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Facilitatory action of 4-aminopyridine on transmitter release from sympathetic nerve terminals in rabbit blood vessels.

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4-Aminopyridine (4-AP) blocks the potassium channel in a variety of excitable tissues and thereby enhances the influx of Ca^{2+} during depolarization of nerve terminals (see Thesleff, 1980). In this study the effect of 4-AP on the overflow of tritium evoked by electrical-field stimulation, nicotine, K^+ and tyramine was studied on the rabbit isolated pulmonary artery and aorta pre-loaded with (–)-[7-[^{3}H](N)]-noradrenaline ([^{3}H]-NA; New England Nuclear Corporation). The methods described in detail (Nedergaard, 1980) were used.

4-AP ($10^{-5} - 10^{-3}$ M) enhanced the electrical stimulation-evoked [^{3}H]-overflow from pulmonary artery up to 912% of control. 3,4-Diaminopyridine (10^{-4} and 10^{-3} M) was equieffective with 4-AP, while the quaternary analogue of 4-AP, 4-aminopyridine methiodide (10^{-6} and 10^{-5} M) was much less so. The enhancing effect of 4-AP (10^{-4} M) was dependent on the frequency (1–30 Hz) of stimulation (300 pulses). At 1–10 Hz, the [^{3}H]-overflow increased about 4–5 fold; at 30 Hz, the enhancement declined to about 1.5-fold. 4-AP (10^{-4} M) enhanced [^{3}H]-overflow about 4–5 fold independent of number of pulses (30–1000) in each stimulus delivered at a low frequency (3 Hz).

Electrical-field stimulation of rabbit arteries preloaded with [^{3}H]-NA induces release of tritium from both neuronal and extraneuronal sites (Schrold & Nedergaard, 1976; 1977). Using a Ca^{2+}-free physiological salt solution, the small stimulation-evoked [^{3}H]-overflow from pulmonary artery which probably represents release from extraneuronal sites, was slightly enhanced by 4-AP (10^{-4} M).

The effect of 4-AP on the pattern of [^{3}H]-NA and its [^{3}H]-metabolites evoked by field stimulation was examined. The overflow from untreated artery *during* stimulation consisted of [^{3}H]-NA (36%), [^{3}H]-DOPEG (13%), [^{3}H]-DOMA (3%), [^{3}H]-OMDA (36%), and [^{3}H]-NMN (2%). 4-AP ($10^{-4} - 10^{-3}$ M) in a concentration-dependent manner enhanced [^{3}H]-NA and correspondingly decreased [^{3}H]-OMDA, [^{3}H]-DOPEG and [^{3}H]-NMN.

The ability of various drugs to influence the enhancing effect of 4-AP (10^{-4} M) on stimulation-evoked [^{3}H]-overflow from pulmonary artery was studied. The enhancement was increased markedly by cocaine (3×10^{-5} M) and by cocaine (3×10^{-5} M) plus corticosterone (4×10^{-5} M), while it was not altered by corticosterone (4×10^{-5} M). Neither hexamethonium (10^{-5} M) nor the prostaglandin-synthetase inhibitor, suprofen (3×10^{-5} M) interfered with the enhancing effect of 4-AP (10^{-4} M).

The [^{3}H]-overflow from aorta elicited by either tyramine (3×10^{-7} and 10^{-5} M) or K^+ (60 mM) was not altered by 4-AP (10^{-4} M). In contrast, the [^{3}H]-overflow evoked by nicotine (10^{-4} M) was reduced by 4-AP (10^{-4} M).

The ability of 4-AP ($10^{-6} - 10^{-4}$ M), desmethylimipramine ($3 \times 10^{-9} - 3 \times 10^{-4}$ M), and cocaine ($10^{-8} - 3 \times 10^{-4}$ M) to reduce the neuronal accumulation of [^{3}H]-NA by aorta was examined. Aorta was treated with pargyline (5×10^{-4} M) and U-0521 (3′,4′-dihydroxy-2-methylpropiophenone; 10^{-4} M) in order to inhibit monoamine oxidase and catechol-O-methyltransferase, respectively. The accumulation was markedly inhibited by desmethylimipramine and cocaine, but not altered by 4-AP.

It is concluded that 4-AP enhances the stimulation-evoked release of noradrenaline by an action which does not involve the neuronal re-uptake mechanism (uptake-1). Furthermore, the enhancement is not mediated via nicotine receptors and is prostaglandin-independent. The results are consistent with the view that 4-AP acts by increasing the intraneuronal concentration of Ca^{2+} and thereby facilitates stimulation-evoked transmitter release from sympathetic neurones. It is unlikely that 4-AP can replace Ca^{2+} in nerve terminals.

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A comparison of the effect of azepexole (B-HT 933) and clonidine on preganglionic sympathetic nerve activity of the cat

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Azepexole has been classified as a hypotensive agent of the 'clonidine type' in spite of its different molecular structure (Kobinger & Pichler, 1977). This suggests that azepexole causes its hypotensive effect by reducing sympathetic tone by a central action on the medulla and/or, by stimulating α_2 adrenoceptors on sympathetic nerve terminals. In the case of clonidine, *in vivo* experiments have been unable to demonstrate a peripheral presynaptic action at doses that cause a powerful central sympatho-inhibitory effect and large falls in blood pressure (Haeusler, 1976). However, a presynaptic action may be easier to demonstrate for azepexole as it is considered to be selective for α_2 adrenoceptors while clonidine is a powerful agonist at both α_1 and α_2 adrenoceptors (Timmermans & Van Zwieten, 1980). Therefore a comparison was made between the effects of

of infusions of low doses of azepexole and clonidine on preganglionic sympathetic activity.

Cats were anaesthetized with a mixture of α-chloralose (70 mg/kg i.v.) and pentobarbitone (12 mg). Atropine (1 mg i.v.) was also given, the animals vagotomized and artificially ventilated after paralysis with gallamine. A pneumothorax was performed and blood gases monitored. Simultaneous recordings were made of blood pressure, heart rate, femoral blood flow (from which conductance was derived) and sympathetic nerve activity. Sympathetic activity was recorded from filaments of the third or fourth thoracic communicans. The fibres used were those that responded to carotid occlusion and showed a reflex increase in activity after an injection of trimetaphan. Drugs were given by single injection into the jugular vein or by slow infusion into the brachial vein.

In two groups of three animals an infusion of clonidine (total dose 12 μg/kg) or azepexole (total dose 135 μg/kg) over a 3 h period caused similar falls in BP of approximately 18%. During this period sympathetic acitivity in the clonidine group declined by 77% while in the azepexole group there was an equivalent increase (73%). Further, azepexole caused only a 7% reduction, while clonidine caused a 30% reduction, in heart rate. Both caused a decrease in femoral arterial conductance. Saline infused (4 ml $kg^{-1}h^{-1}$) over the same period in three animals caused little change in BP and heart rate but an increase in sympathetic activity (27%) with a decrease in femoral arterial conductance. The response elicited by carotid occlusion, at hourly intervals during the azepexole infusion, showed no gross changes. However, with clonidine there was a large reduction in this response, due to the failure of the occlusion to elicit a rise in sympathetic activity by the end of the infusion. Injections of large doses of clonidine (30 μg/kg) or azepexole (300 μg/kg) caused a similar biphasic effect on BP with a large reduction in sympathetic activity. Azepexole differed from clonidine in that its effect returned to normal within 45 minutes.

These results suggest that the hypotensive effect of azepexole is mainly due to a peripheral action, probably on presynaptic α_2 receptors.

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On the nature of the catecholamine receptor mechanisms mediating relaxation and contraction of circular smooth muscle of guinea-pig stomach

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Both noradrenaline and dopamine have dual actions to cause relaxation and contraction of the circular smooth muscle strip obtained from the body of the guinea-pig stomach. A preliminary analysis of the receptor types involved showed the relaxant effects to be antagonized by both phentolamine and propranolol which would indicate both α- and β-adrenoceptor involvement (Costall, Naylor & Sahyoun, 1980). However, the nature of the receptor(s) mediating contraction is less clear and was therefore the subject of present experiments.

Male Dunkin-Hartley guinea-pigs, 350–400 g, were killed by cervical trans-section and smooth muscle strips (15 mm × 5 mm) isolated from the body region of the stomach. The strips were dissected in a plane allowing investigation of tension changes in the circular muscle layer. The mucosal layer was removed and the tissue bathed in 15 ml oxygenated (95%, O_2, 5% CO_2) Krebs-Henseleit solution at 37°C containing 100 mg/l ascorbic acid. Propranolol (5×10^{-7} M, a dose shown to abolish isoprenaline relaxation) was

routinely included in the Krebs solution to eliminate the β-mediated relaxation responses to noradrenaline and dopamine. Tension changes were detected by Grass tension transducers and the response areas integrated (Illingworth & Naylor, 1980) in addition to display on a multichannel Grass recorder. One gram tension was applied to the tissue which was allowed to equilibrate for 30–45 min before the addition of drugs.

Dopamine and noradrenaline ($4.3 \times 10^{-7} - 4.3 \times 10^{-5}$ M and $3.9 \times 10^{-8} - 3.9 \times 10^{-7}$ M respectively) caused contractions of the body strip, whilst relaxations were recorded at the respective concentrations of $5 \times 10^{-5} - 10^{-3}$ M and $5 \times 10^{-7} - 10^{-5}$ M; relaxation was also recorded for phenylephirine ($10^{-7} - 10^{-5}$ M). Prazosin ($10^{-9} - 10^{-7}$ M) inhibited the relaxation caused by noradrenaline, dopamine and phenylephrine (IC_{50}: 10^{-8} M) but failed to affect the contraction responses. In contrast, yohimbine ($10^{-8} - 10^{-5}$ M) and rauwolscine ($10^{-9} - 10^{-5}$ M) inhibited the contraction responses (IC_{50}: 10^{-7}M and 3×10^{-8} M respectively) to both noradrenaline and dopamine with only a small reduction, approximately 10% at 10^{-5} M, in the dopamine, noradrenaline and phenylephrine induced relaxations. Phentolamine ($10^{-8} - 10^{-5}$ M) antagonized both the relaxation and contraction to both noradrenaline and dopamine (with some preference for the contraction response IC_{50}: contraction 8×10^{-8} M, relaxation 6×10^{-7} M), and the relaxant effects of phenylephrine. Haloperidol, domperidone and spiroperidol caused concentration related and selective antagonism of the relaxation responses (IC_{50}: 5×10^{-7} M, 10^{-6} M, 4×10^{-6} M respectively), to noradrenaline, dopamine and phenylephrine. Metoclopramide ($10^{-8} - 10^{-5}$ M) was ineffective against either response. Clonidine ($3 \times 10^{-8} - 3 \times 10^{-6}$ M) caused contractions of the body strip (relaxation was not observed) which were inhibited by yohimbine, rauwolscine and phentolamine but resistant to prazosin, haloperidol, domperidone and metoclopramide.

Thus, classical neuroleptic receptors do not appear to be involved in either the mediation of relaxation (dopamine, noradrenaline and phenylephrine antagonized alike, indicating α_1 action) or contraction (resistant to neuroleptics) of the body strip of stomach. The data would indicate that the mechanisms of relaxation are β and α_1 whilst those of contraction are of the α_2-adrenoceptor type.

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Hyperreactivity of guinea-pig isolated airway smooth muscle

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The airways of asthmatic individuals are hyperreactive in that they respond with greater than normal constrictions to stimuli of various kinds. The mechanism underlying this hyperreactivity is unknown. The airway smooth muscle of ovalbumin-sensitized guinea-pigs possesses Ca^{2+} binding characteristics that are markedly different from normal (Martorana & Rodger, 1980), and we were interested to test whether this might be a factor in any increased reactivity to autacoids that airways smooth muscle from sensitized guinea-pigs might exhibit. Since the airways of the guinea-pigs are especially sensitive to histamine, which is one of the principal mediators of anaphylaxis in this species, we chose to study this autacoid in relation to calcium ion sensitivity.

Single open-ringed preparations of tracheae taken from normal and from post-anaphylactic (ovalbumin-sensitized) guinea-pigs were suspended in Krebs-Henseleit solution (KHS) at 37°C and gassed with 5% CO_2 in O_2. After a 60 min equilibration period, the tissues were made to contract with a concentration of histamine that induced either a 25% (1×10^{-6} M; EC_{25}) or a 90% (2.5×10^{-5} M; EC_{90}) maximal response. The tissues were then incubated in Ca^{2+}-free KHS until histamine (EC_{90} or EC_{25}) failed to elicit a contraction. Cumulative concentration-effect curves to Ca^{2+} ions were then constructed in the presence of either the EC_{25} or EC_{90} histamine.

Ca^{2+} ions (1×10^{-5} to 5×10^{-3} M) produced concentration-related contractions in both normal and

post-anaphylactic tissues. The resulting concentration-effect curves for the two types of tissue were not parallel. The post-anaphylactic airways were always more sensitive to Ca^{2+} ions over the initial two-thirds of the concentration-effect curve, although there was no significant difference between the maximal contractions of each tissue. In the presence of EC_{90} histamine, the EC_{50} values for Ca^{2+} ions calculated from the mean curves were 9×10^{-5} M ($n = 22$) in post-anaphylactic tissues and 2×10^{-4} M ($n = 14$) in normal airways. This represents a 2.2-fold separation between the concentration-effect curves when calculated at the 50% maximum effect level. At the 25% level, the separation between the curves was slightly, but statistically significantly ($P < 0.05$), greater, being 2.8-fold. Similar results were obtained in the presence of EC_{25} histamine.

Verapamil (1×10^{-8} M to 1×10^{-5} M) produced concentration-related inhibitions of the Ca^{2+} ion-induced contractions. There was no significant difference between its effects on post-anaphylactic and normal tissue.

These results provide a possible explanation of airway post-anaphylactic hyperreactivity based upon an increased sensitivity of the smooth muscle cells to extracellular Ca^{2+} ions. Whether this increased sensitivity reflects a generalized increase in the membrane permeability to Ca^{2+} ions or an enhancement of the membrane Ca^{2+}-transport system, or both, remains to be determined.

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An erythrocyte-associated antagonist of inhibitory mechanisms in the bovine retractor penis muscle

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We have recently shown that the smooth muscle inhibitory factor that can be extracted from the bovine retractor penis muscle is inactivated by contact with blood (Bowman, Gillespie & Martin, 1981). The experiments reported here arose as part of a continuing study of this inactivation mechanism.

Relaxations of the bovine isolated retractor penis muscle elicited either by field stimulation or by the inhibitory factor were blocked by blood (2–5 μl/ml). Washed intact erythrocytes resuspended in phosphate buffered isotonic saline were also effective, possibly by virtue of the fact that they were partially haemolysed in the organ bath. Plasma was almost devoid of activity. Haemolysis of the erythrocytes in 20 volumes of dilute phosphate buffer (pH 7.35, 20 milliosmoles) augmented the antagonistic potency. When the haemolysed erythrocytes were centrifuged at 20,000 *g* for 40 min to remove the erythrocyte ghost membranes, the antagonistic activity remained in the supernatant; the ghosts themselves were virtually ineffective. Further studies of the properties of the antagonistic substance were made using the 20,000 *g* supernatant of the haemolysed cells, subsequently referred to as the haemolysate. The antagonistic substance passed through a molecular filter that retained particles of 300,000 daltons or more, but it did not pass through a filter retaining particles of more than 50,000 daltons, and it was inactivated by boiling. At present time we have not been able to distinguish the antagonistic substance from haemoglobin.

