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COMMUNICATIONS

Effects of some cholecystokinin (CCK)-like peptides on gastric emptying of a liquid meal in the rat

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Some CCK-like peptides were tested for their effects on gastric emptying in the rat, taking into account their spasmogenic action on the gastroduodenal junction observed in previous papers (Bertaccini, Impicciatore & De Caro, 1973; Bertaccini, Impicciatore, De Caro, Chiavarini & Burani, 1974). Gastric emptying of a phenol red meal was evaluated by a modification (Scarpignato and Bertaccini, 1980) of the method described by Reynell & Spray (1956). Male rats weighing 200 g were used after a fasting period of 24 h. Peptides used were: caerulein, C-terminal heptapeptide of CCK (hepta-CCK) and pentagastrin. They were injected i.p. in a constant volume of 1 ml/kg 5 min prior to administration of the meal. Rats were killed at various time intervals to evaluate the duration of the effect. A fixed time (20 min) was selected for the study of the dose-response relationship.

All the peptides delayed gastric emptying in a dose-dependent fashion: threshold doses were 0.075 μg/kg for caerulein and hepta-CCK. Both peptides caused a complete block of gastric emptying with doses of 5 μg/kg. Pentagastrin had a threshold dose at 10 μg/kg; the maximum dose (80 μg/kg) caused an inhibition of 36% ($P < 0.01$ in comparison with controls). Thus not only the 'potency' but also the 'efficacy' of pentagastrin were remarkably lower than those of caerulein and hepta-CCK. The 'characteristics' of emptying patterns (according to Hunt & Spurrell, 1956) in controls and in treated rats are shown in Table 1. It is evident from the table that hepta-CCK, which has a half-life very close to that of caerulein, shows a calculated emptying time apparently higher than that of caerulein, thus suggesting a longer lasting effect. Pretreatment of the animals with cimetidine (20 mg/kg i.p., 10 min prior to the administration of the peptides) or with sodium bicarbonate (200 μEq p.o. 5 min before the meal) did not modify the effect of caerulein and hepta-CCK. This suggests an action independent of gastric hypersecretion, which in its turn could affect gastric emptying (Hunt & McDonald, 1954). As a consequence also, the effect of secretin, released by acid bathing the proximal part of the duodenum (Cooke, 1974), could be excluded. Our results

Table 1 'Characteristics' of emptying patterns

Treatment	*Half-life* (*min*)	*Calculated emptying time* (*min*)
Controls	15.3 ± 6.2[1]	120.9 ± 6.1
Caerulein (1 μg/kg)	49.0 ± 14.3[2]	220.6 ± 14.3[2]
Hepta-CCK (1 μg/kg)	53.3 ± 14.3[2]	289.3 ± 14.2[2]
Pentagastrin (80 μg/kg)	23.8 ± 7.7[3]	146.0 ± 7.7[3]

[1] Mean value ± 95% confidence limits.
[2] Statistically significant difference ($P < 0.001$) in comparison with control values.
[3] Statistically significant difference ($P < 0.01$) in comparison with control values.

suggest that the delay in gastric emptying induced by CCK-like peptides is connected with the contraction of the pylorus pointed out in previous studies. Therefore the pyloric sphincter appears to play an important role in the regulation of emptying of liquids in rats, unlike cats and dogs (Stemper & Cooke, 1976) in which gastric emptying of liquids was shown to be independent of the gastroduodenal junction.

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Terpene therapy for gallstones—effects of individual monoterpenes on bile flow, bile composition and hepatic cholesterogenesis in the rat

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The terpene preparation Rowachol (Rowa Ltd., Bantry, Ireland) effectively treats biliary duct stones and is a useful adjunct to chenic acid therapy for gallbladder stones (Ellis & Bell, 1979). It contains menthol (32%), pinene (17%), menthone (6%), camphene (5%), borneol (5%) and cineole (2%) in olive oil and is marketed as a choleretic (Morsdorf, 1965). The combination reduces hepatic cholesterol synthesis, by inhibition of S-3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) (Middleton, Middleton, White & Bell, 1979). Curiously, small doses lower biliary cholesterol saturation, while larger doses increase it (Doran, Keighley & Bell, 1979; Ellis & Bell, 1979).

We investigated the effects of individual monoterpenes on bile flow, bile composition and HMGR in rats. Hepatic HMGR activity was measured, 17 h after dosing by intragastric tube, either at the peak of the diurnal cycle or later, following bile diversion for 2 h. Maximum activity was reduced by all but pinene and camphene (Table 1). Post-cannulation activity did not correlate with cholesterol output ($r = 0.09$, $P > 0.10$), providing further evidence for dissociation of hepatic cholesterol synthesis and biliary cholesterol secretion (Turley & Dietschy, 1978; Long, Jakoi, Stevens & Quarfordt, 1978).

Table 1 records two experimental series, each with its own control group; this was necessary because of a change in the method of cholesterol estimation. All the terpenes produced marked, bile salt-independent choleresis and the beneficial effect of Rowachol on cholesterol saturation was demonstrated. We confirm that the related compound *d*-limonene, a recommended treatment for retained stones (Igimi, Hisatshu & Nishimura, 1976), increases saturation (Kodama, Inone, Noda & Ide, 1976), as does the acyclic monoterpene citronellal.

Studies using chronic dosing and alternative models are now in progress in order to establish more clearly which terpenes are likely to be cholelitholytic agents in man.

Table 1 Bile flow, bile composition and hepatic HMGR activity in rats dosed 17 h prior to cannulation with 2 ml/kg of fluid (individual terpene dose 3 mmol/kg)

Dosing	*Bile flow (ml/100 g body wt. per 24 h)*	*Cholesterol secretion (μmol/100 g per 24 h)*	*Bile salt secretion (μmol/100 g per 24 h)*	*Saturation index (0 to 30 min bile sample)*	*HMGR activity maximal, 17 h post-dose (nmol/min per μg microsomal protein)*	*HMGR activity 2 h post-cannulation (nmol/min per mg microsomal protein)*
Series A						
Control I (olive oil)	10.75 ± 2.27 (11)	5.20 ± 2.03 (11)	319 ± 91 (11)	0.278 ± 0.096 (11)	1.08 ± 0.30 (10)	0.47 ± 0.26 (13)
Rowachol liquid	26.60 ± 5.66**** (6)	3.46 ± 2.05 (5)	429 ± 13 (6)	0.167 ± 0.086* (6)	0.40 ± 0.12*** (6)	0.18 ± 0.04 (3)
Menthol	17.13 ± 1.13*** (5)	2.96 ± 1.36* (5)	315 ± 66 (5)	0.216 ± 0.034 (5)	0.26 ± 0.13*** (7)	
Series B						
Control II (olive oil)	11.13 ± 2.80 (9)	2.99 ± 0.66 (9)	379 ± 135 (9)	0.162 ± 0.070 (9)	As group I	As group I
Camphene	24.73 ± 5.60**** (6)	4.48 ± 1.24*** (6)	479 ± 116 (6)	0.203 ± 0.085 (6)	0.76 ± 0.11 (6)	0.24 ± 0.08 (5)
Cineole	24.95 ± 3.64**** (5)	4.15 ± 2.06 (5)	463 ± 61 (5)	0.238 ± 0.098 (6)	0.27 ± 0.10**** (6)	0.16 ± 0.08** (5)
Menthone	20.47 ± 1.95**** (4)	3.94 ± 0.80* (4)	395 ± 49 (4)	0.220 ± 0.016 (5)	0.45 ± 0.21**** (7)	0.32 ± 0.18 (5)
Pinene	19.90 ± 3.13**** (4)	5.90 ± 2.93** (4)	383 ± 4 (4)	0.280 ± 0.167 (4)	0.92 ± 0.29 (4)	0.23 ± 0.09 (4)
Citronellal	16.52 ± 1.73*** (4)	8.15 ± 1.83**** (4)	482 ± 182 (4)	0.310 ± 0.036*** (4)	0.30 (2)	
d-Limonene	14.66 ± 2.01* (5)	4.48 ± 2.06 (5)	282 ± 45 (5)	0.274 ± 0.095* (5)	0.61 ± 0.05* (3)	
Control III (25% ethyl acetate)	12.67 ± 3.69 (5)	5.08 ± 1.68*** (5)	407 ± 122 (5)	0.230 ± 0.041 (5)	0.93 ± 0.26 (3)	0.57 ± 0.10 (5)
Borneol (in 25% ethyl acetate)	21.93 ± 0.99**** (6)	2.80 ± 1.12* (6)	331 ± 68 (6)	0.172 ± 0.033* (6)	0.44 ± 0.15*** (3)	0.23 ± 0.07****

Values given as mean ± s.d. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$, compared with relevant control group by Student's t test. Borneol requires 25% ethyl acetate for dissolution at this concentration, thus requires a further control group.

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Age-related changes in the antilipolytic action of nicotinic acid

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The correlation between age and the development of metabolic disorders is well known. Many changes occur in adipose tissue with age: a decrease of carbohydrate flux through the pentose cycle, a reduced lipogenesis from acetate, a decrease in FFA esterification et al. (Gellhom & Benjamin, 1965; Holm, Jacobsson, Björntorp & Smith, 1975).

Nicotinic acid, the prototype of a very important family of hypolipaemic drugs, is mainly known and used for inhibiting FFA release from adipose tissue. However, a review of the literature indicates that nicotinic acid exerts other metabolic effects some of which are opposite from those above mentioned, induced by aging (Lee, Ellis & Sigal, 1961; Solyom & Puglisi, 1966). This observation prompted us to investigate whether the antilipolytic effect of nicotinic acid on rat adipose tissue, *in vitro*, is modified by aging.

Male Wistar rats were divided according to age: 4 to 6 weeks (young), 9 to 12 weeks (adult), 16 to 20 weeks (old). Epididymal adipose tissue obtained from fed animals, under ether anaesthesia, was excized and minced. Aliquots of 100 ± 5 mg were incubated in 2.0 ml of Krebs–Ringer bicarbonate buffer pH 7.2, containing 2.5% bovine serum albumin. Nicotinic acid was added at the beginning of the incubation and noradrenaline or theophylline was added 30 min later. The reaction was stopped, after 180 min of incubation, with 0.1 ml of 2.5 N H_2SO_4. FFA were determined according to Dole (1956) and glycerol was determined by the colorimetric method of Lambert & Neish (1950).

In adipose tissue from young animals nicotinic acid (10^{-5} to 10^{-3} M) did not significantly modify FFA release stimulated by noradrenaline (10^{-5} M) or theophylline (3×10^{-3} M). In adipose tissue from adult rats nicotinic acid (10^{-4} M) inhibited by 42 ± 5% the release of glycerol stimulated by theophylline but did not antagonize the lipolytic effect of noradrenaline. In adipose tissue from old animals nicotinic acid antagonized the effect of both theophylline and noradrenaline by 62 ± 5% and 23 ± 9%, respectively.

The present data indicate that the antilipolytic action of nicotinic acid depends upon the age of the animals. During the first weeks of life the drug is ineffective, but its action becomes evident in adult and even more in old animals. These changes in adipose tissue sensitivity to nicotinic acid are probably related to fine metabolic alterations which occur with aging and this point is now under investigation. This new finding on the age-related nicotinic acid activity will hopefully be useful for answering an important question: are there any significant therapeutic involvements directly related to these observations?

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Mechanism of the antileukaemic action of dimethyltriazenes in mice

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Daily i.p. treatment for 5 days of BDF1 mice bearing P388 lymphocytic leukaemia with 4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide (100 mg/kg, DTIC) and *p*-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt (100 mg/kg, DM-COOK) increased the life span over the controls by 107 ± 11 and 58 ± 15%, respectively. This treatment reduced the number of peritoneal tumour cells, 8 days post-implant, to 67.2 ± 5.9 and 45.5 ± 5.5%, respectively, as compared with untreated controls. This reduction indicates that these dimethyltriazenes do not act exclusively by a cytotoxic mechanism, since the treatment with cytotoxic agents increasing survival time by about 60% caused the absence of detectable peritoneal tumour cells. Similar results have been obtained in CBA/LAC mice bearing TLX5 lymphoma.

The mechanism responsible for increase in survival time of leukaemic mice caused by dimethyltriazenes might be their antimetastatic activity, which has already been observed in mice bearing a solid metastasizing tumour, the Lewis lung carcinoma (Giraldi, Houghton, Taylor & Nisi, 1978). Indeed, the survival time of normal mice implanted i.p. with minced brains obtained from treated leukaemic animals was greater than 60 days, as compared with 26.9 ± 0.67 for untreated tumour bearing brain donors. On the other hand, the survival time of normal mice injected i.p. with 10^6 peritoneal tumour cells obtained from treated donors was 13.2 ± 0.5, as compared with 13.3 ± 0.6 for untreated tumour bearing controls.

These data indicate that, in the experimental system used, dimethyltriazenes do not cause cytotoxic effects on peritoneal tumour cells and that the absence of clonogenic tumour cells in the brains of treated animals is presumably due to the prevention of their arrival in that organ. These data also suggest the possible use of dimethyltriazenes in combination with cytotoxic agents for the treatment of early leukaemias, in order to prevent the formation of leukaemic cell reservoirs in sites where cytotoxic agents have little efficacy.

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Comparison of the bradycardia produced by oxymetazoline and clonidine in urethane and pentobarbitone anaesthetized rats

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Kobinger and Pichler (1975) reported that intravenous oxymetazoline failed to elicit bradycardia in vagotomized rats anaesthetized with urethane. This they attributed to poor penetration of the compound into medullary cardiovascular centres. However, we have previously reported that intravenous oxymetazoline, like clonidine, was highly effective in lowering the heart rate of rats anaesthetized with pentobarbitone (Armstrong, Cavero & Lefèvre-Borg, 1980). Moreover, oxymetazoline and clonidine have been shown to inhibit the sympathetic tachycardia evoked by stimulation of the spinal cord of rats pithed during anaesthesia with ether (Armstrong & Boura, 1973), halothane (Drew, 1976; Doxey & Everitt, 1977) or pentobarbitone (Pichler & Kobinger, 1978). We have now investigated whether bradycardia produced by oxymetazoline is influenced by the anaesthetic agent used.

Normotensive male rats (200 to 300 g, Sprague–Dawley, C. River) were anaesthetized either with ure-

thane (1.2 to 1.5 g/kg, i.p.) or sodium pentobarbitone (55 mg/kg, i.p.) or pithed during a brief period of ether anaesthesia. Blood pressure was recorded from a cannulated carotid artery and heart rate computed using a cardiotachometer triggered by the arterial pressure waves. Agents were injected, during a 2- to 5-min period, into a cannulated femoral vein.

In bilaterally vagotomized rats anaesthetized with pentobarbitone, oxymetazoline (1.0 μg total dose, i.v.) decreased heart rate by 62 ± 4 beats/min without 30 s of its injection. This effect lasted for more than 45 min. Clonidine (1.0 μg, i.v.) produced a more slowly developing bradycardia. The heart rate was reduced by 44 ± 3 beats/min, 30 s after injection and by 89 ± 14 beats/min, 25 min later. However, in bilaterally vagotomized rats anaesthetized with urethane the maximal decrease in the heart rate (ΔHR) produced by oxymetazoline and clonidine were markedly less (oxymetazoline: $\Delta HR = 25 \pm 2.2$ beats/min, initial HR: 428 ± 9, $n = 5$; clonidine: $\Delta HR = 38 \pm 4$ beats/min, initial HR: 421 ± 10, $n = 5$).

In rats pithed during a brief period of ether anaesthesia the sympathetic tachycardia (approx. 100 beats/min) evoked by thoracic spinal cord stimulation was reduced by either clonidine or oxymetazoline (1.0 μg/kg, i.v.) but was not modified by intravenous infusion of urethane (100 mg/kg per min/12 min). Oxymetazoline decreased this tachycardia by 66 ± 1 beats/min in rats given saline and by 39 ± 4 beats/min in rats given urethane. Graphs of the falls in heart rate produced by cumulative intravenous doses of oxymetazoline (0.01–30.0 μg) or clonidine (0.1–100.0 μg) were parallel and to the right of controls (ratio of $ED_{50} = 3$) after i.p. urethane (1.2 g/kg).

Thus, the observation that oxymetazoline was poorly active in lowering heart rate in rats anaesthetized with urethane is in accord with the report of Kobinger & Pichler (1975). However, oxymetazoline was highly effective in producing bradycardia in intact pentobarbitone anaesthetized rats and in animals pithed during ether anaesthesia in which the heart rate had been experimentally elevated by electrical stimulation of thoracic spinal cord. Therefore, the failure of oxymetazoline to lower heart rate in urethane anaesthetized rats is more likely to be due to an interaction between the anaesthetic and the mechanisms responsible for the bradycardia induced by the imidazoline than to poor penetration of the compound into the central nervous system, as originally suggested by Kobinger & Pichler (1975).

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Lysophosphatidylserine-induced mast cell activation *in vivo*

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Phosphatidylserine administration to mice resulted in mobilization of the carbohydrate reserves and in glucose accumulation in the brain (Bruni, Toffano, Leon & Boarato, 1976). The efficacy to elicit these effects was increased eight- to ten-fold by phospholipase A_2-catalysed conversion of phosphatidylserine to lysophosphatidylserine (Bigon, Boarato, Bruni, Leon & Toffano, 1979). In the presence of mouse plasma, lysophosphatidylserine promoted the release of histamine from isolated mast cells and, in keeping with this *in vitro* observation, the administration of adrenoceptor blocking agents and antihistamines in mice prevented the lysophosphatidylserine effect (Bigon, Bruni, Mietto & Toffano, 1980). Data presented here suggest that the kininogen–kinin system constitutes

a part in the mechanism of these effects of lysophosphatidylserine. Lysophosphatidylserine purified according to Bigon *et al.* (1979) was dissolved in 50 mM Tris.HCl pH 7.8 at 20°C and injected i.v. into male albino mice. Peritoneal mast cells were obtained from rats according to the conventional procedures. Histamine release were quantified by the biological test on guinea-pig ileum.

In the presence of either 10 mg mouse plasma or 0.4 mg mouse serum protein, 5×10^5 mast cells incubated in 1 ml saline solution released 60 to 70% of their histamine content in the medium that contained lysophosphatidylserine (1 nmol). Antimycin (1 μg) abolished the histamine liberation, indicating the involvement of a non-cytotoxic release mechanism. In addition, the 20-fold elevation in the histamine releasing activity of plasma as consequence of blood-clotting reactions, suggested that this rise in activity was contingent upon an activation of a precursor protein.

The plasma proteins in 0.05 M Tris-HCl, 6 mM EDTA (pH 8.0) were passed through a column of DEAE-Sephadex A 50 at 20°C. Under these conditions only the unadsorbed fraction showed full histamine releasing activity in the presence of lysophosphatidylserine. The active fraction was precipitated by ammonium sulphate at 50% saturation. This indicated that the effectiveness of mouse plasma was due to high molecular weight cationic components.

In support of the *in vitro* experiments showing the promoting effect of lysophosphatidylserine on the mast cell secretory activity, the administration *in vivo* of agents that decrease plasma kininogen and kinin levels has been found to exert a concomitant influence on the pharmacological action of the phospholipid. Mast cells are known to release kinin-generating proteases together with histamine.

Chymotrypsin, known to reduce plasma kininogen levels without a release of kinins (Haberman, 1968), has been found to be most effective. In mice pretreated with purified chymotrypsin (40 mg/kg), followed at 30 min by lysophosphatidylserine (5 μmol/kg), the rise in brain glucose was reduced from 220 to 184% ($P < 0.001$) and that in blood glucose from 69% to between 0 and 20% ($P < 0.001$). As the chymotrypsin effect was particularly pronounced on the blood glucose level, it is possible that a kinin induced discharge of catecholamines from the adrenals is responsible for the hyperglycemic effect of lysophosphatidylserine.

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Muscarinic cholinoceptors in rat mast cells: demonstration by direct binding

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Acetylcholine has been shown to interact with muscarinic cholinoceptors in rat isolated mast cells, causing sequential exocytosis of histamine containing granules (Fantozzi, Masini, Blandina, Mannaioni & Bani-Sacchi, 1978). Recently, the presence of muscarinic receptors has been demonstrated in chicken erythrocyte membranes and in murine lymphocytes by a direct assay (Aronstam, Abood & MacNeil, 1977; Gordon, Cohen & Wilson, 1978). The properties of muscarinic cholinoceptors in rat mast cells were studied by the binding of tritium labelled 3'-quinuclidinylbenzilate ([^{3}H]-QNB, a potent muscarinic antagonist).

Rat mast cells were obtained as peritoneal and pleural washings from male Wistar albino rats, and isolated through density gradient centrifugation in Ficoll.

Murine neoplastic mast cells (HC-clone) were obtained as ascitic fluid from LAF_1 mice (Jackson Laboratory).

Mast cells were homogenized in Tris-HCl (50 mM) buffer containing $MgCl_2$ (10 mM, pH 7.4 at 37°C) and mast cells membranes were separated by ultracentrifugation (25,000 rev/min for 15 min, 0 to 4°C). [^{3}H]-QNB binding was evaluated through vacuum filtration (Whatman GF/C Filters).

Specific binding of [^{3}H]-QNB to membrane preparations of rat mast cells is a rapid and saturable process (Figure 1); the rate and the maximal levels of [^{3}H]-QNB binding were markedly influenced by the temperature. No evidence of any specific [^{3}H]-QNB binding was present in murine neoplastic mast cell membranes. Accordingly, murine neoplastic mast cells do not show any significant histamine release when exposed to acetylcholine.

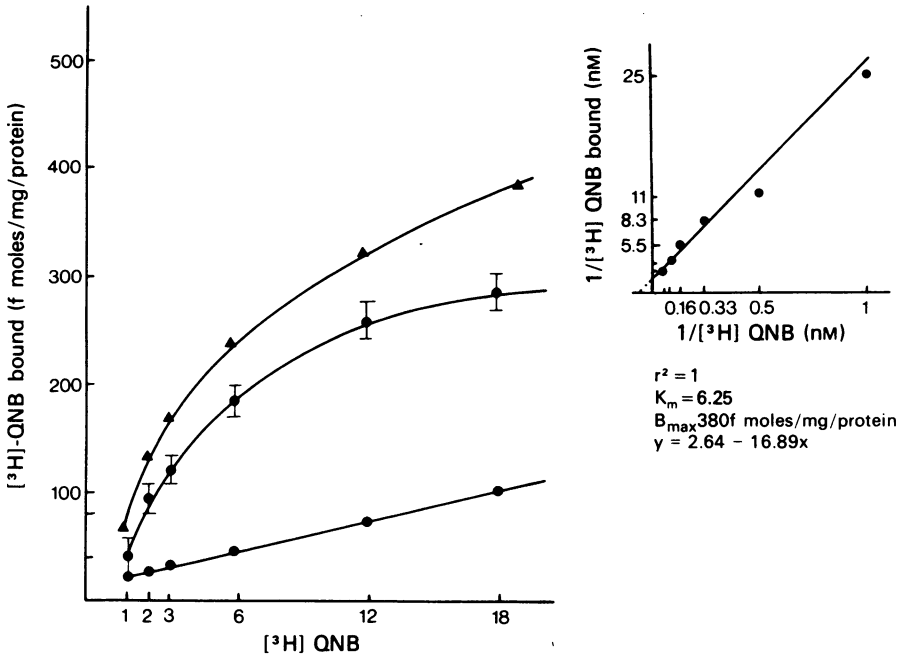

Figure 1 Total, specific and nonspecific binding of [^{3}H]-Quinuclidinilbenzilate ([^{3}H]-QNB; specific activity 16 Ci/mM) with increasing concentration of [^{3}H]-QNB. Mast cells membrane suspensions (100 to 150 μg of protein per sample) were incubated for 20 min at 37°C without (total binding ▲) and with addition of atropine (10^{-5} M, nonspecific binding ○). The results are the means ± s.e. mean of six experiments performed in duplicate. Lineweaver–Burk analysis of the saturation data of specific binding. K_m is the constant of affinity and B_{max} is the number of specific binding sites (receptor density).

