POSTER COMMUNICATIONS

Prostaglandins and SRS-A in anaphylactic contractions of the guinea-pig isolated stomach

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We have previously shown that anaphylaxis in the guinea-pig *in vivo* produces complex changes in gastric motility mediated in part by histamine, with contributions from SRS-A and prostaglandins (Chadwick, Francis & Goadby, 1980). Further investigations of the effect of anaphylaxis *in vitro* have examined the role of different mediators involved in these changes in motility.

Changes in gastric motility were measured in the isolated stomach preparation described by Spedding (1977). Drugs were injected into the stomach vasculature via a cannula positioned in the coeliac artery at the junction of the hepatic and gastrosplenic arteries. Stimulation of the attached vagus nerves was used to obtain an indication of the maximal response of the stomach (designated 100%) and provided a standard with which other responses could be compared. Acetylcholine (25–100 ng), histamine (25–100 ng), SRS-A (50–200 U) and PGE_2 (10–100 ng) produced dose-related displacements, the maxima being close to that produced by vagal stimulation. Administration of antigen (1 mg, Egg albumin) to stomachs from actively sensitized animals produced a displacement of 76 ± 7% (Mean ± s.e. mean) that produced by vagal stimulation.

Mepyramine (100 ng/ml) caused a significant reduction (62 ± 3%; $P < 0.001$) in the response to a dose of histamine (100 ng), which in the absence of antagonist gave a contraction greater than 75% of the maximum response to vagal stimulation. This concentration of mepyramine had no effect on equivalent responses of other agonists or on anaphylactic contractions. Similarly, atropine (100 ng/ml) significantly reduced the responses to acetylcholine and vagal stimulation without affecting the responses to other agonists or to anaphylaxis. However, the presence of FPL 55712 (100 ng/ml) caused a significant reduction (71 ± 9%) in the responses to SRS-A (100 U) and a similar reduction (71 ± 7%; $P < 0.05$) in the anaphylactic contractions, whilst leaving the responses to equivalent doses of the other agonists unaffected. Higher concentrations (250–500 ng/ml) of all three antagonists caused reductions in the responses to all four control agonists and in the responses to anaphylaxis.

The anaphylactic contraction of the stomach was virtually abolished by the presence of Indomethacin (1 μg/ml) although at this concentration there was no effect on the responses to the control agonists.

Thus, although this isolated stomach preparation proved to be susceptible to non-specific depression by high concentrations of antagonist drugs, the results do support previous findings (Chadwick, Francis & Goadby, 1980) that prostaglandins and SRS-A probably make a considerable contribution to anaphylactic contractions of the guinea-pig stomach.

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Prostacyclin-induced bradycardia is dependent on the basal heart rate and is antagonized by sodium cromoglycate

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Prostacyclin, although hypotensive, produces either tachycardia (Armstrong, Lattimer, Moncada & Vane, 1978; Warrington & O'Grady, 1980; Dusting, Chapple, Hughes, Moncada & Vane, 1978) or bradycardia (Chapple, Dusting, Hughes & Vane, 1980; Hintze, Martin, Messina & Kaley, 1979). The bradycardia is a vagal reflex (Chapple *et al.*, 1980; Hintze *et al.*, 1979), the afferent arc of which might be mediated by stimulation of pulmonary 'C'-fibres (Armstrong & Miller, 1980). Dixon, Jackson & Richards (1980) found that sodium cromoglycate (SCG) attenuated capsaicin-induced excitation of 'C'-fibre sensory nerve endings in the canine lung. We have now shown that in anaesthetized beagles the effect of prostacyclin on heart rate is dependent upon the basal state and that the reflex bradycardia is attenuated by SCG.

Beagles of either sex were anaesthetized initially with thiopentone (20–25 mg/kg, i.v.) and maintained with chloralose (50 mg/kg, i.v.) supplemented as required. The animals were ventilated mechanically and blood pressure and heart rate (ECG standard lead II) were recorded.

Prostacyclin given intravenously by injection (0.1–1.0 μg/kg) or infusion (0.05–1.0 μg^{-1} min^{-1}) caused tachycardia in dogs with a low basal heart rate (< 110 beats/min). The heart rate in 3 dogs was then increased to 150–200 beats/min with isoprenaline (100–200 ng kg^{-1} min^{1}) and prostacyclin produced a substantial bradycardia. Similar effects were observed in propranolol-treated dogs when the heart rate was increased during hypotension induced by sodium nitroprusside (10–20 μg kg^{-1} min^{-1}). Furthermore, dogs with a spontaneously high basal heart rate (150–180 beats/min) exhibited bradycardia to prostacyclin, while those with an intermediate rate (120–130 beats/min) showed a biphasic response.

Prostacyclin-induced tachycardia could be reduced or reversed by β-adrenoceptor blockade with propanolol (2 mg/kg, i.v.). Male beagles were 2.5–5 times more sensitive than females to the hypotensive and heart rate effects of prostacyclin.

SCG (100 μg/kg) given intravenously induced a short lasting bradycardia and hypotension (–3 min). 20 min after dosing the response to prostacyclin was changed from a bradycardia to tachycardia or in those dogs which show tachycardia before treatment this was significantly increased. The change in the response to prostacyclin by SCG was slow to develop. When prostacyclin was given within 20 min after SCG an enhancement of the bradycardia or a biphasic effect consisting a small tachycardia followed by bradycardia was observed. Accompanying these changes, there was a small reduction in the hypotensive response to prostacyclin.

Hypotension and tachycardia elicited by sodium nitroprusside (10–20 μg kg^{-1} min^{-1}) was not altered by SCG, neither was the increased heart rate induced by prostacyclin after vagotomy.

Prostacyclin activates two reflexes with opposing actions on heart rate. One, possibly a hypotension-induced baroreceptor reflex, is mediated by the sympathetic nervous system for it is blocked by propranolol. The second is a vagal reflex (Chapple *et al.*, 1980; Hintze *et al.*, 1979), presumably mediated by stimulation by prostacyclin of afferent 'C'-fibres (Armstrong & Miller, 1980) and is attenuated by SCG.

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The effect of 5-hydroxytryptamine on gastric acid secretion by the rat isolated stomach

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5-Hydroxytryptamine (5-HT) has been widely reported to inhibit basal, pentagastrin-, histamine- and cholinergically-stimulated gastric acid secretion *in vivo* but there is a lack of general agreement regarding its mode action (Jaffe, 1979). It may act directly on the stomach or indirectly via modification of central nervous activity or mucosal blood flow. In an attempt to clarify the situation we have investigated the effect of 5-HT on acid secretion by a rat isolated stomach preparation.

