[^{125}I]iodoLSD LABELS A 5-HT$_2$ RECEPTOR IN HUMAN PLATELET MEMBRANES SIMILAR TO THAT IN FRONTAL CORTEX

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5-HT receptors on human platelets mediate shape-change and aggregation responses and have previously been labelled by the non-selective ligand [^{3}H]-LSD (lysergic acid diethylamide) (Geaney *et al.*, 1984). Stimulation of these receptors by 5-HT induces breakdown of membrane polyphosphoinositides (de Chaffoy de Courcelles *et al.*, 1985), the second messenger associated with 5-HT$_2$ and 5-HT$_{1c}$ receptors. [^{125}I]-iodoLSD selectively labels the 5-HT$_2$ and 5-HT$_{1c}$ receptors although the binding in human frontal cortex appears to be exclusively to 5-HT$_2$ receptors (Elliott & Kent, 1987). This study describes the binding of [^{125}I]-iodoLSD to human platelet membranes and the comparison to binding in human frontal cortex in an attempt to further characterise the 5-HT receptor sub-type found on human platelets.

Human platelet membranes were prepared by a method similar to that of Geaney *et al* (1984). Tissue was incubated with [^{125}I]-iodoLSD at six concentrations in the range 60-600 pM for 90 min at 37°C. Non-specific binding was defined as that oberved in the presence of 1μM ketanserin. Incubations were terminated by rapid filtration through Whatman GF-B filters.

Specific binding of [^{125}I]-iodoLSD to human platelet membranes identified a single site of high affinity (K_d = 0.34 ± 0.03 nmol/l) and low capacity (B_{max} = 49.9 ± 5.5 fmol/mg protein, n=6). This is similar to the binding capacity previously identified for [^{3}H]-LSD (57.1 ± 5.6 fmol/mg protein, Geaney *et al*, 1984), suggesting that the iodinated and tritiatd ligands label the same receptor population on human platelet membranes.

Displacement of specific [^{125}I]-iodoLSD binding by serotonergic agonists and antagonists occurred with the order of potency spiperone > ketanserin > methysergide > mianserin > quipazine > 5-HT > RU24969 > 80H-DPAT. Non-serotonergic compounds showed low affinity for this binding site. Comparison of the IC_{50} values for inhibition of [^{125}I]-iodoLSD binding and [^{3}H]-LSD binding to human platelet membranes showed a significant positive correlation ($r = 0.88$, $p<0.001$, n=12). Similar comparison of IC_{50} values for inhibition of [^{125}I]-iodoLSD binding to membranes from human platelets and human frontal cortex also showed a significant positive correlation ($r = 0.96$, $p<0.001$, n=16).

These results demonstrate that the site previously identified by [^{3}H]-LSD on human platelet membranes is also labelled by the iodinated derivative [^{125}I]-iodoLSD. Comparison of the inhibition of [^{125}I]-iodoLSD in human platelet and frontal cortex membranes indicates that this site closely resembles the 5-HT$_2$ sub-type of receptor. The high specific activity of the iodinated ligand, coupled with the low receptor density, suggest that [^{125}I]-iodoLSD may be particularly useful to quantify 5-HT$_2$ receptors on human platelets in clinical studies.

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CORRELATION OF [^{125}I]-LSD BINDING AND 5-HT INDUCED SHAPE CHANGE IN HUMAN PLATELETS

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Pharmacological evidence suggests that binding of the 5-HT receptor ligands ^{125}I-LSD and ^{3}H-Ketanserin to blood platelets is analogous to that seen at central 5-HT$_2$ receptors(Leysen et al, 1983). Furthermore, the platelet shape change and subsequent aggregation induced by 5-HT may also be a 5-HT$_2$ mediated event, so providing a functional assay for this receptor subtype (de Clerk et al, 1984). Unfortunately the human platelet response to 5-HT is weak and reversible and the traces produced by commercially available aggregometers make the determination of shape change inaccurate. Many studies have therefore resorted to the 5-HT amplification of aggregation induced by low concentrations of ADP to measure 5-HT$_2$ receptor function (McBride et al, 1987). The present study describes the advantage of using analogue to digital conversion (ADC) to determine platelet shape change alone. The correlation of 5-HT induced shape change with ^{125}I-LSD binding in human platelets has also been determined.

Blood was taken from healthy, drug-free volunteers. Platelets for shape change determination were pre-incubated (37^{o}, 30 min) and 500 ul samples (1-2 x 10^{8} platelets) were assayed within 2 hrs. Platelet shape change was measured in a Bio-Data Corporation PAP-4 platelet aggregometer, connected via the ADC port to an IBM PC. The ADC translated the percentage aggregation into numerical form which was then stored as BASIC computer data. Maximum platelet shape change was calculated before and after addition of the test drug to the platelet suspension. 5HT induced platelet shape change in a dose-dependent manner, (EC_{50} 1.8 uM) and IC_{50} values for a range of compounds were obtained by adding drug 5 mins prior to addition of 5-HT (3 uM). Binding indices were measured by a microassay using ^{125}I-LSD (2nM) and ketanserin (10 uM) to define specific binding. 6 x 10^{6} platelets were routinely added per tube. A single receptor population was found, characterised by a K_D of 1.10 $\pm$ 0.12 nM and Bmax of 14.5 $\pm$ 6.0 pm/g protein.

IC_{50} values for inhibition of ^{125}I-LSD platelet binding and 5-HT induced shape change are shown below. Competition binding studies confirmed that ^{125}I-LSD labelled a site characteristic of the 5-HT$_2$ receptor. The significant correlation of the two IC_{50} parameters validates the measurement of platelet shape change by the method described, as a functional assay for the platelet 5-HT$_2$ receptor.

DRUG	SHAPE CHANGE IC_{50} (uM)	^{125}I-LSD BINDING IC_{50} (uM)
Spiperone	0.010	0.022
Methysergide	0.011	0.060
LSD-25	0.020	0.030
Ketanserin	0.056	0.250
RU24969	0.070	1.0
MK212	0.4	1.93
5-HT	0.8	24.8
Mescaline	1.1	10.0
Mianserin	1.3	0.047
8-OH-DPAT	7.0	8.85
Alaproclate	10.5	10.8
Sulpiride	>100	>100

Correlation coefficient $r = 0.70$ $t = 2.95$ $p < 0.02$ $n = 2\text{-}5$

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COMPARISON OF BENZYLAMINE, N,N-DIMETHYLPHENETHYLAMINE AND N,N-DIMETHYLTYRAMINE AS SUBSTRATES FOR MONOAMINE OXIDASE B

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Monoamine oxidase (MAO) occurs in two forms with different substrate specificities. 5-Hydroxytryptamine (5HT) is highly selective for MAO-A and benzylamine (BZ) for MAO-B, whilst both deaminate tyramine (Fowler et al, 1981). In addition to BZ some tertiary amines are selective substrates for MAO-B (Barwell, 1985, Inoue et al, 1985). We have compared the selectivity of N,N-dimethylphenethylamine (DMP) and N,N-dimethyltyramine (DMT) with BZ, in order to assess the tertiary amines as substrates for the assay of MAO-B.

Mitochondria were isolated from livers of male Wistar rats (200-250g) by homogenisation in 0.3M sucrose and differential centrifugation. Human placenta was washed free of blood and the membrane and major blood vessels removed. Tissue was homogenised in 0.05M phosphate pH 7.4. The material sedimented at 10,000xg for 20 min was washed twice and used as placental MAO. MAO activity was measured at pH 7.4 and 37°C with an oxygen electrode. The selective irreversible inhibitor of MAO-A, Lilly 51641 (Fuller, 1968), was used to determine which form of rat liver MAO utilised BZ, DMP and DMT. Mitochondria were incubated at 37°C for 15 min with Lilly 51641, after which the activity remaining was assayed with 1mM BZ, 0.2mM DMP, 5mM DMT and 5mM tyramine. Values are mean ± S.E.M., n=4.

The MAO of rat liver mitochondria exhibited similar specific activities with DMP and DMT (114 ± 20, 128 ± 10 nmol.(mg protein)$^{-1}$, h^{-1} respectively) which were half that with BZ (250 ± 20 nmol.(mg protein)$^{-1}$, h^{-1}). The Michaelis constant for DMP (23 ± 2 µM) was six times lower than for BZ (149 ± 20 µM) and twenty times lower than for DMT (479 ± 40 µM). Inhibition studies with Lilly 51641 yielded a biphasic inhibition curve with a distinct plateau between $5x10^{-7}$M and $5x10^{-6}$M inhibitor with tyramine as substrate and showed that the mitochondria contained MAO-A and MAO-B activity, in the proportion 42% MAO-A to 58% MAO-B. With BZ, DMP and DMT as substrate, no inhibition occurred at Lilly 51641 concentrations which selectively inhibited MAO-A. However, a monophasic curve was obtained at concentrations which inhibited MAO-B. The preparation of human placenta MAO had no detectable activity with BZ (1mM), DMP (0.2mM) and DMT (5mM) under conditions where the activity with 1mM 5HT was 376 µM, h^{-1}, in the assay.

The results show that DMP and DMT are as selective for rat liver MAO-B as BZ and that they are apparently not substrates for human placenta MAO-A. BZ is normally used to assay MAO-B but is also an excellent substrate for semicarbazide sensitive amine oxidase (SSAO) which like MAO occurs in most mammalian tissues (Callingham & Barrand, 1987). Since N-methylated amines are not substrates for SSAO either DMP or DMT could be used to assay MAO-B in preparations which also contain MAO-A and SSAO.

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CHANGES IN EXTRACELLULAR DOPAC AND 5HIAA AFTER ELECTRICAL STIMULATION OF THE RAT DORSAL RAPHE OR SUBSTANTIA NIGRA

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In an earlier pharmacological study we demonstrated the capacity of in vivo differential pulse voltammetry (DVP), with specifically electrically pretreated carbon fibre electrodes, to detect interactions between dopamine (DA) and serotonin (5HT) systems in the rat striatum in vivo (Crespi, 1986) via the simultaneous detection of their respective extracellular catabolites 3,4-dihydroxyphenylacetic acid (DOPAC: Peak 2 at +100 mV) and 5-hydroxyindoleacetic acid (5HIAA: Peak 3 at +300 mV) (Crespi et al, 1984).

In the present study we electrically stimulated (1V, 5V or 10V at 20 Hz for 10 mins) the dorsal raphe (DRN), rich in DA cell bodies, of anaesthetised (500 mg/kg ip chloral hydrate) rats. Changes in striatal extracellular DOPAC and 5HIAA were monitored using DPV (Crespi et al, 1984). No significant effect on either of these acids were observed when the nuclei were stimulated at 1 or 5V. However, stimulation of the DRN at 10V produced a rise in striatal 5HIAA during the period of stimulation, reaching significance (compared to sham stimulated controls n = 5) 20 mins after the end of stimulation, with a maximum increase of 169 $\pm$ 19% (n = 5 $\pm$ SD). Extracellular DOPAC in contrast decreased to 57 $\pm$ 7% of control values, but the decrease started only 20 mins after the end of the stimulation of the DRN, and was significant 50 mins later. Previous studies have shown that electrical stimulation of raphe 5HT neurones increases tryptophan hydroxylase activity and the synthesis and release of 5HT while the time-course of the present increase in the level of 5HIAA corresponds to that of enzyme activation (Boadlebiber et al, 1986), suggesting that the change in extracellular 5HIAA is related to the release of 5HT. DRN stimulation decreased extracellular DOPAC in the striatum, but this was later than the rise in 5HIAA, indicating that an increase in 5HT release initiates a process which results in decreased DOPAC production, supporting the DA-5HT interaction model in which activation of 5HT neurones reduces DA release in striatum (Crespi, 1986; Faull et al, 1984).

Conversely, stimulation of the SN (10 V) increased striatal extracellular DOPAC (+20 $\pm$ 8%, n = 5, at the end of the stimulation), while extracellular 5HIAA started to decrease 35 mins after the end of the period of stimulation (-23% at 120 mins). These results indicate a reciprocal effect following stimulation of the SN compared to DRN stimulation, with an increase in striatal DA release inhibiting 5HT release and metabolism.

In conclusion, this study provides in vivo evidence that stimulation of 5HT cell bodies (DRN), with increased 5HT release, inhibits striatal DA metabolism while stimulation of DA cell bodies (SN) decreases striatal 5HT metabolism. It remains to be determined which of the DA and 5HT receptor sub-types are involved in the reciprocal regulation of the release and metabolism of these two neurotransmitters.

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IS ROTATIONAL BEHAVIOUR IN THE RAT A 5-HT_{1A} OR 5-HT_{1B} MEDIATED RESPONSE?

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5-HT_1 agonists produced contralateral rotation in rats with unilateral 5,7-DHT lesions of the dorsal raphé nucleus (DRN, Blackburn et al., 1984a). Both 8-OH-DPAT and RU24969, agonists for the 5-HT1A and 5-HT1B receptor subtypes respectively, induced comparable rotational responses which implies that they act through a common 5-HT receptor. 5-HT_1 receptors, measured by the binding of [^{3}H]5-HT, showed a 3-fold increase in the substantia nigra (SN) on the lesioned side (Blackburn et al., 1984a, & b). In this study, we have attempted to characterise the 5-HT_1 receptor subtype involved in the turning response.

Brains were removed form rats with a unilateral 5,7-DHT lesions in the DRN and which turned consistently to 8-OH-DPAT (2mg.kg s.c.; >200 turns/2h). The brains were frozen, sectioned and the glass-mounted sections were incubated in Tris HCl buffer (170 mM, pH 7.6, 30 min). This was followed by incubation for 1 h in buffer containing 4mM $CaCl_2$, 0.01% asorbic acid and 2nM (propyl-[^{3}H]-8-OH-DPAT at room temmerature. The sections were washed, dried and exposed to tritium film (4 weeks). Specific binding, defined by the addition of 1μm 5-HT_1 averaged 95%.

The autoradiographs showed high binding of [^{3}H]8-OH-DPAT in several brain areas, including the hippocampus and dorsal raphé nucleus. By contrast, there were no detectable 5-HT_{1A} sites in the striatum, globus pallidus or SN on either the lesioned or the non-lesioned side of the brain. Thus, the increased [^{3}H]5-HT binding arising in the SN after 5,7-DHT lesion of the DRN (Blackburn et al., 1984b) cannot be explained by a proliferation of the 5-HT_{1A} receptor subtype. Others have reported that the SN contains 5-HT_{1B} sites (Pazos et al., 1984; Vergé et al., 1986), although following a 5,7-DHT lesion both a loss and an increase in these receptors were found (Verge et al., 1986; Weissman et al., 1986). Ligand binding studies with striatal membranes have revealed a lower affinity site for [^{3}H]8-OH-DPAT (Gozlan et al., 1983; Hall et al., 1985) which cannot be detected autoradiographically (Vergé et al., 1986). Thus the circling response to the 5-HT_{1A} agonist 8-OH-DPAT might be explained by activation of supersensitive low affinity 5-HT_{1A} type binding sites in the SN or in other parts of the basal ganglia. Further studies are in progress to clarify the 5-HT_1 receptor subtype involved in the 8-OH-DPAT behavioural response.

