Sensitivity to arachidonic acid and the metabolism of purines in the rabbit

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Intravascular injections of arachidonic acid (AA) produce physiopathological effects, particularly arterial hypotension, by conversion of AA to active derivatives, such as prostacyclin and thromboxane A_2. In rabbits anaesthetized but not otherwise manipulated, the AA_{50} (intravenous dose of AA necessary to produce a 50% fall of arterial pressure) is about 400 μg/kg. In a study on 456 rabbits, we have observed that the AA_{50} can be reduced to 10–15 μg/kg, by the combined action of three factors: heparinization (10 mg/kg) followed, after an interval of 50 min, by an injection of autologous haemolyzed blood (in sufficient amount to obtain a plasma haemoglobin concentration of 0.5 mg/ml) and, finally, by 10 min of hypoxia (12% O_2 in N_2). 81% of the animals are in this way sensitized to AA (Deby, Noël *et al.*, 1978); the remaining 19% are resistant to this sensitization, probably because inhibitory factors are present in the circulating blood (Saeed *et al.*, 1977, Danon & Assouline, 1978, Deby, Van Caneghem *et al.*, 1978, Deby *et al.*, 1979).

In an attempt to explain the mechanism of sensitization by hypoxia, we have investigated, in 12 rabbits, the role of purines, the plasma concentration of which is markedly increased during hypoxia (Saugstad *et al.*, 1977). We observed a correlation between the intensity of sensitization to AA and the level of uric acid but not of hypoxanthine in the blood (Figure 1). The increase of uricaemia results from conversion of hypoxanthine to uric acid by xanthine-oxidase, after the return to normoxia.

In another experimental series, we injected intravenously various purines in heparinized rabbits injected with haemolyzed blood, but not subjected to hypoxia (AA_{50}: 25 μg/kg). The following results were observed: the AA_{50} was lowered to 12 μg/kg by adenine (3 rabbits; 25 mg/kg), to 9 μg/kg by hypoxanthine (9 rabbits; 15 mg/kg), to 11 μg/kg by xanthine (3 rabbits; 10 mg/kg) and to 8 μg/kg by uric acid (12 rabbits; 10 mg/kg).

The effects of hypoxanthine, adenine and xanthine were suppressed by allopurinol (20 mg/kg), the AA_{50} returned to the value observed before the purine injection (around 26 μg/kg). Allopurinol also sup-

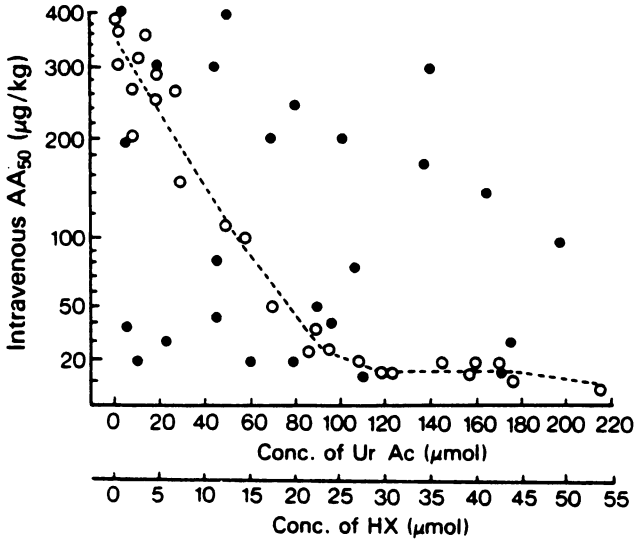

Figure 1 Correlations between hypoxanthine (HX) and uric acid (UrAc) plasma levels and the dose of arachidonic acid necessary to produce a 50% fall in blood pressure (AA_{50}) in anaesthetized rabbits subjected to hypoxia after previous sensitization with heparine and haemolyzed blood. The concentrations of hypoxanthine and uric acid were determined in venous blood taken at various times before and after a period of hypoxia (12% O_2 in ventilated air mixture). ●—individual values for Hx, ○—individual values for UrAc.

pressed the hypersensitization due to hypoxia (the AA_{50} remained at 25–30 μg/kg), in 9 rabbits. Since uric acid can be metabolized to allantoin by uricase, in rodents, we tried this metabolite on 4 rabbits, sensitized by heparin and haemolyzed blood: no changes of AA_{50} were observed. On the contrary, inhibition of uricase by oxonate (10 mg/kg) enhanced the action of uric acid, in 6 rabbits heparinized and injected with haemolyzed blood; the AA_{50} was lowered from 28 μg/kg to 7 μg/kg, with only 7 mg/kg uric acid. Thus, uric acid appears to be the active agent in the sensitization of rabbits to AA by purine metabolites.

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Synthesis of arachidonate cyclo-oxygenase products by rheumatoid and non-rheumatoid synovial tissue

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Stable prostaglandins (PGs) have been detected in synovial fluids from patients with rheumatoid arthritis (Higgs, Vane, Hart & Wojtulewski, 1974) and proliferative cultures of human synovial tissue synthesise prostaglandins (Robinson, Smith, McGuire & Levine, 1975). The cyclic endoperoxide precursors of prostaglandins can also be converted to thromboxane A_2 (TXA_2) or prostacyclin (PGI_2) and there is evidence that these unstable products of arachidonate cyclo-oxygenase are produced in the inflammatory response (Higgs & Salmon, 1979). We have now compared cyclo-oxygenase activity in rheumatoid and non-rheumatoid synovial organ culture.

Synovial membrane was removed, in a bloodless field, at the time of synovectomy (for rheumatoid) and arthrotomy for internal derangements (for non-rheumatoid) mainly of the knee joint. The specimens were removed within 10 min of the application of the tourniquet and placed on a gauze, moistened with Trowell's T8 culture medium, in a sterile vessel. The tissue was cut into segments, each of approximately 4 × 4 mm planar surface, and of the thickness of the membrane (not exceeding 4 mm). These were maintained individually in Trowell's non-proliferative adult organ maintenance culture at the relevant pH (Poulter, Bitensky, Cashman & Chayen, 1970). For some segments, the culture medium contained indomethacin (10^{-4} M). They were maintained, under an atmosphere of 95% oxygen:5% carbon dioxide, at 37°C for 20 hours. The medium was then withdrawn, snap-frozen to −70°C and stored at −20°C. After the removal of excess water, each segment was weighed. Histological confirmation of the pathology was obtained from suitably stained cryostat sections of the segments.

The synthesis of TXA_2 and PGI_2 was measured by the detection of their stable products TXB_2 and 6-oxo-$PGF_{1\alpha}$ respectively. PGE_2, TXB_2 and 6-oxo-$PGF_{1\alpha}$ in culture media were estimated by specific radioimmunoassay following extraction and thin layer chromatographic purification (Higgs & Salmon, 1979). Cyclo-oxygenase activity was expressed as ng of product synthesised during the 20 h culture period per mg wet weight of synovial membrane.

Synovial tissues from five rheumatoid patients produced PGE_2 (2.11 ± 0.88 ng/mg; mean ± s.e. mean),

6-oxo-$PGF_{1\alpha}$ (0.69 ± 0.15 ng/mg) and TXB_2 (0.35 ± 0.15 ng/mg). Ten non-rheumatoid specimens produced PGE_2 (1.95 ± 0.90 ng/mg), 6-oxo-$PGF_{1\alpha}$ (0.38 ± 0.13 ng/mg) and TXB_2 (0.03 ± 0.01 ng/mg). Indomethacin (10^{-4} M) reduced the generation of each cyclo-oxygenase product by at least 85% in six tissues. The production of PGE_2 and 6-oxo-$PGF_{1\alpha}$ was not significantly different in the two groups of tissues but synthesis of thromboxanes by rheumatoid synovia was 14-fold greater ($P < 0.025$) than non-rheumatoid tissues.

These results support the theory that prostacyclin, as well as PGE_2, is an important inflammatory mediator (Higgs, Higgs & Salmon, 1979). The potent vasodilator and hyperalgesic properties of PGI_2 are likely to contribute to inflammatory symptoms whereas thromboxanes may have a separate role in the development of rheumatoid conditions.

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ICI 55897 (CLOZIC), a novel anti-arthritic agent

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Rheumatoid arthritis is a chronic inflammatory disease resulting in the destruction of articulating joints through erosion of the cartilage and bone by invasive, proliferating synovial tissue. Progression of the disease is marked by periods of exacerbation and remission of the inflammatory symptoms, which distinguish this disease from other forms of arthritis and degenerative disease.

Consequently, the search for drugs to treat rheumatoid arthritis has mostly been directed at the inflammatory symptoms of the disease, an approach which has led to the introduction of the non-steroidal anti-inflammatory drugs (NSAIDS). Clinical experience however (McConkey, Crockson, Crockson & Wilkinson, 1973; Amos, Crockson, Crockson, Walsh & McConkey, 1978) has shown that while the NSAIDS are effective inhibitors of the inflammatory symptoms of rheumatoid arthritis, they do not influence the erosive progression.

Several years ago we began a programme of research to identify novel agents which would inhibit chronic inflammatory arthritis, but which did not possess acute anti-inflammatory and analgesic properties. It was speculated that such agents might have a fundamental inhibitory effect on the rheumatoid disease process itself. ICI 55897 (2-(4-p-chlorophenyl benzyloxy)-2-methyl propionic acid) is the outcome of this programme of research.

A model of chronic inflammatory arthritis, based on the method of Newbould (1963), was developed in the Alderley Park strain of rat which, unlike most rat strains, exhibits a prolonged phase of chronic inflammation. An acute phase reactant protein, α_1GP (α_1 acid glycoprotein) measured by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) was used to determine the time course of the disease and the effect of potential therapeutic agents (Billingham & Gordon, 1976).

ICI 55897 effectively inhibited both established chronic arthritis and the developing lesion, the minimum effective dose being 2.5 mg/kg, given once daily P.O. In models of acute inflammation and analgesia ICI 55897 had little activity, for example carrageenin rat paw oedema was not effectively inhibited at doses up to 1000 mg/kg p.o. In further contrast to the NSAIDS ICI 55897 did not induce gastro-intestinal ulceration and irritation when given for fourteen days at doses up to 400 mg/kg p.o. This lack of conventional properties may be due to a relative lack of inhibitory activity against cyclo-oxygenase. ICI 55897 does not inhibit prostaglandin production by rheumatoid synovial tissue in culture (Woolley, Gadher, Foster & Billingham, 1980).

Clinical experience (McConkey, Amos, Billingham, Constable, Crockson, Crockson & Forster, 1980) has demonstrated that ICI 55897 has an affect in rheumatoid arthritis similar to that produced by penicillamine and gold. Clinical benefit is accompanied by a

substantial lowering of the ESR and acute phase reactant proteins, the biochemical markers of disease activity. ICI 55897 thus belongs to the disease modifying class of anti-rheumatic drugs, but its mode of action in rheumatoid arthritis, like that of gold and penicillamine, remains to be clarified.

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AH 19437, a specific thromboxane receptor blocking drug?

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We have suggested the existence of four types of prostanoid receptor mediating smooth muscle contraction (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980a; Coleman, Kennedy, Levy & Penning, 1980c) and have shown that one of these, characterised by a high sensitivity to PGE_2, is specifically blocked by SC 19220 (Coleman, Kennedy & Levy, 1980). We now report that another one of these receptors, characterised by a high sensitivity to the PGH_2 analogue U-46619, is specifically blocked by AH 19437, [1α(Z), 2β, 5α]-methyl 7-[2-(4-morpholinyl)-3-oxo-5-(phenylmethoxy) cyclopentyl]-5-heptenoate.

