activity, the severity of which was dose related. Despite its ability to produce a dose-related coronary vasoconstriction LTD_4 induced arrhythmias only at very high doses (20 µg).

The results of this study show that noradrenaline, LTC_4, LTD_4 and U46619 all induce changes in perfusion pressure when administered directly into the ischaemic areas of the left ventricular wall and that the arrhythmias seen during administration may to some extent be related to these.

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THE EFFECTS OF PROSTAGLANDINS ON UNIDIRECTIONAL SODIUM FLUXES IN THE ILEUM OF THE ANAESTHETISED RAT

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It has previously been shown that prostaglandins stimulate the net secretion of sodium in rat ileum (Bunce & Spraggs, 1982). However, such a secretion of sodium may be caused either by increased unidirectional flux from plasma to lumen (secretion), by decreased unidirectional flux from lumen to plasma (absorption), or a combination of both effects. In the present investigation we have compared the effects of 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on these unidirectional fluxes of sodium in rat ileum *in vivo*.

Female rats weighing 100-120g were anaesthetised with pentobarbitone (50mg/kg i.p.) and treated with indomethacin (14μmol/kg s.c.) to inhibit endogenous prostanoid formation. An 8-10cm segment of terminal ileum was prepared in each rat, and 0.3ml of isotonic NaCl solution containing ^{22}Na (1mCi/mol) instilled into its lumen; prostaglandins were added to this instillate as required. After 30min the intraluminal contents were collected, the net fluid flux was measured gravimetrically, the net flux of sodium was measured by flame photometry, and the unidirectional sodium absorption was measured from the flux of ^{22}Na as described by Matuchansky et al. (1972). The unidirectional secretion of sodium was then calculated from the following relationship:-
Net sodium flux = unidirectional secretion - unidirectional absorption.

The results are shown in Table 1.

Table 1. The effects of $dmPGE_2$ and $PGF_{2\alpha}$ on sodium fluxes in rat ileum.

	Sodium fluxes (μEq/30min)					
	$dmPGE_2$			$PGF_{2\alpha}$		
Prostanoid dose (μmol/kg)	net[a]	US[b]	UA[c]	net[a]	US[b]	UA[c]
0	-18.4±1.8	58.9± 2.4	77.3±2.5	-14.3±2.7	69.1±2.7	83.5±2.5
0.3	+ 6.6±2.1*	69.7± 3.1*	63.1±3.1*	-	-	-
0.1	+31.4±2.1*	96.2± 4.5*	64.9±3.8*	-	-	-
0.3	+42.3±6.7*	110.2± 6.4*	68.0±2.4*	-	-	-
1	+54.8±9.2*	116.0±11.0*	61.1±2.8*	-	-	-
3	-	-	-	- 0.7±2.0*	75.8±3.3	76.5±3.5
10	-	-	-	+13.8±2.9*	75.7±3.3	61.9±2.0*
30	-	-	-	+35.3±5.5*	57.7±6.1	22.4±1.1*

[a] For net fluxes : + = net secretion, - = net absorption.
[b] US = unidirectional secretion. [c] UA = unidirectional absorption.
* $P<0.05$ by unpaired t-test. Results expressed as mean ± s.e. mean. n = 6-12.

These results show that although both prostaglandins stimulated net secretion of sodium, they achieved this effect through different mechanisms. $DmPGE_2$ caused a small inhibition of unidirectional absorption but a marked stimulation of unidirectional secretion, whereas $PGF_{2\alpha}$ had no effect on the latter parameter but markedly inhibited unidirectional absorption.

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ELECTROPHYSIOLOGICAL EFFECTS OF LEUKOTRIENE D_4 IN GUINEA PIG AIRWAY SMOOTH MUSCLE

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Leukotriene (LT)D_4-induced contractions of airway smooth muscle from both guinea pigs and man are resistant to reductions in extracellular Ca^{2+} concentrations, are poorly antagonised by Ca^{2+} channel blocking drugs and are not associated with increases in the La^{3+}-resistant Ca^{2+} content of the smooth muscle cells (Raeburn & Rodger, 1984; Roberts et al, 1985; Raeburn et al, 1986). These data suggest, therefore, that LTD_4 utilises principally an intracellular source of Ca^{2+} to initiate contraction. Electrophysiological effects of LTD_4 have not been described. In this study we have compared the effects of LTD_4 on resting membrane potential, slow wave discharge and tension development in guinea pig trachealis with those of methacholine and KCl.

Segments of trachea were opened and pinned to the base of a recording chamber. Single smooth muscle cells were impaled using glass microelectrodes (resistance 60-80MΩ filled with 0.5M KCl). Resting membrane potential and slow wave characteristics were recorded either in a single cell during exposure to the agonists or in a number of cells sampled before and after drug treatment. Changes in isometric tension were recorded separately using single ring preparations of trachea from a segment adjacent to that used for electrophysiological studies. Both sets of experiments were performed in Krebs-Henseleit solution at 37^0C containing flurbiprofen ($1\mu mol\ l^{-1}$).