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SEROTONIN-STIMULATED PHOSPHATIDYLINOSITOL METABOLISM IN THE IMMATURE RAT BRAIN: PHARMACOLOGICAL CHARACTERISATION

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It has been demonstrated recently that serotonin (5-HT) or 5-HT agonists stimulate inositol phospholipid hydrolysis in the adult rat CNS and that 5-HT_2 receptors mediate this biochemical response in the cerebral cortex (Kendall and Nahorski, 1985; Conn and Sanders-Bush, 1985). With some neurotransmitters (e.g. glutamate or carbachol) known to stimulate brain phosphatidylinositol turnover, the intensity and pharmacology of this biochemical response varies as a function of postnatal age (Nicoletti et al,1986). The recognition of this phenomenon prompted us to characterize the effects of 5-HT on phosphoinositide hydrolysis in the immature (8 day-old) rat brain.

In the presence of LiCl (5 mM), 5-HT caused a marked incrase in total ^{3}H-inositolphosphate levels in cortical slices (maximal effect + 420%, EC_{50} = 7 µM)and a less pronounced increase in hippocampal and striatal slices preprared from the immature rat; the cortical 5-HT-induced phosphoinositide response was tetrodotoxin resistant. The maximal increase in the phosphoinositide response caused by 5-HT was 6 fold higher in the immature than in the adult cortex.

After incubation of immature rat cortical slices for 2.5 min with 100 µM 5-HT (in the absence of LiCl), inositol monophosphate, inositol bisphosphate, inositol trisphosphate and inositol 1,3,4,5-tetrakisphosphate levels increased about 2 fold.

A variety of 5-HT_2 or mixed 5-HT_1/5HT_2 agonists stimulated total ^{3}H-inositolphosphate formation in the immature rat cortex and hippocampus with a rank order or potency (α(+)-methyl-5-HT> quipazine > MK 212 > 5-HT) which resembles their potencies at the 5-HT_2 binding site. In contrast, the 5-HT_{1A} agonist 8-OH-DPAT, the 5-HT_{1B} agonist TFMPP (1-(m-trifluoromethylphenyl) piperazine) and mCPP (1-(m-chlorophenyl) piperazine) and the 5-HT agonist 2-methyl-5-HT were inactive. The 5-HT stimulated phosphoinositide response in the immature rat cortex was blocked potently by the selective 5-HT_2 receptor antagonists ketanserin (Ki = 0.2 nM), ritanserin (Ki = 2.7 nM) and spiperone (Ki = 4.9 nM) and by the mixed 5-HT_1/5-HT_2 antagonists methiothepine (Ki = 0.5 nM), methysergide (Ki = 14.8 nM) and cyproheptadine (Ki = 15.2 nM) but not by the 5-HT_3 antagonist MDL 72-222. The overall pattern of apparent drug affinities was consistent with a 5-HT_2 receptor mediating 5-HT-stimulated phosphoinositide responses in the immature rat cortex. In contrast, in the hippocampus, selective 5-HT_2 antagonists were much less potent at inhibiting the 5-HT response which suggests that in this brain region, this response is mediated at least in part by a 5-HT receptor distinct from the 5-HT_2 subtype.

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INHBITION BY 5-HT_3 ANTAGONISTS OF HYPERACTIVITY CAUSED BY DOPAMINE INFUSION INTO RAT NUCLEUS ACCUMBENS

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GR38032F, a selective 5-HT_3 receptor antagonist, has been shown to inhibit the hyperactivity caused by a persistent infusion of dopamine into the rat nucleus accumbens (Costall et al. 1987). Since the only other agents known to inhibit this response are the neuroleptic agents and lithium, it has been suggested that GR38032F may represent the first of a new class of antipsychotic agents. In the present studies we show that this property is shared by two other selective 5-HT_3 receptor antagonists, ICS205-930 and BRL43694 (Richardson et al. 1985; Fake et al. 1987).

Female Sprague-Dawley rats were subjected to standard stereotaxic surgery to implant chronically indwelling guides for infusion of dopamine (25 µg/24h) at the centre of the nucleus accumbens via subcutaneously implanted Alzet osmotic minipumps. The 13 day infusions of dopamine into the rat nucleus accumbens lead to biphasic hyperactivity responding with peaks on days 3-4 and 10-11 of infusion. Hyperactivity was measured in individual photocell cages and expressed in counts/60 min. Animals receiving intra-accumbens infusion of dopamine showed peaks of hyperactivity in the range 180-250 counts/60 min (vehicle control values were maintained at 80-95 counts/60 min). The dopamine hyperactivity was reduced by treatment with GR38032F, 0.1 -100 µg/kg i.p. b.d. (e.g. peak on day 3 reduced from 227 ± 26 counts/60 min to 77 ± 8 counts/60 min at 0.1 µg/kg GR38032F, $P<0.001$), ICS 205-930, 0.1 - 1 µg/kg i.p. b.d. (e.g. peak on day 3 reduced from 217 ± 22 counts/60 min to 101 ± 12 counts/60 min by 0.1 µg/kg ICS 205-930, $P<0.001$), or BRL43694, 10 ng/kg (e.g. peak on day 3 reduced from 187 ± 19 counts/60 min to 78 ± 9 counts/60 min, $P<0.001$). The antagonism of the dopamine hyperactivity was maintained across a wide dose range of GR38032F but occasionally at the very high dose of 0.5 mg/kg t.d.s. the dopamine response was not inhibited and values for hyperactivity between days 3 and 13 of infusion ranged from 189 ± 20 to 256 ± 27 counts/60 min ($P>0.05$ compared to dopamine). Failure to inhibit the dopamine response was also recorded for higher doses of ICS 205-930 (10 - 100 µg/kg) and BRL43694 (1 µg/kg - 1 mg/kg). At 0.1 mg/kg i.p. b.d. ICS205-930 there was not only a failure to inhibit the dopamine hyperactivity peaks but also an extension of the hyperactivity into the usual 'trough' between peaks and into the post-infusion period (hyperactivity on days 4-13 ranged from 177 ± 18 counts/60 min to 250 ± 26 counts/60 min, $P<0.001$ compared to vehicle). Generally, treatment with BRL43694 was seen to inhibit the first peak of dopamine hyperactivity but a progressive increase in hyperactivity occurred between days 4 - 13 to actually exceed the usual dopamine response (by 30-37%, $P<0.001$).

Thus, whilst GR38032F, ICS205-930 and BRL43694 are effective in a test which may predict antipsychotic activity, the actions of all compounds, particularly those of ICS 205-930 and BRL43694, diminish at high doses. The reason for this apparent loss of selectivity of action remains to be determined. However, this should receive careful attention in studies designed to analyse the antipsychotic potential of the 5-HT_3 receptor antagonists.

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EFFECTS OF THE 5-HT RECEPTOR ANTAGONISTS GR38032F, ICS205-930 AND BRL43694 IN TESTS FOR ANXIOLYTIC ACTIVITY

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Previous studies using rodent and primate tests have revealed potential anxiolytic properties for GR38032F, ICS205-930 and MDL72222 (Costall et al. 1987; Jones et al. 1987; Tyers et al. 1987). Here we extend these findings to include BRL43694 (Fake et al., 1987) but show differences in the profiles of action of the 5-HT_3 receptor antagonists at higher doses.

Mice taken from the dark and placed in a brightly illuminated area with a dark compartment simultaneously available will elect to avoid the illuminated area. On treatment with an anxiolytic agent this profile of behaviour changes to a preference for the novel, brightly illuminated area and measurements of behaviour reveal a delayed latency to enter the black area, a reduced % of time spent in the black area, and increased exploratory rearings and crossings of lines marked on the test box floor in the brightly illuminated section. Such changes in behaviour were induced by GR38032F (0.05 - 10 µg/kg i.p.), ICS205-930 (0.01 -10 µg/kg i.p.) and BRL43694 (0.01 - 1.0 µg/kg i.p.). At these doses, the magnitude of behavioural change was similar for each compound (for example, rearings in the white were increased from control values of 17 - 26/5 min to 33 - 57/ 5 min, crossings in the white were increased from 33 - 42/5 min to 69 - 86/5 min, latency was delayed from 10 - 15s to 22 - 35s, and % time in black decreased from 53 - 55% to 22 - 33%, $P<0.001$). However, as doses of the 3 5-HT_3 receptor antagonists were increased, differences emerged. The anxiolytic activity of GR38032F was maintained up to 1 mg/kg, but that of ICS205-930 and BRL43694 reduced to control values at 0.1 mg/kg ($P>0.05$). When the dose of BRL43694 was increased further to 1 mg/kg i.p. a profile of behaviour developed which was characteristic of anxiogenesis (rears in black increased from 50 ± 6/5 min to 79 ± 8/5 min, crossings from 66 ± 7/5 min to 89 ± 9/5 min, latency reduced from 12 ± 1s to 4 ± 2s and % time in black increased from 54 ± 4% to 71 ± 7%, $P<0.001$). Further differences between the 5-HT_3 receptor antagonists were seen on longer term treatment (7 days, b.d. dosing). The anxiolytic actions of GR38032F and ICS205-930 (10 µg/kg i.p.) were maintained, and slowly waned to control values on abrupt withdrawal of treatment. In contrast, tolerance developed to the actions of BRL43694 (1 µg/kg - 1 mg/kg) and anxiogenesis followed abrupt cessation of treatment (at 1 µg/kg rearing in the black was increased by 71%, crossings by 37%, % time in black by 35%, and latency was decreased by 80%, $P<0.01$ - $P<0.001$).

Similar differences are being established in a primate model where common marmosets normally respond to a human threat by retreating to the back of the cage and exhibiting postures associated with threat. As with an anxiolytic agent such as diazepam (10 - 25 µg/kg), GR38032F (0.1 - 1.0 µg/kg), ICS205-930 (0.1 - 1.0 µg/kg) and BRL43694 (1 ng - 0.1 mg/kg) increased time spent on the cage front (by 160 - 200%, $P<0.001$) and decreased posturing (by 60 - 87%, $P<0.001$). It is therefore suggested that whilst the 5-HT_3 receptor antagonists have anxiolytic activity in animal models, there are differences in their effects on behaviour at high doses.

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DIFFERENTIAL EFFECTS OF ICI 169,369 AND RITANSERIN ON 24 H EEC SLEEP PATTERNS IN RATS

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The role of 5HT and its receptors in sleep regulation remains complex. Recently, increases in slow-wave sleep (SWS) in rats (Dugovic & Wauquier, 1987) and human volunteers (Idzikowski et al., 1986) have been reported following blockade of 5-HT_2 receptors with ritanserin. In view of the putative selectivity of ritanserin for $5HT_2$ receptors (Leysen et al., 1985), a primary role for these receptors in the regulation of SWS has been suggested. The objective of the present study was to further investigate the involvement of $5HT_2$ receptors in sleep regulation by evaluating the acute effects of a novel, highly selective $5HT_2$ antagonist, ICI 169,369 (Blackburn et al., 1987), and ritanserin on the 24 h sleep cycle in rats.

Male Sprague Dawley rats (275-325 g) were prepared with chronic bipolar frontoparietal cortical EEG and temporalis muscle (EMG) electrodes for standard monitoring of sleep-awake activity. Following surgery, the rats were individually placed in recording chambers which served as their home cages throughout the experiment. The unrestrained animals were allowed several days to adapt to this novel environment during which food and water were provided ad lib. A 12 h light-dark cycle was maintained. EEG and EMG activity were monitored continuously until normal diurnal sleep-waking cycles were established. All the animals served as their own controls, were drug naive and used only once.

Twenty-four h following control injections of vehicle (1 or 2 ml/kg, p.o.), ICI 169,369 (1.0, 10 or 40 mg/kg, p.o.) or ritanserin (1.0 or 10 mg/kg, p.o.) was administered. At the highest dose tested (40 mg/kg) ICI 169,369 increased rapid eye movement sleep (REMS) latency 500% and suppressed REMS up to 12 h post-injection. Lower doses of ICI 169,369 were ineffective. In contrast, both low and high doses of ritanserin increased REMS latency (maximal effect = 965%) and suppressed REMS. Furthermore, the REMS suppressant actions of 10 mg/kg ritanserin were still evident 24 h post-injection. The $5HT_2$ antagonists also produced differential effects on SWS. Regardless of the dose tested, ICI 169,369 failed to effect SWS continuity, while 1.0 and 10 mg/kg of ritanserin induced increases in SWS latency and decreases in SWS for 6-12 h.

The results of this study demonstrate differential effects on sleep regulation produced by ICI 169,369 and ritanserin, antagonists with putative selectivity for $5HT_2$ receptors. While both compounds produced similar effects on REMS latency and suppression, their pharmacological profiles and effects on SWS obviously differ.

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AUTORADIOGRAPHIC LOCALISATION OF 5-HT_2 BINDING SITES IN THE INTERMEDIOLATERAL CELL COLUMN OF THE CAT

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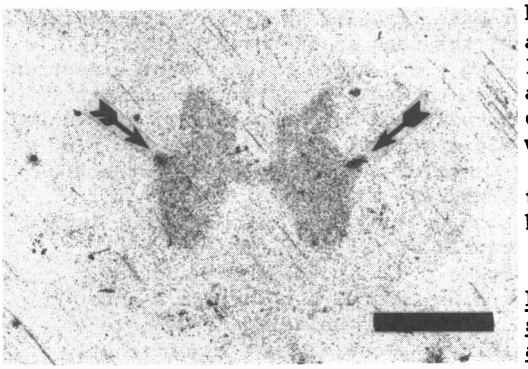
Iontophoretic application of 5-HT to sympathetic preganglionic neurones causes predominantly excitation (Coote et al., 1981). Electrical stimulation of the raphe, from which 5-HT-containing neurones project to the preganglionic sympathetic neurones in the intermediolateral cell column (IML) (Lowey & Neil, 1981) causes sympathoinhibition (Gilbey et al., 1981) as do the putative 5-HT_{1A} receptor agonists 8-OH-DPAT and ipsapirone when administered i.v. (Ramage & Fozard, 1987). These observations suggest the existence of more than one type of 5-HT receptor in the IML. Hence, the present experiments were carried out to determine whether 5-HT_{1A} and/or 5-HT_2 binding sites are present in the cat IML using in vitro autoradiography.

Frozen 20μm sections of various levels of thoracic and lumbar cord were prepared for autoradiography as described previously (Dashwood et al., 1985), and incubated in [^{3}H]5-HT, [^{3}H]8-OH-DPAT and [^{3}H]ketanserin (2nM) in order to identify 5-HT_{1A} and 5-HT_2 binding sites. The degree of binding to non-specific and other possible sites was established by incubating in the presence of excess concentrations (1μM) of the above unlabelled ligands as well as prazosin and mepyramine.