In addition to blocking the effects of nerve stimulation and of inhibitory factor on the bovine retractor penis, the haemolysate in a concentration of 10–40 μl/ml (equivalent to 0.5–2 μl/ml of original blood) also blocked inhibitory responses to sodium nitroprusside (5×10^{-9}–5×10^{-8} M), to isobutylmethylxanthine (IBMX, 5×10^{-7}–5×10^{-6} M) and, though less effectively, to isoprenaline (3×10^{-9}–3×10^{-8} M). In contrast, inhibitory responses of the bovine retractor penis to prostaglandin E_1 ($1–3 \times 10^{-7}$ M) were unaffected by haemolysate. The antagonistic action of the haemolysate was accompanied by a rise in the background tone of the bovine retractor penis. However, we are disinclined to attribute the antagonism of inhibitory mechanisms produced by haemolysate simply to a physiological antagonism, since relaxations produced by PGE_1 were totally unaffected. Furthermore, haemolysate continued to produce a complete block of responses to inhibitory nerve stimulation and to inhibitory factor, even when the control level of background tone had been restored by excess IBMX.

The haemolysate produced related effects on the rabbit isolated aorta in which spasm was produced by

noradrenaline: although the background tone was not increased, the effect of added noradrenaline was enhanced, and the relaxant effects of the inhibitory factor and of sodium nitroprusside were blocked. Preliminary experiments in the guinea-pig isolated taenia coli show that its responses to inhibitory nerve stimulation and to ATP are unaffected by haemolysate.

We tentatively conclude that erythrocytes contain a high molecular weight material which is released by haemolysis; it may in fact be haemoglobin. This material potentiates noradrenaline in its motor effects, and blocks a step that is common to the inhibitory mechanisms evoked by some, but not by all, relaxant substances.

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A simple method for estimation of the negative feedback control of noradrenaline (NA) release from sympathetic nerves in rat vas deferens

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The response of the whole vas deferens of the rat to single pulse stimulation consists of a biphasic contraction, the peaks occurring at approximately 250 ms and 650 ms (McGrath, 1978). These peaks can be separated by division of the tissue into two portions (referred to as the prostatic and epididymal portions) which produce dominant peaks at 250 ms and 650 ms respectively, although vestigial peaks of the other portion are detectable in each. The transmitter responsible for the epididymal (650 ms) twitch appears to be noradrenaline (NA), since it is antagonized by α_1-receptor antagonists and is absent in vasa from reserpinized rats. The transmitter involved in the prostatic (250 ms) twitch is almost certainly non-adrenergic since it is resistant to α_1-blockers and is still present in reserpinized tissues.

Measurement of the responses of the epididymal portion to single pulse stimulation of the motor nerves is complicated by the initial twitch due to remnants of the prostatic portion. However, this initial twitch, as well as the dominant twitch of the prostatic portion, can be preferentially inhibited by concentrations of nifedipine which have negligible effects on the dominant contraction in the epididymal portion (French & Scott, 1981).

In order to assess the extent of negative feedback activity caused by interaction of nerve-released noradrenaline with pre-synaptic α_2-receptors, epididymal portions of rat vas deferens (60% of the total length measured from the epididymis) were set up in a tissue bath containing 50 ml Krebs-Henseleit solution with 5×10^{-6} M nifedipine. The tissues were stimulated every 5 min with twin pulses (each 1 ms, supramaximal voltage) delivered via two platinum wire electrodes running parallel to the tissue. The interval between each pulse was varied from 2 s to 10 s in steps of 2 seconds. In all tissues investigated, the response to the second pulse was always smaller than that to the first pulse, suggesting autoinhibition of noradrenaline release. The ratio of the second twitch to the first twitch increased as the pulse interval was increased: i.e. a 2 s interval resulted in a ratio of 0.21 ± 0.03 ($n = 10$), whereas a 10 s interval gave a ratio of 0.47 ± 0.03 ($n = 10$). The ratio did not approach unity until the pulse interval was at least 60 seconds.

In the presence of the neuronal uptake inhibitor, cocaine, at concentrations between 5×10^{-8} M and 5×10^{-7} M, the ratios at each pulse interval were considerably reduced, while yohimbine, which possesses α_2-receptor antagonistic activity greatly increased the ratios at all pulse intervals.

These results indicate that while depression of the second response to twin pulse stimulation may not be due entirely to negative feedback mechanisms, the fact that inhibition of uptake by cocaine reduces the ratio suggests that the increased concentration of noradrenaline at the synapse causes increased negative feedback: on the other hand, blockade of the α_2-receptors by yohimbine reduces the effects of noradrenaline on the feedback mechanism, and thus increases the ratio of the two responses.

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Feedback regulation of the release of the non-adrenergic transmitter in rat vas deferens

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The release of noradrenaline from sympathetic nerves is modulated by a negative feedback mechanism involving interaction between released noradrenaline and presynaptic (α_2) adrenoceptors (see review by Starke, 1977). In the rat vas deferens, motor nerve activity may involve the release of two transmitter substances: at the epididymal end of the tissue, transmission is almost certainly adrenergic, being antagonized by α_1 receptor antagonists, and by pretreatment of the rat with reserpine. Activation of the prostatic end of the tissue does not appear to be noradrenergic since responses to stimulation of the motor nerves is resistant to both α_1 receptor blockade and is present after reserpine treatment (McGrath, 1978). Ambache & Aboo Zar (1971) suggested that in guinea-pig vas deferens, released noradrenaline may modulate not only its own release, but also that of the other transmitter. The results presented in this paper support the above suggestion.

Sections of vasa deferentia approximately 1.5–2 cm long were removed from the prostatic end of the tissue and suspended under 0.5 g tension in a tissue bath containing 100 ml of Krebs-Henseleit solution at 38–39°C. The motor nerves were stimulated every 5 min with twin pulses (1 ms, supramaximal voltage) via platinum wire electrodes parallel with the tissue. The interval between the twin pulses was varied from 2–10 s, in steps of 2 seconds. In all tissues, the response to the second pulse was invariably smaller than that to the first pulse at all pulse intervals used. This was considered to be due to feedback inhibition of transmitter release. The ratio of the second response to the first was independent of the interval between pulses, being 0.88 ± 0.01 ($n = 16$) at 2 s and 0.91 ± 0.01 ($n = 16$) at 10 seconds. These results are at variance with the ratios obtained in the epididymal end of the tissue, where the degree of inhibition of the second twitch was inversely related to the pulse interval (French & Scott, 1981).

In the presence of the α_2 antagonist yohimbine, the ratios were increased, and reached unity at concentrations of 1×10^{-7} M. Inhibition of neuronal uptake of noradrenaline by cocaine ($1–5 \times 10^{-7}$ M) caused a reduction in the ratios, especially at short pulse intervals eg. in the presence of 5×10^{-7} M cocaine, the ratio at a pulse interval of 2 s was 0.47 ± 0.02 ($n = 6$), whereas with a 10 s pulse interval the ratio was identical with control values (0.91 ± 0.02 ($n = 6$). This effect of cocaine could be antagonized in a dose-related manner by concentrations of 5×10^{-9} M–5×10^{-7} M yohimbine. These results would suggest that noradrenaline can modulate the release of the other transmitter, via activation of α_2 receptors. In vasa from reserpinized rats, evidence for negative feedback was observed only when the pulse interval was 2 s (ratio 0.89 ± 0.01, $n = 10$): however the ratios were greater than unity at all other pulse intervals.

In conclusion, these results do indeed suggest that noradrenaline released from adrenergic nerves does exert a modulatory action on the release of the unknown transmitter, although the result from reserpinized animals may indicate some degree of autoinhibition, especially within 2 s of its release.

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On the structural and functional integrity of autonomic nerves in rats with long-term diabetes mellitus induced by alloxan

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Various workers have reported abnormal responses to autonomic transmitters in chemically diabetic animals (Brody & Dixon, 1964; Foy & Lucas, 1975; Owen & Carrier, 1979), but a correlated structural and functional examination of the autonomic innervation of effector organs which exhibit dysfunction in human diabetes has not been performed.

Male Wistar rats (180–200 g) were given alloxan monohydrate (50 mg/kg^{-1} i.v.) and control animals received an equal volume of saline. Five days after injection and at monthly intervals thereafter 24 h urines were collected. Diabetic animals were characterized by 24 h urine volumes >60 ml and glucose outputs >5 g/24 h.

All animals were sacrificed 7 to 8 months after alloxan treatment. At this time all diabetic rats showed postprandial blood glucose values >15 m M. Right and left atria and vasa deferentia were removed and placed in cold Krebs' solution (see Tomlinson, 1979 for composition). Segments of the left vas and a small piece of the ventral portion of the right atrium were fixed for electron microscopy by the method of Tranzer & Richards (1976). The remainder of the right atria, the left atria and the right vasa were suspended between parallel platinum wire electrodes in organ baths containing Krebs' solution (see Tomlinson, 1979 for details). Left atria were driven at 3 Hz with 10 V, 5 ms pulses. Increasing the voltage to 100 stimulated intramural nerves, thus cholinergic nerves were studied in the presence of propranolol (2×10^{-6} M) and noradrenergic nerves in the presence of atropine (1×10^{-6} M). This gave changes in force of contraction at constant frequency. Right atrial noradrenergic nerves were stimulated in the same fashion to obtain tachycardias. Concentration/effect plots for noradrenaline and acetylcholine for inotropy (left atria) and chronotropy (right atria; noradrenaline only) were also obtained. The right vas was employed to study the response to field stimulation of noradrenergic nerves (200 μs, 140 V pulses for 15 s) and to exogenous noradrenaline.

There was no significant difference between diabetic and non-diabetic rats in the responses of right and left atria and vasa deferentia to stimulation of noradrenergic nerves or to exogenous noradrenaline. Stimulation of cholinergic nerves in left atria of control rats reduced the force of contraction from 3.52 ± 0.27 (mean $\pm$ s.e. mean) mNewtons to 1.51 ± 0.11 mNewtons ($n = 5$ rats; $P<0.001$ by paired t-test). This negative inotropic response was absent in the diabetic rats ($n = 5$). The left atria from diabetic rats were also supersensitive to acetylcholine ($EC_{50} = 1.8 \pm 1.0 \times 10^{-8}$ M; $n = 5$) when compared with control atria ($EC_{50} = 7.2 \pm 1.8 \times 10^{-8}$ M; $n = 5$; $P<0.05$).

Cholinergic terminals were not found in the right atria of diabetic rats in spite of a thorough ultrastructural examination of sections from 5 rats. Some sympathetic terminals were swollen and vacuolated. In the vasa from diabetic rats many noradrenergic terminals were swollen and degenerate but large numbers of terminals of normal appearance were also present.

The findings indicate that rats with alloxan-induced diabetes for 7–8 months exhibit neuropathy of the cardiac vagus. Ultrastructural signs of pathology of noradrenergic terminals were also found, but these did not confer overt dysfunction.

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Analysis of the autonomic neural control of heart rate in awake and anaesthetized dogs

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Intravenous induction of anaesthesia with thiopentone or minaxolone has been shown to elicit a tachycardia in both cats and dogs (Davis, Dodds, Dolamore, Gardner, Sawyer, Twissell & Vallance, 1979; Twissell & Dodds 1979). As the autonomic nervous system exerts important regulatory effects on heart rate, experiments were performed on dogs to determine quantitatively the contributions of the sympathetic and parasympathetic divisions of the autonomic nervous system to the control of heart rate in the resting awake state, and during anaesthesia produced by minaxolone (2.5 mg/kg i.v.) and thiopentone (24 mg/kg i.v.).

The dogs were prepared for recording aortic pressure, cardiac output, left ventricular pressure and electrocardiogram in the conscious animal using the method described by Twissell & Dodds (1979). Each dog received each anaesthetic on four occasions; firstly without pre-treatment, and subsequently 20 min after treatment with methylatropine (0.5 mg/kg i.v.), (±) propanolol (0.5 mg/kg i.v.) or methylatropine and (±) propranolol, with 2–3 day intervals between anaesthetics.

Drug-induced changes in heart rate were analysed employing the 'multiplicative' model of Cavero, Riggenbach, Wall & Gerold (1976) and Gerold, Cavero, Riggenbach, Wall & Haeusler (1976), which permits quantitative assessment of the contributions of both autonomic divisions. In this model the heart rate of the resting conscious animal is defined as the product of the intrinsic heart rate (heart rate following blockade of both sympathetic and parasympathetic influences) and three factors S, V and W, where S and V represent the sympathetic and parasympathetic contributions to heart rate, and W represents any sympathetic-parasympathetic interaction.

Resting heart rate of the conscious dogs before minaxolone administration was 83 ± 10.3 beats/min (mean ± s.e. mean). Following methylatropine heart rate increased to 246 ± 14.2 beats/min, whereas after (±) propranolol it decreased to 70 ± 2.6 beats/min. After blockade with both methylatropine and (±) propranolol, intrinsic heart rate was 189 ± 21.4 beats/min. Resting heart rate was therefore attributable to a marked vagal inhibitory action and a smaller sympathetic accelerator influence, with each division operating independently.

Heart rates decreased after injection of minaxolone when the dogs had been pre-treated with methylatropine, or methylatropine and (±) propranolol, but increased when they had been pre-treated with (±) propranolol alone. Analysis of the chronotropic responses indicated that during minaxolone anaesthesia the parasympathetic influence was reduced (values of %V decreased from −62% before injection to −14% 1 min after injection, $P < 0.05$, paired t-test), and remained significantly lower for at least 30 min; whereas there were no statistically significant changes in the values of S or W. The tachycardia evoked by minaxolone is therefore largely consequent upon the withdrawal of the tonic vagal inhibitory influence normally affecting heart rate in the conscious resting dog. The reduction of heart rate produced by minaxolone after combined autonomic blockade arises from a direct action on the pacemaker.

Similar results were obtained after thiopentone administration. Withdrawal of vagal tone has been reported previously following barbiturate anaesthesia (Greisheimer, 1963; Price, 1960). The mechanism, often referred to as a 'vagolytic' action, is probably not the result of impairment of peripheral parasympathetic function, but a central depressant effect of these anaesthetic drugs.

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The selectivity of yohimbine diastereoisomers for α_1- and α_2-adrenoceptors in the anaesthetized dog

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Yohimbine and its isomers have been shown to be selective for presynaptic α_2- (yohimbine and rauwolscine) or postsynaptic α_1- (corynanthine), adrenoceptors *in vitro* (Weitzell, Tanaka & Starke, 1979). Due to their similar physico-chemical properties, these compounds are useful for analysis ofα-adrenoceptor subtypes in experimental preparations. Since we have recently shown that *in vitro* selectivity data is not always reflected *in vivo* (Langer, Massingham & Shepperson, 1980), we have studied the selectivities of these compounds in an anaesthetized dog preparation.

