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The influence of age and sex on the pharmacokinetics of thiopentone

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Previous studies have indicated that induction of anesthesia with thiopentone can be achieved with smaller doses in elderly patients than in younger patients (Christensen & Andreasen, 1978; Dundee, 1954). In a recent pharmacokinetic study of thiopentone in young women and men (Christensen *et al.*, 1980) a significantly smaller V_3 and a shorter elimination half life was found in the young men. Furthermore, it was suggested that the redistribution rate constant K_{12} was predominant in determining the sleep dose. In the present study a group of eight elderly women and a group of eight elderly men were established with the aim of making a comparison possible between all four groups of patients.

Table 1 shows mean values and ranges of pharmacokinetic parameters in the two groups of elderly patients. Neither the V_3's nor the elimination half lives were significantly different and the same is true for the other calculated parameters. The dose necessary for induction was significantly smaller in the elderly patients (3.9 mg/kg body weight -70% of the dose necessary in the young individuals ($P < 0.05$)). The concentration of thiopentone in the venous blood 20 s after the disappearance of the eyelash reflex was 34.3 μg/ml in the elderly men and 19.2 μg/ml in the elderly women ($P < 0.01$). The volumes of distribution, V_2 and V_3, were significantly larger in the elderly patients (on the average increases between 50% and 400%) ($P < 0.05$). The terminal half lives were increased with increasing age (from 75% to 100% on the average) ($P \leq 0.01$). The clearance value was on the average 50% higher in the elderly women compared to the group of young women ($P < 0.05$). The 30% increase in clearance from the young men to the elderly men was not significant. For all patient groups a significant correlation ($r = 0.65$, $P < 0.001$) was found between the initial venous concentration and the K_{12}-values.

For the group of elderly men a correlation was found between the stroke volume prior to the anes-

Table 1 Pharmacokinetic data from 8 elderly women and 8 elderly men given thiopentone intravenously for the induction of anesthesia. The age of the patients was between 60 and 83 years

	Body weight (kg)	V_1 l	V_2 l	V_3 l	K_{12} min^{-1}	K_{13} min^{-1}	K_{10} min^{-1}	$T_{\frac{1}{2}\alpha}$ min	$T_{\frac{1}{2}\gamma}$ min	$T_{\frac{1}{2}\beta}$ min	Cl $l \times min^{-1}$
Women	60 (47–72)	13.0 (4.5–19.9)	20.9 (10.6–36.4)	161 (83–337)	0.081 (0.043–0.210)	0.039 0.015–0.85	0.019 (0.009–0.026)	5.8 (2.0–8.9)	46 (29–69)	990 (616–2223)	0.19 (0.14–0.28)
Men	80 (73–97)	9.8 (3.3–16.9)	28.3 (15.0–47.7)	125 (60–283)	0.138 (0.050–0.260)	0.037 (0.068–0.017)	0.029 (0.015–0.046)	3.7 (1.8–7.3)	63 (41–94)	791 (440–1580)	0.22 (0.15–0.28)

thesia, measured by impedance cardiography, and the K_{12} value ($r = 0.89$, $P < 0.001$).

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Polymorphism of phenformin 4-hydroxylation in Saudi females

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The metabolic oxidation of several drugs is under single gene control in man (Ritchie, Sloan, Idle & Smith, 1980), the two discernible phenotypes extensive metaboliser (EM) and poor metaboliser (PM) having been defined by the use of debrisoquine (Mahgoub, Idle, Dring, Lancaster & Smith, 1977) in several populations including Saudis (Islam, Idle & Smith, 1980). Phenformin 4-hydroxylation has recently been shown to mirror debrisoquine disposition in a panel of volunteers of established phenotype (Shah, Oates, Idle & Smith, 1980), but until now, polymorphism of phenformin metabolism has not been demonstrated on a population basis.

It is the purpose of this communication to report the metabolic 4-hydroxylation of phenformin in a population of Saudi females of unknown oxidation phenotype.

One hundred and eighty-eight apparently healthy Saudi females, aged 15–65 and resident in the Jeddah area, entered into the study. They were given phenformin (Dibotin, 25 mg tablet) orally around noon and asked to collect all urine until breakfast next day (20 h). No dietary restrictions were imposed. Urines were collected, volumes recorded and aliquots frozen at −20°C until transported frozen to London for analysis of their content of phenformin(P) and 4-hydroxy-phenformin(HP) by h.p.l.c. (Oates, Shah, Idle & Smith, 1980).

Of these 188 subjects, 25 were eliminated from the study because of either low urinary recovery of P + HP (<5%) or excessive urine volume. The remaining 163 urines all contained measurable quantities of P and HP. The following median (range values were obtained: P, 25.5% (2.7–103); HP, 7.8% (0.5–74); P + HP, 39.7% (5–109); P/HP, 2.3 (0.1–104). The ratio %dose as P/%dose as HP eliminated in

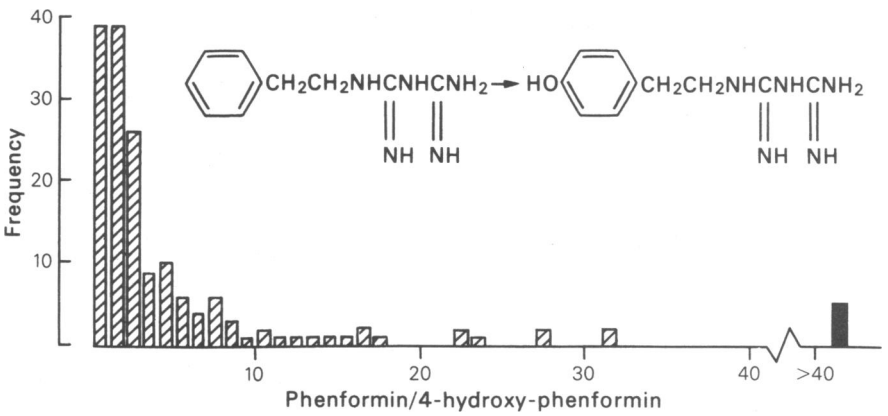

Figure 1 Frequency distribution histogram of phenformin/4-hydroxy-phenformin in 163 Saudi females showing the metabolic reaction under investigation.

urine was discontinuously distributed in the population (Fig. 1). Five subjects had ratios in excess of 40 (43–104), for whom only 1% or less metabolite could be detected.

Thus, the metabolism of phenformin in Saudi females was polymorphic. Family and cross-over studies using debrisoquine will reveal whether or not the relatively defective metabolisers of phenformin constitute the debrisoquine PM phenotype in this population.

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The effect of imipramine and SKF 525 A on the first pass elimination of propranolol in the rat

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The extensive first pass elimination (F.P.E.) of propranolol is probably responsible for the inter-individual variation in plasma concentrations which has been reported (Shand, Nuckolls & Oates, 1970). A reduction in F.P.E. of propranolol by co-administration of chlorpromazine has been shown both in man (Vestal, Kornhauser, Hollifield & Shand, 1979) and in rats (Barber, Kitteringham & Petrie, 1980). We have studied F.P.E. of propranolol following the administration of imipramine and following SKA 525 A.

Rats were pithed under halothane anaesthesia and ventilated with 100% oxygen. The sympathetic outflow to the heart was stimulated, via the pithing rod, for 3–5 s every minute (1 Hz, 0.05 ms, 20–30 V) which resulted in discrete increases in heart rate of approx. 25 beats per minute. [^{3}H]-Propranolol (0.1 mg/kg, 200 μCi/kg) was injected over 30 s via either the jugular vein or a cannulated side branch of the hepatic portal vein. Equimolar doses of imipramine (8.0 mg/kg) or SKF 525 A (9.8 mg/kg), or saline, were infused, according to a Latin square design, via the hepatic portal vein for 15 min before injection of propranolol. Blood samples were obtained from one carotid artery. Propranolol was separated from its metabolites by liquid chromatography (Pritchard, Schneck & Hayes, 1979) and quantified by liquid scintillation counting. Arterial blood pressure was measured in the opposite carotid artery and the heart rate recorded (Grass tachograph).

Both imipramine and SKF 525 A reduced the F.P.E. of propranolol following portal vein administration. This was associated with higher blood concentrations of propranolol (Figure 1a) and reduced concentrations of conjugated metabolites. The area under the concentration-time curve was increased from 74 ± 33 ng ml^{-1} min^{-1} (mean ± s.e. mean) in control animals to 1256 ± 287 ng ml^{-1} min^{-1} following SKF 525 A and 1297 ± 250 ng ml^{-1} min^{-1} following imipramine. The respective peak blood concentrations of propranolol were dissimilar and the mode of inhibition of the F.P.E. of propranolol by imipramine and SKF 525 A may also differ, at least in part. The increased concentration of propranolol following SKF 525 A pre-treatment was associated with an enhanced inhibition of electrically induced tachycardia (Figure 1b).

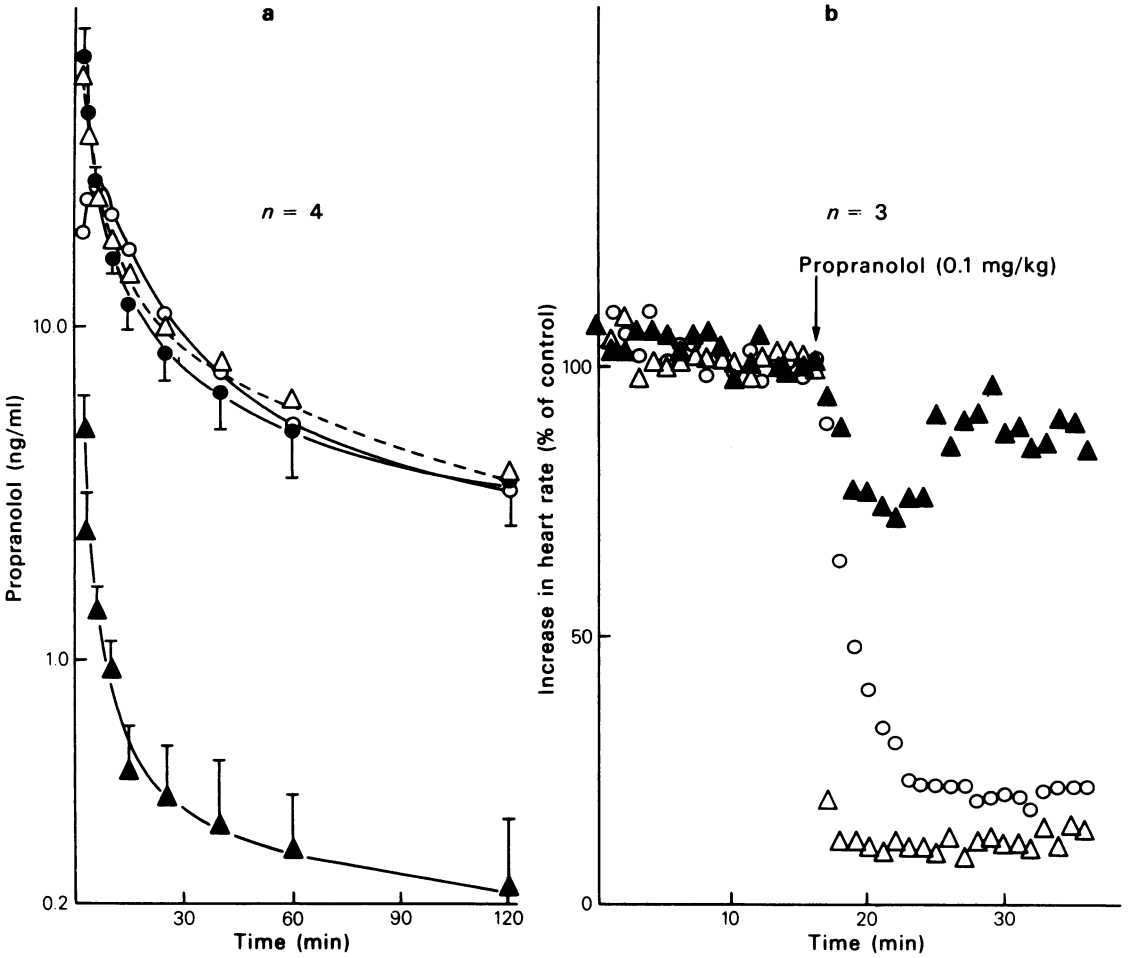

Figure 1 Blood propranolol concentrations (a) and inhibition of stimulation induced tachycardia (b) following intravenous (△) or intraportal (mean ± s.e. mean) (▲) injection of propranolol in the pithed rat. The effect of pre-treatment with imipramine (mean ± s.e. mean) (●) (blood concentrations only) and SKF 525 A (○) on intraportally administered propranolol is also shown. The standard error bars of intravenous and SKF 525 A curves in graph (a) were similar to those of imipramine and have been omitted for clarity of presentation.

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Dose-dependent formation of 1'-hydroxyestragole from estragole in the mouse

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The carcinogenic potential of a chemical is normally evaluated using lifetime feeding studies in rodent species. The results, and in particular their interpretation, pose problems when they are extrapolated to the human situation since the doses used in animal studies are usually much higher than those to which man is exposed. In animals therefore, the detoxication pathways which enable it to eliminate the compound safely may become saturated so that secondary pathways come into operation, which may result in adverse effects. Difficulties may also occur as to whether a 'threshold level' exists, that is, a level below which 'no' tumours are induced in the treated animals. This communication presents some biochemical data on the naturally occurring weak carcinogen estragole which is relevant to these issues.

Estragole (p-methoxyallylbenzene) is a major component of the oils of Basil, Fennel and Tarragon. Like safrole, another allylbenzene, it is an hepatocarcinogen (Drinkwater, Miller, Miller & Pitot, 1976) when given to weanling mice at high doses (ca 500 mg/kg) and its carcinogenicity is related to the formation of a reactive metabolite, 1'-hydroxyestragole. The question of the dose-dependent formation of this metabolite has therefore been investigated since the level of human intake of estragole has been calculated to be

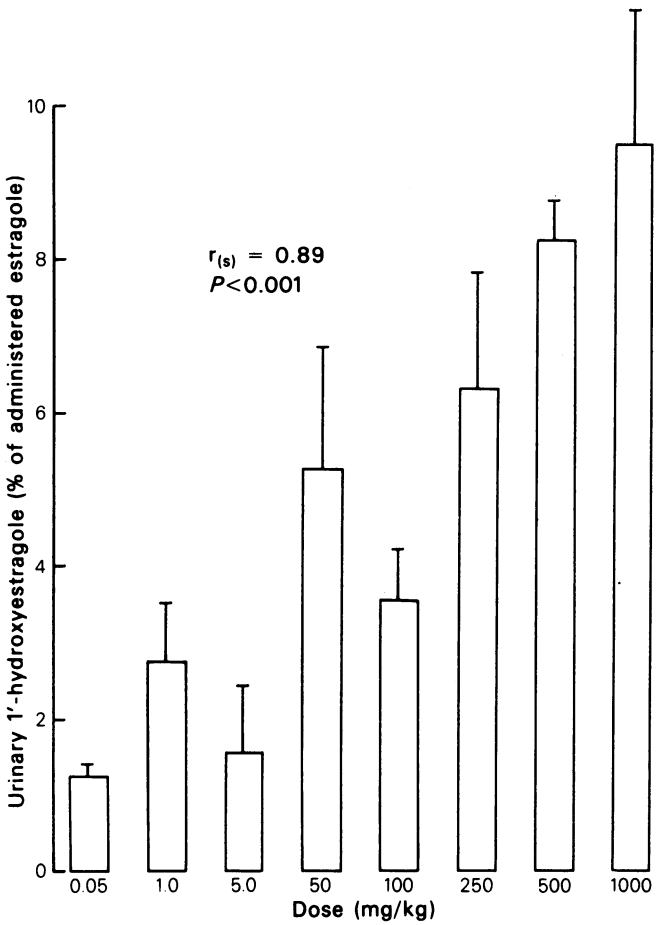

Figure 1 Dose-dependent formation of the carcinogenic metabolite of estragole in the mouse.

much lower (ca 70 μg/day) than that used in animal studies.

