ELECTRICAL AND MECHANICAL RESPONSES TO NERVE STIMULATION IN MOUSE VAS DEFERENS, EVIDENCE FOR CO-TRANSMISSION.

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In the mouse vas deferens the contractile response to field stimulation is biphasic, comprising an initial twitch followed by a slower tonic component which lasts throughout the stimulation period (Farnebo & Malmfors, 1971). Both components are believed to be neurally mediated, being abolished by TTX, but the transmitters involved have not been clarified. However, in the guinea-pig vas deferens, the initial component of the biphasic contraction to field stimulation and the accompanying e.j.p.s are believed to be mediated by ATP or a related nucleotide (Sneddon & Westfall, 1984; Sneddon & Burnstock, 1984). The secondary contractile component is mediated by NA, but unaccompanied by any detectable membrane change.

The present study was undertaken to examine the relationship between the electrical and mechanical responses of the mouse vas deferens to field stimulation, and so determine the role of transmitter-mediated membrane potential changes in the contractile response.

Both contractile components to field stimulation (0.5ms, supramaximal voltage, 5-20Hz) were abolished by TTX ($1x10^{-6}$M) and guanethidine ($1x10^{-6}$M). αβ-methyleneadenosine-5'-triphosphate (αβMeATP, $1-10x10^{-6}$M), which blocks P_2-purinoceptors by desensitization, selectively antagonized the rapid twitch component, leaving the tonic contraction unaffected. The selective α_1 adrenoceptor antagonist prazosin ($1x10^{-7}$M) abolished the tonic phase. ATP, added exogenously produced a rapid transient contraction resembling in time course, the initial phase of the neurogenic contractile response. The contraction produced by NA was slower in onset and more prolonged, resembling the tonic phase of the neurogenic response.

Membrane potential changes (mean resting value -73.1±6.6, n=120) were measured intracellularly, with capillary, glass microelectrodes filled with 3MKCl (20-40x $10^{-6}\Omega$) simultaneously with contractions in response to field stimulation (0.01-0.5ms, supramaximal voltage, via chlorided Ag/AgCl ring electrodes, O.D. 2mm). Contractile responses were accompanied by e.j.p.s (and action potentials) which facilitated above 0.2Hz. In contrast to the rabbit ear artery (Allcorn et al, 1985) and rat tail artery (Cheung, 1982), where the same substances, NA and ATP, appear to be involved, no subsequent noradrenergic slow depolarization after the e.j.p.s was observed. Prazosin ($1x10^{-7}$M) potentiated the e.j.p.s, in the mouse vas deferens and reduced the mechanical contractions; αβMeATP ($1-10x10^{-6}$M) abolished the e.j.p.s, together with the residual contractile responses.

ATP ($1-10x10^{-4}$M), applied locally from a micropipette using a pressure controlled ejection device (Picospritzer II General Valve Corporation) produced a dose-dependent depolarization which was abolished by αβMeATP ($1-10x10^{-6}$M). NA ($1-10x10^{-4}$M) similarly applied produced no significant membrane potential changes.

These results suggest, that in the mouse, as in the guinea-pig vas deferens, NA and ATP act as co-transmitters. Both transmitters play a role in the mechanical response to field stimulation, but only the nucleotide appears to mediate an electrical event.

K.A.W. acknowledges financial support of Glasgow University Medical Faculty.

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A DIACYLGLYCEROL KINASE INHIBITOR, R59022, POTENTIATES THROMBIN-INDUCED PLATELET AGGREGATION

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Platelet activation by thrombin is mediated in part by a concentration-dependent increase in membrane 1,2-diacylglycerol (DG) (Rittenhouse-Simmons, 1979). This molecule activates protein kinase C, the most prominent substrate of which in platelets is a 40,000 dalton protein of uncertain function (Kaibuchi et al., 1983). It is thought that phosphorylation of this protein is involved in platelet activation. The project was undertaken to further test the concept that DG is a second messenger in platelets by preventing its metabolism and looking for a potentiation of response. This is analogous to cAMP phosphodiesterase inhibition by the methylxanthines resulting in potentiation of cAMP responses.

To inhibit the metabolism of DG, we used R59022 - a recently described 1,2-diacylglycerol kinase inhibitor (de Chaffoy de Courcelles et al., 1985). DG kinase phosphorylates DG to form phosphatidic acid (PA). [32P] incorporation into PA was used to indicate the activity of the DG kinase; the [32P] was separated from other lipids by thin layer chromatography (Lapetina et al., 1984), and the radioactivity was counted by liquid scintillometry. Activation of platelets was followed in a Lumi-aggregometer giving aggregation traces and showing ATP secretion. 20,000 and 40,000 dalton protein phosphorylation was measured by gel chromatography and liquid scintillation counting (Lapetina et al., 1984).

In five out of six experiments with duplicate or triplicate samples, platelet aggregation by threshold concantrations of thrombin was potentiated in the presence of R59022 (10 μM). This was accompanied by a significant ($P < 0.05$) decrease in [32P] PA, and increase in [32P] labelling of the 40K protein ($P < 0.01$). No significant change in 20,000 (myosin light chain, MLC) phosphorylation was observed. The table shows data from four experiments with identical methodology:

	Thrombin (mean ± S.E.M.)	Thrombin + R59022 (mean ± S.E.M.)	P
PA (n=4)	173 ± 17.3	126 ± 11.5	< 0.05
40K (n=4)	139 ± 20.8	232 ± 25.7	< 0.01
20K (n=4)	124 ± 13.5	130 ± 9.3	n.s.

These data support the view that DG is involved in platelet activation, that it leads to 40,000 dalton phosphorylation, and is not involved to any appreciable extent in the phosphorylation of MLC. Since PA formation is decreased, and aggregation is potentiated, this is further evidence that PA is not a second messenger involved in platelet activation (Watson et al., 1985).

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ATROPINE REDUCES THE DECAY PHASE OF GIANT POTENTIALS INDUCED BY ESERINE IN THE RAT NEUROMUSCULAR JUNCTION

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The presence of inhibitors of acetylcholinesterase, like eserine, neostigmine and diisopropylfluorophosphate (DFP), in the superfusion medium of isolated mammalian skeletal muscle preparations causes an increase in amplitude as well as a prolongation of the decay phase of spontaneous miniature endplate potentials (MEPPs).

In the present study we have investigated the effect of eserine in detail on the cut muscle phrenic nerve-hemidiaphragm preparation of the rat, using atropine as an antagonist. Superfusion of the isolated diaphragm with eserine, 0.1 to 1 µmol/l, raised the frequency in particular of giant MEPPs.
Giant MEPPs are normally present in small numbers, 1 to 5 %, in recordings of untreated neuromuscular preparations, while under normal physiological circumstances they are found to dominate in embryonic tissue and during regeneration of the neuromuscular junction. Giant MEPPs are characterized by slow and variable rising and decay times.
After treatment with 1 µmol/l eserine, MEPPs with a prolonged decay phase dominated, although normal unitary MEPPs were also found in the same recordings. The half decay time increased significantly from 1.11 (± 0.09,SEM) ms in the controls (at a temperature of 30 ± 0.3° C) to 3.47 (± 0.44) ms in the presence of 1 µmol/l eserine.

Atropine, in concentrations of 1 to 30 µmol/l, caused a small, dose dependent reduction of the half decay time, from 1.11 (± 0.09) ms in the controls to 0.99 (± 0.07) ms in the presence of 30 µmol/l atropine.
The prolonged half decay time, caused by eserine, is significantly reduced by atropine from 3.47 (± 0.44) ms to 2.44 (± 0.30) ms in the presence of 30 µmol/l atropine. Atropine also reduced the variability of the duration of the decay phase, owing to the reduction of the number of slow and giant MEPPs.

Since, after treatment with eserine, both types of MEPPs with normal rising and decay times, as well as, slow and giant MEPPs were found in recordings from the same endplate, it might be assumed that the effect of eserine on the decay phase is caused by a presynaptic effect.
The effect of atropine, thereupon, cannot be fully explained by a postsynaptic effect of atropine, because atropine selectively reduced the prolonged decay time of slow and giant MEPPs.

We tentatively conclude that eserine prolongs the decay phase of a special class of slow and giant MEPPs and that this effect is mediated by presynaptic muscarinic receptors, which can be antagonized by atropine.

CISPLATIN INDUCES BIOCHEMICAL AND HISTOLOGICAL CHANGES IN THE SMALL INTESTINE OF THE FERRET

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The antineoplastic agent cisplatin causes severe nausea and emesis in man which can result in treatment refusal. In ferrets, the selective 5-HT_3 receptor antagonists MDL72222 (Miner and Sanger, 1986) and GR38032F (Brittain et al; Costall et al; both this meeting) inhibit cisplatin-induced emesis implicating serotoninergic mechanisms in the response. In the present series of experiments, the possibility that cisplatin may induce changes in 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels was investigated in the gastrointestinal tract and centrally in the hypothalamus, areas known to contain large quantities of this amine.

Male ferrets, 1.4-1.9kg, were dosed with either cisplatin (Lederle), 9mg/kg i.p., or vehicle i.p. on two consecutive days. On the second day the mean number of emetic episodes was 10±1.9 within the 3h study period. Three hours after the second dose of cisplatin or vehicle the animals were killed. Stomach mucosae (taken from the fundic region), ileal mucosae (taken 30cm from the pylorus) and the hypothalamus were dissected from the animals for analysis of 5-hydroxyindoles by H.P.L.C. with electrochemical detection. Segments of stomach and intestine were also dissected from adjacent areas for histological examination. In cisplatin-treated animals there were increases ($p<0.05$) in the ileal mucosal levels (ng/mg protein) of 5-HT (control 29.4±1.8, cisplatin 58.5±7.2) and 5-HIAA (3.5±1.8 to 7.4±1.7); noradrenaline levels were unaffected (5.2±2.1 to 4.4±1.0). In the gastric mucosa or hypothalamus there were no significant changes in 5-HT or 5-HIAA levels.

In the stomach, macroscopic examination revealed no apparent differences between cisplatin-treated and control ferrets. However, cisplatin treated ileum exhibited marked congestion of the lamina propria, haemorrhaging and glandular cell necrosis. A good correlation was observed between the emetic response of a particular animal and the histological picture. An absolute count of the number of enterochromaffin cells in ileal sections revealed no apparent difference between cisplatin-treated and control animals.

Cisplatin treatment clearly induced selective biochemical and histological changes in the small intestine. The ability of cisplatin to induce degenerative changes of intestinal epithelium has been noted in other species (see review by Vermorken and Pinedo 1982). Whether, the observed changes in 5-HT levels in the ileum are dependent on intestinal degeneration or whether changes can occur in brain areas other than the hypothalamus which may be involved in cisplatin induced emesis remains to be established. It is tempting to speculate that cisplatin induces intestinal degeneration resulting in 'protective emesis' which involves serotoninergic mechanisms.

We wish to acknowledge J. Cook, B. Reed and C. Pick for histological examinations.

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NEUROMODULATORY ACTIONS OF BOMBESIN-RELATED NEUROMEDINS IN THE VAS DEFERENS PREPARATION

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The nerve stimulated vas deferens preparation has proved of great value in the study of neuromodulatory properties of peptides, since the neurally mediated contractile response may readily be measured, and potentiation (eg by substance P-related kinins) or depression (eg by opioids) may be used as a simple measure of effects on neurotransmitter release. We have made use of the preparation in preliminary experiments with bombesin-related peptides with the aim of learning something of the mechanisms and receptors involved, in parallel with similar experiments using substance P-related neurokinins.

In the guinea-pig vas deferens mounted for isometric recording in Krebs solution at 37 °C with field nerve stimulation via platinum electrodes using square wave pulses of 1 ms duration, bombesin (10-100 nM) was found to give dose-dependent slowly reversible potentiation of nerve-mediated contractions in the 10-30 Hz frequency slowly range with sub- or supra-maximal stimulation voltages. This potentiation appears to be at a presynaptic site, presumably the sympathetic nerve endings, since in this concentration range noradrenaline- and carbachol-induced contractions were not potentiated, and bombesin itself had only minimal contractile activity in some preparations at higher concentrations (1 μM).

The guinea-pig vas deferens preparation stimulated at 20 Hz has been used to compare the potencies of neurokinin [NK] (or tachykinin [TK]) agonists (Watson et al, 1983), and on this basis the receptors involved appear similar to those originally designated 'SP-P' (Lee et al, 1982) or more recently 'TK-1' or 'NK-1' (Bailey et al, 1986; Neurokinins Symposium, 1986; this being in interesting contrast to potentiation in the rat preparation which possesses 'SP-E' ('TK-2' = 'NK-2') receptors.

Using these stimulation parameters in the guinea-pig preparation we have been able to assay neurokinins and bombesin-related kinins in parallel in terms of potentiation of nerve-mediated responses. A number of naturally occurring bombesin analogues have been tested, and those found active in a concentration range (10-100 nM) similar to the neurokinins include, in addition to bombesin itself, mammalian neuromedin C.

Thus it appears that the vas deferens preparation may profitably be used to compare substance P-related and bombesin-related peptides with respect to their mechanisms for facilitation of neurotransmitter release, the receptor types involved, and testing of antagonist selectivity.

We gratefully acknowledge a Wellcome Trust Vacation Scholarship to A.J.F. and an M.R.C. Scholarship to J.M.H.

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BIPHASIC ACTIONS OF BRADYKININ IN THE GUINEA-PIG TAENIA CAECI PREPARATION

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Bradykinin (BK) shows an interesting spectrum of actions in gastrointestinal smooth muscle preparations, contracting the majority, relaxing the minority, and in others showing a biphasic action. Mechanistically, this may reflect a balance between two opposing actions, so to throw light on this possibility we have examined particularly the clear biphasic response of the guinea-pig taenia caeci preparation. In preparations where the tone is naturally high, bradykinin (10-100 nM) causes a marked initial relaxation lasting about 20s that gives way to a contraction that takes about a further 10s to peak. Neither phase is sensitive to TTX, atropine, guanethidine, or adrenergic antagonists. A similar response is seen in low tone preparations pre-contracted with carbachol, histamine, substance P, or eledoisin.

Under all conditions we find the relaxant action of BK to be abolished by apamin (10 nM), as also reported by Gater et al (1985) for physalaemin contracted preparations, which suggests the involvement of Ca^{2+}-activated K^+-channels in the inhibitory phase. We have gone on to show that the subsequent contractile phase is largely insensitive to apamin and, further, appears after a similar latency to that before application of the toxin. This suggests that the slow ('brady') contractile response to bradykinin in G.I. muscle in general is not simply a consequence of an initial inhibitory action, though certainly this phase can be demonstrated if the tone is raised in preparations such as the guinea-pig ileum, and here again it is sensitive to K^+-channel blockers.