The preparation used has been previously described to the Society (Langer *et al.*, 1980) but briefly, it uses blockade of the pressor response to intravenous phenylephrine as the index of postsynaptic α-antagonism, and reversal of the inhibition induced by clonidine of neural tachycardia at the level of the heart, as the index of presynaptic α-adrenoceptor effects.

All three isomers potentiated the response to stimulation of the ansa subclavia *per se*. Yohimbine was the most potent isomer in this respect, the threshold dose producing this effect being 10 μg/kg, that for rauwolscine being 30 μg/kg, corynanthine was much less potent, the threshold dose being 300 μg/kg.

Clonidine (15 μg/kg + 2 μg kg^{-1} h^{-1}) inhibited the tachycardia produced by stimulation of the ansa subclavia in a frequency dependent manner, inhibition being greatest at 0.25 Hz. Both yohimbine and rauwolscine reversed the clonidine-induced inhibition at a dose of 3 μg/kg and totally blocked clonidine's effect in doses greater than 30 μg/kg. Both drugs at doses of 30 μg/kg potentiated the responses to ansa subclavia stimulation at 0.5 and 1 Hz above control levels. Corynanthine significantly reversed the effect of clonidine at a dose of 1,000 μg/kg and totally blocked its effects at 3,000 μg/kg. Post-synaptically, all three compounds were approximately equipotent, inhibiting the pressor responses to phenylephrine at a threshold dose of about 100 μg/kg.

The similarity in the postsynaptic blocking effect of the isomers was also seen in anaesthetized dogs prepared for iv injections of drugs and blood pressure measurement only. These animals were ganglion blocked (chlorisondamine 1 mg/kg, atropine 1 mg/kg) and β-blocked (propranolol 0.5 mg/kg), and artificially respired with room air using a Bird Mark 7 or 8 pump. In this preparation the ID_{50} for all the antagonists in inhibiting the mean pressor response to phenylehrine was approximately 300 μg/kg.

These results show *in vivo*, as in the *in vitro* studies of Weitzell *et al.* (1979), that yohimbine and rauwolscine are preferential presynaptic α_2-adrenoceptor antagonists, whilst corynanthine behaves as a preferential postsynaptic α_1-adrenoceptor antagonist. Yohimbine and rauwolscine had the greatest selectivity ratio being approximately 33 fold more potent at α_2-adrenoceptors, whilst corynanthine was 10 fold more potent at α_1- than α_2-adrenoceptors. The most striking feature of these results is that all three drugs blocked vascular smooth muscle α_1-adrenoceptors at similar dose levels. This parallels *in vitro* findings which showed only a five fold difference in the Kb values of the antagonists on postsynaptic α_1-adrenoceptors. Therefore the selectivity of these compounds is entirely due to their relative potencies at α_2-adrenoceptor sites. In conclusion, the yohimbine isomers can only be used experimentally to define α_2-adrenoceptors and not α_1-adrenoceptors, since they possess similar affinities for the latter receptor site.

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The effects of chronic (±) propranolol treatment on blood pressure and tissue reactivity in spontaneously hypertensive rats

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The introduction of propranolol for use as an antihypertensive drug was described by Prichard & Gillam (1964), yet its blood pressure lowering effect on the spontaneously hypertensive rat (SHR) remains debatable, Forman & Mulrow (1974), Roba, Lambelin, DeSchaepdryver (1972), Conway, Darwin, Hilditch, Loveday & Reeves (1975).

Female SHR's (185 ± 5 g, $n = 6$) were treated with (±) propranolol 8 mg kg^{-1} for 8 weeks given daily by intraperitoneal injection; age matched SHR ($n = 6$) were injected with normal saline (0.4 ml i.p.) daily as a control for the same period. Blood pressures of the conscious rats were measured weekly using the tail cuff method. After 8 weeks the animals were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}) and the blood pressure (B.P.) measured directly from the common carotid artery. The animals were then pithed by the method of Gillespie & Muir (1967) and the blood pressure responsiveness to electrical stimulation of the sympathetic outflow (pulse width 0.1 ms, 20 V at 3–25 Hz) and resting blood pressures assessed.

Anococcygeus muscle (Gillespie, 1972), stomach fundic strip (Vane, 1957) and mesenteric artery (McGregor, 1965) preparations were removed and their responsiveness to noradrenaline (NA), 5-hydroxytryptamine (5HT), tyramine and sympathetic electrical stimulation (0.3 ms, 40 V, 3–50 Hz) assessed.

The mean body weights were not altered by chronic propranolol treatments. The mean conscious systolic BP of the propranolol treated hypertensive SHR's was reduced to 150 and 125 mmHg after 1 and 8 weeks of treatment; the initial pretreatment systolic pressures were 170 mmHg. The antihypertensive effect of propranolol was also confirmed in the anaesthetized rats in which both the systolic and diastolic pressures of the propranolol treated SHR's were significantly ($P < 0.01$–0.02) lower than those of the age matched untreated SHR's (145/120 mmHg compared with 215/160 mmHg respectively). Moreover, the systolic and diastolic blood pressures of the SHR treated pithed rats were also lower than their age matched controls (45/25 compared to 80/45 mmHg respectively $P < 0.02$). No significant difference was seen between the treated and untreated SHR's in their blood pressure responsiveness to electrical stimulation of the sympathetic outflow.

The responsiveness of perfused mesentery preparations removed from the propranolol treated rats to NA (0.1–0.8 μg), 5HT (0.1–0.8 μg), tyramine (10 μg) and electrical sympathetic nerve stimulation (3–50 Hz) were significantly reduced ($P < 0.05$) compared to age matched untreated preparations. Furthermore, the responsiveness of the fundic strip preparations removed from treated SHR's to field electrical stimulation (3–50 Hz) 5HT (4.54–7.2×10^{-7} μmol) and tyramine (1.45×10^{-9} μmol) was also significantly reduced ($P < 0.05$, $n = 6$) compared to the untreated preparations. Propranolol treatment had no effect on the response of the isolated anococcygeus muscle preparations to NA (0.47–60.5×10^{-5} M), 5HT (0.72–11.6×10^{-4} M), whilst the responses to electrical field stimulation 3 Hz–50 Hz and tyramine (1.16 μmol) were significantly potentiated ($P < 0.05$, $n = 6$).

These non-specific changes in tissue reactivity may be associated with at least part of the well demonstrated anti-hypertensive effect of (±)-propranolol.

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Vascular reactivity in the experimentally diabetic rat and the effect of treatment with sulphonylureas

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It has been previously reported that the blood flow to various organs of experimentally diabetic rats differs from that of controls (Foy & Lucas 1977), and that treatment with certain sulphonylurea compounds may prevent or reverse some of these changes (Foy & Purvis, 1980). The altered blood flow patterns observed may be caused directly by diabetes-induced changes in blood chemistry or blood vessel wall structure. Another possibility is that these observations are mediated by changes in vascular smooth muscle reactivity to vasoactive neurotransmitters and/or circulating hormones. The purpose of the present study was to observe the effects of various vasoactive agents on the blood pressure and heart rate of alloxan and streptozotocin induced diabetic rats and of similar animals treated with sulphonylurea compounds.

Male rats (200–250 g) were pithed under pentobarbitone anaesthesia and prepared for stimulation of the spinal sympathetic outflow according to the method of Gillespie & Muir (1967). Blood pressure and heart rate responses to tyramine (10 μg and 50 μg/kg), angiotensin II (500 mg/kg), noradrenaline (0.05 μg–10 μg/kg) and sympathetic nerve stimulation (30v, 1 ms, 1–25 Hz for 10 s) were measured.

Diabetes was induced by rapid injection of 1 ml/kg of a freshly prepared solution of alloxan (50 mg/kg, saline), or streptozotocin (60 mg/kg pH 4.5 citrate buffer) via the caudal vein of the tail. Control animals received vehicle only. All rats were subsequently allowed free access to food and water for a period of 36 h, during which time a permanent hyperglycaemia developed. Animals were then divided up into groups according to their treatment regime, i.e. gliclazide 50 mg/kg daily, glibenclamide 5 mg/kg daily or tolbutamide 100 mg/kg daily; all drugs were incorporated in the diet.

Alloxanized, alloxan and sulphonylurea and control animals were pithed 3 days after the initial injection with the diabetogen; streptozotocin, streptozotocin and sulphonylurea treated rats and their controls were pithed 14 days after injection. Further chronic studies were performed on streptozotocin diabetic rats of 60 days duration.

Preliminary results indicate that no changes in vascular reactivity occur in the 3-day alloxan and 14-day streptozotocin diabetic models, however, a significant decrease in sensitivity to noradrenaline was seen in the 60-day streptozotocin diabetic rats. Studies are currently in progress to determine the effect of sulphonylurea treatment on reactivity and these results will also be presented.

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On the mechanism of action of prazosin at the sympathetic nerve-muscle junction of arterioles of the guinea-pig

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Prozosin is an antihypertensive agent and a selective α_1 adrenoceptor antagonist (Cambridge, Davey & Massingam, 1977). It has recently been used in several studies to demonstrate that vascular smooth muscle has both α_1- and α_2-adrenoceptors (Drew & Whiting, 1979; Langer, Massingham & Shepperson, 1980; Docherty & McGrath, 1980). We have examined the effect of prazosin on the responses of arterioles from the guinea-pig small intestine both to exogenous noradrenaline and to stimulation of the periarteriolar nerves. In addition to observing any constriction of the arteriole we have also made intracellular recording of the membrane potential changes in the arteriolar smooth muscle.

Arterioles were isolated from the small intestine of young guinea-pigs and mounted in a small chamber where they were continuously superfused with a warm oxygenated physiological saline (see Hirst, 1977 for further details). The periarteriolar nerves were stimulated using a pair of fine platinum wires placed close to the arteriole. A single stimulus to the nerves gave rise to an excitatory junction potential (e.j.p.) in the arteriolar smooth muscle. If a short train of stimuli was given successive e.j.p.s could combine to generate a depolarization that exceeded the threshold for the generation of an action potential in the muscle, and this would cause the muscle to contract. Contraction of the muscle was seen as a constriction of the arteriole, and was recorded for subsequent measurement using a closed circuit television system with a video tape recorder.

Prazosin in concentrations from 5×10^{-8} M to 10^{-5} M readily abolished the constriction of the arteriole caused by concentrations of noradrenaline up to 2×10^{-5} M. However, even the highest concentration of prazosin did not reduce the amplitude of the e.j.p.; phentolamine (3×10^{-6} M) was equally ineffective. This indicates that although there are α-adrenoceptors on the arteriolar smooth muscle they are not the receptors that are activated by neuronally released noradrenaline.

Prazosin could, however, under certain circumstances, abolish the constriction caused by nerve stimulation. This was caused by an increase in the threshold for generation of an action potential in the muscle. Prazosin at a concentration of 5×10^{-8} M increased the threshold by 5 mV after 30 min., and this small change was sufficient to abolish the constriction caused by a train of three stimuli at 10 Hz. It could be seen from the intracellular recording of membrane potential that the e.j.p.s were not reduced in amplitude and that there was therefore no blockade of postsynaptic receptors, but that they were no longer causing an action potential in the muscle. Higher concentrations of prazosin caused greater increases in threshold.

We therefore conclude that prazosin is capable of blocking the responses of these arterioles to sympathetic nerve stimulation, but that this action is quite unrelated to any blockade of adrenoceptors on the smooth muscle. It is known that prazosin can act as a 'direct vasodilator' (Constantine, McShane, Scriabine & Hess, 1973) and its effect on the threshold for a muscle action potential may be the basis of that action.

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The involvement of central α_1 and α_2 adrenoceptors in the cardiovascular effects of clonidine and R28935 in the conscious cat

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Both clonidine and R28935 are centrally acting hypotensive agents. Clonidine acts via central α-adrenoceptors (Kobinger, 1978) whilst R28935 has been reported both to act via central α-adrenoceptors (Kwa, Timmermans & Van Zwieten, 1979) and not to have a central adrenergic mechanism (Finch, 1975). We have attempted to determine the possible α_1 or α_2-adrenoceptor subtypes involved in the hypotensive effect of clonidine and R28935 given intracerebroventricularly (I.C.V.) in the conscious renal hypertensive cat. The selective α_1-adrenoceptor antagonists prazosin and UK-33,274 (Timmermans, Kwa & Van Zwieten, 1980) and yohimbine and its stereoisomers corynanthine and rauwolscine (Weitzell, Tanaka & Starke, 1979) were given 30 min prior to clonidine or R28935 in antagonism studies.

Clonidine (25 μg I.C.V.) caused a marked hypotension and bradycardia which were maximal 40–60 min after administration. The hypotensive effect was significantly reduced by 100 μg I.C.V. of each antagonist, prazosin being the most effective. Although the clonidine-induced bradycardia was also reduced, this did not achieve statistical significance (Table 1). R28935 (50 μg I.C.V.) also caused a hypotension and bradycardia and these were maximal about 30 minutes after administration. Whereas the hypotension and bradycardia were both significantly antagonized by prazosin (100 μg I.C.V.), yohimbine (200 μg I.C.V.) had no effect (Table 1).

These results indicate that in the conscious cat, the action of clonidine is susceptible to both α_1 and α_2-adrenoceptor antagonism, whilst that of R28935 is only antagonized by prazosin which is α_1-adrenoceptor selective. There is surprisingly little difference in the antagonism of clonidine by yohimbine and its stereoisomers rauwolscine (α_2-selective) and corynanthine (α_1-selective) considering the great differences in selectivity of these agents.

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Table 1 The interaction of clonidine and R28935 with α-adrenoceptor antagonists in the conscious cat.

	Max % Δ from resting levels		
Dose given I.C.V.	B.P. *(Mean ± s.e. mean)*	*H.R. (Mean ± s.e. mean)*	*n*
Clonidine (25 μg) alone	−38.7 ± 1.9	−34.3 ± 7.9	6
+ Prazosin (100 μg)	−13.1 ± 4.9*	−27.7 ± 4.0	7
+ UK-33,274 (100 μg)	−20.6 ± 2.9*	−27.3 ± 1.7	4
+ Corynanthine (100 μg)	−19.4 ± 3.7*	−29.2 ± 2.8	6
+ Yohimbine (100 μg)	−20.0 ± 4.1*	−25.3 ± 4.1	6
+ Rauwolscine (100 μg)	−20.4 ± 2.4*	−27.9 ± 4.2	5
R28935 (50 μg) alone	−27.7 ± 4.2	−25.8 ± 3.5	6
+ Prazosin (100 μg)	−9.5 ± 3.5*	−11.3 ± 5.3*	6
+ Yohimbine (200 μg)	−26.9 ± 2.1	−25.2 ± 7.5	4

* Significantly different from controls ($P < 0.05$), unpaired Students *t*-test. Resting M.A.P. and H.R. = 121.6 ± 5.1 mmHg and 173.3 ± 9.6 beats/min respectively. (Mean ± s.e. mean n = 20.)