[^{14}C]-estragole labelled at the p-methoxy carbon atom was dissolved in trioctanoin and administered to male CD-1 mice (25 g) by intraperitoneal injection at doses ranging from 0.05–1000 mg/kg. The animals were housed in Metabowls permitting the separate collection of urine, faeces and $^{14}CO_2$ in the expired air. The qualitative and quantitative aspects of the urinary metabolites were determined by various techniques including solvent extraction, thin layer chromatography, HPLC, enzyme treatment, reverse isotope dilution and mass spectrometry. This showed that estragole underwent metabolism along three major competing pathways, namely, oxidative *O*-demethylation, 1′-hydroxylation and side chain oxidation. The formation of the carcinogenic 1′-hydroxy metabolite was dose-dependent. At the higher dose level (1000 mg/kg) its excretion accounted for 9.5% of the administered dose whereas at the 0.05 mg/kg level only 1.0% of the dose was eliminated as this metabolite. Over the dose range employed the relationship between dose size and 1′-hydroxyestragole formation (Fig. 1) was highly significant by the Spearman rank correlation test ($r_{(s)} = 0.89$; $P < 0.001$).

These studies show that the formation of the carcinogenic metabolite in the CD-1 mouse is dose-dependent and that a threshold may exist in relation to its formation. Its formation is favoured at high dose levels, this presumably reflecting the saturation of the primary pathways.

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Metabolism of canrenone *in vitro*: evidence for the formation of a glutathione conjugate via epoxidation

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Canrenone is the primary metabolite of potassium canrenoate in man (Vose *et al.*, 1979), and an important intermediate in the metabolism of spironolactone in rat, dog, monkey and man (Karim, 1978). 1-[^{3}H]-canrenone was extensively metabolised by rat liver enzyme preparations *in vitro* to a number of chloroform extractable compounds formed by Δ^4-3-keto reduction, hydroxylation and a combination of these pathways.

However, some 17% of the 1-[^{3}H]-canrenone was converted to a water soluble product, only 0.6% as tritiated water. This water soluble metabolite was not hydrolysed by β-glucuronidase. Both the microsomal and soluble fractions of rat liver were needed for its formation, which was oxygen and NADPH dependent, and inhibited by carbon monoxide and SKF 525 A. Depletion of glutathione by pre-treatment of the liver preparations with diethyl maleate or 1,1,1-trichloropropene-2,3-oxide (TCPO) inhibited the formation of this water-soluble metabolite, and enhanced the formation of dihydrodiol metabolites. Pre-treatment of glutathione free liver enzyme preparations with TCPO inhibited dihydrodiol formation. Bromobenzene, itself metabolised by an epoxidation pathway, also decreased the formation of the canrenone metabolite. These results are compatible with the formation of a glutathione conjugate of canrenone *in vitro*, via an intermediate epoxide formed by cytochrome P-450 oxidation.

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Na^+, K^+-ATPase activity in brown adipose tissue: its relationship to resting metabolic rate and the effects of ciclazindol

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The 'cafeteria rat' is considered to be a valuable tool for the study of diet-induced thermogenesis. Although the biochemical basis of this or any other thermogenic response is incompletely understood, Rothwell & Stock (1979) obtained substantial evidence to link brown adipose tissue (BAT) to diet-induced metabolic changes. We have compared the activity of certain noradrenergic processes in BAT from 'cafeteria' and control rats in order to gain some insight into the mechanisms underlying the apparent energy-dissipating property of this tissue.

The turnover of noradrenaline (NA) in BAT was 55% ($P < 0.05$) greater in 'cafeteria' than in normal rats. The results of experiments involving MAO inhibitors and measurements of reuptake of NA indicated that this difference probably reflected an increase in NA release.

Noradrenaline and isoprenaline (but not phenylephrine) markedly stimulated Na^+, K^+-ATPase activity in BAT; the degree of stimulation was significantly greater in microsomal material derived from 'cafeteria' than control animals (Table 1). This difference did not appear to be associated with a greater β-adrenoceptor density in 'cafeteria' rats because β-adrenoceptor adenyl cyclase activation was similar in both groups of animals. Qualitatively similar effects on Na^+, K^+-ATPase have been described for non-shivering thermogenesis (Horowitz & Eaton, 1975).

'Cafeteria' rats displayed a higher resting metabolic rate (RMR) than controls and there was a close positive correlation ($r = 0.97$, $P < 0.001$) between RMR and BAT homogenate Na^+, K^+-ATPase activity. The RMR and enzyme activity of all rats were increased by NA, isoprenaline and the NA uptake inhibitor ciclazindol (Sugden, 1974). This effect of ciclazindol was limited to homogenates retaining a degree of cellular organisation (Table 1) and appears to be related to the inhibition of extraneuronal and intraneuronal uptake of NA rather than to direct β-adrenoceptor stimulation. The possible relevance of these results to diet-induced thermogenesis will be discussed.

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Table 1 Na^+, K^+ ATPase activity in brown adipose homogenates and microsomal fraction

Animal type	*Drug addition (M)*	*Na^+, K^+ ATPase activity (μmol Pi mg protein^{-1}h^{-1})*		
		Homogenate	*Disrupted homogenate*	*Microsomal fraction*
Control	None	0.23 ± 0.01 (4)	0.18 ± 0.01 (4)	1.32 ± 0.04 (4)
Cafeteria	None	0.32 ± 0.02 (6)††	0.19 ± 0.01 (6)	1.30 ± 0.05 (6)
Control	Isoprenaline (10^{-4})	0.28 ± 0.04 (4)	0.24 ± 0.02 (4)	1.70 ± 0.05 (4)
Cafeteria	Isoprenaline (10^{-4})	0.45 ± 0.03 (3)†††	0.33 ± 0.03 (3)†	2.23 ± 0.03 (6)†††
Control	Ciclazindol (10^{-4})	0.27 ± 0.01 (4)	0.18 ± 0.03 (4)	1.31 ± 0.04 (4)
Cafeteria	Ciclazindol (10^{-4})	0.40 ± 0.02 (3)†††	0.20 ± 0.03 (4)	1.28 ± 0.06 (6)

Na^+, K^+ ATPase activity was measured colorimetrically as outlined by Gilbert & Wyllie (1975). The disrupted homogenate was ultrasonicated by cytoplasm separated from membraneous material by high speed centrifugation (100.000 *g*, 30 min). Enzyme activity was measured in the resultant pellet.
† or ††, ††† activity significantly higher in 'cafeteria' animals.
† $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

A comparison of the kinetics of ifenprodil in the rat and dog

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The kinetic and metabolic profile of ifenprodil (α-4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidineethanol) have been examined in the rat and dog using the compound labelled with [^{14}C] in the Cα of the piperidine-ethanol moiety.

Following intravenous administration (10 mg/kg, 50 μCi/kg in the rat and 10 mg/kg, 20 μCi/kg in the dog), ifenprodil plasma levels appeared to decay according to a one compartment open model in the rat and to a two compartment model in the dog. Apparent terminal plasma half lives for the unchanged compound were 1.05 h in the rat and 2.0 h in the dog. Total radioactivity disappearance rate was considerably slower with apparent plasma half lives of 4 h in the rat and 23 h in the dog. Following oral administration peak plasma levels of total radioactivity were observed at 10–20 min in the rat and 40–60 min in the dog. After either intravenous or oral administration the 0–72 h urinary excretion of total radioactivity accounted for 28% of the dose in the rat and 49% in the dog. The remainder of the administered dose was recovered in the faeces in the two species.

After intravenous administration to both species, unchanged ifenprodil accounted for only a small portion of total plasma radioactivity (less than 12% in the rat and than 7% in the dog). The major compound detected in plasma of the rat and dog was the glucuronide derivative of ifenprodil which represented 32–56% of total radioactivity in the rat and 43–48% in the dog. Similarly the glucuronide of ifenprodil represented the major metabolite found in urine and bile or faeces of the rat and dog. Other metabolites previously identified in the bile and urine of the rat by Nakagawa *et al* (1975) (α-(-4-hydroxyphenyl)-4-[(4-hydroxyphenyl)methyl]-β-methyl-1-piperidineethanol); α-(4-hydroxy-3-methoxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidineethanol) and α-(-4-hydroxy-3-methoxyphenyl)-4-[(4-hydroxyphenyl)-methyl]-β-methyl-1-piperidineethanol) were found present in the rat and dog plasma at very low concentrations mainly as glucuronides.

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The affinities of pirenzepine and atropine for functional muscarinic receptors in guinea-pig atria and ileum

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Hammer, Berrie, Birdsall, Burgen & Hulme (1980) have found that the affinity of pirenzepine for 'muscarinic' receptors, measured by inhibition of radioligand binding, was much higher in some tissues than others. This supports the idea that there are different subclasses of muscarinic receptors and it is therefore important to know whether similar estimates of affinity can be obtained with functional receptors, in experiments in which dose-ratios are measured with agonists acting on intact cells. Such experiments with pirenzepine on guinea-pig isolated atria and ileum were made independently in Bristol and Greenford and it was a discrepancy between the value for atropine, included as a control in the work at Greenford, from the value previously published (Barlow, Berry, Glenton, Nikolaou & Soh, 1976) that led to contact between us. It seemed to us important to try to evaluate the differences which may be expected when the affinity of an antagonist for muscarinic receptors in atria is measured in different laboratories. There were some differences in experimental conditions; the temperature used was 30° in Bristol and 34° in Greenford. The agonist used was carbachol in Bristol, as in previous work (Barlow *et al.*, 1976); in Greenford it was (±)-muscarine and dose-response curves in the pres-

Table 1 Affinity of atropine and pirenzepine for muscarinic receptors in guinea-pig atria and ileum. Mean values of log K are shown ± s.e. mean and number of estimates, together with the range of concentrations tested. In experiments made in Greenford (G) the preparations were set up in Krebs solution at 34°C; in the experiments made in Bristol (B) the atria were set up in Locke's solution at 30°C and the ileum in Tyrode's solution at 37°C; this was also used in the experiments made in Edinburgh (E; Mustafa, 1967). Hexamethonium (0.3 mM) was present in all experiments with carbachol as agonist. The asterisk indicates that the results did not fit the Gaddum-Schild equation and the intercept on the Schild plot (Arunlakshana & Schild, 1959) was 6.54.

The previous reported value of log K for atropine (Barlow *et al.*, 1976) was 9.13 ± 0.04 (3), with methacholine as agonist (not carbachol as stated in the paper). Hammer *et al.* (1980) obtained values of log K of 6.08 for pirenzepine with receptors in atria and 6.10 with receptors in ileum

	Atria		*Ileum*	
	G	*B*	*G*	*E*
Atropine	8.67 ± 0.14 (8) 7–75 nM	8.68 ± 0.03 (24) 10–1000 nM	9.2 ± 0.12 (4) 10 nM	9.01 ± 0.02 (12) 100 nM
	G	*B*	*G*	*B*
Pirenzepine	6.71 ± 0.05 (12) 1–50 μM	6.20 ± 0.04 (22)* 2–200 μM	6.70 ± 0.07 (9) 1–10 μM	6.65 ± 0.02 (24) 2–100 μM

ence of the antagonist were obtained in separate experiments from the controls. Some other differences are indicated in Table 1, which presents the results as mean estimates of log affinity constant.

The results for pirenzepine on atria are complicated by apparently noncompetitive behaviour but, as with the results for the ileum, the affinity appears higher than obtained by Hammer *et al.* (1980). This may be a species difference. Our results confirm that pirenzepine does not differentiate between muscarinic receptors in atria and ileum in the way that some other drugs do (Barlow *et al.*, 1976). It does, however, distinguish between the receptors in these sites and muscarinic receptors in sympathetic ganglia (Brown, Forward & Marsh, personal communication).

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β-Bungarotoxin in studies of neuroeffector transmission in the guinea-pig

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Because they observed no structural distinction between cholinergic and non-adrenergic inhibitory (NAI) neurones of the intestine, Daniel *et al.* (1977) entertained the possibility that the NAI transmitter is liberated from the same axons as acetylcholine. If this is so, and if the release mechanisms are identical, then a drug which interferes with acetylcholine release should also interfere with the release of the NAI transmitter.

Reports (Chang *et al.*, 1973; Kato *et al.*, 1977; Muramatsu *et al.*, 1980) that β-bungarotoxin can inhibit the neuronal release of acetylcholine led us to test its effects on NAI transmitter release. We used β-bungarotoxin as supplied by BCL, The Boehringer Corporation (London) Ltd. The toxin (1–10 μg/ml) was incubated with the guinea-pig isolated tissues for at least 1 hour.

In the phrenic nerve hemidiaphragm preparation the toxin abolished twitches evoked by nerve stimulation but not the directly elicited twitches. The

neuromuscular blockade commenced after a latency of about 5 min, was often associated with a slight rise in muscle tone and took more than 1 h to become complete. Experiments with cremaster muscle were next performed to check whether the toxin had postjunctional activity. The toxin caused no change in cremaster tone or in contractions induced by potassium chloride (10^{-2}–4×10^{-2} M). However, contractions evoked by suxamethonium (0.63×10^{-5}–2.5×10^{-5} M) were insurmountably reduced.

In the transmurally stimulated ileum the toxin caused no change in tissue tone, responsiveness to acetylcholine (2.2×10^{-8}–2.2×10^{-5} M) or to stimulation of intramural cholinergic nerves. There was however some reduction in contractile responses to nicotine (0.64×10^{-6}–41×10^{-6} M).