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Acetylcholine receptor degradation in rat myotubes *in vitro*: effect of myasthenic sera and of drugs used in the treatment of myasthenia gravis

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Rat myotubes in tissue culture contain nicotinic receptors which have the same pharmacological properties as adult muscle nicotinic receptors. Myotubes cultivated *in vitro* are very useful experimental models for studying the synthesis and breakdown of cholinoceptors both in normal condition or after drug treatment (Devreotes & Fambrough, 1975; Kao & Drachman, 1977). We have monitored the appearance and the quantity of nicotinic receptors in myotubes at different times after plating in tissue culture and found that the number of receptors reaches a plateau at about the seventh day of culture. The turnover of cholinoceptors was measured by labelling the receptor with iodinated α-bungarotoxin and by following in the medium the release of degradation product of the toxin (Devreotes & Fambrough, 1975).

We found that the receptor breakdown at an even rate after the seventh day of culture.

It has been shown that IgG from myasthenic patients can increase acetylcholine receptor AChR degradation (Kao & Drachman, 1977), but no systematic investigation on this matter has been done. We have studied more than 110 different myasthenic sera and found that the large majority of them increase significantly the breakdown of the receptor. It was possible to establish a correlation between the increase of the breakdown and the severity of the disease. Immunofluorescence study revealed that myasthenic sera bind to the myotube plasma membranes. However, a strict correlation between the total titre of antibodies against acetylcholine receptor and the increase of the receptor breakdown was not found. We think that only a small fraction of the anti-AChR antibodies are acting on the degradation of AChR. We have also tested the effect of some immunodepressive drugs and found that they inhibited it. These data are relevant in explaining the pathogenesis and the maintenance of myasthenic symptoms and are important for a rational therapy of myasthenia gravis.

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Studies on the interaction of methohexitone and suxamethonium or acetylcholine on the chick biventer cervicis preparation

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Methohexitone (8.8×10^{-5} M, MH) markedly potentiated tetraethylammonium (TEA)-induced contractures of the chick biventer cervicis muscle preparation (Elliott, 1979). In the present experiments the effects of MH (8.8×10^{-5} M) on the contractures elicited by suxamethonium (SUX), or by acetylcholine (ACh) were studied. The methods and drugs used have been previously described (Elliott, 1979).

A full contracture to low concentrations of SUX only developed in 4 min but with this duration of exposure the preparation was desensitized by high concentrations of SUX, hence only the lower dose range of SUX was used to produce a partial dose-response curve. In the control period 2.46 ± 0.18 (s.e. mean) $\times 10^{-7}$ M SUX produced 1 g tension whilst after 30 min immersion in Krebs solution containing MH (8.8×10^{-5} M, MH–Krebs) the corresponding value was $1.82 \pm 0.24 \times 10^{-7}$ M. The difference was significant at the 1% level. The mean of the ratios ED 1 g for SUX, control:MH–Krebs was $1:0.73 \pm 0.06$, $n = 14$.

Full responses to ACh were obtained with a 1-min exposure, desensitization was avoided permitting completion of full dose-response curves. The mean control $ED_{50\%}$ maximum response was $1.07 \pm 0.18 \times 10^{-4}$ M while the ED_{50} after 30-min exposure to MH–Krebs was $2.60 \pm 0.59 \times 10^{-4}$ M, a significant difference at the 2% level. The mean of the ratios ED_{50} control:ED_{50} MH was $1:2.54 \pm 0.43$ ($n = 6$).

The effect of MH on contractures elicited by indirect repetitive stimulation of the preparation was also studied. The preparation was stimulated (5 V, 0.1 ms) at frequencies between 5 and 15 Hz, the duration of stimulation was 1 min and the interval between periods of stimulation 30 min. The frequency which gave a 50% maximum response was 9.88 ± 0.33 Hz ($n = 8$) in the control period and after immersion in MH–Krebs for 30 min it was 9.28 ± 0.48, $n = 8$. There was no significant difference between these frequencies. There was a small shift of the frequency-response curve to the left in MH–Krebs but there was no significant difference between tensions produced at corresponding frequencies on the two curves.

In separate experiments a single frequency, either 6 or 7 Hz, was used which gave a $29.4 \pm 5.2\%$ maximum response (maximum = response at 15 Hz). MH–Krebs gave a slight potentiation of this response. The control response was $1.35 \text{ g} \pm 0.23$ g and in MH–Krebs 1.64 ± 0.30 g ($n = 8$) the difference was significant at the 1% level. The mean ratio, tension in the control period:tension in MH–Krebs was $1:1.17 \pm 0.04$. Thus depending on the measure used MH (8.8×10^{-5} M) either had no effect or produced a very small potentiation (17%) of the contractures elicited by endogenous ACh.

The slight potentiation (17%) of the response to endogenous ACh could not account for the $\times 90$

potentiation by MH of TEA induced contractures (Bell & Elliott, unpublished observations) but might perhaps be involved in the potentiation of SUX induced contractures if SUX releases ACh (Miyamoto, 1979).

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Effects of emetine on rat skeletal muscle fibres after chronic application to the nerve

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Muscle weakness is a recognized side effect of the prolonged administration of (−)-emetine in the treatment of amoebiasis (Fewings, Burns & Kakulas, 1973). There is still no agreement on the pathogenesis of this complication. Salako (1970) reported a tubocurarine like action of (−)-emetine on neuromuscular transmission. Recently, Bradley, Fewings, Harris & Johnson (1976) reported that (−)-emetine directly affects the muscle fibres at a subcellular level. In an earlier paper we showed a reduction of the amplitude of the action potentials induced by (−)-emetine at the Ranvier node of frog sciatic nerve fibres (Mitolo-Chieppa & Marino, 1974). In this paper an attempt has been made to clarify the role of the nerve in the emetine myopathy.

Silicon polymer cuffs containing 0.2% (−)-emetine were placed around the right peroneal nerve (Conte-Camerino & Bryant, 1977) of eight rats. The cuffs were covered with a small sheet of thermoplastic seal (Parafilm). After 13 to 40 days of treatment the extensor digitorum longus (EDL) muscles of the treated and control legs of each rat were removed and analysed for their electrical parameters with two microelectrodes as described in detail earlier (Conte-Camerino & Bryant, 1976; Conte-Camerino & Bryant, 1977).

The mean resting potentials of the treated muscles were not different from the control value (eight preparations). Action potential generation was affected by (−)-emetine treatment: the rheobasic current was lowered from 47.8 ± 2 nA (mean ± s.e. mean from 10 fibres from four control muscles) to 21.4 ± 1 nA (mean ± s.e. mean from 16 fibres from six treated muscles; $P < 0.01$).

Furthermore in the treated fibres very small increase in current produced four or more action potentials of equal amplitude (six preparations), as in Figure 1.

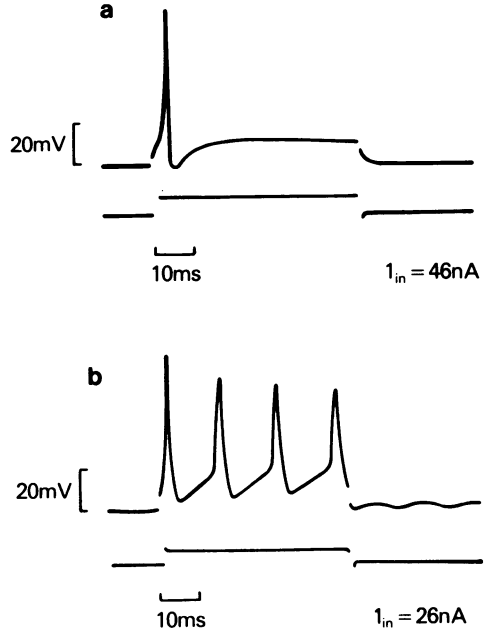

Figure 1 Intracellular recordings of membrane response to a constant current pulse in (a) control and (b) emetine treated, isolated extensor digitorum longus muscle of rat. Duration and magnitude of currents indicated below each potential trace. Time calibration 10 ms, temperature 25°C; normal Ringer, resting potentials −70 mV (A) and −65 mV (B).

Adrian & Bryant (1974) recently described a similar behaviour in intercostal muscle fibres from myotonic goats: the characteristic excitability of these fibres corresponded to a decreased chloride membrane conductance. In our treated EDL the mean resting chloride conductance decreased ($P < 0.01$) from 2989 ± 272 μmhos/cm² (mean ± s.e. mean of 12 fibres from three preparations) to 2251 ± 124 μmhos/cm² (mean ± s.e. mean of 43 fibres from eight preparations). The mean resting potassium conductance increased ($P < 0.01$) from 205 ± 38 μmhos/cm² (mean ± s.e. mean control value) to 600 ± 128 μmhos/cm² (mean ± s.e. mean).

We conclude that (−)-emetine interfering with some neural substance causes alterations in the muscle component conductance and consequently in its excitability.

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L-cysteate mimics the action of L-aspartate on lobster muscle

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L-glutamate is likely to be the excitatory transmitter at the crustacean neuromuscular junction while L-aspartate might be a co-excitatory transmitter at this synapse (Nistri & Constanti, 1979). Complex interactions betweeen glutamate and aspartate can occur on crustacean muscle; for example, combinations of bath-applied aspartate and glutamate can evoke membrane depolarizations not explained by a simple agonist summation mechanism. Furthermore, we have seen another type of interaction whereby a conditioning dose of glutamate enhances *subsequent* aspartate responses (but *not* vice versa), indicating a long-term modulatory interplay between these two amino acids. Of several analogues tested, only D- or L-aspartate were affected by conditioning doses of glutamate. We now report that the depolarizing action of L-cysteate, the sulphonic acid analogue of L-aspartate, can also be modified by glutamate pretreatment.

Experiments were conducted on the claw-opener muscle of the lobster (*Homarus vulgaris*) at 17 to 20°C using conventional intracellular recording methods (Constanti & Nistri, 1978) and bath-application of drugs. L-cysteate (1 to 8 mM) reversibly depolarized the lobster muscle membrane with only a small accompanying increase in input conductance. As with aspartate, cysteate (1 to 2 mM) depolarizations at the *beginning* of an experiment often exceeded 10 mV but following repeated applications they declined to a stable plateau level (2 to 5 mV). These latter responses had a slow onset and offset and did not 'fade' during continued application. On a molar basis, cysteate was approximately twice as potent as aspartate (but 1/20th as potent as glutamate) and the cysteate and aspartate dose/depolarization curves were parallel. Conditioning doses of aspartate did not enhance subsequent cysteate responses or vice versa; however, pre-conditioning with glutamate (100 to 150 μM; 2 to 5 min) markedly enhanced subsequent cysteate responses and increased their rates of onset and offset. The enhancement was not affected by interposing a depolarizing dose of KCl (5 to 8 mM; 2 min); hence, if conditioning concentrations of glutamate were indeed 'trapped' by the lobster preparation, they could not be released by K^+. Depolarizations produced by mixtures of cysteate and aspartate could be explained by mere agonist summation; on the other hand, mixtures of cysteate and glutamate had a typical synergistic effect not predicted from their respective dose/response curves.

In conclusion, we propose that L-cysteate has an action similar to that of L-aspartate on lobster muscle: responses to these agents were enhanced by either simultaneous or prior applications of glutamate, hence demonstrating that both short and long-term modulation of excitatory amino acid effects can occur on crustacean muscle membranes.

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The antiarrhythmic haemodynamic and metabolic effects of a new antianginal agent, bepridil, in the early stages of canine acute myocardial infarction

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Bepridil (1-[3-isobutoxy-2-(benzylphenyl)-amino] propyl pyrrolidine hydrochloride) has been shown to possess a potentially useful anti-anginal profile in both anaesthetized and conscious dogs (Cosnier, Duchene-Marullaz, Rispat & Streichenberger, 1977; Piris, Beaughard, Cosnier & Labrid, 1978). In addition, electrophysiological studies have demonstrated that bepridil is capable of blocking slow ionic channels in guinea-pig isolated perfused hearts (Vogel, Crampton & Sperelakis, 1979).

It was therefore of interest to study the effects of bepridil on the electrophysiological, metabolic and haemodynamic consequences of acute myocardial ischaemia in a well-documented experimental model (Marshall, Parratt & Ledingham, 1974).

When administered to chloralose-anaesthetized, oxygen-breathing greyhounds ($n = 6$) 20 min before coronary artery ligation, bepridil (5 mg/kg) caused an immediate decrease in arterial blood pressure (55 to 75 mmHg) which was accompanied by increases in both left circumflex coronary blood flow of 40 to 90 ml/min and in cardiac output (3.2 ± 0.3 to 5.0 ± 0.7 l/min). Myocardial oxygen extraction was significantly decreased (60 ± 3 to $41 \pm 5\%$) but heart rate and left ventricular contractility ($dP/dt\ P^{-1}$) were unaffected. These immediate haemodynamic effects were short-lived (3 to 5 min) and, indeed, 20 min after bepridil administration, heart rate (172 ± 7 to 139 ± 5 beats/min) and myocardial oxygen consumption (12.8 ± 0.5 to 10.0 ± 0.7 ml/min) were significantly reduced although stroke volume was unaffected (18.2 ± 1.3 before; 18.9 ± 1.5 ml/beat after).

In an untreated series of greyhounds, the major consequences of ligation of the LAD coronary artery were depression of myocardial contractility, the production of lactate in coronary venous blood draining the developing infarct and the early appearance of premature ventricular ectopic beats which resulted in ventricular fibrillation in 6 out of 20 animals. Similar haemodynamic and biochemical changes were seen after ligation in the bepridil-treated group but none of these dogs fibrillated.

When administered ($n = 5$) 1 to 2 h after coronary artery ligation, bepridil (5 mg/kg) caused similar haemodynamic effects as seen before ligation, i.e. long-lasting bradycardia (166 ± 14 to 138 ± 9 beats/min) with little effect on stroke volume or myocardial contractility. In contrast to blood flow in the normal myocardium which fell (from 69 ± 8 to 46 ± 6 ml/min) in response to decreased oxygen demand, perfusion of the infarcting area (measured by ^{133}Xe clearance) was not impaired by bepridil (22 ± 4 to 26 ± 5 ml $100\ g^{-1}\ min^{-1}$). Bepridil caused a significant decrease in the ST-segment elevation measured in individual silver epicardial electrodes overlying the ischaemic and border zones of left ventricle 46 ± 12 to 35 ± 9 mV ($P < 0.05$).

These results confirm the potential therapeutic usefulness of bepridil for the treatment of ischaemic heart disease and suggest that in contrast to β-adrenoceptor antagonists, bepridil slows heart rate without markedly affecting the pumping ability of the heart. In addition, unlike propanolol (Marshall & Parratt, 1976) bepridil does not impair the already critical blood flow in the acutely ischaemic myocardium.

We should like to thank Dr. N. Busch, C.E.R.M., Riom, for the gift of bepridil, Dr. I. McA. Ledingham, Western Infirmary, for the use of specialised laboratory facilities and Messrs. I. Douglas, A. Fleming, L. Brady and M. MacDonald for excellent technical assistance.

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The positive inotropic effect of taurine and calcium and the levels of taurine in ventricular strips

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Ventricular strips were isolated from guinea-pig hearts. From the same right ventricle two strips were dissected and one used for the control, the other to test the effects of taurine. The normal perfusion medium contained (mM): NaCl 115, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 10. When using media of altered ionic strength the osmolarity was kept constant by adding appropriate amounts of sucrose. The perfusing solution (pH 7.4) was maintained at 30°C and continually oxygenated with a gas mixture of 95% O_2 and 5% CO_2. The preparations were electrically stimulated at a constant rate (120/min) and contractile activity was recorded on a two-pen recorder with a force displacement transducer.

The ventricular strips were processed for calcium determinations using an atomic absorption spectrophotometer and for taurine determinations by high pressure liquid-chromatography (Baskin, S.I., personal communication). Perfusion with normal medium was performed for an hour in order to stabilize the strips. Then the control strip was incubated with different concentrations of $CaCl_2$ for an hour and the treated strip with different concentrations of $CaCl_2$ and taurine. The normal perfusion medium did not contain taurine, and thus, under our experimental conditions a 70% decrease in the taurine level was observed and the presence of taurine 4 mM in the external medium was not able to restore the original levels. External taurine (10 mM) restored physiological taurine values while 20 mM doubled them. In the latter case a positive inotropic effect was brought out particularly when the external $CaCl_2$ was 0.9 mM. A significant increase in myocardial calcium (Table 1) was observed only when the external $CaCl_2$ was 1.8 mM. This suggests that taurine increased firmly bound tissue calcium. Dolara, Agresti, Giotti & Pasquini (1973) postulate that taurine increases the calcium pool stored in the non exchangeable compartment, and this could explain the lack of correlation between calcium levels and inotropic effect. Furthermore when the external $CaCl_2$ was 0.9 mM taurine exerted a positive inotropic effect, while myocardial calcium levels were the same as in the control.

In effect Alto & Dhalla (1979) suggest that during calcium depletion the mechanisms responsible for regulating calcium influx are either lost or inactivated. The increase of contractile force, very evident when taurine levels were doubled and the external $CaCl_2$ was 0.9 mM suggests a complex interaction in calcium–taurine-contraction.

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Table 1 Relationship between contractile force, taurine and calcium levels in guinea-pig ventricular strips

$CaCl_2$ mM	1.8			0.9			0.45		
	Contraction (%)	*Taurine (μg/mg)*	*Calcium (μg/mg protein)*	*Contraction (%)*	*Taurine (μg/mg)*	*Calcium (μg/mg protein)*	*Contraction (%)*	*Taurine (μg/mg)*	*Calcium (μg/mg protein)*
Control	100 (7)	2.52 ± 0.56 (7)	1.43 ± 0.16 (7)	65.58 ± 5.48 (8)	2.66 ± 0.17 (8)	0.96 ± 0.04 (8)	32.46 ± 9.66 (4)	2.50 ± 0.19 (4)	—
Taurine (4 mM)	110.99 ± 12.14 (7)	3.41 ± 0.41 (7)	1.85 ± 0.26* (7)	86.08 ± 6.48** (8)	4.61 ± 0.45* (8)	0.89 ± 0.16 (8)	32.46 ± 5.29 (4)	3.32 ± 0.49 (4)	—
Control	100 (7)	—	1.37 ± 0.06 (7)	61.95 ± 10.48 (7)	—	1.01 ± 0.13 (7)	27.35 ± 14.1 (4)	—	1.53 ± 0.59 (4)
Taurine (10 mM)	106.75 ± 4.89 (7)	12.77 ± 0.83*** (7)	1.68 ± 0.11* (7)	77.57 ± 13.67 (7)	9.86 ± 1.37*** (7)	0.99 ± 0.11 (7)	29.82 ± 14.3 (4)	10.89 ± 4.4 (4)	1.40 ± 0.26 (4)
Control	100 (6)	—	1.55 ± 0.22 (4)	65.55 ± 13.24 (5)	—	1.71 ± 0.31 (3)	28.84 ± 2.74 (4)	—	0.93 ± 0.42 (3)
Taurine (20 mM)	126.3 ± 4 (6)	19.8 ± 1.9*** (4)	1.86 ± 0.34 (4)	109.5 ± 9.95*** (5)	19.42 ± 1.9*** (4)	1.80 ± 0.28 (3)	45.19 ± 4.55* (4)	20.00 ± 2.11 (3)	0.93 ± 0.13 (3)

Values are mean + s.e. mean.
* $0.05 \leqslant P \leqslant 0.02$; ** $0.02 \leqslant P \leqslant 0.01$; *** $0.01 \leqslant P \leqslant 0.001$.

A pharmacological study of the oscillatory current in cardiac Purkinje fibres

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We have previously reported that an oscillatory current (I_{os}) can be recorded in normal Tyrode's solution (Vassalle, Mugelli & Gibbons, 1978). The induction of this oscillatory current requires a depolarization to -20 mV or more positive potentials and subsequent repolarization to -40 mV or more negative potentials (Vassalle *et al.*, 1978).

To ascertain whether I_{os} depends on other currents activated during the clamp, the I_{os} was studied in sheep Purkinje fibres by the two-microelectrodes voltage-clamp method, in the absence and in presence of 4-aminopyridine (4-AP) and caesium (Cs). In fact it has been shown that, in Purkinje fibres, Cs abolishes the pacemaker current I_{k2} and the background current I_{k1} (Isenberg, 1976) and that 4-AP almost completely eliminated the early outward current (I_{qr}) (Kenyon & Gibbons, 1979).

4-AP (0.5 mM) reversibly abolished I_{qr}, but I_{os} not only persisted but became larger (5 experiments, 63 tests). 4-AP increased I_{os} independently of the voltage at which it appeared, and did not modify the appearance of I_{os} as a graded phenomenon (3 experiments, 16 tests). The magnitude of I_{os} following repetitive depolarizations (10 clamps, 500-ms duration, 1 Hz) was enhanced by 4-AP. Cs (20 mM) abolished the pacemaker current but not I_{os} (5 experiments, 38 tests). In the presence of Cs, the magnitude of I_{os} was often reduced but this effect was unrelated to the block of I_{k2}. The voltage dependence of I_{os} (depolarization threshold) was still present in the presence of Cs, but the amplitude of I_{os} was decreased after each of the different voltages tested. The reduction in amplitude of I_{os} raises the question as to whether other currents are also modified by Cs. Cs (10 mM) affected the plateau current I_{x1} and its effect on I_{os} amplitude seemed to be dependent on a decrease of slow inward current, which may result in a reduction of cellular calcium stores. The I_{os} appears, in fact, largely dependent upon the contractile state of the tissue, and interventions which increase the contractile force enhance I_{os} or make it appear when absent. Actually, noradrenaline (3×10^{-5} M) and strophanthidin (3×10^{-7} M) greatly enhanced I_{os}.

It is concluded that the oscillatory current is a physiological event which can be enhanced by certain procedures and appears to be important in drive-induced arrhythmias under different conditions. Also I_{os} can be separated from either the early outward current I_{qr} and the pacemaker current I_{k2} and it appears doubtful that 4-AP and Cs act specifically on these two currents only.

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Effect of chemical sympathectomy on the changes in adrenergic function caused by chronic Cd^{2+} treatment in the rat

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Chronic and acute treatment of rats with cadmium (Cd^{2+}) increases blood pressure (Schroeder & Vinton, 1962; Fadloun & Leach, 1980a; Perry, Erlanger, Yunice, Schoepfle & Perry, 1970). The responses of *in vivo* and *in vitro* preparations to electrical stimulation, noradrenaline (NA) and potassium ions (K^+) were potentiated following chronic treatment with Cd^{2+} (Fadloun & Leach, 1980a), whilst acute treatment inhibits the responses of *in vitro* preparations to the same tests (Fadloun & Leach, 1979, 1980b; Hayashi & Toda, 1977). The present studies were, therefore, designed to study the effect of chemical sympathectomy on the Cd^{2+}-induced hypertension and potentiation of the responsiveness of *in vitro* preparations.

Newborn male Sprague–Dawley rats were injected subcutaneously on alternate days from birth to the age of 13 days with 6-hydroxydopamine (6-OHDA, 100 mg/kg) dissolved in normal saline containing ascorbic acid (0.5 mg/ml). Control animals received equivalent volumes of normal saline (Clark, Jones, Phelan & Devine, 1978). The 6-OHDA- and saline-treated groups were further subdivided after 14 weeks; one half receiving Cd^{2+} (25 parts/10^6) in drinking water for 4 weeks, the other half tap water. The animals were then anaesthetized (sodium pentobarbitone, 60 mg/kg i.p.) and blood pressure was measured directly via the carotid artery and the effects of lower sympathetic outflow stimulation (Gillespire & Muir, 1967) and NA intravenously administered (i.v.) on blood pressure were assessed. After the determination of blood pressure responses, isolated kidney and vas deferens preparations were then obtained and perfused/superfused with Kreb's solution (37°C) at a rate of 2 ml/min and the effects of perimural stimulation, NA and K^+ were assessed. Minimal group sizes were 8. Results were analysed using Student's 't' test; P values for assessing significance were $>0.05 < 0.001$.