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Dextran-haemoglobin complex as a possible blood substitute

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For many years there has been interest in haemoglobin solution as a possible plasma expander or blood substitute (see De Venuto, Friedman, Neville & Peck, 1979) since a solution of haemoglobin would require no crossmatching prior to use and would have a greater storage life than bank blood. However, free haemoglobin is rapidly removed from the circulation, therefore to reduce its rate of excretion Tam, Blumenstein & Wong (1978) linked haemoglobin with dextran. Although this complex had a higher oxygen affinity than haemoglobin, Tam *et al* (1978) reported survival in a small number of dogs exchange transfused with this complex. The purpose of this work was to extend the preliminary results of Tam and his co-workers.

Dextran-haemoglobin complex (Dx-Hb) was prepared by the method of Chang & Wong (1977) using dextran 20 (20,000 M.W.) and haemoglobin solution prepared from citrated human blood. A solution contining 6 g% Hb as the Dx-Hb complex was used. The electrolyte composition of this was: (mM) NaCl 98.5; $NaHCO_3$ 35.7; KCl 4.02; $CaCl_2$ 2.5; $MgCl_2$ 0.74; Glucose 1.4.

Beagle dogs (6–8 kg) of either sex were anaesthetized with pentobarbitone (30–40 mg/kg i.v.) and catheters were inserted into the cephalic vein and, via the saphenous artery, into the femoral artery. Exchange transfusion was carried out at a rate of 1 ml kg^{-1} min^{-1} using a Watson Marlow pump. The first 200 ml of blood removed was centrifuged and a volume of plasma equivalent to 10% of the total blood volume was reinfused at the end of the exchange transfusion to restore partially the plasma protein concentration.

Of 6 dogs exchange transfused with Dx-Hb, 5 survived; the mean haematocrit ± s.e. mean of these was 3.4 ± 0.7% at the end of the exchange. The final total Hb concentration was 6.8 ± 0.3 g% and the final concentration of plasma Dx-Hb was 5.7 ± 0.3 g%; the difference between these two values represents the residual red cell Hb. The plasma half life of Dx-Hb was found to be 34 hours. The haematocrit and the haemoglobin concentration returned to normal values 17–21 days after the exchange, the dogs were then killed and post mortem examination was carried out.

Two dogs were also exchange transfused with dextran 110 in 5% dextrose (Dextraven 110, Fisons Ltd); these both died when the haemoglobin concentration was lowered to 2.8 g% (haematocrit 6.5%).

Thus, in conclusion, Dx-Hb complex appears to be effective as a blood substitute in the dog. Further work, however, is required to compare it with existing plasma expanders and blood substitutes.

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Effect of 5-hydroxytryptamine on canine isolated coronary arteries

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It is generally accepted that 5-hydroxytryptamine (5-HT) is a potent vasoconstrictor in many vascular beds. In the skeletal muscle and coronary areas, however, it exerts myorelaxant effects. According to Mena & Vidrio (1976) 5-HT produces coronary vasodilatation in anaesthetized open chest mongrel dogs by directly stimulating specific 5-HT receptors. This effect is independent of cardiac stimulation which 5-HT mediates via the release of endogenous noradrenaline (Fozard & Mwaluko, 1976).

The aim of the present work is to characterize under *in vitro* conditions the receptors in the dog

coronary artery strips which are stimulated by 5-HT.

The circumflex and interventricular arteries were isolated from the heart of mongrel dogs. Strips were cut spirally from the proximal (diameter: $\geq$ 2 mm) and distal (diameter: $\leq$ 0.5 mm) portion of the two arteries and suspended in a 20 ml chamber containing Tyrode-bicarbonate solution maintained at 37°C. The bathing fluid was continuously bubbled with a gas mixture of 95% O_2 + 5% CO_2. The strips were connected to an isotonic myograph transducer and subjected to a tension of 1.5 g for the proximal portion and 0.2 g for the distal portion. Contraction was recorded on a polygraph. Cumulative concentration-response curves to noradrenaline, 5-HT, and tryptamine were constructed. Each concentration of agonist was kept in contact with the tissue for a 10 min period. At least 30 min elapsed between challenge with different agonists. In all experiments, the responsiveness of the preparation was tested with a supra-maximal concentration of KCl (35 mM) at the beginning and end of the study. In one series of experiments, the release of endogenous noradrenaline from adrenergic neurons was induced using K-free physiological saline solution (Bonaccorsi, Hermsmeyer, Smith & Bohr, 1977). In another series of preparations sympathetic denervation was produced with 6-hydroxydopamine as described by Aprigliano & Hermsmeyer (1976).

Noradrenaline caused concentration related contractions in the proximal and distal portions of the coronary artery. 5-HT contracted the proximal portion without modifying the basal tension of the distal portion. The maximal contraction was greater with 5-HT than with noradrenaline. This effect was still present after sympathetic denervation with 6-hydroxydopamine. The concentration responses curves to 5-HT obtained experimentally were very similar to those predicted theoretically on the basis of the mass action law and exhibited a classical hyperbolic shape. The calculated Hill coefficient was approximately 1.

These findings show that 5-HT does not relax potassium contracted canine coronary arterial strips. Furthermore, the contraction produced by 5-HT in this preparation was not due to the release of endogenous noradrenaline. Finally, these results suggest that the responses elicited by 5-HT are mechanistically consistent with receptor occupancy theory and that the proximal portion of the left coronary artery of the dog appears to possess receptors specific for 5-HT and noradrenaline. Thus, the coronary vasodilatation produced by 5-HT under *in vivo* conditions (Mena & Vidrio, 1976) is probably due to an action of this amine on very small resistance vessel.

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An apparent dissociation between the release of coronary vasodilator mediator and perfusate pO_2 in guinea-pig isolated hearts

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The increase in coronary flow accompanying cardiac stimulation by drugs and by electrical pacing has been attributed to the release of vasoactive mediators (Berne & Rubio, 1979). A link in the sequence of events is thought to be hypoxia of the myocardium during the cardiac hyperactivity (Schaper, 1979). The release of coronary vasodilator material from guinea-pig isolated hearts stimulated with isoprenaline (10 ng) was demonstrated by the use of donor and recipient hearts perfused in series (Broadley, 1976). The direct β-adrenoceptor effects in the recipient heart of isoprenaline carried over in the perfusate were antagonized by propranolol (20 μg) administration to the recipient. The isoprenaline-induced coronary vasodilatation and increases in rate and tension of the donor heart were then followed by coronary vasodilatation of the recipient, without any myocardial responses.

Tachycardia of donor hearts, produced by electrical pacing (5 Hz, 10 V, 5 ms) for 2 min, also caused the release of vasodilator material. However, this release was abolished by propranolol (20 μg) and practolol (20 μg) administration to the donor hearts. Furthermore, when donor hearts were removed from animals pretreated with reserpine (0.5 mg/kg i.p. at 24 h) to deplete endogenous catecholamines, in none of the 5 experiments was vasodilator activity recorded in the recipient hearts during pacing. Thus, it would appear that the pacing-induced release of vasodilator mediator was a consequence of the release of endogenous catecholamines, which presumably then released vasoactive material in a similar fashion to isoprenaline. Without this interference, it could be concluded that tachycardia produced by pacing does not cause vasodilator mediator release.

Next we examined the oxygen tension (pO_2) in the perfusate leaving donor hearts, which was collected by cannulation of the pulmonary artery and continuously taken through a Clark oxygen electrode chamber (Corning-Eel, Model 16) at 37°C by means of a Watson-Marlow flow inducer. Positive inotropic and chronotropic responses and coronary vasodilatation to isoprenaline were associated with dose-related falls in perfusate pO_2. This provided an index of increased oxygen utilization by the heart. Pacing also increased oxygen utilization which was related to the frequency of stimulation. Propranolol (50 μg) reduced the resting pO_2 which is probably associated with its membrane stabilizing properties (Papp & Vaughan-Williams, 1969). However, propranolol did not affect the spontaneous release of mediator from donor hearts, since there was no vasoconstriction in recipient hearts on its administration. Spontaneous mediator release and pO_2 levels are therefore apparently dissociated. After propranolol, pacing still caused increases in oxygen consumption, although all the responses to isoprenaline were antagonized.

In contrast, practolol (100 μg) did not alter resting pO_2 levels, but it too failed to antagonize the increase in oxygen utilization accompanying pacing. Thus, after both β-adrenoceptor antagonists there was no release of vasodilator material following pacing-induced tachycardia, yet the perfusate pO_2 still fell. This suggests that an increase in oxygen utilization during cardiac hyperactivity is not a prerequisite for the release of coronary vasodilator mediator.

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The calcium dependence of ventricular fibrillation thesholds in the rat isolated heart and the effects of verapamil

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We have previously reported that extracellular calcium has concentration dependent effects on cyclic nucleotide levels in isolated perfused rat heart (Daugherty & Woodward, 1980). The present study investigated whether vulnerability to ventricular fibrillation could be related to extracellular calcium or its metabolic consequences.

The method of producing vetricular fibrillation was similar to that described by Lubbe *et al.* (1975), using a comparable criterion for quantification of arrhythmias. Briefly, the method involves pacing Langendorff perfused rat hearts at 300 beats/min with one channel of a Grass S88 stimulator whilst recording developed tension, left ventricular pressure, perfusion pressure and epicardial ECG. The second channel of the stimulator was used to deliver extra pulses, via a Grass CCU1 constant current unit, at a preset current and delay. Scanning of the vulnerable period was carried out in order to obtain the ventricular fibrillation threshold (VFT) at the nadir

of the vulnerable period. After initial instrumentation had been completed a stabilization period of 15 min was allowed. Thereafter, 5 min equilibration time was allowed on changing perfusates before recommencement of VFT determinations.

The control VFT at a Ca^{2+} concentration of 1.23 mM was 20.7 ± 1.5 mA. This was significantly ($P<0.001$) reduced in the presence of 2.46 mM Ca^{2+}, and further reduced in the presence of 4.96 mM Ca^{2+} ($P<0.01$). Associated with the reduction in VFT, the nadir of the vulnerable period was displaced nearer to the peak of the R wave with increasing calcium concentration (see Table 1). Verapamil failed to reverse fully the calcium-induced lowering of VFT, or to increase significantly the interval between the R wave and the nadir of the vulnerable period, although the highest concentration used (1×10^{-7} M in the presence of 2.46 mM Ca^{2+}) produced a greater depression of developed tension than perfusion with 1.23 mM Ca^{2+} alone.

The present data, and that of the previous report would seem to suggest that in our model the extracellular calcium concentration affects vulnerability independently of cAMP levels. This dissociation between cAMP and ventricular fibrillation is in agreement with a recent communication (Thandroyen *et al.*, 1980) which has shown a disparity between cAMP levels and arrhythmia protection afforded by calcium slow channel antagonist.

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Table 1 Effects of calcium and verapamil on:
a) VFT at the nadir of the vulnerable period;
b) Interval between the peak of the R wave and the nadir of the vulnerable period.

a)		*VFT (mA)*			
A	Ca^{2+} (1.23 mM)	20.7 ± 1.5	(21)	AvB	$P<0.001$
B	Ca^{2+} (2.46 mM)	7.8 ± 0.7	(19)	AvC	$P<0.001$
C	Ca^{2+} (4.96 mM)	3.4 ± 0.9	(7)	BvC	$P<0.01$
				AvD	$P<0.001$
D	Verapamil (1×10^{-8} M), Ca^{2+} (2.46 mM)	9.1 ± 0.9	(9)	AvE	$P<0.01$
E	Verapamil (1×10^{-7} M), Ca^{2+} (2.46 mM)	13.2 ± 1.3	(9)	DvE	$P<0.02$
b)		*Nadir of vulnerable period (ms)*			
A	Ca^{2+} (1.23 mM)	42.4 ± 1.1	(21)	AvB	$P<0.001$
B	Ca^{2+} (2.46 mM)	32.4 ± 1.4	(19)	AvC	$P<0.001$
C	Ca^{2+} (4.96 mM)	18.9 ± 1.8	(7)	BvC	$P<0.001$
				AvD	$P<0.01$
D	Verapamil 1($\times 10^{-8}$ M), Ca^{2+} (2.46 mM)	32.1 ± 1.4	(9)	AvE	$P<0.01$
E	Verapamil (1×10^{-7} M), Ca^{2+} (2.46 mM)	34.9 ± 1.8	(9)	DvE	NS

Values expressed as mean ± s.e. mean. Numbers in parentheses represent the number of experiments.

Ventricular fibrillation in the rat isolated heart and the effects of antiarrhythmic drugs

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Hearts from male Wistar rats (University of Bath strain) were perfused at 10 ml/min and 37° using a modified Langendorff technique with Krebs solution of the following composition (mM) NaCl 118; KCl 4.75; $CaCl_2$ $6H_2O$ 2.46; KH_2PO_4 1.2; $MgSO_4 \cdot 6H_2O$ 1.2; $NaHCO_3$ 25 and glucose 11, gassed with 95% O_2; 5% CO_2. Ventricular fibrillation could be induced by increasing the $CaCl_2 \cdot 6H_2O$ concentration from 2.46 to 4.92 mM while at the same time omitting KCl or reducing the NaCl concentration from 118 to 59 mM (osmolarity maintained with sucrose). Fibrillation could also be produced at a calcium concentration of 2.46 mM if KCl was omitted and NaCl was reduced from 118 to 59 mM. Fibrillation routinely produced by increasing the $CaCl_2 \cdot 6H_2O$ concentration and lowering the NaCl concentration can be prevented by the following drugs—lignocaine (5, 10 μg/ml), mexiletine (5, 10 μg/ml), disopyramide (5, 10 μg/ml) and propranolol (2 μg/ml). β-blocking drugs without local anaesthetic properties—practolol, sotolol and atenolol did not prevent fibrillation at doses of 10 μg/ml. The calcium slow channel antagonist verapamil (50 ng/ml) prevented fibrillation in 5 out of 6 hearts; nifedipine at a dose (34.6 ng/ml) which depressed contractility more than verapamil was less effective in preventing fibrillation.

The cause of the fibrillation seen in these experiments is not clear, however, an elevated intracellular calcium level is one possibility. Lowering the potassium and/or sodium concentration of the perfusate would be expected to reduce the sodium concentration gradient across the cell, this in turn would lead to an inhibition of sodium calcium exchange and an increased intracellular calcium level (Reuter, 1974). This raises the possibility that Class I antiarrhythmic drugs may be affecting calcium movements within the cell.

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Which properties of β-adrenoceptor blocking drugs are important in the prevention of early postinfarction dysrhythmias?

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Both acute and prolonged treatment with a number of β-adrenoceptor blocking drugs reduce the incidence and severity of the early ventricular dysrhythmias that result from experimental coronary artery ligation (reviewed by Parratt, 1980). Clinical studies have also shown that myocardial infarction patients treated long-term with practolol, alprenolol and oxprenolol have a lower incidence of sudden cardiac death on reinfarction. It is uncertain, however, whether β-adrenoceptor blockade *per se* is the most important property in this prevention of early postinfarction ventricular fibrillation, or indeed whether other properties of these drugs (e.g. cardioselectivity, 'membrane stabilization', intrinsic activity) contribute to this protective effect. An attempt to answer this question has been made using the rat coronary artery ligation model (Kane, McDonald & Parratt, 1979; Clark, Foreman, Kane, McDonald & Parratt, 1980). All the drugs were given intravenously 15 min pre-ligation.