In the pre- and postganglionically stimulated vas deferens preparation the toxin at 1 μg/ml caused no change in tissue tone but selectively suppressed contractions evoked by preganglionic stimulation. Contractions evoked by postganglionic stimulation or nicotine (10^{-5}–10^{-4} M) remained unchanged by the toxin. Pilot experiments showed that at 10 μg/ml the toxin had a less selective effect.

In the hyoscine (3×10^{-5} M) and guanethidine (10^{-5} M) treated taenia caeci, the toxin was without effect on tissue tone and did not modify relaxations evoked by noradrenaline, ATP or field stimulation of the intramural NAI nerves.

We conclude:

(i) that the β-bungarotoxin as supplied by Boehringer had antagonist activity at the nicotinic cholinoceptors of skeletal muscle and this may contribute to its neuromuscular blocking activity

(ii) that the toxin's failure to prevent postganglionic cholinergic transmission to intestinal smooth muscle and its failure to prevent NAI transmission render it of little use as an analytical tool in studies of the NAI neurone.

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The interaction between tetraethylammonium and methohexitone in the chick biventer cervicis muscle preparation: experiments with caffeine and xylocaine

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Tetraethylammonium (TEA) which evoked large contractures of the chick biventer cervicis muscle preparation in Krebs-Henseleit solution containing methohexitone (8.8×10^{-5} M, METHO-KREBS), had no contractile activity in the dose range tested, in the absence of METHO (Elliott, 1979). Previous experiments (Elliott, 1979; 1980) indicated that the release of acetylcholine (ACh) by TEA was probably a minor factor in TEA-METHO contractures.

Contractures of the chick muscle were recorded as described previously (Elliott, 1979). Concentration response curves were obtained for caffeine induced contractures in Krebs solution, METHO-KREBS and after washing with Krebs solution. Caffeine (3.9–7.7 mM) was applied for 5 min at 30 min intervals. In experiments with xylocaine (=XYLO) and TEA the same procedure was adopted except that a 4 min exposure for TEA was used. The xylocaine concentration in the Krebs was 1.07 mM (= XYLO-KREBS).

METHO-KREBS potentiated the caffeine induced contractures of the chick muscle. The ED_{50}s determined from concentration response curves for the cumulated data for nine experiments were control 6.85 mM, and in METHO-KREBS, 5.25 mM. There

was evidence that caffeine tended to desensitize the preparation.

XYLO-KREBS markedly increased TEA induced contractures; the ED_{50}s determined from concentration response curves for the cumulated data from seven experiments were control, 0.56 M and for the preparations in XYLO-KREBS, 0.9 mM.

The TEA induced contractures in XYLO-KREBS were reduced by 85% ± 3.3% $n = 5$ by gallamine (1×10^{-4} M). XYLO-KREBS blocked ACh induced contractures in the presence of eserine (1.6×10^{-6} M), the antagonism was not surmountable by doses of ACh over 3000× the control ED_{50} for ACh which was $2.95 \pm 0.05 \times 10^{-7}$ M, $n = 5$, the ACh induced contractures were restored on washing the preparation for 1 hour.

Bianchi & Bolton (1967) found that caffeine, in intermediate concentrations, acted by releasing Ca^{2+} from the sarcoplasmic reticulum (Bianchi & Bolton, 1967). TEA could act intracellularly like caffeine, but TEA induced contractures were blocked by gallamine, and both TEA and gallamine are quaternary compounds. It seems probable that their action was exerted at the post-synaptic membrane.

A tentative explanation for the interaction of TEA and METHO is that TEA acts on a post-synaptic nicotinic receptor as a weak agonist, it causes a rise in the intracellular free Ca^{2+} level which is insufficient to cause a contracture. METHO by facilitating the release of Ca^{2+} or by preventing its re-uptake allows a contracture to occur. ACh is not involved in this contracture which can probably occur in the absence of depolarization.

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Channel opening time by external and internal recording in rat diaphragm

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Focal recording with external electrodes from rat diaphragm is possible, as shown by the occurrence of inverted miniature end-plate potentials (mepps). These inverted mepps could be located readily at 37°C (Liley, 1956), and were continuously monitored whilst cooling to room temperature. Electrode resistance for external recording was 8–10ΩM, and for internal recording was 15–30 ΩM with end end-plate region clamped at −80 mV. The Krebs-bicarbonate solution contained K^+ (4 mM) and acetylcholine (10 μM), physostigmine (3 μM), and tetrodotoxin (350 nM) were used. Channel opening times were computed by spectral noise analysis (Anderson & Stevens, 1973), the data being digitized at 1 or 4 kHz. For miniature end-plate currents (mepcs) or external mepps at 37°C it was necessary to digitize at 16 or 32 kHz.

At room temperature (20 or 22°C) the channel opening time by spectral noise analysis of acetylcholine-induced current fluctuations from internal recording was 1.04 ms (median of 8 cells, with at least two estimates from each cell, range 0.95–1.20 ms), which agrees with Colquhoun, Large & Rang (1977). Similar values were found from the time-constant of mepcs. At 37°C the channel opening time from clamped end-plates was in the range 280–320 μsec from spectral analysis, and estimates from mepcs and spectral noise analysis from external recordings gave comparable values. The time-constant of mepps, recorded externally, was consistently longer than the channel opening time estimated by spectral analysis (Katz & Miledi, 1973).

Focal reading with external electrodes may have advantage over clamping of the end-plate for long-continued recording, and the method appears to be feasible at body temperature.

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Differential effects of non-specific perturbers on the acetylcholine receptor from *Torpedo* and its ionophore

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Acetylcholine receptor-rich membranes isolated from *Torpedo* electroplaques contain both the acetylcholine binding site (AchR) and its ionophore (AchI). The effect of perturbers on these subunits may indicate how tightly coupled they are.

AchR containing membranes were obtained from *Torpedo californica* by the method of Cohen *et al* (1973). Specific [^{3}H]-acetylcholine (Ach) binding was measured by centrifugation assay in the presence and absence of α-bungarotoxin following pre-treatment with diisopropyl fluorophosphate (Cohen, Weber & Changeux, 1974). Membrane vesicles were loaded with [^{86}Rb]Cl by overnight incubation and the external radio-activity removed by passing through a Sephadex G-50 column. Stimulated [^{86}Rb] efflux was measured by filtration.

The effect of non-specific perturbers on the ratio of bound to free [^{3}H]-Ach (under conditions where half the receptors are occupied) is shown in Table 1. Ethanol and octanol both increased Ach binding whilst pentobarbitone, phenytoin and (−)-ketamine all decreased binding. (+)-Ketamine was ineffective. Full [^{3}H]-Ach binding curves show these effects are non-competitive reflecting changes only in the underlying dissociation constants. All cations were rapidly released by carbachol (100 μM). Four agents blocked cation efflux (Table 1), but phenytoin and ethanol did not. The latter actually increased efflux. Thus, carbachol (12 μM) released half the trapped ions in 10 s, whereas in the presence of ethanol (400 mM) only carbachol (6 μM) was required. This increase in efflux was blocked by pentobarbitone. When spare receptors were blocked with α-bungarotoxin the ID_{50} of pentobarbitone was shifted to 25 μM, a concentration which caused no change in Ach binding. Thus changes in [^{3}H]-Ach binding fail to correlate with changes in [^{86}Rb] efflux.

Table 1 Effects of perturbers on [^{3}H]-acetylcholine binding and on stimulated cation efflux of cholinergic membrane vesicles

	Acetylcholine Binding		*Stimulated cation efflux*
Perturber	*Concentration of perturber* (mM)	*(Bound Ach/ Free Ach) (Control = 1.0)*	*Concentration of perturber reducing efflux 50% (ID_{50})* mM
Ethanol	400	1.4	Increases
	560	1.5	Efflux
Octanol	0.2	1.0	0.2
	2.5	2.0	
Pentobarbitone	0.02	1.0	0.4
	0.15	0.8	
Phenytoin	0.05	0.5	Ineffective
(−)-Ketamine	0.20	1.0	0.2
(+)-Ketamine	0.20	0.7	0.2

The perturber induced conformational changes revealed by Ach binding do not appear to be consistently coupled to AchI function.

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No evidence for an inhibitory pre-junctional effect of hydralazine *in vivo*

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Although the antihypertensive actions of hydralazine are still not clearly understood, it has been suggested that the major effect of this drug is exerted post-synaptically on the vascular smooth muscle of resistance vessels (Uchida & Bohr, 1969). However, the results of recent *in vitro* studies have suggested that hydralazine also exerts a significant additional inhibitory effect at sympathetic nerve terminals by preventing the release of noradrenaline (Worcel, 1978; Chevillard, Mathieu, Saiag & Worcel, 1980). The purpose of the present study was to determine whether hydralazine could preferentially inhibit the vasoconstrictor responses induced by sympathetic nerve stimulation using the pithed rat preparation.

Groups of 5 or 6 normotensive male Wistar rats (300–400 g) were pithed and prepared for stimulation of the spinal sympathetic outflow according to the method of Gillespie & Muir (1967). Vasoconstrictor responses and the tachycardia induced by sympathetic nerve stimulation (30 V, 1 ms, 0.25–10 Hz for 15s), noradrenaline (0.05–1 μg/kg, i.v.) or angiotension II (5 or 500 ng/kg, i.v.) were measured before, 2 min and 45 min after administration of hydralazine (0.25, 0.5 or 1 mg/kg, i.v.).

Hydralazine significantly inhibited the vasoconstrictor responses to exogenous noradrenaline (NA) and to sympathetic nerve stimulation ($P < 0.01$). This effect was apparent after 2 min exposure to hydralazine and was still evident after 45 minutes. A high degree of correlation ($r = 0.99$, slope = 0.97) was seen between the inhibitory effects of hydralazine on the responses to sympathetic nerve stimulation (1 Hz) and on those to exogenous NA (0.5 μg/kg, i.v.). Vasoconstrictor responses to angiotensin II were also significantly inhibited by hydralazine ($P < 0.05$).

Hydralazine had no effect on the tachycardia associated with sympathetic nerve stimulation or on that observed following administration of NA or angiotensin II.

These results show that hydralazine is similarly effective in antagonizing the vasoconstrictor responses to exogenous NA and angiotensin II and those following sympathetic nerve stimulation. No evidence was obtained to support the idea of a preferential pre-synaptic inhibitory action of hydralazine against responses induced by neuronally-released NA (Chevillard *et al.*, 1980). It is therefore suggested that the antagonism of vasoconstrictor responses by hydralazine can be explained on the basis of an inhibitory action at a post-synaptic site.

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Stereoselective antagonism of pre-junctional dopamine receptors in the rabbit ear artery by sulpiride

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The dopamine receptor antagonist sulpiride exists in two enantiomeric forms and has been reported to exhibit stereoselectivity in certain dopaminergic systems (Spano, Stefanini, Trabucchi & Fresia, 1979). We have used the rabbit isolated ear artery preparation to compare the antagonism by (±)-sulpiride and its enantiomers of pre-junctional dopamine receptors, and both pre- and post-junctional α-adrenoceptors. The respective agonists used were ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) a potent agonist at pre-junctional dopamine receptors in this preparation (Brown, Brown, O'Connor & Solca, 1979), clonidine and noradrenaline.

Rabbit central ear arteries were isolated and perfused with Krebs-Henseleit solution (3 ml/min, 37°C, 95% O_2/5% CO_2) containing cocaine (5×10^{-5} M). Vascular sympathetic nerves were stimulated via bipolar platinum ring electrodes for 10 s every 2.5 min (1–2 Hz, supramaximal voltage, 0.5 ms pulse width). Agonists were injected into the extraluminal bath fluid. Pre-junctional activities were measured as inhibitions of stimulation-induced vasoconstriction, whereas activity at post-junctional α-adrenoceptors was assessed as noradrenaline-induced vasoconstriction in the absence of neural stimulation. Antagonists were evaluated using pA_2 determinations (Arunlakshana & Schild, 1959) derived from cumulative log. dose-response curves for each agonist obtained under control conditions and in various concentrations (3×10^{-8}–10^{-5} M) of each antagonist. Results are summarized in Table 1.

(±)-Sulpiride and (−)-sulpiride were potent, selective antagonists of pre-junctional dopamine receptors with the (−)-enantiomer demonstrating approx. 1.7 times the activity of the racemate. By contrast, (+)-sulpiride was relatively ineffective and approximately 100 times less potent than (−)-sulpiride. Unlike some other neuroleptics e.g. haloperidol (Ennis & Cox, 1980) neither (±)-sulpiride nor its enantiomers exhibited antagonist activity at pre- or post-junctional α-adrenoceptors at concentrations up to 10^{-5} M.

The antagonism by sulpiride of pre-junctional dopamine receptors in the rabbit ear artery is therefore stereoselective, with the (−)-enantiomer being preferred. In this respect, the dopamine receptor in this model is similar to those in the central nervous system where (−)-sulpiride also demonstrates the greater activity (Spano, Stefanini, Trabucchi & Fresia, 1979), but contrasts with the dopamine vascular receptor at which (+)-sulpiride is the more potent antagonist (Goldberg, Kohli, Listinsky & McDermed, 1978).

Sulpiride enantiomers were generously donated by Professor P. Fresia, Ravizza, Research Laboratories, Milan.

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Table 1 Rabbit ear artery. pA_2 values for sulpiride and its enantiomers against ADTN, clonidine and noradrenaline

	(±)-*Sulpiride*	(−)-*Sulpiride*	(+)-*Sulpiride*
ADTN	7.79 ± 0.06	8.03 ± 0.08*	5.98 ± 0.09**
Clonidine	<5.00	<5.00	<5.00
Noradrenaline	<5.00	<5.00	<5.00

Values are means ± s.e. means of 6 determinations.
pA_2 value differed significantly from that of (±)-sulpiride (t-test) * $P < 0.05$, ** $P < 0.01$.

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5-Hydroxytryptamine and noradrenaline on human isolated mesenteric blood vessels

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The mechanisms controlling splanchnic circulation are poorly understood although it is widely recognised that abnormalities of gastrointestinal blood flow may cause various pathological conditions in man (Jacobson & Lanciault, 1979). 5-Hydroxytryptamine might contribute to the regulation of portal venous tone as it is produced in the gastrointestinal mucosa. It is a potent constrictor in many vascular beds but dilates skeletal muscle and coronary vessels. Its effect on human mesenteric vessels has now been compared with that of noradrenaline.

Specimens of gastric or colonic vessels were obtained at surgical operations and placed in pregassed Krebs' solution. Connective tissue and fat were dissected away and the vessels cut into spiral strips 2–3 mm wide and 30–40 mm long. The strips were then suspended under a load of 0.5 g, in Krebs' solution at 37°C, bubbled with 5% CO_2 in O_2. Responses were recorded through an isotonic transducer connected to a pen recorder.

5-Hydroxytryptamine creatinine sulphate (5-HT) and (−)-noradrenaline bitartrate (NA) were dissolved in saline containing ascorbic acid (100 μg/ml). Both substances were added for 1 min at intervals of 8–15 minutes.