Bradykinin antagonists should help decide if different receptor types are involved for each phase and allow separation for mechanistic analysis. The antagonist [des-Arg , Leu]BK reported as active on B_1 BK receptors in vascular tissues was inactive against either phase in the taenia caeci. Of a series of BK antagonists synthesised by Vavrek and Stewart (1985), on which some initial biological data are available, the more recent compound [D-Arg, $Thi^{5,8}$, Hyp^3, D-Phe^7]BK is of particular interest since it inhibits BK induced contractions in guinea-pig ileum (pA_2>6). However, at 10 μM it was inactive against either phase in taenia caeci, so we are proceeding to examine other new members of the series.

Thus it seems the relaxant phase of bradykinin's action in guinea-pig taenia caeci involves Ca^{2+}-activated K^+-channels, but it is not clear why the contractile phase has a long latency and is slow. We have no information yet as to the receptor type(s) involved in the biphasic action.

We are grateful for the gift of BK analogue B4162=B3824 from Prof. Stewart and Dr Vavrek. We thank the Wellcome Trust for supporting this collaborative project and the MRC for Research Scholarships for T.D.C., J.M.H and D.V.M.

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EFFECTS OF TACHYKININS ON CHOLINERGIC NEURAL RESPONSES IN GUINEA-PIG TRACHEA.

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The tachykinins, Substance P (SP) and Neurokinin A (NKA) have been identified in afferent nerves and parasympathetic ganglia in mammalian airways and appear to activate different receptor subtypes (SP-P and SP-E respectively). As these sensory peptides may be released during inflammation of the lung, the effects of SP and NKA and two other tachykinins Eledoisin (E) and Physalaemin (P) were studied on pre- or post-ganglionic cholinergic nerve induced contraction of guinea pig trachea.

Guinea-pigs (200-300g) were anaesthetised with urethane (1.5g/kg) and the trachea removed with the right vagus attached for pre-ganglionic stimulation (Blackman and McCaig, 1983).The trachea was suspended in Krebs solution oxygenated with 95%02/5%CO2 at 37°C. Contractions of the trachealis muscle were elicited either by vagal nerve (preganglionic) or transmural (postganglionic) stimulation (30 Hz, 0.2ms, 150pulses). Responses were measured as increases in tube pressure with a Statham (P23AC) transducer. Indomethacin (10 μM) was present.

NKA, P, E and SP caused concentration dependent contractions of the trachealis muscle, (NKA >P >E >SP), suggesting that a SP-E receptor mediates contraction of the smooth muscle. The peptides had no effect on contraction elicited by supramaximal preganglionic or transmural stimulation except at higher concentrations (>10 μM) when they inhibited nerve-induced contractions. In the same experiments, two drugs known to increase acetylcholine output, gallamine (10 μM; Fryer and Maclagan, 1984) and the stable thromboxane analogue, U46619 (1 nM; Chung et al.,1986) potentiated the transmurally-elicited response by 30%. This lack of effect of the peptides contrasts with the potentiation of nerve-induced contractions reported by Tanaka and Grunstein (1986) for SP in rabbit trachea.

In a second series of experiments, submaximal stimulation voltages were used (50% maximal). Under these conditions, the peptides potentiated the contractile responses to both pre-ganglionic and transmural stimulation in a concentration-dependent manner. (Maximum increase, P (10 nM) 42%; E (10 nM) 36%; NKA (10 nM) 11%; SP (1 μM) 4%.) However, the response to exogenous acetylcholine (1 μM) was not potentiated indicating a pre-junctional site of action for the peptides.

These results indicate that tachykinins may activate receptors both on airway smooth muscle and on cholinergic nerves. The mechanism by which tachykinins increase cholinergic nerve transmission only at submaximal electrical stimulation remains to be determined.

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EFFECT OF THE ENDOTHELIUM ON THE EXTRUSION OF CYCLIC GMP FROM RAT ISOLATED AORTA.

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Relaxant responses mediated by an endothelium derived factor (EDRF), are associated with an increase in the tissue content of cyclic (c) GMP due to the activation of soluble guanylate cyclase (GC) (Förstermann et al., 1986). Increased levels of cyclic nucleotides are often associated with their extrusion from the tissue (Barber & Butcher, 1983). These extracellular nucleotides and their metabolites may affect smooth muscle and other cells directly, or modulate the effects of hormones and drugs on the tissue.

The appearence of cGMP in a physiological solution bathing segments of rat isolated aorta with (+) and without (-) endothelium (ENDO) was measured. Aortae were divided into 4 segments, two of which were rubbed to remove ENDO. Experiments were performed on groups of aortic segments + and -ENDO, placed in 1 ml of oxygenated solution at 37° C. After 30 min equilibration, either carbachol (a stimulator of the release of EDRF), atriopeptin II (AP II, an ENDO-independent activator of particulate GC, Winquist et al., 1984) or sodium-nitroprusside (an ENDO-independent stimulator of soluble GC), was added. Sometime later 75 ul of solution was assayed for cGMP (Miller et al., 1984).

Without ENDO the quantity of cGMP in the medium increased with time, plateauing after 30 min. After 120 min, extrusion amounted to 26.6 $\pm$ 11.1 fmol/mg of tissue wet wt (n=6). However, +ENDO there was a greater ($P < 0.005$) increase to 659.7 $\pm$ 146.4 fmol/mg (n=7) over 120 min (means $\pm$ SEM). This demonstrates an ENDO-dependent increase in tissue cGMP extrusion that may be related to basal release of EDRF. Basal cGMP levels in this tissue are increased by about 2 fold +ENDO (Miller et al., 1984). Carbachol 10 uM, did not affect basal extrusion of cGMP -ENDO, but extrusion was increased +ENDO ($P < 0.001$) reaching 3441 $\pm$ 414 fmol/mg tissue (n=8). Sodium nitroprusside (10 uM) significantly increased extrusion -ENDO to 4085 $\pm$ 374 fmol/mg tissue (n=4) and +ENDO to 5239 $\pm$ 195 fmol/mg tissue (n=4). Total extrusion in the presence of sodium nitroprusside was slightly ($P < 0.05$), greater +ENDO, a difference that could be explained by increased basal extrusion in the presence of endothelium. AP II (1 uM) increased extrusion -ENDO to 406 $\pm$ 50 fmol/mg tissue (n=6) and +ENDO to 4421 $\pm$ 365 fmol/mg tissue (n=8). This increase +ENDO, about 11 fold of that -ENDO, could not be explained by differences in the basal level of extrusion.

The results demonstrate a basal extrusion of cGMP that is enhanced +ENDO. Carbachol increased this ENDO-dependent extrusion, perhaps by stimulating release of EDRF. However, AP II, which stimulates a different GC to EDRF, also provoked an ENDO-dependent increase in cGMP extrusion. It is concluded that an ENDO-derived factor might be able to alter the sensitivity of particulate GC to stimulation by AP II, or that AP II and carbachol stimulate the extrusion of cGMP from ENDO cells.

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THE INFLAMMATORY RESPONSE OF THE AIR POUCH TO BACTERIAL LIPOPOLYSACCHARIDE

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The inflammatory potential of bacterial lipopolysaccharides is well documented (Morrison and Ulevitch, 1978). Studies however show that this ability varies between species and between strains of a species, and the type of response depends on where these endotoxins localise. We examined here the response of preformed subcutaneous air pouches to lipopolysaccharide. These pouches have inner linings with histological similarities to normal synovium (Edwards et al, 1981) and respond in a comparable manner during development of polyarthritis to mycobacterial adjuvant (De Brito et al, 1986)

Random bred male Wistar rats weighing 150-200 gm were used. Subcutaneous air pouches were raised on the backs of rats by injection of 20 ml of air and maintained by further injections of 5-10 ml every 3-4 days. Six days after the first injection (designated day 0) half the number of rats were inoculated intradermally (day 0) in the base of the tail with 0.1 ml of paraffin oil containing 0.5 mg dead Mycobacterium tuberculosis (adjuvant). On day 6, pouches of all rats were challenged with 0, 10 or 100 µg Salmonella typhimurium lipopolysaccharide suspended in 2.0 ml sterile saline by direct injection into the cavity.

Table 1

	Increase in air pouch skin fold thickness (mm) on post-inoculation day		
	7	9	11
NI control	0	0	0
AI control	-0.01 ± 0.11	0.73 ± 0.23	0.37 ± 0.11
NI X 10µg Endo	0.07 ± 0.09	0.13 ± 0.21	0.19 ± 0.12
AI X 10µg Endo	0.31 ± 0.24	1.28 ± 0.37	0.57 ± 0.20
NI X 100µg Endo	0.48 ± 0.09 *	0.25 ± 0.22	0.28 ± 0.10
AI X 100µg Endo	0.73 ± 0.29 *	1.94 ± 0.32 **	1.07 ± 0.23 *

NI = non-inoculated; AI = adjuvant-inoculated; Endo = endotoxin; Each result represents the mean ± SEM of six rats; * $p<0.05$, $p<0.01$ in comparison with control (Student's T-test)

Challenge of the pouches of non-inoculated rats with endotoxin resulted in a small but significant increase in the thickness of skin of the pouch (see table). Histological sections of pouch skin taken on day 9 showed moderate numbers of inflammatory cells present around blood vessels in the lining and along its surface. Pouch cavities were however free of inflammatory fluid at this time. Endotoxin challenge of pouches of adjuvant-inoculated rats resulted in a much greater increase in pouch skin thickness - there was marked potentiation of the skin reaction induced by adjuvant.

It would appear thus, that although endotoxin can induce an inflammation in the air pouch, its full potential can best be demonstrated in rats about to develop adjuvant polyarthritis, suggesting that its actions may be of enhancing movement of cells into the pouch and/or increasing their activity following their arrival into the pouch.

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This work was supported by a grant from the Arthritis and Rheumatism Council.

NON-SPECIFIC AIRWAY HYPERREACTIVITY FOLLOWING BRONCHIAL ANAPHYLAXIS IN ANAESTHETISED GUINEA-PIGS

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Non-specific enhanced responsiveness (hyperreactivity) to bronchoconstricting stimuli is a recognised feature of asthma and may occur as a result of underlying airway inflammation (Robinson and Holgate 1985). In guinea-pigs, anaphylactic bronchospasm provokes inflammatory sequelae such as oedema, cellular infiltration and disruption of bronchial epithelium, similar to that described in human asthmatic airways (Dunnill 1973). For this reason, we have investigated whether direct inhalational challenge of anaesthetised guinea-pigs with aerosolised antigen (Payne and Nucci 1987), leads to the subsequent development of hyperreactivity to either inhaled or intravenous bronchoconstrictor agonists.

Male Dunkin Hartley guinea-pigs (300-350g) were actively sensitised to ovalbumin (O.A., 50 mg i.p. and 50 mg s.c.). After 14 to 21 days, the animals were anaesthetised with sodium pentobarbitone (40 mg/kg i.p.), both cervical vagi were cut, the trachea cannulated, and the lungs ventilated mechanically (54 strokes min^{-1} of 1 ml laboratory air per 100g body wt) Pulmonary inflation pressure (PIP) was measured from a lateral port in the afferent limb of the ventilator circuit. A jugular vein was cannulated for i.v. drug administration. Each animal was challenged with a 5 sec aerosol of antigen generated by a DeVilbiss Pulmosonic Ultrasonic Nebuliser from a solution of OA at either 0.03% or 1%. This challenge represented a submaximal (microshock) and supramaximal (macroshock) stimulus inducing a rise in PIP of 20.2±2.6 and 57.4±1.8 cm/H_2O (n=5) respectively. In the latter instance, isoprenaline (1µg/kg/min) was infused for 10 min (starting 10 min after antigen challenge) to reverse substantially the prolonged increase in PIP following anaphylactic macroshock.

An approximate 3-fold leftward shift in the location of the dose-response curve for bronchoconstriction induced by 5HT (1-30µg/kg i.v., n=6) was apparent in challenged animals 60 min after anaphylactic macroshock, when compared to non-challenged control animals. (DR=2.6, $P<0.005$) A significant shift in the dose-response curve to i.v. 5-HT was also seen 60 min following anaphylactic microshock (DR=1.9, $P<0.005$). This hyperreactivity was not specific to 5-HT since the dose-response curve for the bronchoconstrictor effect of acetylcholine (3-100µg/kg i.v.) following anaphylactic microshock was also located to the left of that in control animals, and to the same extent (DR=2.0, $P<0.005$). Furthermore, bronchial reactivity to inhaled 5-HT (100µg/ml aerosol for 2-5 sec) was likewise increased 2-3 fold ($P<0.05$) following anaphylactic microshock.

These results indicate that non-specific and route-independent airway hyperreactivity follows anaphylactic micro- or macro-shock in anaesthetised guinea-pigs. It is not known whether this phenomenon is associated with the initial pulmonary release of anaphylactic mediators including the eicosanoids, or results from specific pathological events such as the subsequent infiltration of inflammatory cells into the bronchial tissue.

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THE EFFECT OF OXYGEN-DERIVED FREE RADICALS ON AIRWAY SMOOTH MUSCLE RESPONSES

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Bacterial infections and inflammation of the lung are associated with the accumulation of phagocytic cells whose activation results in the release of oxygen-derived free radicals. Pulmonary macrophages induce a deterioration of guinea-pig ß-adrenoceptor function which is prevented by free radical scavengers (Engels et al,1985).In addition hydrogen peroxide (H_2O_2) contracts bovine tracheal smooth muscle (Stewart et al, 1981).We have examined the effects of the free radical generating systemn ß-glucose / glucose oxidase and of H_2O_2 on guinea-pig tracheal smooth muscle.

Guinea-pig tracheas were dissected into transverse strips and were mounted in 10 ml organ baths containing Krebs-Henseleit solution at 37°C. Contractile effects of oxygen - derived free radicals were examined by the administration of cummulative doses of H_2O_2 or glucose oxidase in the presence of excess ß-glucose. Matched strips were preincubated with either $1x10^{-5}$ M indomethacin or free radical scavengers. To assess the effect of free radicals on ß-adrenoceptor function dose- response curves to isoprenaline were constructed following precontraction with 10^{-5} M histamine. Strips were then incubated with either 10^{-3} M H_2O_2 or 10^{-3} ß-glucose / 10^{-1} U ml^{-1} glucose oxidase for one hour before repeating isoprenaline dose-response curves. Indomethacin was present in the latter experiments to prevent a direct contractile effect of free radicals.