Isolated stomachs were set up as described previously (Canfield, Curwain & Price, 1978) and randomly allocated to either:

a) the test group which were pre-incubated with 5-HT, methysergide (MS) or 5-HT and MS for 1 h before addition of secretagogues

b) the control group which received only secretagogues.

Secretory responses are expressed as the mean secretory ratio (R ± s.e. mean) calculated by dividing the response by the preceding spontaneous secretion in each stomach. Differences in R were considered significant when $P < 0.05$ by the Mann-Witney U-test.

5-HT at 10^{-5} M (Table 1, A) significantly inhibited secretory responses to pentagastrin, isoprenaline and histamine but not to dibutyryl cyclic AMP (db cAMP) ICI 63197 (a phosphodiesterase inhibitor) or bethanechol. Increasing 5-HT to 5×10^{-5} M (Table 1B) still did not alter the responses to bethanechol or dbcAMP but did cause some further inhibition of isoprenaline and histamine. This inhibition was prevented by the selective 5-HT antagonist methysergide (Table 1, B) at 2.5×10^{-5} M. Methysergide alone did not alter the resonse to either pentagastrin or isoprenaline. Neither 5-HT nor methysergide had any effect on the rate of spontaneous secretion. Mean values in μmole $H^+ cm^{-2} h^{-1}$ were: control 3.31 ± 0.21 ($n = 17$), with 5-HT (5×10^{-5} M) 3.22 ± 0.25 ($n = 23$) and with the methysergide series (Table 1C) control value was 3.86 ± 0.44 ($n = 4$) and test 4.10 ± 0.20 ($n = 5$).

In summary, 5-HT inhibits the secretory responses of the rat isolated stomach to pentagastrin, isoprenaline and histamine but, unlike the reported *in vivo* studies, it has no apparent action on spontaneous or cholinergically-stimulated secretion. The lack of action of methysergide on spontaneous or stimulated secretion suggests that endogenous 5-HT does not normally restrain the response of this isolated stomach preparation.

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Table 1 The effect of 5-hydroxytryptamine (5-HT) and methysergide (MS) on acid secretion by the rat isolated stomach in response to pentagastrin (P, 1.1×10^{-7} M), isoprenaline (I, 6.25×10^{-7} M), histamine (H, 1.65×10^{-4} M), dibutyryl cyclic AMP (db cAMP, 1.3×10^{-4} M), phosphodiesterase inhibition (ICI 63197, 2.1×10^{-4} M) and bethanechol (B, 8.5×10^{-6} M).
Values are mean secretory ratio R (response/spontaneous secretion) with s.e. means and number of observations in brackets. Asterisk denotes $P < 0.05$ for comparison test with control by Mann–Witney U-test.

	P	*I*	*H*	*dbcAMP*	*ICI63197*	*B*
A. Control	1.75 (6) ±0.12	2.19 (6) ±0.13	1.94 (6) ±0.11	1.89 (6) ±0.08	2.20 (6) ±0.21	1.88 (6) ±0.07
+10^{-5} M 5-HT	1.39 (6)* ±0.04	1.79 (6)* ±0.05	1.66 (6)* ±0.08	1.77 (6) ±0.05	2.00 (6) ±0.11	1.82 (6) ±0.14
B. Control	1.73 (6) ±0.07	1.85 (4) ±0.06	1.75 (6) ±0.07	2.07 (5) ±0.19	—	1.68 (6) ±0.08
5×10^{-5} M 5-HT	1.41 (4)* ±0.09	1.41 (4)* ±0.06	1.43 (9)* ±0.05	1.96 (5) ±0.08	—	1.72 (9) ±0.09
+5×10^{-5} M 5-HT 2.5×10^{-5} M MS	1.68 (4) ±0.08	1.96 (4) ±0.05	—	—	—	—
C. Control	1.84 (4) ±0.13	1.91 (4) ±0.20	—	—	—	—
+2.5×10^{-5} M MS	1.74 (5) ±0.08	1.78 (5) ±0.13	—	—	—	—

Antagonism by α-adrenoceptor antagonists of the responses to 2(N,N-dimethyl)-amino,6,7-dihydroxy-1,2,3,4 tetrahydronapthalene in the guinea-pig ileum and the pithed rat preparation

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In the pithed rat, the peripheral cardiovascular effects of two different series of tetrahydronapthalenes (ATN), N,N-dialkylated derivates of 5,6-diOHATN (Hicks & Cannon, 1979; Drew, 1980) and N,N-diMe-6,7-diOHATN (Hicks & Cannon, 1980) have been shown to be mediated through α_2-receptors.

We have further examined the α_2-receptor effects of N,N-diMe-6,7-diOHATN in the guinea-pig transmural stimulated ileum preparation and the pithed rat, using different α-receptor antagonists.

Guinea-pig terminal ileum was set up according to the methods of Drew (1978) and stimulated at 60v; 1 ms; 0.1 Hz. The inhibitory effects of N,N-diMe-6,7-diOHATN were obtained before or after increasing concentrations of α-adrenoceptor antagonists in the Krebs.

Normotensive male Wistar rats (300–400 g) were pithed under pentobarbitone anaesthesia (60 mg/kg; i.p.). The diastolic blood pressure (DBP) increase to submaximal doses of N,N-diMe-6,7-diOHATN (5 μg/kg; i.v.); phenylephrine (5 μg/kg; i.v.), or electrical stimulation of the entire sympathetic chain (30 v; 2.5 Hz; 1 ms) was used to assess post-synaptic α-receptor effects. Cardio-inhibitory effects were obtained in separate pithed rats during continuous thoracic spinal cord stimulation (60 v; 0.3 Hz; 0.3 ms).

Table 1 shows the antagonist potencies of different α-adrenoceptor antagonists against the pre- or post-synaptic effects of N,N-diMe6,7,diOHATN. The order of potency in the guinea-pig ileum was:

phentolamine > yohimbine
= rauwolscine ⩾ corynanthine.

Corynanthine was not a competitive antagonist in this preparation, and prazosin was inactive. In the pithed rat the order of potency against cardioinhibition was:

yohimbine ≥ phentolamine > rauwolscine.

Rauwolscine did not antagonize the cardioinhibitory effects of N,N-diMe-6,7-diOHATN by 50% and prazosin was again inactive.

The order of potency at post-synaptic α_2-receptors was:

rauwolscine > yohimbine ≥ phentolamine.

Corynanthine or prazosin did not act as antagonists over the dose range studied.