The resting blood pressure of rats treated with Cd^{2+} (25 parts/10^6) were observed to be significantly higher than those receiving tap water only (126/172 compared to 101/127 mmHg for control). However, the blood pressure of the 6-OHDA/Cd^{2+}-treated rats did not differ significantly from the 6-OHDA/tap-water-treated rats (97/122 and 102/123 mmHg respectively). Blood pressure changes to sympathetic outflow stimulation at 12 and 25 Hz were enhanced in the control Cd^{2+}-treated rats whilst the responses to stimulation of lower sympathetic outflow of the 6-OHDA/control- and 6-OHDA/Cd^{2+}-treated rats were abolished, and emphasized the extent of chemical sympathectomy. Cd^{2+} treatment of the control animals did not significantly affect responses to NA, whilst the blood pressure responses of 6-OHDA/control-treated rats were significantly potentiated. There was no significant difference between NA responses of 6-OHDA/control- and 6-OHDA/Cd^{2+}-treated rats except at the highest NA dose (0.5 μg/kg) when the response was reduced in 6-OHDA/Cd^{2+}-treated rats. Cd^{2+} pretreatment potentiated the responses of kidney preparations to periarterial electrical stimulation, whilst the effects on the vas deferens were dependent on the frequency and the nature of the response. Cd^{2+} treatment enhanced the responses of both preparations to NA and K^+. Cd^{2+} treatment of the 6-OHDA treated rats increased the extent of the inhibition of stimulation caused by 6-OHDA, and decreased the extent of potentiation of NA and K^+ responses caused by 6-OHDA.

Since the hypertensive action of Cd^{2+} and its ability to potentiate the responses of isolated tissue was abolished after sympathectomy, it is suggested that these effects of Cd^{2+} may be mediated via an action on presynaptic sites of sympathetic adrenergic nerve endings.

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The effect of 6-hydroxydopamine on rabbit peripheral alpha adrenoceptors *in vivo* and *in vitro*

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While 6-hydroxydopamine destroys the terminal ground plexus of peripheral noradrenergic neurons, physiological responses recover before reinnervation is complete (Finch, Haeusler, Kuhn & Thoenen, 1973). This may reflect 6-hydroxydopamine induced changes in number or sensitivity of peripheral postsynaptic alpha adrenoceptors, either of the classical (α_1) type or the proposed α_2-receptor (Drew & Whiting, 1979; Hamilton & Reid, 1980). We have studied the effect in rabbits of intravenous 6-hydroxydopamine (50 mg/kg) on peripheral α_1-receptors *in vivo*, and *in vitro* using radioligand binding techniques.

In the *in vivo* studies pressor dose-response curves to intravenous phenylephrine (25 to 500 μg) and noradrenaline (2.5 to 50 μg) were constructed in conscious rabbits before and 20 min after alpha adrenoceptor blockade with prazosin (0.1 and 0.5 mg/kg), phentolamine (0.1 mg/kg) or yohimbine (1.0 mg/kg). Mean arterial pressure was measured directly from the central artery of the ear and arterial blood was used for measurement of plasma noradrenaline. Radioligand binding studies were carried out using [^{3}H]-prazosin 33 Ci/mmol (Karliner, Barnes, Hamilton & Dollery, 1979). The affinity constant and maximum number of binding sites were calculated by Scatchard analysis.

Twenty-four to 48 h after 6-hydroxydopamine treatment plasma noradrenaline was reduced by 82% from 3.3 ± 2.1 nM to 0.6 ± 1.1 nM ($P < 0.01$) but mean arterial pressure was reduced by only 8.5% from 79 ± 10 mmHg to 67 ± 10 mm Hg. In the binding studies no change was found in the number or affinity constants of α_1-adrenoceptors in heart, spleen or brain after 6-hydroxydopamine treatment. However, there was a shift to the left in dose-pressor-response curves to the α_1 agonist phenylephrine and the mixed α_1/α_2-agonist noradrenaline. This shift was more marked after α-adrenoceptor blockade with prazosin. In intact animals prazosin (0.5 mg/kg) caused a fall in mean arterial pressure of 12 ± 3 mm Hg and in 6-hydroxydopamine-treated animals a similar fall of 18 ± 4 mmHg despite the absence of a baroreflex-mediated rise in plasma noradrenaline in the latter group. In normal control rabbits plasma noradrenaline rose by 2.2 ± 0.6 nM 20 min after receiving 0.5 mg/kg prazosin ($P < 0.05$) but in 6-hydroxydopamine-treated rabbits by only 0.1 ± 0.2 nM. In previous studies in 6-hydroxydopamine-treated rabbits no changes were found either *in vivo* in pressor responses to α_2-agonists (guanabenz and clonidine), or *in vitro* in number or affinity of α_2-adrenoceptors in spleen or brain for the radioligand [^{3}H]-clonidine (Hamilton & Reid, 1980).

Thus, while 6-hydroxydopamine causes changes in responsiveness to α_1-agonists and antagonists, it does not alter responses to drugs acting on α_2-receptors, suggesting that the pressor response mediated via postsynaptic α_1- and α_2-receptors is controlled by different mechanisms. The changes in α_1-responses do not appear to be mediated by alteration in receptor binding under the conditions studied and might involve other sites in excitation–contraction coupling. The difference in regulation of alpha receptor responsiveness may be of relevance in drug therapy.

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An investigation of pre- and postsynaptic α-adrenoceptors in the rabbit hindlimb

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It has recently been demonstrated that, in vascular tissue of the cat and rat, two types of postsynaptic α-adrenoceptor may be present (Drew & Whiting, 1979), one of which resembles the α_2-receptor of adrenergic terminal axons (Docherty, MacDonald & McGrath, 1979), the other being the conventional postsynaptic α_1-receptor (Langer, 1974).

We have now investigated this possibility in the rabbit autoperfused hindlimb, employing agonists and antagonists with varying selectivities for α_1- and α_2-adrenoceptors. Rabbits were anaesthetized with pentobarbitone and artificially ventilated with room air. Both hindlimbs were perfused with blood at a constant rate, and vasocontrictor responses were elicited by stimulation of both lumbar sympathetic chains (L4–L5, supramaximal pulses, 3 ms, at frequencies of 1 and 2 Hz) and by intra-arterial injection of agonists (Steppeler, Tanaka & Starke, 1978). Test antagonists were infused intra-arterially. Two types of experiment were carried out. (1) The effects of the antagonists rauwolscine (α_2-selective) and corynanthine (α_1-selective; Weitzell, Tanaka & Starke, 1979) were examined on responses to noradrenaline, phenylephrine and nerve stimulation. (2) The effects of rauwolscine and prazosin (α_1-antagonist, more selective than corynanthine; Tanaka & Starke, unpublished observations) were examined on the vasoconstrictor responses to four α-agonists, viz. phenylephrine (α_1-selective), noradrenaline (NA) and α-methylnoradrenaline (mixed agonists) and xylazine (α_2-selective) (Starke, Endo & Taube, 1975; Wikberg, 1978).

(1) Rauwolscine (10^{-6} M) potentiated nerve-mediated responses, decreased the response to NA but did not affect the response to phenylephrine. Even a lower concentration of rauwolscine (10^{-7} M) significantly reduced the response to NA. Corynanthine caused only inhibition of responses. These results suggest that, while endogenous feedback inhibition mediated by α_2-adrenoceptors can be demonstrated in this preparation, rauwolscine is apparently more potent post- than presynaptically, since it reduced responses to NA in a concentration which failed to potentiate nerve-mediated responses.

(2) Rauwolscine (10^{-6} M) markedly inhibited the vasoconstrictor response to xylazine, inhibited the responses to α-methylnoradrenaline and NA less markedly, and failed to inhibit the response to phenylephrine. Conversely, prazosin (10^{-8} M) markedly inhibited the response to phenylephrine, inhibited the responses to NA and α-methylnoradrenaline less markedly, and failed to inhibit the response to xylazine.

The results from (2) suggest that both α_1- and α_2-receptors are present postsynaptically in the rabbit hindlimb. This fact can be used to explain why, in (1), rauwolscine was so potent postsynaptically against NA but not against phenylephrine, and why rauwolscine potentiated nerve-mediated responses at only one concentration.

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An explanation of the resistance of the noradrenaline pressor response to prazosin blockade *in vivo*

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In a previous communication to the Society (Langer, Massingham & Shepperson, 1980) we reported that prazosin inhibited the diastolic pressor response to adrenaline (AD) more than that to noradrenaline (NA) in the anaesthetized dog. The pressor response is however complex and can involve receptors other than α-adrenoceptors. We have now therefore examined the question of this NA resistance in dogs anaesthetized with pentobarbitone, ganglia blocked with chlorisondamine (1 mg/kg) and atropine (1 mg/kg), and β-adrenoceptors blocked with propranolol (0.5 mg/kg plus 0.25 mg/kg/h). The right femoral artery was cannulated and blood passed via a roller pump to the left femoral artery. The effects of NA (0.1 to 1.0 μg/kg), AD (0.1 to 1 μg/kg) phenylephrine (1.0 to 10.0 μg/kg) were examined upon the hind limb perfusion pressure at constant flow. Similar increases in perfusion pressure were obtained to each agonist at these doses (see Table 1).

Prazosin (10 μg/kg) significantly reduced the responses to all doses of phenylephrine but only the responses to 1.0 μg/kg AD and NA. Rauwolscine (10 μg/kg) significantly reduced the response to NA but not those to AD or phenylephrine (see Table 1).

A combination of rauwolscine and prazosin significantly reduced all the agonist responses from control levels. However, the responses obtained with phenylephrine in the presence of both antagonists were not significantly different to those obtained in the presence of prazosin alone. The responses to NA and AD in the presence of both antagonists were significantly less than those obtained in the presence of either rauwolscine or prazosin alone.

In conclusion these results support the concept that there are α_2-adrenoceptors located postsynaptically on the smooth muscle of blood vessels. NA and AD are known to be non-selective α-agonists stimulating both α_1- and α_2-receptors (Starke, Endo & Taube, 1975). Thus the pressor responses to these amines are the result of stimulating both receptor types. Prazosin, a selective α_1-adrenoceptor antagonist (Cambridge, Davey & Massingham, 1977), blocks only part or none of these responses, depending upon the ratio of α_1/α_2 stimulation. This is evident from these results where the combination of both prazosin and rauwolscine produces the most effective blockade of the NA and AD responses. The pressor response to phenylephrine, a preferential α_1-adrenoceptor agonist (Starke *et al.*, 1975) is mediated predominantly by α_1-receptors and is thus blocked by prazosin. The fact that the combination of α_1 and α_2-adrenoceptor antagonists does not produce a significantly greater blockade of phenylephrine's responses than prazosin alone is further support for the α_1-selectivity of this compound.

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Table 1 The effects of rauwolscine and prazosin on the hindlimb pressor responses (mm Hg) to NA, AD and phenylepherine

Antagonist		*Noradrenaline (μg/kg)*			*Adrenaline (μg/kg)*			*Phenylepherine (μg/kg)*		
	n	*0.1*	*0.3*	*1.0*	*0.1*	*0.3*	*1.0*	*1.0*	*3.0*	*10.0*
Control	15	15.2 ± 1.4	34.1 ± 2.7	69.0 ± 4.6	27.4 ± 2.7	53.8 ± 4.8	95.7 ± 4.8	17.3 ± 2.6	42.0 ± 3.4	83.6 ± 4.9
Prazosin (10 μg/kg)	10	12.1 ± 1.4	27.0 ± 3.2	52.5 ± 5.5*	21.7 ± 2.3	43.4 ± 3.8	75.0 ± 5.2*	7.4 ± 1.6*	20.2 ± 2.6*	50.8 ± 4.6*
Rauwolscine (10 μg/kg)	5	8.8 ± 1.5*	25.5 ± 1.8*	54.0 ± 5.3*	27.2 ± 7.8	55.3 ± 10.6	86.9 ± 12.5	13.3 ± 2.5	33.3 ± 3.1	65.3 ± 6.0*
Rauwolscine (10 μg/kg) and Prazosin (10 μg/kg)	6	4.0 ± 0.6*	12.2 ± 2.1*	31.7 ± 5.6*	12.1 ± 2.8*	26.1 ± 4.2*	53.2 ± 7.1*	4.8 ± 1.2*	15.7 ± 3.1*	39.4 ± 6.4*

* Significantly different from controls ($P < 0.05$, Student's t test).

Differential sensitivity to prazosin blockade of endogenously released and exogenously administered noradrenaline: possible relationship to the synaptic location of α_1- and the extrasynaptic location of α_2-adrenoceptors in dog vascular smooth muscle

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Prazosin is a selective α_1-adrenoceptor antagonist (Cambridge, Davey & Massingham, 1977) but *in vivo* responses to exogenous noradrenaline (NA) have been found to be resistant to prazosin blockade (Drew & Whiting, 1979). We have recently examined the effects of low doses of prazosin on dog hindlimb constrictor responses to neuronally released and exogenously administered NA in an attempt to clarify whether or not these prazosin resistant α-adrenoceptors are of the α_2-subtype (Starke & Langer, 1979).

Dogs were anaesthetized with pentobarbitone, intubated and artificially respired. Both femoral arteries were then cannulated and blood from the right femoral used to autoperfuse the left leg via the left femoral artery using a roller pump. Low frequency lumbar sympathetic stimulations (0.1 to 2 Hz, 1 ms, 15 V for 1 min) were carried out and then a dose-response curve constructed to intravenous NA (0.1 to 3 μg/kg) on hind limb perfusion pressure and diastolic blood pressure (DBP). When responses were stable, prazosin (10 μg/kg i.v.) was injected and 10 min later the series of nerve stimulation and injections of NA were repeated. Similar experiments were conducted in β-adrenoceptor-blocked dogs (propranolol 0.5 mg/kg i.v.) and in dogs pretreated in addition with rauwolscine (10 μg/kg i.v.).

In control animals prazosin significantly inhibited, by 50 to 60%, the increases in hindlimb perfusion pressure to all frequencies of lumbar sympathetic stimulation. In these dogs prazosin did not significantly affect the peak responses of the hindlimb or DBP to intravenous NA but did however promote a faster recovery of the responses to injected NA, especially in the hind-limb.

Propranolol pretreatment slightly reduced DBP responses to injected NA but the responses remained resistant to prazosin blockade. In contrast the increases in hindlimb perfusion pressure to the higher doses of NA (1 and 3 μg/kg i.v.) were increased by β-adrenoceptor blockade and prazosin then caused a significant inhibition of these responses.

Pretreatment of dogs with the selective α_2-adrenoceptor antagonist rauwolscine (Tanaka, Weitzell & Starke, 1978) in addition to propranolol, produced a diminution of the DBP responses to NA but nevertheless unmasked a significant inhibitory effect of prazosin on the DBP responses to NA.

These results demonstrate a very high sensitivity of neuronally released NA to prazosin suggesting that α_1-adrenoceptors in the dog hindlimb vasculature have a synaptic location. The fact that hindlimb perfusion pressure responses to exogenous NA were resistant to prazosin suggests that the injected amine acts predominantly on α_2-adrenoceptors. However hindlimb responses to the higher doses of NA were significantly greater after propranolol and were then sensitive to prazosin, indicating that after β-blockade more NA might be diverted on to α_1-adrenoceptors. Finally since rauwolscine pretreatment rendered the effects of NA on DBP sensitive to prazosin blockade, these results suggest that α_2-adrenoceptors are present postsynaptically but predominate extrasynaptically in vascular smooth muscle (see also Langer, Massingham & Shepperson, 1980).

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Stereotyped behaviour in fowls elicited by apomorphine given into the optic ventricle and into the nucleus spiroformis lateralis

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Evidence exists that in birds the meso-paleostriatal system is homologous to the mammalian dopaminergic nigro-striatal pathway (Nisticò & Stephenson, 1979). In fowls, dopamine given into the terminal region of this pathway, the paleostriatum augmentatum (homologous with the mammalian corpus striatum), produced contralateral head–neck deviation on which were superimposed stereotyped head–neck movements (Marley & Nisticò, 1972). A similar pattern was evoked by intraventricular injections of apomorphine (Koc & Marley, 1977; Nisticò & Stephenson, 1979). Dopamine and apomorphine given into the nucleus mesencephalicus profundus (possibly homologous to the mammalian substantia nigra) produced ipsilateral head–neck deviation and stereotyped head–neck movements. The above effects of dopamine and apomorphine were prevented or antagonized by haloperidol and other neuroleptics.

The present experiments were performed to determine whether other areas of the bird brain were involved in the control of stereotyped behaviour. Thus the effects of apomorphine, microinfused into several nuclei of the brain-stem (in 0.5 µl) and into the ventricle (in 5 µl) of the optic tectum (homologous with the superior colliculus in mammals) were studied on stereotyped behaviour. The distribution of horseradish peroxidase given into the paleostriatum augmentatum was also followed.

A dose-dependent increase in stereotyped movements (head–neck movements, preening and pecking) was observed after apomorphine (from 0.05 to 0.1 µmol) given into the optic ventricle. This stereotyped behaviour was prevented by prior administration of haloperidol (0.15 µmol/kg i.v.).

Apomorphine (0.05 µmol) given into nucleus spiriformis lateralis (a posterior commissural nucleus) produced vocalization and an intense pattern of stereotyped movements which were prevented by prior administration of haloperidol (0.15 µmol/kg i.v.).

The injection of horse-radish peroxidase into the paleostriatum augmentatum of adult fowls resulted in the appearance of labelled neurones in the optic tectum, nucleus spiriformis lateralis and nucleus mesencephalicus profundus.

In conclusion, the experiments show that stereotyped behaviour in birds is elicited through activation of dopaminergic mechanisms from areas of the brain homologous to the mammalian nigro-striatal pathway as well as from the optic tectum and other brainstem stations anatomically linked to the paleostriatal complex.

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Characterization and localization of dopamine-D_2 central receptors

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We have recently proposed that dopamine (DA) receptors may be classified on the basis of their association with (D_1) or independence from (D_2), adenyl cyclase activity (Spano, Memo, Stefanini, Fresia & Trabucchi, 1980). This definition was founded on biochemical and pharmacological experiments. We have, indeed, previously reported that various ergot derivatives, endowed with DA-mimetic activity do not stimulate cyclic AMP formation in homogenates of brain dopaminergic areas (Trabucchi, Spano, Tonon & Frattola, 1976).

In addition we have shown that sulpiride and other substituted benzamides, endowed with antipsychotic properties and DA-antagonistic activity, fail to block the stimulation of adenyl cyclase activity elicited by DA or apomorphine (Trabucchi, Longoni, Fresia & Spano, 1975). However, both ergot derivatives and substituted benzamides are able to displace radioactive ligands for DA receptors from their specific binding sites. Therefore sulpiride has been suggested to be a selective antagonist at D_2-receptors.

In the present study we report the characteristics of [^{3}H](−)-sulpiride binding in the CNS (Table 1). In

Table 1 Kinetic characteristics of [^{3}H](−)sulpiride and [^{3}H]-spiperone specific binding (S.B.) in rat striatal membrane preparations after different lesions.

	[^{3}H](−)*sulpiride S.B.*			[^{3}H]-*spiperone S.B.*		
	K_D(nM)	B_{max} (*fmol/mg prot.*)	(%)	K_D(nM)	B_{max} (*fmol/mg prot.*)	(%)
Intact	17.4 ± 1.3	180 ± 21	—	1.2 ± 0.1	298 ± 21	
Kainate	18.3 ± 1.2	168 ± 19	−9%	1.3 ± 0.2	184 ± 11†	−38%
Cortical ablation	17.9 ± 1.0	121 ± 12†	−33%	1.1 ± 0.1	175 ± 13†	−41%
6-OH-DA	18.1 ± 1.4	243 ± 18†	+35%	0.9 ± 0.1	389 ± 24†	+30%

† $P < 0.01$ in respect to B_{max} values of intact animals.
Values are the mean ± s.d. of at least 4 experiments using 5 concentrations of radioligand each.

addition the cellular localization of [^{3}H](−)-sulpiride specific binding was investigated in rat neo-striatum by kainate-induced lesions. Specific [^{3}H](−)-sulpiride binding was found saturable, stereospecific and maximally enriched in the synaptic membrane fraction. Scatchard analysis of the results yielded a biphasic curve indicating the presence of two classes of binding sites. The greatest binding was observed in the striatum, hypothalamus and pituitary. The cerebellum and brainstem had no detectable specific binding levels. On the other hand, selective lesions of striatal intrinsic neurons were performed by stereotaxic application of kainic acid. Animals were killed after 21 days and the striata prepared for measurement of specific bindings. We found a reduction in the number of [^{3}H]-spiperone binding sites while the kinetic parameters of [^{3}H](−)-sulpiride stereospecific binding were not modified.

As previously reported, in this experimental condition DA-stimulated adenyl cyclase activity was virtually abolished. In contrast, cortical ablation did not elicit changes in striatal DA-stimulated adenyl cyclase activity while the total number of [^{3}H](−)-sulpiride binding sites was reduced by approx. 30%. In addition, we observed that lesioning the dopaminergic nigro-striatal pathway by intranigral injection of 6-OHDA increased the number of both [^{3}H]-(−)-sulpiride and [^{3}H]-spiperone specific binding sites in the striatum. Our results suggest that dopamine D_2 receptors are not present in the intrinsic neurones of rat neostriatum but exist in the nerve terminals of cortico–striatal pathways. Moreover, striatal D_2 dopamine receptors appear to be sensitive to changes in DA inputs as it has been shown for D_1 dopamine receptors. The physiological significance of these findings needs to be further investigated.

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Thyroid dysfunction and striatal dopamine receptors

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Thyroid dysfunction affects the synthesis of central catecholamines, probably by 'modulating' the sensitivity of their receptors; there is no direct evidence, however, of any effect on the receptors themselves.

The specific binding of [^{3}H]-spiperone to striatal, postsynaptic dopamine (DA)-receptors in 31-day-old, hypo- and hyperthyroid rats was studied. Hypothyroidism was provoked either by feeding pregnant rats and their weaned litters with sodium iodide-rich tap water, or by daily injection of methimazole to high-iodide pups. Hyperthyroidism was obtained by daily injection of T_3 to the pups; euthyroid rats were vehicle-treated newborns. All treatments were continued from birth up to 30 days of age.

The serum levels of TSH and T_4, as well as behavioural and developmental symptoms confirmed that

Table 1 Effects of experimental hypo- and hyperthyroidism on dopamine-receptor binding in the corpus striatum of newborn rats

Treatment	B_{max} *Receptor binding* (*fmol/mg protein*)
Euthyroid	281 ± 21
Hypothyroid (high-iodide)	229 ± 8*
Hypothyroid (high-iodide plus methimazole)	217 ± 12**
Hyperthyroid (T_3)	218 ± 11**

The corpora striata were taken from 31-day-old, male and female rats; no sex differences appeared in the specific binding. [^{3}H]-spiperone was used as a marker of the striatal postsynaptic DA-receptor. Each value is the mean ± s.e. mean of 7 to 11 experiments. Each assay was performed in triplicate.
Comparisons with euthyroid; Student's *t* test; * $P < 0.05$; ** $P < 0.02$.

our rats were slightly (high-iodide), or significantly (methimazole) hypothyroid; T_3 pups were clearly hyperthyroid. Scatchard plot of the saturation curves for [^{3}H]-spiperone to striatal membranes (Table 1) showed that the maximum number of binding sites (B_{max}) was decreased by about 20% in both hypo- and hyperthyroidism; in the latter case, the dissociation constant (K_d) was increased by over 20% versus euthyroidism.

The functional response to drug-induced activation (apomorphine, etc.) of DA-receptors in hyperthyroid rats is unchanged or slightly attenuated (Strömbom, Svensson, Jackson & Engström, 1977); this might be related to the lower binding of [^{3}H]-spiroperidol here reported.

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Effects of pentobarbitone on uptake and release of [^{3}H]-dopamine from a crude striatal synaptosome preparation

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(+)-Amphetamine releases dopamine from neurones in the striatum; this has been established *in vitro* (Kruk & Zarrindast, 1976) and in unanaesthetized *in vivo* preparations (Conti, Strope, Adams & Marsden, 1978). No release, however, was observed *in vivo* in the barbiturate-anaesthetized rat (Conti, Strope, Adams & Marsden, 1978). The present study investigated the effects of pentobarbitone on uptake and release of tritiated dopamine ([^{3}H]-DA) using a crude synaptosome preparation.