Guinea-pig ileum and lung strip, dog iris sphincter muscle and saphenous vein, rat aorta and chick ileum were suspended in organ baths containing modified Krebs solution with antagonists (Coleman, *et al.*, 1980a; Coleman *et al.*, 1980c). Agonist concentration-effect curves were constructed cumulatively and repeated until constant, then a further concentration-effect curve was obtained in the presence of AH 19437 with a contact time of between 20–30 minutes.

AH 19437, 2.4×10^{-5} mol/l, had little or no effect on contractile responses of guinea-pig ileum to PGE_2 or $PGF_{2\alpha}$, chick ileum to PGE_2 or dog iris to $PGF_{2\alpha}$. In contrast AH 19437, 3×10^{-7}–7.2×10^{-5} mol/l, caused concentration-related parallel shifts to the right of concentration-effect curves for U-46619 on guinea-pig lung strip, dog saphenous vein and rat aorta. Analysis of these results (Arunlakshana & Schild, 1959) gave pA_2 values against U-46619 of 6.5 (6.4–6.7), 6.0 (5.5–6.6) and 5.9 (5.2–6.6) on guinea-pig lung strip, dog saphenous vein and rat aorta respectively (95% confidence limits of four or more observations). Slopes of the Schild plots for rat aorta and dog saphenous vein were not significantly different from unity, which is consistent with competitive antagonism, while on guinea-pig lung the slope was significantly greater than unity (at $P = 0.05$). The antagonistic action of AH 19437 was specific in that at 7.2×10^{-5} mol/l it had no effect on responses of guinea-pig lung strip to acetylcholine or histamine or responses of dog saphenous vein and rat aorta to potassium chloride or 5-hydroxytryptamine.

These results show that AH 19437 specifically blocked the receptor characterised by high sensitivity to U-46619. We have previously shown that U-46619 has an identical profile of activity to TxA_2 (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980b) and suggested that the U-46619-sensitive receptor is a thromboxane receptor (Coleman *et al.*, 1980a). Accordingly the effects of AH 19437 on dose-effect curves to TxA_2, generated either enzymatically from PGH_2 (Coleman *et al*, 1980b) or by agitation of guinea-pig chopped lung (Coleman, Kennedy & Sheldrick, 1980) were examined on guinea-pig lung strip,

dog saphenous vein and rat aorta using a cascade superfusion technique (Coleman *et al.*, 1980b). AH 19437, 2.4×10^{-5} mol/l, caused greater than 10-fold shifts to the right of TxA_2 dose-effect curves on all preparations. We therefore suggest that AH 19437 is a specific thromboxane receptor blocking drug.

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Noradrenaline increases prostaglandin E_2 and $F_{2\alpha}$ synthesis in rat cerebral cortex via a β adrenoceptor

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Prostaglandins of the E series (PGEs) inhibit noradrenaline (NA) release from sympathetic nerves in the peripheral (Hedqvist, 1970) and central (Hillier & Templeton, 1980) nervous systems; conversely, inhibition of prostaglandin synthesis leads to increased NA release (Samuelsson & Wennmalm, 1971). Since adrenergic nerve stimulation, and NA, increase prostaglandin production by many tissues (Piper & Vane, 1971), Hedqvist (1969) proposed that PGEs play a regulatory role in adrenergic neurotransmission. If such a role is physiologically important, NA may increase PGE synthesis via a receptor mediated process. Evidence for such a process is, however, insubstantial.

We have estimated PGE_2 and $PGF_{2\alpha}$ synthesis, radioimmunologically, by incubates of rat brain cortex slices (0.2×0.2 mm) in the presence of various α and β adrenoceptor agonist and antagonist drugs.

In control preparations, synthesis of $PGF_{2\alpha}$ was about seven times that of PGE_2 (mean synthesis 5.31 ± 0.25 ng/mg protein/10 min and 0.70 ± 0.05 ng/mg protein/10 min respectively); this ratio was not significantly altered by any of the drugs tested.

Synthesis of PGE_2 and $PGF_{2\alpha}$ was increased in a dose-dependent manner by 1-NA (10–1000 μM), a submaximal 2 to 3-fold increase occurring with 100 μM NA. Increased PGE_2 and $PGF_{2\alpha}$ synthesis was also produced by adrenaline and by the β agonists, isoprenaline, rimiterol and orciprenaline, but not by the α adrenoceptor agonists, oxymetazoline and phenylephrine (all at 100 μM). Those β agonists preferring the β_2 subtype receptor (salbutamol and terbutaline) seemed more effective in increasing prostaglandin synthesis than the β_1 agonist H80/62 (±1[4 hydroxyphenoxy]-3-isopropylamino-2-propanol). The effect on prostaglandin synthesis was stereospecific, d-isomers of both NA and isoprenaline being less effective than the corresponding l-isomers (see Table 1).

Effect of antagonist drugs (100 μM) was tested in the presence of NA (100 μM). The α antagonists, phentolamine and dihydroergotamine, did not alter NA induced stimulation but the β antagonist, propranolol, inhibited the response to NA (PGE_2 0.76 ± 0.17 ng/mg protein/10 min, $PGF_{2\alpha}$ 8.65 ± 1.22 ng/mg protein/10 min in the presence of propranolol + NA). The selective β_1 antagonists, atenolol and practolol, also reduced NA stimulated prostaglandin synthesis but less potently than propranolol.

These experiments suggest that NA induced increases in PGE_2 and $PGF_{2\alpha}$ synthesis in cortex are mediated by a β adrenoceptor, possibly of the β_2 subtype. The location of such a receptor is uncertain but may be on glial or cerebrovascular tissue (U'Pritchard, Reisine, Yamamura, Mason, Fibiger, Ehlert & Yamamura, 1980).

W.W.T. is an MRC student.

Table 1 Synthesis of PGE_2 and $PGF_{2\alpha}$ (mean ± s.e. mean) by rat cerebral cortex slices in the presence of α and β adrenoceptor agonists (100 μM)

Drug	PGE_2 ng/mg protein/ 10 min	P	$PGF_{2\alpha}$ ng/mg protein/ 10 min	P
None	0.70 ± 0.05	—	5.31 ± 0.25	—
Noradrenaline (l)	1.53 ± 0.21	0.001	14.79 ± 1.63	0.001
Noradrenaline (d)	0.49 ± 0.09	NS	7.88 ± 0.71	0.02
Isoprenaline (l)	2.12 ± 0.52	0.001	12.60 ± 1.81	0.001
Isoprenaline (d)	0.84 ± 0.19	NS	6.76 ± 1.25	NS
Adrenaline	1.78 ± 0.33	0.001	12.89 ± 2.96	0.001
Orciprenaline	1.41 ± 0.23	0.001	7.50 ± 0.75	0.05
Rimiterol	1.95 ± 0.23	0.001	10.72 ± 1.79	0.001
Salbutamol	1.48 ± 0.13	0.001	10.53 ± 1.52	0.001
Terbutaline	1.56 ± 0.48	0.001	7.35 ± 1.25	0.05
Oxymetazoline	0.78 ± 0.08	NS	8.92 ± 2.41	0.01
Phenylephrine	0.84 ± 0.18	NS	5.93 ± 1.59	NS

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Effects of yohimbine analogues on neuronal and smooth muscle α-adrenoceptors in rat vas deferens

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It has recently been shown that three stereoisomers of yohimbine have different 'selectivities' as antagonists at α_1 and α_2-adrenoceptors in the vasculature of the rabbit (Weitzell, Tanaka & Starke, 1979; Docherty, Madjar & Starke, 1980). Although yohimbine itself is considered to be selective for α_2-adrenoceptors, rauwolscine was even more selective for α_2-, and corynanthine, in contrast, was selective for α_1-adrenoceptors.

The present study compares the effects of yohimbine, rauwolscine, corynanthine and the yohimbine derivative apoyohimbine on the α-adrenoceptors involved in neurotransmission in the rat, isolated, bisected vas deferens; field stimuli (0.5 ms) were applied either singly or in trains of 40 pulses at 8 Hz separated by at least 5 min (McGrath, 1978; Brown *et al*, 1979).

In the epididymal portions of vasa single stimuli produce biphasic contractile responses, the second and dominant part of which is susceptible to α-adrenoceptor antagonists, their order of potency corresponding to that at post-junctional α_1-adrenoceptors found elsewhere (McGrath, 1978). Against this response, compared with yohimbine, apoyohimbine was 10 times more potent, corynanthine was marginally more potent and rauwolscine was half as potent. The potency of apoyohimbine was comparable with that of prazosin.

In the prostatic portions of vasa from reserpinised (1 mg/kg; i.p.; 18 h) rats single stimuli produced monophasic contractile responses which were inhibited in a dose-dependent manner by xylazine. The yohimbine analogues antagonised this inhibition with an order of potency: apoyohimbine > rauwolscine = yohimbine > corynanthine. Apoyohimbine was thus more potent as an antagonist at nerve terminal α_2-adrenoceptors than yohimbine, which hitherto had been among the most potent drugs known to possess this effect.

α-Adrenoceptor antagonists which are relatively more potent at α_1-adrenoceptors on the smooth muscle than on the α_2-adrenoceptors at nerve terminals reduce the response to trains of pulses, particularly in the epididymal portion. Conversely antagonists with relatively more α_2-potency may, at low concentrations, increase the response (Brown *et al*, 1979). In epididymal portions responses to trains could be most clearly increased by rauwolscine, less so by yohimbine, not at all by apoyohimbine and were only reduced by corynanthine. This corresponds to the declining order of 'selectivity' for α_2-adrenoceptors.

The relative selectivity of each stereoisomer of yohimbine for α_1- and α_2-adrenoceptors in the rat vas deferens was similar to that found in rabbit pulmonary artery, where they are 'post- and pre-synaptically' (Weitzell *et al.*, 1979) and pithed rat blood pressure where each is located on vascular smooth muscle (Flavahan & McGrath, 1980).

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Effects of nerve stimulation and agonist drugs on the vas deferens of immature rats

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After castration responses of the rat vas deferens to adrenoceptor agonists or to field stimulation are altered and these changes can be reversed by testosterone (MacDonald & McGrath, 1980). In the present study the responses of vasa from immature rats were investigated.

Isolated vasa deferentia from male Wistar rats 3–6 weeks old were bisected transversely and set up through 'ring and hook' Ag:AgCl electrodes in a 30 ml organ bath containing Krebs' bicarbonate solution at 37°C and gassed with 95% O_2:5% CO_2 (Anton, Duncan & McGrath, 1977). Isometric tension was recorded and responses were elicited by supramaximal pulses of 0.5 ms duration applied either individually or in trains at 8 Hz.

Spontaneous activity. The vasa from immature rats exhibited spontaneous contractions occurring mainly in the prostatic portion (17 out of 33 experiments) but also in the epididymal end (5 out of 33 experiments). Vasa from mature rats never displayed spontaneous activity.

Contractile effects of α-adrenoceptor agonists. In the immature rats the effect of exogenous noradrenaline was similar to that in adults. However the sensitivity of the epididymal portions to oxymetazoline was greater in the immature rats, the threshold for contractions being as low as 10^{-9} M, compared with 10^{-8}–10^{-7} M in adult rats.

Inhibitory effect of exogenous α-adrenoceptor agonist. In immature rats oxymetazoline inhibited the prostatic response to single stimuli, with a threshold similar to that in adult rats (10^{-10} M). Inhibition of epididymal responses to single stimuli was not seen due to potentiation of the response by oxymetazoline. This reflects the increased sensitivity to the post-junctional effects of oxymetazoline described above.