Fifty three vessels from 33 patients contracted to both agonists in a dose related manner. Artery and vein strips from the same patient were approximately equally sensitive to NA (threshold concentrations $\leqslant$ 10 ng/ml). On vein strips NA and 5-HT were approximately equally potent, whereas artery strips were less sensitive to 5-HT (threshold concentration $\geqslant$ 100 ng/ml).

In many experiments there was insufficient time to obtain a full dose response curve to both agonists, so, to make quantitative comparisons, a responsiveness index (RI) has been calculated. This index combines a submaximal concentration of agonist, the response measured on the chart paper and the electrical amplification as indicated by the pen recorder range setting.

Thus:

$$RI = \frac{\text{Response (mm)} \times \text{Range (mV/cm)}}{\text{Concentration (ng/ml)}}$$

The indices calculated for 5-HT on artery and vein respectively were 0.15 (0.03–0.85, $n = 30$) and 4.4 (1.0–14, $n = 18$) (median and semi-quartile ranges). Equivalent values for NA were 1.7 (0.7–2.9, $n = 33$) and 4.3 (2.4–9.0, $n = 19$).

Because of wide inter-patient variation none of these values was significantly different (i.e. $P > 0.05$, Wilcoxon's rank sum test). However, when indices for artery and vein from the same patient were compared using Wilcoxon's matched-pairs signed-ranks test, there was a clear difference in responsiveness to 5-HT ($P < 0.01$, 16 pairs) but not to NA ($P > 0.1$, 15 pairs).

These results are consistent with the possibility that low concentrations of free 5-HT in the portal venous system could cause constriction leading to reduced blood flow and hypoxic tissue damage.

We thank the surgeons of Charing Cross Hospital for their co-operation in providing specimens and Janssen Pharmaceutical Ltd. for financial support.

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Further characterization of pre- and post-junctional receptors for 5-hydroxytryptamine in isolated vasculature

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It has previously been suggested that two types of receptor for 5-hydroxytryptamine (5-HT) exist in the vasculature (Apperley, Feniuk, Humphrey & Levy, 1980). One type, the D-receptor, is found in the rabbit aorta and is potently blocked by the 5-HT antagonists, methysergide and cyproheptadine (Apperley, Humphrey & Levy, 1976; Apperley *et al.*, 1980). The other type, found both pre- and post-junctionally in the dog saphenous vein is only weakly blocked by these antagonists and appears to be stimulated by methysergide (Feniuk, Humphrey & Watts, 1979 (a) & (b); Apperley *et al.*, 1980). In an attempt to characterize these receptors further we have determined the relative potencies of some structural analogues of 5-HT on the rabbit aorta and dog saphenous vein *in vitro*.

Isometric contractions of rabbit aortic strips and dog saphenous vein strips were recorded as described previously using a modified Krebs solution (Apperley *et al.*, 1980). In other experiments dog saphenous vein strips were mounted to allow electrical stimulation of noradrenergic nerves (Feniuk, Humphrey & Watts, 1979a). Cumulative concentration-effect curves were obtained to both 5-HT and a 5-HT analogue in each preparation. The relative potencies of the compounds examined are shown in Table 1.

N-methyl 5-HT was almost equipotent with 5-HT on all three preparations while α-methyl 5-HT was of similar potency on the rabbit aorta but weaker on the dog saphenous vein at both pre- and post-junctional sites. In contrast the 5-carboxamide tryptamine derivatives were potent both pre- and post-junctionally in the dog saphenous vein but inactive or markedly weaker in the rabbit aorta. These results provide further evidence for our postulate that the 5-HT receptors which exist pre-junctionally in the dog saphenous vein are similar to those which exist post-junctionally in the same preparation but both are different to the D-receptor which occurs in rabbit aorta and dog femoral artery (Feniuk *et al.*, 1979., Apperley *et al.*, 1980).

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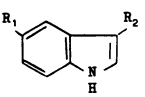
Table 1 Relative potencies of some structural analogues of 5-hydroxytryptamine as agonists on isolated vasculature



Compound R_1	Compound R_2	$\frac{EC_{50}\ value}{EC_{50}\ value\ for\ 5\text{-}HT}$ Rabbit Aorta Contraction*	Dog Saphenous Vein Contraction*	Dog Saphenous Vein Inhibition**
-OH	$-CH_2CH_2NHCH_3$	2.2 (1.0–4.7)	0.8 (0.7–0.9)	0.8 (0.6–1.1)
-OH	$-CH_2CH(CH_3)NH_2$	2.2 (1.1–4.1)	13 (5–32)	25 (13–46)
$-CONH_2$	$-CH_2CH_2NH_2$	26† (14–49)	0.4 (0.1–0.9)	0.3 (0.2–0.5)
$-CON(CH_3)_2$	$-CH_2CH_2NH_2$	130† (86–197)	2.1 (1.1–4.0)	3.4 (2.4–4.7)
$-CONHC_2H_5$	$-CH_2CH_2NH_2$	>350†	1.1† (0.5–2.2)	1.2 (0.9–1.6)

Mean values (95% confidence limits) from 4 or more preparations were calculated by comparing the molar concentration (EC_{50} value) of each compound necessary to produce 50% of its maximal effect with that for 5-HT.
* In presence of atropine, mepyramine, phentolamine (all at 1.0×10^{-6} mol/l) and iproniazid (5.0×10^{-5} mol/l).
** Inhibition of electrically stimulated contraction in presence of indomethacin (2.8×10^{-6} mol/l), cocaine (3×10^{-5} mol/l), cyproheptadine, atropine, mepyramine (all at 1.0×10^{-6} mol/l) and iproniazid (5×10^{-5} mol/l).
† Maximum effect less than 60% of 5-HT maximum effect.

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The presynaptic effects of cholinoceptor and adrenoceptor agonists and antagonists *in vivo* in cat heart

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It has been postulated that presynaptic α and β-adrenoceptor sites exist on sympathetic nerve terminals mediating a negative and positive feedback respectively of transmitter release (Langer, 1977). However most of the evidence relies on biochemical methodology and there are few reports measuring organ responses *in vivo*. Recently Kalsner (1979, 1980) and Chan & Kalsner (1979) have questioned the hypothesis and particularly the parameters of stimulation, therefore the stimulus-response relationships have been examined in the anaesthetized cat measuring heart rate following stimulation of the cardio-accelerator nerve at frequencies of 0.05 to 10 Hz using either 5 or 10 stimuli. A maximal response was produced at approximately 2.5 Hz using 5 stimuli and 1.0 Hz using 10 stimuli. The effects of various agonists and antagonists on the responses to different rates of stimulation have been investigated and compared to the effect on the response to 10 ng/kg i.v. isoprenaline. All results represent responses in at least two cats.

Atropine (1.0 mg/kg i.v.) doubled the response to all frequencies without affecting the response to isoprenaline or causing any marked or consistent change in the resting heart rate, and edrophonium (2.5 $\mu g.kg^{-1}.min^{-1}$) almost halved the responses. The effects of adrenaline (5, 10 and 25 $ng.kg^{-1}.min^{-1}$ i.v.), bufuralol (0.5 $\mu g.kg^{-1}.min^{-1}$ i.v.), and yohimbine (10 and 25 $mg.kg^{-1}.min^{-1}$ i.v.) were measured. Adrenaline affected the response to lower frequencies of stimulation to a greater degree than the higher frequencies. The lowest concentrations (β-agonist) potentiated and the higher concentration (α-agonist) depressed the responses.

The α-antagonist yohimbine and the β-antagonist bufuralol affected the responses to the higher frequencies of stimulation to a greater extent than the lower frequencies of stimulation; yohimbine produced potentiation and bufuralol depression of the responses.

Clonidine (0.25 and 0.5 $\mu g.kg^{-1}.min^{-1}$ i.v.) produced similar responses to the higher doses of adrenaline. Sotalol (10 $\mu g.kg^{-1}.min^{-1}$ i.v.) and clenbuterol (10 and 25 $ng.kg^{-1}.min^{-1}$ i.v.) produced only marked post-synaptic depression. Phenoxybenzamine produced variable responses which could not be attributed to presynaptic effects.

The results obtained therefore support the hypothesis of presynaptic α and β-adrenoceptors mediating a positive and negative feedback of transmitter; agonists having the greatest effect at lower frequencies of stimulation and antagonists at higher frequencies of stimulation. The effect of atropine, which does not occupy the same receptors as the transmitter, was not frequency dependent.

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Alpha adrenoceptors in two models of experimental hypertension in the rabbit

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An increased vascular reactivity to intravenous noradrenaline (NA) has been reported in essential hypertension (Vlachakis, 1979). This may be a result of changes in vascular α adrenoceptors. We have investigated α adrenoceptors in two types of experimental hypertension in the rabbit, a renal model (cellophane perinephritis) and a neurogenic model (bilateral sinoaortic denervation).

Mean arterial pressure had increased by 45 mmHg and 21 mmHg in the renal and neurogenic models respectively 3 weeks after operation. Plasma NA was similar in the renal hypertensive and control groups but elevated after sinoaortic denervation (Table 1). Responses to the specific α_1 agonist phenylephrine, the mixed α_1/α_2 agonist NA, and the α_1 antagonist prazosin were also examined at this time. In both models of hypertension there was a shift to the left of dose response curves to phenylephrine. The response to NA was enhanced in renal hypertension but not sinoaortic denervated animals. However prazosin blocked phenylephrine responses to a similar extent in all groups. Radioligand binding studies in which [^{3}H]-prazosin was used as the specific ligand (Karliner, Barnes, Hamilton & Dollery, 1979) revealed no changes in the maximum number of binding sites (B_{max}) or their dissociation constant (K_D) in heart, spleen, forebrain and hindbrain. Thus the increased responses to phenylephrine is unlikely to be mediated directly by changes at the post synaptic α_1 adrenoceptor. Prazosin caused similar falls in mean arterial pressure in renal hypertensive and control animals but a greater fall in denervated rabbits in whom no baroreceptor mediated changes in heart rate or plasma NA were observed (Fraser, Hamilton, Reid & Zamboulis, 1980).

Although changes in responses to α_1 adrenoceptor agonists were observed in both models of hypertension, these may involve several mechanisms and could not be correlated with changes in alpha$_1$ receptor binding in heart, spleen or brain.

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Table 1 Alpha adrenoceptor responses after 3 weeks in two models of experimental hypertension in the rabbit (n = 8–12)

		Renal hypertensive	*Uninephrectomised controls*	*Sinoaortic denervation*	*Sham operated controls*
Mean arterial pressure (mmHg)		**127 ± 14	82 ± 3	**93 ± 17	71 ± 9
Plasma noradrenaline (nM)		1.3 ± 0.8	2.2 ± 2.0	*4.4 ± 3.9	2.5 ± 2.1
Phenylephrine dose ratio relative to controls		**3.0	—	*1.7	—
Noradrenaline dose ratio relative to controls		**3.1	—	1.0	—
Fall in MAP after prazosin 0.5 mg/kg (mmHg)		24 ± 12	20 ± 11	**32 ± 11	17 ± 7
Increase in plasma noradrenaline after prazosin 0.5 mg/kg (nM)		3.3 ± 2.0	2.7 ± 1.3	*1.4 ± 0.4	2.2 ± 0.9
Phenylephrine dose ratio after prazosin 0.5 mg/kg		10.8	13.0	14.8	12.1
[^{3}H]-prazosin binding					
B_{max} (fM/mg protein)	heart	143 ± 52	176 ± 34	140 ± 60	110 ± 37
	spleen	212 ± 74	172 ± 31	103 ± 30	150 ± 33
	forebrain	100 ± 13	89 ± 12	117 ± 25	165 ± 35
	hindbrain	160 ± 58	160 ± 61	129 ± 32	194 ± 60

All results expressed as mean ± s.d.
** $P < 0.01$, * $P < 0.05$ when hypertensives compared to controls.

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Is there a change in post-synaptic α-receptors in hypertension? *In vitro* and *in vivo* studies

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A subdivision of post-synaptic α-receptors has recently been demonstrated in the intact cardiovascular system of several species (Drew & Whiting, 1979; Docherty, Madjar & Starke, 1980). In order to determine whether there is a change in the sensitivity, or density of peripheral post-synaptic α-receptors in hypertension, as recently demonstrated in the brain of spontaneously hypertensive rats (Gheyouche, Le Fur, Collotte, Burgerin & Uzan, 1980), we have compared vasoconstrictor responses to α-receptor agonists *in vivo*, in pithed spontaneously hypertensive rats (SHR) with normotensive controls (WKY) or normal Wistar rats (NR), before or after treatment with prazosin. Binding studies, using [^{3}H]-prazosin (33 Ci/mmole) as a selective α_1-adrenoceptor ligand (Greengrass & Bremner, 1979), were carried out *in vitro* from membranes prepared from spleen, ventricle and kidney of SHR or NR. The affinity constant and maximum number of binding sites were determined by Scatchard analysis.

Male SHR (Okamoto Aoki) and age matched WKY or NR (I.C.I., Alderley Park) were used at 13–15 weeks of age (body wt. 280–310 g). Systolic blood pressure (SBP) was measured by a tail cuff method. SHR or NR (in groups of 6–9) were used if the SBP exceeded 185 mmHg or was less than 130 mmHg respectively.

Hypertensive and normotensive rats, set up as paired experiments, were pithed under pentobarbitone anaesthesia (55 mg/kg, i.p.) and respired with room air. Blood pressure of SHR (82 ± 5/39 ± 4) was not significantly different from NR (80 ± 6/40 ± 5) after pithing.

In SHR, the vasoconstrictor responses (DBP) induced by higher doses of NA, phenylephrine (PE) or angiotensin (AII) lay to the left of controls, and the maximum responses were increased. In contrast, the pressor curves to the α_2-agonists N,N-dimethyl-6,7-diOHATN (Nicks & Cannon, 1980) or BHT 933 (Timmermans & Van Zwieten, 1980) were superimposable in SHR and NR.

The antagonist potency (DR10) of prazosin (0.01–0.25 mg/kg, i.v., 15 min) at α_1-receptors was assessed at the ED_{100} dose of NA or PE. Prazosin was 2.5× more potent against PE and 1.75× more potent against NA in SHR, than against the same agonists in NR. Furthermore, in SHR an inhibitory effect of prazosin was demonstrated against all doses of NA, whereas in NR 60–70% of the NA pressor curve was prazosin resistant. WKY and NR did not differ significantly in their sensitivity to prazosin. Treatment with cocaine (5 mg/kg, i.v.) and propranolol (1 mg/kg, i.v.) did not influence the ratio of increased prazosin potency (SHR/NR) and the shape of the NA pressor curve was not altered by this treatment.