Uptake of [^{3}H]-DA was measured (Kruk & Zarrindast, 1976) on striatal synaptosomes (100 µl) incubated for 5 min at 37°C with 1.8 ml oxygenated Krebs–Henseleit medium containing pargyline (0.125 mM) before the addition of drugs or control medium (100 µl) and [^{3}H]-DA (0.1 µM final concentration) and further incubation for 5 min. Uptake was terminated by vacuum filtration (Whatman GF/B), the washed filter paper was solubilized and radioactivity was then counted. The Po_2 of the incubates was monitored (Corning-EEL digital 160 Po_2 analyser). Release was measured from synaptosomes preloaded with [^{3}H]-DA, by incubating synaptosomes (3 ml) with incubation medium (17 ml) in the presence of [^{3}H]-DA (0.1 µM) for 5 min. After centrifugation (50,000 g at +4°) the synaptosomal pellet was resuspended and 100 µl samples were incubated with oxygenated medium (1.8 ml) in the presence of drugs. Release was terminated by filtration as in the uptake studies.

Dopamine uptake correlated linearly with Po_2 ($r = 0.875$; $P < 0.001$) and inadequate Po_2 control markedly increased sample variation in uptake and release studies. (+)-Amphetamine (6×10^{-8} M) reduced [^{3}H]-DA uptake by 24% ($n = 6$, $P < 0.01$) as did benztropine (2×10^{-7} M, −19%, $n = 6$, $P < 0.05$; 2×10^{-6} M, −79%, $n = 6$, $P < 0.001$). While pentobarbitone (1×10^{-6}, 10^{-5} and 10^{-4} M) had no effect.

(+)-Amphetamine (2.5×10^{-7} and 2.5×10^{-6} M) increased [^{3}H]-DA release (+48% and +111%, $P < 0.001$, $n = 6$, respectively) and this effect was blocked by benztropine (2×10^{-6} M, $n = 5$) in agreement with previous studies (Kruk & Zarrindast, 1976; Raiteri, Bertollini, del Carmine & Levi, 1976). Pentobarbitone (1×10^{-6}, 10^{-5} and 10^{-4} M, $n = 5$) produced small but not significant increases in [^{3}H]-DA release. The same concentration of pentobarbitone.

however, significantly ($P < 0.01$) reduced the release of [^{3}H]-DA by (+)-amphetamine (2.5×10^{-7} and 10^{-6} M, $n = 6$).

The results support *in vivo* findings (Conti, Strope, Adams & Marsden, 1978) that barbiturate anaesthesia reduces (+)-amphetamine-induced release of striatal dopamine and indicate that anaesthetics other than barbiturates should be used with *in vivo* anaesthetized preparations. Furthermore, the results show the need to determine the effect of any anaesthetic used for *in vivo* studies on transmitter release processes.

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Dopamine reuptake as a major feedback mechanism controlling dopamine synthesis

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Dopamine (DA) synthesis is generally believed to be modulated by autoreceptors located on presynaptic terminals. It was proposed that, following chronic neuroleptic treatment, DA autoreceptors become supersensitive to DA agonists (Nowycky & Roth, 1977). However, synaptosomes from haloperidol-treated rats showed no supersensitivity towards apomorphine as synthesis inhibitor (Raiteri, Cerrito, Casazza & Levi, 1980). The existence of autoreceptors controlling DA synthesis was therefore reinvestigated.

Synaptosomes (P_2) were prepared from adult Wistar rats. DA synthesis was measured by monitoring $^{14}CO_2$ formation from L-[1-^{14}C]-tyrosine (Kuczensky & Segal, 1974). Superfusion experiments were performed as previously described (Raiteri, Angelini & Levi, 1974).

Extracellular DA (5×10^{-7} M) inhibited DA synthesis by 60% (Table 1). However, the effect was almost abolished by the DA uptake blocker nomifensine, indicating that DA acted intracellularly.

Table 1 also shows that: (a) $^{14}CO_2$ evolution was accelerated by nomifensine; (b) the uptake blocker abolished the synthesis inhibition caused by pargyline.

These results suggest that the newly taken up DA plays a key role in the control of DA synthesis. According to this hypothesis, which is alternative to that of autoreceptors: (a) in 'control' conditions, DA synthesis is inhibited by the DA recaptured following spontaneous release; (b) nomifensine disinhibits synthesis by preventing reuptake; (c) pargyline inhibits synthesis mainly because a larger amount of DA reaches the biosynthetic enzymes through reuptake. Interestingly, in superfusion (i.e. in the absence of reuptake) nomifensine was inactive and pargyline caused only minimal synthesis inhibition.

The kind of synthesis inhibition here considered and the classical 'end-product' inhibition are probably identical. What differs in our model is that the 'end-product' comes directly from the synaptic cleft, in amounts proportional to the actual synaptic concentration. Thus the role of DA reuptake would be twofold: inactivation of the synaptic transmitter and direct modulation of its synthesis.

Table 1 Effect of extracellular dopamine, pargyline and nomifensine on dopamine synthesis in striatal synaptosomes

Incubation conditions	*DA synthesis (pmol $^{14}CO_2$. mg protein^{-1}. min^{-1})*	*% Control*
Controls	3.87 ± 0.26	100
Dopamine (5×10^{-7} M)	1.55 ± 0.15*	40
Nomifensine (10^{-5} M)	6.56 ± 0.60*	169
Dopamine + nomifensine	5.90 ± 0.49**	152
Pargyline (5×10^{-4} M)	2.23 ± 0.20*	58
Pargyline + nomifensine	5.80 ± 0.27**	150

Aliquots of the striatal P_2 fraction were incubated 10 min at 37°C either in Krebs-Ringer medium or in the presence of the drugs; 2×10^{-5} M L-[1-^{14}C]-tyrosine was then added and the incubation continued for 20 min.
The results are the means ± s.e. of 10 observations.
* $P < 0.001$ vs. controls (Student's t test). ** Not significantly different compared to corresponding nomifensine-treated samples in the absence of dopamine or pargyline.

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Cortical influences on striatal function in the rat

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Among the different pathways afferent to the striatum and regulating its functions, an excitatory pathway originating from the frontal cortex has been described which appears to use glutamic acid (Glu) as a transmitter (Spencer, 1976). Lesions of this corticostriatal pathway have been shown to increase amphetamine-induced stereotypies (Iversen, Wilkinson & Simpson, 1971). However, the exact role of this cortical projection and the mechanism whereby it affects striatal function are as yet unclear.

In this study, we have investigated the effect of the surgical destruction of the corticostriatal projections on haloperidol (Hal)-induced catalepsy, apomorphine (Apo)-induced stereotypies and the Hal-induced increase in striatal homovanillic acid (HVA) levels. In addition, the effect of kainic acid (KA), a putative Glu releasing agent (McGeer, McGeer & Hattori, 1978), on Hal catalepsy, was studied.

Male Sprague–Dawley rats (Charles River, France), weighing 250 to 300 g, were used. Lesions of the corticostriatal projections were performed according to McGeer, McGeer, Scherer & Singh (1977). Catalepsy was measured using the four-cork test (Worms & Lloyd, 1979). Stereotypies were scored using a rating scale from 0 to 5 (Worms & Scatton, 1977). Striatal HVA levels were measured fluorimetrically according to Westerink & Korf (1977).

Bilateral lesions virtually abolished the cataleptogenic action of Hal (0.75 mg/kg i.p.) injected 3, 6, 9 or 21 days after surgery (% catalepsy = 10 ± 4, 4 ± 2, 13 ± 5 and 13 ± 6% of that in sham operated controls, respectively; $P < 0.001$). Also, the effect of a higher dose of Hal (e.g. 2 mg/kg i.p.) was blocked 9 days after lesions, the mean % catalepsy representing 15 ± 5% of that in sham controls ($P < 0.001$).

These results show that Hal-induced catalepsy is prevented as soon as 3 days after lesions. In a parallel group of rats, 3 weeks after lesions, the maximal efficacy of Apo (0.2 to 2 mg/kg, s.c.) for inducing stereotypies was not affected whereas the duration of stereotyped behaviours was markedly enhanced. Thus, 20 min after Apo (0.2 mg/kg), stereotypy scores were 4.1 ± 0.3 and 3.9 ± 0.3 for sham and lesioned rats, respectively, whereas at time 40 min, scores were 1.1 ± 0.1 and 2.3 ± 0.3 ($P < 0.01$), respectively; 30 min after 2 mg/kg of Apo, scores were 4.9 ± 0.1 for sham and 5.0 ± 0.2 for lesioned rats, and 1 h later, these scores were 0.7 ± 0.2 and 2.4 ± 0.4 ($P < 0.01$), respectively.

The increase in striatal HVA levels induced by Hal (2 mg/kg i.p.) was not affected after unilateral lesion of the corticostriatal projections (344 and 338% versus saline controls, respectively). Finally, injections of KA (1.0 to 3.0 mg/kg, i.p.), 30 min before Hal (0.6 mg/kg, i.p.) potentiated Hal-induced catalepsy in normal rats (% catalepsy: Hal = 39 ± 11%; Hal + KA, 1 mg/kg = 67 ± 11, $P < 0.05$; Hal + KA, 3 mg/kg = 73 ± 7, $P < 0.01$) but were without effect in lesioned animals.

These results indicate that the excitatory corticostriatal (glutamatergic) pathway plays an important role in behavioural events connected with changes of dopaminergic transmission. As the lesions affected behavioural patterns elicited by either blockade or stimulation of dopamine receptors, it is likely that the site of this action is distal to dopaminergic neurons.

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Effect of drugs on [^{3}H]-sulpiride binding in rat striatal synaptic membranes

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The substituted benzamide, sulpiride, has actions which are similar yet distinct from those of classical neuroleptics (Spano, Trabucchi, Corsini & Gessa, 1979; Theodorou, Crockett, Jenner & Marsden, 1979). We have investigated the effects of drugs on striatal sulpiride binding.

[^{3}H](±)-sulpiride (specific activity 26.2 Ci mmol^{-1}) binding to rat striatal synaptic membranes was measured in the presence and absence of S(−)-sulpiride (1 μM) to define specific binding. The preparation of synaptic membranes and receptor binding assays were according to Woodruff, Davis, Andrews & Poat (1979), with an additional lysis in 5 mM Tris-Kreb's buffer (pH 8.0) before final resuspension. Free and bound ligand were separated by filtration (Millipore HAWP 02400 filters). Potencies of drugs in displacing specific sulpiride binding are expressed as IC_{50} values (concentrations causing 50% inhibition of binding).

Table 1. Inhibition of specific striatal [^{3}H]-sulpiride binding by drugs

Compound	IC_{50}(nM)
Fluphenazine	0.5
Cis-clopenthixol	0.6
(+)-Butaclamol	0.6
Cis-flupenthixol	1.8
Haloperidol	4.5
(±)-Sultopride	7.0
Chlorpromazine	9.0
S(−)-Sulpiride	11.0
Trans-clopenthixol	40.0
Tiapride	110.0
*ADTN	120.0
Trans-flupenthixol	140.0
Apomorphine	220.0
R(+)-sulpiride	240.0
Dopamine	3000.0

(−)-Butaclamol, 5-hydroxytryptamine, (−)-noradrenaline, (±)-propranolol and glutamic acid all gave IC_{50} values greater than 10,000 nM.
Each drug was tested at four different concentrations in triplicate in 2 to 4 experiments.
* 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

Saturable specific binding of (±)-sulpiride to striatal synaptic membranes was detected, giving a linear Scatchard plot. Analysis gave an affinity constant of 7.4 nM and a maximum specific binding of 240 fmol/mg protein. Using [^{3}H](±)-sulpiride (15 nM), specific binding was 137 ± 13 fmol/mg protein (mean ± s.e. mean, n = 16) which was approximately 55% of total binding (253 ± 17 fmol/mg, n = 16). In the absence of tissue there was an apparent specific binding of 1.4 ± 0.3 fmol per filter (n = 7) compared with a typical result of 12.9 ± 1.4 fmol (n = 16) in the presence of tissue. This blank rate was independent of ligand concentration and essentially unaffected by drugs at their IC_{50} concentrations.

Specific sulpiride binding was potently inhibited both by classical neuroleptics and benzamides, and less potently, by dopamine receptor agonists (Table 1). S(−)-sulpiride was about 22 times more active than R(+)-sulpiride in displacing binding, in good agreement with behavioural studies (Andres & Woodruff, 1979).

Our results indicate another area of similarity between substituted benzamides and other neuroleptics.

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Reduction of specific [^{3}H]-sulpiride binding sites in rat striatum following decortication or striatal kainic acid lesions

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Substituted benzamide drugs, such as sulpiride, preferentially inhibit cerebral D_2 dopamine receptors since they do not antagonize dopamine stimulation of adenylate cyclase (Jenner, Elliott, Clow, Reavill & Marsden, 1978). Lesion studies indicate that adenylate cyclase in rat striatum is associated with striatal neurones while dopamine receptors identified by [^{3}H]-spiperone, which binds to D_1 and D_2 receptors, lie both on striatal neurones and on the terminals of cortico-striate glutamate fibres (Kebabian & Calne, 1979). We now report the effect of kainic acid or 6-hydroxydopamine (6-OHDA) lesions of striatum or decortication on the specific striatal binding of [^{3}H]-spiperone [0.125 to 4.0 nM; as defined using 5×10^{-6} M (+)-butaclamol] and [^{3}H]-sulpiride [5 to 40 nM; as defined using 5×10^{-6} M (−)-sulpiride].

Binding studies were performed in tissue from animals with unilateral destruction of the nigro-striatal pathway induced by 6-OHDA lesions of striatum (4 μg in 2 μl saline) or the medial forebrain bundle (8 μg in 3 μl saline) or with unilateral destruction of striatal neuronal cell bodies induced by kainic acid (2 μg in 1 μl saline) lesions of striatum or in animals with unilateral destruction of the cortico-striate glutamate pathways induced by removal of the frontal and parietal cortex. Ligand binding to striatal tissue from lesioned forebrains was compared with that from the intact side (100%) in all experiments. In striatal tissue from the control hemisphere the number of binding sites (B_{max}) and the dissociation constant (K_d) for [^{3}H]-spiperone varied between 27.3 to 37.0 pmol/g wet wt. tissue and 0.20 to 0.59 nM, respectively, and for [^{3}H]-sulpiride between 18.3 to 25.7 pmol/g wet wt. of tissue and 21.8 to 29.4 nM respectively.

Unilateral lesioning of the striatum or the medial forebrain bundle with 6-OHDA 22 days previously did not decrease K_d or B_{max} values for [^{3}H]-spiperone or [^{3}H]-sulpiride binding. Kainic acid lesions of striatum 22 days previously caused a 52 and 67% reduction in [^{3}H]-spiperone and [^{3}H]-sulpiride binding sites, respectively, and Kd for [^{3}H]-sulpiride fell. Ablation of frontal and parietal cortex 5 days previously reduced B_{max} for [^{3}H]-spiperone and [^{3}H]-sulpiride by 22 and 37% respectively compared to control tissue preparation. K_d values were unchanged.

The data suggests neither [^{3}H]-sulpiride nor [^{3}H]-spiperone binds predominantly to presynaptic receptors on dopamine neuronal terminals (although alterations in postsynaptic receptor numbers resulting from denervation may have masked this) and confirms the existence of cyclase independent D_2 receptors on the terminals of cortico-striate glutamate fibres. However, kainic acid lesions suggest that D_2 receptor sites may lie on the same or separate striatal cell bodies as D_1 receptors. Alternatively, the linkage between specific ligand binding sites and dopamine sensitive adenylate cyclase in lesion studies may be coincidental to their occurrence at the same neuronal location.

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Effects of cimetidine and ranitidine on some non-invasive indices of cardiac function

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Cardiovascular adverse effects, particularly bradycardia, have been reported after cimetidine administration, but the functional significance of histamine H_2-receptors in the human heart remains uncertain. Cohen, Weetman, Dargie & Krikler (1979) suggested that arrhythmias following cimetidine might be due to the rise in plasma prolactin concentration which occurs after the drug is given intravenously. In two separate studies of randomized, balanced, double-blind and cross-over design, we have compared the cardiovascular effects of (1) oral cimetidine and placebo, and (2) intravenous cimetidine, ranitidine (a potent new H_2-receptor antagonist) and saline, using non-invasive methods.

Oral study. Six healthy men took cimetidine 400 mg four times daily, or matching placebo tablets for 7 days. On the morning of the 7th day, the subject attended the laboratory in the basal state; after 30 min rest, measurements of heart rate, arterial BP, systolic time intervals (STI), high speed surface ECG and the normalised first derivative of the apexcardiogram (ACG) were made using methods described previously (Burgess, Turner & Wadsworth, 1978; Denef, Popeye, De Geest & Kesteloot, 1975). Recordings were made in triplicate and a single blood sample taken, approx. 2 h after the subject had taken the last dose of tablets.

Intravenous study. Six healthy men received either cimetidine (3.5 mg/kg) ranitidine (1.5 mg/kg), or an equivalent volume of saline intravenously over 5 min, and recordings of the heart rate, BP, STI and ECG were made, and blood samples taken for estimation of prolactin concentrations, at 0, 5, 10, 15, 30 and 60 min after administration.

All tracings were analysed 'blind' and corrected for changes in heart rate using regression equations derived in this laboratory (Burgess *et al.*, 1978; Burgess, Wadsworth & Warrington, 1979). Statistical analysis was by parametric analysis of variance (first study) and co-variance (second study).

Compared with placebo, oral cimetidine significantly reduced the heart rate by 3.3 beats/min ($P < 0.05$) but no change was noted in BP, STI, ACG or ECG. In the second study, cimetidine and ranitidine caused no significant change in any of the cardio-vascular variables. Plasma prolactin concentrations rose significantly after intravenous cimetidine but were unchanged after ranitidine.

Oral cimetidine caused a bradycardia, but intravenous cimetidine did not in spite of the marked rise in prolactin concentration which followed its administration. The effect on heart rate might be due to a metabolite of cimetidine rather than the parent compound. Since ranitidine had no influence on prolactin concentration, the increase noted after intravenous cimetidine is unlikely to be due only to its H_2-receptor antagonist activity.

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Comparison of the peripheral anticholinergic activities of DL-308, a potential new neuroleptic, of thioridazine and atropine in healthy volunteers

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DL-308 (1-(3-chlorophenyl)-3-[2-(3,3-dimethyl-1-azetidinyl)ethyl]) is a new compound that has a similar pharmacological activity to established neuroleptics (Lepetit Pharmaceuticals Ltd., unpublished). In a previous study, we have compared the effects of approximately equisedative doses of DL-308 (20 mg) and thioridazine (50 mg) on some autonomic functions in healthy volunteers; both drugs caused a decrease in salivation, suggesting a possible antimuscarinic action (Szabadi, Bradshaw & Gaszner, 1980). In the present study, we have further investigated this possibility by comparing the effects of the two neuro-

Table 1 Percentage changes in measures of cholinolytic activity (mean ± s.e. mean)

	Placebo (n = 8)	*DL-308* (n = 8)		*Thioridazine* (n = 8)		*Atropine* (n = 8)	
		10 mg	*20 mg*	*25 mg*	*50 mg*	*0.6 mg*	*1.2 mg*
Resting pupil diameter[1]	+1.71	−1.21	−1.96	−1.53	−3.82**	+5.52**	+9.46**
	(±0.95)	(±1.52)	(±1.16)	(±0.78)	(±0.72)	(±0.57)	(±1.78)
Pupillary constriction	—	−10.41**	−14.75**	−8.74**	−20.13***	−15.43***	−21.59***
to pilocarpine[2]		(±2.52)	(±3.15)	(±2.16)	(±3.22)	(±2.66)	(±2.86)
Baseline sweating[1]	+8.11	+44.08	+52.53	+43.33	+123.58*	−23.55*	−38.90***
	(±4.71)	(±38.71)	(±35.62)	(±27.88)	(±50.27)	(±6.80)	(±4.41)
Sweat gland response	−1.22	−8.60*	−33.91**	−6.98	−29.24**	−34.75***	−55.00***
to carbachol[1]	(±2.23)	(±3.32)	(±8.08)	(±3.00)	(±5.41)	(±3.20)	(±2.63)
Salivation[1]	+11.51	−5.81	−17.74*	−13.02	−14.09*	−39.84**	−66.21***
	(±3.29)	(±7.39)	(±6.08)	(±6.34)	(±5.10)	(±7.55)	(±4.85)
Pulse rate[1]	−4.73	+0.08	+8.39*	+1.12	+10.21	+3.98	+4.49
	(±2.21)	(±3.97)	(±2.48)	(±2.93)	(±4.89)	(±5.10)	(±5.24)

[1] Measurements made before treatment were taken as 100%.
[2] The response to pilocarpine in the presence of placebo was taken as 100% and was used as basis of reference for measurements taken in the presence of the drugs.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Student's t-test; paired comparison).

leptics on tissue responses to cholinoceptor agonists. Atropine was also included as a control.

Eight healthy volunteers (3 males, 5 females: 20 to 22 years) participated in nine experimental sessions [three sessions: placebo; two sessions: DL-308 (10 mg and 20 mg); two sessions: thioridazine (25 and 50 mg); two sessions: atropine (0.6 mg and 1.2 mg)]. Resting pupil diameter and pilocarpine-evoked miosis, baseline and carbachol-evoked sweating, and salivation were measured in each session as described previously Gaszner, Szabadi & Bradshaw, 1979).

The results are summarized in Table 1. Atropine displayed the full profile of a cholinolytic drug: it increased resting pupil diameter and reduced the pilocarpine-evoked miosis, baseline and carbachol-evoked sweating, and salivation. Both DL-308 and thioridazine reduced the pilocarpine-evoked miosis, carbachol-evoked sweating and salivation, suggesting an antimuscarinic activity for both drugs. Both DL-308 and thioridazine decreased resting pupil diameter, although the miosis was statistically significant only after the bigger dose (50 mg) of thioridazine. The miosis caused by the neuroleptics may reflect a possible α-adrenoceptor blocking effect of these drugs (for discussion see Szabadi *et al.*, 1980). Pulse rate did not seem to be a sensitive index of cholinolytic activity: although all the drugs tested caused tachycardia, this reached statistical significance in only one case (DL-308: 20 mg).

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A standardized method for assessing the effect of β-adrenoceptor blocking drugs upon isoprenaline-induced physiological tremor and tachycardia: a test for cardioselectivity?

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Intravenous administration of isoprenaline (IPNA) is known to increase the amplitude of physiological tremor in normal subjects. The effect, which can be blocked by propranolol, is considered to be mediated by stimulation of β-adrenoceptors located in skeletal muscle (Marsden, Foley, Owen & McAllister, 1967). Since the receptors involved are probably of the β_2-type (Thiringer & Svedmyr, 1976), it might be possible to use the comparative ability of β-adrenoceptor blocking drugs in reducing IPNA-induced tremor and tachycardia as a measure of cardioselectivity. In order to evaluate the latter hypothesis, we have developed a simple method for the simultaneous quantitative determination of the effect of antagonists upon physiological finger tremor and tachycardia following graded injections of IPNA in normal volunteers.

Physiological tremor was recorded with an accelerometer attached to a middle finger, and the output quantitated in 0.5 Hz band widths by frequency spectrum analysis. Repeated bolus injections of IPNA were administered under continuous ECG monitoring according to the procedure described by Cleaveland, Rangno & Shand (1972), the heart rate response being determined by the three shortest consecutive R–R intervals on the electrocardiogram. Dose–response curves were constructed by plotting the absolute increase in heart rate and tremor amplitude against log-IPNA dose. Rectilinear relationships were usually found for both of these parameters (Figure 1). The dose-response curves for tremor and tachycardia were shifted to the right to a greater extent by oral propranolol (40 mg) than by the cardioselective drugs atenolol (100 mg) and metoprolol (100 mg), even though these doses of the cardioselective agents had greater β_1-blocking activity as assessed on exercise tachycardia. These data are consistent with the hypothesis that the ability of β-blockers in reducing IPNA-induced tremor is inversely related to their relative cardioselectivity.