Both [^{3}H]5-HT and [^{3}H]ketanserin showed a marked binding to the IML at all levels. The displacement of [^{3}H]5-HT and [^{3}H]ketanserin by unlabelled compounds indicated that these compounds were binding to the 5-HT_2 site (fig.1). [^{3}H]8-OH-DPAT only showed marked binding to the superficial laminae of the dorsal horn.

Fig.1. Section of cat spinal cord (T3) showing dense binding of [^{3}H]ketanserin at the IML (arrowed). Scale bar 2mm.

These results suggest that 5-HT_2 rather than 5-HT_{1A} receptors predominate in the IML. Furthermore, it is suggested that these 5-HT_2 receptors mediate the excitation caused by the iontophoretic application of 5-HT. However, the precise physiological function of this receptor subtype in sympathetic control at the level of the IML remains to be determined.

The support of the BHF and the MRC is gratefully acknowledged and also Janssen Phamaceutical Ltd for supplying [^{3}H]ketanserin.

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THE ATTENUATION OF 8-OH-DPAT-INDUCED HYPOTHERMIA IN THE MOUSE BY SELECTIVE INHIBITORS OF 5-HT UPTAKE

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The highly selective 5-HT_{1A} binding ligand 8-hydroxy-N, N-dipropylaminotetralin (8-OHDPAT) induces a moderate hypothermia in the mouse; an effect attributed to the activation of central 5-HT_{1A} autoreceptors, leading to an inhibition of 5-HT release (Goodwin et al., 1985). Here we report the effects of selective 5-HT uptake inhibitors on 8-OHDPAT-induced hypothermia in the mouse.

Uptake inhibitors or vehicle were administered orally to female Tuck T/O mice (20-27g) 60min. prior to the subcutaneous administration of 8-OHDPAT or vehicle (isotonic saline). Rectal body temperatures were measured immediately before each of these treatments and at 15 and 30 min. following 8-OHDPAT administration. The hypothermic response was recorded as the maximum decrease in body temperature occurring in this latter period. In experiments of similar design, the effects of uptake inhibitors on apomorphine (0.25 and 1.0 mg/kg s.c.)-induced hypothermia were also examined. All experiments included appropriate control groups and were performed at an ambient temperature of 20°C. The animals were housed in groups of six and were assigned randomly to treatment groups. Treatment groups comprised 8 animals and data were analysed using Student's unpaired t-test.

8-OHDPAT dose-dependently decreased body temperature, with a maximum response (3.5-4.0°C decrease) occurring at doses greater than 1mg/kg s.c. A sub-maximal dose of 0.8mg/kg inducing a 2-3°C decrease, was used in subsequent drug interaction studies. Desipramine (3-30 mg/kg p.o.) had no significant effect on 8-OHDPAT-induced hypothermia; however, the selective 5-HT uptake inhibitors citalopram, fluvoxamine and panuramine markedly and dose-dependently attenuated 8-OHDPAT-induced hypothermia, with ED_{50} values of 2.9, 8.0 and 8.7 mg/kg p.o. respectively. Citalopram and fluvoxamine completely blocked the hypothermic response at the top dose of 30 mg/kg p.o., whereas the maximal effect of panuramine was a 62% inhibition at 15 mg/kg p.o. ($p<0.01$). Apomorphine (1mg/kg s.c.)-induced hypothermia was not significantly affected by citalopram or fluvoxamine except at the top dose of 30 mg/kg p.o. (inducing 52% and 39% inhibitions respectively; $p<0.05$). Apomorphine-induced hypothermia was not modified by panuramine up to a dose of 45 mg/kg p.o. Relative to vehicle-pretreated control animals the uptake inhibitors alone did not significantly modify body temperature, except for the top dose of desipramine (1.4°C. decrease 60min. following drug administration).

These results are consistent with the suggestion that 8-OHDPAT-induced hypothermia in the mouse is a result of an inhibition of 5-HT release, possibly due to autoreceptor activation. Inhibition of 5-HT re-uptake may at least partially counteract the impairment of transmission imposed by this mechanism.

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BRL 34915 AND RELAXATION OF RAT UTERUS - ROLE OF K^+ CHANNELS

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BRL34915 is a potent relaxant of a number of smooth muscles, an effect which is associated with the opening of potassium (K) channels (Hamilton et al., 1986). However, the relaxant action of BRL34915 in the uterus of the day 22 pregnant rat is not accompanied by the marked hyperpolarisation or increase in ^{86}Rb efflux observed in other tissues (Hollingsworth et al., 1987). In an attempt to clarify these anomalies, further studies have been conducted using ^{42}K efflux and by assessment of the ability of K channel blockers to antagonise BRL34915.

Endometrium-free myometrial strips were loaded with ^{42}K for 90 min and exposed to BRL34915 (1 or 10 μM) between the 16th and 24th min of efflux. BRL34915 failed to modify ^{42}K efflux (control 7.7 ± 0.3 %/min, n = 6; 1 μM 7.2 ± 0.3 %/min, n = 6; 10 μM 7.0 ± 0.3 %/min, n = 7; mean rate coefficient for 16 to 24 min ± s.e.m.).

Using uterine strips, with phasic tension development enhanced by the addition of oxytocin (0.2 nM), cumulative concentration-effect curves were constructed to BRL34915 or salbutamol in the absence and after 30 min incubation with a K channel blocker.

Table 1 Antagonism of BRL34915 and salbutamol by K channel blockers

Blocker	Conc.	BRL34915	Salbutamol
Procaine	1 mM	1.55 ± 0.31	0.17 ± 0.18
Quinine	100 μM	1.22 ± 0.37*	-0.25 ± 0.09
Quinidine	100 μM	1.53 ± 0.23*	0.44 ± 0.17
Tetraethylammonium	10 mM	1.12 ± 0.21*	0.58 ± 0.18*
4-aminopyridine	10 mM	1.52 ± 0.19*	0.54 ± 0.23

Antagonism is expressed as change in potency as $\log_{10}$ concentration ratio. Values are means ± s.e. mean, n = 5-9 * = significant reduction in slope of concentration-effect curve.

The potency of BRL34915 or salbutamol did not alter significantly in time-matched controls. Procaine (1 mM), quinine (100 μM), quinidine (100 μM), tetraethylammonium (10 mM) and 4-aminopyridine (10 mM) all antagonised BRL34915. Salbutamol was either not antagonised by these K channel blockers or the degree of antagonism was less than that seen against BRL34915 (Table 1).

The K channel blockers had different effects on tension development. Quinine and quinidine had little effect. Procaine and tetraethylammonium increased the frequency and amplitude of phasic tension waves. 4-aminopyridine initially produced a complete abolition of tension development followed by a tonic spasm before the return of phasic tension waves.

The selective antagonism of BRL34915 by 5 proposed K channel blockers supports the idea that K channel opening is involved in its relaxant action in rat uterus. The failure of BRL34915 to increase either ^{86}Rb (Hollingsworth et al., 1987) or ^{42}K efflux (present study) is compatible with a selective action of BRL34915 on pacemaker cells in rat uterus. The enhancement of spontaneous tension development in the presence of some K channel blockers but not others and their qualitatively different effects suggests the presence of more than one functional K channel in uterus.

We thank Dr. T. Hamilton (Beecham) for BRL34915.

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EFFECT OF CROMAKALIM (BRL 34915) AND PINACIDIL ON BARORECEPTOR SENSITIVITY IN THE ANAESTHETISED CAT

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The effects of two new antihypertensive agents, cromakalim and pinacidil (PIN), on baroreceptor sensitivity (BS) were investigated in the anaesthetised cat.

Cats of either sex were anaesthetised with alpha-chloralose (80 mg/kg iv). A femoral artery and vein were cannulated to facilitate blood pressure measurement and intravenous administration of drugs respectively. Heart rate (HR) was derived from the phasic pressure signal with a BRL phase lock loop ratemeter. The animals were artificially respired and blood gas status was periodically measured with a Corning 158 blood gas analyser. After a 1h period, increasing doses of phenylephrine (PHE; 0.3-3.0 µg/kg) or sodium nitroprusside (NIP; 1.0-10.0 µg/kg) were injected, intravenously, predose (twice) and then 10 and 90 min after infusion (1 ml/min, 1 ml/kg) of cromakalim (10 µg/kg) or PIN (60 µg/kg). The peak changes in systolic pressure (SBP; mmHg) and heart period (HP; mS) to either PHE or NIP were measured and BS assessed by the 'steady state' method of Korner, West, Shaw and Uther (1974). In the same animals BS was also assessed from a single dose of PHE (1.0 µg/kg) by the 'ramp' method of Smyth, Sleight and Pickering (1969).

Reproducible BS responses were obtained in 2 cats receiving vehicle (1 ml/kg) infusion instead of cromakalim or PIN. Cromakalim (10 µg/kg) and PIN (60 µg/kg) elicited similar effects on SBP (-9 mmHg and -11 mmHg 10 min post dose) and HR (+15 beats/min and +10 beats/min 10 min post dose). Cromakalim significantly ($p<0.05$) increased 'steady state' BS to PHE at 10 and 90 min post dose and slightly decreased BS to NIP at 10, but not 90, min post dose (table 1). PIN (60 µg/kg) slightly, but not significantly, reduced BS to PHE and increased ($p<0.05$) BS to NIP (table 1).

Table 1. 'Steady state' BS (Δ HP/Δ SBP)

	cromakalim		PIN	
	PHE	NIP	PHE	NIP
Predose	3.17 ± 0.58	1.82 ± 0.20	2.93 ± 0.67	0.85 ± 0.21
10 min	6.55 ± 1.55*	1.32 ± 0.15	2.78 ± 0.80	1.74 ± 0.46*
90 min	6.45 ± 2.09*	2.25 ± 0.29	2.23 ± 0.54	2.24 ± 0.35*

values are mean ± sem for 4 cats;* = $p<0.05$ (Dunnett's test).

Neither cromakalim nor PIN affected BS assessed by the 'ramp' method.

Although only single, low doses of each compound were used in this study, this preliminary data suggests that cromakalim may possess a desirable effect in sensitising baroreceptors to pressor challenge. This property, however, is not shared by PIN and since BS in human essential hypertension is altered (Korner et al. 1974) this may confer some advantage on cromakalim over PIN.

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COMPARISON OF THE CARDIOVASCULAR AND RENAL EFFECTS OF BRL 34915 WITH THOSE OF NITRENDIPINE IN DOGS AND SHR

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BRL 34915 and nitrendipine (NIT) are both antihypertensive and renal vasodilating agents. Whereas NIT relaxes vascular smooth muscle by inhibiting the entry of calcium into cells, BRL produces this effect by hyperpolarizing cell membranes through activation of K^+-channels (Buckingham et al. 1986 ; Weir and Weston, 1986). In view of their different mechanisms of action we have now looked for differences in their cardiovascular and renal effects.
In anesthetized (pentobarbitone 35mg/kg i.v +5mg/kg/h) normotensive mongrel dogs both agents (1-30 ug/kg i.v.) produced dose-related decreases in blood pressure of 10-60%. Cardiac output was acutely increased (9-64%) after each injection of NIT, but was not changed by BRL at any time point. Renal blood flow was increased 9 ± 2 and 21 ± 7 % after BRL (3 and 10 ug/kg) and was maintained at larger doses despite a marked fall in mean blood pressure. A net decrease in renal vascular resistance (10-40 %) was seen with both compounds over the dose range studied.
The results obtained using conscious SHR are shown in Table 1. In animals given a saline load (40 ml/kg i.p.), NIT (10 mg/kg p.o.) increased urine volume (UV) and urinary Na^+-excretion ($U_{Na}V$) but did not change the output of potassium (U_KV) over 6 h. In SHR with indwelling arterial catheters, the same dose of NIT lowered diastolic blood pressure for 7h (DBP; initial value of all groups: 156 ± 2 mmHg; n=29). In contrast, BRL (0.1 or 0.3 mg/kg p.o.) caused only short-lasting (1-2 h) falls in DBP but a pronounced anti-diuretic effect over 6 h. Furthermore, after administration of BRL (0.3mg/kg), $U_{Na}V$ decreased and U_KV was unchanged when compared to controls. All parameters had returned to control values at 24h. At 3 mg/kg BRL was both diuretic and kali-natriuretic.

Table 1 . Effects of BRL 34915 and nitrendipine in conscious SHR

Drug mg/kg p.o.	n	UV ml/kg/6h	$U_{Na}V$ mmol/kg/6h	U_KV mmol/kg/6h	n	% Δ DBP 1h	2h	4h
vehicle (1% Tween 80)	36	9.2±0.9	1.3 ±0.2	0.44±0.08	14	-4±2	-3±2	-2±2
BRL 0.1	36	5.6±0.8*	1.0 ±0.2	0.42±0.04	4	-24±4*	-15±3*	- 8±1
BRL 0.3	24	4.6±0.8*	0.8 ±0.14*	0.39±0.07	6	-31±4*	-20±4*	- 1±4
BRL 3.0	36	23.6±1.8*	2.6 ±0.2*	0.8±0.08*	4	-53±1*	-40±4*	-36±5*
NITR 10.0	36	14.6±1.5*	2.3 ±0.2*	0.45±0.03	5	-36±4*	-35±5*	-31±5*

* Significantly different from control $p<0.05$ (ANOVA and t-test).

In conclusion, both BRL and NIT caused hypotension and renal vasodilator effects in normotensive dogs however, unlike NIT, BRL did not change cardiac output. At low doses in SHR, BRL caused the excretion of a hypertonic urine of reduced volume while NITR produced diuresis and natriuresis as previously shown (Vemulapalli et al.1987). At higher doses BRL was a long acting antihypertensive with diuretic and kali-natriuretic properties. It remains to be determined whether the antidiuretic effects of BRL are a consequence of intra-renal K^+-channel activation or are indirectly related to other mechanisms such as vasopressin release.

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INHIBITION OF BRL 34915-STIMULATED $^{86}RB^+$ EFFLUX IN RABBIT AORTA BY FRACTIONATED LEIURUS QUINQUESTRIATUS HEBRAEUS SCORPION VENOM

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The ability of the drug BRL 34915 to inhibit agonist-induced responses in smooth muscle is associated with the opening of membrane K^+ channels (Weir & Weston, 1986; Quast, 1987). Preliminary electrophysiological studies have shown that BRL 34915 is capable of prolonging the open time of a large conductance $K^+(Ca)$ channel (Kusano *et al*, 1987). *Leiurus quinquestriatus hebraeus* scorpion venom is known to contain at least two different toxins which block distinct $K^+(Ca)$ channels (Castle & Strong, 1986). In the present investigation, we have studied the effects of crude venom and its isolated fractions on BRL 34915-stimulated $^{86}Rb^+$ efflux in rabbit aorta.