LTD_4 ($0.1\text{-}500nmol\ l^{-1}$) induced concentration-dependent contractions of the isolated trachealis. Maximum contraction amounted to 82.6 ± 5% (mean ± s.e.mean, n=5) of the methacholine-induced maximum (at $0.1mmol\ l^{-1}$). Mean EC50 value for LTD_4 was 6.7 ± 2.1nmol l^{-1}.

Table 1. Effects of LTD_4 on resting membrane potential (Em) and electrical slow wave characteristics

Drug concentration	Em(mV)	Slow wave amplitude(mV)	Slow wave frequency(Hz)
Control	-40.0 ± 0.7 (102)	5.3 ± 0.4 (96)	0.69 ± 0.02 (55)
$5nmol\ l^{-1}$	-38.1 ± 0.5 (42)	5.4 ± 0.7 (26)	0.77 ± 0.04 (21)
$100nmol\ l^{-1}$	-33.0 ± 0.5* (132)	2.7 ± 0.3* (56)	0.96 ± 0.02* (41)

Figures in parenthesis indicate the number of cells impaled in 5 separate experiments.
* indicates significant difference from control values, $p < 0.001$.

The data of Table 1 show that LTD_4 at a concentration close to its EC50, calculated in tension studies, produced no significant electrophysiological changes. In contrast, LTD_4 ($100nmol\ l^{-1}$; EC90) depolarised the cells but to a significantly lesser extent than either methacholine or KCl. Slow wave discharge was altered similarly by all three agonists and abolished at high concentrations.

These results show that contraction of guinea pig airway smooth muscle elicited by LTD_4 may occur independent of changes in membrane potential and are thus consistent with the view (Raeburn & Rodger, 1984) that LTD_4 mobilises Ca^{2+} from an intracellular source to initiate contraction.

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400P

IS ASPIRIN A PRO-DRUG FOR SALICYLATE?

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The anti-inflammatory potency of aspirin and salicylate is similar but aspirin is considerably more potent in inhibiting prostaglandin synthesis in vitro (Vane, 1971). This has led to speculation that the mechanism of anti-inflammatory action of these drugs is not related to inhibition of prostaglandin synthesis (Smith, 1975). We have now investigated the pharmacokinetics of these drugs in relation to their effects on prostaglandin (PG)E_2 production in experimental inflammation.

Inflammatory exudates were collected 6h after the subcutaneous implantation of carrageenin soaked polyester sponges in rats (Higgs and Salmon, 1979). Aspirin (200mg/kg) or salicylate (200mg/kg) was administered orally 5-360 min before sponge removal and the levels of both these drugs in plasma and exudate were determined using high pressure liquid chromatography (Lo and Bye, 1980). PGE_2 in the exudate was measured by specific radioimmunoassay (Higgs and Salmon, 1979).

Plasma levels of aspirin were highest 5 min after dosing (2.3±1.1µg/ml; mean± s.e.mean;n=5) then declined to undetectable levels after 120 min. Exudate levels of aspirin peaked at 30 min (2.0±0.5µg/ml) but were undetectable at 360 min. After administration of aspirin, the levels of salicylate in plasma (127±10µg/ml) and exudate (73±10µg/ml) were highest 120 min after dosing; these concentrations decreased by approximately 50% after 360 min. After administration of salicylate, aspirin was not detected in the plasma or exudate but salicylate levels in plasma peaked at 10 min (149±11µg/ml) and remained constant up to 360 min, whilst salicylate levels in the exudate increased steadily reaching a maximum 360 min after dosing (160±14µg/ml).

Inflammatory exudates from control animals contained 25.6±2.8ng PGE_2/ml and exudates collected 10-360 min after administration of either aspirin or salicylate contained significantly less. With aspirin, PGE_2 levels were reduced to 43.9±4.0% of control values 120 min after dosing and with salicylate to 21.9±1.9% 360 min after dosing. The greatest effect of each drug coincided with peak salicylate levels in the exudate.

In a separate series of experiments the inflamed tissue around the sponge implants was removed and maintained in non-proliferative culture (Poulter et al, 1970) in the presence of aspirin (0.25-25µg/ml) or salicylate (2.5-100µg/ml). The production of PGE_2 by these explants was inhibited dose-dependently by aspirin (IC_{50}=3.9µg/ml) and the potency of salicylate was surprisingly similar (IC_{50}=8.5µg/ml).

The low levels of aspirin and high levels of salicylate in the sponge exudate suggest that aspirin is a pro-drug for salicylate (Dreser, 1899) and that the anti-inflammatory activity of both drugs is through the inhibition of PGE_2 synthesis by salicylate.