The vasoconstrictor responses to phenylephrine or electrical stimulation were preferentially blocked by prazosin but rauwolscine was more potent than yohimbine against stimulation, but less potent against phenylephrine. This may suggest a post-synaptic α_2-receptor involvement in the stimulation induced effects, or might reflect a difference in pre-synaptic activity of these compounds.

The effects of N,N-diMe-6,7-diOHATN were antagonized only by α_2-blocking drugs, but the potencies of these drugs differed in the guinea-pig ileum and pithed rat.

Table 1 Effect of N,N-dimethyl-6,7-diOHATN at pre- and post-synaptic α_2-adrenoceptors: influence of α-adrenoceptor antagonists

	Pithed Rat		*Guinea-pig Ileum*
Antagonist	*Antagonism of vasoconstrictor effect*	*Antagonism of cardioinhibitory effect*	pA_2
	(mg/kg)		*(−Log M)*
Rauwolscine	0.32 (0.39–0.24)	non competitive antagonism	8.06 (8.59–7.53)
Yohimbine	0.65 (0.81–0.49)	0.37 (0.56–0.18)	7.82 (8.28–7.36)
Corynanthine	no antagonism	no antagonism	<6.0 —
Phentolamine	0.85 (1.3–0.4)	0.39 (0.52–0.2)	9.7 (10.2–9.17)
Prazosin	no antagonism	no antagonism	no antagonism

Antagonist potency in pithed rats = dose (95% confidence limits) required to inhibit the agonist response by 50%. In guinea-pig ileum, antagonist potency = pA_2.
(n = 5–8).

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2-(2-imidazolyl methyl)-1,4-benzodioxans, a series of selective α_2-adrenoceptor antagonists

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α-Adrenoceptors have been classified as either α_1 or α_2 depending upon their relative affinity for various agonists and antagonists (Starke & Langer, 1979). Rauwolscine has been reported to be the most selective α_2-adrenoceptor antagonist available (Weitzell, Tanaka & Starke, 1979). In the present study a series of 2-(2-imidazolyl methyl)-1,4-benzodioxans has been compared with some standard α_2-adrenoceptor antagonists for α_2/α_1 selectivity using the rat, isolated, transversely bisected vas deferens (Michel & Whiting, 1981).

Brown, McGrath & Summers (1979) have shown that the α-adrenoceptors associated with the dominant contractile response in epididymal portions of vasa are of the α_1 type and those on the nerve terminal of the prostatic portion are of the α_2 type. In the present study α_1-adrenoceptor antagonist activity was assessed by determination of the pA_2 value using the preferential α_1-adrenoceptor agonist, amidephrine (Flavahan & McGrath, 1980), in epididymal portions of vasa. α_2-adrenoceptor antagonist activity was assessed by determination of the pA_2 value against the inhibitory effect of the preferential α_2-adrenoceptor agonist, xylazine, on the contractile response of prostatic portions of vasa to single pulse stimuli.

All the compounds examined were more selective α_2-adrenoceptor antagonists than rauwolscine (see Table 1) although the potency at α_2-adrenoceptors was reduced compared to that of rauwolscine. RS-21361 exhibited the most pronounced selectivity for α_2-adrenoceptors.

We wish to acknowledge the valuable help and criticism of Professor J.C. Gilbert, both during the experimental design and preparation of the abstract.

Table 1 Relative selectivities for α_2-adrenoceptors of α-adrenoceptor antagonists as determined in the rat, isolated, transversely bisected vas deferens preparation.

Compound	pA_2 (α_2) ± *s.e. of mean*	pA_2 (α_1) ± *s.e. of mean*	† α_2/α_1 *selectivity ratio*
Prazosin	5.56 ± 0.18	8.76 ± 0.07	0.00063
Phentolamine	8.10 ± 0.12	8.10 ± 0.22	1.0
Rauwolscine	7.92 ± 0.09	6.27 ± 0.08	45.0
RS-84663 (1)	6.10 ± 0.13	4.22 ± 0.22	76.0
RS-49640 (2)	6.20 ± 0.28	4.33 ± 0.08	75.0
RS-21361 (3)	6.79 ± 0.21	4.00 *	>616.0

n = 6 in all cases.

† α_2/α_1 selectivity ratio is the antilog of the difference between the pA_2 values at α_2- and α_1-adrenoceptors.

* No α_1-adrenoceptor blocking activity at 10^{-4} M.

(1) 2-(2-imidazolyl methyl)-1,4-benzodioxan.

(2) 2-(1-methyl-2-imidazolyl methyl)-1,4-benzodioxan.

(3) 2-(1-ethyl-2-imidazolyl methyl)-1,4-benzodioxan.

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The rat, isolated, transversely bisected vas deferens; a preparation for determining the potency of antagonists at both α_1- and α_2-adrenoceptors

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Although the rat isolated vas deferens has been extensively used for determining the potency of antagonists at α_1- and α_2-adrenoceptors, it is now apparent that the prostatic and epididymal portions of this tissue differ considerably in both their sensitivity to agonists and their response to nerve stimulation (McGrath, 1978; Kawuya & Suzuki, 1979). Due to these differences we have avoided the use of whole vasa and have assessed a method for determining potency of antagonists at α_1-and α_2-adrenoceptors using transversely bisected vasa.

α_2-Adrenoceptor antagonist activity was assessed in prostatic portions of vasa by determination of the pA_2 value against the inhibitory effect of the α_2-adrenoceptor agonist, xylazine, on the contractile response of vasa to single pulse field stimulation (0.3 msec, supramaximal voltage, McGrath, 1980). Brown, McGrath & Summers (1979) have shown that this response is predominantly non-adrenergic in nature, but is subject to prejunctional α_2-adrenoceptor mediated inhibition.

α_1-Adrenoceptor antagonist activity was assessed by determination of the pA_2 value against the contractile effect of amidephrine, a selective α_1-adrenoceptor agonist (Flavahan & McGrath, 1980), in epididymal portions of vasa.

In both studies contralateral portions of vasa were used and dose-response curves to the agonist were determined in the presence of antagonist in one portion and in its absence in the contralateral portion.

The pA_2 values for prazosin at α_1-adrenoceptors and yohimbine at α_2-adrenoceptors were independent of the agonist used (see footnote, Table 1).

Table 1 Relative selectivities of α-adrenoceptor antagonists for α_1- and α_2-adrenoceptors as determined in the rat, isolated, transversely bisected vas deferens preparation.