Single pulse stimulation. In rats aged 3–5 weeks both portions of the vas gave monophasic responses unaffected by prazosin (6×10^{-6} M). Biphasic re-

sponses began to emerge in the epididymal end at 5–6 weeks; the second phase of these responses was reduced by prazosin (6×10^{-6} M).

Trains of pulses (*8 Hz, 5 s*). Before 5 weeks, responses in each portion were resistant to prazosin (6×10^{-6} M). At 5–6 weeks, prazosin reduced the response to a train and a subsequent dose of yohimbine (6×10^{-7} M) produced a potentiation to beyond the initial level; this indicates that the pre- and post-junctional effects of endogenous noradrenaline appear at the same time.

It is concluded that, although adrenergic nerves containing noradrenaline are present in the rat vas deferens at 3 weeks (Owman, Sjöberg & Swedin, 1971), functional adrenergic transmission cannot be demonstrated until 5–6 weeks, corresponding to the age at which testosterone secretion increases (Knorr, Vanha-Perttula & Lipsett, 1970).

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The effect of ethanol on motor and inhibitory responses of the anococcygeus and bovine retractor penis muscles

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We have previously reported that ethanol (200 mM) reduced the response to stimulation of non-adrenergic non-cholinergic nerves in the rat anococcygeus muscle (Gillespie & McKnight 1978). More recently we have been studying the properties of an inhibitory factor isolated from the bovine retractor penis (BRP) which might be the neurotransmitter of these nerves (Gillespie & Martin, 1980). If ethanol is acting post-synaptically it should reduce both inhibitory effects. We have investigated ethanol's effects on the response to inhibitory nerve stimulation and inhibitory extract; we have also determined the specificity of its inhibitory action on these responses by comparing its effect on other inhibitory agents.

Ethanol 200 mM reduced the response of the BRP and the rabbit and rat anococcygeus to field stimulation of the inhibitory nerves by between 60% and 90% depending on frequency. The specificity of ethanol was investigated by examining its effects on six other inhibitory stimuli, isoprenaline, carbachol, bradykinin, isobutylmethylxanthine (IBMX), and ATP on the rabbit anococcygeus and isoprenaline, IBMX and sodium nitroprusside on the BRP. In the rabbit anococcygeus carbachol-induced inhibition was reduced by 50–90% depending on the carbachol dose. In contrast inhibition by IBMX or bradykinin was unaltered and that by isoprenaline or ATP was potentiated. In the BRP the inhibitory effect of IBMX or sodium nitroprusside was unaltered though that to isoprenaline was reduced. The effect of ethanol on the response to inhibitory extract was variable. In most preparations inhibition was reduced but in a minority ethanol had no effect or even rarely potentiated the response. In both preparations the development of inhibition was slowed by ethanol even where the final response was undiminished. With agents such as the inhibitory extract which are rapidly inactivated in the organ bath the slow development of inhibition could prevent them developing their maximum effect and give an erroneous appearance of a reduced response. This possibility was investigated by continuously infusing extract into Krebs' saline passing over BRP preparations arranged as a cascade until a plateau of inhibition was reached. In these conditions ethanol (200 mM) still produced variable results but commonly reduced the inhibitory response to the extract.

Effects on motor responses were examined in the rat anococcygeus. Ethanol (200 mM) reduced the motor response to field stimulation of the adrenergic nerves or to noradrenaline by 25–30% but reduced that to carbachol by 45–70%.

Since the reduction by ethanol of the inhibitory effect of the extract is smaller and less consistent than the reduction in the response to inhibitory nerve stimulation there must be some doubt on the role as inhibitory transmitter for the inhibitory factor in extracts from the BRP.

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The effect of extracellular sodium ion concentration on the action of opiates to inhibit [^{3}H]-noradrenaline release from the mouse vas deferens

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The binding affinity of opiate agonists in brain homogenates is decreased by the presence of sodium ions (Simon, Hiller & Edelman, 1973). To determine whether the extracellular sodium concentration influences the pharmacological actions of opiates we have examined the effect of changes in extracellular sodium ion concentration on the inhibition, by opiates, of potassium-evoked noradrenaline (NA) release from the mouse vas deferens.

Isolated vasa deferentia were labelled with [^{3}H]-NA as described by Hughes (1973). [^{3}H]-NA release was evoked in the presence of phentolamine (100 μM) and cocaine (7 μM) by raising the extracellular potassium concentration to 50 mM for 2 minutes. Choline or sucrose were used to substitute for sodium in the Krebs solution bathing the tissues.

Normorphine (0.3–10 μM) inhibited the potassium-evoked release of [^{3}H]-NA in a dose-dependent manner. The maximum inhibition produced by normorphine was 60% of the total [^{3}H]-NA released. The concentration of normorphine which produced 50% of the maximal inhibition was 1.5 μM. The inhibition was stereospecific and reversed by naloxone (0.3–1 μM).

As the sodium concentration of the bathing solution was reduced (from 143 mM to either 25 or 0 mM) the amount of [^{3}H]-NA release evoked by potassium increased. This increased release probably reflects an increased calcium entry into the nerve terminals (Kelly, 1965). The ability of normorphine to depress [^{3}H]-NA release decreased as the sodium concentration decreased. This attenuation of the effect of normorphine may have been a consequence of the increased amount of [^{3}H]-NA released. By decreasing the extracellular calcium concentration (from 2.54 to 0.5 mM) the amount of [^{3}H]-NA release evoked by potassium in low sodium Krebs (25 mM) could be reduced to that evoked in normal Krebs (sodium 143 mM, calcium 2.54 mM). However, the depression of [^{3}H]-NA release by normorphine in low sodium, low calcium Krebs was still significantly lower than the depression observed in normal Krebs.

Thus it appears that the removal of sodium attenuates rather than potentiates the action of opiates in the mouse vas deferens.

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Adrenoceptors in pregnant rat uterus

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In the non-pregnant rat uterus, the response to catecholamines is inhibitory throughout the four stages of the natural oestrous cycle (Digges & Boyle, 1979). This inhibition is mediated via β-adrenoceptors. An α-adrenoceptor mediated motor response was seen only rarely and only in the presence of a β-adrenoceptor antagonist. In the present investigation the response to catecholamines in the pregnant rat uterus was examined. The gestation period in the rat is approximately 21 days, and the uterus was examined midway through gestation at 10 days, just before parturition at 20 days and on the 1st day post-partum.

Uterine horns were removed from rats at the appropriate stage of pregnancy, and a longitudinal incision was made along each horn, to allow removal of the foetuses and attached placentae. 2–3 cm lengths of mid-uterus were mounted in paired 10 ml organ baths containing Tyrode's solution, aerated with 95% O_2:5% CO_2, and maintained at 37°C. Isometric tension was recorded via Grass FT03 force-displacement transducers and displayed on a Grass Model 7D Polygraph. The β-inhibitory effects of the catecholamines were measured as a percentage reduction in the motor response to a concentration of acetylcholine which gave 70–80% of the maximum response. The tension developed in response to ACh increased throughout pregnancy, being greatest immediately pre-partum and returning towards non-pregnant values, 1 day post-partum.

Adrenaline (Adr), noradrenaline (NA) and isoprenaline (Iso) inhibited the standard ACh-induced contraction throughout pregnancy, with the same order of potency (i.e. Iso > Adr > NA) as seen in non-pregnant animals. The degree of inhibition produced by the catecholamines was the same throughout pregnancy (i.e. ~80%) except for NA in post-partum rats, which produced only 50% inhibition. EC_{50} values for the catecholamines in 10 day pregnant rats were not significantly different from those of non-pregnant rats. In 20 day pregnant rats, however the mean EC_{50} values for both Iso and NA were significantly greater than those of non-pregnant rats. In 1 day post-partum rats, the mean EC_{50} value for Iso returned towards non-pregnant values, but the mean EC_{50} value for NA was further increased.

This apparent reduction in β-activity in 20 day pregnant and 1 day post-partum uteri, may be due to an increase in α-adrenoceptor responsiveness, since in these animals, NA and Adr sometimes produced motor responses. Indeed, in the presence of a β-receptor antagonist, propranolol (10^{-5} M), Adr and NA produced consistent motor responses, so much so that dose-response curves could be constructed. No such consistent motor responses were found in non-pregnant rats.

In conclusion, towards the end of pregnancy there is an increase in α-receptor motor responsiveness in the rat uterus, which may play some role in the process of parturition.

KGD is an MRC Student.

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Nicotine-induced relaxation of guinea-pig trachealis

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Electrical field stimulation of guinea-pig isolated trachea can evoke relaxant effects which are tetrodotoxin-sensitive yet are partly resistant to sympatholytic drugs. This suggests a non-adrenergic inhibitory innervation of the tissue (Coburn & Tomita, 1973; Coleman & Levy, 1974; Clark, 1978). In gastrointestinal tissue (Small & Weston, 1979) and vascular tissue (Hughes & Vane, 1970) nicotine directly or indirectly stimulates non-adrenergic inhibitory fibres. We have now investigated whether nicotine can activate such structures in trachealis muscle.

Segments of trachea were isolated from guinea-pigs (350–650 g, either sex) and set up for recording mechanical activity (Coburn & Tomita, 1973) under isotonic conditions (load 200 mg). All experiments were carried out using Krebs' solution containing atropine

(10^{-6} M). Concentration/effect curves to nicotine were constructed taking care to minimise tachyphylaxis by the use of adequate inter-dose intervals and washing procedures. Relaxant responses to nicotine (2.5×10^{-5} to 2×10^{-4} M) were measured in terms of the maximal relaxation evoked by sodium nitrite (2×10^{-2} M).

Hexamethonium (5×10^{-4} M) produced approximately twenty five-fold antagonism of nicotine. Tetrodotoxin (3×10^{-7} M) and guanethidine (5×10^{-5} M) each almost abolished the effects of nicotine. Propranolol (2×10^{-8} to 5×10^{-6} M) produced a rightward shift in the concentration/effect curve to nicotine and markedly reduced the maximal response. There was little residual response to nicotine following the highest concentration of propranolol used.

Using cocaine and ergotoxine as pharmacological tools Hawkins and Paton (1958) generated data to suggest that nicotine relaxed the trachea by releasing transmitter from adrenergic neurones. Our findings support this earlier suggestion. Nicotine's transmitter-releasing action involves the activation of neural nicotinic cholinoceptors and a subsequent process involving Na^+ activation (probably the discharge of nerve action potentials).

If the trachealis is innervated by non-adrenergic inhibitory neurones as the results of field stimulation experiments suggest, then nicotine does not activate their terminal axons. The possibility exists, however, that nicotine-sensitive parts of such neurones or nicotine-sensitive structures providing them with excitatory input may be located outside the tracheal wall.

L.C. is a CASE student.

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An *in vitro* comparison of haloalkylamine activity upon rat anococcygeus and guinea-pig trachealis

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Kalsner & Nickerson (1969) used GD 131 to dissociate two of the pharmacological properties of haloalkylamines; potentiation of noradrenaline on the rabbit aorta and α-adrenoceptor blockade.

Iversen, Salt & Wilson (1972) used phenoxybenzamine (PB), SKF 625A and SKF 550, among other haloalkylamines, to dissociate inhibition of $Uptake_1$ and inhibition of $Uptake_2$ on the rat heart and α-adrenoceptor blockade. We have examined the association between the activities of the same three haloalkylamines as α-adrenoceptor blocking agents on the rat isolated anococcygeus and their ability to potentiate noradrenaline and isoprenaline on the guinea-pig isolated trachealis.