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CARTILAGE DEGRADATION IN A MOUSE AIR POUCH MODEL

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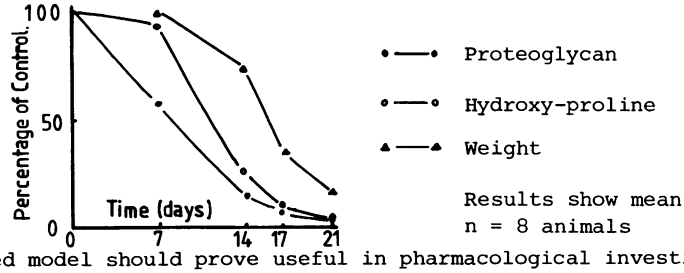
Cartilage destruction is the major pathological consequence of rheumatoid arthritis and involves loss of both major structural components of the tissue, proteoglycan and collagen. The assessment of cartilage damage in animal models of arthritis is usually made by histological or radiological techniques which are complex and only semi-quantitative. A novel in vivo model of studying cartilage destruction has been described (Sin et al, 1984, Sedgwick et al, 1985) which involves implanting cartilage into sub-cutaneous tissues. Recently De Brito et al (1985) have demonstrated that a granulomatous reaction around the cartilage, induced by a cotton pellet, enhances the rate of proteoglycan loss in this model. We have extended these findings to include measurement of the collagen content and weight of the cartilage.

Femoral head cartilage was removed from male Sprague-Dawley rats (150-200g), weighed, and if required wrapped in cotton pellets (5mg). The cartilage was implanted into an air pouch formed six days earlier on the dorsal surface of female Charles River CD-1 mice (20-25g) (Sin et al, 1984). The cartilage was removed 7-21 days later, weighed, and the proteoglycan content and hydroxy-proline levels (as an index of collagen content) determined by the methods of Farndale et al (1982) and Berg et al (1982) respectively.

Rat femoral head cartilage implanted into the mouse air pouch in the absence of a cotton pellet lost proteoglycan (-13, -21 and -30% at 7, 14 and 21 days respectively) but not hydroxyproline (-2% at 21 days) nor weight (+12% at 21 days, n=8 animals). Cartilage wrapped in cotton to induce granuloma formation not only lost proteoglycan at a faster rate, confirming the results of De Brito et al (1985), but also lost hydroxy-proline and weight (Figure). Significant proteoglycan loss occurred prior to hydroxy-proline or weight loss which agrees with the results of studies on cartilage degradation in tissue culture (Saklatvala & Dingle, 1980)



This modified model should prove useful in pharmacological investigations of drugs designed to prevent cartilage erosion since both proteoglycan and collagen degradation can be measured.

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GRANULATION TISSUE INDUCED BT CROTON OIL - A SUITABLE MODEL FOR WOUND HEALING?

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In a previous communication it was noted that 5-hydroxytryptamine (5-HT) had no effect on the myofibroblast population present in rat testicular capsule (Lal & Naylor, 1985). This finding was unexpected due to the reported sensitivities of this cell type to 5-HT which has been reported for other myofibroblast containing tissues (Majno et al, 1971; Gabbiani et al, 1972; Ryan et al, 1974; Baker et al, 1981; Garcia-Valdecasas et al, 1981). One reason for this lack of sensitivity could have been that such cells were only present in animals undergoing an inflammatory response of a severe nature and consequently were absent in 'natural' population of myofibroblasts. To investigate such a possibility, three groups of rats (Bradford strain, 390±11g) were used. Group 1 control, Group 2 subcutaneous (s.c.) implantation of perspex rods (5 x 20 mm) and Group 3 Selye's technique. At given times (see table) the rat testicular capsule and granulation tissue, where present, was removed and tested in vitro as previously described (Lal & Naylor, 1985). Concentration response curves were constructed for three agonists: (n=6 in all groups)

Group	Testicular Capsule			Tissue	Granulation tissue			Tissue
	5-HT*	M**	D***	Wt.(g)	5-HT*	M**	D***	Wt.(g)
1 Control	0	↑	↑	0.09±0.01	-	-	-	-
2 s.c.perspex rods (14-28) days	0	↑	↑	0.11±0.01	0	↗	↗	0.24±0.1
3a Selyes pouch (10-20 days)	↑	↑	↑	0.11±0.01	↗	↗	↗	3.54±0.9
b Pouch absent (10-20 days)	↑	↑	↑	0.10±0.02	-	-	-	-

5-HT (2.83×10^{-4} - 2.27×10^{-3}M), **Mepyramine (1.75×10^{-5} - 1.4×10^{-4}M), ***Diphenhydramine (1.96×10^{-5} - 1.57×10^{-4}M), 0 = insensitive at dose levels tested (2.83×10^{-4} - 2.27×10^{-3}M), ↑ contractile response and ↗ contractile response but at different concentration range to testicular capsule.

In all samples examined the granulation tissue from the perspex rods (Group 2) was insensitive to 5-HT and the testicular capsules had sensitivities identical to control animals. In contrast spiral strips (3 x 20 mm) of granulation tissue (group 3a) were responsive to 5-HT in a dose dependent fully reversible manner. In the croton oil treated animals (group 3a) the testicular capsule also responded to 5-HT. This finding was also made in animals lacking pouches (group 3b) which suggests that croton oil may produce a stimulating effect on myofibroblasts present in sites distant from the injection site by either direct or indirect means. The nature of such 'transformed' myofibroblasts is presently being further investigated from electron microscopical and histochemical view points.

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