Flux assays were performed as follows: segments of rabbit thoracic aorta were incubated in Krebs-Henseleit solution (KHS) for 45 min before the transfer to KHS containing $^{86}Rb^+$ (5μCi/ml). After 2h, $^{86}Rb^+$ was allowed to efflux from the tissue into normal KHS for 10 min. Tissues were then exposed to KHS containing PN200-100 (0.5 μM) for 20 min before the transfer to the same solution supplemented with the flux blocker under test. After an additional 20 min, the tissues were then challenged with BRL 34915 (1 μM). The erythrocyte assay was performed as previously described (Castle & Strong, 1986).

Crude venom (200 mg batches) was extracted with H_2O (4 × 5 ml; 4°C; 20 min). The supernatant (15 ml) was chromatographed on a column of Sephadex G-50 (90 × 2.6 cm dia) equilibrated with 50 mM ammonium formate buffer pH 3.5. The major peak (as monitored by A_{278} nm absorbance), eluting at ≈7000-10,000 mol.wt. possessed > 90% of the $^{86}Rb^+$ blocking activity of crude venom. This fraction (from 2 × 200 mg batches of the crude venom) was diluted with an equal volume of H_2O and applied to an S-Sepharose ion-exchange column (10 × 1.6 cm dia). The column was equilibrated with 50 mM ammonium acetate (NH_4OAc) buffer pH 6.0 and the material which bound to the column was eluted with a NH_4OAc linear salt gradient (50-600 mM, pH 6.0; total volume 800 ml). Twelve fractions were collected and assayed for biological activity. Fraction 12 (charybdotoxin; 85% pure by HPLC) showed absolutely no ability to block BRL 34915-induced $^{86}Rb^+$ efflux although it was a potent inhibitor of A23187-stimulated K^+ flux from human erythrocytes. In contrast, both fractions 10 & 11, although completely devoid of activity in the erythrocyte assay, were effective blockers of BRL 34915-stimulated $^{86}Rb^+$ efflux in the aorta assay.

A novel toxin(s), distinct from charybdotoxin, is responsible for the ability of *Leiurus quinquestriatus hebraeus* scorpion venom to inhibit the BRL 34915-stimulated $^{86}Rb^+$ efflux from rabbit aortic tissue.

This work was performed while PNS was a visiting scientist at Sandoz, Basle.

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FURTHER STUDIES OF THE ANTIVASOCONSTRICTOR EFFFECT OF THE K^+ CHANNEL OPENER BRL 34915 ON THE RABBIT AORTA

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Previous studies have shown that BRL 34915 (BRL) can non-competitively inhibit angiotensin II (AII) contractions of the rabbit aorta by around 55%, a significantly greater inhibition than is seen with Ca^{2+} antagonists such as the dihydropyridine (DHP), PN 200-110 (PN) (Cook & Quast, 1987). This effect of BRL is still evident in the presence of a saturating concentration of PN. Hence, BRL would appear to have antivasoconstrictor activity in this vessel in addition to an indirect inhibition of Ca^{2+} entry through DHP-sensitive Ca^{2+} channels. However, with noradrenaline (NA) as the agonist, BRL shifted the concentration-response curve slightly to the right, but did not affect the tissue maximum response to NA (Cook & Quast, 1987).

The present study considers 3 points arising from these studies: (1). Is the inhibition of AII contractions by BRL a consequence of its ability to open K^+ channels and thereby modify the membrane potential (see Hamilton et al, 1986)? (2). Would reducing the number of functional α-adrenoreceptors by phenoxybenzamine (PBZ) treatment render the NA contractions sensitive to BRL, as has been reported to occur with Ca^{2+} antagonists (Timmermans et al., 1985)? (3). Could inhibition of Ca^{2+} entry through the electrogenic Na^+/Ca^{2+} exchanger (as a consequence of membrane hyperpolarization) in part explain the inhibitory effects of BRL on the aorta? Preparation of the rabbit aortic rings, recording of isometric tension and the experimental protocol were as described by Cook et al (1987).

Concentration-response curves to AII in the presence of 35mM KCl and 0.1μM PN were comparable to those seen in the presence of PN alone, the maximum AII response being inhibited by 15-20%. However, BRL (0.03-10μM) failed to further inhibit AII contractions in these partially depolarized aortic rings. This suggests that even with the DHP-sensitive Ca^{2+} channel blocked, the additional antivasoconstrictor effect of BRL remains voltage dependent. In contrast, inhibition of AII contractions of the aorta by atriopeptin III (0.01μM) and sodium nitroprusside (0.1μM) were only minimally reduced in depolarized vessels.

In the presence of PBZ (3-10nM), BRL (0.3-3μM) was able to partially inhibit NA contractions of the aorta in a non-competitive manner (maximal contractions to NA in the presence of 10nM PBZ being reduced ≈ 50% by 3μM BRL). Hence, like the inhibition of AII responses by BRL, PBZ treatment reveals a component of the NA contraction which is also sensitive to inhibition by BRL.

Finally, in the presence of dichlorobenzamil (3-10μM), an inhibitor of the Na^+/Ca^{2+} exchanger, the ability of BRL to inhibit AII contractions was indeed reduced. However, these experiments do not rule out other possible actions of dichlorobenzamil, such as a direct blocking action on K^+ and Ca^{2+} channels. Further studies are clearly required to test the hypothesis that Na^+/Ca^{2+} exchange might be involved in contractile responses of the aorta, with antivasoconstrictor effects of BRL partly due to inhibition of this pathway.

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POTENT INHIBITORS OF THE EFFECTS OF THE K+ CHANNEL OPENER BRL 34915 IN VASCULAR SMOOTH MUSCLE

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BRL 34915 is a novel vasorelaxant agent proposed to act by opening $^{86}Rb^+$-permeable K^+ channels (Hamilton et al; 1986). The antivasoconstrictor effects of BRL 34915 in vascular smooth muscle (VSM) and the associated increase in $^{86}Rb^+$ efflux are inhibited to varying degrees by K^+ channel blockers like tetraethylammonium (TEA) and the aminopyridines and by procaine at concentrations above 0.1 mM (Quast, 1987; Wilson, 1987). Total suppression of BRL 34915-stimulated $^{86}Rb^+$ efflux from VSM has been observed at 10 µM of trifluoperazine (TFP), gallopamil and nifedipine (Kreye et al; 1987). In the search for potent synthetic inhibitors of the effect of BRL in VSM we have investigated glybenclamide (GBC, a potent inhibitor of ATP-sensitive K^+ channels), dichlorobenzamil (DCB, an inhibitor of Na^+/Ca^{2+} exchange) and TFP (an antagonist at dopamine receptors and inhibitor of calmodulin).

The vasorelaxant action of BRL 34915 was assessed against contractions induced by angiotensin II (AII) of rabbit aortic rings (Cook et al, 1987) or against the myogenic activity (MA) of rat portal veins (Quast, 1987). $^{86}Rb^+$ efflux from rat portal vein or aortic strips was measured as described earlier (Quast, 1987) and is quantitated by the rate constant, k. Drug effects are expressed by the area under the curve of the k versus time plot.

GBC (1 µM) had no effect on resting tension or AII-induced contractions of rabbit aorta but partially inhibited the relaxant effect of 3 µM BRL 34915. In rat portal veins, 0.3 µM GBC increased basal values of MA and k by $\approx$ 5 % but shifted the inhibition of MA by BRL 34915 to tenfold higher concentrations. In this preparation and in rat aorta, BRL 34915-stimulated $^{86}Rb^+$ efflux was inhibited by GBC (rat aorta: $IC_{50} \approx 0.3$ µM).

DCB ($> 10^{-5}$M) concentration-dependently increased resting tension and inhibited noncompetitively AII-induced contractions in rabbit aorta. The relaxant effect of BRL 34915 was inhibited by DCB (3 - 10 µM). In rat portal vein and aorta, DCB concentration-dependently inhibited the $^{86}Rb^+$ efflux stimulated by BRL 34915 with IC_{50} values of approximately 5 µM.

TFP (10 µM), which had no effect on resting tension and only slightly inhibited the AII-concentration-effect curve in rabbit aortic rings, inhibited the BRL-induced vasorelaxation. Inhibition of BRL 34915-stimulated $^{86}Rb^+$ efflux from rat portal vein and aorta occurred with IC_{50} values of about 30 µM.

It is concluded that GBC is the most potent synthetic inhibitor of the effects of BRL 34915 in VSM described thus far. Interestingly, this substance only marginally affects the basal values of tension and $^{86}Rb^+$ efflux at the relevant concentrations. Hence, if inhibition by GBC were at the level of the K^+ channel opened by BRL 34915, these results would suggest that this channel is not normally involved in the maintenance of resting tone (or membrane potential) in these vascular tissues.

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COMPARISON OF THE INHIBITORY EFFECTS OF BRL34915 AND NIFEDIPINE ON NORADRENALINE- AND KCL-INDUCED CONTRACTIONS IN RABBIT AORTA

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BRL34915 belongs to a new class of antihypertensive agent, the effects of which are associated with the opening of membrane K^+-channels (Hamilton et al., 1986; Weir & Weston, 1986). Such an action should reduce any agonist-induced depolarisation and is currently believed to be responsible for the blood pressure- lowering properties of BRL34915. However, in some blood vessels, contraction is not associated with depolarisation and in the present investigation, the effects of BRL34915 have been studied using rabbit aorta, a tissue in which the action of noradrenaline is electrically silent (Cauvin et al., 1984).

The thoracic aorta was removed from male Half-lop rabbits (2.5-3.5 kg) and divided into segments each approximately 1cm in length. Each segment was opened along its longitudinal axis and the endothelium carefully removed by rubbing the intimal surface with a moist cotton bud.

The resting membrane potential of rabbit aorta was -52±3 mV (mean ± s.e.mean, n=43). Exposure to noradrenaline (NA, 0.0125-39 μM) for up to 10 min produced a concentration-dependent contraction which was not accompanied by detectable changes in membrane potential. Using the lanthanum technique (Weir & Weston, 1988) no increase in calcium influx (measured as the lanthanum-resistant calcium fraction - LRCF) was detected during the first 3 min of exposure to NA (300 nM). However, a small but significant increase in the LRCF was observed following 5 and 10 min exposure to NA (300 nM). This increase was not antagonised by nifedipine (300 nM). Exposure of tissues to KCl (20-80 mM) was accompanied by contraction and a marked increase in the LRCF, events which were antagonised by nifedipine (30-300 nM).

Pre-incubation of aortic segments with BRL34915 (0.3-10 μM) or nifedipine (30-300 nM) for 40 min had no effect on the initial (phasic) component of NA (0.0125-39 μM) -induced contractions. However, in tissues pre-contracted with NA (100-300 nM), cumulative addition of BRL34915 (0.3-10 μM) at 10 min intervals produced a concentration-dependent reduction in the maintained (tonic) phase of the contraction whilst exposure to nifedipine (30-300 nM) was without inhibitory effect. BRL34915 (0.3-10 μM) produced no inhibition of the maintained phase of KCl (40 mM) -induced contractions, whilst nifedipine inhibited these responses in a concentration-dependent manner.

It is concluded that BRL34915 can inhibit a component of NA contractions which is associated with calcium influx via a nifedipine-insensitive mechanism. Whether this action is a consequence of BRL34915-induced K^+-channel opening and subsequent hyperpolarisation is the subject of a further investigation (Bray et al., 1987, this meeting).

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ANALYSIS OF THE INHIBITORY ACTION OF BRL34915 ON RESPONSES TO NORADRENALINE IN RABBIT AORTA

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We have previously shown (Bray et al., 1987, this meeting) that BRL34915 can inhibit the nifedipine-insensitive, maintained phase of noradrenaline (NA)-induced contractions in rabbit aorta. The objective of the present study was to analyse this inhibitory action of BRL34915 using endothelium-free segments of rabbit aorta prepared as described previously (Bray et al., 1987, this meeting).

Using microelectrodes, BRL34915 (0.3-10 μM) produced a slowly-developing, concentration-dependent maintained hyperpolarisation. BRL34915 (10 μM) produced a hyperpolarisation of 17 ± 2 mV (mean ± s.e.mean, n = 6); no increase in membrane potential was observed at concentrations of BRL34915 < 0.3 μM. Over a similar concentration range, BRL34915 produced an increase in ^{86}Rb efflux (Kreye & Weston, 1986).

In a series of tissue bath experiments pre-incubation of aortic strips with nifedipine (30-300 nM) for 40 min produced a concentration-dependent inhibition of KCl (20-80 mM)-induced contractions. However, nifedipine (300 nM, the highest concentration used) had no effect on responses to NA (0.0125-39 μM). As reported for other vascular smooth muscles (Hamilton et al., 1986; Weir & Weston, 1986) pre-incubation with BRL34915 produced no inhibition of responses to KCl (40-80 mM). Aortic segments were exposed to nifedipine (300 nM) for 40 min followed by exposure to KCl (40 mM) for 10-15 min. A small contraction ensued. Subsequent exposure to NA (100-300 nM) produced further contraction which was maintained during cumulative addition of BRL34915 (0.3-10 μM). In parallel experiments, in which tissues were not depolarised by exposure to KCl, BRL34915 (0.3-10 μM) inhibited the maintained phase of NA-induced contractions.

It is concluded that although NA contractions in rabbit aorta are electrically silent (Bray et al., 1987), the supply of calcium for tension maintenance is reduced by BRL34915-induced hyperpolarisation This property of K-channel openers is not shared by calcium-entry blocking agents like nifedipine. It may be of special relevance in vivo in which blood vessels are continuously exposed to excitatory mediators. Such an inhibitory action, together with the reduction in depolarisation-induced excitation (Hamilton et al., 1986) may account for the differences in the inhibitory profiles of K-channel openers and calcium entry blockers (Buckingham et al., 1986). The inhibitory effect of BRL34915 on tension maintenance may also explain why this agent can inhibit the spontaneous tone of bronchial smooth muscle (Allen et al., 1986), a mechanical event which is calcium-dependent yet resistant to calcium entry blocking drugs (Foster et al., 1984).

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INTERACTION BETWEEN CROMAKALIM (BRL 34915) AND PROTEIN KINASE C ACTIVATION IN RABBIT MESENTERIC ARTERY

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Cromakalim stimulates rubidium efflux from rabbit isolated mesenteric artery (RIMA) but the time course of the response suggests that it does not interact directly with the potassium channels it opens (Coldwell, 1987). This report examines a possible intermediate step in the opening of these channels. Protein kinase C (PKC) activation may inhibit a K^+ conductance in neuronal cells (Higashida & Brown, 1986). It was therefore of interest to investigate whether cromakalim might be reversing the inhibition of potassium currents by PKC. The interaction between cromakalim and phorbol esters (PE), some of which activate PKC directly (Castagna et al, 1982), has therefore been studied.