Tissues were kept at 38°C in Krebs solution containing ascorbic acid (0.1 mM) and gassed with 5% CO_2 in O_2. In every experiment the aqueous (38°C) dilution of haloalkylamine from an acidified ethanolic stock solution occurred 30 min before introduction to the tissues.

Isolated paired anococcygeus muscles from male Wistar rats (250 to 350 g) were mounted under 1 g resting tension. Sequential (−)-noradrenaline (NA) log. concentration isometric response curves were constructed before and after 1 h incubation with concentrations of PB, SKF 625A or SKF 550 which produced depressions of the response to NA 1 mM of approximately 20 to 80%.

Four tracheal segments were isolated from each guinea-pig (300 to 600 g, either sex), opened and mounted under 150 mg tension. Cumulative log. concentration isotonic response curves to NA or (−)-isoprenaline (ISO) were constructed before and 1 and 4 h after incubation of three segments with various concentrations of the three haloakylamines. An aminophylline-induced maximum relaxation was superimposed on each catecholamine curve.

Table 1 shows that no quantitative correspondence exists between the α-adrenoceptor blocking, NA-

Table 1 Potencies of haloalkylamines

	Phenoxybenzamine	*SKF 625A*	*SKF 550*
α-blockade[1]	8.21 ± 0.14	7.41 ± 0.12	7.06 ± 0.11
NA-potentiation[2]	6.31 (6.13, 6.58)	4.88 (4.65, 5.30)	3.97 (2.62, 4.34)
ISO-potentiation[2]	5.40 (4.77, 5.92)	7.25 (6.92, 7.71)	7.13 (6.85, 7.45)

[1] $-\log_{10}$ molar concentration halving the response to 1 mM NA (mean ± s.e. mean, $n = 6$) on rat isolated anococcygeus, 1 h treatment.
[2] $-\log_{10}$ molar concentration causing two-fold potentiation of the catecholamine (mean, 95% confidence limits, n at least 6) on guinea-pig isolated trachealis, 1 h treatment.

potentiating and ISO-potentiating potencies of these haloalkylamines.

The potencies found here for potentiation of catecholamines closely resemble those found by Iversen *et al.* (1972) for inhibition of $Uptake_1$ and $Uptake_2$ in the isolated perfused rat heart. This resemblance contributes to the circumstantial evidence (Foster, 1969; Anning, Bryan & O'Donnell, 1979) that there is a causal connection between inhibition of $Uptake_2$ (extraneuronal uptake) and potentiation of ISO.

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Intrinsic activity of labetalol on guinea-pig trachealis muscle *in vitro*

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The mixed α- and β-adrenoceptor antagonist labetalol has been reported to be devoid of intrinsic activity (Farmer, Kennedy, Levy & Marshall, 1972; Brittain & Levy, 1976). However, labetalol has since been shown to stimulate β-adrenoceptors of rat uterus (Whalley, 1977; Carey & Whalley, 1979). To determine whether such intrinsic activity could be detected at β-adrenoceptors at another site, labetalol was tested for its ability to relax the guinea-pig trachealis muscle *in vitro*.

Segments of guinea-pig trachea were set up and allowed to develop tone in Krebs' solution gassed with O_2 + 5% CO_2 at 37°C, as described by Coburn & Tomita (1973). Responses were recorded using isotonic transducers (Washington type T2) with a tissue loading of 150 mg. Relaxations to drugs were measured as percentages of the maximum relaxation that could be induced in that tissue by aminophylline, (10^{-3} M). Cumulative log concentration-effect curves were produced using 4-fold increments either in the absence of or after 30 min exposure to propranolol (10^{-7} M, 10^{-6} M or 10^{-5} M).

The resulting curves (Figure 1) indicate that labetalol has intrinsic activity at guinea-pig tracheal β-adrenoceptors.

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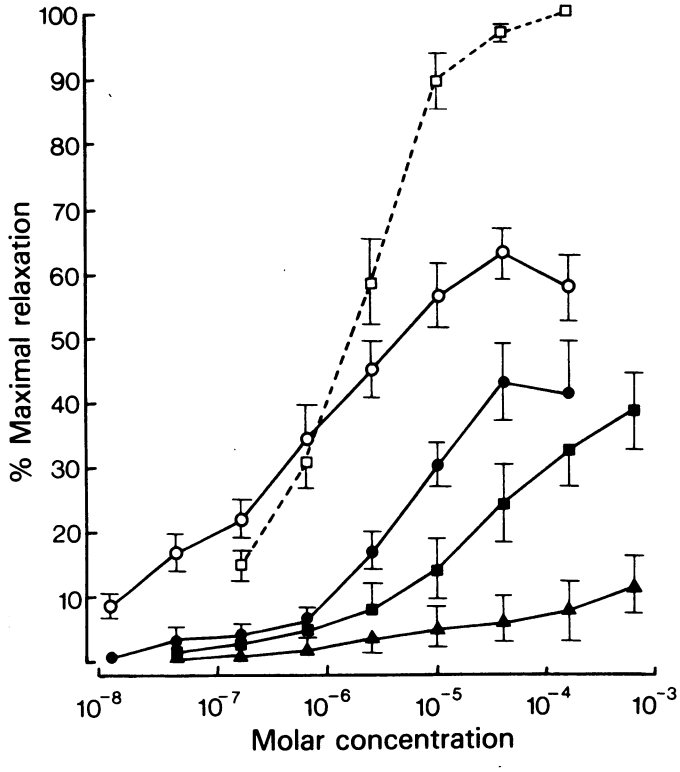

Figure 1 Responses (measured as % of maximal relaxation to 10^{-3} M aminophylline; mean ± s.e. mean) to (□) noradrenaline and (○) labetalol, and to labetalol in the presence of propranolol 10^{-3} M (●), 10^{-6} M (■) and 10^{-5} M (▲). n = 5–14.

A comparison *in vitro* of guinea-pig central and peripheral airways smooth muscle

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A number of isolated preparations have been devised to examine the direct action of drugs on bronchial smooth muscle. These have included tracheal tubes (Farmer & Coleman, 1970), rings (Castillo & de Beer, 1947) and spirals (Coburn & Tomita, 1973), all of which indicate possible actions on large, central airways. The more recent development of the lung parenchymal strip (Lulich, Mitchell & Sparrow, 1976; Burns & Doe, 1978; Drazen & Schneider, 1978) has enabled the investigation of the action of agents on the smaller airways. Therefore, it has been possible to compare the responses of constrictor and relaxant

drugs on peripheral and central airways in the same species (Lulich *et al.*, 1976). Lulich & Paterson (1980) concluded that the airways of the rat responded in a different manner to human bronchial muscle. In this light, we have investigated the isolated lung strip and tracheal strip of the guinea-pig as a possible model of human airways muscle and attempted to characterize the action of selected agents on these preparations.

Lung parenchymal strips (Drazen & Schneider, 1978) and tracheal strips (Coburn & Tomita, 1973) were mounted in McEwens' solution at 37°C for isotonic recording. Both preparations exhibited an intrinsic tone, allowing both contractile and relaxant responses to be monitored.

Adrenaline (0.5–50 μM) produced concentration-related relaxations of tracheal strips but the maximum effect on the lung strips was only 15% of total relaxation. Salbutamol and terbutaline were similarly ineffective on the lung strip. However, both preparations were found to be totally relaxed by the phosphodiesterase inhibitors papaverine, isobutylmethylxanthine, ICI 63197 and theophylline. The EC_{50} values were respectively 22, 28, 177 and 335 μM on the lung strip and 3, 2, 0.4 and 110 μM on the tracheal strip. Propranolol, in a concentration (0.3 μM) which antagonized adrenaline-induced relaxations of the tracheal strip, did not change the sensitivity to these spasmolytic agents in either of the tissues.

Lung strips were contracted in a concentration dependent manner by histamine (0.5–500 μM) and acetylcholine (0.4–400 μM). The tracheal strip was also contracted by these agonists, although the sensitivity to both histamine (10–1000 μM) and acetylcholine (10–2000 μM) was considerably reduced. However, the maximum contraction caused by histamine was significantly ($P < 0.05$) greater than that which could be induced by acetylcholine in the lung strip, whereas in the trachea the converse was true, acetylcholine producing a greater maximum contraction.

The results of this investigation indicate that in the guinea-pig, the peripheral airways are more sensitive to the constrictor agents, histamine and acetylcholine than are the central airways. This does not seem to be the case with the spasmolytic agents used which were more effective on central airways.

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Parasympathectomy increases β-adrenoceptor density in the rat parotid gland

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In the rat parotid gland denervation of either division of the autonomic nervous system results in an increased sensitivity to agonists at cholinoceptors and adrenoceptors (Ekstrom, 1980). We have attempted to correlate this change in adrenoceptor agonists sensitivity with changes in the binding of the antagonist [^{3}H]-dihydroalprenolol, in rat parotid glands.

One parotid gland was parasympathectomized by unilateral section of the auriculo-temporal nerve under pentobarbitone (60 mg/kg i.p.) anaesthesia. The contralateral gland acted as a control. After 14 days the animals were used either to assess the degree of supersensitivity in terms of gland secretory response or for binding studies.

Denervation reduced both the wet and dry weight of the gland (parasympathectomized 123.0 ± 18.7 mg wet weight and 44.6 ± 4.2 mg dry weight compared with 218.7 ± 22.2 and 69.0 ± 7.1 for control gland

$P < 0.001$). Despite this marked atrophy, the parasympathectomized glands showed increased sensitivity to agonists. Thus, the threshold secretory dose of acetyl-β-methylcholine was reduced to 0.21 ± 0.11 from a control value of 0.88 ± 0.13 ($n = 5$–7, $P < 0.01$) μg/kg i.v., and the maximum secretory response increased from 16.0 ± 2.8 μl (control) to 28.0 ± 6.1 μl ($n = 5$–7). Parasympathectomy also increased the response to adrenaline, the threshold dose being 0.40 ± 0.06 compared with 6.25 ± 1.61 μg/kg in control tissue ($n = 5$–7, $P < 0.02$).

[^{3}H]-Dihydroalprenolol (56 Ci/mmol binding was studied in crude membrane fractions of parasympathectomized and control parotid tissue. Glands were homogenized in 50 mM tris-HCl buffer, pH 8.0, and membranes collected by centrifugation for 20 min at 50,000 g following two 10 min spins at 800 g to separate duct material. Membranes were washed at least once. Non-specific binding was assessed with propranolol (10 μM) and all assays were in triplicate. Following incubation for 30 min at 25°C free and bound radioactivity were separated by filtration through GF/C filters. Bound radioactivity was eluted in 2% sodium dodecylsulphate and assayed by liquid scintillation spectrometry.

Membranes from both control and denervated tissue showed a saturable specific binding component (0.1–4 nM range). The affinity constants were similar in control and denervated glands (1.66 nM for parasympathectomized and 1.25 nM for control membranes). Binding was increased in parasympathectomized tissue at all ligand concentrations used (e.g. at 1 nM, specific binding was 0.40 ± 0.04 in control and 0.63 ± 0.06, $n = 3$, $P < 0.05$, fmol/μg protein). Thus, maximum binding was increased from 25 to 50 fmol mg protein^{-1} min^{-1}. However, the total number of binding sites/gland was unaffected by parasympathectomy.

This result is consistent with an increase in the density of β receptor sites and may in part, explain the increase in sensitivity to β agonists in parasympathectomized parotid glands.