Specific binding at α_1-receptors by [^{3}H]-prazosin (defined as the binding displaced by (−)-NA (200 μM)) represented more than 80% of the total binding at 0.2 nM of the ligand in all tissues. Analysis of the binding data using Scatchard plots showed that there was no significant difference in receptor density (B_{max}) or in the affinity constant of [^{3}H]-prazosin (K_D) between SHR and NR in any of the tissues examined.

In conclusion, an increase in the α_1-receptor antagonist potency of prazosin has been demonstrated in SHR. In this hypertensive model NA-vasoconstrictor responses (normally considered to be α_2-receptor mediated) appear more susceptible to prazosin blockade. This suggests that in hypertension, there may be a shift in the proportion of α_1/α_2-postsynaptic receptors. The increased potency to prazosin in SHR does not appear to be due to an increased population of α_1-receptors since [^{3}H]-prazosin binding did not change in this model. However, it remains a possi-

bility that these *in vivo* effects, mediated predominantly through vascular resistance beds, are not fairly represented by the tissues sampled *in vitro*.

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Withdrawal syndrome after continuous infusion of clonidine in the spontaneously hypertensive and normotensive rat

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Despite intensive research the clonidine withdrawal syndrome in man and in laboratory animals has been poorly defined. Various attempts to develop an animal model suitable to study and predict rebound phenomena following cessation of treatment with clonidine have met with limited success. Most of the inconsistencies can, however, be explained by the short action of clonidine, especially in the rat, so that discontinuous administration of the drug can be expected to produce large daily fluctuations of heart rate and blood pressure rather than sustained falls of these parameters (Prop, 1978; Oates *et al.*, 1978; Salzmann, 1979; Dix & Johnson, 1979; Finch & Hicks, 1979). Therefore, we have used the ALZET® osmotic minipump to ensure a permanent subcutaneous infusion of clonidine hydrochloride (500 $\mu g\ kg^{-1}\ day^{-1}$) in male spontaneously hypertensive (SH) and normotensive rats. During the infusion and following removal of the miniosmopump after 12 days, blood pressure and heart rate were measured directly via an indwelling abdominal aortic catheter in conscious, unrestrained animals.

In the SH rats blood pressure and heart rate were significantly reduced throughout the clonidine-infusion period, as compared to saline-treated controls. In the normotensive rats, however, no consistent differences were observed between the clonidine- and saline-treated groups. Following removal of the clonidine-charged minipumps a genuine heart rate overshoot was seen in the SH as well as in the normotensive rats. In both groups blood pressure proved most unstable during the withdrawal period, showing typical short-lasting upswings. Heart rate and number of upswings per hour increased from 1 h after pump removal onwards, reaching a maximum at 8–10 h after cessation of clonidine infusion. No changes were observed after discontinuation of saline infusion.

In the SH rats plasma noradrenaline concentration was significantly lower during and significantly higher at 20 h following discontinuation of the clonidine infusion compared to saline treated controls. In the normotensive rats plasma noradrenaline concentration was significantly elevated towards control levels during the rebound phase.

In summary, the withdrawal syndrome after a twelve-day continuous administration of clonidine in SH and normotensive rat was associated with a marked heart rate overshoot accompanied by a period of blood pressure lability, which was manifest by typical upswings. These animal models seem suitable for the study of the effects of discontinuation of clonidine and other antihypertensive drugs.

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In vitro and *in vivo* effects of a new powerful calcium antagonist

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PY 108-068 is a dihydropyridine derivative (Figure 1). Calcium antagonism on canine coronary artery was assessed *in vitro*. PY 108-068 did not behave as a simple competitive antagonist. We nevertheless calculated apparent pA_2 values using calcium as an agonist. The apparent pA_2 was 10.5 for PY 108-068. The value obtained for verapamil was 7.8. The calcium antagonism was not limited to coronary arteries. On saphenous artery the apparent pA_2 value was 10.4.

In anaesthetized open chest cats PY 108-068 decreased blood pressure and increased coronary flow. These effects were similar to those of the same doses (1–43 μg/kg) of nifedipine. In contrast to nifedipine, PY 108-068 decreased heart rate in this animal model. Cardiac output was increased to the same extent by both drugs, yet right atrial pressure increased less with PY 108-068.

Regional blood flow was measured in various vascular beds with tracer microspheres. Both drugs increased myocardial flow and redistributed it in favour of the outer layer of the left ventricle. PY 108-068 but not nifedipine increased blood flow to the kidneys substantially, whereas nifedipine dilated the small intestinal vessels more than PY 108-068.

PY 108-068 is light stable and therefore easy to use for *in vitro* experiments. As the most potent calcium antagonist presently known it may prove to be a useful pharmacological tool in addition to its possible therapeutic potential.

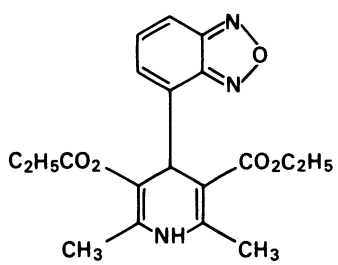

Figure 1 Chemical structure of PY 108-068

Relationship between 'staircase' phenomena and response to cardiac glycosides in rat, guinea-pig and ground squirrel papillary muscles

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It has been postulated (Langer, 1971; 1973) that the positive inotropic responses elicited by cardiac glycosides and by an increase in heart rate are produced by a single common mechanism—an electrically neutral coupled exchange of calcium and sodium. This view would explain why rat myocardium, which exhibits a 'negative staircase' (Blesa, Langer, Brady & Serena, 1970), is particularly insensitive to cardiac glycosides (Repke, Est & Portius, 1965). We have therefore examined the relationship between force-frequency characteristics and sensitivity to the semi-synthetic reversible glycosides actodigin (Thomas, Allen, Pitts & Schwartz, 1979) in isolated papillary muscles from the rat, the guinea-pig and from a hibernating ground

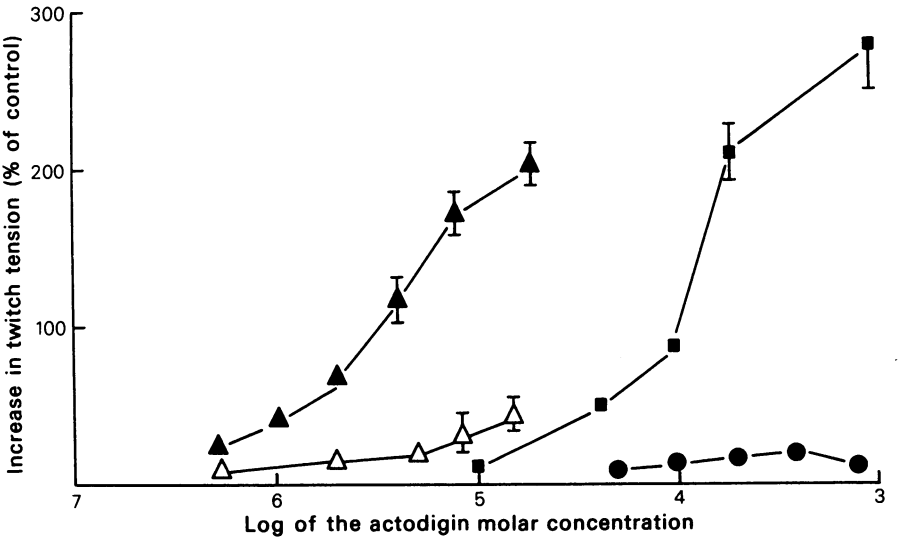

Figure 1 Percentage increase in twitch tension (%ΔT) produced by actodigin in electrically stimulated papillary muscles from the rat (●), the guinea-pig (■) and both the hibernating (△) and fully active (▲) Richardson ground squirrels. Each point is the mean of at least 8 values.

squirrel (*Spermophilus richardsonii*). All preparations were mounted on punctate electrodes, maintained in Krebs-Henseleit solution at 34°C and stimulated at 0.5 Hz using 5 ms square pulses of twice threshold voltage.

No matter the season, ground squirrel papillary muscles exhibited a clear negative staircase, which was similar to that observed in rat preparations but which contrasted with the positive staircase observed in the guinea-pig.

Rat papillary muscles were insensitive to actodigin (up to 10^{-3} M) whereas guinea-pig preparations exhibited concentration-dependent increases in developed tension (Figure 1). The response to actodigin in ground squirrel preparations depended on the resting state of the animals. During hibernation even high toxic concentrations ($>10^{-5}$ M) of actodigin (which caused missed beats) only increased twitches by less than 50% whereas during summer, the preparations were highly sensitive to the glycoside.

In guinea-pig preparations verapamil (2 μM), which decreased twitches by 59 ± 6% (mean ± s.e. mean) abolished the inotropic response caused by increasing the stimulation frequency from 0.5 to 2.0 Hz (131 ± 36 to 4 ± 26%) without modifying the response to 10^{-4} actodigin (117 ± 32 before; 83 ± 24% after).

These data do not support the contention that both Bowditch and glycoside-induced inotropic effects are mediated through a common mechanism. In addition it is unlikely that the insensitivity to glycosides of rat myocardium is related to its negative staircase.

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Effects of L-3,3',5-triiodothyronine on rat cardiac function

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Chronic administration of thyroid hormones to rats causes an increase in spontaneous heart rate (Aronson, 1976) and an increase in cardiac contractility (Rovetto, Hjalmarson, Morgan, Barrett & Goldstein, 1972) in *ex vivo* preparations. In order to reduce possible indirect changes in cardiac function resulting from prolonged cardiac stimulation *in vivo*, the effects of a single dose of thyroid hormone have been investigated 48 h after administration when effects are maximal (Brooks, Flynn & Underwood, 1980).

Thyroidectomized (Tx) rats were injected sub-cutaneously with L-3,3',5-triiodothyronine (L-T_3) in alkaline saline or vehicle and cardiac function investigated 48 h later using a modification of the isolated working heart preparation described by Neely, Liebermeister, Battersby & Morgan (1967). Hearts were perfused via the left atrium at a filling pressure of 10 cms H_2O with modified Krebs-Henseleit bicarbonate buffer containing Ca^{2+} (1.25 mM) at 33°C. The left ventricle ejected fluid against a pressure head of 70 cms H_2O. Contractility was assessed using the maximum value of the first differential of left ventricular pressure (dLVP/dt max). After recording spontaneous heart rate (SHR), measurements of cardiac function were made during electrical pacing, 300 beats/min.

The SHR of hearts from control Tx rats was 184 ± 4 (mean of 7 ± s.e. mean). L-T_3 caused dose-dependent increases in SHR to 194 ± 6, 229 ± 12, 252 ± 7 at 20, 100 and 500 μg/kg respectively (n = 6 in each case). Administration of two doses of L-T_3 (2 mg/kg) at 0 and 24 h caused no further increase in SHR. The effects of L-T_3 on other parameters in hearts paced at 300 beats/min are shown in Table 1 and compared with values obtained in hearts isolated from euthyroid (Eu) rats. SHR of hearts from Eu rats, 252 ± 7 (n = 15), was not significantly different from the maximum obtained in hearts from L-T_3 treated Tx rats. However, the maximum value of dLVP/dt max of hearts from L-T_3 treated Tx rats was significantly higher than that of hearts from Eu rats.

These experiments show that the cardiac function of hearts from Tx rats can be restored to values at least those of hearts from Eu rats, 48 h after a single injection of L-T_3.

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Table 1 Effects of L-T_3 on function of hearts from Tx rats compared with hearts from euthyroid rats. Values are mean ± s.e. mean. Statistical significance between groups was tested using Student's 't'-test and level of significance indicated

Dose L-T_3 (μg/kg s.c.)	*dLVP/dt max (mmHg/s)*	*Coronary Flow (ml min^{-1} gdw^{-1})*	*Cardiac Output (ml min^{-1} gdw^{-1})*
Control (n = 7)	3400 ± 160	27 ± 5	257 ± 19
20 (n = 6)	4060 ± 490	28 ± 3	298 ± 19
100 (n = 6)	5120 ± 360[a]	32 ± 4	322 ± 19
500 (n = 6)	5810 ± 180[a]	45 ± 5[b]	311 ± 16[c]
2 × 2000 (n = 6)	5530 ± 410	50 ± 4	357 ± 23
Eu (n = 15, 5.5)	4180 ± 110[d]	43 ± 3	362 ± 20

gdw—g dry weight of heart.
a—$P < 0.001$, b—$P < 0.025$, c—$P < 0.05$, compared with controls d—$P < 0.001$, compared with Tx + L-T_3 (500 μg/kg).

Effects of alinidine (N-allyl clonidine ST567) on experimental cardiac arrhythmias

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The development of abnormal pacemaker activity in ventricular tissues is an important mechanism causing cardiac arrhythmias in experimental animals and man (Wit, Rosen & Hoffman, 1974). Alinidine caused bradycardia by a direct action on the heart, slowing the sinus node with negligible changes in the PR and QT intervals and the QRS complex (Kobinger, Lillie & Pichler, 1979a and b). In guinea-pig isolated atria the bradycardia was not affected by pre-treatment with phentolamine or atropine, and there was no competitive antagonism of an isoprenaline tachycardia. Since alinidine reduces the rate of the normal cardiac pacemaker we have studied the effects of alinidine on three experimental cardiac arrhythmias (Allen & Shanks, 1974) which are associated with abnormal ventricular pacemaker activity.

Anaesthetized dogs were respired with halothane in room air and the test dose of adrenaline to cause ventricular tachycardia was determined. Alinidine was administered intravenously in increasing doses from 0.5 to 4.0 mg/kg until the test dose of adrenaline failed to cause ventricular ectopic beats. The arrhythmia was abolished in 5 dogs at a mean dose of alinidine of 2.9 ± 0.7 mg/kg (mean $\pm$ s.e.) which also reduced the sinus rate from 174 ± 12 to 118 ± 10 beats/min.

Ouabain-induced ventricular tachycardia in 5 anaesthetized dogs was abolished by the intravenous administration of increasing doses of alinidine after a mean dose of 8 mg/kg (cumulative dose 15.5 mg/kg).

Ventricular tachycardia in conscious dogs 24 h after ligation of the anterior descending branch of the left coronary artery is associated with abnormal pacemaker activity and slow conduction in the Purkinje fibre network over the infarct (Friedman, Stewart & Wit, 1973). However these arrhythmias were not abolished despite the administration of large doses of alinidine (15.5 mg/kg cumulative).

Alinidine can abolish certain experimental cardiac arrhythmias, but relatively large doses of the drug are required, and its mode of action is not clear.

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A comparison of the intensity and duration of the antidysrhythmic effects of mexiletine and Org 6001 in anaesthetized rats

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Although the aminosteroid, Org 6001 has been shown to exert anti-dysrhythmic activity in a variety of experimental models (Vargaftig, Sugrue, Buckett & Van Riezen, 1975; Marshall & Parratt, 1975; Kane, McDonald & Parratt, 1979) there is little information available on its duration of action. The object of this study was to compare the haemodynamic and antidysrhythmic effects of both parenterally and orally administered Org 6001 and mexiletine in pentobarbitone-anaesthetized rats subjected to acute ligation of the main left coronary artery (Kane *et al.*, 1979).