The results (Table 1) allow some tentative conclusions to be drawn:

1. β-adrenoceptor blockade is clearly important in this protection. All the β-adrenoceptor blocking drugs so far examined in this (and similar) models exert some degree of protection (Table 1 and Parratt, 1980). The protection is dose-related (oxprenolol results in Table 1).
2. Inhibition of the rapid Na^+ inward current in cardiac muscle (Vaughan Williams class 1 effect) appears to be less important. Thus practolol is protective and (−)-oxprenolol is much more effective than (+)-oxprenolol (Table 1).

3. Cardioselectivity is of some importance. Thus the cardioselective para-oxprenolol (Vaughan Williams, Bagwell & Singh, 1973) is rather more effective than dl-oxprenolol (Table 1) whilst practolol (Table 1) is more effective than propranolol (Kane *et al.*, 1979).
4. It appears that for this protection, it might be helpful for β-adrenoceptor blocking drugs to possess intrinsic activity. All the drugs possessing this property are effective (Table 1), rather more so than propranolol (Kane *et al.*, 1979).

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Table 1 Total number of ventricular extrasystoles and the duration and incidence of ventricular tachycardia (VT) and of ventricular fibrillation (VF) in the first 30 min following coronary artery ligation in anaesthetized rats. Values are mean ± s.e. mean of *n* experiments

Drug	*Dose (mg/kg)*	*n*	*Ventricular ectopic count*	*Duration(s) and incidence (%) of VT*	*Duration(s) and incidence (%) of VF*
Controls	—	27	1081 ± 147	72 ± 13 (100%)	75 ± 26 (69%)
(±)-Oxprenolol	1	11	964 ± 278	65 ± 27 (91%)	37 ± 16 (45%)
,,	2	10	374 ± 100**	15 ± 7* (90%)	14 ± 13 (20%)*
,,	5	8	278 ± 71**	12 ± 5** (38%)**	7 (13%)**
(−)-Oxprenolol	1	7	324 ± 124*	35 ± 2 (23%)*	0 (0%)**
(+)-Oxprenolol	1	9	986 ± 178	61 ± 17 (100%)	53 ± 48 (23%)
para-Oxprenolol	1	8	238 ± 115**	15 ± 9* (88%)	0** (0%)**
practolol	5	7	270 ± 120**	22 ± 13 (72%)**	86 (14%)*

**$P<0.01$, *$P<0.05$

Benserazide: an inhibitor of clorgyline-resistant amine oxidase in rat cardiovascular tissues

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Rat cardiovascular tissues contain monoamine oxidase (MAO)-A, -B, and a clorgyline-resistant amine oxidase (CRAO) (Coquil, Goridis, Mack & Neff, 1973; Lyles & Callingham, 1975, 1980). CRAO is insensitive to inhibition by clorgyline (10^{-3} M) *in vitro*, at which concentration MAO-A and -B are totally inhibited, and it possesses a very low K_m (around 5 μM) for benzylamine (BZ) as substrate. The physiological function and endogenous substrate(s) of this enzyme are at present unknown. In rat heart homogenates, although both MAO-A and -B metabolize BZ (K_m around 500 μM) (Lyles & Callingham, 1975), CRAO activity can be studied alone by the use of BZ (1 μM) when the contribution by MAO-A and -B becomes negligible. In rat aorta BZ is only metabolized by CRAO (Lyles & Callingham, 1980). These tissues have been used as a source of CRAO to investigate its sensitivity towards inhibition *in vitro* and *in vivo* by benserazide (DL-serine 2-[2,3,4-trihydroxybenzyl]-hydrazide, Ro4-4602).

Hearts and aortae from male Sprague-Dawley rats (weighing around 200 g) were homogenized in potassium phosphate buffer (1 mM, pH 7.8), centrifuged at 600 *g* for 10 min, and the supernatants used for assay of CRAO activity with [^{14}C]-BZ (1 μM, 10 μCi/μmole) (Lyles & Callingham, 1980). Benserazide hydrochloride was dissolved in potassium phosphate buffer (0.2 M, pH 7.8) for use *in vitro*. For *in vivo* studies, rats (4–5 in each group) were given daily intraperitoneal injections of benserazide (5–150 mg/kg) dissolved in saline (0.9% NaCl, w/v) and killed 24 h after either one day or seven days treatment. Control animals received saline alone. Tissues were removed, homogenized and CRAO assayed as described above. Protein contents of all homogenates were measured, to allow expression of results as specific enzyme activities.

After preincubation with homogenates for 20 min at 37°C before addition of substrate, benserazide inhibited CRAO activity with an IC_{50} of 8×10^{-7} M (aorta) and 4×10^{-6} M (heart). These values are dependent upon assay conditions, since inhibition was dependent on the time of preincubation, suggesting irreversible inhibition. Ackermann-Potter plots showing reaction velocities at different enzyme concentrations supported this conclusion. Some evidence for a partially reversible component in the inhibition was also found in these experiments.

Rats treated with benserazide showed dose-dependent inhibition of CRAO in their hearts and aortae compared with controls. After one day, mean percentage inhibition of aortae ranged from 15% (5 mg/kg) to 60% (150 mg/kg), with rat heart CRAO showing slightly less inhibition. After seven days treatment, CRAO in both tissues showed similar inhibition ranging from 30–80% at these doses.

In conclusion, benserazide inhibits rat cardiovascular CRAO both *in vitro* and *in vivo*, a property which may be of interest in addition to its recognized clinical use as an inhibitor of aromatic amino acid decarboxylase in Parkinsonian patients (Barbeau, Gillo-Joffroy & Mars, 1971).

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Monoamine deamination in the anococcygeus muscle of the rat

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There is now a great deal of information concerning the deamination of monoamines in a wide variety of organs in the rat and in other animals (see Fowler, Callingham, Mantle & Tipton, 1978). However nearly all organs are composed of several different tissues that are not separated before assay of enzyme activity. In addition attention has been drawn to the widespread distribution of at least one clorgyline-resistant amine oxidase (CRAO) distinct from monoamine oxidase-A or -B (MAO-A, MAO-B), which preferentially deaminates benzylamine (Lewinsohn, Böhm, Glover & Sandler, 1978).

In an attempt to find a comparatively simple tissue that could serve as a control in the study of more complex systems, deaminating activities have been examined in the anococcygeus muscle of the rat; a tissue whose qualities were first recognized and exploited by Gillespie in 1972.

Muscles were pooled from groups of 12–14 male Sprague-Dawley rats in weight ranges from 200–500 g and homogenized in potassium phosphate buffer (1 mM, pH 7.8). The supernatant fractions following centrifugation at 600 *g* for 10 min were used for the assay of MAO and CRAO activity with [^{3}H]-5-hydroxytryptamine (5-HT), [^{3}H]-tyramine (TYR), [^{14}C]-β-phenethylamine (PEA) and [^{14}C]-benzylamine (BZ) as substrates.

Specific activities for deamination varied with the substrate used and the age of the animals. For example with 5-HT (1 mM) as substrate, specific activity was about 230 and 100 nmoles mg protein^{-1}h^{-1} in 250 and 400 g rats respectively while with TYR (1 mM) they were 188 and 75.

Estimations of enzyme activity after preincubation with clorgyline in concentrations from 10^{-10} – 10^{-3} M produced single sigmoid inhibition curves with 5-HT (1 mM) and TYR (1 mM). Although the curves revealed that both 5-HT and TYR at 1 mM are deaminated by MAO-A with no detectable contribution from MAO-B (IC_{50} for clorgyline of 2×10^{-9} M), about 17% of the metabolizing activity towards TYR remained even in the presence of 10^{-3} M clorgyline, indicating a contribution from CRAO. With PEA (250 μM), MAO-A, MAO-B and about the same proportion of CRAO as seen with TYR were involved. When BZ (1 mM) was used about 70% of its deamination was brought about by CRAO and the rest by MAO-B, although a tiny contribution by MAO-A cannot be ruled out. With BZ (10 μM) all measurable activity was CRAO which could be almost completely inhibited by semicarbazide (10^{-3} M).

Treatment of 200–250 g rats for 7 days with daily subcutaneous doses of benserazide (150 mg/kg) significantly reduced the subsequent *in vitro* deamination of all substrates except 5-HT which was totally unaffected. The greatest effects were seen with BZ and PEA as substrates.

These results indicate that the anococcygeus muscle of the rat should be a reliable tissue for studies of the effects of drugs and other agents on both MAO and CRAO.

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Changes in the responsiveness of intestinal smooth muscle to agonists in rats infected with *Nippostrongylus brasiliensis*

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Nippostrongylus brasiliensis, an intestinal nematode of the rat, has a complex life cycle, and is eventually expelled from the intestine by an immune reaction (Ogilvie & Love, 1974). Exactly how immunologically damaged worms are expelled is unknown, but a factor which may be important is gut motility. Propulsive motility of the intestine is increased during infection (Farmer, in press), and the present study examined the responsiveness of isolated segments of gut to agonists at different times post-infection with the parasite.

N. brasiliensis larvae were cultured as previously described (Jennings, Mulligan & Urquhart, 1963). Male hooded Lister rats (170–200 g) were infected with larvae (5,000/rat, injected s.c.) and killed on days 6, 8, 10, 12, 14 and 20 after infection. A 2 cm segment of small intestine from each rat was suspended in Krebs solution (37°C), gassed with 95% O_2/5% CO_2. The resting tension was adjusted to 10 g and responses were recorded isometrically. Dose response curves to acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) were obtained, and mean pD_2 values (Ariens & van Rossum, 1957), and maximum responses for each group were compared with control means using Student's *t*-test.

Nippostrongylosis did not affect pD_2 values for ACh, but reduced those for 5-HT on days 8, 14 and 20 (Table 1). This specific subsensitivity to 5-HT may be a response to the greatly increased intestinal levels of 5-HT associated with infection (Murray, Miller, Sandford & Jarrett, 1971). The most obvious effect of *N. brasiliensis* infection was the non-specific increase in maximum responses to ACh and 5-HT. The mechanism of this effect is unknown, but it is interesting that the increased responsiveness to these agonists was maximal at day 14 post-infection, when the rate of parasite expulsion is also at a peak (Murray *et al.*, 1971). It is possible that these two processes are linked.

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Table 1 The effect of *N. brasiliensis* infection on pD_2 values and maximum responses of rat small intestine to acetylcholine (ACh) and 5-hydroxytryptamine (5-HT).

Day of infection	*ACh* pD_2	*ACh maximum (g)*	*n*	*5-HT* pD_2	*5-HT maximum (g)*	*n*
6	6.05 ± 0.15	3.11 ± 0.74*	5	7.29 ± 0.13	3.26 ± 0.20***	6
8	6.14 ± 0.16	3.78 ± 0.50***	5	6.83 ± 0.14*	3.65 ± 0.20***	4
10	6.11 ± 0.17	5.11 ± 0.54***	6	7.08 ± 0.06	4.23 ± 0.62***	6
12	6.16 ± 0.04	6.80 ± 0.59***	6	7.00 ± 0.05	4.24 ± 1.09**	4**
14	6.14 ± 0.04	10.23 ± 1.16**	6	6.81 ± 0.05**	5.26 ± 0.64***	5
20	6.09 ± 0.06	5.88 ± 0.72***	6	6.89 ± 0.09**	4.43 ± 0.38***	6
uninfected control	6.03 ± 0.05	1.80 ± 0.17	11	7.28 ± 0.10	1.81 ± 0.14	10

Values are given as mean ± s.e. mean, *n* = number of observations.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

SK&F 93479, a potent and long acting histamine H_2-receptor antagonist

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Although SK&F 92994 (oxmetidine) (Blakemore, Brown, Durant, Emmett, Ganellin, Parsons & Rasmussen, 1980) and ranitidine (Bradshaw, Brittain, Clitherow, Daly, Jack, Prince & Stables, 1979) are more potent H_2-receptor histamine antagonists than is cimetidine (Brimblecombe, Duncan, Durant, Ganellin, Parsons & Black, 1975) they do not consistently show a longer duration of action in inhibiting gastric acid secretion.

SK&F 93479 (2-[2-(5-Dimethylaminomethylfuran-2-ylmethylthio)ethyl-amino]-5-(6-methylpyrid-3-yl-methyl)pyrimid-4-one trihydrochloride, mol. wt. = 522.95) has now been demonstrated to be a potent H_2-receptor histamine antagonist and to have a sustained antisecretory action in all species studied.

SK&F 93479 differs from previously described antagonists in possessing three basic centres (pK_a (25°) = 3.0, 6.1 and 8.5 due to protonation of pyrimidone, pyridine and dimethylamine groups respectively), and an acidic centre (pyrimidone pK_a = 10.2) (Graham, personal communication).

On the guinea-pig isolated atrium SK&F 93479 antagonized the positive chronotropic action of histamine giving a pA_2 value of 7.78 (7.33–8.48) (60 min equilibration) with a Schild plot slope of 0.88 ± 0.20 (mean ± 95% confidence limits), indicating competitive antagonism. A high degree of specificity for the H_2 receptor is indicated by the relatively weak activity against isoprenalinine (guinea-pig atrium, β receptor, pA_2 = ca 3.7), against histamine (guinea-pig ileum, H_1 receptor, pA_2 = ca 4.2) and against carbachol (guinea-pig ileum, muscarinic receptor, pA_2 = 4.8).

SK&F 93479 was approximately ten times more active than cimetidine in inhibiting histamine-stimulated gastric acid secretion in the perfused stomach preparation of the anaesthetized rat; 50% peak inhibition was obtained at a dose of 0.14 (0.06–0.23) μmol/kg i.v. compared with 1.37 μmol/kg for cimetidine. In the acutely fistulated anaesthetized cat the potency relative to cimetidine was approximately sixteen, a dose of 0.5 μmol/kg i.v. giving 98% inhibition (n = 5) (cf. cimetidine, 92% at 8 μmol/kg i.v.). In the rat and cat inhibition was prolonged relative to cimetidine.

Intravenous administration of SK&F 93479 to the conscious Heidenhain pouch dog produced a sustained inhibition relative to cimetidine or ranitidine (Figure 1). Oral administration of SK&F 93479 (0.625 and 1.25 μmol/kg) to the dog gave mean peak inhibitions of 67% and 90% respectively (cf. cimetidine, 82% at 20 μmol/kg and ranitidine 95% at 5 μmol/kg), and there was no significant decline from peak inhibition 4 h after administration. By both routes in the dog SK&F 93479 was approximately sixteen times more potent than cimetidine.

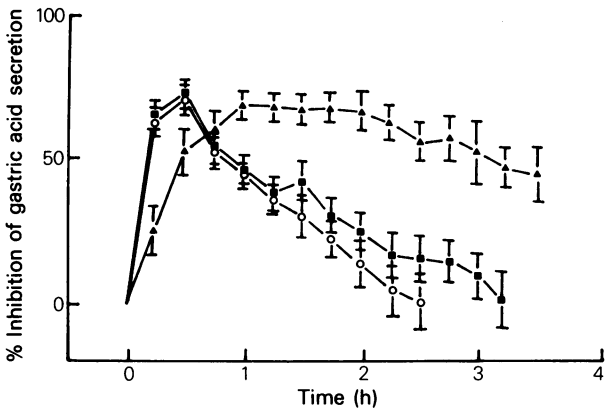

Figure 1 The inhibition of maximal histamine-stimulated gastric acid secretion in the conscious Heidenhain pouch dog by cimetidine (4.0 μmol/kg i.v. = ○), ranitidine (0.5 μmol/kg i.v. = ■) and SK&F 93479 (0.25 μmol/kg i.v. = ▲). Results are means ± s.e. mean (n = 6).