RIMA were prepared as previously described for Rb efflux (Coldwell, 1987) or isometric tension studies (Wilson & Clapham, 1987). In tension experiments, concentration-response effects for phorbol 12,13-dibutyrate (PDBu), phorbol 12-myristate, 13-acetate (TPA), phorbol 12-monoacetate (PMA) and 4αphorbol 12,13-didecanoate (4αPDD) in the range 10 nM to 10 μM were investigated. In other studies, tissues were exposed to cromakalim (10 μM) for thirty min before the concentration-response curve to PDBu.

PDBu inhibited the ^{86}Rb efflux response to cromakalim in a concentration dependent manner with an IC_{50}=70 nM, total inhibition occurring at 300 nM. TPA, PMA and 4αPDD had little effect at concentrations up to 300 nM.

PDBu caused slow contractions of RIMA with an EC_{50}=100nM. In separate experiments, cromakalim significantly antagonised the PDBu contractions with a concentration ratio of 5.0 at the EC_{50} level. TPA also contracted RIMA but at much greater concentrations (reaching only 41% of the PDBu maximum at 10 μM). Contractions to TPA took longer to reach maximum effect than PDBu contractions. At concentrations up to 10 μM, PMA and 4αPDD did not contract the tissue.

Thus PMA and 4αPDD, which do not activate PKC (Castagna et al, 1982), were unable to cause contraction and had only slight effects (at high concentrations) on cromakalim induced Rb efflux. Kinetic studies with PKC have shown that TPA is more potent as an activator of the enzyme than is PDBu (Castagna et al, 1982), but in these experiments TPA only caused contractions at high concentrations and had little effect on cromakalim stimulated Rb efflux. This may be due to a lower ability of TPA to permeate RIMA, as has been suggested for porcine coronary artery (Miller et al, 1986). However PDBu, the phorbol ester which was able to contract RIMA, inhibited the rubidium efflux response to cromakalim, with an IC_{50} similar to the EC_{50} for causing contraction. Conversely, cromakalim was able to inhibit PDBu-induced contractions.

In conclusion, there is an interaction between cromakalim and PDBu but the present data is insufficient to state whether this is a direct or indirect effect of cromakalim on PKC.

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A COMPARISON OF THE EFFECTS OF CROMAKALIM (BRL 34915) ON BASILAR, CORONARY AND MESENTERIC ARTERIES

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Cromakalim (±) 6-cyano-3,4-dihydro-2,2-dimethyl-trans-4-(2-oxo-1-pyrrolidyl)2H-benzo(b)pyran-3-ol) a potassium channel activator (Hamilton et al. 1986) has been examined for effects on basal tension and on contractions induced by K^+ and 5-hydroxytryptamine (5-HT) in segments of rabbit basilar and mesenteric arteries and pig coronary arteries. Experiments were performed with 0.3 cm arterial segments suspended in organ baths in Krebs-Henseleit solution (composition in mM: NaCl 118.4, KCl 4.8, $CaCl_2 \cdot 2H_2O$ 2.0, $MgSO_4 \cdot 7\ H_2O$ 1.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.1) gassed with 5% CO_2 in O_2 at 37°C. Tension was measured with isometric force-displacement transducers; resting tension was 0.5 g for basilar arteries and 2 g for mesenteric and coronary arteries. Cumulative concentration-response curves for 5-HT and K^+ were constructed. The changes induced by cromakalim are presented as means (± s.e. mean) of at least four experiments and significance was determined using Student's t-test ($p<0.05$).

Cromakalim 0.1 µM, 1 µM and 10 µM caused a relaxation of resting tone of 33±7%, 58±6% and 56±6% and 10±1%, 26±4% and 24±2% in basilar and coronary arteries, respectively, 0.01 µM was ineffective. The relaxation was significantly greater at all concentrations in basilar arteries, and was not inhibited by indomethacin (10 µM). In mesenteric arteries, no relaxation occurred.

Concentration-response curves to 5-HT were biphasic in basilar and mesenteric arteries. The first phase of the curve reached maximum at 3µM, this is consistent with mediation via 5-HT receptors. The second section of the 5-HT curve did not level off even at 1 mM. This section of the curve was mediated via α_1 receptors, as judged by its prazosin sensitivity (pK_B 8.3) in mesenteric arteries, but was prazosin insensitive in basilar arteries. Prazosin 1 µM had no effect on responses to low (<3µM) concentrations of 5-HT in either tissue. In coronary arteries, responses to 5-HT reached maximum at 3 µM. Cromakalim caused downward displacement of the 5-HT concentration-response curves in all arteries. Cromakalim 0.1 µM and 1 µM reduced the response to 3 µM 5-HT by 86±3% and 94±3%, 40±14% and 46±16% and 31±8 and 85±3% in basilar, mesenteric and coronary arteries, respectively. The response to 1 mM 5-HT was not inhibited by 0.1 µM cromakalim in basilar arteries, but was inhibited by 61±13% in mesenteric arteries. This second component of the 5-HT response, which is not α_1 mediated, in basilar arteries is relatively insensitive to block by cromakalim.

Cromakalim inhibited the contractions induced by low but not high (>90mM) concentrations of K^+ in all arteries, i.e. there was a non-parallel shift of the concentration-response curves and in contrast to 5-HT, the maximum attainable contraction to K^+ was not reduced. Basilar arteries were more sensitive to K^+ (EC_{50} 15±1 mM) than coronary (EC_{50} 24±1 mM) and mesenteric (EC_{50} 36±1 mM) arteries. Cromakalim 1µM caused a greater shift in the EC_{50} in basilar arteries (1.7±0.2 fold) than in coronary (1.3±0.03 fold) and mesenteric arteries (1.1±0.03 fold). The effects of cromakalim on resting tension, 5-HT and K^+ induced contractions could be inhibited by lidocaine or procaine which block potassium channels. This finding is consistent with the hypothesis that all these effects of cromakalim are mediated via increased K^+ conductance.

This investigation has revealed differences in the sensitivity of different arterial preparations to cromakalim. The order to potency with regard to direct relaxant effects and inhibition of contractions mediated via 5-HT receptors and K^+ is rabbit basilar arteries>pig coronary arteries>rabbit mesenteric artery.

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ERYTHROCYTE STABILIZING EFFECT OF A NEW FLAVONOID BENCIANOL

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Bencianol (Di-O-Diphenylmethylene 3',4'(+) catechin is a semi-synthetic flavonoid. The compound has a protective action on D-galactosamine induced oedema in the rat (Gulati et al., 1983) and we have recently shown that it can inhibit compound 48/80 induced release of histamine from the rat peritoneal mast cells (Sharma & Gulati, 1987).

In the present study we have used human erythrocyte model (Seeman & Weinstein, 1966) to investigate if bencianol has a plasma membrane stabilizing effect. We have also compared its effect with chlorpromazine, cromolyn sodium and two calcium channel antagonists (verapamil & nifedipine). Chlorpromazine is a known stabilizer of the erythrocyte membrane (Sharma & Jande, 1987). Cromolyn sodium (Pearce & Clements, 1982) and calcium channel antagonists (Ennis et al., 1983) have histamine release inhibitory properties.

Heparanised samples of antecubital vein blood were obtained from male volunteers and centrifuged for 10 minutes at 1500 g and at 4°C. The plasma and the buffy coat were carefully removed and cells suspended in 154 mM NaCl in 10 mM sodium phosphate pH 7.0 to give a final erythrocyte concentration of about 2×10^8 cells/ml. Erythrocyte lysis was produced by bringing the molarity of NaCl to 68.5 mM in the above buffer. Cell suspensions were incubated at 37°C for periods ranging from 5 to 60 minutes. They were then centrifuged to obtain supernatant which was measured for haemoglobin content using a Unicam ultraviolet spectrophotometer. Solution of bencianol was prepared as described earlier (Sharma & Gulati, 1987). Mean IC_{50} and C_{max} (concentrations which produced 50% inhibition and almost complete inhibition of erythrolysis) are shown in the table.

Drug (M)	IC_{50}	C_{max}	
Bencianol	2.5×10^{-6}	2×10^{-5}	
Chlorpromazine	4×10^{-6}	6×10^{-5}	
Cromolyn sodium	5×10^{-3}	$> 1 \times 10^{-2}$	) Higher concentra-
Verapamil	1×10^{-3}	$> 2 \times 10^{-3}$	) tions were prolytic
Nifedipine	1×10^{-4}	5×10^{-4}	

The results show that bencianol has a potent erythrocyte stabilizing effect. Maximal stabilizing effect occurred at 2×10^{-5}M. An increase over this concentration, however, had a prolytic effect and there was a gradual increase in erythrocyte lysis with increase in drug concentrations. This is not surprising since chlorpromazine and most other agents with erythrocyte membrane protective effect cause lysis at higher concentrations (Seeman & Weinstein, 1966).

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TOXICITY TESTING OF ATRACURIUM IN THE RAT IN VITRO

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The pharmacology of atracurium has been studied in man and in animals (Payne & Hughes,1981;Hughes & Chapple,1981).However,there are a few reports which indicated the toxic or adverse side-effects of atracurium in man and in animals (Mirakhur,Lyons,Carson,Clarke,Ferres & Dundee,1983;Rowlands,1983;Nigrovic,Klaunig, Smith,Schultz & Wajskol,1986). In the present investigation,the pharmacologic and toxic side-effects of atracurium were studied in the isolated diaphragm,liver, lung,heart and kidney of the rat, following both low and high concentrations of atracurium (0.2 and 100 μM).

Altogether 12 rats were used.The rats were killed by a blow to the head and bled. The internal organs were removed and placed in separate petridishes,containing cold Krebs-Henseleit solution plus atracurium. The tissues were incubated in this solution for 5 min and they then were placed in formalin (4 ml, 10% V/V),for fixation and subsequent staining,with eosin and haematoxylin, and histological sectioning. A total of 24 slides were prepared. Toxicity was assessed in terms of damage to cell membranes,intracellular dissolution of organelles,damage to blood vessels and lymphocyte infiltration.

Atracurium (1 μM) reduced the twitch tension,in the rat diaphragm preparation,by 43$\pm$2.1% of control tension (0.95$\pm$0.1 g, mean$\pm$SEM,n=6),in 4 min exposure.Higher concentrations of atracurium(e.g., 10 and 100 μM),further reduced or blocked the twitch tension,and complete twitch blockade occurred in 1-2 min,depending on the concentration used. The mean IC_{50} values (concentration to produce 50% maximum inhibition) of atracurium-induced inhibition of twitch tension was 2.4$\pm$0.2 μM.

Atracurium(0.2 μM) had no significant effect on cell membrane,intracellular organelles and integrity of all the tissues studied.However,in high concentrations (100 μM)(i.e 10-100 times clinical concentrations),atracurium produced significant damage in the cell membranes and cell contents in the liver,lung and heart.No significant effect was observed in the diaphragm or the kidney. In the liver, atracurium (100 μM),produced lesions in the cell membrane,blood vessels and periportal cell necrosis. In the lung,atracurium caused significant lymphocyte infiltration,with foamy macrophages,and breakdown of alveoli.However,the respiratory ducts remained intact.

There results indicated the lack of toxic effect of atracurium in low concentrations (close to clinical concentrations),and that only at high concentrations,did atracurium produce toxic side-effects in the tissues of the rat.These results are in agreement with those previously reported by Skarpa,Dayan,Follenfant,et.al(1983) and Cato,Lineberry & Macklin(1985),who showed the lack of effect of atracurium in paralytic and supraparalytic concentrations (doses) in man and in animals.

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CALCIUM CHANNEL MODULATORS AND COCAINE LETHAL TOXICITY

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A calcium channel modulator, nitrendipine, prevents cardiac lesions induced by cocaine administration in the rats. Nitrendipine also protects the rat against a lethal dose of cocaine. Cocaine stimulates the sympatho-adrenal system which results in the release of catecholamines which may reach toxic synaptic and tissue concentrations. Nitrendipine prevents such an outcome.

The purpose of this study was to assess the antidote properties of other clinically tested calcium channel modulators on a lethal dose of cocaine administered to the rat. Fifty Sprague Dawley rats weighing 303 ± 29 g were fitted under ether anesthesia with an intra-arterial caudal catheter according to a technique described earlier which uses a computerized system for on-line recording of arterial pressure and heart rate of the intact awake animal. All animals were intra-peritoneally administered a lethal dose of 60 mg/kg of cocaine hydrochloride. Survival time of the 8 control animal was 9'49" ± 4'56". Five minutes after being given a lethal dose of cocaine, the test animals were given one of the following calcium channel modulators : Nimodipine, nicardipine, verapamil, flunarizine and diltiazem. Treatment consisted of administration of a loading dose followed by a constant infusion of the selected calcium modulator according to the following schedule. In the case of nicardipine no loading dose was administered.

Table 1

Calcium Channel Modulator	Number	Survival Time	Amount administered
Control	8	9'49" ± 4'56"	0
Verapamil	7	6: 8'55" ± 3'16" 1: 3 h	20 µg + 5 µg/min/10 min
Nicardipine	8	7: >24 h 1: 7'	0.5 µg/min/6 min
Nimodipine	7	4: >24 h 3: 13'20" ± 3'20"	5 µg + 0.5 µg/min/14 min
Flunarizine	7	7: >24 h	50 µg + 5 µg/min/8 min
Diltiazem	7	7: >24 h	40 µg + 8 µg/min/8 min
Nitrendipine	6	6: >24 h	24 µg + 0.4 µg/min/85 min

Not all calcium channel modulators were equally effective in protecting rats against lethal cocaine toxicity. Verapamil did not prevent the occurence of convulsions and death. Nicardipine, if administered as a bolus, did not prevent convulsions. Nimodipine also failed to prevent lethal convulsions in three of seven animals. Diltiazem, which protected the animal against death, did not adequately control hypertension and tachycardia. Nitrendipine and flunarizine were effective in protecting the animals from a lethal outcome and their administration restored cardiovascular markers to normal. The effectiveness of these two compounds might be related to their multiple effects on cardiac peripheral, pulmonary, renal and cerebral vascular beds.

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DO THE PRESERVATIVES METHYL AND PROPYL HYDROXYBENZOATE ACTIVATE PROTEIN KINASE C?

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The preservatives methyl and propyl hydroxybenzoate are widely used and pharmacologically active. Their presence in Narcan (the commercial preparation of naloxone) has often caused confusion. They have been shown to relax tracheal (Soulioti et al, 1986) and vascular (Brandt et al, 1983) smooth muscle. The mechanism of this relaxation is unknown but it does not involve cyclic AMP dependent processes. A possible direct effect on transmembrane calcium fluxes was suggested by the observation that hydroxybenzoates inhibit calcium induced tracheal contraction. To examine this further the following experiments were carried out.