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Sgd 101/75 (4(2-imidazoline-amino)-2-methyl-indazol-chlorhydrate): a drug that can act as an agonist, partial agonist or antagonist on alpha-adrenoceptors of isolated tissues

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Sgd 101/75 (4(2-imidazoline-amino)-2-methyl-indazol-chlorhydrate) is a derivative of clonidine that differs from the parent drug in (a) not lowering arterial blood pressure and heart rate in anaesthetized animals (dog, cat, rat and guinea-pig), and (b) in not inducing sleep on i.v. injection into chicks. Furthermore, tachyphylaxis developed to the initial vasopressor effect of Sgd 101/75 in the anaesthetized rat and cat, and subsequent examination of the cardiovascular response of these preparations to noradrenaline revealed that alpha-adrenoceptor blockade had occurred (Ismail, Jahn & Weetman, unpublished results). These *in vivo* effects were consistent with a partial agonist action of the derivative on the vascular alpha-adrenoceptors; thus Sgd 101/75 was considered worthy of further investigation *in vitro*.

Guinea-pig taenia, coaxially stimulated ileum (0.1 Hz, 1 ms pulses maximal voltage) and rat anococcygeus preparations were arranged in isolated organ baths containing McEwen's solution maintained at 37 ± 1°C and gassed with 95% O_2:5% CO_2. Responses were measured either kymographically (taenia, magnification 1:4, load 0.5 g; anococcygeus, magnification 1:5, load 0.5 g) or on a Grass polygraph, using a Grass isometric-force-displacement transducer (load 0.5 g).

Concentration-response curves for noradrenaline were obtained initially and then, following the addition of Sgd 101/75 (400 nM–4 mM), the effects of noradrenaline were redetermined. On the taenia, the relaxant effects of Sgd 101/75 were tachyphylactic. From experiments in which only one concentration of Sgd 101/75 was applied to each of a series of preparations, the derivative was shown to be a partial agonist for alpha-adrenoceptors ($\alpha = 0.46$, $n = 23$), and there was a significant correlation between the initial submaximal relaxant effect and the subsequent desensitization to noradrenaline ($r = +0.65$, $P < 0.005$, $n = 17$). In contrast, Sgd 101/75 was a full agonist on the alpha-adrenoceptors of the anococcy-

geus ($\alpha = 1.01$, $n = 6$), and its contractile effect did not exhibit tachyphylaxis. In separate experiments, phentolamine was used to show that Sgd 101/75 acted upon alpha-adrenoceptors of the anococcygeus muscle. The pA_2 values for phentolamine, as determined by the method of Arunlakshana & Schild (1959), were found to be 8.4 (slope 0.7; $n = 12$) with noradrenaline as the agonist, and 8.0 (slope 1.2; $n = 15$) when Sgd 101/75 was used to contract the tissue: these pA_2 values were not significantly different ($P > 0.05$).

Although the subsequent sensitivity of the anococcygeus to noradrenaline in the presence of Sgd 101/75 was not assessed because both drugs contracted the tissue, no desensitization was apparent 15 min after washing out Sgd 101/75 (400 μM, 3 changes of McEwen's solution).

Application of Sgd 101/75 (4–400 μM) to the coaxially stimulated guinea-pig ileum enhanced the electrically-induced twitches in a concentration-dependent and non-tachyphylactic manner. The subsequent responsiveness of the tissue to noradrenaline was reduced (pA_2 for Sgd 101/75 was 6.6, slope 0.8, $n = 5$).

Sgd 101/75 is therefore capable of interacting with alpha-adrenoceptors on various tissues in three different ways, as a full agonist, as a partial agonist, and as an antagonist, and may be of value in examining the postulated sub-divisions of the alpha-adrenoceptor.

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The effects of anaphylaxis on stomach motility in the guinea-pig

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We have previously shown that protracted anaphylaxis in the guinea-pig produced haemorrhagic damage to the gastric mucosa which could be partially inhibited by metiamide (Chadwick & Goadby, 1980). It was also shown that the lesions were not the result of excessive gastric acid production and a possible alternative explanation was that the gastric mucosa may have been disrupted by changes in motility.

Stomach motility was measured in urethane-anaesthetized guinea-pigs (1.5 g/kg, i.p.) by ligating the pylorus and filling the stomach with either normal saline or glycine-mannitol buffer (1:5, 300 m osmol/l) via an oesophageal cannula connected to a reservoir exerting a pressure of 1–2 cm water on the stomach. Changes in air pressure above the solution in the reservoir were monitored by an air pressure transducer (Ether, UP1). Arterial blood pressure was monitored from a cannula in the carotid artery and drugs were administered via cannulae in the jugular vein or the coeliac artery at its junction with the hepatic and gastrosplenic arteries. Isolated stomach preparations were prepared as described by Spedding (1977).

Administration of antigen (1 mg egg albumen, i.v.) to previously sensitized (100 mg egg albumen, i.p., at least three weeks before test) guinea-pigs produced an initial increase in gastric intraluminal pressure followed by a decrease which, in turn, was followed by an even larger, more sustained increase in pressure.

In comparison, histamine (2.5–20 μg i.v.) caused a small initial increase in pressure followed by a decrease although intra-arterial administration produced only increases in pressure.

In isolated stomachs anaphylaxis caused a marked increase in pressure but histamine produced much smaller increases, the maximum being only 20% of that evoked by anaphylaxis which was equivalent to 95% of the maximum evoked by vagal stimulation. Mepyramine (5×10^{-6} M) abolished the effects of histamine but had no effect on the increase in pressure caused by anaphylaxis.

However, *in vivo*, mepyramine (1 mg/kg i.v.) converted the effects of histamine (1–20 μg i.v. or 5–20 μg i.a.) to decreases in pressure which were antagonized by metiamide (5 mg/kg i.v.). Anaphylaxis following mepyramine pretreatment produced a sustained increase in pressure which could be prevented by pretreatment with FPL 55712 (1 mg/kg i.v.) or indomethacin (1 mg/kg i.v.).

These results indicate that histamine may produce

contraction of the guinea-pig stomach *in vivo* by an action on histamine H_1-receptors and relaxation by activating histamine H_2-receptors. Anaphylaxis produces complex gastric motility patterns mediated in part by histamine but with possible contributions from SRS-A and prostaglandins.

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Dopamine inhibition of the transmurally-stimulated gastric fundus of the rat

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Dopamine has been proposed as the endogenous substance mediating vagal gastric relaxation in the canine stomach (Valenzuela, 1976) and inhibitory dopamine receptors were suggested to be present in the guinea-pig stomach (Van Nueten, Ennis, Helsen, Laduron & Janssen, 1978). We have studied the inhibitory effects of dopamine and noradrenaline on the transmurally stimulated rat fundus preparation (Vane, 1957).

Rat fundus strips were set up under a load of 1 g in eserinized (2×10^{-8} g/ml) Tyrode solution at 37°C. Contractions were elicited by transmural stimulation (1 ms, 12 V, 0.5–16 Hz) and recorded auxotonically (Harvard heart/smooth muscle transducer). Frequency response curves were obtained in the absence and presence of dopamine, (−)-noradrenaline, phentolamine, propranolol and domperidone.

Noradrenaline (1, 3 and 10×10^{-6} M, $n = 27$) and dopamine (0.3, 1, 3 and 10×10^{-5} M, $n = 14$) produced dose dependent inhibition of the electrically-evoked contractions. The inhibition was most pronounced for the lower frequencies. The concentrations used had almost no effect on the resting length of the fundus strip. The threshold concentration for inhibition was between 1 and 3×10^{-6} M for noradrenaline and between 0.3 and 1×10^{-5} M for dopamine. Noradrenaline (3×10^{-6} M) and dopamine (3×10^{-5} M) produced a similar degree of inhibition.

Phentolamine (3×10^{-7} M) as well as propranolol (10^{-6} M) partially antagonized the inhibitory effects of noradrenaline (3×10^{-6} M). Higher concentrations of phentolamine and propranolol were not used since they inhibited in themselves the response to transmural stimulation. The combination of phentolamine (3×10^{-7} M) and propranolol (10^{-6} M) did not antagonize the inhibitory effect of noradrenaline (3×10^{-6} M) at 0.5 Hz, partially antagonized it at 1 Hz and completely abolished it at 2, 4, 8 and 16 Hz.

The inhibitory effect of dopamine (3×10^{-5} M) was abolished by the combination of phentolamine (3×10^{-7} M) and propranolol (10^{-6} M) at all frequencies. The separate testing of the antagonists against dopamine showed an almost complete antagonism of dopamine by phentolamine, while propranolol only partially antagonized the inhibitory effect of dopamine.

Domperidone, a peripheral dopamine antagonist (Laduron & Leyson, 1979) with a very high selectivity as a ligand for dopamine receptors (Baudry, Martres & Schwartz, 1979) had in a dose of 10^{-5} M no effect on transmural stimulation. This dose did not antagonize the inhibitory effect of dopamine.

Our study does not provide evidence for the existence of specific inhibitory dopamine receptors in the rat fundus and indicates that the inhibitory effect of dopamine on contractions evoked by transmural electrical stimulation is mainly mediated by alpha-adrenoceptors.

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Metabolism of norethisterone oenanthate *in vitro*

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Norethisterone oenanthate (17α-ethinyl-17β-oestr-4-en-3-one-17β-heptanoate; NET OEN), is a depot contraceptive which produces prolonged contraceptive protection when injected intramuscularly (i.m.). A consistent finding in women is that plasma concentrations of NET OEN fall rapidly and exponentially from a peak soon after injection to low levels by about 2 months after injection. Norethisterone (NET) concentrations are always higher than NET OEN (Gerhards, Hecker & Bellmann, 1976; Saxena, Shrimanker & Fotherby, 1977). In an attempt to find a suitable animal model for predicting the pharmacokinetics of long acting steroid esters in humans, the disposition of NET OEN was studied in the rabbit following i.m. injection (Fotherby, Shrimanker & Saxena, 1978). No circulating NET OEN was present at any time in the study, although NET was detected for 60 days. It was therefore suggested that hydrolytic enzyme activity towards NET OEN is greater in the rabbit than in the human.

We have investigated the enzymic cleavage of NET OEN first in the plasma of six species (rabbit, rat, guinea-pig, dog, goat and human) and secondly in the tissues of rabbit and human.

When plasma (2 ml) was incubated at 37°C with [^{3}H]-NET OEN (0.4 μCi) there was a marked species variation in the extent of hydrolysis of the steroid ester. Rabbit, rat and guinea-pig plasma possessed high esterase activity (99.9%, 76.1% and 46.0% NET OEN hydrolysed in 90 min respectively) whereas plasma from the dog, goat and human caused only minor breakdown of NET OEN (less than 2.5% hydrolyzed in 90 min).

Rabbit muscle, fat, liver and kidney, human muscle and fat were incubated in Krebs-Henseleit buffer (pH 7.4) plus a NADPH regenerating system (NADP, 4 μmol; glucose 6-phosphate, 10 μmol; glucose 6-phosphate dehydrogenase, 2 units; $MgCl_2$, 20 μmol) and [^{3}H]-NET OEN (0.1 μCi; 10 μg). The percentage breakdown of NET OEN was determined at 60 minutes. Rabbit muscle, liver and kidney showed marked hydrolytic activity towards NET OEN (45.7%, 95.7% and 89.7% hydrolysed respectively). In contrast, in human muscle only 1.1% of NET OEN was hydrolysed. There was little hydrolysis by either rabbit or human fat (6.4% and 1.1% hydrolysed respectively).