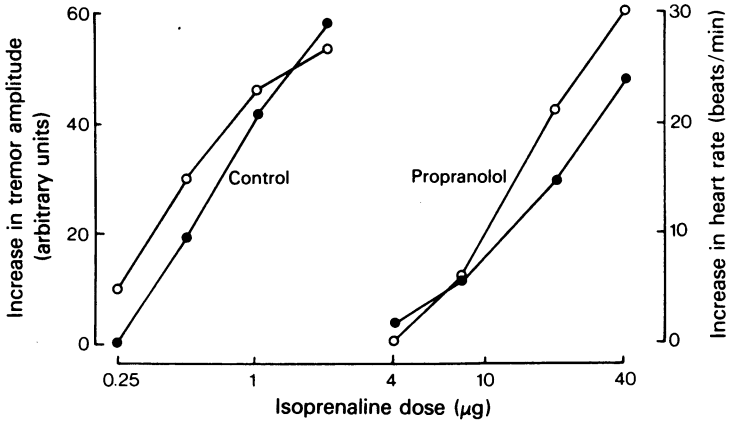

Figure 1 Dose-response curves for isoprenaline-induced increase in heart rate and physiological tremor in a representative subject, in the control state and 1½ to 2 hours following 20 mgm oral propranolol. Tremor amplitude was determined for 2½ Hz centred around the peak frequency of physiological tremor. ● = tremor; ○ = heart rate.

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Effects of atenolol and propranolol on finger tremor in man

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Adrenaline increases tremor by an action on peripheral β-adrenoceptors which is antagonized by propranolol (Marsden, Foley, Owen & McAllister, 1967). McDevitt & Nelson (1978) reported the tremor of hyperthyroidism to be reduced, as assessed by scoring on a three-point scale, by oral propranolol but less by atenolol.

In a clinical trial of the long-term effects of β-adrenoceptor blockade after myocardial infarction, the opportunity was taken to measure finger tremor in 29 patients selected randomly. The patients received propranolol (40 mg three times daily), atenolol (50 mg twice daily) or placebo allocated randomly and double-blind. During the last month of treatment and one month following cessation of treatment, measurements of tremor were taken from an accelerometer on the middle finger (Birmingham, Williams, Wilson & Wright, 1977). Recordings were made on each hand with (i) and the forearm supported and hand relaxed ('rest' tremor), (ii) the arm outstretched ('postural' tremor), (iii) the middle finger exerting an upward thrust against a strain gauge transducer ('work' tremor), and (iv) the arm again outstretched ('post-work postural' tremor). For each measurement the root mean square (r.m.s.) of tremor amplitude was calculated.

Changes in tremor r.m.s. amplitude for one hand are shown in Figure 1. The effects on the other hand were similar. Compared with the response to placebo propranolol produced significant decreases in postural tremor ($P < 0.01$) and work tremor of both hands ($P < 0.01$ left, $P < 0.05$ right); the reductions produced by atenolol reached statistical significance ($P < 0.05$) only for right hand work tremor and left hand post-work postural tremor.

The r.m.s. of tremor acceleration seems to be an objective measurement capable of detecting changes in tremor. The differences in the effects on tremor produced by propranolol and atenolol, in doses which produced similar changes in heart rate, emphasise the cardio-selectivity of atenolol and the β_2 classification of peripheral receptors associated with muscle tremor.

H.J.W. was supported by the S.R.C. We are grateful to our clinical colleagues for allowing us to make measurements on their patients.

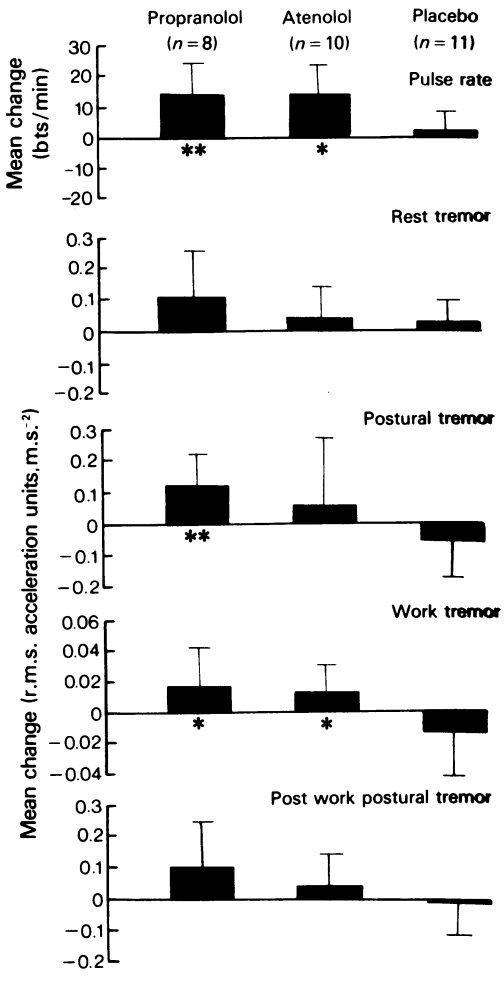

Figure 1 The means (+1 s.d.) of the changes in pulse rate and in right hand tremor amplitude (r.m.s. acceleration, units ms^{-2}) obtained by subtracting the measurement made whilst the patient was receiving treatment from the measurement made one month after withdrawal of the tablets. ** $P < 0.01$, * $P < 0.05$: Mann–Whitney 'U' test of group differences.

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Mexiletine elimination: influence of urinary pH and volume

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The influence of urinary pH on mexiletine elimination is established. However, the degree to which mexiletine excretion is affected by changes in urinary pH is difficult to quantitate. Kaye, Kiddie & Turner (1977) demonstrate a three-fold increase in mexiletine half-life by altering the urinary pH in four volunteers from pH 5 to pH 8, and spontaneous changes in urinary pH have also been shown to affect mexiletine excretion (Johnston, Burgess, Warrington, Wadsworth & Hamer, 1979). In this study we have repeated the work of Kaye *et al.*, increasing the number of subjects to nine and following the plasma mexiletine levels for a further 4 h. Additionally we have included control of the subjects' water intake and a study period during which urinary pH was uncontrolled.

Each subject received mexiletine 4 mg kg^{-1} i.v. over a 15-min period and blood samples were then taken at 0.75 h and then hourly until 12 h. Urinary pH was altered by either ammonium chloride or sodium bicarbonate. Water intake was set at 50, 100 and 300 ml per hour and randomized between urinary pH treatments, so that each subject had a period of acid, uncontrolled and alkaline urine and three different water intakes making a total of 27 half-life determinations in the nine subjects. Urine was collected for mexiletine estimation in 2-h periods up to 12 h and volume and pH recorded. Multiple regression was used for the statistical analysis of the urinary pH, volume and mexiletine excretion data and the results are tabulated in Table 1. Friedman two-way analysis of variance by ranks was used to compare the half-life data and a significant difference ($P < 0.01$) was found between the mexiletine half-life at low pH (mean 3.94 h), uncontrolled (mean 5.0 h) and high pH (mean 5.7 h). A highly significant correlation was observed between urinary pH and hydrogen ion concentration with mexiletine excretion. Volume of urine was also significantly associated with mexiletine excretion.

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Table 1 Multiple regression of urinary excretion data (* $P < 0.05$). Values are regression coefficients (r values) for each correlation

Time	−0.305*				
Volume	0.164*	−0.004			
pH	−0.632*	−0.065	−0.004		
Water load	0.042	0	0.755*	0.022	
(H^+)	0.660*	−0.008	−0.066	−0.723*	−0.088
	Mexiletine excretion	Time	Volume	pH	Water load

Placental transfer and pharmacokinetics of acebutolol and *N*-acetyl acebutolol in the newborn

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In 11 women treated with acebutolol (Sectral®) for arterial hypertension during the last 2 to 3 months of pregnancy, predelivery plasma concentrations of acebutolol ranged between 14 and 439 ng/ml and *N*-acetyl acebutolol plasma concentrations ranged between 113 and 809 ng/ml. Following delivery the maternal plasma elimination half-life was of the order of 6 to 12 h for the parent compound and 8 to 16 h for the *N*-acetyl metabolite. These values are approximately twice those reported in healthy volunteers (Melfin, Winkle, Peters & Harrison, 1977; Melfin, Harapat, Yee & Harrison, 1977).

The umbilical venous and maternal venous plasma concentration ratio ranged between 0.5 and 1.0 (mean ratio 0.8) for acebutolol and between 0.3 and 0.8 (mean ratio 0.6) for *N*-acetyl acebutolol, indicating an efficient transplacental transfer of drug.

The umbilical arterial and umbilical venous plasma concentration ratio ranged from 0.3 to 1.0 (mean ratio 0.6) for acebutolol and from 0.4 to 0.8 (mean ratio 0.7) for *N*-acetyl acebutolol, suggesting an uptake of the drug and its metabolite by the foetus.

The plasma elimination rate of acebutolol in the newborn appeared mono-exponential, with half-lives ranging between 6 and 14 h. The elimination rate of the *N*-acetyl metabolite, however, was non-linear and the half-life within the first 24 h ranged between 24 and 30 h and 12 and 16 h for the remaining 24- to 48-h investigation period. The urinary excretion of the drug and its metabolite was maximum during the first 24 h of life of the newborn. The total amount of drug excreted in urine during 60 h ranged from 40 to 347 μg for acebutolol and from 400 to 4150 μg for metabolite. The ratio of total urinary excretion of parent drug and metabolite ranged between 7 and 13 in the various newborn. Acebutolol and *N*-acetyl acebutolol were present in urine when no longer detectable in plasma.

The concentration of acebutolol and *N*-acetyl acebutolol in breast milk was several times higher than corresponding values in maternal plasma, suggesting that an important quantity of drug could be transferred to the newborn by breast feeding.

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Storage and steroid-induced release from rat leucocytes of a phospholipase inhibitor

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There is increasing evidence that the inhibition of prostaglandin (PG) biosynthesis by glucocorticoids requires receptor occupancy and RNA/protein synthesis (Danon & Assouline, 1978; Flower & Blackwell, 1979; Di Rosa & Persico, 1979; Russo-Marie, Paing & Duval, 1979; Tsurufuji, Sugio & Takemasa, 1979; Carnuccio, Di Rosa & Persico, 1980).

We have previously demonstrated that hydrocortisone prevents PG generation by rat peritoneal leucocytes and that this inhibition is mediated by a non-dialysable inhibitor which acts like a 'second messenger'. We now report that this inhibitor is stored within leucocytes and that it is released by anti-inflammatory steroids. For production of the inhibitor, rat peritoneal leucocytes (80% macrophages) were collected and incubated in Krebs (enriched with albumin) with or without steroids as described by Carnuccio *et al.* (1980). The inhibitory activity thus released was assayed using PG release from leucocytes phagocytosing killed bacteria (*B. pertussis*) as previously described (Di Rosa & Persico, 1979). To determine the time course of inhibitor release cells were incubated with hydrocortisone (20 μM) for different times.

Table 1. Time course of the release of the hydrocortisone (20μM) induced inhibitor from rat leucocytes

Time (min)	n	*% total inhibitory activity recovered (± s.e. mean)* *Intracellular*	*Extracellular*
30	9	46.5 (±5.2)	53.5 (±3.9)
90	5	32 (±6.5)	68 (±1.4)
150	5	10.1 (±6.5)	89.9 (±5.8)

At the end of incubation the inhibitory activity present in the medium as well as within the cells were separately determined. After removing the cells by centrifugation, the medium was dialysed and tested. The centrifuged cell pellet was resuspended in an equivalent volume of Krebs, disrupted by repeated (4 ×) feeeze–thawing and centrifuged (100,000 g for 60 min). The supernatants were tested for the inhibitory activity. Within 30 min of adding hydrocortisone to suspensions of rat peritoneal leucocytes, approximately one-half of the total inhibitory activity was released into the medium whereas the other half remained within the cells. At 90 min most inhibitory activity was extracellular, and by 150 min release was complete, for no detectable inhibitory activity remained in the cells. To establish whether the inhibitor was synthesized *de novo* in response to the hydro-

cortisone, or was stored preformed and secreted by a steroid dependent process we tested the inhibitory activity in lysed preparations of untreated rat peritoneal leucocytes. The lysates were first heated to 70°C for 5 min to provide some protection from proteolysis. Dilutions of the lysates displayed high inhibitory activity on the rat phagocytosing leucocyte system strongly suggesting that the inhibitor was stored preformed in the cells.

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Serum copper concentration and ceruloplasmin activity during carrageenan foot oedema in rat

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Endogenous copper is involved in both human and experimental inflammation as suggested by a large body of data some of which have been obtained in our laboratories. A rise of copper concentration and/or ceruloplasmin activity in biological fluids (usually blood) or tissues has been measured in man and animals under a wide variety of inflammatory conditions (Milanino, Conforti, Fracasso, Franco, Leone, Passarella, Tarter & Velo, 1980; Milanino & Velo, 1980). Moreover, copper is involved in a large number of biochemical processes, some of which, like the immune reaction, PG and collagen biosynthesis and free-radical metabolism, are closely linked with inflammation (Milanino & Velo, 1980). Our previous results show that the acute inflammatory process is enhanced in copper-deficient rats (Milanino, Mazzoli, Passarella, Tarter & Velo, 1978); this has been later confirmed by Denko (1979).

To achieve a better understanding of the role played by copper in acute inflammation, we have measured the concentration of copper and the activity

Table 1 Changes in foot oedema volume, serum copper level and ceruloplasmin activity during carrageenan oedema in rats

Hours	n†	*Oedema Volume* (*s.d.*)† (*ml*)	*Serum* Cu^{2+} (*μg/100 ml*) (*s.d.*)‡	*Serum Cp*§ *Units* (*s.d.*)‡
0	7	–	153.29 (18.38)	402.50 (74.57)
1	7	0.43 (0.14)	135.00 (18.72)	384.14 (46.84)
3	7	0.73 (0.13)	153.00 (17.91)	360.00 (40.56)
5	7	0.77 (0.16)	143.43 (16.30)	348.14 (48.64)
22	8	0.66 (0.26)	251.63 (35.28)*	767.00 (141.59)*
48	8	0.59 (0.19)	220.25 (29.15)*	604.44 (111.50)*
72	9	0.42 (0.12)	237.89 (27.56)*	642.00 (113.84)*
96	8	0.31 (0.08)	207.00 (35.26)*	530.19 (137.70)

† n = number of animals.
‡ (s.d.) = standard deviation.
§ Cp = ceruloplasmin.
* $P < 0.01$ (Dunnet's test).

of ceruloplasmin in serum at different times during carrageenan oedema in normal rats (0.1 ml of 1% carrageenan Viscarin Rex[7191] in sterile saline). The results are summarized in Table 1. A rise of both copper and ceruloplasmin activity after 22, 48 and 72 h was observed. Except for the readings after 22 h, the ratio between ceruloplasmin activity and copper concentration was constant; this latter observation confirms the data obtained in the serum of rheumatic patients by Scudder, Al-Timmi, McMurray, White, Zoob & Dormandy (1978).

We have previously shown that a copper-deficient diet dramatically reduces the serum copper level to below 10% of the control animals. Since over 90% of the copper measured in serum by atomic absorption spectrophotometry is ceruloplasmin-bound, we can reasonably assume that copper-deficient animals are also ceruloplasmin deficient. Therefore the enhancement of the inflammatory reaction seen in this experimental condition sustains the hypothesis of a protective role for endogenous copper, probably in its ceruloplasmin-form, in inflammation (Milanino *et al.*, 1980; Scudder *et al.*, 1978; Denko, 1979).

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Lipoxygenase products derived from blood elements within the decidua of the rat pregnant uterus inhibit myometrial prostacyclin formation

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The myometrium of the rat pregnant uterus when separated from the decidual tissue synthesizes large amounts of prostacyclin (PGI_2) (Williams, Dembinska-Kiec, Zmuda & Gryglewski, 1978; Williams & El Tahir, 1980). Conversely the decidual tissue synthesizes high amounts of prostaglandins (PGs) and thromboxane (Downing & Williams, 1977). It seemed possible that in the whole uterus cyclic endoperoxides produced by the high cyclo-oxygenase activity in the decidua may be utilized by the myometrium and converted to PGI_2. Thus experiments have been carried out to compare PGI_2 production by the isolated myometrium with that of whole uterine fractions (i.e. myometrium plus attached decidua).

Rats were killed on day 22 of pregnancy (day of delivery). Separated myometrial and decidual fractions or whole uterine fractions were prepared, incubated and PGI_2 generation estimated by inhibition of platelet aggregation (Williams & El Tahir, 1980). Statistical significance was calculated by the Student *t*-test.

PGI_2 production by separated myometrium was found to be 4.75 ± 0.39 ng/mg (mean ± s.e. mean; $n = 4$) over 15 min at 20°C but in the whole uterine samples production was only 2.01 ± 0.24 ng/mg, a significant difference ($P < 0.05$). As the decidual fraction produced 1.82 ± 0.24 ng/mg of PGI_2 it appeared that in the whole uterus myometrial PGI_2 was largely inhibited by some factor produced or released from decidual tissue. This was confirmed by incubating myometrial samples together with decidual tissue (2:1 ratio by weight approximating the tissue ratio in the uterus). Under these conditions myometrial PGI_2 generation was significantly inhibited by $48 \pm 9\%$ ($P < 0.05$; $n = 7$). Preincubation of decidual tissue for 10 min at 37°C with indomethacin (30 μg/ml) did not abolish the inhibitory effect exerted on myometrial PGI_2 production but preincubation with eicosatetraynoic acid (TYA) (30 μg/ml) significantly reduced the inhibitory effect. These data indicated a lipoxygenase product may be responsible for the inhibitory effect. Indeed preincubation of myometrial tissue with soyabean lipoxygenase (0.5 to 2 mg/ml) resulted in a dose-dependent inhibition at 1 mg/ml of myometrial PGI_2 production from 4.05 ± 0.23 ng/mg to 2.37 ± 0.08 ng/mg ($P < 0.05$; $n = 5$). This inhibition was reversed by TYA.

In separate experiments pregnant rats were anaesthetized with pentobarbitone sodium (70 mg/kg s.c.) and the uteri perfused free of blood via the dorsal aorta. The decidual preparations from these perfused uteri exerted no inhibitory action on myometrial PGI_2 formation, indicating that generation of the inhibitory factor(s) was associated with trapped decidual blood elements. Incubation of myometrial tissue and perfused decidua in the presence of pregnant rat blood platelets (0.75×10^9 platelets/ml) restored the inhibitory activity.

The experiments demonstrate that lipoxygenase products generated from the trapped blood elements (possibly the blood platelets) within the decidual blood vessels can suppress uterine PGI_2 formation.

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Vascular actions of prostaglandins on the isolated perfused stomach of the rabbit

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The action of vasoactive substances on the gastric microcirculation is an important aspect in the study of gastric function. We now describe a novel *in vitro* technique which measures such actions in the isolated vascular-perfused whole-stomach of the rabbit.

Male rabbits (2.5 kg body weight) were starved overnight, anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.) and the stomach, abdominal aorta and portal vein exposed and freed from adherent tissue. Catheters were inserted into the gastric lumen via the oesphagus and duodenum for gastric lavage. All branches of the splenic artery and vein were carefully ligated, leaving free the left gastro-epiploic artery and vein, and the spleen was removed. The branches of the coeliac artery supplying the liver, pancreas and duodenum were also tied. Following heparin administration (1000 i.u./kg, i.v.), the abdominal aorta was ligated above the coeliac axis and a catheter inserted into the coeliac artery, Krebs' solution (37°C, gassed with 95% O_2, 5% CO_2) containing 3% w/v dextran was perfused (12 ml/min) through the vasculature. The hepatic portal vein was cannulated, then ligated close to the liver, and the perfusate allowed to drain from the portal cannula. After transferring the isolated stomach to a plexiglass chamber (at 37 C), vascular resistance was measured as changes in perfusion pressure via a transducer connected to the coeliac catheter, whilst drugs were administered into the catheter close to the stomach.

Under basal perfusion conditions (perfusion pressure 30 to 50 mm Hg) bolus injections of noradrenaline (0.5 and 4 μg) caused a dose-dependent increase in perfusion pressure (PP) of 17 ± 4 and 42 ± 10 mm Hg over basal respectively (mean ± s.e. mean; $n = 5$). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 5 to 20 μg) likewise elevated PP but was less potent and produced a shallow dose–response relationship, a dose of $PGF_{2\alpha}$ (20 μg) increasing vascular resistance by 10 ± 2 mm Hg ($n = 4$).

The epoxy-methano endoperoxide-thromboxane analogue (U-44069; 2 μg) also produced vasoconstriction, whereas the endoperoxide, PGH_2 (0.4 to 2 μg) produced a biphasic response with a transient increase being followed by a more prolonged small decrease in PP. Under these basal perfusion conditions, PGE_2 and prostacyclin (PGI_2) caused only a small decrease in PP. Therefore, the effects of these vasodilators were investigated under conditions of elevated vascular resistance.

Infusion of noradrenaline (10 μg/min; 0.8 μg/ml) increased PP, which reached steady levels (33 ± 12 mm Hg over basal; $n = 7$) within 3 min. Under these conditions bolus injections of both PGE_2 (0.5 to 8 μg) and prostacyclin (PGI_2; 0.025 to 0.4 μg) cause dose-dependent decreases in vascular resistance. PGE_2 (4 μg) caused a fall in PP of 18 ± 3 mm Hg ($n = 5$) whereas prostacyclin (0.2 μg) caused a fall of 24 ± 3 mm Hg ($n = 5$). Arachidonic acid (1 to 2.5 μg) likewise decreased vascular resistance. Indomethacin (5 μg/ml), in a concentration which itself caused a two-fold increase in the vasconstrictor response to noradrenaline, abolished the vasodilator effect of arachidonic acid.

As in the rabbit isolated mesentery (Malik, Ryan &

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McGiff, 1976) the cyclooxygenase inhibitor, indomethacin (Vane, 1971), potentiated the vasoconstrictor response to noradrenaline, supporting a modulator role for endogenous prostaglandins in the regulation of the gastric circulation. Such an action *in vivo* would reduce mucosal blood flow and hence could contribute to the processes involved with gastric damage induced by aspirin-like drugs (Whittle, 1977). In previous studies on gastric mucosal blood flow *in vivo* in the rat, both PGE_2 (Main & Whittle, 1978) and prostacyclin (Whittle, Boughton-Smith, Moncada & Vane, 1978) were potent mucosal vasodilators following intravenous infusion. In the present *in vitro* preparations following close arterial administration, prostacyclin was 30 times more active as a vasodilator than PGE_2. Thus, the isolated vascular-perfused stomach of the rabbit provides a convenient technique for the study of vasoactive responses under readily-controllable conditions.

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Relationship between bronchoconstriction and thromboxane A_2 formation in the guinea-pig: effect of aspirin

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The ability of histamine and slow reacting substance of anaphylaxis to generate and release thromboxane A_2 (TXA_2) from isolated lungs of normal and ovoalbumin-sensitized guinea-pigs has been previously established (Berti, Folco, Nicosia, Omini & Pasargiklian, 1979). Guinea-pigs (400–600 g) of either sex were anaesthetized with pentobarbitone sodium (60–80 mg/kg i.p.). The trachea was cannulated for artificial ventilation, and for measuring bronchoconstriction, according to the method originally described by Konzett and Rössler (1940). The right carotid artery was cannulated to provide blood for the extracorporeal circulation via a constant-output roller pump. Assay tissues (2–3 rabbit aortas) were superfused with blood at 5 ml/min. An external jugular vein was also cannulated for return of blood to the animal via a second channel in the pump.

In most of the experiments a small catheter was passed down the left carotid artery; small aliquots (0.2–0.3 ml) of arterial blood were taken and quickly subjected to excess methanol treatment in order to convert the circulating TXA_2 into its mono-O-methyl TXB_2 derivative, which was measured by a radioimmunoassay.

In experiments carried out with anaesthetized guinea-pigs, the bronchospasmogenic activity of histamine, bradykinin and acetylcholine has been studied in relation to TXA_2 generation. The results obtained clearly indicate that bronchoconstriction induced by histamine (2.5, 5, 10, 20 μg/kg) and bradykinin (0.5, 1, 2 μg/kg) is concomitant with an increased formation of TXA_2, whereas acetylcholine (10, 20 μg/kg), or vagal electrical stimulation enhance the airway resistance without affecting the basal rate of formation of TXA_2.

Aspirin treatment (20 mg/kg i.v.) prevented the formation of TXA_2 induced by histamine without altering the spasmogenic activity of the autacoid on bronchial smooth muscle. On the other hand aspirin treatment abolished both the bronchoconstriction and TXA_2 formation caused by bradykinin. Different mechanisms of action of histamine and bradykinin in promoting bronchoconstriction in guinea-pigs will be discussed.