In the presence of excess ß-glucose, glucose oxidase (10^{-4}-1 U ml^{-1}) elicits a dose-dependent contraction of tracheal strips (-log EC_{50} 1.61±0.21 U ml^{-1}, maximal contraction 34.5±6.4 % of carbachol maximum, n=7), which is inhibited by 100 U ml^{-1} catalase (an H_2O_2 scavenger) but not by 25mM mannitol (a hydroxyl ion scavenger). H_2O_2 (10^{-6}-10^{-2} M) causes a similar contraction of tracheal strips (-log EC_{50} 3.65±0.14M, maximal contraction 49.5±7.8 % of carbachol maximum, n=9) which is inhibited by indomethacin. Removal of the epithelium increases the sensitivity of strips to H_2O_2 (-log EC_{50} 4.94±0.38M, maximal contraction 187.1± 35.7% of maximal contraction in the presence of epithelium, n=6). Relaxation of tracheal strips to isoprenaline was similar before and after incubation with ß-glucose/glucose oxidase (-log EC_{50} 7.36±0.03M versus 7.39±0.04M, maximal relaxation 103.4±0.03 % versus 107.7±3.1 % reversal of precontraction, n=6), or H_2O_2 (-log EC_{50} 7.19±0.10M versus 6.92±0.19M, maximal relaxation 104.2±2.7% versus102.8±0.2% reversal of precontraction, n=5).

Inflammation of the airways leads to the release of oxygen - derived free radicals which may contribute to bronchoconstriction via the generation of cyclooxygenase products. This bronchoconstriction may be enhanced by pathological damage to the epithelium.

This work was supported by the Asthma Research Council.

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ANTITUSSIVE EFFECTS AND INHIBITION OF VACAL SENSORY NERVE ACTIVITY BY TYR.D.ARG.GLY.PHE (^{4}-NO_2).PRO.NH_2. (443C)

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Stimulation of sensory nerve endings in the respiratory tract of animals and man leads to a variety of vagally mediated reflexes, including cough (Widdicombe 1954a&b; Nadel et al. 1965). Tyr.D.Arg.Gly.Phe(4-NO_2).Pro.NH_2 (443C) is a novel enkephalin analogue with restricted penetration of the blood brain barrier and we have examined the effects of 443C on "cough-like" reflexes and the activity of rapidly adapting "irritant" receptors in the respiratory tract.

Male guinea-pigs (Dunkin-Hartley, 375-575g) were placed in a 20l glass vessel and exposed for 5 min to an irritant vapour generated by nebulising an aqueous solution of citric acid (30% w/v). Coughs were recorded over a 12.5 min period. ED_{50}s were calculated from linear regression analysis. Male cats (3.1-4.1kg) were anaesthetised with chloralose (60-80mg/kg iv) following induction with 5% halothane. Respiratory parameters were recorded using an online analogue computer (Buxco Electronics, model 4). "Cough-like" reflexes were induced, in spontaneously breathing animals, by inflating with air a small rubber balloon inserted in the region of the larynx and recorded as an increase in respiratory flow (ml/sec). Animals used for nerve recording were paralysed with dimethyl tubocurarine and artificially ventilated. Thin nerve filaments were dissected from the left vagus (cut at the central end) and impulse discharges in single fibres from "irritant" receptors were recorded and counted. Drugs were dissolved in saline and injected by iv bolus or infusion (0.1ml/min).

443C was antitussive in both species tested. In guinea-pigs, the ED_{50} values for 443C, morphine and codeine were 0.7, 1.6 and 8.7mg/kg iv respectively. In 4 cats, "cough-like" reflexes were significantly reduced from 180.9 ± 17.5 to 61.6 ± 10.5ml/sec during an infusion of 443C at 30µg/kg/min (1h, total dose 1.8mg/kg). Reflexes were monitored for a further 2h during which time they returned to pre-drug values.

In 10 cats spontaneous discharges of "irritant" receptors were recorded for 20 min before iv infusion of 443C at either 30µg/kg/min (1h, total dose 1.8mg/kg, n=4)) or 100µg/kg/min (1h, total dose 6.0mg/kg, n=6). The spontaneous discharges of the receptors were significantly reduced; with 30µg/kg/min from 1.74 ± 0.16 to 0.73 ± 0.3 impulses/sec and with 100µg/kg/min from 1.64 ± 0.40 to 0.20 ± 0.03 impulses/sec. Spontaneous discharges returned to pre-drug values by 2h after cessation of the infusions of 443C.

In summary, 443C produced effective inhibition of "cough-like" responses in the conscious guinea-pig and the anaesthetised cat. The ability of this compound to reduce the spontaneous activity of "irritant" receptors implies that at least part of the antitussive activity of 443C is due to an effect on sensory nerve endings in the respiratory tract.

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OPIOID INFLUENCES ON THE RESPONSE OF RESPIRATORY-RELATED-NEURONES TO CARBON DIOXIDE

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Chemosensitive medullary areas of the cat have been shown to be sensitive to topical applications of opiates, which cause a reduction in responsiveness to carbon dioxide (CO_2) stimulation (Hurle et al., 1982). We now report that some opioid peptides can decrease the responsiveness of single respiratory-related-neurones (RRN) to CO_2 stimulation in the rat medulla.

Male Wistar rats (250-350g) anaesthetised with urethane (1.75 g/kg, i.p.), were used. The left external carotid artery and the left femoral artery were cannulated, and then the animal was prepared for electrophysiological recording and microiontophoresis as described previously (Bradley & Dray, 1974). Conventional 5-barrelled micropipettes (tip diameter 4-6 μm) were used. The central recording barrel contained 4M NaCl, whilst one of the outer barrels contained the dye Pontamine Sky Blue (PSB) (2.5% in Na acetate, pH 5.6), which was utilised for current balancing and marking the position of the electrode tip. The remaining barrels contained a combination of the following solutions; D-Ala, D-Leu-enkephalin (DADLE) (10mM pH 4.5), D,L-Homocysteic acid (DLH) (200mM pH 8), Ethylketocyclazocine methane sulphanoate (EKC) (100mM pH 4.3), Naloxone hydrochloride (NaL) (20mM pH 4.5), RX 783006 (RX) (10mM pH 4.3). Agents were ejected and retained as cations with the exception of DLH. Carbon dioxide stimulation was achieved by intra-arterial injections of small volumes of CO_2-equilibrated saline (0.025-0.05ml) into the carotid body, which caused hyperventilation and a hypo- or hypertensive response in the animal.

Spontaneously active RRN were studied, a population of neurones that have previously been shown to be depressed by the opioid peptides DADLE and RX, when applied microiontophoretically (Bryant, 1985). CO_2 stimulation caused excitation of 51/57 RRN tested whilst 5/5 non-RRN were not affected. The excitatory response displayed by RRN was characterised by a sharp increase in neuronal activity coinciding with hyperventilation and changes in BP. The μ-, δ- or κ-opioid peptides were applied either just before or concurrently with the CO_2 stimulus. Both DADLE (14/16 RRN) and RX 783006 (17/18 RRN) reduced the response of the cells to CO_2 stimulation, with full recovery occurring within 15 min. EKC (5/5 RRN) had no effect on the excitatory response elicited by CO_2 stimulation. On several neurones naloxone reversed the effects of the opioid peptides. Histological examination showed the RRN recorded from to be located within the ventral portion of the medulla.

These results show that CO_2 stimulation of the carotid chemoreceptors excite ipsilateral medullary RRN and suggest that this response may be modulated by the opioids at the level of the medullary RRN.

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COMPARATIVE EFFECT OF DRUGS ON BRONCHOCONSTRICTION INDUCED BY PAF, HISTAMINE AND ACETYLCHOLINE

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Platelet activating factor (PAF) has been implicated as an important mediator in asthma in view of its pro-inflammatory effects in addition to its ability to induce bronchoconstriction and bronchial hyper-reactivity. (Vargaftig et al 1980, Morley et al, 1984.) Therefore it was of interest to compare the effect of a range of drugs on responses to PAF with responses to other bronchoconstrictor agents.

Guinea pigs (350-500 g) were anaesthetised with urethane (25% w/v, 7ml/kg i.p.) and ventilated (1 ml/100 g b.w., 66 strokes/min). Sodium pentobarbitone was used to inhibit spontaneous respiration. Airway resistance was computed breath by breath from measurements of flow, transpulmonary pressure and tidal volume. Blood pressure was recorded from the carotid artery and drugs injected via the jugular vein. Bronchoconstrictor dose-response curves were constructed to histamine (1-10 μg/kg) and acetylcholine (10-50 μg/kg). Complete dose response curves to PAF could not be obtained in single animals so curves were constructed from groups of guinea pigs given single doses of PAF (50-120ng/kg). Antagonist potency was calculated as the effective shift of the standard control curve.

ANTAGONIST		n	EFFECTIVE DOSE RATIO ± S.E.M. PAF	ACETYLCHOLINE	HISTAMINE
Atropine	50 μg/kg	3	1.92 ± 0.57	>10	
Mepyramine	5 mg/kg	3	2.06 ± 0.09		>500
Dazoxiben	10 mg/kg	3	3.65 ± 0.34	0.97 ± 0.27	
Ketotifen	1 mg/kg	3	2.57 ± 0.71	0.96 ± 0.20	>500
	3 mg/kg	3	5.09 ± 1.06	1.84 ± 0.43	
L652731	1 mg/kg	4	4.01 ± 0.56	0.90 ± 0.13	
	3 mg/kg	3	6.22 ± 0.49	0.73 ± 0.16	
Saline	1 ml/kg	5	0.94 ± 0.14	0.94 ± 0.13	1.19 ± 0.24

As expected from previous data (Vargaftig et al 1980, Lewis et al 1984) atropine and mepyramine had little effect on responses to PAF induced bronchoconstriction at doses which markedly reduced responses to acetylcholine and histamine respectively. Dazoxiben, a thromboxane synthetase inhibitor, reduced but did not abolish PAF responses suggesting that release of thromboxane A_2 by PAF contributes to the bronchoconstrictor response. Ketotifen antagonised PAF responses but only at doses 1000 fold those required to inhibit histamine responses. Similar high doses of ketotifen were required to reduce PAF induced bronchial hyper-reactivity in the anaesthetised guinea pig (Mazzoni et al 1986). In contrast, the PAF receptor antagonist Merck L 652731 (trans-2, 5-bis (3,4,5-trimethoxyphenyl tetrahydrofuran)) (Hwang et al 1985) selectively inhibited PAF induced bronchoconstriction. The effectiveness of this class of drug in asthma remains to be established.

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INOSINE, DIPYRIDAMOLE AND ADENOSINE-INDUCED RESPIRATORY STIMULATION IN THE ADULT RABBIT

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Adenosine is an endogenous nucleoside with varied pharmacological effects which include respiratory stimulation in man (Watt & Routledge, 1985; Reid et al., 1986) and laboratory mammals (Buss et al., 1986a; Monteiro & Ribeiro, 1986). We compared the respiratory effects of adenosine and its deamination product inosine in the rabbit, and examined the modulatory effect of dipyridamole on adenosine-induced respiratory stimulation.

Ten adult New Zealand White rabbits (weighing 2-4 kg) were used for the inosine study and 8 for the dipyridamole study. Adenosine was administered by serial rapid intravenous bolus doses (separated by at least 60 seconds) via a cannula sited in a marginal ear vein. The initial dose was 40 $ug.kg^{-1}$, increasing in steps of 40 $ug.kg^{-1}$, to a maximum of 400 $ug.kg^{-1}$. Respiratory changes were assessed by a semi-quantitative, non-invasive technique using a Lectromed type 4320 respiration transducer as previously described (Buss et al., 1986a). In the inosine study, the series of injections of each nucleoside were given in random order. In the dipyridamole study a series of adenosine boluses was given before and after dipyridamole 10 mg intravenous injection over 60 seconds. Peak ventilatory effects, usually 2-4 seconds after injection, were compared to baseline using Student's paired t test.

Inosine did not increase ventilation at any dose studied between 40 and 400 $ug.kg^{-1}$ ($P > 0.05$ in each case), whereas adenosine increased ventilation by approximately 40% in the dose-range 120-400 $ug.kg^{-1}$.

In the dipyridamole study the effects of adenosine alone appeared slightly smaller with ventilatory increases of approximately 25% in the dose-range 120-400 $ug.kg^{-1}$, but the change did not reach statistical significance at doses of 200 and 320 $ug.kg^{-1}$. Following dipyridamole pretreatment the respiratory stimulant action of adenosine was not apparent, there being no change in ventilation in the dose range 40 - 400 $ug.kg^{-1}$, ($P > 0.05$) in each case.

The abolition of adenosine-induced respiratory stimulation by dipyridamole was unexpected, and contrasts with the potentiation by dipyridamole of adenosine-induced respiratory stimulation in man (Watt & Routledge, 1986). One possible explanation is that in the rabbit exogenous adenosine must be transported by the cell membrane nucleoside transporter to reach its site of action, which lies within the carotid body (Buss et al., 1986b; Watt et al., 1986). Our data also indicate that the respiratory stimulant action of adenosine does not depend on deamination to inosine consistent with our findings in man (Reid et al., 1986).

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EFFECTS OF MILRINONE AND SULMAZOLE ON ANAPHYLACTIC BRONCHOCONSTRICTION AND MEDIATOR RELEASE IN RAT ISOLATED LUNGS

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Accumulating data indicate that two recently developed cardiotonic agents milrinone and sulmazole act by inhibiting phosphodiesterase in cardiac cells. Like theophylline, these drugs are able to alter cyclic nucleotide levels in a variety of cell types. Therefore we investigated whether milrinone and sulmazole possess beneficial effects on anaphylactic bronchoconstriction and mediator release. The experiments were carried out in vitro.

Isolated lungs of actively sensitized male Wistar rats were challenged i.v. with antigen. The lungs were perfused with Krebs solution via the pulmonary artery and rhytmically ventilated. Tracheal pressure and pulmonary artery pressure were continuously measured. Earlier, this method proved to result in a reproducible IgE-mediated bronchoconstriction.