These results indicate that *in vivo* in humans NET OEN is unlikely to be metabolised at the injection site or in the circulation, whereas in the rabbit extensive hydrolysis will occur at both sites. The rabbit is therefore not an appropriate model for predicting the pharmacokinetics of steroid esters in humans. Using the one criterion of the rate of breakdown of NET OEN in plasma, the dog could prove to be a realistic alternative.

We are grateful to Professor G. Bentley and Mr. G. Dowd of the Royal Liverpool Teaching Hospital for obtaining human tissue for the study and to Schering AG for the gift of [^{3}H]-norethisterone oenanthate. This investigation received financial support from the World Health Organization. P.H.R. is supported by Wyeth Laboratories.

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The effect of streptozotocin-induced diabetes on glucuronyl transferase activity in the rat

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Drug metabolism has been shown to be altered in insulin-dependent diabetic subjects. In addition both alloxan- and streptozotocin-induced diabetes in animals cause sex-dependent decreases in the hepatic Phase I metabolism of some substrates (Kato, 1977; Reinke, Stohs and Rosenberg, 1978). However, many drugs are extensively conjugated by Phase II metabolism and this study reports decreases in glucuronyl transferase (GT) activity in streptozotocin-induced diabetes.

Sprague–Dawley rats (200 ± 20 g) were starved for 24 h prior to administration of a single dose of streptozotocin (60 mg/kg i.v.) on day 1. On day 6 the rats were divided into two groups. The first group was killed and liver microsomal suspensions prepared and assayed for GT activity whilst the second group received daily treatment with 16 IU isophane insulin, subcutaneously, for 6 days before hepatic GT activity was measured on day 12. GT activity was measured both before and after activation of the enzyme, which was achieved by 10 min pre-incubation of the microsomes with an equal volume of 0.5% Triton X-100.

Glucuronidation of p-nitrophenol was assayed by the disappearance of substrate from an incubation mixture containing: p-nitrophenol (500 nmol), uridine diphosphoglucuronic acid (5 μmol, UDPGA), saccharo-1,4-lactone (12.5 μmol), 0.2 ml microsomal suspension (1 ml/g wet weight liver) adjusted to 2.5 ml with 0.1 M Tris–HCl buffer, pH 7.4.

GT activity in 'native' (non-activated) microsomes was significantly decreased in diabetic male rats compared to controls, but was not affected in either the activated preparation or in female rats (Table 1). 'Native' microsomes from control male rats also showed increased glucuronidation over 'native' microsomes from control female rats ($P < 0.02$), as shown in the table. In all experiments UDPGA was present in excess, so any differences in glucuronidation must be due to alterations in GT enzyme activity. This differs from the results of Dajani & Kayyali (1973) and Schriefers, Ghraf & Pohl (1966), who attributed decreased glucuronidation in alloxan diabetes to a deficiency of UDPGA.

Detergents are thought to alter the microsomal membrane structure so unmasking further active sites on the enzyme, resulting in activation of GT (Graham & Wood, 1972). The absence of any effect when activated microsomes were used suggests that the decreased GT activity in diabetic male rats is due to some alteration in the microsomal membrane structure, rather than a transferase deficiency.

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Table 1 Effect of streptozotocin-induced diabetes on glucuronyl transferase activity (nmoles p-nitrophenol glucuronide formed mg microsomal protein^{-1} min^{-1}.)

	Control	*Streptozotocin treated*	*Control + insulin*	*Streptozotocin treated + insulin*
1. Male rats				
'Native' microsomes	1.24 ± 0.13 (7)	0.61 ± 0.09** (7)	1.07 ± 0.06 (6)	1.18 ± 0.07 (6)
Activated microsomes	5.78 ± 0.31 (7)	5.25 ± 0.54 (7)	5.81 ± 0.10 (6)	5.87 ± 0.30 (6)
2. Female rats				
'Native' microsomes	0.84 ± 0.04 (7)	0.71 ± 0.07 (7)	0.76 ± 0.03 (6)	0.63 ± 0.08* (5)
Activated microsomes	5.14 ± 0.35 (7)	5.53 ± 0.22 (7)	5.45 ± 0.22 (6)	5.40 ± 0.42 (5)

Results are expressed as the mean ± s.e. mean with the number of animals used given in parentheses.
* $P < 0.05$; ** $P < 0.005$, using a non-paired Student's t test.
P values refer to differences between treated and control animals.

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Effects of sotalol on lignocaine pharmacokinetics in rat

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The major determinants of hepatic drug elimination include liver blood flow (LBF), hepatic enzyme activity and drug binding. Two mathematical models have evolved to explain the influence of these factors on hepatic drug extraction, namely the 'well stirred' and the 'parallel tube' models. The predictions of both models have recently been compared (Pang & Rowland 1977a). We have studied the effect of reduction in LBF produced by administration of sotalol on the kinetics of lignocaine, a drug for which LBF is the principal factor governing elimination.

Sotalol (100 mg/kg) was given by intraperitoneal injection to male CFY rats at −24 h, −18 h, −12 h and 0 h. At +15 min 6 rats received lignocaine 5 mg/kg i.v. and 6 received 70 mg/kg p.o.; blood was collected from the tail at appropriate intervals and assayed for lignocaine by GLC. Control rats received the same doses of lignocaine ($n = 8$ per group) but were not given sotalol. LBF was measured using the radioactively labelled microsphere method of McDevitt & Nies (1976).

In 10 rats which received no sotalol LBF was 1.71 ± 0.11 ml min^{-1} g liver^{-1} (mean ± s.e. mean) and in 7 rats which received sotalol LBF was 0.83 ± 0.09 ml min ($P < 0.05$). The area under the blood concentrations time curve after i.v. dosing (AUC_{iv}) was 444.7 ± 48.2 μg ml^{-1} min^{-1} in control rats and 822.8 ± 65.5 μg ml^{-1} min^{-1} in sotalol treated rats. AUC_{po} was 103.1 ± 9.5 μg ml^{-1} min^{-1} in control rats and 208.5 ± 27.8 μg/ml in sotalol treated rats.

The increase in AUC_{iv} with decline in LBF was predicted by both models. Only marginal change was observed in systemic availability (F) of lignocaine whereas both models predict a reduction in F with decline in LBF. The 'well stirred' model predicts no change in AUC_{po} and the 'parallel tube' model predicts a decline in AUC_{po} when LBF is reduced. Thus neither model predicted the observed increase in this parameter.

The present findings in the intact animal are in contrast with data obtained using the rat isolated perfused liver (Shand, Kornhauser & Wilkinson 1975) and the perfused rat liver *in situ* (Pang & Rowland 1976b) which support the assumptions of the 'well stirred' model.

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Intestinal absorption of oestrone sulphate in the rat

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The enterohepatic circulation of endogenous and drug conjugates is generally understood to involve hydrolysis of the conjugates in the gastrointestinal tract, before reabsorption (Plaa, 1975; Duggan & Kwan, 1979). There is evidence, however, that some sulphates may be absorbed intact (Fries, Knapstein, Wendlberger & Oertel, 1967; Eriksson, 1971). The question arises as to whether oestrone sulphate, which is present in several hormone replacement therapy preparations, is absorbed intact or if the gut microflora, as a source of hydrolytic enzymes is involved in the absorption process.

Following intravenous administration of oestrone sulphate (10 μCi/kg; 10 μg/kg) to rats, 74.5% of the dose was excreted in bile in 4 h; the highest rate of excretion being in the first hour (61% of the dose). After injection into the small intestine or caecum, 31.5% and 36.5% respectively of the dose of oestrone sulphate was excreted in bile in 4 h, at a more or less constant rate.

Pretreatment of rats with ampicillin (100 mg kg^{-1} day^{-1} for 4 days orally) caused a partial but significant reduction in the absorption of oestrone sulphate from the small intestine (20.5% of the dose excreted in bile in 4 h) and almost total reduction in the absorption from the caecum (3.8% of the dose excreted in bile in 4 h).

When the absorption of the steroid conjugate was studied *in situ* in isolated segments of small intestine, 19.8% of the dose was recovered from the mesenteric vein draining the segment in 1 hour. Pretreatment of rats with neomycin + lincomycin (100 + 100 mg/kg/day for 4 days) caused a significant reduction in drug absorption to 7.7% of the dose.

In rats given [^{3}H]-oestrone [^{35}S]-sulphate (0.2 μCi [^{3}H] and 0.4 μCi [^{35}S]) orally, it was found that ampicillin pretreatment (as above) caused an increase in the 72 h faecal excretion of [^{35}S] from 6.4% to 44.7% of the dose and a concomitant decrease in urinary excretion from 80.8% to 36.8% of the dose. The sulphur label in faeces was associated with oestrone and indicated that by reducing the gut microflora only part of the dose of oestrone sulphate was absorbed.

When [^{3}H]-oestrone sulphate (0.1 μCi) was incubated with rat faeces for 60 h anaerobically, 89.2% of the conjugate was metabolized to either oestrone (50.1%) or oestradiol (39.1%).

The results of this study suggest that in the small intestine, oestrone sulphate can be partly absorbed intact but also undergoes some hydrolysis. In the caecum, deconjugation by the gut microflora is a prerequisite of absorption.

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The effect of reserpine on plasma corticosterone levels and on the incorporation of [^{3}H]-thymidine into regenerating liver in partially hepatectomized rats

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In rats, partial hepatectomy (PH) is followed by liver regeneration, which restores the liver to its normal size within 20 days (Higgins & Anderson, 1931). During the first 24 h DNA synthesis increases rapidly. This stage is inhibited by reserpine, which has been suggested to act indirectly by stimulating the hypothalamus-pituitary-adrenal axis to release corticosteroids, which may inhibit DNA synthesis in the regenerating liver (Cihák & Vaptzarova, 1973). In contrast, corticosteroids released after PH have been suggested to play a part in initiating liver regeneration (Marotta, Witek-Janusek, Yu, Sithichoke & Garcy, 1978).

The effect of reserpine (1 mg/kg, i.p.) and PH on plasma corticosterone levels and [^{3}H]-thymidine uptake into liver were examined. Male Wistar rats (100–150 g) were anaesthetized with ether and either sham operated or partially hepatectomized (Higgins & Anderson, 1931). Rats were killed at intervals of 1, 2, 5, 10, 15 or 20 days after PH having been injected 1 h before death with the nucleic acid precursor, [^{3}H]-thymidine (50 μCi.5 μmoles^{-1}.0.2 ml^{-1}.100 g body wt^{-1}, i.p.). [^{3}H]-Thymidine incorporation into the acid insoluble fraction of liver was measured (Ashrif, Gillespie & Pollock, 1974), and plasma corticosterone levels were determined fluorimetrically (Zenker & Bernstein, 1958). Reserpine was dissolved in acetic acid and administered 24 h before PH. Acetic acid was used as the control solution.

Following PH, rat livers regenerated to normal size in 20 days. The increase in [^{3}H]-thymidine incorporation was maximal 24 h after PH, when [^{3}H]-thymidine incorporation was higher ($15.7 \times 10^4 \pm 2.4 \times 10^4$ d min^{-1} g liver^{-1}, $n = 10$, $P < 0.001$) than in liver from sham operated ($2.8 \times 10^4 \pm 0.2 \times 10^4$ d min^{-1} g liver^{-1} $n = 5$) or intact rats ($3.0 \times 10^4 \pm 0.4 \times 10^4$ d min^{-1} g liver^{-1}, $n = 7$). PH also increased plasma corticosterone levels (76.3 ± 25.8 μg/100 ml, $n = 8$, $0.05 > P > 0.01$) above those in intact rats (20.5 ± 2.2 μg/100 ml, $n = 5$). This peak of this effect occurred 24 h after PH. Sham operations also increased plasma corticosterone levels (92.9 ± 44.4 μg/100 ml, $n = 4$).