Compound	pA_2 (α_2) ± *s.e. of mean*	pA_2 (α_1) ± *s.e. of mean*	† α_2/α_1 *selectivity ratio*
Prazosin	5.56 ± 0.18	8.76 ± 0.07[1]	0.00063
Corynanthine	6.40 ± 0.18	6.84 ± 0.05	0.36
Yohimbine	7.72 ± 0.08[2]	6.25 ± 0.07	29.5
Rauwolscine	7.92 ± 0.09	6.27 ± 0.08	45.0

$n = 6$ in all cases.

† α_2/α_1 selectivity ratio is the antilog of the difference between the pA_2 values at α_2- and α_1-adrenoceptors.

[1] pA_2 against phenylephrine (8.64 ± 0.10; $n = 6$) not significantly different from this value ($P > 0.05$).

[2] pA_2 against guanfacine (7.80 ± 0.12; $n = 6$) not significantly different from this value ($P > 0.05$).

Prazosin and corynanthine were more selective for α_1-adrenoceptors whilst rauwolscine and yohimbine were more selective for α_2-adrenoceptors.

These results indicate that the rat, isolated, transversely bisected vas deferens is a suitable preparation for comparing the potency of antagonists at α_1- and α_2-adrenoceptors. The use of paired tissues eliminates time dependent changes in tissue sensitivity to agonists, whilst the use of specific and selective α_1-or α_2-adrenoceptor agonists which are not subject to neuronal uptake optimizes conditions for the study of α-adrenoceptors.

We wish to acknowledge the valuable help and criticism of Professor J.C. Gilbert and Dr. J.C. McGrath, both during the experimental design and preparation of the abstract.

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In vitro determination of the relative selectivity of ifenprodil for blocking peripheral and central α_1- and α_2-adrenoceptors

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Ifenprodil possesses α-adrenoceptor antagonist properties (Carron, Jullien & Bucher, 1971). With the recent recognition and subdivision of α-adrenoceptors into α_1- and α_2-types (Langer, 1974, 1978; Starke & Langer, 1979) it was considered of interest to investigate the effects of ifenprodil on these subtypes of α-adrenoceptor in tissues taken from the peripheral and central nervous system of the rat and rabbit.

Vasa deferentia were removed from rats and suspended under 0.5 g resting tension in magnesium-free Krebs' bicarbonate solution gassed with 95% O_2, 5% CO_2 in 2 ml baths. Parallel platinum electrodes were used for stimulation of the tissues (supramaximal voltage, 2 ms, 0.1 Hz) and twitch responses measured. Clonidine was then added cumulatively to the bath to produce inhibition of the twitch and this effect was then reversed by step-wise addition of the α-adrenoceptor antagonist.

The effect of ifenprodil on [^{3}H]-noradrenaline ([^{3}H]-NA) release elicited by field stimulation was assessed in rat occipital cortex and rat atrial preparations. Slices of rat cortex were labelled with [^{3}H]-NA, placed in small perspex chambers and superfused with Krebs' solution. Tritium overflow was evoked by two periods of electrical stimulation (S_1 and S_2; 3 Hz, 2 ms, 20 mA for 2 min) separated by an interval of 40 minutes. Ifenprodil was added to the perfusion medium 20 min before S_2 and changes in tritium overflow were expressed as fractional release with respect to S_1. Rat atria were placed in 3 ml organ baths and superfused with Krebs' solution containing atropine (0.3 μM). Parallel platinum electrodes were used (supramaximal voltage, 1 msec, 3 Hz, 30 s) and two periods of stimulation were carried out separated by a 33 min interval. Drugs under study were added to the perfusion medium 26 min before S_2.

Postsynaptic α-adrenoceptor blocking potency was assessed by determining pA_2-values in rabbit isolated aortic ring preparations bathed in Krebs' solution.

The concentrations of yohimbine and ifenprodil required to reverse the α_2-agonist effects of clonidine in rat vasa deferentia were 0.22 μM and 1.29 μM, respectively. In rat cortex, ifenprodil caused a concentration-dependent increase in stimulation-evoked tritium overflow and was almost equipotent to yohimbine (EC_{100} ifenprodil: 0.39 μM, EC_{100} yohimbine: 0.23 μM). Ifenprodil (0.1–10 μM) but not yohimbine (0.01–10 μM), caused significant, concentration-dependent increases in the spontaneous outflow of tritium. Ifenprodil and yohimbine (0.01–1 μM) also caused concentration-dependent increases in the stimulation-induced overflow of tritium from rat atria, yohimbine being more potent

(EC_{100}, 0.007 μM) than ifenprodil (EC_{100}, 0.072 μM). These results would appear to be consistent with an α_2-adrenoceptor antagonist effect of ifenprodil. However, experiments carried out in the presence of cocaine (10 μM) in the rat cortex suggest that inhibition of neuronal uptake mechanisms contribute to the observed results. In fact, in the presence of cocaine, the ifenprodil EC_{100} was increased 25 fold. Arunlakshana and Schild (1959) plots yielded pA_2 values of 6.71 (slope 0.94) and 5.18 (slope 1.02) for ifenprodil and yohimbine respectively in rabbit aorta against 1-NA.

These studies demonstrate that ifenprodil possesses no relative selectivity toward α_1- or α_2-adrenoceptors, since the blocking effects occurred toward both receptor subtypes over similar concentration ranges. However, part of the increase in tritium overflow observed in the presence of ifenprodil is due to an effect of the drug on neuronal uptake. Furthermore, it appears that ifenprodil can displace NA from neuronal storage sites. This may also affect estimations of the α-adrenoceptor blocking potency and selectivity of the drug.

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The effects of α_1 and α_2-adrenoceptor antagonists on endogenous catecholamines in the pithed rat

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Exogenous adrenaline produces its pressor effect by acting on two sets of post-junctional α-adrenoceptors, one of which is resistant to rauwolscine but susceptible to prazosin, the other resistant to prazosin but susceptible to rauwolscine; these can be classified as α_1- and α_2-, respectively (Flavahan & McGrath, 1980a & b). In contrast, noradrenaline liberated from vasopressor nerves produces a pressor response which is relatively more susceptible to prazosin than to yohimbine or rauwolscine (Docherty & McGrath, 1980a & b; Madjar *et al.*, in the press). Although the separation is only relative this might suggest that noradrenaline from pressor nerves acts predominantly via α_1-adrenoceptors, while circulating catecholamines can act at both types. The object of the present study was to determine whether endogenous adrenaline, liberated from the adrenal medulla, would exert its pressor effect via either or both types of post-junctional α-adrenoceptor.