When administered 15 min before ligation, both Org 6001 (2–10 mg/kg) and mexiletine (1.0 mg/kg) significantly reduced the number of ventricular ecto-

Table 1 The effects of Org 6001 and mexiletine administered either i.v. or p.o. on the number of ventricular extrasystoles (VES) and per cent incidence of ventricular fibrillation (VF) induced by acute coronary artery ligation in the anaesthetized rat. All determinations were made within the first 30 min of ligation. Drugs were given either i.v. 15 min prior to ligation or p.o. 60 min before ligation

Intravenous Study					*Oral Study*				
Treatment	*dose (mg/kg)*	*n*	*VES*	*VF (%)*	*Treatment*	*dose (mg/kg)*	*n*	*VES*	*VF (%)*
Control		23	1312 ± 196	52	Control		18	1458 ± 206	44
Org 6001	2	6	*595 ± 213	17	Org 6001	10	6	1400 ± 728	33
	5	8	681 ± 463	0		20	7	*547 ± 161	0
	10	6	**235 ± 63	0		100	7	***142 ± 59	0
Mexiletine	0.4	8	1342 ± 493(6) †4732(1)	38	Mexiletine	20	8	872 ± 252(7) †4943(1)	50
	1.0	6	**319 ± 74	0		50	7	*510 ± 217	0
						100	4	**270 ± 49	0

Values are the mean ± s.e. mean of *n* observations.
* ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$) denote significant differences from the controls.
† Outside range seen in controls.

pic beats (VES) and the incidence of ventricular fibrillation (VF) (Table 1). Either drug caused only transient reductions in diastolic arterial blood pressure, heart rate and left ventricular dP/dt max, the maximum changes being (for Org 6001 10 mg/kg) 44 ± 4, 20 ± 3 and 19 ± 2% and for mexiletine (1.0 mg/kg) 32 ± 6, 8 ± 1 and 24 ± 5% respectively.

When given orally (100 mg/kg 60 min before ligation), both Org 6001 and mexiletine reduced the number of VES and completely abolished VF (Table 1). At this dose, Org 6001 was still active at both 12 and 18 h, the mean number of VES being 23 ± 11 and 211 ± 194 beats respectively and VF being absent. In contrast, ligation 12 h after mexiletine (100 mg/kg. p.o.) resulted in an increased mortality (61% compared with 6% in controls) and no reduction in the number of VES or in the incidence of VF.

Ventricular fibrillation thresholds (VFT's) were determined (using impulse train 50 Hz, 0.8 ms) in blind experiments using animals treated orally with either vehicle (4 ml/kg), Org 6001 or mexiletine (both 100 mg/kg), 12 h prior to ligation. 15 min before ligation, mean VFTs were 549 ± 69, 421 ± 48 and 363 ± 48 μA respectively. 4 min after ligation, mean VFTs in the control and mexiletine groups fell to 332 ± 59 and 279 ± 43 μA whereas those in the Org 6001 group remained unchanged (493 ± 69 μA).

In conclusion, Org 6001 shows comparable oral antidysrhythmic potency with mexiletine but is longer acting.

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An explanation for the cholinomimetic action of Ba^{2+} on sympathetic neurones

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Ba^{2+} ions seem to imitate the muscarinic effects of acetylcholine on sympathetic (Ambache, 1949; Takeshige & Volle, 1964; Tashiro & Nishi, 1972) and cortical (Krnjević, Pumain & Renaud, 1971) neurones. These effects include membrane depolarization, increased input resistance and facilitation of repetitive firing.

Muscarinic responses of frog sympathetic neurones result from the suppression of a time- and voltage-dependent K^+-current, normally activated between −60 and −10 mV, which we have termed the M-current (I_M: Brown & Adams, 1980). We have now observed (Figure 1) that Ba^{2+} (1–4 mM) can also selectively depress I_M in voltage-clamped frog neurones, in preference to the larger outward K^+-currents (voltage-sensitive delayed rectifier and Ca^{2+}-driven currents) activated at more positive membrane potentials. I_M-suppression was accompanied by the expected inward (depolarizing) current within the I_M-activation range. Neither inward current nor membrane depolarization (in unclamped cells) occurred when I_M had previously been suppressed by 10 μM muscarine. Other divalent cations were either inactive (Ca^{2+} or Mg^{2+} at 10 mM; Sr^{2+} at 4 mM) or preferentially depressed the large outward K^+-currents (Cd^{2+} at 100 μM; Mn^{2+}, Ni^{2+} or Co^{2+} at 4 mM), and did not fully replicate 'muscarinic' effect of Ba^{2+} on unclamped neurones.

This muscarine-like action of Ba^{2+} was not due to release of acetylcholine, since it was not blocked by atropine. Instead, we suggest that Ba^{2+} blocks the M-channels directly.

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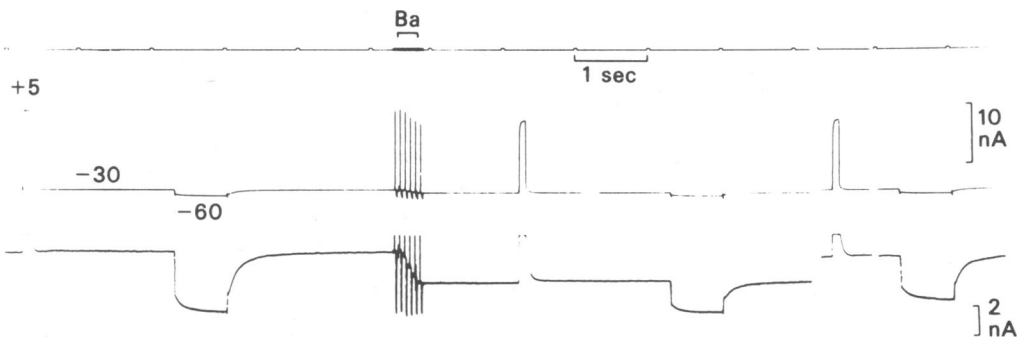

Figure 1 Membrane currents (middle and lower records, two different gains) recorded from a bullfrog voltage-clamped sympathetic neurone (see Brown & Adams, 1980, for details) held at −30 mV and subjected to voltage-commands to +5 mV for 70 ms and −60 mV for 700 ms alternately. The former activated the large outward currents whose threshold lay positive to the holding potential. At the holding potential about 60% of the M-channels are open, giving a steady outward K^+-current. The hyperpolarizing commands inactivate these channels, producing the slow inward relaxation observed on the lower (high-gain) current record; repolarization re-opens the M-channels, producing the slow outward relaxation. $BaCl_2$ (4 mM, applied for about 25 s while the chart recorder was slowed down 100 times) produced a steady inward current of some 2 nA and reduced the repolarizing M-current by about the same amount (−1.9 nA, −63%). The large outward currents (middle record) were reduced by only 11% (−1.6 nA), some of which may be attributed to suppression of that component of I_M activated above −30 mV. The gap in the records represents several minutes washing, after which some recovery of I_M is apparent.

Solubilization of β_1 and β_2 adrenoceptors from rat and rabbit lung

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The presence of both β_1 and β_2 adrenoceptors in lung tissue has been suggested both from biochemical (Rugg, Barnett & Nahorski, 1978) and pharmacological (Zaagsma, Oudhof, van der Heijden & Plantje, 1979) approaches. However, the definitive identification of distinct receptor subtypes requires the separation of β_1 and β_2 adrenoceptors from solubilized preparations. In the present communication we describe the solubilization and successful assay of specific β-adrenoceptor binding sites prepared from rat and rabbit lung, which possess predominantly β_2 and β_1 adrenoceptors, respectively, in particulate preparations (Rugg, Barnett & Nahorski, 1978).

Rat (Wistar) or rabbit (New Zealand white) lung membranes prepared as previously described (Rugg, Barnett & Nahorski, 1978) were solubilized in 0.5% digitonin, 50 mM Tris–HCl, pH 7.8 (digitonin:protein ratio 3:1) for 30 min at 4°C. Following centrifugation at 50,000 *g* for 60 min the supernatant was used as the soluble receptor preparation. Identification of β-adrenoceptors was achieved with the antagonist (−)-[^{3}H]-dihydroalprenolol [^{3}H]-DHA, and a charcoal (0.6% Norit GSX, 0.13% bovine serum albumin in 50 mM Tris–HCl, pH 7.8) centrifugation assay was used to separate free and bound ligand in the soluble preparation. Initial experiments using Polyethylene glycol 6000 and collection of precipitated protein on glass-fibre filters was shown to produce a non-linear precipitation of receptor protein. Binding assays were performed at 22°C.

Specific binding of [^{3}H]-DHA (binding displaced by (−)-isoprenaline (200 μM) was >95% in all soluble preparations. The addition of NaCl (10 mM–1 M) increased the yield of soluble β-adrenoceptors from rat and rabbit lung and protected the sites from loss on standing at room temperature. The characteristics of [^{3}H]-DHA binding to soluble receptors was identical to that found in particulate preparation for both rat and rabbit (rat lung K_D particulate 0.35 ± 0.01 nM, soluble 0.32 ± 0.01 nM; rabbit lung K_D particulate 0.59 ± 0.05 nM, soluble 0.67 ± 0.06 nM).

The yield of soluble receptors and total protein was 37% and 38%, respectively, for rat lung but only 11% and 35% for rabbit lung. The low recovery of β-adrenoceptors from rabbit lung was reflected by a relatively selective loss of the β_1 sites during solubilization. Thus, using computer-assisted iterative curve fitting of the displacement curves generated by the highly selective β_1 selective antagonist (±)-atenolol yielded 20% β_1, 80% β_2, receptors for rat lung membranes but 100% β_2 receptors in the soluble preparation. Likewise, rabbit membranes possess 79% β_1 and 21% β_2 receptors but only 10% β_1 and 90% β_2 sites in solution. It will be important to improve the recovery of β_1 receptor sites before any physical separation of subtypes is attempted.

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Reserpine-induced supersensitivity to the β-adrenoceptor-mediated responses of cardiac, respiratory and uterine tissues of the guinea-pig

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Chronic pretreatment of animals with reserpine produces supersensitivity to sympathomimetic amines in isolated cardiac tissue (Meisheri, Tenner & McNeill, 1979). We have examined the β-adrenoceptor-mediated responses of various tissues from guinea-pigs pretreated with reserpine (5 mg/kg at 72 h, 3 mg/kg at 48 and 24 h i.p. before sacrifice) to compare with the supersensitivity previously described for isolated atria (Broadley & Lumley, 1977).

All preparations were suspended in Krebs-bicarbonate solution gassed with 5% CO_2 in oxygen at 38°C. Isometric tension was recorded. Except for uteri, they were incubated throughout with phentolamine (5×10^{-6} M) and metanephrine (10^{-5} M) to inhibit

α-adrenoceptors and extraneuronal uptake respectively. Cumulative dose–response curves to (−)-isoprenaline were constructed and geometric mean EC_{50} values determined. Isolated papillary muscles were examined initially, paced at 2 Hz with threshold voltage +50% and 5 ms pulse width. Supersensitivity of the positive inotropic effect of isoprenaline was demonstrated by the mean ($n = 13$) dose–response curve after reserpine pretreatment (EC_{50} 5.1×10^{-10} M) being significantly ($P < 0.001$) to the left of that for untreated animals (EC_{50} 5.2×10^{-9} M, $n = 10$).

Tracheal spirals, contracted with carbachol (5.8×10^{-7} M), from reserpine-pretreated guinea-pigs were also supersensitive to the relaxant response to isoprenaline. The mean ($n = 8$) EC_{50} value (4.2×10^{-9} M) was significantly ($P < 0.01$) less than that for untreated animals (1.3×10^{-8} M, $n = 8$). Since the tracheal spiral may not accurately reflect the adrenergic receptor characteristics of the peripheral airways (Lulich, Mitchell & Sparrow, 1976), we have also examined the isoprenaline-induced relaxation of lung parenchymal strips contracted with carbachol (5.8×10^{-6} M). No supersensitivity after reserpine pretreatment occurred, the mean ($n = 5$) EC_{50} value (5.4×10^{-9} M) was not significantly different ($P > 0.05$) from the mean ($n = 4$) control value (7.9×10^{-9} M).

The β-adrenoceptors of lung strips have been classified as the β_2-type (Siegl, Rossi & Orzechowski, 1979) and it has been proposed that β_2-adrenoceptors are not under sympathetic innervation (Russell & Moran, 1980). Although the trachea is often recognized as having receptors of the β_2-type, there is evidence for the presence of β_1-adrenoceptors (Lulich *et al.*, 1976; O'Donnell & Wanstall, 1979), which are presumably innervated. It is possible that the supersensitivity of tracheal and cardiac preparations is of innervated β_1-adrenoceptors and the failure to produce supersensitivity in lung strips is due to a lack of innervation. This possibility was further examined in guinea-pig isolated uteri, the adrenoceptors of which are designated β_2 (O'Donnell, Persson & Wanstall, 1978). Uteri were contracted by replacing the Krebs-bicarbonate solution with one in which the Na^+ ions were substituted by an equivalent amount of K^+ ion (O'Donnell *et al.*, 1978). The mean ($n = 18$) EC_{50} value for the isoprenaline-induced relaxation in untreated uteri (1.2×10^{-8} M) did not differ significantly ($P > 0.05$) from that of reserpine-pretreated animals (1.4×10^{-8} M, $n = 8$). Thus uteri, like lung strips, exhibited no supersensitivity.

As a pharmacological test of sympathetic innervation of lung and tracheal preparations, their responsiveness to indirectly acting sympathomimetic amines was examined. The relaxations of the tracheal spirals by tyramine and β-phenylethylamine at the maximum concentrations were $70.1 \pm 6.4\%$ ($n = 8$) and $87.3 \pm 4.0\%$ ($n = 8$) respectively of the isoprenaline maximum. However, in lung strips the maximum relaxations were only 17.1 ± 4.3 ($n = 8$) and $21.3 \pm 6.9\%$ ($n = 8$) respectively. At higher concentrations of β-phenylethylamine, the lung strip contracted. This suggests that the lung strip is virtually devoid of innervation which might explain the absence of reserpine-induced supersensitivity.

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Effects of monovalent and divalent cations and purine nucleotides on the α-adrenoceptor of human intact platelets

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In rabbit platelet lysates the affinity for α-adrenoceptor agonists is reduced by the presence of Na^+ with little effect on antagonists (Tsai & Lefkowitz, 1978). In human platelet lysates the affinity for α-adrenoceptor agonists is selectively increased by the presence of Mg^{2+} and subsequently reduced by GTP (Tsai & Lefkowitz, 1979). This study investigated the effects of monovalent and divalent cations and purine nucleotides on the binding characteristics of the α-adrenoceptor of intact human platelets and on platelet morphology.