Thus SK&F 93479 is a selective H_2-receptor histamine antagonist *in vitro*, and a potent inhibitor of histamine-stimulated gastric acid secretion *in vivo*. It produces a prolonged inhibition relative to cimetidine or ranitidine which appears not to depend upon species or route of administration. Human studies are being undertaken.

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Excretory ovalbumin, a potent protective factor against fatal anaphylaxis to egg-whites in guinea-pigs

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Fifteen male Duncan-Hartley guinea-pigs were sensitized with ovalbumin (50 mg in 0.5 ml saline), in order to investigate the nature of the heat-labile factor, found in the urine of sensitized guinea-pigs which will protect them against anaphylaxis (Lewis & Wilson, 1980). The experiment was also designed to investigate whether pooled urine would be as effective as individual samples. The animals were divided into three groups of five animals. In groups 'A' and 'B', individual post-immunization urine specimens (Lewis & Wilson, 1980) were collected for 48 hours. In group 'A', the urines were kept separate, and in group 'B', the urines were pooled. Urines collected from group 'C' were discarded, and this group served as the control group. All urine specimens were deep-frozen until required for subsequent use. On day 22, each animal in groups 'A' and 'B' was given an intraperitoneal injection of a 1 in 10 dilution with saline of either its own urine, or of the pooled urine respectively. Animals in group 'C' received 1 ml of saline. Further injections were given on day 23, but the urine specimens were not diluted. This sequence of urine treatments minimized the severity of the side effects following the injection of normal undiluted urine (Lewis & Wilson, 1980). All three groups of animals were challenged 24 h later by intraperitoneal injections of ovalbumin (25 mg in 0.5 ml saline) on day 24.

All the control animals in group 'C' became lethargic, developed severe respiratory depression and died within 30 min after ovalbumin challenge. Animals in groups 'A' and 'B' also become lethargic, but respiratory depression was less severe. One animal died from each of these groups at 60 min and 58 min respectively after ovalbumin challenge. The rest recovered and survived. Using the Ouchterlony gel diffusion technique (Feinberg, 1957), ovalbumin was shown to be present in both the individual and pooled urine specimens, at a concentration equivalent to a 1:1000 dilution of the sensitizing dose, for the 48 h collecting period. The same technique failed to reveal the presence of antibodies to ovalbumin in the urine. It is conceivable, therefore, that the protective factor in the urines of these sensitized animals is associated with the presence of ovalbumin excreted in the urine. The manifestation of mild anaphylactic signs after urine injections (Lewis & Wilson, 1980), is indicative of the presence of ovalbumin antigen in the urine. It is possible that the urinary concentration of ovalbumin for the 48 h collecting period, is at a therapeutic level, and is able to offer protection against anaphylaxis induced by the challenging dose of ovalbumin (Miller, 1977). The results further demonstrate, that pooled urine from specifically sensitized animals is as effective as the urine from individual animals.

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Leucopenia and the dextran anaphylactoid reaction in the rat

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Goth (1968) showed that peritoneal cells obtained from rats rendered leucopenic by pretreatment with nitrogen mustard released less histamine when challenged with dextran *in vitro* compared to control cells, whereas that induced by compound 48/80 was unaltered. Similarly, Anderson, Hanahoe & West (1978) showed that nitrogen mustard inhibited dextran induced histamine release from rat peritoneum *in vivo*. Further, Garcia Leme, Bechera & Ribeiro Dos Santos (1976) reported that the intravenous injection of lymphocyte lysates potentiated the effect of dextran *in vivo* in the rat. These observations suggest that leucocytes are associated with the dextran anaphylactoid reaction. We report here experiments by which this hypothesis has been examined. Leucopenia was produced in rats by the following treatments; nitrogen mustard, methotrexate, cyclophosphamide, antilymphocyte serum and thymectomy and X-ray irradiation (Table 1). The responses of such rats to intraperitoneal, intravanous,

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intrapedal and intradermal injection of dextran was then examined. Nitrogen mustard inhibited the effects of dextran injected by each of these routes, whereas the effects of compound 48/80 were not inhibited. However, pretreatment with each of the other leucopenic agents failed to inhibit the dextran reactions. The inhibition, by nitrogen mustard treatment, of the dextran reaction was not overcome by pretreating the leucopenic animals with viable leucocytes to increase their white blood cell counts to normal values. However, in the nitrogen mustard treated rats, but not in other leucopenic animals, nor in normal rats, dextran injection significantly raised the blood sugar level. Further, injection of glucose (5 g/kg i.p.) into nitrogen mustard treated rats resulted in a prolonged hyperglycaemia (300 mg%), whereas control rats reached only low levels (180 mg%) which recovered quickly. It is well documented (Hanahoe, Tanner & West, 1973) that glucose inhibits the dextran anaphylactoid reaction but not the effects of compound 48/80 in rats. Stress caused by the dextran reaction will release adrenaline which in turn will raise the blood sugar. Nitrogen mustard inhibits glucose utilization (Guyton, 1971). We, therefore, suggest that the inhibition of the dextran reaction caused by nitrogen mustard pretreatment results from an impaired ability of these rats to metabolism the glucose released by the stress of dextran injection and that this hyperglycaemia is the inhibitor factor.

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Table 1 The effect of cytotoxic procedures on peripheral leucocyte numbers in rats

	Total white blood cells	*Lymphocytes*	*Polymorpho-nuclear cells*	*Monocytes*
Nitrogen mustard (n = 80) 1 mg/kg i.v. animals used 2 days after the last injection.	84	87	62	91
Methotrexate (n = 50) 2 mg/kg i.v. 3 injections at 24 h intervals. Animals used 3 days after the last injection.	69	67	84	77
Cyclophosphamide (n = 50) 20 mg/kg i.v. 2 injections at 24 h interval. Animals used 3 days after last injection.	80	82	63	91
Rabbit anti rat lymphocyte serum (n = 7) 1.25 mls i.p. 2 injections at 24 h interval. Animals used 1 h after last injection.	87	91	71	100
Thymectomy and irradiation (n = 6) 700 rads	99	99	98	100

Numbers refer to the percentage reduction in cell numbers after treatment.
n = number of animals.

Effect of some antirheumatic immunoactive and anti-inflammatory drugs on type II collagen arthritis in the rat

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Trentham, Townes & Kang (1977) described the induction of arthritis in rats, following sensitization with type II collagen (but not types I or III) given as an emulsion in Freund's incomplete adjuvant; both humoral and cellular immune responses were stimulated. We now report the evaluation of selected anti-rheumatic, immunoactive and anti-inflammatory drugs on this arthritis using type II collagen prepared from bovine articular cartilage.

In initial studies it was found that 40–50% of the Alderley Park Strain 1 rats sensitized with 0.5 mg of type II collagen developed monolateral or bilateral hind limb swelling. X-ray monitoring of the responder rats showed that by day 22 after sensitization the ankle joints showed soft tissue swelling but no marked change to the bone structures. By day 56 when swelling had waned and there was no systemic inflammation considerable erosion of the joint was evident. Antibodies to type II collagen were measured using a haemagglutination assay with type II collagen coated human red cells. Sensitized rats had high antibody titres (up to $\log_2$ titre of 19) and the titre was similar in responders and non responders. D-penicillamine (30 mg/kg^{-1} p.o.) dosed for 30 days after development of hind limb inflammation did not reduce the swelling nor did it reduce the antibody titre. When the drug was dosed from day 7 after sensitization no effect on the incidence of development of arthritis was noted. In another experiment a range of anti-rheumatic, immunoactive and anti-inflammatory drugs were tested. The drugs dosed were levamisole (20 mg/kg p.o.), gold thiolmalate (20 mg/kg s.c.), tilorone (30 mg/kg p.o.), azathioprine (20 mg/kg p.o.), indomethacin (3 mg/kg p.o.) and dexamethasone (1 mg/kg s.c.). Dexamethasone stood out as the only compound markedly to reduce both hind limb swelling and antibody titre. Delayed hypersensitivity (D.H.) to type II collagen has been measured by a radiometric assay (McCune, Trentham & David, 1980). Dexamethasone (0.5 mg/kg s.c.) dosed after the development of arthritis reduced the D.H. response whereas indomethacin (3 mg/kg p.o.), levimasole and azathioprine (20 mg/kg p.o.) had no effect. Dexamethasone (0.5 mg/kg) significantly protected the joint from radiological changes seen in the arthritic control group.

Though type II collagen arthritis resembles adjuvant arthritis in the time-course to onset of immunologically mediated inflammation, serum from adjuvant rats was not found to contain antibody to type II collagen.

We are grateful to Dr N.A. Roberts and Mr P.A. Robinson for the preparation of type II collagen.

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Salai guggal: a promising anti-arthritic and anti-hyperlipidaemic agent

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Guggal obtained from *Commiphora mukul* is commonly employed for treatment of rheumatism in the Indian system of medicine; some lesser known varieties of guggal have been investigated by us with a view to finding greater activity. Salai guggal, the oleogum of *Boswellia serrata* has shown the most promising results.

Different solvent fractions have been prepared and the defatted alcoholic fraction has shown cholesterol lowering and anti-inflammatory activity and was, therefore, further studied.

In the study of its anti-inflammatory and anti-arthritic activities, Salai guggal treatment caused inhibition of the carrageenin induced rat hind paw oedema by 39–75% and 65–73% in 50–200 and 50–100 mg/kg administered orally (p.o.) and intraperitoneal (i.p.) treatments respectively compared to 47% inhibition seen with phenylbutazone (50 mg/kg p.o.). The anti-inflammatory effect was equally well marked in adrenalectomized rats. In the anti-arthritic study on the mycobacterial adjuvant induced poly-arthritis in rats, Salai guggal showed 34% and 49% inhibition of paw swelling with 50 and 100 mg/kg p.o. doses compared to controls. Phenylbutazone (50 and 100 mg/kg p.o.) showed 26 and 60% inhibition. The development of secondary symptoms was also inhibited and the body weight loss

and rise in serum transaminases were markedly less compared with controls. The P values in the above findings were less than 0.01–0.001.

In the antihyperlipidaemic study, Salai guggal in a dose of 100 mg/kg p.o. decreased the serum cholesterol and triglyceride concentrations as follows: 42% and 60% in triton treated rats, 45% and 48% in weanling rats, 25% and 62% in cockerels fed on a high lipid diet. In the anti-atherosclerotic study on rabbits fed on high lipid diet for 3 months, treatment with Salai guggal showed 32–46% and 53–62% decrease in serum cholesterol and triglyceride levels respectively monitored at weekly intervals. These rabbits were sacrificed and examined for atherosclerotic lesions. The aortae from Salai guggal treated rabbits were either all clear or showed only slight atherosclerotic lesions as compared to atheromateous plaque formation in the region of aortic arch and the occurrence of streaks or patches in the descending aorta in the controls.

Salai guggal did not show any anti-pyretic, analgesic, ulcerogenic, behavioural or cardiovascular effects tested in 100–500 mg/kg p.o. in rats, dogs and rabbits. Mice showed no mortality up to 2 g/kg p.o. Chronic feeding for 4 months in rabbits revealed no toxic effects.

The mechanism of action of Salai guggal is not clear. As an anti-inflammatory agent it seems to be unlike the known steroidal or non-steroidal anti-inflammatory agents in as much as it is free from the undesirable side effects associated with the above agents. Because of the high promise which this plant product offered, controlled clinical trials were initiated in patients suffering from rheumatoid arthritis. Results of the clinical trials on some 120 patients in this study have proved its high therapeutic value in the treatment of rheumatoid arthritis.

A relationship between putrescine and the anti-inflammatory activity of sponge exudate

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The anti-inflammatory effects of the inflammatory exudate obtained by the implantation of polyester sponges into rats are well known (Robinson & Robson, 1966) also the anti-inflammatory component within the exudate has been reported to be synthesized *de novo* by the liver (Billingham, Gordon & Robinson, 1971). We have recently reported (Bird & Lewis, 1979) that sponge exudate was capable of stabilizing the membranes of inflammatory cells and that dialysable molecules are released from it which are anti-inflammatory.

It has been suggested that dexamethasone exerts its anti-inflammatory effect causing *de novo* synthesis of the polyamine putrescine which may be implicated in the production of anti-inflammatory mediators (Bartholeyns, Fozard & Prakash, 1980). In this work we have explored the direct anti-inflammatory properties of putrescine using animal models and investigated the possibility that putrescine is responsible for the anti-inflammatory action exhibited by sponge exudate.

We first assayed sponge exudate for putrescine levels and also for its ability to release putrescine *in vitro*.

Using aseptic techniques, sealed bottles were prepared containing 100 cm^3 of phosphate buffer (pH 7.4) and dialysis sacs enclosing 1 cm^3 of a 100 $mgcm^{-3}$ solution of sponge exudate. After incubation for seven days at 37°C samples of the dialysate and original exudate solution were taken and levels of putrescine determined by coupling with dansyl-chloride and measuring the fluorescene of the DANS-putrescine after separation by thin layer chromatography (Seiler & Askar, 1971).

The sponge exudate was found to contain 6.8 μg/100 mg of putrescine and a 1 cm^3 sample of the dialysate contained 6.1 μg of putrescine.

The carrageenan rat foot oedema test (Winter, Risley & Nuss, 1962) was used to evaluate the action of putrescine on an acute inflammatory model. Groups of rats were dosed with putrescine at levels of 50 μg, 500 μg and 5000 μg per kg body weight 1 h before an injection of carrageenan (0.05 cm^3 of 2% w/v in saline) into the subplanter region of the foot. At both 3 and 5 h after injection of the carrageenan all levels of putrescine were found to be significantly anti-inflammatory ($P < 0.01$), but a dose dependant relationship was not observed. The irritancy of putrescine at these levels was insignificant when compared to saline using the method described by Atkinson & Hicks (1971).

Adjuvant arthritis in the rat was used to evaluate the action of putrescine on a chronic model (Newbould, 1963). In this case putrescine at a dose of 50 μg/kg/day was found to significantly ($P < 0.05$) inhibit the chronic inflammation while a dose of

500 μg $kg^{-1}day^{-1}$ was ineffective. Thus, we have shown that sponge exudate both contains and is capable of releasing putrescine. Furthermore, putrescine has a direct anti-inflammatory effect in both acute and chronic animal models and is not irritant. It is possible, therefore, that the anti-inflammatory activity of sponge exudate may be attributed, at least in part, to the presence of putrescine.