Both milrinone and sulmazole inhibited the bronchoconstriction in a dose dependent manner, the EC_{50} values being 0.15 µmol/ml and 0.05 µmol/ml respectively. Also, the simultaneous rise in pulmonary artery pressure was reduced dose dependently.
In separate experiments we investigated the influence of milrinone on the mediator release during the anaphylactic reaction in vitro. Therefore, milrinone was dissolved in the perfusate in a concentration of 50 µg/ml. This treatment inhibited bronchoconstriction as well as the rise in pulmonary artery pressure significantly ($p < 0.05$, n=17) when compared with control values (n=16). During the experiments, samples of the perfusate were collected for the analysis of histamine and serotonin by means of HPLC. Also the possible effect of the drug on the production of leukotrienes was measured. Leukotriene concentrations were determined by means of a bioassay using the guinea pig isolated ileum. This method has been described previously. Milrinone did not interfere with this assay.

The results indicate that milrinone significantly inhibits histamine release during the antigen-antibody reaction. Moreover, leukotriene production was significantly reduced ($p < 0.05$) in the presence of milrinone.

In conclusion, our study indicates that the phosphodiesterase inhibitors milrinone and sulmazole inhibit the IgE-mediated bronchoconstriction and the rise in pulmonary artery pressure in the rat isolated lung in a dose dependent manner. These beneficial pharmacological effects can be attributed to the ability of the drug to prevent the release of mediators (like histamine) and to inhibit the synthesis of leukotrienes during anaphylaxis. The presented results will be the basis for further inverstigations.

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HISTAMINE RELEASE BY COTTON DUST FROM ISOLATED LUNG CELLS

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The release of histamine in the lungs by a component of cotton dust has, amongst several possibilities, been proposed as a mechanism for the acute reversible bronchoconstriction observed in byssinotic cotton workers (Bouhuys et al., 1960). In an in vitro study with chopped lung, an aqueous extract of cotton dust was shown to release histamine well from human and pig lung but only poorly from that of common laboratory species such as the guinea pig (Evans & Nicholls, 1974). Recently the guinea pig has been selected as a possible animal model for studies of the acute and chronic stages of byssinosis (Karol et al., 1985) and thus it appeared important to re-evaluate the histamine-releasing activity of cotton dust with the lung of this species. Because of the unreliability of chopped lung for this type of investigation (Ainsworth et al., 1979), collagenase-freed cell suspensions of lung were used. Such a preparation is both sensitive and reliable for histamine release studies (Barrett et al., 1983).

Chopped lung from Dunkin-Hartley (400g) guinea pigs was incubated for 90 min at 37°C in a solution (25ml/lung) of collagenase (0.5mg/ml) in Tyrode solution containing 10mM HEPES and bovine serum albumin (1mg/ml). The tissue was disrupted by passing through a syringe, and filtered through gauze. The separated cells were harvested by centrifuging at 4°C for 5 min (150xg). After several washings, the cells were suspended in HEPES-Tyrode ($3x10^5$ cells/ml) and incubated for 30 min at 37°C with extracts of cotton dust or cotton bract in this medium. Released histamine was assessed fluorimetrically.

Extracts of both cotton dust and bracts released histamine from the lung cells in a concentration-dependent manner over the range 10-100mg dust/ml of incubation medium. With both materials the maximum release was 30% of the total in the cells. This is an effect much higher than previously found (1%) for chopped guinea pig lung at such dust concentrations (Evans & Nicholls, 1974). The releasing activity of cotton dust was temperature- and time-dependent, optimal conditions being 25°C for 30 min. The mechanism appeared to be energy-dependent because histamine-release by dust was 60-70% inhibited by the presence of the metabolic inhibitors antimycin-A (1μM) and 2-deoxyglucose (1mM).

The present findings indicate that the guinea pig is likely to be a useful animal model in which to study the role of histamine release in the symptomatology of byssinosis.

PJN is grateful to Cotton Inc., Raleigh, N.C. for financial support.

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BRONCHIAL AIRWAYS RESPONSE OF THE GUINEA-PIG TO COTTON DUST EXTRACT

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The acute symptoms of byssinosis include chest tightness, shortness of breath and a decrease in lung function (McKerrow et al., 1958). These responses can be reproduced in healthy volunteers by inhalation of aqueous extracts of cotton dust (Johnson et al., 1985). Although the aetiology and pathogenesis of the disease remain obscure, the release of histamine (Bouhuys et al., 1960) and of arachidonic acid metabolites (Mundie & Ainsworth, 1985) have been proposed as possible mechanisms underlying the acute bronchoconstrictor effects of inhaled cotton dust. Elucidation of the mechanisms involved requires the availability of a suitable animal model. In view of the finding that exposure of guinea pigs to cotton dust causes perturbations of respiratory patterns (Ellakkani et al., 1984), the present investigation was undertaken to examine the airways response of this species to inhaled extracts of cotton dust.

Specific airway conductance (sGaw) was measured in unanaesthetized guinea pigs with a constant volume plethysmograph, a fall in sGaw reflecting bronchoconstriction. Measurements were made prior to aerosol challenge (baseline) and at 1, 2 and 3.5h after inhalation, results being expressed as % change in sGaw at each time point from the baseline value. An aqueous extract of cotton dust (Johnson et al., 1985) equivalent to 6g dust/50ml saline, was filter-sterilized and nebulized (Wright nebulizer, 20psi, 10ℓ air/min). Animals were exposed to the aerosol for 6 min. In some experiments, FPL 55712 (3% w/v in saline) was administered by the same route for 5 min.

In a group of 13 animals, inhalation of the dust extract caused a significant ($P<0.01$) fall in mean sGaw (i.e. bronchoconstriction) at 1h (22±11% fall) and at 2h (23±11% fall). After 3.5h, sGaw returned to within baseline values. Inhalation of saline produced no significant changes in sGaw. Although the extent of the response to the dust exttact was quite variable, this dose was effective in all 13 animals, the onset and duration of bronchoconstriction being similar to that previously described in human subjects inhaling a comparable dust extract (Johnson et al., 1985). In a group of 6 animals, inhalation of FPL 55712 did not affect sGaw. However, its administration immediately before dust extract inhalation prevented the airways response to the latter.

The results indicate that a bronchoconstriction comparable with that occurring in man is produced in the guinea pig upon inhalation of a cotton dust extract. This gives support to the use of this species as an animal model for byssinosis. The ability of the leukotriene C4 and D4 antagonist FPL 55712 to block the effects of the dust suggests that the synthesis and release of such metabolites may be implicated in the acute bronchoconstrictor response of the guinea pig to cotton dust. This supports the work of El-Mahdy & Nicholls (1986) that the cotton dust-induced bronchoconstriction of guinea pig perfused lung is antagonized by FPL 55712.

PJN is grateful to Cotton Inc., Raleigh, N.C., for financial support and to Fisons, Loughborough, for the gift of FPL 55712.

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A NOVEL METHOD FOR THE EVALUATION OF BRONCHOACTIVE AGENTS IN THE CONSCIOUS GUINEA-PIG

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The various methods available for assessing bronchoconstrictor/dilator activity in conscious guinea-pigs all have drawbacks, being either traumatic and unreliable (Loew et al., 1944; Herxheimer, 1952), invasive (Amdur and Mead, 1958) or complicated and insufficiently versatile (Dorsch et al., 1981)

We describe a simple, non-invasive, non-traumatic and reproducible method, in which lung function may be recorded in 6 guinea-pigs at the same time. It is comprised of 6 'head-out' whole body plethysmographs (WBP): 20cm x 8cm diam. perspex tubes with at one end a rubber neck seal, and at the other a 'plunger' with which to adjust WBP length. Pressure changes within the WBP are measured with a low pressure transducer connected to a side-arm. Each WBP is connected via a head chamber (HC) to an aerosol expansion chamber (AEC). Each HC consists of a 7cm x 8cm diam. perspex tube with a resin insert approximating the profile of a guinea-pig's head to reduce the dead-space. The AEC consists of a 50cm length of 10cm square steel trunking, 3 HCs being mounted on each side. Holes through the walls of the AEC into each HC are gated by sliding valves, which when closed, allow the animals to breathe O_2/CO_2 (95%/5%). A Devilbiss 645 nebulizer at one end of the AEC generates bronchoconstrictor aerosols, the outlet at the other end going to waste. With the guinea-pigs in the apparatus, respiratory rates ($\simeq$ 100 min^{-1}), derived from the cyclic pressure changes in each WBP are recorded. While the animals breathe O_2/CO_2, a bronchoconstrictor aerosol (see Ball et al., 1987) is driven into the AEC for 15s, after which time the nebulizer is switched off and the valves in the HCs opened to the AEC. The resulting bronchoconstriction induces an increase in respiratory rate. Each animal is exposed to the aerosol for 4 min or until a 40% increase in respiratory rate is obtained (whichever comes first) and the valves then shut automatically and independently and the animals breathe the O_2/CO_2 again. During the exposure, a purpose built respiratory computer measures the areas under the respiratory rate curves for all 6 animals simultaneously. These areas are assessed over 4 min and up to a maximum of 40% increase. When challenged at 1-3h intervals, the area for each animal is highly reproducible. After constant control responses have been obtained, a test compound may be administered (e.g. by aerosol or p.o.), and the rate response to the bronchocontrictor re-determined at intervals thereafter. The maximum degree of protection by the test compound and its duration of action can be determined. Protection results in a reduction in the area under the rate curve (complete protection = zero area). Potency can be quantified as the dose of drug giving a 50% inhibition of the area under the curve (IC_{50}). Results using this method are reported in an accompanying communication (Ball et al., 1987)

We wish to thank Mr. A. Newbury and Mr. S. Cranwell of the Bioengineering Unit, Glaxo Group Research Ltd. who developed the exposure chambers and the control electonics respectively.

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EVALUATION OF SOME BRONCHOACTIVE AGENTS IN CONSCIOUS GUINEA-PIGS

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We have described a novel, non-invasive method of evaluating bronchoactive agents in conscious guinea-pigs (Ball et al., 1987). We now present some experimental data obtained using this method with a range of bronchoconstrictor and bronchodilator agents, after aerosol administration.

We have evaluated the bronchoconstrictor effects of aerosolized solutions of histamine (Hist, 0.5-5.0mgml^{-1}, n=4) and acetylcholine (ACh, 0.5-3.0mgml^{-1}, n=4). These two agents were equipotent, with a threshold response at 0.5mgml^{-1} and a maximum or near-maximum response at 1mgml^{-1}; EC_{50} values (95% C.L.) for Hist and ACh of 0.79 (0.62-0.99, n=4) mg ml^{-1} and 0.74 (0.63-0.87, n=4) mgml^{-1} respectively were obtained. The responses to inhaled Hist and ACh were different, in that those to Hist were maintained after cessation of exposure to the aerosol, usually to the end of the 4 min measurement period, whereas those to ACh were extremely labile, with complete recovery within seconds of the cessation of aerosol exposure.

In addition to Hist and ACh, other bronchoconstrictor agents: 5-hydroxytryptamine (5-HT), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), bradykinin (BK), leukotriene D_4 (LTD_4) and the thromboxane A_2 - mimetic, U-46619 (Coleman et al., 1981) have been tested and the concentration which caused a response approximately equal to that of Hist or ACh (1mgml^{-1}) determined. All of the agonists except $PGF_{2\alpha}$ induced increases in repiratory rate, the rank order of potency (approximate equipotent concentration, ACh=1) being: LTD_4 (0.01) > U-46619 (0.1) > Hist (1.0) ≃ ACh (1.0) ≃ BK (1.0) ≃ 5-HT (1.0) > $PGF_{2\alpha}$ (>5.0).

Finally, we have tested the bronchodilator drugs, salbutamol, clenbuterol, N-ethylcarboxamide-adenosine (NECA), prostaglandin E_2 (PGE_2), theophylline, and papaverine, as well as atropine, mepyramine and verapamil against ACh (1mgml^{-1})-induced bronchoconstriction. The agents were all administered by aerosol for 1 min, 3 min prior to ACh challenge, and the concentration required to inhibit ACh-induced bronchoconstriction by 50% (IC_{50}) determined. PGE_2 was the most potent, IC_{50} [range] = 0.03 [0.02-0.08, n=3] mgml^{-1}. The rank order of inhibitory potency (equipotent concentration, PGE_2=1) was PGE_2 (1) > atropine (7.3) > salbutamol (11.1) > clenbuterol (19.3) > NECA (46.7). Mepyramine (1mgml^{-1}), theophylline (25mgml^{-1}), papaverine (20mgml^{-1}) and verapamil (20mgml^{-1}) were all inactive.

These data demonstrate that the method described may be used to quantify in conscious guinea-pigs both bronchoconstrictor and dilator activities of a range of different agents, and therefore represents a simple and reliable means of assessing bronchoactive agents in vivo.

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CHARACTERISATION OF THE HISTAMINE RECEPTOR WHICH MEDIATES EDRF RELEASE IN A MICROVASCULAR PREPARATION.

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Van de Voorde and Leusen (1983) first demonstrated that an intact endothelium was essential for the relaxing effect of histamine on rat thoracic aorta *in vitro*, and was shown to be mediated by an H_1 receptor.

The isolated perfused mesenteric bed of the rat was prepared by a modified McGregor (1965) procedure according to the method of Byfield et al (1986). Methoxamine ($2x10^{-5}M$) was used to increase perfusion pressure from 38±1 to 69±2 mmHg, n=16. Endothelial cells (EC) were removed by perfusion with 1.5 mg ml^{-1} sodium deoxycholate for 30 seconds via a parallel perfusion line. It has recently been shown by scanning electron microscopy (unpublished data) that this procedure was effective in removal or damage of EC in arterioles of 88±12 µM, n=15 without damage to the underlying elastic lamina or smooth muscle.

Histamine and 2(2-amino-ethyl) thiazole dihydrochloride (2-TEA, H_1 agonist) produced dose related EC dependent falls in perfusion pressure. Dimaprit (H_2 agonist) was found not to be EC dependent. Results are expressed in Table 1 as the dose (g) which produced a 20% fall in perfusion pressure (ED_{20}). The effect of mepyramine, on the histamine response in the presence of EC and following EC removal, was also studied.

Table 1 Effect of EC removal on H_1 and H_2 induced vasodilatation

	n	ED_{20} (g) unstripped	stripped	Ratio
Histamine	6	$6.3x10^{-7}$	$3.5x10^{-3}$	5555
2-TEA	6	$1.4x10^{-6}$	$1x10^{-3}$	714
Dimaprit	6	$1.4x10^{-3}$	$1.8x10^{-3}$	1.3
Histamine	5	$8.9x10^{-7}$		
+Mepyramine($10^{-6}M$)	5	$8.9x10^{-5}$	$7.1x10^{-4}$(n=4)	8.0

These experiments confirmed the existence of an H_1 EDRF mediated vasodilatation in a vascular bed, which contains microvascular resistance vessels, as the response was attenuated by EC removal and antagonised by mepyramine. A direct H_2 response was demonstrated as illustrated by the effect at higher doses of histamine in the absence of EC. In the presence of mepyramine the response to histamine as well as the response to dimaprit alone was unaffected by removal of EC.