Reserpine reduced [^{3}H]-thymidine incorporation into regenerating liver examined 24 h after PH ($7.5 \times 10^4 \pm 2.4 \times 10^4$ d min^{-1} g liver^{-1}, $n = 7$, $0.05 > P > 0.01$). Reserpine (21.4 ± 3.3 μg/100 ml, $n = 4$) or acetic acid control solution (24.1 ± 5.6 μg/100 ml, $n = 3$) reduced plasma corticosterone levels measured 24 h after PH.

These results show that the inhibitory effect of reserpine on [^{3}H]-thymidine uptake in regenerating liver is not due to an increase in plasma corticosterone levels. They also suggest that corticosteroid levels need not be elevated for regeneration to occur, since the acetic acid control solution also reduced corticosteroid levels but did not affect [^{3}H]-thymidine incorporation into regenerating liver.

J.M.B. is a University of Glasgow Postgraduate Scholar.

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The effect of cycloheximide on endogenous pyrogen fever

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Cycloheximide which is a protein synthesis inhibitor is known to have an antipyretic action, however it is not clear how this effect is brought about (Seigert, Philipp-Dormston, Radsak & Menzel, 1976; Milton & Sawhney, 1980; Stitt, 1980). In the experiments reported here the effect of cycloheximide on circulating endogenous pyrogen (EP) levels has been measured, and in addition the effects of cycloheximide on the febrile response to EP has been investigated.

EP synthesis and release was initiated in conscious Dutch rabbits by intravenously injecting the lypopolysaccharide from *Shigella flexnari* in a dose of 5 μg/kg. The rectal temperature was measured throughout the procedure and the animals were bled out at either the height of the first or second fever peak. The plasma containing circulating EP was prepared from the whole blood by centrifugation. The EP content of the plasma was bioassayed by infusing the plasma (6–9 ml/kg) into recipient rabbits and measuring the Thermal Response Index (TRI) Milton & Wendlandt, (1971). The recipient rabbits had previously been made tolerant to *S. flexnari* by daily injections of the endotoxin (10 μg/kg) for a period of at least 21 days.

The plasma containing EP, whether collected at the height of either the first or the second fever peak produced a monophasic fever response in the recipient animals. No febrile response was seen when plasma from afebrile rabbits was injected into the recipient animals. If the donor rabbits were treated with cycloheximide (5 mg/kg) 90 min before the injection of *S. flexnari* then suppression of the fever response was observed. These animals were bled out at the time when either the first or the second fever peak would have been expected and the plasma prepared as in non-treated animals. When this plasma was assayed for EP the amount present was the same as in non-treated donors, and the recipients produced the same monophasic febrile response. Plasma from cycloheximide treated donors which had not received *S. flexnari* was without effect when administered to recipient animals. If the recipient rabbits tolerant to *S. flexnari* were treated with cycloheximide (5 mg/kg) 90 min before the infusion of plasma from a *S. flexnari* treated donor animal the monophasic response to EP containing plasma did not occur and no febrile response was observed.

The results show that cycloheximide is not exerting its antipyretic action by inhibiting the synthesis of endogenous pyrogen, a low molecular weight protein, as has been suggested by Norland, Root & Wolff (1970), since circulating EP is still present in an animal in which the febrile response to endotoxin has been suppressed by the drug. In addition, the febrile response to EP is also suppressed by cycloheximide indicating that this drug must be exerting its effect at some other site in the fever pathway, perhaps in the central nervous system.

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Comparison of the depressant effects of leucine- and methionine-enkephalin on spontaneous chemoreceptor activity in cats

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There is evidence that methionine-enkephalin (met-ENK) and leucine-enkephalin (leu-ENK) are present in cat carotid body type 1 cells (Lundberg, Hökfelt, Fahrenkrug, Nilsson & Terenius, 1979; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard & Will, 1980). Intra-carotid injection of met-ENK decreases spontaneous chemoreceptor discharge in cats (McQueen, 1979; McQueen & Ribeiro, 1980). The present study was undertaken to investigate the effects of leu-ENK on the carotid chemoreceptors and to compare them with those of met-ENK.

Experiments were performed on cats anaesthetized with pentobarbitone (42 mg/kg i.p., supplemented every 1–2 h). The animals were artificially ventilated with air and paralysed by gallamine (3 mg/kg i.v.). Chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve (McQueen, 1977). In the majority of experiments the ganglioglomerular (sympathetic) nerves were cut. Drug solutions were injected into the ipsilateral common carotid artery (i.c.) over a 2 s period.

Leu-ENK and met-ENK both decreased spontaneous chemoreceptor discharge frequency in a dose-dependent manner, being of similar potency (see Figure 1). The intensity and duration of the depression evoked by both peptides were reduced after naloxone (0.2–0.4 mg i.c.). Similar responses were obtained in all the experiments, regardless of whether or not the sympathetic nerve supply to the carotid body had been cut.

These results suggest that leu-ENK and met-ENK depress spontaneous chemoreceptor discharge by acting on opiate receptors in the carotid body. Since the enkephalins have potent effects on chemoreceptor discharge and are present in the carotid body, they may function there as neurotransmitters or neuromodulators. Further studies are needed to establish their physiological roles in the carotid body and to identify the opiate receptor(s) (Hughes, Kosterlitz, McKnight, Sosa, Lord & Waterfield, 1978).

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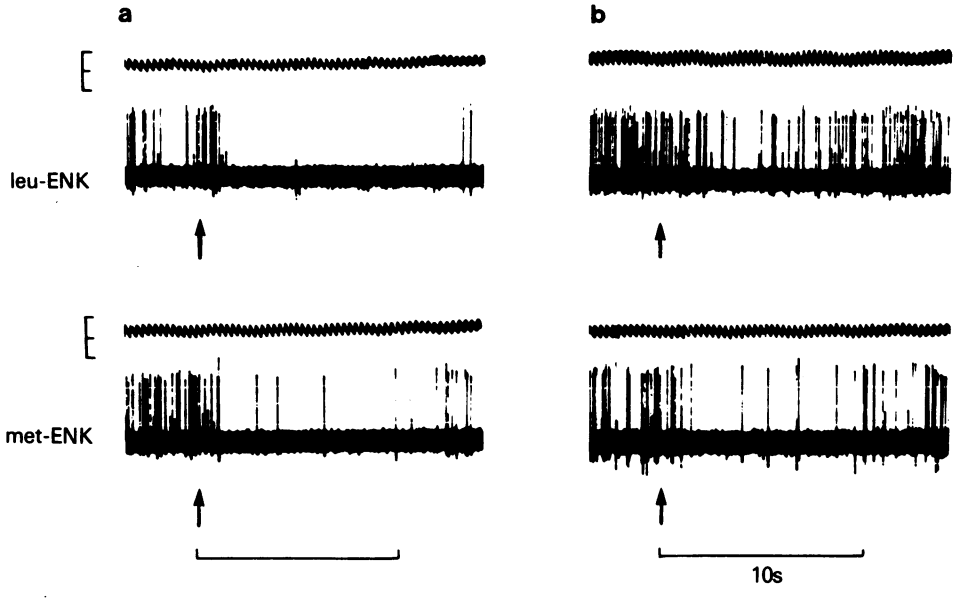

Figure 1 Effects of i.c. injections (arrows) of leu-ENK (10 μg) and met-ENK (10 μg) on spontaneous chemoreceptor activity before (*a*) and after (*b*) naloxone (0.4 mg i.c.)–leu-ENK was injected 5 min after naloxone and met-ENK 10 min later. The upper part of each panel shows the femoral arterial B.P. (calibration 0–100–200 mmHg), and the lower part the neurogram.

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Effects of acetylcholine antagonists on anticholinesterase-induced twitch potentiation in the rat isolated diaphragm preparation

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Anticholinesterase-induced twitch potentiation may arise from: (1) antidromic firing (ADF) in the motor nerve giving rise to repetitive endplate potentials and/or (2) prolongation of single endplate potentials triggering repetitive muscle action potentials. Dithiothreitol (DTT), which reduces the affinity of acetylcholine for the post-junctional cholinoceptor at the neuromuscular junction, preferentially reduces the contribution of mechanism (1) to twitch potentiation produced by the organophosphate anticholinesterase paraoxon (diethyl-4-nitrophenylphosphate) in rat diaphragms (Clark, Hobbiger & Terrar, 1979, 1980). Webb & Bowman (1974) have suggested that ADF involves the action of acetylcholine on prejunctional receptors which can be blocked selectively by hexamethonium but not by pancuronium. We thus compared the effects of these two antagonists in untreated and DTT-treated rat diaphragms on normal twitch and paraoxon-potentiated twitch. The effects of a

Table 1 Effect of acetylcholine antagonists on twitch tension

	Control twitch	*Paraoxon-potentiated twitch*	
	IC_{50} [1]	$IC_{50(a)}$ [2]	$IC_{50(b)}$ [3]
Hexamethonium			
untreated	5.4 ± 0.2 mM	0.6 ± 0.1 mM	5.8 ± 0.3 mM
DTT-treated	1.7 ± 0.1 mM	0.3 ± 0.1 mM	2.2 ± 0.3 mM
Pancuronium			
untreated	4.2 ± 0.2 μM	1.4 ± 0.4 μM	12.3 ± 0.8 μM
DTT-treated	1.9 ± 0.2 μM	0.6 ± 0.1 μM	6.1 ± 1.1 μM
PMCG			
untreated	—	9.9 ± 2.0 μM	202.5 ± 3.8 μM
DTT-treated	—	14.5 ± 5.2 μM	182.5 ± 4.8 μM

Each figure represents mean ± s.e. mean of 4 experiments.
Muscles which had not been treated with DTT (untreated) and muscles which had been treated with 1 mM DTT for 15 min (DTT-treated) were exposed to paraoxon (0.5 μM) for 5 and 10 min respectively and then washed for 10 to 15 min before addition of antagonist.
[1] IC_{50} = concentration of antagonist which reduced the twitch height by 50%.
[2] $IC_{50(a)}$ = concentration of antagonist which reduced the potentiation by 50%.
[3] $IC_{50(b)}$ = concentration of antagonist which reduced the twitch height to 50% of the control (i.e. pre-paraoxon) level.

muscarinic antagonist, N-ethyl-2-pyrrolidylmethyl-cyclopentylphenyl glycollate (PMCG), which reduces anticholinesterase-induced twitch potentiation in cat soleus muscle (Brimblecombe, French & Webb, 1979), were also investigated. Muscles were bathed in Tyrode solution containing 2 mM $CaCl_2$ and 0.1 mM $MgCl_2$ at 37°C and stimulated via the phrenic nerve (0.2 Hz, 200 μsec and supramaximal voltage). The results are summarized in Table 1.

Hexamethonium and pancuronium, in concentrations which had no effect on control twitch height, reduced the paraoxon-induced twitch potentiation and abolished ADF as recorded extracellulary from the phrenic nerve. The ratio $IC_{50(b)}$:$IC_{50(a)}$ for both antagonists was approximately 10:1 (Table 1) and a plot of twtich height against concentration of antagonist failed to show any discontinuity. In DTT-treated muscles, the effectiveness of hexamethonium and pancuronium was enhanced and the ratio $IC_{50(b)}$:$IC_{50(a)}$ for both antagonists was again approximately 10:1. With PMCG the ratio was approximately 20:1 and DTT treatment did not increase its effectiveness.