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Comparative effects of aspirin and diflunisal on platelet and gastric prostaglandin-synthetase in humans

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Aspirin inhibits prostaglandin (PG) and thromboxane (TX) synthesis by covalently acetylating the active site of cyclo-oxygenase in the PG-synthetase pathway (Roth, Stanford & Majerus, 1975). In contrast, diflunisal, a salicylic acid analogue, does not covalently modify the enzyme (Majerus & Stanford, 1977). When used at equally effective doses, aspirin causes endoscopically confirmed gastric lesions with a significantly higher incidence than diflunisal: 50% vs 10%, respectively (Caruso & Bianchi Porro, 1980). Since inhibition of both platelet aggregation and gastric PG synthesis have previously been linked to the ulcerogenic property of aspirin (Vane, 1971), the present study separately examined platelet TXA_2 and gastric PGE_2 synthesis in healthy subjects and arthritic patients receiving single oral doses of aspirin of diflunisal.

Informed consent was obtained from 12 healthy subjects and 15 patients with rheumatoid arthritis or osteoarthritis. Serial blood samples were obtained prior to and 1, 3, 6, 24 and 48 h after oral administration in the healthy subjects. Whole blood was allowed to clot at 37°C for 30 min and TXB_2 (the chemical breakdown product of TXA_2) concentrations were measured by radioimmunoassay (RIA) (Patrono, Ciabattoni, Pugliese, Pinca, Castrucci, De Salvo, Satta & Parachini, 1980). A 2-h collection of gastric juice was performed before drug or placebo administration in arthritic patients. 1 h after oral intake, gastric juice aspiration was resumed and continued for 3 h. Gastric juice collected on a PG-synthesis inhibitor was extracted and assayed for PGE_2 by RIA (Patrono, Ciabattoni & Pugliese, 1979).

In healthy subjects, aspirin (400 mg) caused a rapid and long-lasting suppression of serum TXB_2 levels: more than 95% inhibition at all time intervals ($P < 0.0005$, $n = 6$). In contrast, diflunisal (750 mg) caused a slower and less pronounced inhibitory effect (65% inhibition at 6 h, $P < 0.005$, $n = 6$), fully reversible at 48 h. In arthritic patients, aspirin caused a marked suppression of gastric PGE_2 output which was partially reversible 3 to 4 h after intake; in contrast, an equally effective dose of diflunisal or placebo caused no statistically significant changes (Table 1).

These results provide evidence for a differential inhibition of platelet and gastric cyclo-oxygenase *in vivo*. The combination of a marked inhibitory effect on both enzymes by oral aspirin might be responsible for its striking ulcerogenic activity. In contrast, a less pronounced and reversible effect on platelet cyclo-oxygenase and the apparent lack of effect on the gastric enzyme might contribute to the lower incidence of gastric lesions in patients undergoing diflunisal treatment.

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Table 1 Effects of a single oral dose of aspirin (1 g), diflunisal (0.5 g) and placebo on gastric PGE_2 output in arthritic patients

	Gastric PGE_2 output (pg/min)			
Treatment	*−120 to 0*	*60 to 120*	*120 to 180*	*180 to 240 min from intake*
Aspirin	396 ± 103	60 ± 20**	81 ± 32**	145 ± 40*
Diflunisal	200 ± 80	295 ± 155	257 ± 106	201 ± 105
Placebo	680 ± 110	680 ± 100	690 ± 145	640 ± 125

Values are means ± s.e. means for five patients per treatment group.
The level of significance of the difference between values obtained before and after treatment was determined by the paired Student's *t* test. * $P < 0.05$; ** $P < 0.01$.

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Inhibitors of neuronal GABA uptake potentiate the inhibitory effect of GABA on the field-stimulated guinea-pig vas deferens preparation

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We have reported that γ-aminobutyric acid (GABA) depresses the electrically-evoked twitch of the mouse vas deferens preparation (Bowery, Doble, Hill, Hudson, Shaw & Turnbull, 1979) and have argued that this effect is mediated at a novel bicuculline-insensitive, β-*p*-chlorophenyl GABA (baclofen) sensitive, receptor which is also present on other autonomic terminals and in the central nervous system (Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnbull, 1980). However, on peripheral tissues such as rat atria, guinea-pig ileum and mouse vas deferens, GABA and baclofen produce a maximum inhibitory effect of approx. 40%. This is in contrast to the almost complete inhibition which can be produced by drugs acting at other pre-synaptic receptors, e.g. opiate or α-receptors on the vas deferens. Burnstock and his co-workers have recently presented evidence for the presence of a specific neuronal GABA uptake process in the myenteric plexus of the guinea-pig taenia coli and have also shown that peripheral nerves can synthesize GABA and homocarnosine from glutamic acid (Jessen, Mirsky, Dennison & Burnstock, 1979). We therefore considered the possibility that the action of GABA-like agents on peripheral tissues might be limited by the presence of such an uptake system. Accordingly we have investigated the effect of drugs known to affect GABA uptake on the response to GABA of the guinea-pig vas deferens preparation. We have also investigated the effect of homocarnosine on the vas and of GABA and homocarnosine on the 'purine receptors' of the field-stimulated guinea-pig taenia coli.

Contractile responses of the field-stimulated guinea-pig vas deferens were recorded isotonically in a 5-ml bath according to the stimulus parameters of Shaw & Turnbull (1978). Cumulative dose–response curves to GABA (10^{-7} to 10^{-3} M) were obtained before and after the addition to the bath of β-alanine (10^{-4} M; 10 min), diaminobutyric acid (DABA) (10^{-7} to 10^{-5} M; up to 15 min) or cis-1,3-aminocyclohexane carboxylic acid (ACHC) (10^{-7} to 10^{-5} M; 20 min). Homocarnosine was tested in the concentration range 10^{-7} to 10^{-3} M.

GABA inhibited the guinea-pig vas to a similar extent as that already reported for the mouse vas deferens [guinea-pig 28 ± 2 (10); mouse 33 ± 6 (15) (mean ± s.e. mean (*n*) maximum % reduction in twitch height)]. β-alanine, an inhibitor of glial GABA uptake, did not affect the sensitivity of the vas to GABA.

In the presence of DABA (10^{-5} M, 10 min) the maximum inhibitory effect of GABA was potentiated to 127 ± 4% (3) of the control response and the dose ratio reduced to 0.75 ± 0.14 (3) (mean ± s.e. mean). A similar enhancement was generally seen at all pre-incubation times from 2 to 15 min with DABA (10^{-5} M) and at 20 min with ACHC (10^{-6} M). However, very large enhancements (dose ratios <0.1 and two-fold potentiation of the maximum GABA inhibition) were occasionally seen in the presence of either DABA or ACHC. When DABA (10^{-5} M) and GABA were added simultaneously to the bath in two experiments there was a decrease in the maximum inhibitory effect of GABA (41 and 51% of normal) and an increase in the dose ratio (to 15 and 35, calculated at the EC_{30} point). Homocarnosine was without effect on either the mouse or guinea-pig vas deferens and GABA, DABA and homocarnosine did not affect the 'purinergic' response of the field-stimulated guinea-pig taenia coli.

These results suggest the presence of a neuronal GABA uptake system in the guinea-pig vas deferens, although it is difficult to understand why inhibition of this uptake system should enhance maximum inhibitory effect shown by GABA.

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Potassium contractures in the rat isolated vas deferens: the role of noradrenaline release and of extracellular calcium

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Swamy, Triggle & Triggle (1976) found that both phases of the KCl response in rat isolated vasa deferentia were dependent on $[Ca^{2+}]_0$. We have studied this further and examined the role of the release of endogenous noradrenaline in these responses.

Vasa deferentia from Wistar rats (250 to 450 g body weight) were suspended in Krebs–Henseleit solution ($Na^+ = 144$, $K^+ = 5.8$, $Ca^{2+} = 2.5$, $Mg^{2+} = 1.2$, $HCO_3^- = 25$, $H_2PO_4^- = 1.2$, $SO_4^{2-} = 1.2$, $Cl^- = 129$, glucose = 11.1 mM) at 36 to 37°C and contractions were recorded isometrically.

KCl (40 mM) produced contractions which had an early phasic and a later tonic component. With higher concentrations (up to 400 mM) there was a progressive increase in the tension of both phases and of the rate of rise of the first phase. With 160 mM KCl the tension of the phasic response was 70% of that with 400 mM KCl and the time peak was 17.6 ± 1.1 s. After transverse bisection of the vas (Pennefather, Vardolov & Heath, 1974; Anton, Duncan & McGrath, 1977) it was found that the phasic response was faster and larger in the prostatic half than in the epididymal half (prostatic: phasic response = 3.4 ± 0.5 g, time to peak = 7.0 ± 0.4 s; epididymal: phasic response = 1.98 ± 0.33 g, time to peak = 43.4 ± 4.0 s).

In the complete vas, phentolamine (2 μg/ml) inhibited the phasic with little effect on the tonic response (phasic reduced by 23.6 ± 6.1%, tonic by 9.2 ± 10.5%). However, in complete vasa removed from rats chronically pretreated with guanethidine to destroy adrenergic nerves (Heath, Evans, Gannon, Burnstock & James, 1972) the phasic response was not reduced by phentolamine. After chronic guanethidine treatment, the phasic response was 2.4 ± 0.15 g, a reduction of 30% compared with untreated animals.

In nominally Ca^{2+}-free Krebs–Henseleit, the responses to 160 mM KCl were reduced progressively over a period of 90 min. The phasic and tonic responses were approximately equally sensitive. The contractions were rapidly restored on readmission of Ca^{2+}. In low Ca^{2+} Krebs–Henseleit (0.1–0.5 mM) the phasic response was inhibited more than the tonic response. Both phases increased in parallel with $[Ca^{2+}]_0$ = 1 to 6 mM.

Verapamil HCl antagonized both phases of the response to KCl (160 mM) with some selectivity for the tonic response. With verapamil (0.1 μg/ml) the phasic response was reduced by 15.2 ± 5.4% while the tonic response was reduced by 36.4 ± 5.5%. In the presence of verapamil (0.4 μg/ml), the phasic response was reduced by 36.8 ± 6.2%, and the tonic response was reduced by 55.2 ± 2.5%.

It is concluded that the phasic part of the response to KCl (160 mM) includes a component that is due to noradrenaline release from intramural nerves. Both phases require extracellular calcium, or a membrane store in rapid equilibrium with it. Verapamil has some selectivity for the tonic phase but Triggle, Swamy & Triggle (1979) found a more pronounced difference in the effect of methoxyverapamil on the two phases.

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Marked differences in the effects of 'Ca^{2+}-antagonists' on $CaCl_2$-induced contractions in K^+-depolarized smooth muscle

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All drugs classed as 'Ca^{2+}-antagonists' inhibit Ca^{2+}-induced contractions in K^+-depolarized smooth muscle preparations (Fleckenstein, 1977; Quintana, 1978; Broekart & Godfraind, 1979). However, as some of these drugs exhibit apparent tissue specificity (e.g. Van Nueten, Van Beek & Janssen, 1978), the possibility arises that they may possess different modes of action. In order to investigate this possibility I have compared the effects of several of these compounds in a K^+-depolarized smooth muscle preparation, but using different methods of assessment of their 'Ca^{2+}-antagonist' effects.

Guinea-pig taenia caeci preparations were set up in K^+-depolarizing Tyrode solution (mM: NaCl 97; KCl 40; $NaHCO_3$ 11.9; NaH_2PO_4 0.4; glucose 5.5) maintained at 35°C and gassed with 95% O_2 and 5% CO_2. Cumulative concentration–response curves to $CaCl_2$ (30 to 3000 μM; EC_{50} 134 ± 8 μM, $n = 58$; 3 min contact at each concentration, 20 min washout between curves) were reproducible for >5 h. Furthermore, contractions to single additions of Ca^{2+} (100 μM, 40 to 65% of maximum contraction) were stable for >1 h. Apparent pA_2 values were measured by the method of Arunlakshana & Schild (1959).

The Ca^{2+}-antagonists tested have approximately equivalent antagonist potency when assessed by their ability to displace concentration–response curves to Ca^{2+} to the right following 15 to 20 min incubation in Ca^{2+}-free media. The pA_2 values were: verapamil 7.8 ± 0.1; cinnarizine 7.7 ± 0.2; flunarizine 7.6 ± 0.2; diltiazem 7.6 ± 0.1; pimozide 7.5 ± 0.2; fendiline 7.1 ± 0.2, $n = 4$ to 6). In contrast, the drugs differed markedly in their ability to relax established contractions induced by Ca^{2+} (100 μM). Verapamil (0.1 μM) and diltiazem (1 μM) relaxed the contractions rapidly. The time to 50% relaxation (t50) for the two drugs was <10 min and not significantly ($P > 0.1$) longer than the t50 following washout of Ca^{2+} from the bath (3.4 ± 0.4, min = 20), whereas the other drugs (all at 1 μM) relaxed the contractions slowly (t50 > 30 min; $P < 0.01$ against t50 for Ca^{2+} washout). Interestingly, the effects of 15 to 20 min incubation in Ca^{2+}-free media of these concentrations of verapamil and diltiazem were reversed by washout over 2 h (Ca^{2+} dose ratio <2) whereas the effects of the other drugs were not (Ca^{2+} dose ratio >10). These data are consistent with considerable differences in the association and dissociation constants of the drugs for their site(s) of action.

The relative ineffectiveness of some of the drugs (e.g. cinnarizine) against established Ca^{2+}-induced contractions appears incompatible with their effectiveness in Ca^{2+}-free media. However, if Ca^{2+} (100 μM) was present during the first 15 min of a 20-min incubation period with cinnarizine (1 μM) subsequently obtained dose ratios were lower ($P < 0.05$) than if cinnarizine had been incubated in Ca^{2+}-free media throughout. In contrast, prior incubation with Ca^{2+} (100 μM for 15 min of a 20-min incubation) did not influence the dose ratios obtained after incubation with verapamil (0.2 μM). Thus the presence of low concentrations of Ca^{2+} may affect the 'Ca^{2+}-antagonist' effects of drugs and this may be more apparent if the drugs have a slow onset of action.

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DNA-damaging and mutagenic activity of five hydrazine derivative monoamine oxidase inhibitors†

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Despite general knowledge of the chronic toxic effects produced by hydrazine MAO inhibitors, until now, apart from the recognized tumorigenic activity of iproniazid and phenelzine in mice, little attention has been devoted to their mutagenic-carcinogenic properties. This study was performed to investigate the relative potency of iproniazid, phenelzine, nialamide, isocarboxazid and mebanazine in inducing DNA damage *in vivo*, and in increasing mutation frequency in bacteria; two properties which have often shown a satisfactory qualitative correlation with tumorigenic activity, and have been proposed for the rapid prescreening of carcinogenic chemicals.

Liver and lung DNA damage was evaluated by the alkaline elution technique (Brambilla, Cavanna & Parodi, 1978) in Swiss male mice treated either i.p. or by the oral route with single (or a few repeated) equitoxic doses according to a standardized protocol. Mutagenicity was checked by the *Salmonella*/microsome test (Ames, McCann & Yamasaki, 1975) either in the presence or in the absence of Aroclor-induced rat liver S-9 mix.

By i.p. route, isocarboxazid, nialamide and phenelzine induced a statistically significant ($P < 0.05$) increase in liver and/or lung DNA elution rate; iproniazid and mebanazine were inactive. Similar results were obtained in preliminary experiments performed in mice treated by the oral route. If DNA damaging potency is calculated as the ratio between DNA elution rate over controls (roughly proportional to the number of DNA single-strand breaks) and mmoles/kg of the most active dose, it varied over a ~10-fold range, and the three active hydrazines ranked as follows: isocarboxazid, phenelzine, nialamide.

Nialamide, phenelzine and mebanazine induced a statistically significant ($P < 0.05$) increase of mutation frequency in *S. typhimurium his*$^-$ strains, while iproniazid and isocarboxazid were inactive. Mutagenic activity of nialamide and phenelzine decreased in the presence of S-9 mix. If mutagenic potency is calculated as the ratio [number of revertants over controls in the most sensitive strain/μmoles], it varies over a ~150-fold range, and the three active hydrazines ranked as follows: nialamide, phenelzine, mebanazine. On the basis of their distinctive effects on the five test strains (TA1535, TA100, TA1537, TA1538, TA98), nialamide and phenelzine could be classified as base-substituting agents, while mebanazine induced frameshift errors.

In an analysis performed on 11 hydrazine derivatives we observed that a satisfactory quantitative correlation existed between DNA damaging and carcinogenic potencies, while it was absent between mutagenic and carcinogenic potencies, despite a higher sensitivity of the Ames test in detecting positive hydrazines. In this light, a first conclusion of this work could be that isocarboxazid, which showed a potency of the same order of 1,2-dimethylhydrazine in inducing DNA fragmentation, should be considered strongly suspect of carcinogenic activity. In a more general sense, the results obtained should recommend a careful evaluation of the benefit/risk ratio in the therapeutic use of hydrazine derivatives. In this respect it should be also emphasized that all hydrazines up to now tested in rodents were found to be tumorigenic, even if in very different degrees (Toth, 1975).

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Histochemistry of porcine tissues using antibodies to pig plasma amine oxidase

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We have previously studied the immunological reactivity of various tissues of the pig with rabbit antibodies raised to pig plasma amine oxidase (Buffoni, Della Corte & Hope, 1977). In this paper we present further evidence for the presence of a protein in the connective tissues of pig organs which possesses the same immunological determinant as pig plasma amine oxidase.

Pig plasma amine oxidase was purified and crystallized by the method already described (Buffoni & Blaschko, 1964). A crystalline preparation with a specific activity of 0.2 i.u. per mg (25°C, oxygen as gas phase) was used.

Antibodies were prepared as already described. The immunodiffusion paterns in agar were photographed after 22 h. They gave a single band with pure pig plasma benzylamine oxidase and did not cross-react with the lysyloxidase of pig aorta (Buffoni, Ignesti & Lodovici, 1980), pure diamine oxidase of pig kidney, pure spermine oxidase of beef plasma, human serum diamine oxidase or benzylamine oxidase (Oratore, Banchelli Soldaini, Buffoni, Mondovi' & Finazzi Agro', 1980).

Immunofluorescence and immunoperoxidase histochemistry were carried out as already described (Buffoni, Della Corte & Hope, 1977). In the latter, however, peroxidase conjugated anti-rabbit γ-globulin (*Miles*) was used instead of fluorescein anti-rabbit γ-globulin (*Wellcome*).

Spleen, thyroid and eye were particularly studied.

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Effect of reserpine on met-enkephalin-like material concentrations in the rat adrenal gland

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Despite the fact that the presence of enkephalins in several peripheral tissues is by now well documented little is known about the biochemistry and physiological role of these peptides. However, a small number of experimental conditions have been shown to affect enkephalin concentration in central as well as in peripheral tissues. Here we report a dual effect of reserpine on met-enkephalin-like material (ELM) concentration in the adrenal gland. It has been recently shown that 10 days after a daily treatment with reserpine there is an increase of the enkephalin concentration in the striatum, while a similar effect fails to occur within short term after acute injection of the drug (Costa, Di Giulio, Fratta, Hong & Yang, 1979).

In our experiments ELM was measured by means of a radioimmunoassay method as previously described (Di Giulio, Yang, Fratta & Costa, 1979). Separation by Biogel P-2 column chromatography of the ELM extracted from the adrenal gland has shown the presence of at least four well defined peaks of immunoreactivity. We have called low molecular weight met-enkephalin-like material (LELM) the ELM with a mol. wt. below 1000 and high molecular weight met-enkephalin-like material (HELM) the ELM with mol. wt. higher than 1800.

We have found that 2 to 6 days after acute reserpine injection (5 mg/kg i.p.) the HELM and LELM content of the adrenal gland was increased two- to three-fold over control values. Indirect evidence suggests that central mechanisms may be involved. On the other hand, shortly after reserpine treatment (4, 8 and 24 h) the adrenal HELM concentrations, like those of the catecholamines, were progressively decreased. This latter finding supports *in vitro* evidence suggesting that part of the ELM present in the adrenal gland may be stored and secreted from the chromaffin vesicles together with catecholamines.

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Rat small intestine contains opiate receptor binding and a potent interfering factor

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Pharmacological data, including a recent report that naloxone reverses the antidiarrhoeal action of the locally-effective drug loperamide (Piercey & Ruwart, 1979) suggest that the rat small intestine (SI) contains opiate-specific molecular sites of action. Lately we have presumptively labelled these receptors *in vivo* by injecting the potent narcotic, buprenorphine, tritiated at high specific activity (Tavani, Bianchi & Manara, 1979). Yet it is currently assumed that the rat SI lacks *in vitro* opiate receptor binding (ORB) (Snyder, Pasternak & Pert, 1975; Simantov, Childers & Snyder, 1978). This discrepancy prompted us further to investigate rat SI preparations and we report *in vitro* ORB therein, as well as their possible contamination by a potent locally-occurring interfering factor.

As shown in Table 1, all our preparations from guinea-pig SI were positive for ORB which however was inconsistent in similar rat preparations. Moreover, successful preparations from rats (e.g. experiment 3) when mixed with guinea-pig material did not inhibit ORB though this did happen with rat preparations which showed no ORB (e.g. experiments 1 and 2). To be responsive for ORB rat preparations had to be carefully dissected free from the mesentery; this enabled us to obtain reproducible samples for characterizing ORB in SI and to locate the source of the interfering factor. ORB in rat SI has characteristics similar to that in brain as regards high affinity (K_{D_1} = 1.5 nM), saturability, stereospecificity and Na effect. A main source of the interfering factor was the mesentery root. Crude microsomal fractions prepared from this and diluted to deliver 8 μg protein to the incubation medium (0.5 ml) inhibited by 50% 0.4 nM [^{3}H]-etorphine binding to 0.4 mg protein from rat brain microsomes. This inhibition was reduced in the presence of soybean trypsin inhibitor, but not of EGTA concentrations completely protecting ORB from 50 ng/ml phospholipase A_2. Contamination by pancreatic tissue containing trypsin and by an unidentified interfering factor occurring in the mesentery seems to account for previous failure to demonstrate ORB in rat SI.

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Table 1 [^{3}H]-etorphine opiate receptor binding in guinea-pig and rat intestine

Experiment	*1*	*2*	*3*	*4*	*5*
Guinea-pig	1.92 ± 0.37	2.04 ± 0.04	1.22 (1.18 to 1.26)	Not assayed	2.33 (2.09 to 2.57)
Rat	n.d.	n.d.	0.36 (0.34 to 0.39)	0.81 ± 0.06	0.16 (0.15 to 0.18)
Guinea-pig plus rat	0.45 ± 0.13	0.09 ± 0.09	1.04 (0.90 to 1.19)	Not assayed	Not assayed

Figures (total binding minus binding after pre-incubation with 10^{-6} M etorphine; mol × 10^{-14}/mg protein) are means and s.d. from triplicate assays or mean and range from duplicate assays on crude microsomal fractions prepared from pooled tissue homogenates (small intestine longitudinal muscle) in Krebs Tris pH 7.4 (mM: NaCl, 118; KCl, 4.75; $CaCl_2$ 2.54; $MgSO_4$, 1.2; Tris-HCl, 25). Longitudinal smooth muscles with attached myenteric plexus were obtained according to Rang (1964) and pooled from 8 to 10 animals for each experiment. Portions for assays containing about 0.75 mg protein (1.1 for exp. 3) from guinea-pig, rat or both mixed in equal amounts, were processed as described by Pasternak, Wilson & Snyder (1975) in 0.5 ml buffer containing 0.4 nM [^{3}H]-etorphine (Radiochemical Centre, U.K., 31 Ci/mmol).
n.d. = not detectable

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The effects of electrical stimulation, high K^+ concentrations, atropine and physostigmine on high affinity choline uptake (HACU) in guinea-pig brain slices

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It has been proposed that changes in activity of cholinergic neurones are correlated with those of the sodium-dependent HACU system (Atweh & Kuhar, 1976). In order to test this hypothesis, HACU was measured in cortical brain slices in which neuronal activity was enhanced either by electrical stimulation at different frequencies or by K^+ depolarization.