Intact human platelets were resuspended from platelet-rich plasma in a medium comprising 2.5 mM EDTA and 150 mM NaCl or NH_4Cl (pH 7.5). Platelet α-adrenoceptors were identified by a receptor-binding technique using [^{3}H]-dihydroergocryptine ([^{3}H]-DHEC) as described previously (Boullin & Elliott, 1979). The affinity and capacity of specific [^{3}H]-DHEC binding were determined from Scatchard analysis and the affinities for (−)adrenaline and (−)noradrenaline from competitive inhibition studies.

The platelet α-adrenoceptor binding capacity was similar in media containing NaCl and NH_4Cl, but the affinity for [^{3}H]-DHEC was significantly greater ($P < 0.05$) in the presence of Na^+ than NH_4^+. The affinity for (−)adrenaline and (−)noradrenaline was lower in NH_4Cl than in NaCl, but this difference was not statistically significant.

Addition of Ca^{2+} or Mg^{2+} to platelets resuspended in EDTA/NaCl medium reduced the α-adrenoceptor capacity without altering the affinity for [^{3}H]-DHEC or (−)adrenaline or (−)noradrenaline. Simultaneously the addition of Ca^{2+} or Mg^{2+} altered the platelet shape as detected by changes in transmitted and scattered light, suggesting a shift from a spheroid to a discoid form.

The guanine nucleotides GDP and GTP (10 μM) had no effect either alone or in the presence of Mg^{2+} (0.5 mM) on the binding of [^{3}H]-DHEC or on the affinities of (−)adrenaline and (−)noradrenaline.

We conclude that unlike the situation in rabbit and human platelet lysates, the affinity of α-adrenoceptor agonists on intact human platelets is not reduced by the presence of exogenous Na^+ or GTP. The coincident changes in platelet shape and α-adrenoceptor capacity suggest an association between cell morphology and receptor sensitivity.

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Table 1.

	[^{3}H]-DHEC Binding		Competitive affinity (K_i)	
	Affinity (K_d) (nM)	*Capacity (R_t) (fmol/10^8 platelet)*	*(−)-Adrenaline (μM)*	*(−)Noradrenaline (μM)*
EDTA incubation medium containing				
Na^+ (150 mM) ($n = 4$)	3.45 ± 0.60	72 ± 6	4.1 ± 0.4	14.9 ± 2.0
NH_4^+ (150 mM) ($n = 4$)	9.16 ± 1.56*	68 ± 7	10.5 ± 3.9	34.5 ± 12.9
EDTA/NaCl incubation medium				
Control ($n = 3$)	2.94 ± 0.25	87 ± 3	4.1 ± 0.38	14.9 ± 2.0
Ca^{2+} (1 mM) ($n = 3$)	3.76 ± 0.66	55 ± 5*	4.3 ± 0.51	14.0 ± 1.37
Mg^{2+} (1 mM) ($n = 3$)	3.77 ± 0.83	71 ± 8	4.0 ± 0.46	12.5 ± 2.17

Effects of monovalent and divalent cations on the α-adrenoceptor binding characteristics of intact human platelets.
* Indicates $P < 0.05$ as compared by Student's unpaired t-test.

Pharmacological analysis of opiate receptors in the rat vas deferens

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The rat vas deferens has been found to be rather insensitive to most synthetic narcotic analgesics (Lemaire, Magnan & Regoli, 1978) and it has been suggested that the opiate receptors present in this preparation are of a novel type (Wüster, Schulz & Herz, 1979), different from the already described μ-, δ- and κ-receptors. In this communication the effects of normorphine, which acts selectively through μ-receptors, of D-Ala^2-D-Leu^5-enkephalin, a δ-agonist and of porcine β-endorphin, which interacts equally well with both of these types of receptors, have been studied in the rat vas deferens. (−)-Ethylketazocine, which is a κ-agonist in the mouse vas deferens and in the guinea-pig ileum, has also been tested.

Vas deferentia from hooded rats of the Aberdeen colony (250–350 g) were prepared (Lemaire *et al.*, 1978) and trains, consisting of 2–3 pulses of 0.5–1 ms duration at intervals of 200 ms, were delivered at a frequency of 0.1 Hz, the voltage being 80–95% maximal. Agonist potency is expressed as the IC_{50} value obtained from dose–response curves and antagonist potency as the equilibrium dissociation constant, K_e, obtained by the method of Arunlakshana & Schild (1959).

The IC_{50} values were 2068 ± 98 nM (n = 21) for normorphine, 300 ± 16 nM (n = 52) for D-Ala^2-D-Leu^5-enkephalin and 23.8 ± 1.9 nM (n = 16) for β-endorphin; (−)-ethylketazocine had no agonist activity. Naloxone, MR 2266 and (−)-ethylketazocine were pure antagonists (Table 1).

The present evidence suggests that μ- and δ-receptors are present in the rat vas deferens. It is of particular interest that ethylketazocine which is a pure agonist in the guinea-pig ileum and mouse vas deferens (Hutchinson, Kosterlitz, Leslie, Waterfield & Terenius, 1975), is a pure stereospecific antagonist in the rat vas deferens. No explanation for this species difference is available at present.

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Table 1 Antagonist potencies of naloxone, MR 2266 and (−)-ethylketazocine against normorphine, D-Ala^2-D-Leu^5-enkephalin and β-endorphin

	Equilibrium dissociation constants (K_e, nM)		
Antagonist	*Normorphine*	*D-Ala^2-D-Leu^5-Enkephalin*	*β-Endorphin*
Naloxone	6.3 ± 0.3	33.7 ± 1.3	14.7 ± 1.2
MR 2266	3.7 ± 0.2	37.2 ± 7.7	14.6 ± 2.4
(−)-Ethylketazocine	58.8 ± 6.6	206 ± 29	332 ± 18
(+)-Ethylketazocine	> 5,000	> 5,000	> 5,000

The values are the means ± s.e. mean of 3–4 estimations. MR 2266 is (−)-α-5,9-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan (C.H. Boehringer Sohn).

Dextran and histamine secretion from rat mast cells: effect of glucose and low molecular weight glucose polymers

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Dextran, a high molecular weight glucose polymer has long been known as a releaser of histamine from rat mast cells (Ankier & West, 1964). Its action, *in vitro*, was shown to be dependent on calcium (Foreman & Mongar, 1972) and phosphatidyl serine (Goth, Adams & Knoohuizen, 1971). The activity of dextran as a stimulus to secretion, on a molecular bases, is only slightly dependent on molecular weight over the range 40,000 to 500,000 (EC_{50} about 1 μM) in the range. Below 40,000 the typical S-shaped concentration–effect curves become bell-shaped with a reduced maximum effect. These dextrans may be considered as partial agonists. Below 10,000 all agonist activity disappears (Figure 1). These low molecular weight glucose polymers, and indeed glucose itself, function as pure antagonists of the secretory system stimulated by dextran.

The nature of the antagonists has been studied. Glucose produces a parallel shift of the dextran ($\bar{M}w$ 80,000) log dose response curve over a limited range of concentrations (2–16 mM): a Schild plot is linear with a pA_2 of 2.56 ± 0.081 but the slope is significantly greater than unity (3.84 ± 0.59) so the interaction can hardly be described as a simple one to one competition for the receptor site. Above 20 mM glucose the maximum effect obtainable by the dextran agonist is somewhat reduced. The antagonism of the low molecular weight glucose polymers (1000 and 3000) has also been studied. They produce a parallel shift like glucose but are more active inhibitors (pA_2 3.24 ± 0.04). Again the slope is high (3.12 ± 0.35). The significance of these results will be discussed in terms of a model involving the cross-linking of glucose receptors on the surface of the mast cell.

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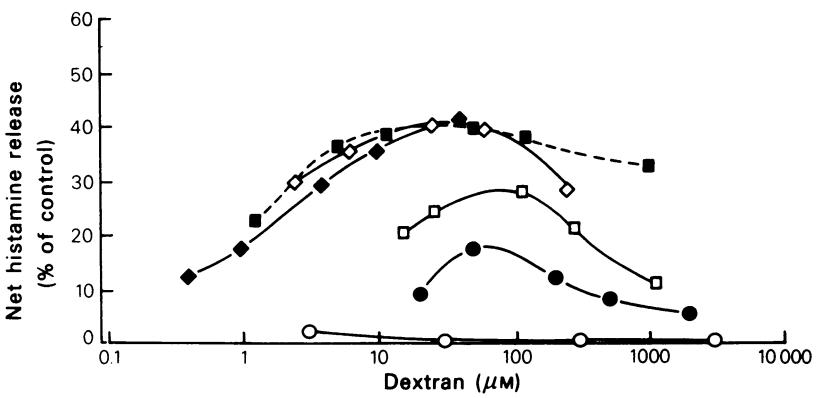

Figure 1 Effect of molecular weight of dextran on histamine release from rat mast cells. ◆ 500,000; ◇ 80,000; —■— 40,000; □ 20,000; ● 10,000; ○ 3,000.

Comparison of the activities of some peptides in human skin and as histamine releasing agents

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An antigen–antibody reaction is the trigger for histamine release in anaphylactic and acute allergic reactions, but several low molecular weight substances, including peptides and adenine nucleotides, also release histamine from mast cells and basophil leucocytes. Endogenous peptides or nucleotides may, therefore, have a role in releasing vasoactive and other mediators from mast cells or basophils.

Histamine release was measured, *in vitro*, from rat peritoneal mast cells and human basophil leucocytes, in response to substance P (SP), physalaemin, eledoisin-related-peptide (ERP), poly-L-arginine (mol. wt. 120,000) and ATP. The wheal and flare responses to these agents were also measured in the skin of the human forearm. SP (0.5 to 13.2 μmol/l), physalaemin (0.16 to 4.4 μmol/l), ERP (1 to 27 μmol/l) and ATP (1 to 100 μmol/l) failed to release histamine from human basophils. ATP (0.3 to 10 μmol/l) potentiated (by up to twofold) imunologically triggered release from basophils. SP (1.7 to 15 μmol/l) and ERP (1.2 to 60 μmol/l) produced a graded increase in histamine release from rat mast cells but physalaemin (0.15 to 4.0 μmol/l) failed to elicit this response. SP (0.02 to 0.2 nmol), physalaemin (0.01 to 0.1 nmol), ERP (0.1 to 1 nmol), ATP (0.02 to 0.5 μmol), histamine (0.01 to 0.1 μmol) and poly-arginine (0.04 to 0.4 nmol) in 25 μl buffered saline (pH 7.4) all induced graded wheal responses in human skin, but the flare responses to these agents were strikingly different. Doses of SP, physalaemin and ERP which produced wheals of approximately equal area (50 mm^2) gave mean flare areas of 900, 150 and 30 mm^2 respectively. For approximately equivalent wheal sizes, histamine, poly-arginine and ATP produced flare areas of 370, 340 and 590 mm^2 respectively. Pretreatment with chlorpheniramine, 16 mg orally or 20 mg intravenously, reduced the area of the flare responses to histamine and ATP by about 65% and the wheal areas by about 50%. The flare response to SP was reduced by about 50% but the wheal response by only 20%. Wheal responses to ERP and physalaemin were reduced by about 15%.

These results confirm previous observations that SP produces wheal and flare reactions in human skin and releases histamine from rat mast cells (Hägermark, Hökfelt & Pernow, 1978; Johnson & Erdös, 1973). Of the four peptides tested only SP and poly-arginine produced marked flare responses and these appeared to be related to histamine-releasing activity. Both peptides release histamine from rat mast cells though only poly-arginine causes release from basophils (Foreman & Lichtenstein, 1980). Furthermore, SP-induced flare is reduced by antihistamine, and the flare response to ATP similarly appears to be mediated by histamine.

Wheal responses produced by SP-related peptides appeared to be largely independent of histamine release, in contrast to ATP. Thus, physalaemin and ERP evoked wheal responses despite their ineffectiveness as histamine releasers. The order of potency for wheal response (physalaemin 2.5:SP 1:ERP 0.04) parallels that for contractile responses in the guinea-pig ileum (physalaemin 2.3:SP 1:ERP 0.55—P. Gater & C.C. Jordan, unpublished observation), but contrasts with that for flare where the order of activities is SP $\gg$ ERP $\ggg$ Physalaemin.

Thus, peptide-induced wheal and flare responses appear to involve independent mechanisms, the former being histamine independent but the latter being at least in part histamine-mediated.

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Dependence of anaphylactic histamine release from rat mast cells on cellular energy metabolism

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In the present investigation the relation between anaphylactic histamine release from rat mast cells and the cellular energy metabolism has been studied. The cells were exposed to metabolic inhibitors (antimycin A, 1 μmol/l; oligomycin, 1 μg/ml; 2-deoxyglucose (2DG), 5 mmol/l) or to glucose (5 mmol/l) in order to change the adenosine triphosphate (ATP) level prior to initiation of the release process by antigen. ATP was determined by the bioluminescence method using luciferin-luciferase from firefly tails (Johansen & Chakravarty, 1975) and histamine was determined by the fluorometric method (Shore, Burkhalter & Cohn, 1959).

In presence of antimycin A alone or in presence of all three inhibitors, histamine release was low although the cellular ATP level was high at the time of initiation of the release process. By contrast, in presence of glucose in addition to the two respiratory inhibitors or in the presence of 2-DG alone, histamine release and ATP levels were high. It has been demonstrated that mast cells were depleted of their ATP after 20 min incubation with antimycin A alone and after 2 min with all three inhibitors present (Johansen, 1979a, b) due, apparently, to reduced ATP-synthesis. The steady state ATP level after incubation with 2DG alone was about 65% of that of untreated cells (Johansen, 1979b) and about 80% after incubation with the inhibitors plus glucose. The latter value corresponded with a synthesis rate of 2 pmol/10^3 cells/min, based on the rate of lactate production (Johansen, 1979b). Furthermore, it was found that a 30 s incubation of antimycin A-treated cells with glucose enhanced both the histamine release and the lactate production (Peterson & Diamant, 1974). Thus secretory competence of mast cells was associated with a high rate of cellular ATP-synthesis, where no relation was found between the cellular ATP level at the time of initiation of the release process and the amount of histamine released.

Anaphylactic histamine release is initiated by the antigen–antibody reaction taking place on the cell surface. This reaction stimulates the cell to release histamine by exocytosis. In order to study if the exocytotic process itself is dependent on production of cellular energy, the mast cells were pretreated with 2DG, which blocks the glycolysis of the mast cells (Johansen, 1980), but does not block the anaphylactic histamine release in presence of oxygen (Chakravarty, 1968). Then antigen was added to the cell suspension followed by addition of respiratory inhibitors (antimycin A and oligomycin). The amount of histamine released and the duration of the release process were both reduced when the respiratory inhibitors were added to the cell suspension 5 s after exposure of the cells to antigen. The inhibition of the exocytotic process after initiation of the release mechanism seems to indicate that exocytosis requires energy, that is produced as the exocytosis takes place.