Rats were pithed by the method of Gillespie *et al.*, (1970) which permits selective stimulation of a restricted portion of the sympathetic outflow. Stimulation was applied at a level (T6–T8) which produced both an immediate response through vasopressor nerves and a delayed second component due to the release of adrenaline from the adrenal medulla (5 or 10 Hz, 10 pulses of 1 ms).

At this relatively high frequency, the direct, vasopressor response was reduced by 50% by prazosin (0.1 mg/kg): it was not reduced by rauwolscine (1 mg/kg) and in some experiments was increased. Since antagonism of feedback via prejunctional α_2-adrenoceptors could complicate interpretation, single stimuli were employed: prazosin (0.1 mg/kg) almost abolished the response whereas rauwolscine (1 mg/kg) produced slight attenuation ($\leq$20%). This confirms that noradrenaline released from pressor nerves acts predominantly via α_1-adrenoceptors.

The delayed, adrenal response could be reversed to a depressor response by either prazosin (0.1 mg/kg) or rauwolscine (1 mg/kg). When the vasodilator β_2-adrenoceptors were blocked by propranolol (1 mg/kg) the adrenal response was increased in height and duration: this response could not be blocked completely by either prazosin (1 mg/kg) or rauwolscine (1 mg/kg) but could be abolished by the combination.

These results were confirmed by isolating the components of the response; the direct response in adrenalectomized rats and the adrenal response after pretreatment with 6-hydroxydopamine. Following 6-hydroxydopamine no evidence was found for a disproportionate increase in the α_1-component to endogenous adrenaline's response as there might have been if α_1-adrenoceptors were located only near sympathetic nerve terminals.

In conclusion, the pressor effects of endogenous adrenaline, like those of exogenous adrenaline, are mediated via α_1 and α_2-adrenoceptors.

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An investigation of the possible function of postsynaptic vascular α_1- and α_2-adrenoceptors

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Langer, Massingham & Shepperson (1980) have proposed that, in the dog hindlimb, postsynaptic vascular α_1-adrenoceptors are located predominantly intrasynaptically, whereas the postsynaptic α_2-adrenoceptors are mainly extrasynaptic. If this is true, α_1- adrenoceptors should respond to neuronally released noradrenaline, whereas the α_2-adrenoceptors may respond to circulating catecholamines released from the adrenal medulla. We have examined this proposal by determining the effect of chemical sympathectomy or adrenal demedullation on the vasopressor responses to the intravenous injection of the α_1-adrenoceptor agonist phenylephrine, and the α_2-adrenoceptor agonist 2-N,N-dimethylamino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene (M-7), (Drew, 1980). Our experiments were carried out in pithed rats, in which there is considerable evidence for the existence of postsynaptic vascular α_2- as well as α_1-adrenoceptors (Timmermans, Kwa & van Zwieten, 1979; Drew, 1980). The rationale for these experiments is that removal of the neuronal or adrenal medullary catecholamine stores might be expected to lead to supersensitivity of the receptors that these transmitters normally act upon, which would be reflected by an increased vasopressor activity of the corresponding exogenous agonist. Non-specific changes in vascular reactivity were assessed by determining the influence of these procedures on pressor responses to 5-hydroxytryptamine (5-HT).

Male hood rats (AH/H strain, 200–350 g) were pithed and prepared for experimentation (Drew, 1980) 8–10 days after bilateral adrenal demedullation or administration of 6-hydroxydopamine (2 × 50 mg/kg i.p., day 1, plus 2 × 100 mg/kg i.p., day 7). Dose response curves to phenylephrine (0.1–3.0 μg/kg i.v.), M-7 (0.1–3.0 μg/kg i.v.) or 5-HT (1.0–100 μg i.v.) were then repeated until responses became constant (n = 12 rats per group).

In comparison with untreated preparations, bilateral adrenal demedullation produced a mean 1.60, 1.64 and 1.71-fold shift to the right in the dose response curves to M-7, phenylephrine and 5-HT, respectively; this appears to be a non-specific effect. 6-OHDA had no effect on the responses to 5-HT, caused a mean 2.3-fold shift to the right in the phenylephrine dose-response curve and mean 1.7-fold shift to the *left* in the M-7 dose-response curve. The differential effect of 6-OHDA on the responses to phenylephrine and M-7 is statistically significant ($P < 0.005$; analysis of variance).

The results provide no evidence that circulating adrenal catecholamines modulate vascular α_1- or

α_2-adrenoceptor function. The differential effect of 6-OHDA on responses to phenylephrine and M-7 may reflect a change in the proportion of vascular α_1- and α_2-adrenoceptors, or a change in their affinities fortheir respective agonists, and suggests some α_2-adrenoceptors may be 'innervated'. The difference between the present results and those obtained in rabbits by Hamilton & Reid (1980) may reflect a species difference in response to sympathectomy.

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Studies on the contribution of peripheral presynaptic β-adrenoceptor blockade to the anti-hypertensive effect of single doses of (±)-propranolol and atenolol in adrenal-demedullated spontaneously hypertensive rats

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The possibility that blockade of facilitatory β-adrenoceptors located on peripheral sympathetic nerve terminals might impair neurogenic transmission and thereby contribute towards the overall anti-hypertensive effects of β-adrenoceptor antagonists has been suggested by a number of workers (see review by Buckingham & Hamilton, 1979). This possibility has now been tested in adrenal-demedullated spontaneously hypertensive (SHAD) rats, a model in which several β-adrenoceptor antagonists have been shown to reduce mean arterial pressure acutely after a single dose (Buckingham & Hamilton, 1980). All animals used in the present study had been subjected to unilateral adrenalectomy and contralateral adrenal-demedullation 2–4 weeks earlier.

In initial experiments to establish dose-time course of effect relationships, (±)-propranolol, (+)-propranolol, atenolol (0.01, 0.03, 0.1 mmol/kg s.c.) or 0.9% w/v saline (2 ml/kg s.c.) were administered to groups (n = 8 or 9) of conscious, male SHAD-rats (300–470 g). Systolic blood pressure (SBP) and heart rate were determined (using a tail cuff method) immediately before dosing and then after 1, 2, 3 and 5 hours.