Cortical slices were prepared from adult guinea-pigs and superfused according to Beani, Bianchi, Giacomelli & Tamberi (1978). HACU was determined according to Simon, Atew & Kuhar (1976) on a crude synaptosomal preparation obtained from the slices after 20-min stimulation with pulses of alternate polarity 5 ms duration, 30 mA/cm^2 intensity, or superfusion with different K^+ concentrations. The influence of physostigmine and atropine added to the superfusion medium on HACU was also investigated.

Figure 1 shows that a direct relationship ($r = 0.99$ $b = 48$) between the increase in stimulation frequency and the percentage increase in HACU exists. The addition of physostigmine (3×10^{-5} M) to the perfusion medium significantly ($P < 0.05$) reduced the effect of electrical stimulation on HACU. Conversely the addition of atropine 1.5×10^{-8} M strongly enhanced the increase in HACU elicited by electrical stimulation. The effect of atropine was potentiated by physostigmine.

A direct relationship ($r = 0.98$ $b = 248$) also existed between the increase in K^+ concentration in the superfusion medium from 25 to 62 mM and the per cent increase in HACU.

These results demonstrate that HACU is activated by depolarization and its increase closely follows the rate of electrical stimulation. The activation was enhanced by atropine which enhances acetylcholine output (Hardhazy & Szerb, 1977) and by atropine and physostigmine together and depressed by physostigmine which prevents acetylcholine destruction. Whether HACU changes can be related to variations

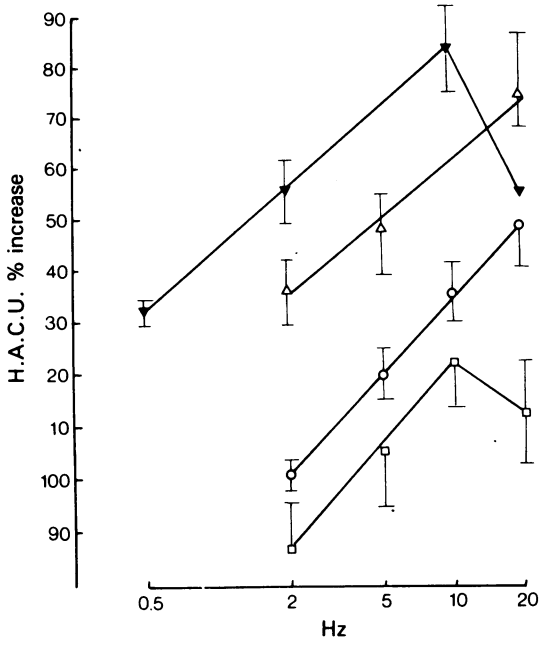

Figure 1 Relationship between stimulation frequency and high affinity choline uptake (HACU) in brain slices from guinea-pigs. Each point is the mean ± s.e. mean (vertical bars) of at least five determinations. (○) controls; (□) physostigmine, 3×10^{-5} M; (△) atropine, 1.5×10^{-8} M; (▼) atropine + physostigmine.

in acetylcholine and choline contents in, or release from, the slices remains to be elucidated.

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The efflux of endogenous GABA and glutamate from the rat parietal cortex *in vivo*

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The measurement of the release of putative neurotransmitters from exposed brain surface may give information on the functional activity of underlying neurones. This has been proved for acetylcholine (Pepeu, 1973) and we attempted to extend the investigations to aminoacid neurotransmission.

GABA and glutamate were measured by a mass-fragmentographic method (Moroni, Tanganelli, Bianchi, Moneti & Beani, 1980) in Ringer solution filling Perspex cylinders applied on the dura in freely moving rats or on to the exposed cortex in urethane (1 g/kg i.p.) anaesthetized rats. Three samples were collected before and after the application of KCl (50 mM); collection time 20 min. In some animals the electrocorticogram was also recorded. Table 1 shows that the removal of the dura did not affect spontaneous GABA efflux but significantly increased glutamate efflux. In rats with intact dura anaesthesia significantly decreased glutamate efflux. KCl (50 mM) placed in the epidural cylinders significantly decreased GABA but increased glutamate efflux. KCl (50 mM) placed in the cortical cylinders greatly increased GABA output while glutamate output decreased. These changes had similar time-courses and were abolished by the addition of tetrodotoxin (3×10^{-5} M). The increase in GABA efflux was associated with high voltage low frequency electrocorticographic activity. The administration of amphetamine (1 mg/kg i.p.) in anaesthetized rats with removed dura was followed by a 43% increase in glutamate efflux and by low voltage high frequency electrocorticographic activity.

These results suggest that GABA and glutamate released from the cerebral cortex originate at least in part from brain tissue and confirm the existence of a relationship between the electrical activity of the cortex and aminoacid output (Jasper, Kahn & Elliott, 1965).

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Table 1 The effects of KCl (50 mM) on GABA and glutamate efflux from the cerebral cortex of the rat

Conditions		*GABA efflux* (*pmol/cm².20 min ± s.e. mean*) Spontaneous	KCl	*Glutamate efflux* (*nmol/cm².20 min ± s.e. mean*) Spontaneous	KCl
Unanaesthetized	Intact dura (3)	104.40 ± 7.72	31.96 ± 6.31†	6.12 ± 0.65[a]	8.76 ± 1.02
Anaesthetized	Intact dura (5)	72.56 ± 9.88	38.96 ± 4.28†	4.13 ± 0.40[b]	7.97 ± 1.56†
Anaesthetized	Removed dura (8)	79.20 ± 14.00	640.00 ± 26.00†	16.44 ± 2.08[c]	9.64 ± 1.44†

Number of rats in parentheses.
* Difference from basal efflux $P < 0.05$.
† Difference from basal efflux $P < 0.01$.
a versus b: $P < 0.01$; b versus c: $P < 0.01$; a versus c: $P < 0.02$.

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Central nervous system derived GABA influences plasma GABA concentrations and prolactin secretion

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Evidence has been given of a dual GABAergic control on prolactin (PRL) secretion in the rat, one stimulatory exerted through a central nervous system (CNS) site, the other inhibitory, occurring at the level of the anterior pituitary (AP) (Locatelli, Cocchi, Frigerio, Betti, Kronsgaard-Larsen, Racagni & Müller, 1979). It has been also demonstrated that GABA is present within the AP probably being synthesized in and released from the hypothalamus and thence conveyed to the gland through the portal circulation (Racagni, Apud, Locatelli, Cocchi, Nisticó, Di Giorgio & Müller, 1979).

In this report we have investigated: (1) the role played by the hypothalamo-pituitary (Hy-AP) GABAergic system in the maintenance of circulating levels of the amino acid; (2) the mechanisms whereby bodily GABA titres may affect PRL secretion. Intraventricular injection of ethanolamine-O-sulphate (EOS), a specific competitive and catalytic inhibitor of GABA transaminase (2 mg/kg per rat), increased 2, 4 and 8 h later, GABA concentrations in the hypothalamus and AP and 4 and 8 h later, plasma GABA titres. After central EOS administration PRL declined in plasma with a time-pattern related to the increase of GABA occurring in the Hy-AP system. However, the inhibition of PRL secretion lasted until 24 h, a time when GABA titres in the Hy-AP system had returned to baseline. On the contrary systemic administration of EOS (200 and 400 mg/lg i.v.) altered 4 h post-drug injection neither GABA in the Hy-AP system and in the plasma nor PRL levels. Only the highest dose of EOS (600 mg/kg i.v.) increased GABA concentrations in the Hy-AP system and lowered plasma PRL levels.

Transplantation of the gland under the kidney capsule markedly decreases its GABA content. Administration of EOS (2 mg/kg i.v.t. or 600 mg/kg i.v.) failed to modify either GABA content in the ectopic gland or GABA and PRL concentrations in plasma. Only hypothalamic GABA concentrations were strikingly or slightly increased respectively after central i.v. administration of EOS.

These data indicate that (1) changes of GABA reflect alterations primarily manifested in the CNS, suggesting that measurement of plasma GABA may be a reliable index of central GABAergic function; (2) GABA in the periphery seems not to exert a functional role on PRL secretion, which could be operated by the centrally derived aminoacid.

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Neuroendocrine studies in a rat model mimicking Huntington's disease

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There is increasing evidence for a variety of hypothalamic abnormalities in patients with Huntington's disease (HD), a neurologic disorder affecting the basal ganglia, in which hyperfunctioning of nigro-striatal dopaminergic (DA) system has been postulated. Previous studies performed in patients with HD with the use of DA agonist drugs had shown the existence in individual subjects of hyper- or hyporesponsiveness to the growth hormone (GH) releasing effect of dopaminergic stimulation (Müller, Parati, Panerai, Cocchi & Caraceni, 1979; Chalmers, Johnson, Keogh & Nanda, 1978). These findings suggested the existence in HD subjects of altered DA receptors involved in GH control.

Neuroendocrine studies have been performed in an animal model which mimics the neurochemical and behavioural alterations present in human HD, e.g. rats injected bilaterally into the head of the caudate nucleus with kainic acid (KA) (0.63 μg/μl), a rigid analog of glutamate. Rats underwent experiments 15 days later when they had recovered from initial body weight loss, and 2 days after having been prepared with chronic indwelling cannulae into the right atrium.

Administration of apomorphine (0.1 mg/kg i.v.) into these unanaesthetized freely moving rats failed to stimulate the release of GH, an effect present in sham-operated control rats. In the same rat model, the GH-releasing effect of muscimol (500 ng rat, intraventricularly) a potent GABA-mimetic drug, was in contrast strikingly potentiated. In another experiment, KA-lesioned rats were injected peripherally with muscimol (2 mg/kg i.p.) and the effect of this GABAergic stimulation on plasma PRL was evaluated. Unlike sham-operated controls, the KA-lesioned rats did not exhibit the initial plasma PRL rise induced by peripherally administered muscimol.

Discreteness of the lesion, which appeared histologically and biochemically confined to the caudate nucleus, makes unlikely the possibility that the altered GH and PRL responsiveness of KA-lesioned rats was due to diffusion of the toxic amino acid from the extrapyramidal system to the hypothalamus.

These findings, would suggest that: (1) the KA-lesioned rat is a suitable model for investigating the neuroendocrine abnormalities present in human HD; (2) there exist important functional relationships between the extrapyramidal system and CNS areas for the control of anterior pituitary function.

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H^2-mediated histamine induced release of thyrotrophin releasing hormone (TRH) from hypothalamic synaptosomes: a neuroendocrine role for histamine

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Histamine is released from neurones and non-neural cells in the hypothalamus and other brain regions and convincing evidence has been presented that this amine may act as a neurotransmitter as well as in immune and inflammatory processes (Schwartz, 1979). The highest levels of histamine in brain are found in the hypothalamic median eminence (Brownstein, Saravedra, Palkovitz & Axelrod, 1974) indicating a possible neuroendocrine function, and histamine has been shown to release TRH from mediobasal hypothalamic slices (Charli, Joseph-Bravo, Palacios & Kordon, 1978). In the present study the effect of histamine on the release of TRH from hypothalamic synaptosomes was examined. Synaptosomes were isolated from either the 'whole' hypothalamus (600 to 700 mg) or separately from the median eminence (80 to 100 mg), the dorsal periventricular hypothalamus (150 to 200 mg) and the remainder of the hypothalamus (370 to 400 mg) of sheep collected at a local abattoir from freshly killed and exsanguinated animals (Bennett, Edwardson, Holland, Jeffcoate & White, 1975). The synaptosomes, in 1 ml suspensions, were incubated for

30 min at half hypothalamus equivalent/ml at 37°C in Krebs bicarbonate medium, containing glucose (10 mM) and gassed with 95% O_2:5% CO_2. Test agents were added for the last 20 min in 10 µl volumes of medium. After incubation the synaptosomes were removed by centrifugation (5 × 1000 g) at room temperature and the supernatant snap-frozen and stored at −20°C prior to measurement of TRH by a sensitive and specific radioimmunoassay (Marcano de Cotte, De Menezes, Bennett & Edwardson, 1980).

Synaptosomes from the undissected hypothalamus released TRH (two- to three-fold; $P < 0.001$, $n = 6$) in response to histamine (10^{-10} M) comparable with the effect of elevated potassium (60 mM) ($P < 0.01$, $n = 9$) and dopamine (10^{-8} M) ($P < 0.001$, $n = 9$) (Bennett, Edwardson, Holland, Jeffcoate & White, 1975) but 10-fold higher or lower concentrations of neurotransmitter were without effect. Control median eminence synaptosomes released 1 to 2 ng/hypothalamic equivalent of TRH whereas synaptosomes from other hypothalamic regions released proportionately less peptide, presumably reflecting the differences in content of TRH-nerve terminals. Incubation with elevated potassium caused a similar enhanced release of TRH with synaptosomes from *all* hypothalamic regions (two-fold, $P < 0.01$, $n = 6$) and the release was prevented in the presence of a calcium chelating agent (EGTA 1 mM). In contrast, histamine (10^{-10} M) ($P < 0.001$, $n = 9$) and dopamine (10^{-8} M) ($P < 0.05$, $n = 6$) released TRH from median eminence synaptosomes *only*, suggesting the presence of receptors for these amines on the TRH-nerve endings in this region. Histamine and dopamine have been shown also to release TRH from rat hypothalamic synaptosomes in similar studies.

The histamine induced release of TRH from median eminence synaptosomes was simulated by a selective H_2-receptor agonist, dimaprit (10^{-8} M, $P < 0.001$, $n = 6$) and blocked by histamine H_2-receptor antagonists, metiamide (10^{-6} M, $P < 0.001$, $n = 5$) or cimetidine (10^{-6} M, $P < 0.001$, $n = 6$) but not an H_1-receptor antagonist, mepyramine (10^{-6} M). Likewise, the response to dopamine (10^{-8} M) was blocked by the dopamine receptor antagonist α-flupenthixol ($P < 0.001$, $n = 6$) but not the inactive isomer, β-flupenthixol. In total, these data indicate that TRH release from the sheep median eminence is mediated by histamine-H_2 and dopamine receptors. Interestingly, preliminary experiments have shown that the histamine induced TRH release was also blocked with α-flupenthixol, although metiamide had no effect on the dopamine response, which suggests that the histamine action on TRH release acts via dopamine release.

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High-affinity binding of [^{3}H]-imipramine to human platelets: differences between untreated depressed patients and healthy volunteers

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Human platelets have been widely used to investigate neurotransmitter-related abnormalites in different mental diseases (Stahl, 1977) and may be considered as a model for monoamine-containing neurones (Sneddon, 1973). Recently a high-affinity binding site for [^{3}H]-imipramine has been described in human platelets (Briley, Langer & Raisman, 1979) with apparently identical characteristics to those in rat brain (Raisman, Briley & Langer, 1979, 1980). We report here that platelets from untreated depressed patients have a lower density of [^{3}H]-imipramine binding sites than those from healthy volunteers.

Washed platelets were obtained from platelet-rich plasma as described by Briley *et al.* (1979), and platelet membranes prepared by hypotonic lysis and

centrifugation. [^{3}H]-Imipramine binding was carried out as described for the rat cortex (Raisman *et al.*, 1979).

The control group consisted of 27 healthy volunteers aged between 20 and 65 years (13 males and 14 females) and receiving no psychoactive medication. The maximal [^{3}H]-imipramine binding (B_{max}) in membranes prepared from the platelets of these volunteers was 559 ± 49 fmol/mg protein and the affinity constant (K_d) 1.9 ± 0.3 nM. The untreated depressed patients (20 to 65 years old) (4 males and 13 females) were suffering from either mono or bipolar endogenous depression or reactive depression, of sufficient severity to require hospitalisation (mean Hamilton rating 48 ± 2; NIMH 24 item scale). This group had a significantly lower maximal binding (B_{max}: 311 ± 29 fmol/mg protein; $P < 0.001$) than the control group. The mean K_d value 3.0 ± 0.5 nM did not vary significantly from that of the control ($P > 0.05$).

Treatment with different tricyclic antidepressant drugs for 9 days changed neither the affinity constant nor the maximal [^{3}H]-imipramine binding in the platelets of depressed patients (B_{max}: 361 ± 63 fmol/mg; K_d = 4.9 ± 0.9 nM, n = 12; $P > 0.25$ compared with values before treatment) although there was a significant improvement in their depression as indicated by a decrease in the mean Hamilton score (40.1 ± 2.1). In addition [^{3}H]-imipramine binding was studied in platelets of 11 of these patients when they were considered to be recovered and were about to be discharged from the hospital (under continuing medication). The [^{3}H]-imipramine binding parameters of this group did not vary significantly (B_{max}: 355 ± 38; $P > 0.25$; and the K_d 5.0 ± 1.1 nM, $P > 0.25$) from the untreated values although the Hamilton scores were now normal, 29 ± 0.9.

It is possible that the lower density of [^{3}H]-imipramine binding sites in platelets from depressed patients under treatment are related to the continued presence of tricyclic antidepressant drugs that prevents the return of the B_{max} values to normal higher levels. Indeed chronic treatment with desipramine has been shown to decrease the maximal binding of [^{3}H]-imipramine in rat cortex (Raisman, Briley & Langer, 1980). Another possibility is that the lower density of [^{3}H]-imipramine binding sites in platelets of both treated and untreated depressed patients may indicate a susceptibility to depression rather than a reflection of mood and thus the improvement in the Hamilton score induced by antidepressant drugs is not followed by changes in [^{3}H]-imipramine binding.

It is tempting to speculate that changes in [^{3}H]-imipramine binding sites in human platelets reflects similar changes in human brain. An indication that changes in [^{3}H]-imipramine binding in platelets may indeed reflect those occurring in the central nervous system is that chronic treatment with imipramine in cats produces a decrease of the binding of [^{3}H]-imipramine in platelets in parallel with changes in the binding in the hypothalamus (Arbilla, Briley, Cathala, Langer, Pornin & Raisman, 1980). Thus [^{3}H]-imipramine binding in human platelets appears to be a parameter which may be related to the pathogenesis of depression and therefore represents a potential tool in the study of affective disorders.

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Parallel changes in [^{3}H]-imipramine binding sites in cat brain and platelets following chronic treatment with imipramine

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High-affinity binding sites for [^{3}H]-imipramine ([^{3}H]-IMI) have been demonstrated in rat brain (Raisman, Briley & Langer, 1979a, b, 1980) and human platelets (Briley, Raisman & Langer, 1979). [^{3}H]-IMI binding in human platelets has been used to investigate the possible relationship between this binding site and the pathogenesis of affective disorders (Briley, Langer, Raisman, Sechter & Zarifian, 1980). To explore the validity of extrapolating from platelets to the brain we investigated the respective changes in [^{3}H]-IMI binding in brain and platelets of the same animals following chronic treatment with imipramine.

Cats of 2.5 kg received 7.5 mg/kg imipramine (IMI) twice daily for 21 days, and were killed after 1 day of withdrawal from the drug. [^{3}H]-IMI binding to membranes prepared from cat brain and platelets was measured according to the method of Raisman *et al.* (1979b) and Briley *et al.* (1979). [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) binding was measured by a modification of the method of Alexander, David & Lefkowitz (1975) and the 5-HT receptor component of [^{3}H]-Spiperone ([^{3}H]-SP) binding by the method of Perutka & Snyder (1979). Specific binding was defined as the difference between the binding in the presence and absence of 100 μM desipramine, 20 μM propranolol and 10 μM 5-HT for [^{3}H]-IMI, [^{3}H]-DHA and [^{3}H]-SP binding, respectively.

As already demonstrated in the rat brain (Banerjee, Kung, Riggi & Chanda, 1978; Sarai, Frazer, Bunswick & Mendels, 1977) the B_{max} for [^{3}H]-DHA binding in the cerebral cortex of cats was significantly reduced after chronic treatment with IMI (control: B_{max}, 82.96 ± 1.48 fmol/mg protein, $n = 8$; IMI-treated: 67.43 ± 2.91 fmol/mg protein ($n = 10$, $P < 0.001$), with no change in the K_d values (control: K_d, 2.26 ± 0.10 nM, $n = 8$; IMI-treated: 2.52 ± 0.34, $n = 10$, $P > 0.25$). The maximum binding and the K_d of [^{3}H]-spiperone to 5-HT receptors in the cortex were, however, unaltered after chronic IMI treatment (B_{max}: controls, 43.33 ± 6.25, $n = 6$, fmol/mg protein; IMI-treated: 41.50 ± 7.14, $n = 6$, fmol/mg protein, $P > 0.25$; K_d: control, 0.79 ± 0.19 nM, $n = 6$; IMI-treated 0.50 ± 0.07 nM, $n = 6$, $P > 0.25$). In the same animals the hypothalamus showed a high density of [^{3}H]-IMI binding sites (B_{max}: 374 ± 56 fmol/mg protein, $n = 3$), the affinity constant (K_d: 6.1 ± 0.9, $n = 3$), was similar to that already described in the rat cortex (Raisman *et al.*, 1980). The hypothalamus of cats chronically treated with IMI had significantly reduced [^{3}H]-IMI binding (B_{max}: 115 ± 12, $n = 3$, $P < 0.05$) with no change in the affinity constant (K_d: 5 ± 1.2 nM, $n = 3$). Platelets from the same cats showed a reduction following chronic treatment with IMI which very closely paralleled that in the hypothalamus (B_{max}: control, 783 ± 76 fmol/mg protein, $n = 4$, IMI-treated: 240 ± 61 fmol/mg protein, $n = 4$, $P < 0.005$; K_d: control, 8.8 ± 1.1 nM, n = 4; IMI-treated 10.0 ± 1.5, $n = 4$, $P > 0.25$).

Thus the chronic administration of IMI to cats produces a similar decrease in the β-adrenoceptor binding to that seen in rats (Banerjee *et al.*, 1978; Sarai *et al.*, 1977). The decrease in [^{3}H]-imipramine binding in the hypothalamus confirms the decrease in B_{max} in rat cortex after chronic treatment with desipramine that was reported recently (Raisman *et al.*, 1980). The parallel decreases in [^{3}H]-IMI binding found in the hypothalamus and in platelets of cats chronically treated with IMI suggests that [^{3}H]-IMI binding sites in platelets undergo similar changes to those in the brain. [^{3}H]-IMI binding in platelets would therefore appear to be a valid tool to investigate changes of this specific high-affinity binding site in the brain. Changes in [^{3}H]-IMI binding in human platelets may therefore reflect similar changes for the same site in the human brain. Thus [^{3}H]-IMI binding in platelets would appear to be a valid potential tool to investigate the role of [^{3}H]-IMI binding in the pathogenesis of the depression.

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Increase of opioid activity in the rat pituitary following the administration of sulpiride

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The present study was undertaken to ascertain whether the administration of a neuroleptic drug may influence the opioid peptidergic system of the rat pituitary.

Rats of the Sprague–Dawley strain weighing 220 to 250 g were used and randomized into groups of 10 animals each. The first, second and third groups of rats were acutely treated, respectively, with: (a) the substituted benzamide neuroleptic sulpiride (HCl, Serpero Pharm.) 5 mg/kg i.p.; (b) the dopaminergic agent 2 Br-α-ergocriptine (methan sulphonate, Sandoz) 5 mg/kg i.p.; (c) 2 Br-α-ergocriptine 5 mg/kg i.p. 1 h prior to sulpiride 5 mg/kg i.p. The fourth group of rats was treated with sulpiride, 5 mg/kg i.p. for 21 days. Control animals received saline only. The experiments were repeated six times on rats of the same strain under the same experimental conditions. The rats were killed 2 h after the last treatment and their pituitary glands quickly removed and pooled according to different groups of treatment. Extracts with opioid activity were obtained according to the method of Teschemacher, Opheim, Cox & Goldstein (1975) and tested on a morphine sensitive model: the longitudinal muscle-myenteric plexus preparation from guinea-pig ileum (Ferri, Reina & Santagostino, 1977). The opioid activity was expressed as a concentration of morphine (HCl, C. Erba) which produced an equivalent inhibiting effect on the bioassay (Santagostino, Cocchi, Giagnoni, Gori, Muller & Ferri, 1978).

The opioid activity shown by pituitary extracts from rats treated with sulpiride was equivalent to a morphine concentration of 12.97 nmol/g pituitary (wet wt.), significantly higher ($P < 0.01$) than that calculated for controls (4.05 nmol/g. Also a 21-day treatment with the neuroleptic resulted in an increased pituitary opioid activity (9.9 nmol/g). Gel filtration analysis (Sephadex G-50 fine column) of the pituitary extracts showed that the increase always occurred in the intermediate/posterior lobe of the gland.