Isolated rat hepatocytes were injected with the photoprotein aequorin as described by Woods et al, 1986. The response to Ca++ mobilising hormones is a series of transients, rising within 3s to a peak Ca++ of at least 600nM and lasting about 7s. The frequency of the transients (0.3-4.0 min) depends on agonist concentration. The hepatocyte was initially stimulated with phenylephrine and methyl and propyl hydroxybenzoate (in the same proportions as in naloxone vehicle and at concentrations known to relax tracheae) added when the transient pattern was well established. Hydroxybenzoates were found to depress intracellular Ca++ mobilisation in a dose dependent manner. The inter transient interval increases progressively until the spikes are completely suppressed. This response is reminiscent of the effect of phorbol dibutyrate, which is thought to activate protein kinase C. It has been proposed (Woods et al, 1987) that protein kinase C provides negative feedback, possibly via phosphorylation of receptors or G proteins, to curtail inositol trisphosphate production during a transient. It is conceivable that hydroxybenzoates act via a similar mechanism.

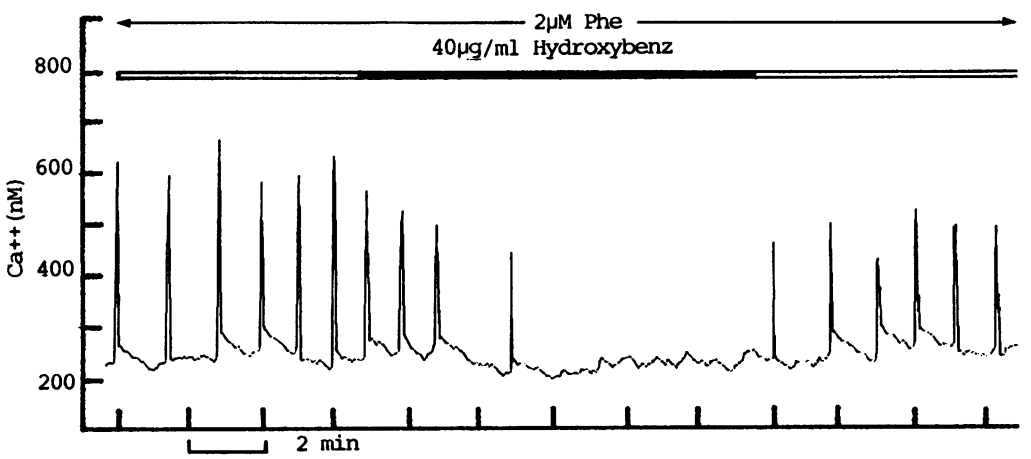

Figure 1. A recording of the aequorin signal from a single hepatocyte, plotted as free Ca++(nM). Hydroxybenzoates reversibly inhibit the transients.

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INTERACTION BETWEEN CALCITONIN AND PIRENZEPINE IN INDOMETHACIN-INDUCED GASTRIC EROSIONS IN THE RAT

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Calcitonin has been shown to have a potent anti-ulcerogenic effect in indomethacin induced gastric erosions in the rat, when given either subcutaneously (s.c.) or intragastrally (i.g.), (Bates et al, 1979). Pirenzepine is an antimuscarinic compound that has been reported to show selectivity for gastric mucosal receptors (Texter et al, 1982) and M_1 receptors (Watson et al, 1983). It also has an anti-ulcer effect. In this investigation, we have compared the effects of salmon calcitonin (sCT) and pirenzepine on indomethacin-induced gastric erosions when administered either s.c. or i.g.

Sprague-Dawley rats of either sex (150 - 180g) were starved overnight before injection of 0.112 mmol kg^{-1} indomethacin intraperitoneally. Ulcers were induced over a 5hr period and then stained by a modification of the method of Robert and Nezamis (1964). Four doses of both sCT and pirenzepine were chosen, ranging from zero to maximum response. All possible combinations of the two agents were administered either s.c. or i.g. at the same time as indomethacin.

Table 1 Effect of sCT (U kg^{-1}) and pirenzepine (mg kg^{-1}) on the number of ulcers obtained after administration of indomethacin

	s.c. Pirenzepine				i.g. Pirenzepine			
sCT	0	0.5	2	16	0	1	2	8
0	11.8±0.5	9.0±0.4	7.0±0.5	2.5±0.8	11.3±0.5	9.0±0.5	8.2±0.3	7.0±0.3
1	8.3±0.4	7.2±0.8	6.7±0.4	3.3±0.8	8.7±0.4	7.5±0.2	7.2±0.3	6.2±0.3
3	5.5±0.9	5.5±0.4	4.3±0.6	2.3±0.7	7.2±0.4	6.2±0.3	6.2±0.5	5.8±0.4
10	2.7±0.8	2.8±0.4	3.3±0.5	2.2±0.7	6.8±0.4	6.2±0.3	5.8±0.6	6.0±0.4

Values shown are mean±SEM. n=6

The results were analysed statistically in three ways. The Mann-Whitney U test indicated that there was a significant inhibitory effect for all the combinations of sCT and pirenzepine, ($p < 0.005$ for both s.c. and i.g. administration). Analysis of variance showed that there was an interaction between the two, for both methods of administration ($p < 0.001$ for s.c. and $p < 0.005$ for i.g.). The nature of the interaction was investigated by plotting isoboles, as described by Busvine (1971). This suggested that for s.c. administration, the interaction was additive,with a possibility of potentiation between sCT and pirenzepine for 50% and 60% inhibition, while i.g. administration indicated potentiation for all inhibition levels tested (50 - 90%).

We therefore conclude that after either s.c. or i.g. administration of sCT, its effect is not mediated by simple inhibition of the same receptors that pirenzepine binds to.

Pirenzepine for this work was kindly donated by The Boots Co.

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SELECTIVE DESTRUCTION OF CYTOCHROME P-450d AND ASSOCIATED MONO-OXYGENASE ACTIVITY BY CARBON TETRACHLORIDE IN THE RAT

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Polycyclic aromatic hydrocarbon (PAH)-inducible cytochrome P-450 isoenzymes (forms c and d in the rat) are of considerable toxicological interest not only because they are inducible by environmental pollutants but also because they are capable of activating otherwise innocuous compounds into toxic or carcinogenic intermediates. Carbon tetrachloride (CCl_4) is both hepatotoxic and nephrotoxic in many species. It depends upon metabolism by the cytochrome P-450-dependent monooxygenase system for its toxicity, and also acts as a suicide inhibitor of the isoenzyme(s) involved.

The effects of CCl_4 treatment *in vivo* on PAH-inducible forms of cytochrome P-450 in the rat have now been investigated using both immunochemical and enzymological techniques. Rats were treated with 3-methylcholanthrene (MC) (80 mg/kg in corn oil by i.p.injection) and 62 h later they received CCl_4 as a 5% solution in corn oil (800 mg/kg) by i.p. injection. Groups of three rats were killed at 0, 1, 3 and 6 h after CCl_4 treatment and microsomal fractions isolated from liver and kidney. Cytochrome P-450 forms c and d were immunoquantified following Western blotting with specific monoclonal antibodies.

Total cytochrome P-450 was reduced by 60% in hepatic microsomal fractions from rats exposed to CCl_4 for 6 h. However, whereas the immunochemically determined content of cytochrome P-450 form c in liver was increased from 252±60 pmol/mg to 311±43 pmol/mg, the content of form d was reduced from 285±18 pmol/mg to 4.4±0.9 pmol/mg (98% reduction) during the same period. Total renal microsomal cytochrome P-450, in the same animals, was reduced by 30%, with no change in the content of form c (40-50 pmol/mg). Cytochrome P-450 form d was undetectable in kidney (<2.0 pmol/mg), even before treatment with CCl_4.

Aryl hydrocarbon hydroxylase (AHH) and phenacetin O-deethylase (POD) activities were determined as representative of activities mediated by hepatic forms c and d, respectively. AHH activity was reduced in both hepatic and renal microsomes by 15% at most, whereas POD activity was selectively reduced, by 95%, only in hepatic microsomes with no change in renal microsomal POD activity. The time-course for the loss of hepatic POD activity was parallel to the loss of immunochemically detectable form d.

Thus, CCl_4 treatment of PAH-induced rats selectively destroys cytochrome P-450d, and confirms earlier studies (Boobis *et al.*, 1987) that POD activity of liver is catalysed almost entirely by form d, whereas this isoenzyme appears to make no contribution to activity in the kidney. Although form c catalyses the O-deethylation of phenacetin both when reconstituted and in renal microsomes (Boobis *et al*, 1987), it cannot support this activity in hepatic microsomes, even in the absence of form d.

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TISSUE DISTRIBUTION OF AMINOGLUTETHIMIDE IN THE FEMALE RAT

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Aminoglutethimide (AG) is an aromatase inhibitor used in the management of oestrogen receptor positive advanced breast cancer in post-menopausal women. However, it also inhibits another cytochrome P-450 system, adrenal cholesterol side chain cleavage enzyme (CSCCE), and this necessitates the coadministration of hydrocortisone. In a premenstrual rat model, AG (50mg/kg) reduces plasma oestradiol by 94% 3h after administration by inhibition of ovarian aromatase (Pourgholami et al.1987). This dose also has a marked inhibitory effect on CSCCE as judged by increases in adrenal cholesterol levels. However, in vitro, AG is a weaker inhibitor of CSCCE than of aromatase (Nicholls et al. 1986). The possibility that the differences between the in vivo and in vitro data may be related to drug distribution pattern has been examined by measuring the concentration of AG in various tissues of female Wistar rats (200-250g).

Animals received AG (50mg/kg suspended in 1% w/v carboxymethylcellulose) orally. Blood was collected by cardiac puncture under terminal ether anaesthesia at either 1, 3 or 9h after dosing and various tissues were rapidly dissected. From the latter, homogenates (20% w/v) were prepared in 0.1M acetate buffer (pH 5.6) for the extraction of AG into dichloromethane. AG was assayed by reverse phase hplc.

Table. Concentration of aminoglutethimide (μg/g) in various tissues of the female rat

Time (h)	Adrenal	Brain	Fat	Kidney	Liver	Ovary	Plasma*
1	16.4 (±2.1)	6.3 (±3.1)	1.5 (±0.5)	13.6 (±1.7)	5.4 (±0.8)	1.1 (±0.1)	7.0 (±1.6)
3	25.9 (±3.4)	4.5 (±0.8)	1.9 (±0.2)	5.0 (±0.6)	6.2 (±1.2)	2.2 (±0.1)	9.1 (±2.2)
9	13.5 (±1.8)	1.6 (±0.6)	0.6 (±0.1)	2.5 (±0.2)	2.3 (±0.6)	1.2 (±0.1)	2.1 (±0.3)

Values are means ± s.d., n=4, *μg/ml

AG was present in the tissues examined at each of the time points, the highest concentrations being found at 3h (Table). Levels of AG in the tissues were generally lower than the concurrent plasma level. The AG levels present in the ovary suggest that the drug is an effective aromatase inhibitor at low concentration in this species. However, at the three times investigated, the adrenal gland possessed a significantly ($P<0.05$) higher concentration of AG than at other sites. This may, in part, account for the marked inhibition of adrenal CSCCE observed at this dose of AG. It is quite likely that a similar pattern of distribution of the drug exists in women taking AG, and thus may contribute to the clinically-significant degree of adrenal suppression observed at conventional dose regimens.

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COMPARISON OF EXUDATION, VASCULAR PERMEABILITY CHANGES AND CLEARANCE PROPERTIES IN TWO MODELS OF ACUTE INFLAMMATION IN RAT

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The measurement of oedema, exudation and vascular permeability changes is commonly used for the assessment of acute inflammatory responses. Although the formation of inflammatory exudate reflects the accumulation of extravasated fluid counterbalanced by venular reabsorption and lymphatic drainage (Zanin and Ferreira, 1978), little consideration appears to have been given to the clearance properties of these lesions. In the present study these events were compared in two rat models of acute inflammation. The selected models were inflammation induced over a 24 hour period either by subcutaneous implantation of saline-soaked polyester sponges (Doyle et al. 1983) or injection of carrageenan (10mg in 1ml saline) into 6 day subcutaneous air pouches (Sedgwick et al. 1984). At various times exudates were harvested in 5ml 0.1% EDTA-saline and the exudate volume was calculated. Protein content in cell-free exudate was determined by the method of Lowry et al. (1951). Vascular permeability changes were assessed using a radiolabelled-albumen pulse technique (Udaka et al. 1970). The recovery of radiolabelled-albumen incorporated in the saline-sponges or carrageenan solution was measured as an index of the clearance properties.

Exudate volume was not considered to be a meaningful parameter since in the sponge model it remained constant throughout, and in the air pouch model retention of the viscous carrageenan solution partly accounted for the apparent exudate, particularly in the early stages. Protein levels increased throughout the time courses of both responses although near maximum levels were achieved earlier in the sponge model. The pattern of vascular permeability changes was also similar in both models with a peak response at 4-6 hours followed by a decline by 24 hours. A major difference between the two models was observed for the clearance results. In the sponge model 90% of the initially incorporated radiolabel was recovered at 3 hours. This decreased slowly to 82% at 6 hours and 48% at 24 hours. In contrast, in the air pouch there was a rapid loss of radiolabel with only 61% recovered after 30 minutes, 30% at 6 hours and 10% at 24 hours.

In conclusion, despite similarities in patterns of protein accumulation and vascular permeability changes in the two models, marked differences were observed with respect to the clearance properties. The high degree of clearance of radiolabel in the air pouch model may indicate an underestimation of the magnitude of the vascular permeability changes. The low clearance in the sponge model may reflect intrinsic fluid retaining properties and the relatively small surface area for reabsorption. These observations underline the dynamic nature of acute inflammatory responses and emphasise the need for caution in extrapolating from data obtained at single time points in apparently similar models.

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EFFECT OF SENSORY NEUROPEPTIDE DEPLETION ON CUTANEOUS RESPONSES TO INFLAMMATORY MEDIATORS IN MAN

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The role of neurogenic mechanisms in inflammatory responses, in man, is uncertain. The flare response to histamine is mediated by an axon reflex involving the retrograde release of sensory neuropeptides (Bernstein et al. 1981). We have studied whether the cutaneous responses to other inflammatory mediators, such as platelet-activating-factor (PAF), prostaglandin E_2 (PGE_2) and to allergen are also mediated by release of sensory neuropeptides. We used local application of capsaicin to deplete sensory neuropeptides, by repeatedly painting a solution of capsaicin in increasing concentrations (0.05% to 0.5%) over four days, on the forearm of six normal subjects, the other arm receiving ethanol, the diluent. On the fifth day, histamine (1ug) and PAF (0.2ug) were injected intradermally. Flare area 15 min after histamine was significantly reduced from 17.6 $\pm$ 3.1 cm^2 (mean $\pm$ SEM) to 3.5 $\pm$ 1.7 cm^2 ($p<0.05$, Wilcoxon matched pairs test) and after PAF from 11.8 $\pm$ 2.2 cm^2 to 3.1 $\pm$ 1.3 cm^2 ($p<0.05$), by capsaicin treatment. There was no effect on wheal volume. In seven non-asthmatic atopic subjects, we injected 1 to 10 BU of housedust mite or grass pollen (Pharmacia AB, Upsalla, Sweden) after similar capsaicin treatment, and compared the response to those of histamine (1ug) and PGE_2 (0.5ug). Capsaicin, again, significantly reduced the histamine flare ($p<0.02$). The flare induced by PGE_2 was also significantly reduced, from 6.0 $\pm$ 1.2 cm^2 to 3.6 $\pm$ 0.4 cm^2, at 15 min ($p<0.05$) but the wheal responses remain unchanged. The early flare response to allergen was significantly reduced from 15.5 $\pm$ 1.6 to 10.4 $\pm$ 2.5 cm^2 at 15 min ($p<0.05$), but neither the early nor the late inflammatory response was affected.