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Evidence for two types of vascular permeability-increasing mediators: the direct action of histamine and bradykinin; the polymorph-dependent action of C5a, leukotriene B_4 and formyl tripeptide

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We have found that complement activation of rabbit plasma by zymosan results in vascular permeability-increasing activity corresponding to C5a, the major leucotactic substance present. This has led us to evaluate a general hypothesis that substances with chemotactic activity *in vitro* for polymorphonuclear (PMN) leucocytes increase vascular permeability *in vivo* by a mechanism dependent on these cells. Three different leucotactic substances were investigated, rabbit C5a (des arg form), leukotriene B_4 (LTB), and N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP). Bradykinin and histamine, both with established vascular permeability-increasing activity, were employed for comparison. Plasma exudation was measured using the 30 min accumulation of intravenously-injected [^{125}I]-albumin in rabbit dorsal skin in response to 0.1 ml injections of agents (Williams, 1979).

Unlike bradykinin and histamine which produce plasma exudation, the leucotactic substances alone produced very little exudation. This may be because bradykinin and histamine have some intrinsic vasodilator activity (as measured by [^{133}Xe] clearance) whereas the leucotactic substances do not. With the addition of vasodilator prostaglandin (PGE_2, 100 ng/0.1 ml) to simulate conditions pertaining in an inflammatory reaction (Williams & Peck, 1977) the potent permeability-increasing activity of the leucotactic substances emerged; C5a and FMLP producing significant exudation at doses as low as 10^{-12} to 10^{-13} mol and LTB at 10^{-11} to 10^{-12} mol. Locally-injected mepyramine abolished responses to histamine + PGE_2 but had little effect on responses to the other substances.

In experiments designed to measure onset times of exudation responses, first significant changes were observed at 1.5 min for bradykinin and histamine and at 6 min for the leucotactic substances (all with PGE_2). Duration of action in the skin was found to be short for bradykinin and histamine (e.g. response $t_{\frac{1}{2}}$ for bradykinin = 4.5 min), but remarkably protracted for the leucotactic substances (e.g. $t_{\frac{1}{2}}$ for C5a = 95 min).

Rabbits were depleted of PMN-leucocytes using single ear vein injections of nitrogen mustard (1.75 mg/kg). Four days later the blood PMN-leucocyte count was $4.0 \pm 1.4 \times 10^4$ cells/ml compared with $3.5 \pm 1.1 \times 10^6$ cells/ml for controls. In the depleted animals normal responses were produced by histamine, histamine + PGE_2, bradykinin, and bradykinin + PGE_2. However, in these animals no responses could be produced by C5a + PGE_2, FMLP + PGE_2, or LTB + PGE_2.

A temporary clamp applied to the terminal descending aorta during, and for 10 min after, injection of nitrogen mustard (which has a short half-life in blood) prevented access of the drug to the bone marrow of the hind limbs. These animals did respond to injections of leucotactic substances (+PGE_2) into back skin, showing that the action of nitrogen mustard was not on the skin itself.

In 3 of 6 experiments, circulating PMN-leucocyte count was elevated to within normal range by cross-blood transfusion between depleted and normal rabbits (6 ml/min for 60 min). In these 3 experiments responses in the skin to leucotactic substances (+PGE_2) were elicited.

From these results we conclude that there are two types of mediator which increase vascular permeability: those acting directly on vascular endothelial cells such as bradykinin and histamine; and those such as C5a, FMLP, and LTB, whose action, described here for the first time, depends on the rapid involvement of PMN-leucocytes.

We thank Dr A. W. Ford-Hutchinson, King's College Hospital, London, for LTB_4.

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Differing biological potencies of isomers of leukotriene B_4

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Leukotriene B_4 (LTB_4; 5,12-dihydroxy-eicosatetraenoic acid) is formed from leukotriene A_4 (LTA_4) in the presence of polymorphonuclear leucocytes (PMNs) either enzymatically or by non-enzymatic hydrolysis (Rådmark, Malmsten, Samuelsson, Clark, Goto, Marfat & Corey, 1980). Chemical hydrolysis of LTA_4 produces two diastereoisomers of LTB_4 in which all the double bonds within the conjugated triene possess the trans configuration (isomers I and II). A further isomer, in which the triene structure contains one cis and two trans double bonds, is formed enzymatically (isomer III) (Borgeat & Samuelsson, 1979).

LTB_4, generated from rat PMNs stimulated with ionophore A23187, was separated into the isomers by reverse phase high pressure liquid chromatograph (HPLC) (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980). The isomers were further purified by rechromatography on the same HPLC column using a more polar solvent system (methanol/water/acetic acid; 72/28/0.01, v/v/v). They had identical HPLC retention times and U.V. spectra to those previously reported for the three isomers of LTB_4 (Borgeat & Samuelsson, 1979). Analysis of isomers I and III by gas chromatography-mass spectrometry showed characteristic C values and mass spectra for these compounds (Borgeat & Samuelsson, 1979). There was insufficient isomer II for mass spectrometric analysis. Final yields from a bulk preparation of 3×10^9 rat PMNs were 7.0, 3.7 and 18.5 μg of isomers I, II and III.

The relative potencies for the three isomers in the following assays: chemokinesis of human PMNs (Ford-Hutchinson *et al.*, 1980); aggregation of rat peritoneal PMNs (Ford-Hutchinson *et al.*, 1980); vascular permeability changes in rabbit skin in the presence of PGE_2 (Williams, 1979; Bray, Cunningham, Ford-Hutchinson & Smith) are shown in Table 1. The results show that the isomers (I and II) derived by the non-enzymatic route are less active than the enzymatically produced isomer (III). Assessment of the significance of LTB_4 production in biological systems will therefore depend on the separation, chemical characterization and biological assay of the individual isomers of LTB_4.

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Table 1 The effects of isomers of leukotriene B_4 on the chemokinesis of human PMNs, the aggregation of rat PMNs and vascular permeability changes in the rabbit skin in the presence of PGE_2 (100 ng)

	ED_{50} Isomer III ng/ml	Relative potency III	II	I
Chemokinesis of human PMNs	0.035	100	4.7	0.6
Aggregation of rat PMNs	0.76	100	38.5	3.7
Plasma exudation in rabbit	8.8	100	23.3	2.2

Relative potency obtained by comparison of ED_{50} with ED_{50} for Isomer III (expressed as 100) determined in the same experiment.

C5a generation in blood plasma of man, pig, guinea-pig, rabbit and rat: a link between complement activation and inflammatory oedema

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Blood plasma has the capacity to kill micro-organisms. During activation of the complement system, the enzyme system responsible for microbial lysis, several protein fragment byproducts are formed. We have evidence that one of these byproducts has become adapted as an extracellular messenger in order to control the supply of complement to tissues infected with micro-organisms. In our model, zymosan (yeast cell walls) is injected intradermally in the rabbit and local plasma exudation measured as the accumulation of intravenously-injected [^{131}I]- or [^{125}I]-albumin, (Williams, 1979). The response depends on the generation of two types of mediator; one mediator increases vascular permeability, but plasma exudation is only observed when a second mediator is present—a vasodilator prostaglandin (Williams, 1979). We propose that increased vascular permeability in this model is dependent on the extravascular generation of C5a, a fragment of the fifth component of complement.

Incubation of rabbit, pig, guinea-pig or rat plasma with zymosan (1 mg/ml, 37°C for 30 min) resulted in the generation of a histamine-independent vascular permeability-increasing substance which only produced significant plasma exudation in rabbit skin after the addition of a vasodilator prostaglandin (Williams, 1978). Generation of activity was prevented by inhibitors of the complement system, but not by inhibitors of the kallikrein system (Jose, Peck, Robinson & Williams, 1978). The active substance from rabbit plasma has been purified using cation exchange (CM Sephadex C-25) and gel filtration (Sephadex G-100), and shown to correspond to the major leucotactic substance in rabbit complement-activated plasma, C5a.

Human plasma, unlike plasma from other species, was highly active in rabbit skin without incubation with zymosan. For this reason, human C5a activity was only demonstrable after purification of zymosan-activated plasma. C5a from human activated plasma, like that of rabbit, corresponded to 18,000 M.W. by gel chromatography (Sephadex G-75) and 13,000 M.W. by gel electrophoresis (SDS-PAGE). Human C5a activity in the skin was dependent on added prostaglandin, but independent of histamine release.

Plasma contains a highly active carboxypeptidase B which cleaves carboxyl terminal arginine from C5a leaving a product, C5a des arg, with low anaphylatoxic activity (spasmogenic, histamine release) but high leucotactic activity. Incubation of human plasma with zymosan and a carboxypeptidase inhibitor (1 M epsilon amino caproic acid) followed by purification, yielded C5a with both spasmogenic activity on guinea-pig ileum and permeability-increasing activity in rabbit skin. Incubation of this material with rabbit plasma (37°C, 30 min) abolished spasmogenic activity, but had little effect on permeability-increasing activity. Since permeability-increasing activity is stable in plasma in all species tested, this supports the idea that C5a is active in its des arg form.

We suggest that the presence of micro-organisms in extravascular tissue fluid can lead to the generation of a vasodilator prostaglandin and C5a, both of which control the further supply of complement to facilitate microbial lysis. This could be a basic mechanism underlying inflammatory oedema.

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Blockade of the anti-inflammatory action of dexamethasone by DL-α-difluoromethylornithine; comparison with actinomycin D and cycloheximide

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The effect of the intravenous administration of corticosteroids encapsulated in intact erythrocytes on adjuvant arthritis in the rat

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Many attempts have been made to encapsulate drugs in biodegradable materials including erythrocytes (e.g. Tyrell & Ryman, 1976; Ihler & Glew, 1977). However, when administered intravenously all these preparations are rapidly removed from the circulation by the reticuloendothelial system. The method used here is unique in that the loaded cells survive for their normal lifespan.

The encapsulation procedure involves a gradual swelling of the cells which does not result in rupture of the red cell membrane but which makes it permeable to the substance to be encapsulated. Blood is obtained by cardiac puncture and the separated cells are initially swollen in reversed K^+ Hanks physiological medium (Hanks, 1948) adjusted to 0.66 tonicity. By protecting these cells with a layer of haemolyzed blood, the drug in aqueous solution can be added gradually in small portions until the point of haemolysis is reached, recognized by a sudden alteration in the transparency of the solution and difficulty in sedimenting the cells. By rapid addition of the calculated quantity of 10 times eutonic reversed K^+ Hanks medium, the cells are resealed. After washing in eutonic Hanks medium, the cells are resuspended in saline ready for injection.

By this technique fluorescein isothiocyanate (FITC) has been encapsulated in rat erythrocytes and the cells returned intravenously. We have been able to show that the normal lifespan of the erythrocyte is not significantly altered, with some FITC-labelled erythrocytes surviving for 50 days (the normal lifespan for the erythrocyte in the rat). This is grossly different from the survival time of FITC encapsulated in erythrocyte ghosts where, in our hands, cells were not detectable after 12 hours. We have also been able to treat adjuvant arthritis in the rat using cortisol phosphate or prednisolone succinate encapsulated in erythrocytes. Approximately 8% v/v of the rat's total erythrocytes were loaded and returned intravenously to the circulation. Corticol phosphate, encapsulated at three dose levels, and prednisolone succinate, at two levels, were compared against the effects of free steroids administered either daily (s.c.) or as a single dose (i/v). The treatments were as follows:

1. Encapsulated steroid: Day 0, Day 7, Day 0 and 10.
2. Free steroid i/v (Equivalent to amount encapsulated): Days 0 and 10.
3. Daily steroid s.c. (Equivalent to 1/10th amount encapsulated) i.e. 1 mg/kg day: Day 0 to completion. Day 7 to completion.

N.B. Administration of adjuvant = Day 0.

In all cases the encapsulated preparations proved to be significantly superior to all other treatments in suppressing inflammation as assessed by foot volume measurements.

We believe we have demonstrated that loaded erythrocytes administered intravenously are a useful vehicle for the prolongation of the pharmacological action of an anti-inflammatory steroid.

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Some actions of natural and synthetic slow-reacting substances on isolated preparations

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The structures of slow-reacting substance of anaphylaxis (SRS-A) from guinea-pig lung and SRS released by the ionophore A23187 from rat basophilic leukaemia (RBL-1) cells have recently been elucidated as 5-hydroxy-6-cysteinylglycinyl-7,9,11,14-eicostatetraenoic acid (Morris, Taylor, Piper & Tippins, 1980; Morris, Taylor, Piper, Samhoun & Tippins, 1980), and subsequently referred to as leukotriene D (LTD) (Örning, Hammarström & Samuelsson, 1980).

We have compared pure natural SRS-A from lung and SRS from RBL-1 cells with synthetic 5-hydroxy-6-cysteinylglycinyl-7,9,11,14-eicosatetraenoic acid and 5-hydroxy-6γ-glutamyl-cysteinylglycinyl-7,9,11,14-eicosatetraenoic acid (LTC) (Hammarström, Murphy, Samuelsson, Clark, Mioskowski & Corey, 1979) prepared by the method of Rokach, Girard, Guindon, Atkinson, Larve, Young, Masson & Holme (1980).

The actions of the SRSs were compared on the following tissues from guinea-pig: longitudinal smooth muscle from ileum (GPISM), trachea (GPT), and strips of lung parenchyma (GPP), superfused with Tyrode solution and on ileum (GPI) suspended in a 10 ml organ bath.

Natural SRS-A and LTD produced identical responses on GPI and GPISM. Five ng synthetic LTD were equivalent to 1 unit pure SRS-A. Responses to LTC differed from those of LTD; in the organ bath responses to LTC were slower in onset (30 s compared to 10 s) and took longer to reach maximum (2.5 min compared to 1 min). Responses to LTC were maintained longer than 5 min, whereas those to LTD decayed to 50% of maximum in 3 minutes. Similar differences in response to LTC and LTD were seen on superfused GPISM. In both systems LTC was less potent than LTD by at least one order of magnitude. When two banks of GPISM were superfused in series, the contractions due to LTC were greater in the second bank than in the first, relative to control doses of SRS-A.

Lung parenchyma strip contracted to SRS-A, LTD and LTC, LTD being more potent than LTC. Contractions to SRS-A and LTD were antagonized by indomethacin (1 μg/ml) and FPL 55712 (0.5–1 μg/ml). LTD and LTC were equipotent on GPT but LTC produced slower contractions than LTD or SRS-A.

The increase in potency of LTC on GPISM with time suggest that LTC is converted to the more active LTD on contact with the tissue, probably by γ-glutamyl transferase present in the tissue. Inhibition of LTC- or LTD-induced contraction of GPP by indomethacin suggests at least part of the contraction is due to release of cyclo-oxygenase products (Engineer, Morris, Piper & Sorois, 1978).

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