We would like to acknowledge Dr. H. Jones for the SEM studies performed at Smith Kline and French Research Laboratories.

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DESENSITISATION OF RESPONSES TO NORADRENALINE AND POTASSIUM CHLORIDE IN THE ISOLATED PERFUSED RAT TAIL ARTERY

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It is common practice with vascular smooth muscle preparations to activate the tissue several times in order to stabilise the tissue or establish the experimental parameters. The first concentration-response curve (CRC) or the first few responses are generally discarded and are never used for the analysis of data. Since desensitization of rat perfused tail artery is particularly marked and seemed to depend on $[Ca^{2+}]_o$ ([free Ca^{2+}]), we have compared the first with the subsequent 'Ca^{2+} CRCs' in an attempt to incorporate the first responses in an analysis of the properties of the tissue.

1-2 cm lengths of the proximal segment of tail artery (male Wistar, 300-350g) were perfused at 2-3ml/min with a Krebs' bicarbonate saline ($[Ca^{2+}]$ 2.5mM, pO_2 580-650mmHg, pH 7.2-7.3 at 37°C), using a pulsatile flow pump and was immersed in a similar medium. Maximal changes in the peaks of the pulsatile perfusion pressure waves were measured for calculation of vasoconstrictor responses. CRCs for Ca^{2+} were constructed by activating the tissue with noradrenaline (NA) (3uM) or potassium chloride (KCl) (100mM) with or without Bay K 8644 (0.1uM). NA was added when $[Ca^{2+}]_o$ was low (1uM) and $[Ca^{2+}]_o$ was increased in steps allowing construction of 'Ca^{2+} CRCs' as an estimate of $[Ca^{2+}]_o$ sensitivity. $[Ca^{2+}]_o$ was buffered with NTA (nitrilotriacetic acid) and EGTA (2.5mM of each), so that 'total Ca' = 2.5mM to 10mM but $[Ca^{2+}]_o$ = 1uM to 5mM. The buffers allow accurate determination of $[Ca^{2+}]_o$ (otherwise impossible <0.1mM).

NA 3uM The 1st CRCs lay further left than subsequent ones and were "bell-shaped" peaking at 0.3mM or 1.25mM with 30% of its maximum (approx. 50mmHg) attained by 30uM. 2nd and subsequent curves showed a direct correlation of log$[Ca^{2+}]$ and response and had not attained a true maximum by 5mM. The 2nd curve had reached 50mmHg (approx 30% of its 'maximum') by 300uM, the 3rd by 600uM with the rightward shift slowing thereafter eg. 1mM for the 6th. This desensitization could be accelerated by a single 5min pre-exposure to NA 3uM in 2.5mM Ca^{2+}, which shifted the 1st CRC almost to the position of the usual 2nd curve. Conversely, if the CRC was taken only to a highest $[Ca^{2+}]_o$ of 300uM the rate of shift was partially arrested. These results suggest that desensitization requires activation in the presence of a high $[Ca^{2+}]_o$.

KCl 100mM The results were essentially similar to those with NA except that the $[Ca^{2+}]_o$ required for any given response was approximately 10 times higher, i.e. all curves lay further to the right (including the declining leg of the 'bell-shaped' first curve. One consequence of this is that, in the first CRC at physiological $[Ca^{2+}]_o$ (1.25mM), an increase in $[Ca^{2+}]_o$ causes a steep fall in response whereas in the subsequent curves it produces a steep rise. Furthermore, the $[Ca^{2+}]_o$ for a 50mmHg response changes from just above 100uM (1st) to 1.25mM (2nd).

Bay K 8644 0.1uM Ca^{2+} CRCs (NA or KCl) were shifted to the left. This resulted in clear potentiation of responses in low $[Ca^{2+}]_o$ but little change in their size at physiological levels. For NA a response of 50mmHg required only $[Ca^{2+}]_o$ = 10uM. However, desensitisation, although delayed, still occurred. This considerably complicates any longitudinal analysis of the effects of such drugs on a single tissue and makes time controls essential. This probably accounts for our earlier difficulty in demonstrating significant effects of Bay K 8644 on this tissue, at these oxygen tensions (McGrath & Ugwu, 1986).

In conclusion we are left with a dilemma over the actual physiological $[Ca^{2+}]_o$ sensitivity of this tissue and hence of the likely effects in vivo of drugs which modify this. Should we believe the first curve and regard the desensitised state as an experimental artefact, or vice versa ?

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COMPARISON OF THE EFFECTS OF SOME M_1-MUSCARINIC AGONISTS OF THE HEART AND SYMPATHETIC GANGLIA.

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McN-A-343 (4-(m-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride) is commonly used as a selective M_1- agonist. However, its usefulness and selectivity may be questioned, because McN-A-343 may act allosterically with the receptor (Birdsall et al. 1983), and because of its low efficacy (Eglen and Whiting, 1985). It was the aim of the present study to compare in vivo and in vitro the characteristics of McN-A-343, pilocarpine and aceclidine. The following muscarinic antagonists were included in this study: atropine, pirenzepine, dicyclomine, 4-DAMP (4-diphenyl-acetoxy-N-methylpiperidine methbromide) and AF-DX 116 (11-2[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3,-b][1,4]benzodiazepine-6-one). In vivo the pithed rat model was used to investigate the agonist mediated bradycardia (M_2) and their ganglionic activity (M_1). Radioligand binding experiments were performed with ^{3}H-N-methylscopolamine as a ligand to M_2-binding sites in rat atrial membranes. ^{3}H-Pirenzepine was used as a ligand for the M_1-binding sites in cortical membranes. The order of potency for the agonists in vivo with respect to their increase in blood pressure and bradycardic activity was as follows: McN-A-343 > pilocarpine > aceclidine. All three compounds showed a higher potency for the sympathetic ganglia. The binding studies, however, revealed no differences in preference of the agonists between M_1- and M_2-binding sites. Besides pirenzepine also 4-DAMP and dicyclomine show high affinity for M_1-binding sites compared to those in the heart (De Jonge et al. 1986). However, 4-DAMP and dicyclomine proved to be non-selective in vivo when McN-A-343 was used as an agonist for the M_1-receptor present in the sympathetic ganglia (see Table 1). In contrast to the results obtained by McN-A-343 when pilocarpine or aceclidine were employed, 4-DAMP and dicyclomine appeared to possess higher affinity for the M_1-receptor and these results are therefore in agreement with the binding experiments. The present study indicates that pilocarpine and aceclidine might be better tools for the investigation of the M_1-receptor in vivo.

Table 1: comparison of the -log ID_{50} (mol/kg) values for the antagonists studied.

	M_2	M_1		
	oxotremorin	McN-A-343	pilocarpine	aceclidine
atropine	6.95	6.92	6.70	6.60
pirenzepine	4.56	5.77	6.24	5.08
4-DAMP	5.60	5.65	6.80	6.44
dicyclomine	4.21	4.56	5.34	4.92
AF-DX 116	5.62	4.37	4.80	5.38

S.E.M. < 5%

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We would like to thank Dr. R.B. Barlow for a gift of 4-DAMP.

BIOCHEMICAL AND PHARMACOLOGICAL CHARACTERISATION OF ALL FOUR ISOMERS OF THE MUSCARINIC AGONIST AF-30

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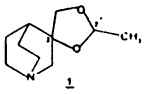
AF-30 has been reported to be a selective M-1 muscarinic ligand (Fisher et al, 1976). Although the compound has previously been separated into two diastereomers, the characterisation of all four isomers has not been reported. The synthesis and absolute configuration of all four isomers has been recently achieved.



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We have examined these four isomers in binding assays using rat cerebral cortex (M1) and heart membranes (M2) and in functional assays; depolarization of the rat superior cervical ganglion, contraction of the guinea-pig ileum and inhibition of the electrically stimulated guinea-pig atria. The results are shown below.

Compound	Heart (M-2) [3H]NMS	Cortex (M-1) [3H]Pirenzepine	Ganglion pEC_{50}(FE)	Ileum pEC_{50}(FE)	Atria pEC_{50}(FE)
	pKapp Molar	pKapp Molar	Molar	Molar	Molar
Carbachol	5.72	4.35	-	6.99(1.0)	6.68(1.0)
Muscarine	5.64	4.55	6.77(1.1)	-	-
AF-30	4.89	5.17	4.83(0.7)	5.2 (0.8)	4.08(0.8)
CIS (A + C)	5.08	5.23	5.24(0.7)	5.48(0.8)	4.66(0.8)
3(S),2'(S),A	4.19	4.26	<4	*	<4
3(S),2'(R),B	4.04	4.18	<4	<3.5	-
3(R),2'(S),C	5.66	5.57	5.25(0.8)	5.66(0.8)	4.83(0.8)
3(R),2'(R),D	5.07	5.39	4.56(0.7)	4.70(0.7)	≈4.0(0.2)

n = 3-5, F.E. functional efficacy, maximum responses relative to that of carbachol or 1μM muscarine (1.0), NMS:N-methylscopolamine, * inconsistent, weak variable responses, pKapp: negative logarithim of the apparent affinity constant.

The 3(R),2'(S) isomer was the most potent in binding and pharmacological assays, though all four isomers displayed little receptor selectivity in the binding assays. Functionally each isomer was most potent in the ileum, followed by the ganglion and least potent in the heart. All isomers were partial agonists (FE ≈0.8) compared with the full agonists, with the exception of 3(R),2'(R) which displayed low efficacy in the heart (FE 0.2 ± 0.2).

Intraperitoneal injection of muscarinic agonists into rats produced increased mouth movements (Salamone et al, 1986). The 3(R),2'S isomer showed significantly ($P < 0.001$) more activity in this in vivo model of central muscarinic receptor activity than the 3(R),2'(R) isomer.

These studies demonstrate the most potent isomer of AF-30 and show that the four isomers exhibit little selectivity in their affinity and potency on muscarinic receptors, with the possible exception of 3(R),2'(R).

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SELECTIVE LIGANDS FOR THE [^{3}H]-PIRENZEPINE BINDING SITE IN RAT CEREBRAL CORTEX

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Based upon the anomolous binding properties of pirenzepine two subtypes of the muscarinic receptor have been proposed (Hammer et al, 1980). Muscarinic receptors with high pirenzepine affinity are classified as M_1 receptors, those with low affinity for pirenzepine are termed M_2. Recent studies have indicated that [^{3}H]-pirenzepine ([^{3}H]-pir) can bind to high affinity sites in rat cerebral cortex indicating its suitability for labeling the M_1 muscarinic receptor (Watson et al., 1982). In the present study we report on several muscarinic ligands that are selective for the [^{3}H]-pirenzepine binding site in rat cortex.

In all experiments EDTA washed rat cerebral cortical and cardiac membranes were assayed in a 3 ml volume of assay buffer (50 mM Tris; 0.5 mM EDTA; 5 mM $MgCl_2$; pH 7.4 at 32°C). M_1 receptors in cortex were labeled using 0.5 nM [^{3}H]-pir. M_2 receptors in heart were labelled using 50 pM [^{3}H]-N-methyl scopolamine ([^{3}H]-NMS). [^{3}H]-NMS (50 pM) was used to label M_1 and M_2 receptors in cortex. Reactions were for 3 hrs. Bound ligand was separated from free by vacuum filtration. Data were analysed using iterative curve fitting techniques.

	CORTEX		HEART	M_1 selectivity	
	[^{3}H]-pir	[^{3}H]-NMS	[^{3}H]-NMS	A	B
Adiphenine	8.07 (0.94)	7.35 (0.96)	6.60 (0.95)	6	30
dicyclomine	9.17 (1.07)	8.97 (0.93)	7.69 (1.03)	19	30
HA	9.31 (1.11)	8.89 (0.95)	7.80 (1.01)	12	32
pirenzepine	8.27 (1.13)	7.52 (0.74)+	6.69 (0.90)	7	38

Values are pKi (Hill coefficient). n=4. SEM <5%. + 2 site fit indicated 76% M_1 receptor (pKi=7.89); 24% M_2 receptor (pKi=6.71). A is Ki [^{3}H]-NMS(heart)/Ki Ki [^{3}H]-NMS(cortex). B is Ki [^{3}H]-NMS(heart)/[^{3}H]-pir.

In addition to pirenzepine and dicyclomine, both adiphenine and hexahydroadiphenine (HA) were selective ligands for the M_1 receptor of rat cortex labeled using [^{3}H]-pir (column B). However the selectivity of the compounds for M_1 receptors differed depending upon whether M_1 receptors in rat cortex were labeled using [^{3}H]-pir or [^{3}H]-NMS (cf columns A and B). This was not due to the heterogeneity of muscarinic receptors labeled in rat cortex by [^{3}H]-NMS. Indeed, with the exception of pirenzepine, none of the ligands were able to identify hetrogeneity of muscarinic receptors labeled by [^{3}H]-NMS in rat cortex even though all ligands were of comparable M_1 selectivity. The reasons for the failure of HA, dicyclomine and adiphenine to identify heterogeneity of cortical [^{3}H]-NMS binidng sites in rat cortex is uncertain but may indicate that the M_2 receptors of cortex and heart differ. Further work is necessary to establish such speculation, however.

Additional binding as well as functional studies are required to determine the reasons for the differences in M_1 affinity estimates obtained using [^{3}H]-pir and [^{3}H]-NMS.

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APPLICATION OF THE CHENG-PRUSOFF RELATIONSHIP TO THE ESTIMATION OF ANTAGONIST AFFINITY CONSTANTS FROM ISOLATED TISSUES

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The Cheng-Prusoff relationship (Cheng and Prusoff, 1973) is routinely used with data in which the concentration of antagonist, inhibiting the binding or second messenger turnover by 50%, is related to the affinity. However, there may be problems in applying the expression to agonist responses characterized by a high effective receptor reserve, since the affinity of such agonists differs greatly from the potency. The aim of the study was to apply the Cheng-Prusoff relationship to the estimation of an antagonist affinity using contractile responses of the guinea-pig trachea to agonists with high and low efficacy.