It is likely that release of sensory neuropeptides is involved in causing the flare response to histamine, PAF, PGE_2, and also to antigen. Because the early flare response to antigen is mediated partly by histamine release, our results suggest that antigen causes the release of histamine which, in turn, stimulates the release of sensory neuropeptides to cause vasodilatation. By contrast, sensory neuropeptides are not involved in either the early wheal or late phase response to antigen in human skin.

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PAF DOES NOT CONTRIBUTE TO BRONCHIAL HYPERREACTIVITY INDUCED BY INDOMETHACIN OR PROPRANOLOL

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Several drugs induce airway hyperreactivity, including the beta adrenoceptor antagonist propranolol and the non-steroidal anti-inflammatory drug (NSAID) indomethacin, although the mechanisms underlying this are not clearly understood. Recently, platelet activating factor (PAF) has been shown to induce a long-lasting, non-selective increase in airway reactivity (including histamine and ACh) in experimental animals (Robertson & Page, 1987; Barnes et al, 1987) and man (Cuss et al, 1986). The present study has investigated the role of PAF in airway hyperreactivity induced by propranolol and indomethacin, by the use of the PAF antagonists CV 3988 (1 mg/kg i.v.) and WEB 2086 (1 mg/kg i.v.).

Guinea pigs were anaesthetised with urethane (7ml/kg, 25% soln.) and after tracheal cannulation the pulmonary inflation pressure (PIP) was measured using the method of Konzett-Rossler. Dose-response curves to acetylcholine (Ach) (1-40 ug/Kg i.v.) were constructed before and after the infusion of PAF (600 ng/kg for 1 hour) in vehicle and drug treated animals. The results are summarised in Table 1.

Table 1 ED50 values in response to i.v. Ach (ug/kg)

	Before		After	
vehicle	11.8 ± 2.1	(n=7)	**5.9 ± 1.8	(n=7)
CV 3988 1 mg/kg	9.1 ± 1.2	(n=5)	7.9 ± 2.2	(n=5)
vehicle	9.8 ± 1.0	(n=5)	**4.2 ± 0.9	(n=5)
WEB 2086 1mg/kg	16.0 ± 2.9	(n=5)	14.6 ± 6.5	(n=5)

Results are expressed as mean ± S.E.M. of (n) experiments. **P<0.05 (Wilcoxon matched pairs test).

In a second series of experiments, the response to histamine (4 ug/kg i.v.) was measured before, 15, 30 and 60 min after injection of propranolol (0.1 mg/kg), indomethacin (5 mg/kg) or vehicle. The results at 30 min were maximal and are shown in Table 2.

Table 2 % increase in PIP induced by Histamine (4 ug/kg)

	PROPRANOLOL				INDOMETHACIN			
	Before	(n)	After	(n)	Before	(n)	After	(n)
vehicle	110 ± 30	(5)	263 ± 39	(5)	85 ± 14	(7)	274 ± 56	(4)
CV 3988	120 ± 23	(5)	264 ± 32	(5)	80 ± 21	(5)	222 ± 51	(4)
WEB 2086	96 ± 13	(5)	233 ± 26	(6)	90 ± 22	(5)	220 ± 30	(4)

The results are expressed as the mean ± S.E.M. of (n) experiments.

These results confirm previous reports that both propranolol and indomethacin will induce bronchial hyperreactivity in the guinea pig, but that this is not inhibited by the PAF antagonists CV 3988 or WEB 2086 at concentrations which clearly inhibit PAF-induced bronchial hyperreactivity.

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PLATELET ACTIVATING FACTOR DOES NOT CAUSE HYPERALGESIA AFTER SUBPLANTAR INJECTION IN RATS

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Platelet activating factor (PAF) has been shown to induce hyperalgesia and oedema when injected subplantar in rats (Bonnet et al, 1981; Vargaftig and Ferreira, 1981). However, there are discrepancies between these reports regarding the dose required to induce hyperalgesia and inhibition of this response by cyclo-oxygenase inhibitors. PAF has also been reported to cause hyperalgesia after intradermal injection in man (Basran et al, 1983) but a recent study has contradicted this finding (Sciberras et al, 1987). In view of this confusion, we have reexamined the ability of PAF to induce hyperalgesia in rat paws.

Nociceptive pressure threshold and paw volume were measured in female Alderley Park (AP) rats (60-100g) as previously described (Haworth and Carey, 1985). Subplantar injection of octadecyl PAF (C_{18}PAF, Bachem) induced oedema which increased to a maximum at 1hr post-injection and then gradually declined. PAF, 0.03, 0.3, 1.0, 3.0, 10 and 30 nmole increased paw volume at 1hr by 0.02±0.02, 0.19±0.02 ($p<0.001$), 0.40±0.01 ($p<0.001$), 0.39±0.02 ($p<0.001$), 0.37±0.02 ($p<0.001$) and 0.26±0.02ml ($p<0.001$) respectively (mean±sem, n=6-30). At the same doses, PAF did not induce hyperalgesia between 5 min and 4hr post-injection but a hypoalgesia was observed which correlated with the extent of oedema (max. at 1hr, 5±11; 42±5, $p<0.001$; 62±7, $p<0.001$; 50±6, $p<0.001$; 59±11, $p<0.001$ and 32±16 gcm resepctively). In contrast, subplantar injection of 20%(w/v) yeast and PGE_2 (3 nmole) as positive controls gave maximal hyperalgesia of 123±13 ($p<0.001$) and 45±11 gcm ($p<0.01$) respectively. Injection of PAF with either 0.01% (w/v) human serum albumin or 0.25% (w/v) bovine serum albumin (BSA) as phospholipid carriers did not influence the nociceptive response but BSA increased the potency of PAF to induce oedema by approx. 3 fold. Responses to hexadecyl PAF (Bachem) or bovine heart PAF (Sigma) were not significantly different from C_{18} PAF. Injection of C_{18} PAF (3 nmole) in male Sprague Dawley rats (140-180g) gave qualitatively similar responses to AP females (1 hr max: oedema, 0.79±0.04ml, $p<0.001$; hypoalgesia, 118±22 gcm, $p<0.001$) while yeast produced the expected hyperalgesia (max. 157±18 gcm, $p<0.001$).

We conclude that PAF, despite inducing a prolonged oedema, does not cause hyperalgesia after subplantar injection in rats.

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220P

EFFECT OF INDOMETHACIN AND BW A137C ON ANAPHYLACTIC MEDIATOR RELEASE FROM GUINEA-PIG LUNGS

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There is increasing evidence that PAF-acether as well as oxidative products of arachidonic acid metabolism may be involved in the pathogenesis of asthma (Barnes and Chung, 1987). We have previously shown that anaphylactic challenge of sensitized guinea-pig lungs perfused through the airways provokes the release of PAF-acether, leukotriene B_4 (LTB_4) and thromboxane B_2 (TXB_2) (Parente et al., 1987). In the present study, we have investigated the effects of selective inhibition of either cyclo-oxygenase or lipoxygenase on the release of such mediators in this experimental model. For this purpose we have used indomethacin, and the novel 5-lipoxygenase inhibitor, BW A137C [N-(4-benzyloxybenzyl)- acetohydroxamic acid] (Bhattacherjee et al., 1987; Jackson et al., 1987) respectively.

Male Dunkin-Hartley guinea-pigs (250-350g) were sensitized by injecting ovalbumin (OA) 50mg s.c. and 50 mg i.p. Three weeks later, the lungs were removed and perfused with Krebs bicarbonate solution, containing 0.25% BSA, warmed (37°C), and gassed (95% O_2, 5% CO_2) at 5 ml/min through the trachea as previously described (Fitzgerald et al., 1986). Drugs or vehicle were infused at 0.1 ml/min for 20 min prior to challenge and subsequently for a further 20 min. OA was administered as an infusion for 5 min (500 ng/min). Two 1 min samples of pulmonary effluent were collected prior to challenge and then every min after challenge for a total of 20 min. Samples were either collected directly into cold acetone for the extraction and bioassay of PAF-acether (Fitzgerald et al., 1986) or were collected for radio-immunoassay of eicosanoids.

Infusion of OA provoked the release of PAF-acether, TXB_2, LTB_4 and an increase in airway perfusion pressure, all reaching a maximum within 6 min of starting the challenge. Indomethacin (5.6 μM) completely suppressed ($p<0.01$) the anaphylactic release of TXB_2 (maximal release, 64 ± 12 ng/ml, n=7) but significantly ($p<0.01$) increased the maximal release of LTB_4 from 0.6 ± 0.1 ng/ml (n=7) to 1.4 ± 0.2 ng/ml (n=5). Although indomethacin reduced the levels of PAF-acether released 4 min after the start of the antigen infusion from 0.28 ± 0.02 ng/ml (n=7) to 0.12 ± 0.01 ng/ml (n=5, $p<0.001$), it did not significantly alter its maximal release. BW A137C (1μM) substantially reduced the maximal release of LTB_4 from 1.6 ± 0.4 ng/ml (n=4) in control lungs to 0.5 ± 0.1 ng/ml (n=4, $p<0.05$) in treated lungs, but did not significantly reduce the maximal release of either TXB_2 or PAF-acether ($p>0.05$). Indomethacin (5.6μM) and BW A137C (1μM) were without significant effect on the antigen-induced increase in airway perfusion pressure. These results suggest that the anaphylactic release of PAF-acether from the lungs of sensitized guinea-pigs perfused through the trachea is not secondary to the release of TXB_2 or LTB_4.

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EVIDENCE THAT THE INHIBITORY RESPONSE TO PGE_2 IN THE HUMAN MYOMETRIUM IN VITRO IS MEDIATED VIA THE EP_2-RECEPTOR SUBTYPE

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The response to prostaglandin-E_2 (PGE_2) in the spontaneously active human myometrium 'in vitro' is biphasic and consists of an initial contraction followed by an inhibition of spontaneous activity. The first component of this response does not appear to be dependent upon concentration, however, the subsequent inhibition of activity is clearly concentration related. It has recently been proposed that there are three subtypes of PGE-sensitive (EP) receptors (Coleman et al 1987) designated EP_1, EP_2 and EP_3. The antagonists SC19220 and AH6809 block the action of PGE_2 at the EP_1-receptor (Kennedy et al. 1982; Coleman et al. 1985) but not at the EP_2 or EP_3 subtypes. Also, the PGE_2 analogues AY23626 and sulprostone are potent agonists at the EP_2/EP_3-receptors and EP_1/EP_3-receptors respectively. The purpose of this study was to characterise the EP-receptors involved in the inhibitory myometrial response to PGE_2.

Samples of human myometrium were obtained from the anterior wall of the corpus uteri from non-pregnant pre-menopausal patients at hysterectomy. Histological examination of the endometrium ensured that all tissues used were in the luteal (secretory) phase of the cycle. Myometrial strips were set up as previously described (Massele and Senior 1981) and superfused with Krebs solution at a rate of 2 ml min^{-1}.

PGE_2 (2.8×10^{-7}mmol-3.63×10^{-5}mmol) and the selective EP_2/EP_3-receptor agonist AY23626 (2.3×10^{-6}mmol-2.9×10^{-4}mmol) evoked a qualitatively similar response, producing initial transient contractions followed by a dose related inhibition of spontaneous activity. The inhibitory responses to either compound were not blocked by either SC19220 (10^{-5}M) or AH6809 (10^{-6}M). The EP_2EP_3-receptor agonist sulprostone did not inhibit spontaneous activity in concentrations up to 6.0×10^{-4}mmol. It did however, evoke a contractile effect which appeared independent of antagonist presence. In all cases the occurrence of contractions after agonist challenge was >85%. The sum of the areas of contractile responses (cm sec^{-1}) over the complete agonist concentration range used was not significantly lower when responses in the presence of the EP_1-receptor antagonists were compared to controls. This does not suggest a role for the EP_1-receptor in this response.

These results support a role for the EP_2-receptor subtype in the inhibitory response elicited by PGE_2. The concentration-independent nature of the contractile response does not allow reliable conclusions to be drawn, however it would appear that the EP_1-receptor is not involved.

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THE PRODUCTION OF THE INFLAMMATORY MEDIATOR PROSTAGLANDIN E_2 BY EQUINE SYNOVIAL CELLS IN RESPONSE TO BONE FRAGMENTS AND SILICA

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It is well recognised that trauma, particularly that resulting in an intra-articular fracture, may lead to degenerative joint disease. Cellular mechanisms which underlie this process are poorly understood. However, the observation that synovial fluid from some traumatised joints contains large numbers of small bone fragments led us to investigate the direct effect of bone fragments and a relatively inert particle, silica, on synovial lining cells.

Synovial tissue was obtained from mature horses and ponies killed at the Royal Veterinary College for conditions which did not involve the joints. Bone filings were obtained from the second phalanx of one of these animals by the use of a small file and bone shavings were obtained with the aid of a scalpel blade.

Synovial lining cells were isolated from synovial tissue by a sequential enzyme digestion method, and grown in monolayers in DMEM with HEPES buffer, gentamicin, amphotericin B and 5% FCS (Gibco), with changes of medium every 3-4 days until confluent. Cells at passage 4 were used for this experiment.

Synovial cell cultures were each treated with one of the following stimuli (four replicates): bone shavings, bone filings and silica (Sigma) for 48 hrs at concentrations of 0, 0.1, 1, 10 and 100 µg/ml. The conditioned medium was harvested at the end of this period and stored at -20°C until analysed by radioimmunoassay for PGE_2.

The bone shavings, bone fragments and silica each increased significantly the level of PGE_2 production by equine synovial cells in culture. This response was dose dependent (Table 1), and related to particle size (mean max. diameter of particles (µm)-silica:2.2±0.3, bone filings:56.3±4.6, and bone shavings:359.5 ±33.3). It was not related to increased cell death as judged by LDH release.