SBP was lowered by all doses of (±)-propranolol after 1 h, and these anti-hypertensive effects were sustained throughout 5 hours. Heart rate, however, was reduced by (±)-propranolol only at doses of 0.03 and 0.1 mmol/kg. At these two higher doses, atenolol reduced SBP and heart rate at all time intervals. At the lowest dose (0.01 mmol/kg) of atenolol, SBP was reduced at 2–5 h, though a bradycardia was detected only at 2 hours. By contrast, (+)-propranolol lowered SBP (at 1–2 h) only at a dose (0.1 mmol/kg) which caused a pronounced bradycardia (1–5 h). This latter effect is probably the result of myocardial depression evoked by the membrane stabilizing (or 'quinidine-like') activity of the drug (Howe & Shanks, 1966). The results confirm that (±)-propranolol and atenolol produce an acute anti-hypertensive effect in male SHAD-rats, but that the non-β-adrenoceptor blocking isomer of propranolol is devoid of such activity at doses which do not reduce heart rate.

Experiments to determine the effects of (±)-propranolol and atenolol on peripheral sympathetic neurotransmission were performed in pithed, male SHAD-rats. Groups (n = 6–11) of animals received (±)-propranolol, atenolol (0.03 mmol/kg s.c.) or 0.9% w/v saline (2 ml/kg s.c.). After 2 h they were pithed under methohexitone sodium (45 mg/kg i.p.) anaesthesia, and the total peripheral sympathetic outflow was electrically stimulated via the pithing rod (0.4–8.0 Hz, supramaximal voltage, 1 ms pulse duration). The results showed that log frequency–diastolic blood pressure response curves were unchanged by pretreatment with the β-adrenoceptor antagonists. It is suggested, therefore, that the acute anti-hypertensive effect of these drugs in conscious, male

SHAD-rats is unlikely to be mediated by an impairment of peripheral sympathetic neurotransmission.

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The effect of Cd^{2+} on the output of noradrenaline and dopamine-β-hydroxylase activity from the rat isolated vas deferens

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Cd^{2+} has been found to inhibit the responsiveness of the vascular and non-vascular preparations to noradrenaline (NA), potassium ions (K^+), tyramine and sympathetic nerve stimulation (Fadloun & Leach, 1979, 1980). An experiment has therefore been designed to study the effect of Cd^{2+} on the resting and nerve stimulated release of NA and DBH from an isolated organ.

Male Sprague-Dawley rats (200–300 g) were killed and the vasa deferentia removed and incubated in Krebs solution containing pargyline (100 μM), desmethylimipramine (DMI) (10 μM) and ascorbic acid (20 mg/l) at pH 7.4. In studies using tyramine the DMI was omitted. The medium was aerated with 95% O_2 + 5% CO_2 at a temperature of 37°C. The effect of Cd^{2+} (1, 10 and 100 μM) on the resting and nerve stimulated (20 V, 0.3 ms, 3 or 30 Hz for 15 min) release of NA and DBH following electrical sympathetic stimulation. The addition of K^+ (30 and 60 mM for 15 min) and tyramine (0.5 mM for 5 min) were also studied. NA and DBH activity were measured as previously reported (Fadloun & Leach, 1978).

Cd^{2+} (1–100 μM) was found to reduce the stimulation-induced output of NA and DBH in a concentration dependent manner. The 3 Hz-induced output of NA and DBH following Cd^{2+} (100 μM) treatment were reduced to 28 and 38% respectively of their respective control values ($P < 0.01$) whilst the 30 Hz-induced NA and DBH release represented 37 and 53% respectively of the control values (Table 1). The K^+ (30 and 60 mM)-induced release of NA and DBH were inhibited in a concentration-dependent manner by the addition of Cd^{2+} (1–100 μM), the amounts released by higher K^+ dose (60 mM) being consistently inhibited to a greater extent than that of the lower dose (30 mM) (Table 1). Cd^{2+} (1 μM) significantly ($P < 0.05$) increased the tyramine-induced release of NA; higher concentrations of Cd^{2+} (10 and 100 μM) significantly ($P < 0.01$) reduced the release of NA. Cd^{2+} did not significantly

Table 1 The release of noradrenaline (NA) from the rat vas deferens following nerve stimulation (20 V, 0.3 ms, 15 min) and treatment with K^+ (30 and 60 mM, 15 min) and tyramine (0.5 mM, 15 min), (n = 6–8)

	NA release (ng/15 min/g tissue)				
Cd^{2+} concentration (μM)	*Frequency (Hz)*		*K^+ concentration (mM)*		*Tyramine concentration*
	3	*30*	*30*	*60*	*0.5 mM*
0	465 ± 36	495 ± 54	490 ± 136	509 ± 100	348 ± 28
1	253 ± 35**	342 ± 51*	396 ± 71	282 ± 55**	548 ± 72*
10	186 ± 10***	207 ± 37***	294 ± 52*	205 ± 32**	191 ± 40**
100	130 ± 9***	184 ± 22***	96.5 ± 17**	107 ± 21**	112 ± 25**
Cysteine (0.5 mM)	210 ± 39*	286 ± 37*	156 ± 29*	189 ± 32*	170 ± 20

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

influence the output of DBH activity following tyramine treatment.

It is concluded, therefore, that Cd^{2+} inhibits the release of NA and DBH by affecting exocytosis possibly through the inactivation of -SH groups and antagonism of Ca^{2+} permeability.

Z.F. is sponsored by the Scientific Studies and Research Centre in Syria.

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Assessment of the α-adrenoceptor selectivity of WB 4101: a comparison with prazosin and phentolamine

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WB 4101 is reported to be a potent, selective α_1-adrenoceptor antagonist (Kapur & Mottram, 1978) and has been extensively used as a radioligand to characterize α-adrenoceptors in binding studies (U'Prichard, Greenberg & Snyder, 1977). Recently, Langer, Massingham & Shepperson (1980) observed that *in vivo* the selectivity of WB 4101 for postsynaptic α_1-adrenoceptors was much less than that of prazosin. We have presently re-examined the selectivity of WB 4101 for α-adrenoceptors in isolated tissue, radioligand binding and *in vivo* experiments.

The pre- and postsynaptic α-adrenoceptor antagonist activities were assessed *in vitro* by determining pA_2 values against clonidine in the rat vas deferens and against noradrenaline in the anococcygeus muscle, respectively (Doxey, Smith & Walker, 1977).

Inhibition by the 3 antagonists of the binding of [^{3}H]-clonidine, [^{3}H]-prazosin and [^{3}H]-WB 4101 to α-adrenoceptors in rat cerebral membranes was assessed (see U'Prichard *et al.*, 1977).

In vivo presynaptic α_2-adrenoceptor antagonist activity was determined in the pithed rat. Stimulation (6.0 Hz, 50 μs, 40 v for 2 s every 30 s)—evoked contractions of the vas deferens were inhibited with clonidine (100 μg/kg, i.v.) and the cumulative intravenous dose of antagonist causing 25% reversal of this inhibition was used to express antagonist activity.