The potency and affinity of carbachol and pilocarpine was assessed at muscarinic receptors in the guinea-pig trachea, using methods described previously (Furchgott and Bursztyn, 1967). The affinity of atropine was assessed, in separate experiments, by the method of Arunlakshana and Schild (1959) and by applying the Cheng-Prusoff relationship in the form $pK_B = -\log K_B = IC_{50}/(1 + [A]/EC_{50})$ (where the EC_{50} = concentration of agonist which produces 50% of the maximum response). All values quoted are mean $\pm$ sem, n = 5.

The affinities ($-\log K_A$) of carbachol and pilocarpine were 4.54 $\pm$ 0.03 and 5.32 $\pm$ 0.05, respectively. The potencies ($-\log EC_{50}$) of carbachol and pilocarpine were 6.77 $\pm$ 0.03 and 5.85 $\pm$ 0.02, respectively. Pilocarpine possessed an efficacy, relative to carbachol, of 0.02. The pA_2 values for atropine determined when either carbachol (9.2 $\pm$ 0.12) or pilocarpine (8.9 $\pm$ 0.11) were used as the agonist were not significantly different ($p < 0.05$) and the Schild slopes were unity. The pK_B values estimated with the Cheng-Prusoff relationship, using the EC_{80} concentration of either carbachol (9.1 $\pm$ 0.14) or pilocarpine (9.2 $\pm$ 0.10) were not significantly ($p < 0.05$) different from each other or in comparison to the pA_2 values above. In contrast, the pK_B values were significantly ($p < 0.05$) different when the concentrations of either carbachol (10.3 $\pm$ 0.11) or pilocarpine (9.9 $\pm$ 0.13) were substantially supramaximal, i.e. EC_{300}. In tissues, pretreated with phenoxybenzamine, the maximal responses to carbachol were reduced and the concentration - response curve shifted to the right. The affinity of atropine, estimated by the Cheng-Prusoff relationship, in these tissues, using the EC_{80} concentration of carbachol was no different to that observed previously (9.1 $\pm$ 0.11).

In summary, application of the Cheng-Prusoff relationship to the estimation of affinity constants from contractile responses agrees well with those obtained by Schild analysis. The affinity can be estimated using agonists of high and low efficacy, indicating that the effective receptor reserve does not influence the estimated value. However, caution should be exercised when agonist concentrations used are substantially supramaximal, since the method can then overestimate the antagonist affinity constant.

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HUMAN COLON MUCOSAL MAST CELLS: ISOLATION AND CHARACTERIZATION

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Mast cell heterogeneity in the rat is well established, but demonstration of human mast cell subtypes is at present preliminary. Enzymatic dispersion techniques have allowed functional characterization of human lung mast cells (Church et al, 1982) and, more recently, human skin mast cells (Benyon et al, 1986). The aim of this study was to develop a dispersion technique for human colon mucosa and to functionally characterize colon mucosal mast cells with respect to releasing agents.

Colectomy specimens, obtained from patients undergoing resection for colo-rectal carcinoma, were carefully dissected to separate histologically normal mucosa from the underlying muscle layers. After weighing, mucosal strips were chopped finely with scissors and the fragments washed once in Eagles minimum essential medium (MEM) to remove faeces, and twice in MEM + 1mM dithiothreitol (DTT) to remove mucus. Two 1h digestions were then carried out in MEM, each containing collagenase (Type I, 1.5mg/ml) and hyaluronidase (0.75mg/ml), 20% foetal calf serum and 1mM DTT. Dispersed cells were isolated at each stage by filtration through nylon gauze and after two washes, mast cell and total nucleated cell numbers were estimated using Kimura's metachromatic stain. Prior to challenge with secretagogues, cells were washed once in HEPES buffered salt solution with 2.8mM $CaCl_2$ (HBSS), and were pre-incubated for 10min at 37°C. Challenge was then carried out for 15min, and histamine release terminated by addition of ice-cold HBSS. Histamine in the supernatants was analysed spectrofluorimetrically and release expressed as a % of total cell histamine corrected for spontaneous release.

The dispersal method yielded 6.2 ± 0.6 x 10^5 mast cells/g mucosa (mean ± SEM, n = 17) with a purity of approximately 1.5-2%. Mast cell histamine content was 2.7 ± 0.3pg/cell (n = 10). Histamine release was detected in response to goat anti-human IgE (range 0.03%-10%) and calcium ionophore A23187 (0.03μM-3μM), both in a concentration-dependent fashion. Peak histamine release was 15.1 ± 1.1% of total cell histamine (n = 6) with 0.1% anti-IgE and 49.6 ± 5.1% with 1μM A23187 (n = 6). In five experiments, no release was seen with substance P, f-met-leu-phe, compound 48/80 or poly-L-lysine. In all challenge experiments, spontaneous release was low with an average of 4.8 ± 0.7% of total cell histamine (n = 8).

The absence of release to substance P, compound 48/80 and poly-L-lysine is similar to that of mast cells of human lung but not those of human skin, which release to these agents (Benyon et al, 1986). The failure to respond to f-met-leu-phe and poly-L-lysine also distinguishes colon mast cells from human basophils.

In conclusion, we report a dispersion method for human colon mast cells which is less involved and gives higher yields than previously described (Fox et al, 1985). We also present evidence that intestinal mucosal mast cells are functionally similar to those dispersed from human lung parenchyma, in the tests performed.

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DESENSITIZATION OF β-ADRENOCEPTOR DEPENDENT ADENYLATE CYCLASE IN A-431 CELLS

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A-431 cells (EIII subclone) are a human epidermoid cell line which express β_2-adrenergic receptors (Strosberg et al., 1984). We have found that incubation of these cells with 10^{-5}M isoprenaline for 30 min at 37°C resulted in a homologous-type desensitization of adenylate cyclase. Cells were grown to 70% confluency in medium supplemented with 10% horse serum and the medium was replaced by serum-free medium 12 hr prior to experiments. Intact cell binding assays were performed using the hydrophilic ligand [^{3}H]-CGP 12,177 (s.act. 46 Ci/mmol). Membranes were prepared following cell disruption by nitrogen cavitation. Intact cells and nuclear material were removed by centrifugation at 500 x g for 15 min. Receptor binding in membranes was measured using [^{3}H]-CGP 12,177 and [^{125}I]-cyanopindolol (s.act. 2,000 Ci/mmol). Displacement of [^{125}I]-cyanopindolol (40 pM) binding was carried out using isoprenaline (10^{-9} - 10^{-4}M) in the presence and absence of 10^{-4}M GTP. Adenylate cyclase was assayed according to Salomon et al. (1974).

Following treatment with isoprenaline we observed a marked reduction of hormone-stimulatable adenylate cyclase activity in membranes prepared from these cells while stimulation of the enzyme by Gpp(NH)p, NaF or forskolin was not significantly altered. Receptor coupling to the GTP-binding protein as assayed by the GTP-induced shift of agonist binding was also reduced after isoprenaline treatment. To investigate the role of receptor downregulation in this process, we examined the binding of β-adrenoceptor ligands to whole cell and membrane preparations. After desensitization there was a 50% reduction in the number of cell surface binding sites as determined by [^{3}H]-CGP 12,177 binding, without a change in KD (0.35 nM). A similar downregulation of receptor number was measured by [^{3}H]-CGP 12,177 binding to a crude membrane preparation. However using the more lipophilic ligand [^{125}I]-cyanopindolol, no similar reduction could be demonstrated in this preparation.

We conclude therefore that short-term desensitization in A-431 cells is accompanied by a downregulation of cell surface β-adrenoceptors since the reduction of β-receptor number is detectable only by the hydrophilic ligand [^{3}H]-CGP 12,177. The downregulated receptors remain however in the membrane fraction, as the hydrophobic ligand [^{125}I]-cyanopindolol measures the full complement of β-receptors in membranes prepared from desensitized cells.

This work was carried out at the University of Wurzburg, during the tenure of a Boehringer-Ingelheim fellowship (by D.C.). The support of the SFB 176 is acknowledged.

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IN VIVO POTENCIES OF SELECTIVE MUSCARINIC ANTAGONISTS SUPPORT THE SUBDIVISION OF M-RECEPTORS INTO M_1, M_2 AND M_3-SUBTYPES

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On the basis of affinity constants obtained with pirenzepine, AF-DX 116 (11-2[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one) and 4-DAMP (4-diphenylacetoxy-N-methylpiperidine methbromide) we concluded that there are three different muscarinic binding sites. M_1-binding sites are present in neuronal tissue, M_2-binding sites in the heart and M_3-binding sites are considered to be present in exocrine glands (de Jonge et al., 1986).

In the present study we investigated whether this novel subclassification, based on radioligand binding experiments, also holds true in vivo. Therefore we studied the antagonistic potencies of atropine, pirenzepine, 4-DAMP and AF-DX 116 in the following in vivo experiments: A) bradycardia elicited by oxotremorine, B) increase in blood pressure produced by pilocarpine, which is a result of stimulation of muscarinic receptors in the sympathetic ganglia, C) pilocarpine-mediated salivation and D) vasodilatation evoked by methacholine, due to stimulation of muscarinic receptors in the endothelium. Experiments A and B were performed in pithed rats and C as well as D in pentobarbitone-anesthetized rats.

Table 1. Comparison of -log ID_{50} (mol/kg) values for muscarinic antagonists for muscarinic receptors in the heart, sympathetic ganglia, salivary glands and endothelial cells.

	heart bradycardia	symp.ganglia blood pressure ↑	salivary glands salivation	endothelial cells vasodilatation
atropine	6.95 ± 0.05	6.70 ± 0.02	7.52 ± 0.03	7.07 ± 0.01
pirenzepine	4.56 ± 0.02	6.24 ± 0.04	5.94 ± 0.03	5.27 ± 0.03
4-DAMP	5.60 ± 0.03	6.80 ± 0.06	7.73 ± 0.05	6.79 ± 0.05
AF-DX 116	5.62 ± 0.06	4.80 ± 0.03	4.88 ± 0.04	4.54 ± 0.04

Values represent means ± S.E.M., n = 5-8.

A comparison of the relative potencies (atropine = 1) showed that pirenzepine was highly selective for the muscarinic receptors in the sympathetic ganglia. 4-DAMP possessed high affinity for the muscarinic receptors in the sympathetic ganglia, salivary glands and on vascular endothelial cells and a low affinity for cardiac muscarinic receptors. AF-DX 116 appeared to be selective for cardiac muscarinic receptors. The results obtained are in agreement with the presence of three different muscarinic receptor subtypes. Pirenzepine is selective for M_1-receptors and AF-DX 116 for M_2-receptors. 4-DAMP shows high potency for both M_1- and M_3-receptors. Because none of the antagonists studied can clearly discriminate between muscarinic receptors present in the salivary glands (M_3) and those on endothelial cells, our results indicate that the muscarinic receptor on the endothelial cells resembles the M_3-receptor subtype.
We conclude that the present in vivo results strongly support the existence of at least three subtypes of muscarinic receptors.

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We are greatly indebted to Dr. Barlow for a gift of 4-DAMP.

THE INTERACTION BETWEEN BAY-K-8644 AND NITRENDIPINE ON RENIN RELEASE FROM RAT KIDNEY SLICES

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Inhibition of calcium entry by calcium channel blockers leads to a stimulation of renin release from rat kidney slices (Churchill, 1981). In contrast the calcium channel activator Bay-k-8644 is reported to inhibit renin release (May and Peart, 1985; Matsumura et al, 1985). Studies of the interaction between nifedipine and Bay-k-8644 upon renin release has produced conflicting results (May and Peart, 1985; Matsumura et al, 1985). This study examines the effects of nitrendipine, Bay-k-8644 and their combination on renin release from rat kidney slices, where renin release has been suppressed by depolarisation with 60mM K+.

Rat cortical kidney slices (200 μm), were incubated in 5ml of oxygenated Krebs-Henseleit solution (containing 2g/l BSA) at 37^{o}C and this medium was replaced at 20 minute intervals with fresh solution. Following preincubation with drug (vehicle, nitrendipine or Bay-k-8644), slices were incubated with a solution containing 60mM K+ and aliquots of discarded solution were retained for renin determination. Results were calculated as the ratio of the renin release during pre-drug incubation and release during exposure to high K+ and drug.

Incubation with 60mM K+ significantly ($p<0.001$) reduced renin release to 22% of control. Nitrendipine (0.01-1μM) produced a significant and dose-related increase in renin release in the presence of 60mM K+; 1μM produced complete restoration to control. Bay-k-8644 (0.01-1μM) also stimulated release, although it was 100 fold less potent than nitrendipine, with 1μM producing an increase in renin release to 32% of control. In contrast, when the two drugs were co-administered, Bay-k-8644 antagonised the nitrendipine-induced rise in renin release in a dose related manner. The combination of Bay-k-8644 (0.1-1μM) and nitrendipine (0.1μM) produced renin release rates significantly below those observed with Bay-k-8644 alone.

Decreased calcium entry into juxta-glomerular cells is reported to stimulate renin release and the effect seen with calcium channel blocking drugs is consistent with this hypothesis. In the presence of 60mM K+, the calcium channel activator (Bay-k-8644) also behaved as a weak calcium channel blocker. However, the presence of nitrendipine appeared to unmask its calcium channel activator activity, leading to an inhibition of renin release to levels below those observed with Bay-k-8644 alone. This potentiation of the action of Bay-k-8644 by nitrendipine is not consistent with a simple agonist/antagonist interaction. Alternative explanations are that nitrendipine or one of its enantiomers could be acting as partial calcium channel activator (agonist) or that there is complex interaction involving two dihydropyridine receptors. A 'two-receptor hypothesis' has been proposed to explain a similiar potentiation of Bay-k-8644 by nitrendipine in vascular tissue (Dube et al, 1985).

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PRESYNAPTIC AUTORECEPTORS REGULATE RELEASE OF ENDOGENEOUS DOPAMINE (DA) IN BOTH LOBES OF THE RAT NEUROINTERMEDIATE LOBE (NIL)

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Presynaptic autoreceptors by which a transmitter substance can regulate its own release have been demonstrated in different neuronal systems. Thus, the release of DA from nigrostriatal DA neurons is modulated by DA receptors (e.g. Starke et al., 1978;). In vivo turnover studies on tuberohypophyseal DA neurons suggested that only DA neurons innervating the intermediate lobe (IL), but not those innervating the neural lobe (NL) may be regulated by DA receptors (Lookingland et al., 1985). In the present study the effect of the DA receptor antagonist sulpiride on the in vitro release of endogenous DA from the combined NIL or isolated NL was studied.