We conclude that under our experimental conditions, the reduction in twitch potentiation by both hexamethonium and pancuronium can be explained by a postsynaptic action. A comparison of IC_{50} and $IC_{50(b)}$ values for the two drugs reveals differences which may reflect an action additional to cholinoceptor antagonism.

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Effects of sea *Anemonia sulcata* toxin II on normal and chronically denervated mammalian neuromuscular junctions

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Considerable work has been devoted to investigate the action of the polypeptide neurotoxin, *Anemonia sulcata* toxin II on excitable membranes (for review see, Rathmayer, 1979). The present study concerns the effects of *Anemonia sulcata* toxin II (ATX II) on quantal spontaneous transmitter release and on muscle membrane potential on mouse neuromuscular junctions with conventional electrophysiological methods. On the isolated nerve-diaphragm preparation, the toxin (10^{-7} to 3.2×10^{-6} M) induced a dose-dependent increase in miniature end-plate potential (mepp) frequency with a simultaneous depolarization of the muscle membrane. The membrane depolarization was slowly reversible by washing, depending on the concentration of ATX II and on the contact time with the preparation but the increase in mepp frequency did not decline. The two effects of the toxin were also observed in the virtual absence of calcium (no calcium added and 1 mM EGTA in the medium) and were completely suppressed or prevented by tetrodotoxin (TTX) 2×10^{-7} M. The ATX II-induced depolarization of the muscle membrane was not reduced by d-tubocurarine (6 μg/ml). In normal and denervated (20–40 days after denervation) isolated extensor digitorum longus muscles (EDL) ATX II decreased the membrane potential and induced contractile activity; TTX suppressed the contractile activity in both cases. ATX II completely reversed the depolarization in normal muscles but only partially in denervated muscles.

In normal muscles, the TTX-sensitive depolarizing effect of ATX II can be attributed to an increase in the muscle membrane sodium permeability; in denervated muscles, the TTX-resistant depolarizing effect of TTX II may be related to the presence of TTX-insensitive sodium channels described by Redfern & Thesleff (1971) after denervation.

The increase in spontaneous quantal transmitter release induced by ATX II, both in the presence or absence of extracellular calcium, can be explained by the ability of the toxin to increase sodium per-

meability in the nerve terminals via a TTX-sensitive pathway which can lead to an increase in the intracellular calcium concentration.

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Radioligand binding studies using [^{3}H]-cimetidine and [^{3}H]-ranitidine

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The binding of [^{3}H]-cimetidine to rat and guinea-pig brain membrane has been attributed to an interaction with the histamine H_2 receptor (Burkard, 1978; Devoto, Marchisio, Carboni and Spano, 1980). The binding of [N-methyl-^{3}H]-cimetidine (17 Ci/mmol) and a second H_2 receptor antagonist, [^{3}H]-ranitidine (2.4 Ci/mmol) to guinea-pig heart membranes has been compared with their effects on histamine-induced cAMP elevation in the isolated rabbit gastric cell.

Guinea-pig heart membranes were prepared and the radioligand receptor binding studies performed by the method of Barnett, Rugg & Nahorski (1978). Non-specific [^{3}H]-cimetidine binding was determined in the presence of metiamide (10^{-4} M).

Rabbit isolated gastric cells were prepared essentially by the method of Berglindh & Öbrink (1976), the collagenase digestion being extended to 2.5 hours. Histamine (10^{-6}–10^{-3} M) in the presence of the phosphodiesterase inhibitor isobutylmethylxanthine (10^{-4} M) produced a concentration-dependent elevation of cAMP in rabbit isolated gastric cells. The selective histamine H_2 antagonists cimetidine and ranitidine (Bradshaw, Brittain, Clitherow, Daly, Jack, Price & Stables, 1979) both inhibited the histamine (10^{-4} M) induced elevation in gastric cell cAMP giving respectively ID_{50} (mean ± s.e. mean) values of $6.44 \pm 3.4 \times 10^{-6}$ M ($n = 5$) and $2.24 \pm 0.40 \times 10^{-7}$ M ($n = 3$).

Sewing, Ruoff, Gunther, Gladziwa & Frisch (1979) have also shown that ranitidine is more potent than cimetidine as an inhibitor of histamine induced elevation of cAMP in the parietal cell.

Cimetidine, metiamide and impromidine displaced [^{3}H]-cimetidine (9.5–11.0 nmol) from the guinea-pig heart membrane preparation with ID_{50} values of 5.2×10^{-8} M, 2.6×10^{-7} M and 2.8×10^{-7} M respectively. The ID_{50} values for the displacement of [^{3}H]-cimetidine by ranitidine and histamine were respectively 4.8×10^{-4} M and 5.2×10^{-4} M. The 10^{-4} fold difference in ID_{50} values for cimetidine and ranitidine is inconsistent with the cAMP inhibition data and suggests that ranitidine and cimetidine may bind to different sites in the guinea-pig heart membrane preparation.

Ranitidine displaced [^{3}H]-ranitidine from the guinea-pig heart membrane binding site but there was no displacement of [^{3}H]-ranitidine by metiamide, cimetidine, mepyramine and chlorpheniramine at 10^{-4} M concentration.

The membrane bound [^{3}H]-cimetidine was not displaced by prazosin, practolol, isoprenaline or noradrenaline or by the H_1 antagonists chlorpheniramine and mepyramine but phentolamine and tolazoline. which both contain an imidazole moiety, displaced the bound radioligand as described previously (Burkard, 1978). The binding of [^{3}H]-cimetidine to the guinea-pig heart membrane preparation was saturable above 40 nmol. The affinity constant for [^{3}H]-cimetidine compares favourably with those reported previously (Burkard, 1978; Devoto, Marchisio, Carboni & Spano, 1980) but is significantly different from the value of 282 nmol determined pharmacologically (Bradshaw, Brittain, Clitherow, Daly, Jack, Price & Stables, 1979).

One binding site on the membrane appears to be selective for compounds containing the imidazole moiety and another for ranitidine: therefore, radioligand binding studies using the vacuum filtration technique cannot be used to compare the potency of H_2 receptor antagonists of differing chemical structure.

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[^{3}H]-Cimetidine binding in guinea-pig gastric mucosa

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Minimal data have been published concerning radioligand binding studies on the histamine H_2 receptor. However, investigations using [^{3}H]-metiamide (Rosenfeld, Jacobson & Thomson, 1976) and [^{3}H]-cimetidine (Burkard, 1978; Rising, Norris, Warrander & Wood, 1980) suggested that these H_2 antagonists might have a high affinity binding sites unrelated to the pharmacological receptor. In this present communication we describe a study to examine ^{3}H-cimetidine binding compared to the effects on H_2 dependent adenylate cyclase system in membranes prepared from guinea-pig gastric mucosa.

Male Hartley guinea-pigs (400–700 g) were killed and a fundic gastric mucosal particulate preparation prepared by homogenisation and centrifugation. [^{3}H]-cimetidine binding was determined using a rapid filtration assay. Specific and non-specific components of binding were calculated from the difference in binding obtained in the presence and absence of 10 μM metiamide. [^{3}H]-Cimetidine bound saturably to a finite number of sites with an equilibrium dissociation constant (K_D) of 283 ± 67 nM. The K_i values for a variety of known H_2 agonists and antagonists were calculated from displacement curves (Table 1).

Adenylate cyclase activity was measured, in the fundic mucosal preparations, by a modification of the method of Brown, Albano, Ekins & Sgherzi (1971). The K_i values for the antagonists were calculated from inhibition of histamine stimulated adenylate cyclase, and the K_A values of the agonists from their ability to stimulate adenylate cyclase. (Table 1).

It can be concluded from the present study that [^{3}H]-cimetidine binding does not represent labelling of the H_2-receptor. The nature and possible function of the [^{3}H]-cimetidine binding site identified remains to be established.

Table 1

	[^{3}H]-Cimetidine binding	*Adenylate Cyclase Activity*	
	K_I (M)	*K_I or K_A (M)*	*Stimulation relative to histamine*
Cimetidine	$4.0 \pm 1.2 \times 10^{-7}$	$3.9 \pm 0.6 \times 10^{-6}$	—
Metiamide	$3.4 \pm 2.4 \times 10^{-6}$	$1.3 \pm 0.1 \times 10^{-6}$	—
ICI 125,211	$4.3 \pm 0.8 \times 10^{-4}$	$3.9 \pm 1.1 \times 10^{-7}$	—
Ranitidine	$>10^{-3}$	$1.0 \pm 1.6 \times 10^{-6}$	—
Impromidine	$2.0 \pm 0.5 \times 10^{-6}$	$2.0 \pm 0.9 \times 10^{-7}$	1.0
2-Methylhistamine	$1.4 \pm 0.3 \times 10^{-5}$	$3.9 \pm 1.3 \times 10^{-5}$	1.0
4-Methylhistamine	$7.4 \pm 2.3 \times 10^{-5}$	$1.1 \pm 0.8 \times 10^{-5}$	1.0
Histamine	$3.0 \pm 1.3 \times 10^{-4}$	$4.9 \pm 0.8 \times 10^{-6}$	1.0
Dimaprit	$>10^{-3}$	$5.1 \pm 1.6 \times 10^{-6}$	0.8

Compounds were analysed in duplicate on three separate occasions and the values expressed as means ± standard deviation.

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Disodium cromoglycate and mast cell calcium-ATPase

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The allergic response reaction results from an antigen-antibody interaction which causes the release of chemical mediators. Histamine is one of these mediators and it is stored within membrane-bound granules in mast cells. The release of histamine appears to be triggered by calcium ion influx followed by fusion of granules with the mast cell membrane. Since mast cells contain a calcium-activated ATPase (Cooper & Stanworth, 1976), we have determined the effect of the anti-allergy drug disodium cromoglycate (DSCG) on mast cell calcium-ATPase to ascertain if the drug might act by influencing this enzyme.

Mast cells were prepared from rat peritoneal exudates. Following the injection of 10 ml Tyrode solution containing 0.5% bovine serum albumin and 25 i.u. per ml heparin into the peritoneal cavity, the abdomen was massaged and the fluid removed. The exudate was centrifuged at 110 *g* for 2 min in an M.S.E. bench centrifuge and the pellet of cells washed twice and resuspended in Tyrode solution (modified as above). 8.3 ml suspension was layered onto 11.5 ml metrizoate solution (6 vol sodium metrizoate, density 1.200, to 1 vol modified Tyrode solution) and centrifuged at 4°C for 20 mins at 240 *g* using an M.S.E. super 65 centrifuge. The cell pellet was washed in modified Tyrode solution and resuspended in either (i) modified Tyrode solution or (ii) the ATPase assay medium (final vol 1 ml) comprising 50 mM imidazole/HCl buffer, pH 7.8 containing calcium in the required concentration, and Tris ATP (4 mM). ATPase activity was measured over a 45 min period at 37°C. The reaction was stopped with 1 ml 0.8% sodium lauryl sulphate and the phosphate released estimated by the method of Baginski, Foa & Zak (1967).

The structural and functional properties of the purified mast cells were assessed by light microscopy, trypan-blue exclusion and histamine-release studies.

The cells exhibited calcium-ATPase activity which increased as the calcium concentration of the medium was increased up to 8 mM. DSCG at concentrations from 0.1 to 10 μg/ml did not significantly influence the activity of the enzyme in the presence of 4 mM or 8 mM calcium. The drug (1 μg/ml and 3 μg/ml) also failed to influence the activity of the enzyme as the calcium concentration of the medium was changed between 1 and 10 mM. In the presence of vanadium-free Tris ATP, DSCG (3.0 μg/ml) increased the Ca-ATPase activity of the mast cells by 36%.

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