Results from *in vitro* experiments indicated that prazosin was the most selective agent for postsynaptic α_1-adrenoceptors followed by WB 4101. Phentolamine was slightly selective for α_2-adrenoceptors (Table 1). Phentolamine was a potent antagonist of the α_2-adrenoceptor stimulant effects of clonidine in pithed rats. An inhibitory effect for WB 4101, but not prazosin, could be demonstrated against clonidine.

In conclusion, a high selectivity for α-adrenoceptors could not be demonstrated for WB 4101 and, as such, care should be taken when using this compound to characterize α-adrenoceptors.

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Table 1 α-Adrenoceptor antagonist profiles in isolated tissue and radioligand binding studies and pithed rats. In the binding studies, tubes containing rat cerebral membranes, [^{3}H]-ligand and 10 concentrations of competing drugs were incubated in duplicate according to established methods (see U'Prichard *et al.*, 1977). The data are the mean of 3–4 experiments ± s.e. mean. Ki values were calculated according to $Ki = IC_{50}/1 + C[[^3H]\text{-ligand}]/K_D$

Antagonist	*Isolated tissue studies*			*Radioligand binding studies*				
	Postsynaptic (α_1) pA_2 value versus noradrenaline	*Presynaptic (α_2) pA_2 value versus clonidine*	α_1/α_2 *ratio (α_1)*	*Ki (nM)* [^3H]-*WB 4101 (α_2)*	[^3H]-*prazosin*	[^3H]clonidine	α_1/α_2 *ratio*	*Pithed rats* *Cumulative I.V. concentration to reverse clonidine by 25%*
Phentolamine	7.7 ± 0.2	8.4 ± 0.1	0.2	5.0 ± 0.5	5.4 ± 0.2	4.9 ± 0.2	0.9	34 ± 13 μg/kg
WB 4101	8.2 ± 0.2	6.8 ± 0.1	25	3.2 ± 0.4	2.9 ± 0.7	143 ± 10	50	3.1 ± 0.4 mg/kg
Prazosin	8.1 ± 0.1	5.9 ± 0.1	160	2.5 ± 0.8	0.19 ± 0.05	880 ± 33	4600	no effect at 1.3 mg/kg

Alpha adrenoceptors and contractile responses in isolated arterial smooth muscle

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The existence of presynaptic α-adrenoceptors (α_2) and their differences from postsynaptic α-adrenoceptors (α_1) in terms of sensitivity to α-adrenoceptor agonists and antagonists is now well established (Starke, 1977). More recently the existence of an α-receptor located postsynaptically but possessing the pharmacological characteristics of the α-adrenoceptor has been postulated in vascular smooth muscle (Drew & Whiting, 1979; Docherty & McGrath, 1980; Hamilton & Reid, 1980) and in the rat anococcygeus muscle (Docherty & McGrath, 1980). However, only one electrophysiological study (Hirst & Neild, 1980) has demonstrated *in vitro* that two populations of excitatory receptors for noradrenaline (NA) exist in arteriolar smooth muscle. Our study was designed to investigate postsynaptic α-adrenoceptor-mediated responses *in vitro* in helical strips of rabbit abdominal aorta and left renal artery.

All experiments were performed in vessels from male New Zealand White rabbits. Spiral strips were prepared in the manner described by Furchgott & Bhachakom (1953) and suspended in a Krebs-bicarbonate solution at 37°C containing cocaine (10^{-5} M), 17 β-oestradiol (10^{-5} M) and propranolol (10^{-6} M). Both phenylephrine (PE) and NA in the dose range 10^{-9}–3×10^{-4} M) produced dose-dependent contractions of aorta and renal artery strips. The ED_{50}'s for NA and PE in aorta were $1.53 \pm 0.17 \times 10^{-7}$ M (mean ± s.d.) and $2.63 \pm 0.07 \times 10^{-6}$ M respectively, and $1.87 \pm 0.11 \times 10^{-6}$ M and $4.66 \pm 0.04 \times 10^{-6}$ M respectively in renal artery. Guanabenz, an α_2-adrenoceptor agonist *in vivo* (Hamilton & Reid, 1980), 10^{-8}–10^{-3} M did not produce consistent contractions of either tissue.

Prazosin, an α_1-adrenoceptor antagonist (Cambridge, Davey & Massingham, 1977) caused a parallel concentration-dependent shift to the right of the dose-response curves to NA and PE. In renal arteries, the pA_2 to prazosin with PE as agonist was 7.6 ± 0.12, significantly ($P < 0.01$) different from the pA_2 with NA as agonist of 7.22 ± 0.21.

Pretreatment of rabbits with phenoxybenzamine, which is more potent at α_1 than α_2-adrenoceptors (Dubocovich & Langer, 1974), 5 mg/kg i.v. 30 min before sacrifice caused an almost 100-fold increase in the ED_{50} of PE ($4.66 \pm 0.04 \times 10^{-6}$ M to $1.02 \pm 0.15 \times 10^{-4}$ M; but a much smaller shift of the NA dose-response curve (ED_{50} $1.87 \pm 0.11 \times 10^{-6}$ M to $4.48 \pm 0.14 \times 10^{-6}$ M after phenoxybenzamine). Guanabenz still did not cause a contractile response. 24 h after phenoxybenzamine the contractile response to NA had nearly returned to control, whilst the PE response remained markedly attenuated. These responses *in vitro* closely mirrored the pressor responses to i.v. NA and PE in intact rabbits, in whom the guanabenz pressor response was present unaltered. Thus phenoxybenzamine may result in a selective and long-lasting loss of α_1-adrenoceptor mediated responses with sparing of α_2-responses.

Although α_2-adrenoceptor mediated responses were not observed, the NA responses *in vitro* which are resistant to prazosin or phenoxybenzamine (in the presence of a β-adrenoceptor antagonist and blockers of $uptake_1$ and $uptake_2$) suggest that post-synaptic α-adrenoceptors similar to α_2-receptors occur in arterial smooth muscle.

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Clonidine-induced potentiation of reflex vagal bradycardia in anaesthetized cats

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Clonidine facilitates the vagally mediated reflex bradycardia elicited by intravenous injection of angiotensin in β-adrenoceptor blocked anaesthetized dogs (Kobinger & Walland, 1972a). The facilitation occurs centrally and can be prevented by α-adrenoceptor antagonists (Kobinger & Walland, 1972b). The agonists alone, depress the reflex bradycardia to angiotensin in normal, but not in catecholamine-depleted dogs (Kobinger & Walland, 1973), which suggests that endogenous catecholamines modulate, rather than mediate, the reflex. In contrast to its action in dogs, clonidine is reported to have little effect on the vagal reflex in cats (Kobinger & Pichler, 1978); we have reinvestigated this interaction.