Isolated NILs or NLs with their stalks held in a platinum wire electrode were incubated in 80 µl Krebs-HEPES solution which contained pargyline (10 µM) and the DA uptake inhibitor GBR 12921 (1(2-(diphenylmethoxy)-ethyl)-4-(3-phenyl-2-propenyl)-piperazine, 200 nM). The medium was changed every 10 min and DA determined by HPLC with electrochemical detection. The pituitary stalks were stimulated after 50 min (S1) and after 90 min (S2) of incubation with pulses of 0.2 ms; 10 V; 3, 5 or 15 Hz; 3 times for 1 min with 1 min intervals. As the release of DA from the NL is under inhibitory control of endogenous opioids (Racké et al., 1986) naloxone (Nal 1 or 10 µM) was added to the medium when DA release from the NL was studied.

DA release from the NIL evoked by S1 at 3, 5 or 15 Hz amounted to 31±1.6 (n=41), 58±6.6 (n= 13) and 142±9.6 (n=25) pg (means±S.E.M). DA release from the NL evoked by S1 at 15 Hz was 6.4±1.0 pg (n=4) in the absence of Nal and 23±1.6 (n=31) and 23±2.5 (n=30) pg in the presence of Nal 1 and 10 µM. The effect of sulpiride, added 30 min before S2, on DA release from the NIL or NL is shown in Table 1.

Table 1: Effects of (+)- and (-)-sulpiride (Sul) on the electrically evoked DA release from the NIL or NL (S2/S1).

	Ctr.	10 nM	100 nM	1 µM	10 µM	
NIL 15Hz	1.11±0.08	1.09±0.04	1.66±0.03**	2.21±0.08**	2.41±0.30**	(-)Sul
		-	1.18±0.18	1.44±0.16	1.57±0.09	(+)Sul
NIL 5Hz	1.22±0.04	1.33±0.06	-	3.54±0.35**	-	(-)Sul
NIL 3Hz	1.18±0.04	1.30±0.04	2.22±0.25**	3.45±0.45**	3.89±0.90*	(-)Sul
NL 15Hz						
Nal 1 µM	1.19±0.04	-	1.02±0.08	1.76±0.06*	2.16±0.34**	(-)Sul
		-	1.28±0.02	1.34±0.07	1.29±0.10	(+)Sul
Nal 10 µM	1.11±0.09	-	1.48±0.06*+	1.59±0.04*	2.16±0.30**	(-)Sul

Means±S.E.M. of 3-7 experiments. Significance of differences from the respective control group (=absence of sulpiride) *P < 0.05, **P < 0.01; from the corresponding value in the presence of Nal 1 µM +P < 0.05.

In conclusion, sulpiride increased the evoked release of endogenous DA from both lobes of the NIL in a stereo-specific manner, suggesting that inhibitory DA autoreceptors may regulate the release of DA in the NL as well as in the IL. However, as endogenous DA appears also to modulate the release of opioids in the NL which in return regulate the release of DA, the analysis of presynaptic DA receptors in the NL requires a complete blockade of opioid receptors by naloxone.

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Supported by the Deutsche Forschungsgemeinschaft.

EFFECTS OF INTRACEREBROVENTRICULAR BRADYKININ ON CARDIAC OUTPUT AND TISSUE BLOOD FLOW IN ANAESTHETISED RATS

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There is evidence for the existence of an endogenous kallikrein-kinin system in the central nervous system (Chao et al, 1983; Perry & Snyder, 1984). Intracerebroventricular (i.c.v.) injections of bradykinin (BK) are known to bring about increases in BP in the rat (Correa & Graeff, 1976) and activation of endogenous kallikrein has similar effects in the dog (Thomas et al, 1984). The contributions to this pressor response from changes in cardiac output and vascular resistance are not known so we have determined the effect of i.c.v. injection of BK on cardiac output and tissue blood flow in anaesthetised rats.

Male Wistar rats (210-250g; Bantin & Kingman) were anaesthetised with 120mg/kg thiobutabarbitone (Inactin-BYK). Both femoral arteries were cannulated to allow withdrawal of blood (at a rate of 0.5ml/min) during the microsphere injection and continuous recording of blood pressure and heart rate (HR). Injection of 30ug BK (Sigma) into the lateral cerebral ventricle over 10s in a volume of 30ul artificial cerebrospinal fluid (Merlis, 1940) was made through a cannula (diameter 0.45mm) inserted to a depth of 3.5mm at co-ordinates 1.5mm lateral and 1mm caudal to bregma. Control animals received 30ul vehicle only. After 2min a stable response was obtained with BK and 60000-80000 ^{113}Sn-labelled microspheres (15um diameter; NEN) were injected through a cannula into the left cardiac ventricle. After 90s the animals were killed, the organs removed, weighed and placed in vials for radioactivity counting. Cardiac output and tissue blood flow were determined by the method of McDevitt & Nies (1976). Statistical comparisons were made by Student's unpaired t-test.

In 6 rats, BK increased mean arterial pressure (MAP) from 99±3 to 114±3 mmHg ($P<0.001$) with no effect on HR (384±11 beats/min before; 381±11 after BK). In control rats (n=6) the respective values before and after injection of vehicle were, for MAP, 103±5 and 99±5 mmHg and, for HR, 392±17 and 391±17 beats/min. Cardiac output was not different in the two groups being 30.8±3.3 (control) and 28.1±2.6 (BK) ml/min/ 100g body wt. Its distribution was significantly greater in the BK rats (values given second) to the heart (4.9±0.5%; 7.0±0.8%: $P<0.05$), hepatic artery (4.3±0.9%; 6.7±0.6%: $P<0.05$) and brain (1.2±0.1%; 2.0±0.1%: $P<0.001$) and lower to the stomach (0.98±0.1%; 0.72±0.08%: $P<0.05$), large intestine (1.9±0.1%; 1.5±0.1%: $P<0.05$) and pancreas/mesentery (2.3±0.1%; 1.7±0.2%: $P<0.01$). There was significantly greater blood flow in the liver (0.32±0.05, control; 0.48±0.03 ml/min/g, BK: $P<0.01$) and brain (0.54±0.04, control; 0.74±0.1 ml/min/g, BK: $P<0.05$) of the BK rats with lower flows in the spleen (1.2±0.2, control; 0.7±0.1 ml/min/g, BK: $P<0.05$) and skin (0.15±0.02, control; 0.09±0.02 ml/min/g, BK: $P<0.05$).

Thus the increase in MAP induced by BK appears to be accompanied by constriction of vasculature in the skin and gastrointestinal tract but there is also vasodilatation in the brain, liver and heart.

This work was supported by the British Heart Foundation (Grant No. 84/49)

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AFFERENT C-FIBRE AND A-DELTA ACTIVITY IN MODELS OF INFLAMMATION.

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Two groups of primary afferent sensory nerve fibres, A–delta and C–fibres, are known to be responsible for the transmission of nociceptive information from peripheral tissue injury. Under normal conditions nociceptive afferents have a low level of spontaneous background activity, but following sensitisation possess a lower nociceptive threshold and an increased level of background activity. We have used a simple method to monitor background C–fibre and A–delta activity in a discrete bundle of the saphenous nerve innervating the dorso–lateral surface of the hind paw of the anaesthetised rat. Fine surface stimulating electrodes placed on the skin receptive field were used to evoke activity in several units while recording from the whole nerve bundle. Under these recording conditions afferents identified (by conduction velocity) as C–fibres had spike amplitudes <20 μV, whereas A–delta fibres produced spike amplitudes between 20 and 54 μV. Identified A–beta amplitudes were always >54 uV. Background activity was recorded for subsequent analysis using a spike processor where differences in spike amplitude were used to count action potentials falling within the range of C–fibres (<20 μV) and A–delta fibres (20–54 μV). This distinction was consistently found throughout this series of experiments.

Following topical application of the irritant mustard oil (5% in paraffin) to the hind paw there was an increase in spontaneous impulse activity in each group of afferents. The A–delta component was of short duration whereas the C–fibre component was maintained at a high level throughout the 30 min recording period. No activity of afferents falling within the expected spike amplitude range for A–beta units were recorded, though they could still be evoked electrically via the stimulating electrodes. 18 to 21 days after topical application of capsaicin to the saphenous nerve the C–fibre response to mustard oil was reduced by 80%. In contrast, the A–delta component was significantly enhanced. An intraplantar injection of the acute inflammatory agents carrageenan (1%) or yeast (20%) caused no significant increase in spontaneous activity up to 3 hours post injection, despite the formation of a substantial paw oedema. An injection of formalin (1%) evoked an immediate and intense stimulation of C–fibre, but not A–delta afferents. Intraplantar injection of Freund's adjuvant significantly increased both A–delta and C–fibre activity at 2 days through to 14 days after injection with a depression of spontaneous activity on days 6 to 8 which correlates with the time courses of the phasic changes in paw volume and body weight seen in this model of inflammation.

The differential response of the C–fibre and A–delta components following mustard oil application and capsaicin pretreatment is supporting evidence that the simple method used here can substantially separate C–fibre activity from A–delta activity. Analysis of these parameters can provide electrotrophysiological evidence of the changes in afferent impulse activity during inflammation.

TETRODOTOXIN INHIBITS THE SECRETORY/ANTIABSORPTIVE EFFECTS OF VIP, PGE_1 AND BETHANECHOL IN THE SMALL INTESTINE

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The selective nerve inhibitor tetrodotoxin (TTX) is used increasingly to help investigate the potential role of nerves innervating the intestinal epithelium. Despite this, TTX has not been investigated for potential direct effects on the absorptive and secretory functions of the intestine *in vivo*. This possibility has been investigated using the directly-acting secretagogues prostaglandin (PG) E_1 vasoactive intestinal peptide (VIP) and bethanechol.

Water transport rates were measured by a previously described method whereby the small intestine of anaesthetized rats is perfused with a saline solution containing phenol red as a non-absorbable marker and intestinal fluid secretion is stimulated by infusion of secretagogues into the left common carotid artery at constant rate (Coupar 1985). Groups of animals receiving TTX were injected with 20 µg/kg and were respired by an air pump.

Contractions induced by transmural stimulation of jejunal segments taken from anaesthetized animals pretreated with TTX were significantly reduced compared to controls (1 msec, 10 and 20 Hz, $P < 0.05$). However, responses to acetylcholine were unaffected. TTX given 15 min before perfusing the jejunum did not alter the rate of water absorption. Intra-arterial (i.a.) infusion of PGE_1 (5.6 nmol/min) or VIP (0.24 nmol/min) starting 5 min before and continuing for the duration of the jejunal perfusion both produced large net secretions. The values attained were near maximal responses achievable with each of these secretagogues. TTX blocked PGE_1-induced secretion and inhibited the VIP-induced response. In separate experiments bethanechol (500 nmol/min) produced an antiabsorbtive effect which was greater in the ileum than jejunum. TTX inhibited the bethanechol induced response in the ileum (Table 1).

Table 1. Effect of TTX on changes in water transport rates induced by secretagogues.

i.v. INJECTION	i.a. INFUSION			
	SALINE	VIP	PGE_1	BETHANECHOL
	Values from the jejunum			ileum
SALINE	+ 216 ± 27	- 396 ± 24]	- 82 ± 35]	+ 6 ± 9]
TTX	+ 258 ± 46	- 3 ± 25	+ 267 ± 26	+ 173 ± 24

Transport rates (+ = net absorption, - = secretion) are expressed in µl/g wet weight of tissue in 20 min. Means joined by brackets are significantly different (Student's unpaired t test, $P < 0.05$). The control absorption rate in the ileum was 350 ± 38.

The results show that TTX inhibits the effects of directly-acting secretagogues without affecting the physiological rate of net water absorption. Further experiments are therefore required to elucidate the mechanism of the TTX antisecretory effect. Until such results are available interpretations of secretagogue action using TTX should be made with caution.

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EFFECTS OF ADRENOCEPTOR AGONISTS ON THE BIPHASIC RESPONSE TO PROLONGED FIELD STIMULATION IN ISOLATED RAT VAS DEFERENS

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Prolonged field stimualtion of rat vas deferens results in a biphasic contractile response. The first phase is an immediate rapid contraction known as the "twitch" response which quickly falls until the contractile response levels out, producing a second "plateau" phase of contraction. In 1971, Swedin proposed that both phases of contraction are mediated by release of noradrenaline (NA) the difference in the responses being attributable to location of the receptors through which NA interacts. Doubt as to whether NA is the sole transmitter released from adrenergic nerve terminals was expressed by Ambache & Zar (1971). More recently Gillespie & Macrae (1983) suggested that NA is responsible for the second phase of contraction but that the twitch component is produced by a second neurotransmitter.

It is well established that neurotransmitter release is modulated by drug interaction at prejunctional receptors. The present study was undertaken to investigate the effect of several adrenoceptor agonists on the biphasic response of rat vas deferens following prolonged field stimulation to establish whether a differential effect on the two phases of contraction could be shown.

Vasa deferentia from Wistar rats (200-300g) were set up in Tyrodes containing cocaine (3μM), propranolol (1μM) and prazosin (0.3μM) and aerated with 95% O_2 : 5% CO_2. Field stimulation of frequency 10Hz, 0.3msec duration and strength 80v was applied in trains of 10s duration every 100s using Grass stimulators. Contractions were recorded via Lectromed strain gauge transducers and recorders. Dose-response relationships to agonists were obtained following cumulative addition to the bath. The drugs used in the study were: isoprenaline (β agonist) N.A.(α), phenylephrine (α_1-selective), U.K.14304, clonidine, α-methyl noradrenaline (α-M.N.A.), B-HT920 (all α_2-selective).

Results showed that the twitch phase of contraction was inhibited dose dependantly in the order:- α_2 agonists $>> \alpha_1 > \beta$. The plateau phase was only inhibited by α_2-selective agonists. $I.C._{50}$ values showed that for α_2-selective agonists inhibition of the plateau response required approximately a ten-fold higher concentration than those required to inhibit the twitch. The order of potency against the twitch response was U.K.14304 > clonidine > N.A. ≈ α-M.N.A. > B-HT920. The same order of potency was seen against the plateau phase of contraction though B-HT920 was inactive against this phase.

The agonists based on the imidazoline structure (U.K.14304 & clonidine) and B-HT920 (previously shown to have clonidine-like effects, Mottram, 1983) produced complete inhibition of the twitch phase whilst the β-phenethylamines, N.A. and α-M.N.A., were only partial agonists against this phase. On the plateau phase neither the imidazolines nor the β-phenethylamines achieved a complete inhibition of the response.