Table 1 Levels of PGE_2 and LDH in tissue culture media following stimulation of cells with silica, bone filings and bone shavings.

Particles (µg/ml)	Silica		Bone filings		Bone shavings	
	PGE_2 (ng/ml)	LDH (units/l)	PGE_2 (ng/ml)	LDH (units/l)	PGE_2 (ng/ml)	LDH (units/l)
0	2.1±0.35	23.0±1.2	1.53±0.34	16.8±1.8	2,1±0.45	17.0±1.2
0.1	2.4±0.31	21.5±0.5	1.28±0.10	15.8±1.3	1.65±0.18	19.8±1.0
1	2.8±0.29	20.3±1.2	1.42±0.29	17.0	1.27±0.28	18.5±0.9
10	9.0±0.60**	20.8±2.8	7.33±1.13	16.3±2.5	3.2±0.53	18.3±1.8
100	103±9.10**	18.5±0.9	44.25±4.89**	15.8±2.8	22.13±2.76*	15.5±0.9

*p<0.01
**p<0.005

If these results can be extrapolated to the whole animal they have implications for the conservative treatment of traumatised joints and the fixation of intra-articular fractures such as condylar fractures without opening the joint. It would support the view that flushing such joints with balanced salt solutions to remove intra-articular debris is likely to have beneficial results by minimising the production of inflammatory mediators.

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THE FORMATION OF 13-HYDROXYOCTADECADIENOIC ACID (13-HODE) BY ENDOTHELIAL CELLS (EC) FROM DIFFERENT SPECIES

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Buchanan et al (1985a, 1985b) have found that linoleic acid (18:2ω6) is metabolised by a lipoxygenase in cultured human endothelial cells (EC) to 13-hydroxyoctadecadienoic acid (13-HODE). Production of 13-HODE is suppressed by salicylate or 30 min exposure to aspirin and Buchanan et al correlate this with an increase in adhesion of platelets to EC (if PGI_2 synthesis is also inhibited). They suggest that 13-HODE is an important anti-adhesive compound which in some way acts intracellularly.

We have investigated the production of 13-HODE from the unstimulated aorta of various species and human and bovine EC. Samples were extracted into ice cold methanol, homogenised, sonicated and after concentration, analysed by HPLC, using a Techsphere 5ODS column, with a solvent system of methanol, water, acetic acid 80:20:0.2 v/v/v. The flow rate was maintained at 1.0ml/min. UV absorbance was monitored at 229 and 280nm.

Bovine aorta (either whole tissue or EC) did not generate 13-HODE, but rabbit aorta produced a compound that co-eluted with 13-HODE on HPLC and whose identity was confirmed by gc-ms analysis. The amounts of 13-HODE generated by the aorta (assessed by the UV peak height) increased with the time of incubation in Krebs'solution at $4^{o}C$ after removal from the animal. Whereas after 5 min incubation, the amount released was 30 ± 10 ng/g of tissue, at 20 hrs the amount was 154 ± 68 ng/g tissue and at 60 hrs, 1155 ± 160ng/g. Preincubation of aortae with the lipoxygenase inhibitors NDGA (100μM) or ETYA (50μM) for 30 min did not reduce the amount of 13-HODE extracted.

After 5 min incubation, dog and porcine aorta also contained 13-HODE (70ng/g and 15ng/g respectively) as did human umbilical vein EC (9.3 ± 5.8ng/10^{6} cells).

Our results show that the amount of 13-HODE extracted from aorta or EC varies from species to species and that the high concentrations found after long incubations are most likely produced by a non-enzymic process. The lack of effect of lipoxygenase inhibition on the amounts extractable after short (30 min) incubation suggests that this may represent content rather than *de novo* production

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EDRF INHIBITS PLATELET AGGREGATION IN VIVO AND LEUCOCYTE AGGREGATION IN VITRO

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Endothelium derived relaxing factor (EDRF) is a chemically unstable, locally acting vasodilator substance released from vascular endothelium by various substances including ACh. EDRF mediates the ACh induced relaxation of preconstricted rings of isolated arteries and veins. EDRF is also responsible for the vasodilator effect of this substance on resistance blood vessels of the perfused rat lung and rabbit heart. Additionally, preliminary reports have suggested that EDRF inhibits platelet aggregation in vitro (Bhardwaj & Moore, 1987). In the present study we have investigated the effect of EDRF on platelet aggregation in the anaesthetised rat in vivo and the effect of EDRF on human leucocyte aggregation in vitro.

In these studies leucocytes were prepared from heparinised (2 U/ml) human blood by centifugation (400g for 30 min) on Mono Poly resolving medium. Leucocyte layers were washed once and resuspended in Krebs' solution ($2x10^7$ cells/ml). Aggregation in response to A23187 (5 ug/ml) was assessed turbidometrically in 0.1 ml aliquots (37°C, 800 rpm). EDRF was generated in vitro by challenge of indomethacin (14 uM) pretreated rat aortic rings (10 mg) with ACh. Platelet aggregation in vivo was determined by monitoring the pulmonary accumulation of 111-Indium oxine labelled platelets in anaesthetised rats (urethane 25% w/v, i.p.) following i.v. injection of ADP (1-40 ug/kg) as described previously (Page et al, 1982). EDRF was generated in vivo by i.v. administration of carbachol (0.5-5 ug/kg) in rats pretreated with hexamethonium (10 mg/kg; i.v.) and/or indomethacin (3 mg/kg; i.v.).

Indomethacin (14 uM, 30 min) pretreated rat aortic rings did not inhibit leucocyte aggregation in response to A23187 (PGI_2 concentration measured by radioimmunoassay of 6-oxo-PGF1 alpha < 19 $\pm$ 2.2 pg/ml; n=5). In the presence of ACh (0.1 uM, 1.1 uM) similar aortic rings inhibited leucocyte aggregation by 10.5 $\pm$ 1.8 % and 65.3 $\pm$ 5.8 % respectively (n=4-8; $p<0.05$). No such inhibition was observed in rat aortic rings rubbed to remove the endothelium or in rings pretreated with mepacrine (10 uM) or methylene blue (10 uM). In all cases the concentration of PGI_2 measured in leukocyte suspensions as 6-oxo-$PGF_{1\alpha}$ was less than 40 pg/ml. I.v. administration of ADP (20 ug/kg) induced a 31.9 $\pm$ 2.1 % (n=5) increase in the content of 111-indium oxine labelled platelets in the thoracic region of anaesthetised rats. Carbachol (0.5 & 5 ug/kg) injected 5 sec before ADP administration produced a dose related inhibition of ADP-induced platelet accumulation (increase in labelled platelet count of 28.2 $\pm$ 0.9% and 23.0 $\pm$ 1.1% respectively n=5; $p<0.001$). Carbachol-induced inhibition of platelet accumulation was not modified in rats pretreated with indomethacin or with indomethacin plus hexamethonium. The present results suggest that EDRF or a like material inhibits both leucocyte aggregation in vitro and platelet accumulation in vivo.
These effects are unlikely to be accounted for by the generation of cyclo-oxygenase metabolites of arachidonic acid such as PGI_2. These results suggest that EDRF may be involved in the regulation of the activity of blood elements in the circulation.

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THE BIOTRANSFORMATION OF A SYNTHETIC PROSTAGLANDIN DERIVATIVE BY RAT WHOLE BLOOD

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M&B 33,153 ((+)2-[7-hydroxyheptyl]-3-[3-oxo-4-phenoxybutyl] cyclopentanone), has been under investigation as an antipeptic ulcer agent. Kinetic studies following oral and i.v. administration of the product had failed to detect measurable levels of unchanged parent compound in plasma. This non-detection of M&B 33,153, despite blood sampling, minutes post i.v. administration, had implied rapid and complete metabolism by an extrahepatic mechanism, possibly in blood itself.

To test the metabolic potential of blood, M&B 33,153 spiked with [13,14-$^{3}H_2$] M&B 33,153 was incubated in vitro with heparinised rat whole blood for 3h at 37°C. Chromatographic analysis of the plasma resulting from the incubate revealed extensive metabolism, with six radiolabelled products (M1-M6) detected. Preparative reverse phase hplc successfully isolated the four major products of the incubation (M2-M5), which were characterised by ^{1}H nmr and mass spectrometry and assigned structures presented in Figure 1. Trace metabolites M1 and M6 were not identified.

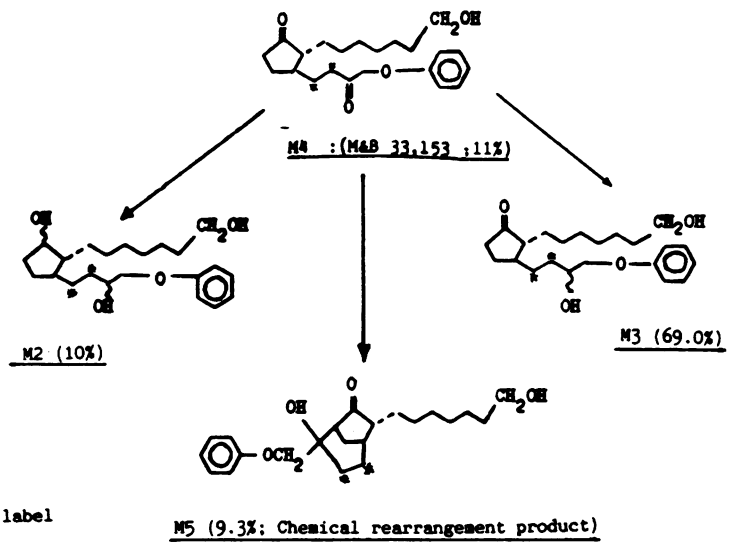

Figure 1 Products isolated from the incubation of M&B 33,153 with whole blood.

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DUAL EFFECT OF 12-HYDROXYEICOSATETRAENOIC ACID (12HETE) ON RABBIT PLATELET AGGREGATION

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13-Hydroxyoctadecadienoic acid (13HODE), 12HETE and 15HETE inhibit blood platelet activation, provided that plasma is absent (Coene et al., 1986). These hydroxyfatty acids (HOFAs) may act as weak antagonists of the thromboxane A2 mimetic 15(S)-hydroxy-11α,9α-(epoxymethano) prosta-5Z,13E-dienoic acid (U46619) in vascular smooth muscle. Therefore we investigated whether these HOFAs also interfere with platelet aggregation induced by U46619, and whether the inhibition was structure-dependent.

15HETE, 13HODE and washed rabbit platelets were prepared as described (Coene et al., 1986). The platelet suspension (PS) in calcium free Krebs' was kept under 5 % CO2 and 95 % 02. Prewarmed PS (280 µl) and HOFA (0.01 to 30 µM, given in 35 µl) were preincubated for 1 min in a Chronolog M540 aggregometer before injection of 1 µM U46619 (in 35 µl). Aggregation was expressed as percentage of the control (saline). 9HODE was prepared by incubation of linoleic acid with a cytosolic fraction of a tomato homogenate and 12HETE by incubation of arachidonic acid with the cytosolic fraction of washed platelets. Products were extracted and purified by TLC and HPLC. Their purity was checked with gaschromatography (GC) and their identity confirmed by GC-mass spectrometry of the methylester, trimethylsilyl-ether derivatives.

All HOFAs tested as well as linoleic acid inhibited platelet aggregation dose-dependently. All products were equipotent, the concentrations producing 50 % inhibition ranged between 3 and 7 µM. None of the HOFAs induced platelet aggregation, but 12HETE enhanced the aggregation induced by U46619 (Table 1) and ADP, without affecting aggregation induced by arachidonic acid (data not shown). The threshold for stimulation was 10 nM and it was not shared by the other HOFAs (Table 1).

Table 1. Percent platelet aggregation in the presence of fatty acids (1 µM).

	linoleic acid	ricino-leic acid	9HODE	13HODE	5HETE	12HETE	15HETE
mean (%)	93	94	92	87	83	177*	112
s.e mean	8	6	7	12	21	25	7
n	5	3	3	9	3	6	10

* Significantly different from control, paired Student's t-test.

In conclusion, high concentrations of hydroxyfatty acids cause a non-specific inhibition of U-46619 induced platelet aggregation, which does not depend on the presence of a hydroxygroup. On the other hand, 12HETE may selectively promote aggregation in concentrations which could be reached in the platelet. These results may help to explain the conflicting reports on pro- or anti-aggregating effects of 12HETE. Its pathophysiological relevance has to be determined as 12HETE biosynthesis is a sluggish process relative to the dynamics of platelet activation.

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TWO SITES OF ACTION FOR COLONIC ELECTROGENIC SECRETAGOGUES

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Calcium ionophore A23187 has been widely used to study the role of Ca^{2+} in Cl^- secretion of intestinal epithelium. We have previously shown that A23187 induces release of PGE_2 from rabbit mucosal colonic sheets and suggested that this might contribute to the observed secretory response in addition to the ionophore's direct effects on the epithelial secretory mechanism (Hoult & Phillips, 1986a). The present studies extend these findings and compare A23187 with lysylbradykinin (LBk). Colonic sheets stripped of both muscle layers were mounted in Ussing chambers and the I_{sc} and p.d. monitored by a computer-driven voltage clamp, and the PGE_2 release measured by RIA.

The electrogenic I_{sc} response to 10^{-6}M A23187 in rabbit colon was sided in that a significant increase in I_{sc} was only observed when the ionophore was applied serosally (72 ± 15 $\mu A/cm^2$) but not mucosally (4 ± 8 $\mu A/cm^2$). In addition, when a sheet of rabbit colon with epithelial cells removed was challenged with 10^{-6}M there was increased release of PGE_2 (2.6 ± 0.9 $ng/cm^2/min$) into the serosal bathing medium, a change larger than that in epithelial-intact tissue (0.8 ± 0.2 $ng/cm^2/min$). A23187 did not cause increased release of TXB_2 or 6keto-PGF_{1a} in either rabbit colonic preparation. When piroxicam (10^{-5}M), a cyclo-oxygenase inhibitor lacking direct inhibitory effects on epithelial transport was used, the release of PGE_2 was completely blocked but the increase in I_{sc} was only reduced to ca. 50%. Both the increases in I_{sc} and the release of PGE_2 caused by ionophore were abolished in calcium-free conditions (EGTA chelation).

Taken together, these results indicate that the subepithelial cells of the lamina propria are the principal source of the A23187-induced PGE_2 and that the PG may contribute substantially to the electrogenic response. Both steps are dependent upon extracellular Ca^{2+}. Thus the release of mediators such as PGE_2 from the cells of the lamina propria contributes to the colonic secretory response to A23187, but there is also a direct component (likely due to raised Ca^{2+} within the epithelial cell activating chloride secretion).

This dual site of action of colonic secretagogues is also the case for LBk: we have shown that cells of the lamina propria release PGE_2 in response to LBk (Hoult & Phillips, 1986b), although a PG-independent component can be discerned under certain circumstances (Cuthbert et al., 1984).

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