2 Br-α-ergocriptine did not significantly change, *per se*, the opioid content of pituitary but completely prevented the sulpiride-induced effect (4.45 nmol/g).

Up to now little is known about the physiological mechanisms which regulate endorphin stores in the pituitary. However, the results of the present investigations support the view that there is dopaminergic control of endorphin stores in the intermediate/posterior lobe of pituitary. Work is now in progress in order to ascertain whether the neuroleptic effect is due to a direct action on dopamine receptors of pituitary or is the result of an impact on higher areas, such as the hypothalamus.

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Tolerance and cross-tolerance between D-Ala^2 methionine-enkephalinamide and morphine on their action on motor behaviour and on the metabolism of dopamine and serotonin in some rat brain areas

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Opioid peptides mimic both the pharmacological and biochemical effects of morphine (Calderini, Consolazione, Garattini & Algeri, 1978). When [D-Ala^2] methionine-enkephalinamide (DALA 50 μg/rat i.c.v.) or morphine (15 mg/kg i.p.) are administered to rats, they had a catatonic effect and enhanced dopamine (DA) metabolism as indicated by increases in the concentrations of the metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) in striatum, s. nigra, and limbic area, the three brain regions considered in the present study.

The development of tolerance with repeated use of narcotics and the demonstration of cross-tolerance between their actions is a characteristic common to these drugs. After a dose of morphine, rats chronically treated with this drug showed tolerance to the appearance of catatonia. Tolerance was also present with regard to the biochemical effect, morphine failing in these animals to enhance DA and 5-HT metabolism in any of the three areas, with the exception of DOPAC in striatum.

When DALA was given to morphine dependent rats, cross tolerance was not present either for the effect on motor behaviour or for the effect on DA metabolism with the exception of DOPAC in limbic area and s. nigra where this metabolite was not affected.

When DALA was given to rats repeatedly treated with this peptide [50 μg/rat (i.c.v.) × 4 times/24 h × 5 days] a tolerance to the cataleptic effect was noticed. However no tolerance was evident for its biochemical effect. Similarly the administration of an acute dose of morphine to DALA chronically treated rats gave cross tolerance to the cataleptic effect but not cross tolerance to the enhancement in DA metabolites.

These results suggest that although enkephalins have in common many pharmacological effects with morphine they differ from this narcotic in their potency to induce tolerance.

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Investigations into baclofen analgesia: effect of naloxone, bicuculline, atropine and ergotamine

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(±)-Baclofen has various pharmacological effects in rodents. After intraperitoneal doses of 2 to 4 mg/kg it has a potent antinociceptive and diuretic action, while at higher doses (>5 mg/kg) it is a strong centrally-acting muscle relaxant. The muscle relaxing (Bein, 1972) and antinociceptive (Cutting & Jordan, 1975) effects of baclofen are well known; however, we are so far unaware of reports concerning its diuretic action, which is, nevertheless, very significant ($P < 0.01$) on male Wistar rats.

(±)-Baclofen analgesia, detected by the various common analgesic tests, is not antagonized by naloxone (Levy & Proudfit, 1977) except in mice in the jump-latency hot-plate test (Frederickson, Burgis & Edwards, 1977). Since these findings were fully reproduced by us, we have postulated that (±)-baclofen may induce analgesia by stimulating a new type of GABA receptor, insensitive to bicuculline and muscimol, recently described by Bowery, Doble, Hill, Hudson, Shaw & Turnbull (1979).

Experiments were carried out on 350 white Swiss–Webster mice, 80 Wistar male albino rats and 25 guinea-pigs.

Our hypothesis is supported by the following data: (1) Analgesia induced in mice by (±)-baclofen HCl (2.5–4 mg/kg s.c.) and tested with writhing and hot-plate licking tests, was not antagonized by naloxone (10 to 100 mg/kg s.c.) nor by bicuculline HCl (2 mg/kg i.p.), nor by atropine sulphate (1 mg/kg s.c.) which, however, antagonized oxotremorine analgesia.

(2) (−)-Baclofen (5 mg/kg i.p.) induced a very high degree of analgesia (>cut-off time) in mice, whereas (+)-baclofen did not.

(3) Ergotamine tartrate (0.1 to 0.5 mg/kg s.c.) greatly ($P < 0.01$) increased (±)-baclofen analgesia in mice (from +56 to +65% depending on the analgesic test), while it did not increase opioid analgesia.

(4) The increase in GABA content in the CNS pro-

duced by treating both mice and rats with aminooxyacetic acid (AOAA) 25 mg/kg i.p., 1 and 6 h, respectively, before the analgesic test, induces an analgesia (+43% in mice and +29% in rats) which was significantly increased ($P < 0.05$) in mice (+91%) and in rats (+67%) by bicuculline. HCl (2 mg kg^{-1} i.p.) injected 75 min after AOAA. Thus, the antagonism by bicuculline of the muscimol-sensitive GABA receptors, which are known to antagonize morphine analgesia (Mantegazza, Tammiso, Vicentini, Zambotti & Zonta, 1979), may leave endogenous elevated GABA free to stimulate the baclofen-sensitive receptors alone. This results in analgesia.

From these data it appears likely that the analgesic and, possibly, diuretic effects of (−)-baclofen might depend on its selective stimulation of a new type of GABA-receptor.

Experiments are now in progress to establish whether (+)-baclofen—by inhibiting carboxypeptidase A activity—might contribute to the analgesic effect of (−)-baclofen. This possibility is supported by the structural analogies between (+)-baclofen and (+)-phenylalanine, a carboxypeptidase inhibitor, and by the fact that naloxone (10 mg/kg i.p.) in the hot-plate jumping test can partially antagonize (±)-baclofen analgesia (5 mg/kg i.p.); moreover (+)-baclofen (3×10^{-5} g/ml), like (+)-phenylalanine, potentiates the inhibition evoked by metenkephalin of longitudinal muscle contractions of field-stimulated guinea-pig ileum.

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Naloxone excitation of spinal nociceptive units fails to occur in hypophysectomized cats

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(introduced by J.W. COMMISSIONG)

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Naloxone, administered intravenously in doses of 0.1 mg/kg, causes an increase in the spontaneous rate of discharge of single dorsal horn units in lumbar spinal segments 5 to 7 in the spinal cat, an effect observed with units responding to noxious cutaneous stimuli but not with units responding only to innocuous cutaneous stimuli (Henry, 1979). The fact that this response is observed even after ipsilateral dorsal root section (Henry, 1979) and that it occurs during daytime experiments but not during night-time experiments (Henry, 1980) suggests that naloxone may be preventing the action of a circulating factor which is inhibitory specifically on nociceptive units, and that this circulating factor may originate from the pituitary gland. Experiments were therefore done on hypophysectomized cats to determine whether the effects of naloxone were dependent upon an intact pituitary gland. The pituitary glands were removed from six cats using suction via a trans-buccal approach. After survival times of 7 to 26 days, blood samples were taken from each cat and measured for cortisol by the method of Murphy (1967).

After blood samples were taken, each animal was prepared as before for the daytime experiments. Sixty mg/kg of alpha chloralose were given i.v. after induction with halothane/oxygen. Spinal segments L_5–L_7 were exposed for recording and covered with warm mineral oil. The cords were transected at L_1. Extracellular unit spikes were recorded with multibarrelled micropipettes.

Only units excited by noxious cutaneous stimuli were selected for study because of the selective effects of naloxone on these units found in the earlier study (Henry, 1979). Five cats were found to be without detectable basal levels of cortisol. In each cat, naloxone administered in doses up to 0.5 mg/kg failed to alter the on-going rate of discharge or the response to noxious cutaneous stimulation. On the other hand, in the experiment on the sixth hypophysectomized cat, in which basal levels of 10 ng/ml of cortisol were detected, naloxone (0.1 mg/kg) increased both the on-going rate of discharge and the response to auto-

matically controlled periodic applications of noxious radiant heat to the cutaneous receptive field. Similar excitatory effects of naloxone were observed with two unoperated cats done during the same sequence of experiments: basal levels of cortisol in these cats were 12 and 20 ng/ml.

These results demonstrate that an intact pituitary gland is necessary for the expression of the excitatory effects of naloxone on spinal nociceptive units in the spinal cat. It is therefore proposed, that naloxone causes excitation by its antagonism of an endogenous opioid agent, that this agent reaches the spinal cord from the circulation and that it is present in the circulation only when the pituitary gland is operating. Whether the origin of this agent is the pituitary gland itself, or is another endocrine gland regulated by pituitary output, remains to be determined.

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Naloxone reversal of morphine inhibition on electrically-evoked ACh release from guinea-pig thalamus *in vitro*

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The effect of morphine (Mo) on acetylcholine (ACh) release from superfused slices of guinea-pig brain was examined according to a method recently described (Beani, Bianchi, Giacomelli & Tamberi, 1978). Mo 1×10^{-6} to 1×10^{-5} M did not change ACh release from unstimulated slices of parietal cortex, caudate nucleus and thalamus. At higher concentrations (1×10^{-5} M to 3×10^{-6} M) and stimulation rate (5 Hz of pulse duration 5 ms, intensity 30 mA/cm^2, alternate polarity) Mo produced a naloxone-sensitive inhibition of evoked ACh release in the parietal cortex, caudate nucleus and thalamus, whereas at low concentration (3×10^{-6} M) and stimulation rate (1 Hz) it significantly enhanced ACh extra release in thalamus slices. This facilitatory effect was unaffected by naloxone (1×10^{-5} M) which, on the contrary, changed to facilitation the inhibition caused by the high doses of Mo.

When Mo (1×10^{-5} M) was tested in the presence of increasing naloxone amounts (Figure 1), ACh release inhibition was reduced at first and then progressively reversed, maximum enhancement of extra release reaching +65%.

Both effects, i.e. inhibition by Mo and facilitation by Mo plus naloxone, were counteracted by doubling Ca^{2+} concentration in the superfusing medium. These findings show that Mo was able both to inhibit and to enhance evoked ACh release in the thalamus. Since only the inhibitory effect was prevented by naloxone, two different receptor sites may be postulated. The guinea-pig thalamus differs from the rat striatum,

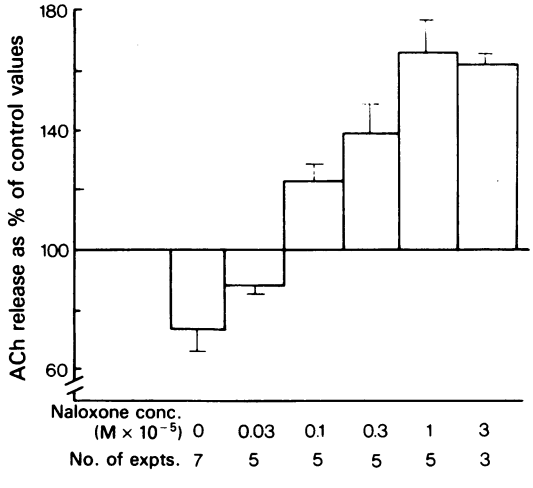

Figure 1 Effect of Morphine, 1×10^{-5} M, alone or in the presence of different concentrations of Naloxone, on ACh outflow from slices of guinea-pig thalamus, electrically stimulated at 2 Hz. The values are given as percentages of control (pre-drug) release. Vertical bars: s.e. mean. All the differences from the control values were statistically significant (Student's *t*-test for paired data, $P < 0.05$ or less).

where Mo facilitation on ACh release has been reported to be naloxone-sensitive (Vizi, 1979).

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Presynaptic actions of GABA on rat isolated sympathetic ganglia demonstrated in the presence of 4-aminopyridine

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Presynaptic receptors for GABA exist in rat (Brown & Marsh, 1978; Brown & Higgins, 1979) and frog (Kato & Kuba, 1980) sympathetic ganglia. We have investigated the nature of these receptors under conditions of enhanced presynaptic excitability induced by 4-aminopyridine (4-AP; Lemeignan, 1972).

Intracellular recordings were made from 35 neurones in rat isolated superior cervical ganglia maintained in flowing Krebs' solution at 25° or 30°C. Drugs were bath-applied. 4-AP (0.1 to 1 mmol/l) usually induced spontaneous excitatory postsynaptic

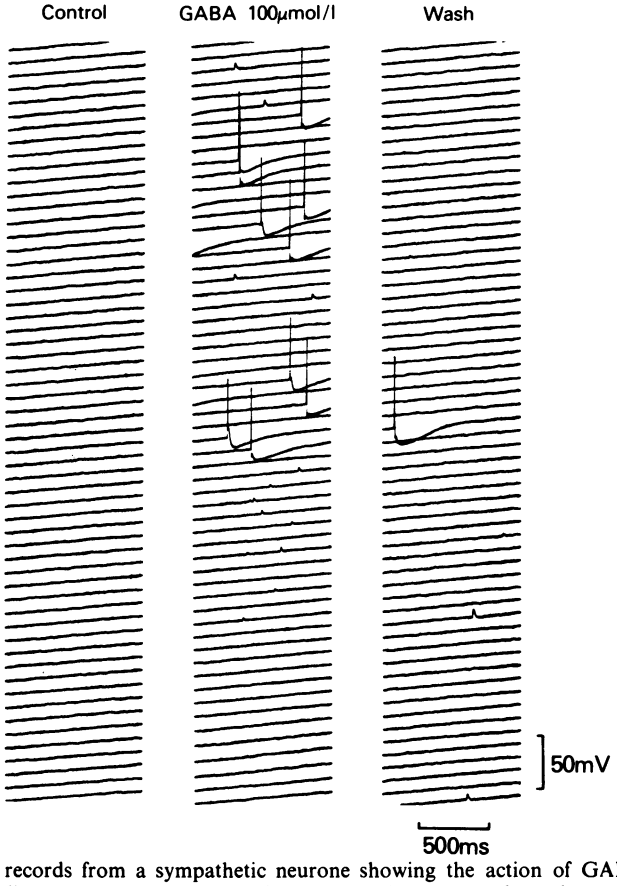

Figure 1 Intracellular records from a sympathetic neurone showing the action of GABA (100 μmol/l) in the presence of 4-aminopyridine (100 μmol/l). The records are successive sweeps of membrane potential (d.c. coupled); 4-aminopyridine was added 10 min before the recording commenced. The three traces (which read from the bottom upwards) are continuous in time from left to right, and show the membrane potential before, during and after a 1-min application of GABA.

potentials (EPSPs) and/or action potentials, which were abolished by hexamethonium (1 mmol/l). Direct actions of 4-AP on soma membrane potential and resistance were slight. In the presence of 4-AP, GABA (100 μmol/l) evoked a small membrane depolarization on which bursts of action potentials and EPSPs were superimposed (Figure 1). These action potentials and EPSPs were antagonized by either hexamethonium (1 mmol/l), tetrodotoxin (350 nmol/l) or bicuculline methochloride (30 μmol/l). The ability of GABA to induce action potentials or EPSPs in 4-AP solution was mimicked by 3-aminopropanesulphonic acid (30 μmol/l) and muscimol (50 μmol/l) but not by (±)-baclofen (200 μmol/l), glutamate, glycine or taurine (all 1 mmol/l).

We conclude that 4-AP probably induces action potentials in ganglionic presynaptic structures leading to release of acetylcholine. During this condition, a further GABA-induced axonal depolarization (Brown & Marsh, 1978) most likely leads to a burst of action potentials, which were recorded postsynaptically as EPSPs or spikes. From experiments with GABA-analogues and bicuculline, it appears that the GABA-receptors responsible for this *presynaptic* action are similar to those present on the postsynaptic membrane of mammalian peripheral and central neurones.

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Dopamine: spontaneous and drug-evoked release from presynaptic nerve endings of the tuberoinfundibular system

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The tuberoinfundibular dopaminergic (TIDA) nerves play an important role in the regulation of prolactin release. In the present experiments we have examined the characteristics of dopamine (DA) release from the terminals of TIDA neurones in a synaptosomal preparation of rat median eminence (ME) by a superfusion system (Raiteri, Angelini & Levi, 1974). The crude synaptosomal preparation was prelabeled with [^{3}H]-DA and spontaneous or drug-induced release was measured through collection of 1-min fractions.

Superfusion of ME synaptosomes with increasing concentrations of KCl (10 to 30 mM) produced DA

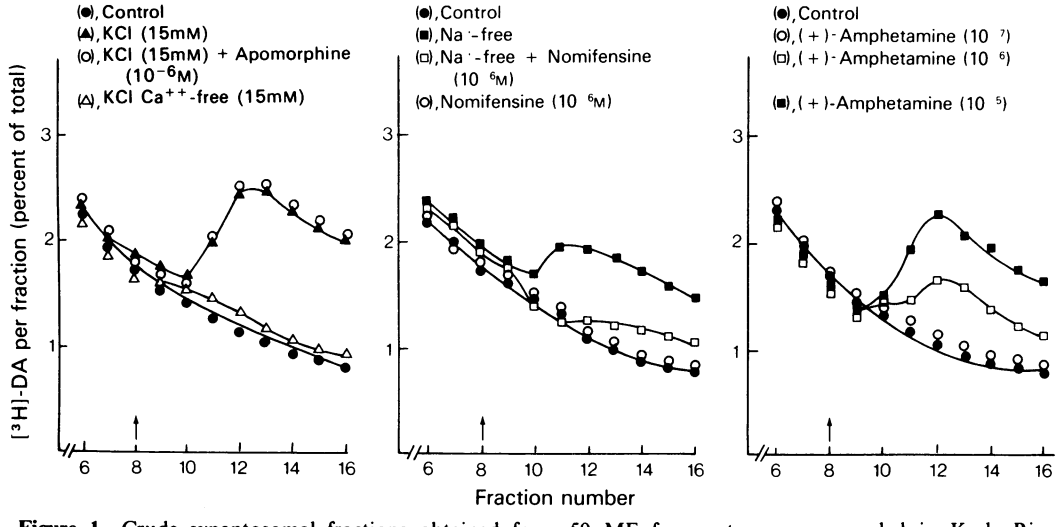

Figure 1 Crude synaptosomal fractions obtained from 50 ME fragments were suspended in Krebs-Ringer medium and prelabeled with 0.1 μM [^{3}H]-DA for 10 min at 37°C. Aliquots of the suspension were placed on 0.80 μM Millipore filters laying at the bottom of parallel superfusion chambers. Fractions were collected every minute. [^{3}H]-DA present in the fractions and remaining in the filter was isolated by ion exchange chromatography utilizing Biorex 70 columns. Where indicated by the arrow, the superfusion medium was replaced either with identical medium (controls) or with medium containing the compounds under study.

release in a dose-dependent manner (Figure 1). This K^+-evoked release is Ca^{2+}-dependent since the absence of this ion from the extracellular medium strongly reduced the K^+-induced DA release.

Removal of Na^+ from the superfusion medium caused a release of [^{3}H]-DA, which was completely antagonized by the DA carrier blocker Nomifensine, suggesting a carrier-mediated exit of cytoplasmic DA. Nomifensine (1–10 μM) by itself was devoid of any effect on DA spontaneous release from ME synaptosomes.

(+)-Amphetamine (1 to 10 μM) stimulated the release of 'newly taken-up' DA in ME in a dose-related way. Interestingly under identical experimental conditions DA release was consistently increased by a lower dose of (+)-amphetamine (0.1 μM) in striatal synaptosomes. This lower sensitivity of ME synaptosomes to (+)-amphetamine gives further support to the evidence that the TIDA neurones present remarkable differences from the nigrostriatal system (Annunziato, 1979).

The release of [^{3}H]-DA elicited by KCl (10 to 20 mM) was unaffected by the DA agonist apomorphine (1 μM). This result is not compatible with the existence of presynaptic autoreceptors controlling DA release from TIDA neurones.

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Effects of anorectic drugs on the hyperphagic response induced by insulin or 2-deoxy-d-glucose glucoprivation

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The anorectic activity of drugs is usually evaluated by experimental models in which an increased food intake is obtained by fasting the rats prior to the experiment (rats trained to eat 4 h a day). Under these experimental conditions two different classes of drugs have been shown to be active in reducing food consumption, i.e. dopamine-mimetics, such as amphetamine, mazindol and ergot derivatives and serotonin (5-HT) agonists such as fenfluramine and quipazine.

Hypoglycaemic doses of insulin or administration of 2-deoxy-d-glucose (2-DG), a glucose analogue that induces intracellular glucopenia, activate feeding behaviour in rats as a result of cerebral glucoprivation.

In the present contribution we report on the ability of dopamine and serotonin mimetic anorectics to antagonize the hyperphagic response to insulin- or 2-DG-induced glucoprivation. Amphetamine (2 mg/kg i.p.), mazindol (2.5 mg/kg i.p.), lisuride (0.05 mg/kg i.p.), bromocriptine (3 mg/kg i.p.), fenfluramine (3 mg/kg i.p.), *p*-chloromethamphetamine (2 mg/kg i.p.) and quipazine (5 mg/kg i.p.) all antagonized the hyperphagic response to 2-DG (750 mg/kg i.p.). On the other hand the hyperphagia induced by insulin (6 i.u./kg i.p.) was antagonized only by fenfluramine, *p*-chloromethamphetamine and quipazine (doses as before) but not by amphetamine, lisuride and bromocriptine (doses as before). Thus, both dopamine and 5-HT agonist drugs, at dose levels active in reducing food intake in rats trained to eat 4 h a day, can counteract the hyperphagic response to 2-DG, whereas only 5-HT agonists can antagonize insulin-induced hyperphagia, dopamine agonists being ineffective.

These results, demonstrating the existence of a different effectiveness of dopamine and serotonin mimetic anorectics in the two experimental models of hyperphagia investigated, suggest that different neuronal circuitries underly the hyperphagic responses to insulin and 2-DG glucoprivation.

The potential importance of these findings for the clinical pharmacology of disturbances of feeding behaviour deserves consideration.

Effects of crinia-angiotensin II on drinking behaviour of rats and pigeons

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Crinia-angiotensin II (CAII) is a naturally-occurring angiotensin which differs from the other known angiotensins II in that it has the tripeptide Ala-Pro-Gly attached to the N-terminal Asp-residue and in that an Ile-residue replaces the usual Val_3-residue (Erspamer, Melchiorri, Nakajima, Yasuhara & Endean, 1979).

Data of Fitzsimons (1971) and Evered & Fitzsimons (1976) demonstrate that the receptor for angiotensin II induced drinking is highly specific, since shortening or minor alterations of angiotensin II structure produce, both in rats and in pigeons, a tremendous reduction of its dipsogenic effect. We studied in Wistar rats and pigeons (*Columba livia*) the effect of this new angiotensin on water intake in comparison to that of Ile_5-angiotensin II (AII). The substances, dissolved in 1 µl of isotonic saline, were injected into the brain of conscious animals through indwelling cannulae stereotaxically implanted into the lateral (rats) or third (pigeons) ventricle.

After a short latency (30 to 300 s) CAII caused rats and pigeons to drink. Drinking was absolutely normal and was not accompanied by other behavioural alterations. In rats the effect was poor, but pigeons drank large amounts of water in a dose-dependent fashion (Figure 1).

We can conclude that in the rat the high specificity of the drinking receptor for AII is confirmed, while in pigeons the molecular requirements for the dipsogenic activity of CAII are similar to those for the dipsogenic effect of AII. Thus, although similar, the AII receptors for drinking in the pigeon are not identical to those of the rat.

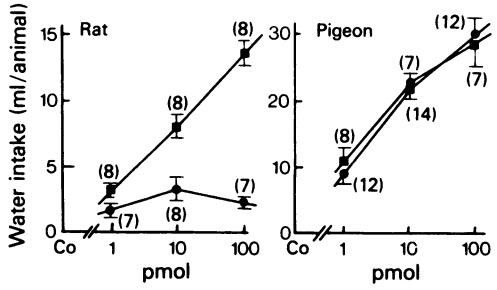

Figure 1 Mean water intakes during 15 min following intracranial injections of various doses (picomoles per animal) of (■) AII or (●) CAII to rats and pigeons. Controls (Co) received 1 µl of isotonic saline. The number of observations is given in parentheses. Vertical lines are s.e. mean.

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