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The effect of aspirin on the release of thromboxane following acute myocardial ischaemia; relation to early arrhythmias

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Histamine receptors mediating vasoconstriction and vasodilatation in the gastric vasculature of the rabbit

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Prostacyclin is stablized by serum albumin

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Prostacyclin (PGI_2) is rapidly hydrolysed in aqueous solution at neutral or acidic pH (Cho & Allen, 1978; Dusting, Moncada & Vane, 1978). However, in plasma PGI_2 is relatively stable (El Tahir, Betteridge, Reckless & Williams, 1980). We have performed experiments demonstrating that this effect can be mimicked by solutions containing serum albumin.

Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared from citrated human blood (1 vol 3.15% citrate: 9 vol blood). PGI_2 (40 ng) was added to 4 ml of either PPP or Krebs solution containing bovine or human serum albumin (BSA or HSA) aerated with 5% CO_2 in O_2 and incubated at 37°C. The pH of the incubates was 7.4 ± 0.1 throughout. The biological activity of the PGI_2 was measured at intervals by bracket assay on ADP-induced platelet aggregation.

The biological half-life of PGI_2 increased linearly (r = 0.98) with increasing concentration of Cohn fraction V BSA over the range 0–4.5% w/v (Table 1). Incubation with Cohn fraction V HSA (3% w/v) produced only a modest increase in stability of PGI_2. However, fatty acid free HSA (HSA FAF) 1.5 or 3.0% w/v considerably prolonged the biological half-life of PGI_2 (Table 1). PPP was also effective in increasing the half-life of PGI_2 ($T½$ ranged from 14.2 to 23.3 min).

The ability of fatty acids to decrease the stabilization of PGI_2 suggests that the protective effect of albumin may be due to binding to the PGI_2. PG-albumin interactions are likely to involve the carboxylate group and the ω-terminus of the PG (Spector, 1975; Gueriguian, 1976). Binding could prevent the carboxylate anion acting as an electrostatic catalyst or the protonated carboxylic acid group providing an intramolecular proton source (Chiang, Kresge & Cho, 1979).

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Table 1 Biological half-life of PGI_2 in Krebs solutions at pH 7.4 and 37°C

Addition	*Initial Concn. of PGI_2* (ng/ml)	$T½$ (min)	*Correlation Coefficient*
None	12.0	2.6	0.999
	6.5	2.5	1.000
1% BSA V	13.0	6.8	0.998
	13.5	7.5	1.000
2% BSA V	12.0	12.1	1.000
	15.0	10.4	0.974
3% BSA V	13.0	12.9	0.975
	14.0	14.4	0.974
4.5% BSA V	11.0	16.6	1.000
	11.0	19.2	0.987
3% HSA V	14.5	6.0	0.900
	9.5	13.7	0.998
	11.0	5.9	0.982
1.5% HSA FAF	11.0	12.7	0.988
	11.0	14.2	0.999
3.0% HSA FAF	10.0	43.0	0.962
	11.5	38.2	0.984
3.0% BSA FAF	15.0	14.1	0.968

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Prostaglandins and human lung cancer

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The amounts of prostaglandin-like material (PG-1m) extracted from human mammary carcinomas correlate with tumour invasiveness, spread to bone, and early death from cancer (Bennett *et al.*, 1977, 1979).

We now report measurements of PG-1m extracted from human lung carcinomas and lung tissue obtained fresh from lobectomy and pneumonectomy specimens from 108 men and 26 women. Each tissue was divided, and one part processed histologically. The other part was cut into small pieces, washed in Krebs solution, and samples were homogenized either in acid ethanol (basal PG-1m) or in Krebs solution which allows PG synthesis from released endogenous precursors (total PG-1m) (Bennett, Stamford & Unger, 1973). The PG-1m was extracted (Unger, Stamford & Bennett, 1971) and assayed as ng PGE_2 equivalents/g moist tissue, using rat gastric fundus. The results were analyzed using the Mann-Whitney U test, Wilcoxon matched pairs sign-ranked test, and the Kruskal-Wallis one way analysis of variance. Tumours were classified according to their histological type and degrees of differentiation (Table 1).

Overall, tumours yielded more PG-1m than did normal tissue (Table 1). However, the amounts from the individual tumour types varied, unlike normal tissue which showed no significant differences between the groups (basal $P = 0.59$; total $P = 0.36$). Well-differentiated adenocarcinomas yielded highest amounts of PG-1m, whereas the anaplastic tumours yielded the lowest amounts ($P < 0.01$ compared with large cell anaplastic tumours). In contrast, well differentiated (best prognosis) tumours yielded less PG-1m than did poorly differentiated squamous tumours, although large-cell anaplastic tumours (mainly undifferentiated squamous tumours) yielded low amounts of PG-1m. The various tumours arise from different cell types and cannot be compared, and the extent to which the PG-1m values reflect tumour type, cellularity, degree of differentiation, or other histological features such as host cells, remains to be determined.

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Table 1 The amounts of tumour PG-1m (ng PGE_2 equivalents/g) are shown as median values with semiquartile ranges in parentheses, or ranges where $n \leq 4$. The tentative prognosis, based on tumour histology, is shown as 1 = best, 4 = worst; with squamous and adenocarcinomas, prognosis is shown as equal but opinion on this is divided (Campobasso, Invernizzi, Musso & Berino, 1974; Hyde, Yee, Wilson & Patno, 1965.) In some cases basal values were not obtained due to shortage of tissue.

Tumour type	*Tentative prognosis*	*n*	*Basal PG-1m*	*P*	*Total PG-1m*	*P*
Squamous, well differentiated	1	48	21 (12–47)	c	47 (22–110)	c
Squamous, poorly differentiated	2	28	48 (19–190)		145 (55–460)	
Adenocarcinoma, well differentiated	1	13	100 (49–830)	b	170 (88–1800)	a
Adenocarcinoma, poorly differentiated	2	7	41 (34–62)		86 (59–110)	
Adenosquamous		3	32–55		120–300	
Large cell anaplastic (mostly undifferentiated squamous)	3	30	15 (5–39)		37 (17–145)	
Small cell anaplastic	4	4	0.8–14		3–44	
Overall tumour values		133	28 (11–79)	d	70 (24–190)	d
Normal tissue		132	16 (6–38)		33 (14–63)	

a, $P = 0.1$; b, $P < 0.05$; c, $P < 0.01$; d, $P < 0.0001$

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Enhanced anti-cancer effect by combining cytotoxic drugs with the prostaglandin synthesis inhibitor flurbiprofen

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Prostaglandin synthesis inhibitors can reduce tumour size (see Bennett, 1979). Our preliminary data (Berstock, Houghton & Bennett, 1979) indicate that flurbiprofen given with chemotherapy to mice with resected primary tumours, prolonged survival and lessened local tumour recurrence. These results combined with three other experiments show benefit with flurbiprofen alone, and particularly when combined with low doses of cytotoxic chemotherapy.

Approximately 10^6 NC carcinoma cells were injected s.c. into the left flank of 250 female WHT/Ht mice (Hewitt, Blake & Walder, 1976). Drug treatments by mouth commenced on day 13; primary tumours were excised the next day under anaesthesia. Each experiment contained four groups: controls (0.1 ml syrup twice daily); chemotherapy (melphalan 1.4 mg/kg, methotrexate 2 mg/kg); flurbiprofen (2.5 mg/kg twice daily); chemotherapy and flurbiprofen. Treatment continued until the animals died, or were killed if death from cancer was imminent or at day 120. Post-mortem examinations assessed the incidence of distant metastases (mainly in lungs) and recurrence at the excision site.

Survival was similar in the control and chemotherapy groups, but longer in mice given flurbiprofen ($P = 0.04$), and even longer with flurbiprofen plus chemotherapy ($P = 0.02$ compared with chemotherapy). Compared to controls, mice given flurbiprofen alone or with chemotherapy had a lower incidence of distant metastases, since at day 120 there were more disease-free animals (Table 1). Local recurrence at the excision site tended to be less in the flurbiprofen group, and was clearly less with combined treatment (Table 1). No tumour growth occurred in a contralateral excision made at the time of tumour removal (10 mice in each group). Thus

Table 1 Mouse survival and tumour recurrence at the excision site

Treatment group	*n*	*Survival in days (median and semi-quartiles)*	*Survivors*		*Mice with local tumour recurrence*
			Disease-free	*Total*	
Syrup (control)	51	45 (35–57)	4	7	31
Chemotherapy (days 1–3, 8–10, 15–17 after excision)	72	49 (38–69)	9	10	33
Flurbiprofen	56	53.5 (42–118)*	15*	15	26
Chemotherapy and flurbiprofen	71	63 (46–109)*	16*	16	15**

* $P < 0.05$.

**$P < 0.005$ compared with controls using life-table analysis or Fishers' exact probability test.

tumour in the excision site does not seem due to trapping of circulating malignant cells within a sutured wound.

We conclude that flurbiprofen combined with cytotoxic chemotherapy prolongs survival time of mice after excision of primary NC tumours, and reduces the recurrence at the excision site.

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Predictions and performance in pharmacology in a pharmacy course: a correlative study

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Teachers of undergraduate courses have attempted from time to time to determine the degree of correlation existing (if any) between entry qualifications to a course and performance during and even after that course. (Wankowski, 1978; Tinkler, 1978; Black & Stenhouse, 1976; Veloski, *et al.*, 1979.) In the study reported here we have attempted to correlate all academic subjects taken during the three years of a pharmacy course, not only between the subjects themselves but with such other features as sex and headteachers predictions. In order to do so all relevant data from 99 students presenting for third year examinations in 1980 were prepared for inter-correlation analysis by computer as previously described (Foy & Senior, 1974). The resultant print-out was checked for level of significance by two-sided '*t*' test within the programme, the minimum level being taken as $P > 0.05$. From this mass of data, the more interesting and important correlations will be discussed.

From the whole matrix it is clearly shown that as the student proceeds through the course the correlations between subjects become increasingly significant, revealing the degree of interrelationship which develops in the course between the contributing subject areas.

The predicted average 'A' level grade, taken from the UCCA form, shows a significant correlation between 'A' level performance, success in first year examinations and the average mark in third year. The *actual* average 'A' level grade correlates only with examination results in physiology and physical chemistry at the end of year 1 and seems to be a less useful measure of future success or otherwise. Individual 'A' level results, however, are more useful especially those obtained in chemistry which are significantly related to all first year subjects and the average for second year subjects. It is interesting to note that in the third year, where applied subjects are studied, the biology 'A' level result gives a significant correlation with the results in all subjects. The other two 'A' levels studied, physics and mathematics were not good predicters of future performance. Physiology in year 1 correlates with all other first year subjects and with second year subjects excepting organic chemistry. In the third year of the course, where subjects become more applied to the practice of pharmacy, the first year physiology performance loses its significance. There is no correlation between physiology and applied pharmacology, perhaps suggesting that these subjects require different skills. The second year subject, experimental pharmacology, correlates with all subjects and averages for all years of the course. The third year result in applied pharmacology correlates significantly with physical chemistry in year 1, all second year subjects, except pharmacognosy, and all third year subjects.

In summary, we suggest that emphasis should be placed on 'A' level predictions and actual scores in chemistry and biology when selecting candidates for a degree course containing a high proportion of pharmacology.

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Naloxone enhances neophobia

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Several studies report that naloxone, an opiate receptor antagonist, reduces deprivation induced eating and drinking. However, in the present study, naloxone (5 mg/kg, i.p.) did not reduce food intake of rats maintained on a 22 h deprivation—2 h feeding schedule. In contrast, naloxone (5 mg/kg, i.p.) progressively reduced water intake in deprived animals to 46% of saline treated controls. No effects of naloxone (1.5 mg/kg) on established bar pressing for food or water were observed with either continuous or fixed ratio schedules of reinforcement. However, naloxone (5 mg/kg) accelerated extinction of responding when food and water were no longer available.

Animals treated with naloxone (5 mg/kg) during training of the bar-pressing ate only 26% of the pellets delivered whereas controls ate all pellets delivered. Since the animals had not previously experienced the pellets or the operant apparatus, the possibilities arose that naloxone effects were due to enhanced neophobic effects of the novel food pellets or novel apparatus cues, or were due to conditioned taste aversion. Therefore, food novelty, apparatus novelty and timing of injections were independantly varied in different groups of 8–10 rats treated with saline or naloxone. Rats were maintained at 85% body weight with 12 g lab chow per day. On experimental days 46 small pellets (Cambden instruments) were placed on a small petri dish in the home cage of some groups or released from a pellet dispenser in an operant box for other groups. The dependent variable was the number of pellets eaten over 15 minutes.

Naloxone (1.5 mg/kg, i.p.) injected 5 or 20 min before test almost completely suppressed pellet eating if the animals had not been previously exposed to the pellets ($P < 0.01$, 't' test vs saline groups). This occurred independently of whether tests were carried out in the home cage or novel operant box. Naloxone induced suppression of pellet eating was almost abolished in either environment if animals had been exposed to the pellets for the five preceeding days in the same or different environment to the test environment. Naloxone (5 mg/kg, i.p.) administered immediately after pellet eating tests failed to suppress subsequent pellet eating.

Thus, naloxone suppressed pellet eating if the pellets were novel and if naloxone was administered before eating tests. The results suggest naloxone enhances neophobic effects of novel foods and that suppression of novel pellet eating is not due to enhanced effects of novelty of apparatus cues or to conditioned taste aversion.

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Naloxone reversal of the chronic effects of morphine on rat liver and brain tryptophan metabolism

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Despite controversy, there is considerable evidence that chronic morphine administration enhances brain 5-hydroxytryptamine (5-HT) synthesis and turnover (see Way, 1972 and references cited therein). The mechanism of this enhancement has, until recently, been little understood. Messing, Flinchbaugh & Waymire (1978) reported that chronic morphine administration increases brain tryptophan concentration and that this effect is not reversed by naloxone, which blocks the above enhancement (Shen, Loh & Way, 1970). We have recently (Badawy, Punjani & Evans, 1981) confirmed the finding that chronic morphine administration increases brain tryptophan concentration, and obtained evidence strongly suggesting that the drug acts by increasing the availability of circulating tryptophan to the brain secondarily to the previously reported (Badawy & Evans, 1975) NADPH-mediated inhibition of liver tryptophan pyrrolase activity. In view of these latter findings and the reported (Messing *et al.*, 1978) failure of naloxone to reverse the morphine effect on brain tryptophan concentration, we have re-examined the naloxone effects and found that it reverses all the morphine effects on liver and brain tryptophan metabolism.

Locally bred male Wistar rats (150 g ± 7% at the start of experiments) were given morphine sulphate in drinking water for 3 weeks as described previously (Badawy & Evans, 1975). At 2 h before death, both control and morphine-treated rats received an intraperitoneal injection of either naloxone hydrochloride (1 mg/kg) or an equal volume (2.5 ml/kg) of 0.9% NaCl. All determinations were performed by standard procedures (for references, see Badawy, Punjani, Evans & Evans, 1980).

Chronic morphine administration inhibited the total pyrrolase and apoenzyme activities by 44 and 71% respectively, and increased the concentrations of liver, free serum, total serum and brain tryptophan and brain 5-HT and 5-HIAA by 24, 22, 26, 31, 20 and 24% respectively. Tryptophan binding to serum proteins was not affected. These findings (and supporting evidence) therefore strongly suggest that chronic morphine administration enhances brain 5-HT synthesis by increasing the availability of circulating tryptophan to the brain secondarily to the inhibition of liver tryptophan pyrrolase activity.