Results indicate that the pre-junctional mechanisms for the control of neurotransmitter release are complex and that, following prolonged stimulation, the two phases of contraction may involve two distinct release mechanisms and possibly, therefore, two neurotransmitters.

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MODULATION OF THE SECRETION OF CORTICOTROPHIN RELEASING FACTOR (CRF) BY OPIOID SUBSTANCES

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Opioid substances have been shown both to stimulate (Buckingham, 1982) and inhibit (Buckingham, 1986) the secretion of CRF by the rat hypothalamus in vitro. Their stimulatory effects appear to be mediated by μ- and κ- opioid receptors (Buckingham & Cooper, 1986). In an attempt to characterize the receptors effecting their inhibitory actions, the effects of some selective agonists and antagonists of opioid receptors on the basal and neurochemically-stimulated release of CRF from isolated hypothalami have been investigated. Hypothalami, removed from male Sprague-Dawley rats, were incubated in conditions described previously (Buckingham & Hodges, 1977a). The CRF released into the incubation medium after a 10 min contact time with the agonist was determined (Buckingham & Hodges, 1977b). Where appropriate, antagonists were added to the incubation medium 10 min (naloxone or the δ-opioid receptor antagonist, ICI 154129) or 90 min (the μ-receptor antagonist, β-funaltrexamine, β-FNA) before the agonists.

Although morphine (10^{-8}-10^{-6}M) and the long-acting enkephalin analogue Tyr-D-Ala-Gly-Phe-Ser (B_z1) (10^{-8}-10^{-6}M) caused significant ($P < 0.01$, Duncan's test) dose-related increases in the release of CRF from isolated rat hypothalami in vitro the selective δ-opioid receptor agonist, [D-Pen^2-D-Pen^5]-enkephalin (2×10^{-10} - 2×10^{-7}M), did not influence the resting secretion of the hypothalamic hormone. In low concentrations (10^{-10}-10^{-9}M), β-endorphin also stimulated CRF release ($P < 0.01$, Duncan's test) but, when its concentration was increased, its effects were diminished and, in concentrations above 10^{-7}M, it reduced significantly ($P < 0.01$, Duncan's test) not only the spontaneous release of CRF but also, like [D-Pen^2-D-Pen^5]-enkephalin (2×10^{-7}M), the normal secretory response to acetylcholine. The inhibitory actions of β-endorphin (10^{-7}-10^{-6}M) and [D-Pen^2-D-Pen^5]-enkephalin (5×10^{-6} - 5×10^{-5}M) were abolished by the δ-opioid receptor antagonist, ICI-154129 (5×10^{-6}M), but were unaffected by naloxone (10^{-7}M) or the μ-receptor antagonist, β-FNA (10^{-9}M). In contrast, the stimulatory actions of morphine (10^{-8}-10^{-6}M), β-endorphin (10^{-10}-10^{-9}M) and Tyr-D-Ala-Gly-Phe-Ser (B_z1) (10^{-7}M) on CRF secretion were antagonised readily ($P < 0.01$, Duncan's test) by naloxone (10^{-8}-10^{-6}M) and β-FNA (10^{-9}M) but not by ICI-154129 (5×10^{-6}M).

The results add further support to the concept that μ-opioid receptors are involved in the initiation of CRF-release and suggest that stimulation of δ-opioid receptors may lead to inhibition of the secretion of the releasing factor.

We are grateful to the MRC for financial support, to Dr A. Hayes, Glaxo Group Research Ltd for β-FNA, to Mr R. Cotton and Dr M. J. Rance, ICI plc for ICI-154129 and to Dupont (UK) Ltd for naloxone.

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BINDING OF [^{3}H]-ANGIOTENSIN II TO BASOLATERAL MEMBRANES FROM THE PROXIMAL RENAL TUBULE OF THE RAT

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Angiotensin II (AII) stimulates the reabsorption of sodium and water by the proximal renal tubule (Harris & Navar, 1985). Receptors for ^{125}I AII have been identified in mixed rat epithelial membranes (Cox et al, 1983) and in purified brush border (Brown & Douglas, 1982) and basolateral membranes (Brown & Douglas, 1983). Using ^{3}H AII in a basolateral membrane preparation of proximal tubular origin, we have encountered substantial degradation of AII tracer which could not be inhibited without adversely affecting binding. Measurement of free and bound AII by TLC and analysis of results by curve fitting techniques have demonstrated a considerably higher affinity receptor in this tissue than previously reported.

Membranes were prepared according to Scalera et al, 1981. Their purity was confirmed by EM and by enrichment of K dependent phosphatase but not alkaline phosphatase marker enzymes. Their predominantly proximal tubular origin was confirmed by measurement of hormone stimulated cAMP, showing a ten-fold increase with PTH and small increases (1.5 fold) with AVP, calcitonin and isoprenaline (Charbardes et al, 1975).

Binding was measured by incubation at 24°C for 90 min in 50mM tris pH 7.4 containing NaCl 100mM, $MgCl_4$ 4mM, BSA 0.5% and membrane protein 10-25µg tube^{-1}, in a final volume of 250µl. Tracer and unlabelled peptide were added in variable concentrations. Non-specific binding was defined by 10^{-6}M Cold AII. Incubations were terminated by dilution and rapid filtration through Whatman GF/B filters. Peptide degradation was measured by TLC of both free and bound ^{3}H AII at equilibrium (Goodfriend & Simpson, 1981). Curve fitting was carried out using the Harwell Library non-linear regression programme VB01A.

^{3}H AII binding reached a maximum at 90 min remaining stable for 180 min, and increased linearly with protein concentration to 100µg ml^{-1}. Binding was saturable and reversible. Structural specificity was demonstrated by competitive inhibition by unlabelled AII, angiotensins I and III and the substituted AII analogues Sar^{1}ala^8 AII and Sar^{1}leu^8 AII, but not by ACTH or bradykinin. Analysis showed Hill slopes not significantly different from unity, consistent with a single class of receptor. After 90 min incubation 80.4±0.6% (n=6) of bound radioactivity was intact ^{3}H AII, and 16.8±1.8 (n=6) of free radioactivity was intact ^{3}H AII. The protease inhibitors DTT, EDTA and PMSF were used to try to reduce tracer degradation but were found to reduce measurable binding (EDTA 5mM = 66.4±8.6% control; DTT 5mM = 10.2±5% control) or to be impossible to solubilize (PMSF). Saturation curves were therefore constructed by direct measurement of the concentration of free and bound ^{3}H AII at equilibrium and estimates of K_D and B_{max} by direct curve fitting of these values. This gave K_D = 0.21±0.01nM, B_{max} 485.8±81.9 fmolmg^{-1} (n=5), suggesting a ten-fold higher affinity for the receptor at this site than previously reported (Brown & Douglas, 1983).

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NPL is a British Heart Foundation Junior Research Fellow.

EFFECT OF ANGIOTENSIN II ON ADENYLATE CYCLASE IN BASOLATERAL MEMBRANES FROM THE PROXIMAL RENAL TUBULE OF THE RAT

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Angiotensin II (AII) has been reported to inhibit basal and hormone stimulated adenylate cyclase in crude homogenates of rat renal cortex (Woodcock & Johnston, 1982).

We have tried to correlate data obtained from ^{3}H AII binding to basolateral membranes from the rat proximal renal tubule (Lewis & Ferguson, 1986) with an effect of AII on adenylate cyclase. The preliminary results obtained are at variance with those of Woodcock & Johnston.

cAMP was assayed by incubation of basolateral membranes (10-20μg Protein), prepared as described (Lewis & Ferguson, 1986), for 10 mins at 37°C in a final volume of 250μl containing Tris 20mM pH 7.6, NaCl 150mM, $MgSo_4$ 3mM, KCl 5mM, EDTA 0.2mM, caffeine 10mM, ATP 0.8mM, GTP 10^{-5}M, BSA 1mg ml^{-1}, adenosine deaminase 10μg ml^{-1}, creatine phosphokinase 10μg ml^{-1} and phosphocreatine 20mM. Incubations were terminated by rapid cooling and centrifugation. A 50μl aliquot of the supernatant was assayed. cAMP increased linearly with protein concentration in the range 5-30μg tube^{-1}. Accuracy of the assay was confirmed by accounting quantitatively for additions of exogenous cAMP to incubations. The results were assessed by analysis of variance.

Figure 1 cAMP shown pmol mg protein^{-1} per 10 mins (mean ± S.E.M.)

Concn. AII (M)	Basal	PTH 5×10^{-7}M
0	252.2±7.6	1660.5±27.8
10^{-10}	242.6±16.8	1676.9±67.1
5×10^{-10}	235.4±6.3	1732.5±52.3
10^{-9}	200.4±10.9	1770.1±27.8
5×10^{-8}	210.4±33.2	1806.1±63.8
10^{-8}	278.4±5.9	1753.8±50.7
5×10^{-7}	246.4±11.3	1765.2±16.4
10^{-7}	238.2±8.7	1773.4±19.6
5×10^{-6}	238.2±7.9	1709.6±37.6
10^{-6}	238.8±8.0	1644.2±36
	n=5	n=3

Basal cAMP production was not significantly altered by AII in the range 10^{-10} - 10^{-6}M (Fig. 1). Initial results suggested that in the presence of PTH 5×10^{-7}M, AII potentiated cAMP production, but this could not be substantiated on further investigation (Fig. 1). AII 5×10^{-9}M did not affect the generation of cAMP in basolateral membranes with time either alone or in the presence of PTH 5×10^{-7}M. The log dose response curve of cAMP generation by PTH (10^{-11} - 10^{-9}M) in basolateral membranes was not altered by the presence of AII 5×10^{-9}M. In conclusion, AII does not appear to have stimulatory or inhibitory effects on adenylate cyclase in this preparation.

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NPL is a British Heart Foundation Junior Research Fellow.

THE EFFECT OF PHORBOL ESTERS ON THE GUINEA-PIG PARENCHYMAL STRIP

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The transduction mechanism in many cell types involves hydrolysis of phosphatidylinositol bisphosphate with generation of two intracellular messengers - diacylglycerol, which activates protein kinase C, and inositol trisphosphate, which mobilizes intracellular calcium (Nishizuka, 1984; Berridge and Irvine, 1984). Inositol trisphosphate has been shown to mobilize calcium and to cause tension development in vascular smooth muscle (Suematsu et al., 1984 and Somlyo et al., 1985, respectively), and the protein kinase C activator, phorbol 12-myristate 13-acetate (PMA), to cause tonic contraction in both vascular smooth muscle (Rasmussen et al., 1984; Danthuluri and Deth, 1984) and guinea-pig parenchymal strip (Dale and Obianime, 1985). We have suggested that tonic spasm produced by inappropriately activated protein kinase C may contribute to the bronchospasm of the delayed phase of asthma. In this study we examined the effects of various active and inactive phorbol esters on the guinea-pig parenchymal strip, measuring the contraction with an isotonic transducer and expressing the results as a percentage of the maximum histamine contraction.

Phorbol esters known to be inactive on protein kinase C, 4α-phorbol 12,13-didecanoate (4αPDD) and 4α-phorbol 12,13-dibutyrate (αPDBu), tested at 10^{-5}M, had no effect on the strip, nor did the diluent used, dimethylsulphoxide, in the relevant concentrations. Of those phorbol esters known to activate protein kinase C, 4β-phorbol 12,13-dibutyrate (βPDBu) was most active, causing dose-dependent contraction over the concentration range 3×10^{-9}-10^{-5}M, the maximum contraction being 188% of the histamine maximum (n=17). PMA and 4β-phorbol 12,13-didecanoate (4βPDD) both caused dose-dependent contraction over 10^{-7}-10^{-5}M, the PMA maximum being 112% of the histamine maximum (n=20) and the 4βPDD maximum being 70% (n=8). The response to 4βPDBu started promptly but the contraction was slow, taking approximately 40 minutes to reach maximum, and though sustained for up to 30 mins, eventually subsided. The response to PMA (which is very lipid soluble) was very slow indeed, taking 1½-2 hours to reach maximum and was best seen if the tissue was immersed in a large volume (50ml) of Krebs solution. On washing, the PMA contraction increased still further, and with the high concentrations (10^{-6}-10^{-5}M) was maintained for the rest of the experiment (40-60 mins).

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THE EFFECT OF HISTAMINE ON AXONAL CONDUCTION

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The tetrodotoxin (TTX)-sensitive subunit of the sodium channel can be selectively phosphorylated by a cyclic AMP-dependent protein kinase (Costa et al, 1982). Since histamine can increase cyclic AMP through H_2-histamine receptors in several tissues (Schwartz et al, 1986), it was decided to investigate the effect of histamine on compound action potentials recorded from the frog sciatic nerve in the presence of TTX.

The experiments were carried out at room temperature (22-25^{o}C) on the partially desheathed frog sciatic nerve trunk. The preparations were arranged so that the solutions containing the drugs could be applied to the desheathed part of the trunk. The nerve was stimulated supramaximally with rectangular pulses of 0.02 ms duration applied once every 5 s. Throughout the experiments compound action potentials were recorded in the conventional way and photographed. Ringer solution contained (mM): NaCl 117; KCl 2.5; NaH_2PO_4 1; Na_2HPO_4 1; $CaCl_2$ 1.8; $MgCl_2$ 1.2 (pH=7.0).

In the presence of TTX (20-80 nM), which reduced the compound action potential to 20-30% of its initial value in normal Ringer, histamine (1-100 uM) decreased the amplitude of the compound action potentials in a concentration-dependent manner. A similar effect was also obtained with the H_2-histamine receptor agonist, dimaprit (7.5-75 uM). In both cases the full effect was usually observed within 7-10 min after its application to the nerves. The concentration needed to obtain 50% decrease in the action potential amplitude recorded in the presence of TTX (20-80 nM) was 34.4±9.4 uM (n=4) for histamine and 56.3±6.3 uM (n=4) for dimaprit. Both substances were devoid of effect on nerve conduction when applied to the nerve in the absence of TTX. Cimetidine, an H_2-histamine antagonist used in a concentration of 0.2 uM which was devoid of effect on preparations that respond to histamine (5-50 uM) or dimaprit (5-50 uM), antagonized the inhibitory effect of these substances shifting their concentration-response curves to the right.

The results suggest that histamine enhances the TTX inhibitory action on nerve conduction by activating H_2-histamine receptors and are consistent with previous suggestions that cyclic AMP is involved in nerve conduction (e.g. Ribeiro & Sebastião, 1984).

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