If this is the mechanism of action of morphine on 5-HT synthesis, then the naloxone reversal of the latter should also be associated with reversal of all the morphine effects on liver tryptophan metabolism and tryptophan disposition. That this is so will be presented. The naloxone reversal of the morphine inhibition of pyrrolase activity was also achieved as early as 15 min after antagonist administration. Finally, the morphine-induced increase in liver [NADPH] (which causes the pyrrolase inhibition) was also reversed by naloxone. These findings with naloxone therefore not only provide further support to our proposed mechanism of action of morphine on 5-HT synthesis, but also illustrate a new and unique aspect of the actions of this opiate antagonist.

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Effects of nicotine on intracranial self-stimulation in non-tolerant rats

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In non-tolerant rats, nicotine may depress or facilitate intracranial self-stimulation (Olds & Domino 1969; Newman 1972; Pradhan & Bowling 1971). The findings may reflect an action of nicotine on central reward mechanisms or an effect on the animals' ability to perform motor tasks.

Male hooded rats (n = 11) were trained to shuttle between two selected ('ON') arms of a Y maze in order to obtain electrical stimulation of the medial forebrain bundle. A pulse train (duration 200 ms) was delivered when the rat broke an infra-red photobeam at the end of an 'ON' arm. Repetitive entries into the same 'ON' arm were not rewarded. Every 67 s, stimulation was made available from a different pair of arms. Six current levels equally spaced on a logarithmic scale (25, 35.3, 50, 70.7, 100, 141 μA) were presented in random order four times during each session.

Undrugged subjects responded faster, and with a greater proportion of rewarded responses, the higher the stimulation current. Two independent measures served to control for drug effects upon motor performance: unrewarded entries into the third ('OFF') arm, and any responses made during signalled periods when current was unavailable in any arm (time-out). Test sessions lasted for 80 min and were begun immediately after subcutaneous injection of nicotine tartrate (0, 25, 50, 100, 200, 400 μg/kg base: BDH; neutralized with NAOH). Each rat was tested under all drug conditions, given in a randomized sequence. Test days were three or four days apart. Each rat was treated as its own control. Data were analyzed by multivariate analysis of variance, and comparisons of trends over dose and current were made.

In the first 20 min after injection, all responding, whether rewarded or unrewarded, was depressed by nicotine in a dose-related manner ($P < 0.001$) and ataxia was observed at higher doses. In the second half of the session (40–80 min after injection), nicotine tended to enhance the rate of rewarded responding at low or medium current levels ($P < 0.02$); but at the highest current level, rewarded responding was actually depressed ($P < 0.02$); both effects were dose-related. This result is consistent with a rate-dependent action of nicotine (Pradhan & Bowling 1971). Responding during time-out was also increased 40–80 min after injection in a dose-related manner ($P < 0.0005$).

The effects of nicotine on the rats' ability to detect, and respond for, electrical brain stimulation were assessed by measuring the proportion of rewarded entries out of the first ten responses that were made each time the 'ON' arms changed round in the second half of the session. The rats' accuracy of responding was not enhanced in conjunction with the increased activity which was produced by nicotine.

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Effects of chronic nicotine administration on plasma corticosterone and brain 5-hydroxyindoles in stressed rats

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Previous studies (Benwell & Balfour 1979, 1980) have shown that the chronic administration of nicotine to unstressed rats causes a reduction in the levels and rate of synthesis of 5-hydroxytryptamine (5-HT) in the hippocampal formation, changes which have been related, tentatively, to the rebound increase in plasma corticosterone levels observed when the nicotine is withdrawn. However, behavioural studies indicate that nicotine-dependence is best demonstrated in stressful situations (Morrison 1974) and, therefore, in the present study, the effects of chronic nicotine administration in a stressful situation have been investigated.

Rats were treated chronically with nicotine (0.4 mg/kg/day s.c.) or saline (controls) for 39 days as described previously (Benwell & Balfour 1979). On day 40, half the nicotine-treated rats received their

normal nicotine injections while half (withdrawn group) received saline. The controls received saline as usual. In some of the experiments the rats were exposed to psychological stress for 30 min after each injection (i.e. on 40 occasions) by being placed individually on elevated platforms (Balfour & Reid 1979) whereas, in other experiments, they were exposed to the stress only once on day 40. Immediately after the final session on the platform (and 30 min after the last injection) the rats were killed by cervical dislocation and the 5-HT and 5-hydroxyindole acetic acid levels in hippocampus, hypothalamus and the remainder of the brain measured by the method of Curzon & Green (1970). The plasma corticosterone levels were measured by the method of Mattingly (1962).

Neither chronic nicotine administration nor its withdrawal had any significant effects on plasma corticosterone or the levels of 5-hydroxyindoles in any of the brain regions studied when the rats were exposed to psychological stress for the first and only time on day 40.

If rats were exposed to the stressful situation repeatedly, by day 40 they had adapted so that the plasma corticosterone levels in saline-treated rats ($14 \pm 1\ \mu g/100$ ml) was not significantly different from those found in unstressed controls ($11 \pm 2\ \mu g/100$ ml). However, the administration of nicotine, prior to each session on the platform, blocked this adaption to the extent that the corticosterone levels of the nicotine-treated ($25 \pm 4\ \mu g/100$ ml) and withdrawn rats ($26 \pm 4\ \mu g/100$ ml) remained high and were significantly different ($P < 0.05$) from those found in the saline-treated rats. Neither chronic nicotine nor its withdrawal significantly altered the 5-hydroxyindole levels in the brains of these rats although chronic nicotine did reverse the positive significant correlation ($P < 0.01$) found to exist between hippocampal 5-HT and the plasma corticosterone concentration in the saline-treated rats repeatedly exposed to the stress procedure. The significance of these changes to the development of nicotine-dependence remains unclear.

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5-Hydroxytryptamine-dependent behaviours induced by (+)-amphetamine have different relationships with dopamine

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(+)-Amphetamine at high dosage causes little classical dopamine (DA) behaviour but provokes numerous 5HT-dependent behaviours: (a) backward walking and circling which depend on concurrent DA and 5HT release (Curzon, Fernando & Lees, 1979), (b) reciprocal forepaw treading, hind limb abduction and body shakes; involvement of DA here is controversial (Heal, Green, Boullin & Grahame-Smith, 1976; Sloviter, Drust & Connor, 1978; Fernando, Lees & Curzon, 1980). We have therefore investigated the effects of bilateral lesions of DA tracts on these behaviours.

Male Sprague-Dawley rats (190–210 g) were given desipramine (25 mg/Kg i.p.) to protect noradrenergic neurones and stereotaxically injected 30–60 min later (under pentobarbitone anaesthesia, 60 mg/Kg) with 6-hydroxydopamine HBr (6OHDA) in ice-cold NaCl (0.9%) containing ascorbic acid (1 mg/ml) at 1 μl/min. 6OHDA was injected into the following sites (coordinates from König & Klippel, 1963) in

Table 1 Effects of 6OHDA lesions on regional DA concentrations and 5HT dependent behaviours provoked by amphetamine (25 mg/kg i.p.)

	% of control values					
	DA		*Behavioural scores*			
Lesion	*Striatum*	*Nucleus accumbens*	*Backward walking*	*Forepaw treading*	*Hindlimb abduction*	*Body shakes*[1]
Substantia nigra	30***	65***	3*	6*	128	1425**
A10	45***	44***	0***	26**	187**	186
Striatum	39***	88	18**	154*	190**	105
Nucleus accumbens	85	51***	86	103	126	500

DA values: n/group = 6–14 rats. Behavioural Scores: n/group = 4–8 cages of 2–3 rats/cage (12–16 rats/group). Differences from scores of concurrently studied unlesioned rats: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
[1] Only marginally present in unlesioned rats.

amounts indicated: substantia nigra (8 μg/4 μl), ventral tegmental area (A10) or nucleus accumbens (8 μg/2 μl), striatum (16 μg/4 μl). Controls were treated identically except that they were stereotaxically injected with medium only. Two weeks later, (+)-amphetamine sulphate (25 mg/kg as base) was given i.p. and behaviour scored 'blind' (Fernando & Curzon, 1981). Rats were killed one week later for 5HT and DA determinations in striatum and nucleus accumbens as previously indicated (Fernando & Curzon, 1981). Decreases in DA levels are shown in Table 1; 5HT levels were unaffected.

Backward walking was virtually abolished when striatal DA was decreased but unaltered by specific nucleus accumbens DA depletion. Forepaw treading decreased when DA was reduced in both regions but increased when only striatal DA fell. Hindlimb abduction increased in 2 of the 3 groups in which striatal DA was lowered. Body shakes were increased after nigral lesions.

These different influences of 6OHDA lesions on components of 5HT-dependent behaviour together with the effects of DA antagonists (Fernando *et al.*, 1980) and spinal lesions (Jacobs & Klemfuss, 1975) indicate that individual components reflect distinct 5HT-DA interactions.

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Withdrawal from chronic treatment with 5-hydroxytryptamine antagonists enhances the behavioural response to 5-methoxy-N'N'-dimethyltryptamine

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It has recently been shown that a number of antidepressants can block central 5-hydroxytryptamine (5HT) receptors (Ögren, Fuxe, Agnati, Gustafsson, Jonsson & Holm, 1979) and these drugs take 10–20 days to have a clinical effect. It is therefore of interest to determine whether prolonged pharmacological blockade of 5HT receptors causes adaptive changes in post-synaptic 5HT mechanisms which may be related to the delay in onset of therapeutic activity of the antidepressants. In the present study we compare the effects on a 5HT receptor mediated behavioural syndrome of the 5HT receptor antagonist metergoline (Fuxe, Agnati & Everitt, 1975) with those of propranolol which also blocks 5HT receptors (Middlemiss, Blakeborough & Leather, 1977) and the antidepressant amitriptyline following acute, chronic, and on withdrawal from chronic, treatment.

Groups of 12 male Wistar rats (200–250 g) received twice daily intraperitoneal injections of metergoline (2 mg/kg) propranolol (15 mg/kg) amitriptyline (10 mg/kg) fluoxetine (2 mg/kg) or vehicle (2 ml/kg i.p.) for 14 days. Control animals received either saline or saline containing 0.6% ascorbic acid (metergoline control group only). 30 min after the first and in half the animals after the last injection, the 5HT agonist 5-methoxy-N',N'-dimethyltryptamine was administered (2.5 mg/kg i.p.) and the resulting behavioural syndrome was scored using a subjective scoring technique (Aldridge, Glithero, Hull, Jarmann, Johnson & Marsden, 1979). Locomotor activity was monitored simultaneously using a doppler shift radar activity monitor (Marsden & King, 1979). In the case of metergoline, propranolol and amitriptyline the other half of the group of 12 were tested 3 days after cessation of drug treatment.

The behavioural syndrome observed following 5 MeODMT administration was significantly reduced ($P < 0.01$ MannWitney U test) by acute administration of metergoline (71%) amitryptyline (31%) and propranolol (55%). Fluoxetine was without significant effect at the dose used. Following 14 days treatment the behavioural syndrome was still significantly ($P < 0.05$) attenuated in the metergoline and amitriptyline treated groups, but not in animals treated with propranolol or fluoxetine. Three days after cessation of drug treatment a significantly ($P < 0.05$) potentiated response to 5 MeODMT was observed in the metergoline (41%) propranolol (70%) and amitriptyline (24%) treated groups compared with appropriate controls. Although metergoline reduced the 5 MeODMT induced behavioural syndrome, ambulatory activity was enhanced following acute and chronic metergoline administration. In contrast, acute and chronic treatment with propranolol and amitriptyline reduced ambulatory activity following 5 MeODMT administration.

The effects of metergoline on ambulation after 5 MeODMT confirm previous studies (Deakin & Green, 1978), while the absence of this effect with either propranolol or amitriptyline may reflect known actions of these drugs on other receptors.

The results show that 5HT antagonists, including the antidepressant amitriptyline, produce behavioural supersensitivity in response to a 5HT agonist after a period of drug withdrawal from chronic treatment. Fluoxetine, on the other hand inhibits 5HT re-uptake, has no effect on 5HT receptor binding *in vitro* (Ögren, *et al.*, 1978) and failed to alter the response produced by 5 MeODMT. It remains to be determined whether the effect of amitryptyline on 5HT receptor responsiveness is important for its clinical actions.

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Core temperature changes following electrical stimulation of the midbrain raphe nuclei and intrahypothalamic injection of tryptamine in the rat

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We have previously reported that intrahypothalamic injection of tryptamine caused a hyperthermia in the rat, whereas intrahypothalamic injection of 5-HT caused a hypothermia. Since these two different types of response also had different susceptibilities to blockade by indoleamine-receptor antagonists it seems possible that there are two different receptor populations within the preoptic anterior hypothalamus (PO/AH) which mediate the effects of 5-HT and tryptamine (Cox, Lee & Martin, 1981). Recently, an endogenous indoleamine other than 5-HT has been suggested to be involved in the heat production pathway in the sheep (Bligh, Silver & Smith, 1979). Therefore in the present study, we have investigated the possibility of the existence of a similar pathway in the rat.

Male Alderley Park SPF rats were used at an ambient temperature of 21 ± 1°C. Drugs were injected into the PO/AH through previously implanted guide cannulae in a dose volume of 1 μl. The raphe nuclei were stimulated electrically by chronically implanted electrodes. Core temperature was measured by means of a rectal probe.

Tryptamine (1 μg) was injected at different points throughout the hypothalamus. The most sensitive site was found to be located within the PO/AH (co-ordinates: AP 2.0–2.8 mm, L. 0.5–2.0 mm and D. 7.0–8.0 mm, according to the atlas of Pellegrino & Cushman, 1967). Injections with their perimeters outside this sensitive region were ineffective. The sensitive area for 5-HT was found to be approximately the same as that for tryptamine (co-ordinates: AP 2.2–3.0 mm, L. 0.5–1.5 mm and D. 7.0–8.0 mm).

Continuous electrical stimulation (40 min) of midbrain raphe nuclei caused a current-related hyperthermia in the rat, which could be blocked by methergoline (1.25 mg/kg, i.p.). This antagonist has been shown to exert some selectivity towards tryptamine when compared with 5-HT (Cox, Lee & Martin, 1981).

These results suggest that there may be an endogenous tryptamine-like system in the heat production pathway in the rat since (i) there is an area sensitive to tryptamine located in the PO/AH, an area known to be involved in thermoregulation (Hardy, 1961); (ii) electrical stimulation of the midbrain raphe nuclei produced a tryptamine-like hyperthermia. It is of course possible that an indoleamine other than tryptamine is involved and it is of interest that a non 5-HT indoleamine pathway from the midbrain raphe has been previously described (Bjorklund, Falck & Stenevi, 1971). One of the candidates for this unknown transmitter was 5-methoxy-tryptamine, a compound known to be present in rat hypothalamus (Green, Koslow & Costa, 1973). It is of interest that 5-methoxytryptamine also caused a dose-related hyperthermia after intrahypothalamic injection in the rat.

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