After pretreatment with propranolol (1 mg/kg i.v.) submaximal doses of angiotensin (0.7–10 ng/kg) were injected intravenously into chloralose (60 mg/kg i.p.) anaesthetized cats (1.6–2.95 kg) at intervals of 10 min throughout the experiment.

When the resulting vasopressor and reflex vagal bradycardia responses had become constant saline (10–40 μl; $n = 5$) yohimbine (200 μg in 40 μl; $n = 5$) or prazosin (2 × 100 μg, each in 40 μ; $n = 4$) was injected intracisternally (i.c.i.). 30 min later, clonidine (1 or 3 μg in 10 μl) was injected i.c.i.; subsequent doses of clonidine were administered cumulatively at intervals of 40 min to give a final dose of 10 or 30 μg.

Saline did not alter the reflex bradycardia to angiotensin. Clonidine (1–10 μg i.c.i.) had little effect on the peak reflex bradycardia but caused a dose-dependent prolongation of it; thus the overall bradycardia was increased by clonidine.

The selective α_1- and α_2-adrenoceptor antagonists prazosin and yohimbine (Starke, Barowski & Endo, 1975; Cambridge, Davey & Massingham, 1977; Doxey, Smith & Walker, 1977), alone, did not affect the reflex bradycardia to angiotensin. Yohimbine, however, greatly reduced the effect of subsequently injected clonidine on the bradycardia whereas prazosin was without effect.

These results confirm that the reflex vagal bradycardia in response to increases in blood pressure in cats differs in some respects from that seen in dogs. For example, it is not modulated by endogenous catecholamines and its duration, rather than its magnitude, is increased by clonidine. This effect of clonidine appears to be mediated via α_2- rather than by α_1-adrenoceptors since it was prevented by yohimbine but not by prazosin.

This work was supported jointly by Glaxo Group Research Ltd. and the Science Research Council.

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Exchange transfusion with dextran-haemoglobin, haemoglobin and dextran solutions in anaesthetized cats

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Recently Tam, Blumenstein & Wong (1978) have described a dextran-haemoglobin complex which was capable of supporting life in the dog following complete exchange transfusion. We have compared this complex (Dx-Hb) with haemoglobin (Hb) and dextran solutions on the maintenance of cardiovascular function during exchange transfusion in the chloralose anaesthetized cat.

Cats of either sex (1.5–2.5 kg) were anaesthetized with chloralose (80 mg/kg i.v.) following induction with halothane in N_2O/O_2. Blood pressure was recorded from a catheter in the femoral artery and heart rate obtained from the blood pressure pulse. For the exchange transfusion, blood was taken from the right carotid artery and Dx-Hb, or dextran administered via the right femoral vein. The rate of exchange was 1 ml kg^{-1} min^{-1}. The following parameters were also recorded: total and plasma haemoglobin concentrations, haematocrit, arterial and venous pH, PCO_2 and PO_2.

Hb was prepared from citrated human blood by lysis of red cells in a 5mOsm phosphate buffer, pH 7.4 (Doczi, 1976), and Dx-Hb complex was produced from this by the method of Chang & Wong (1977), using dextran 20 (20,000 M.W.). The following solutions were used for exchange transfusion: 6g% Hb as the Dx-Hb complex in Tyrode; 6g% Hb in Tyrode; 6% Dextran 20 (Dx-20) in Tyrode; 6% Dx-20 in saline; 6% Dx-110 in 5% dextrose (Dextraven 100, Fisons Ltd.); and 0.9% NaCl (isotonic saline).

Of 9 cats exchange transfused with Dx-Hb, 8 survived after the haematocrit (Hct) had been lowered to 1% or below. With Hb exchange, however, only 1 of 5 animals survived to a Hct of 1% and the mean final Hct of the 5 cats was 1.5 ± 0.5%. Cats transfused with the remaining solutions all failed to survive to a Hct of 1% or below. The mean haematocrits at which death occurred were 7 ± 1.5%; 3.5 ± 0.5%; 2.5 ± 0.5%; 2.4 ± 0.6% for saline, Dx-110, Dx-20 in saline and Dx-20 in Tyrode respectively (n = 5). Mean arterial blood pressure and heart rate were best maintained by Dx-Hb and least by saline. The ascending order of efficacy was found to be saline; Dx-110; Dx-20 (either in saline or Tyrode); Hb; Dx-Hb.

During the exchange transfusion the venous PO_2 fell as the Hct was lowered; the greatest fall was observed with Dx-Hb for which the venous PO_2 7–10 mm Hg at the end of the exchange transfusion. This fall in PO_2 may be related to the high oxygen affinity of Dx-Hb. Since the Dx-Hb complex has a P_{50} of 8–10 mm Hg a low PO_2 would be necessary for Dx-Hb to deliver oxygen to the tissues.

In conclusion, therefore, Dx-Hb complex appears to support cardiovascular function at a lower haematocrit than Hb or dextran solutions in the anaesthetized cat following exchange transfusion.

The authors would like to thank Prof. J. T. Wong for his co-operation and supply of bromodextran for the preparation of Dx-Hb.

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Low incidence of retinopathy in dogs with sustained renovascular hypertension

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In a group of 15 mongrel dogs, comprising 7 with hypertension of 1–10 years' duration following bilateral constriction of the renal arteries (Goldblatt, Lynch, Hanzal & Summerville, 1934) and 8 normotensive dogs, the fundus was examined for evidence of vascular damage. Blood pressure was measured in a percutaneously punctured femoral artery on the same day as examination of the fundus (Table 1).

Table 1 Arterial blood pressure in normotensive and renal hypertensive, mongrel dogs

	Systolic (mm Hg)	*Diastolic (mm Hg)*	*Mean (mm Hg)*
Normotensive	176 ± 6	89 ± 4	113 ± 4
Renal hypertensive	248 ± 3	133 ± 1	167 ± 2

Results are mean ± s.e.

The fundus was photographed with a Kowa RC2 camera on kodachrome film after administration of a mydriatic.

Alterations of the fundus were found in only one dog with hypertension of over 5 years' duration. These changes consisted of relatively widespread, fresh haemorrhages in the retina. No vascular damage to the retina was observed in the other hypertensive or normotensive dogs, including 2 dogs with hypertension of over 5 years' duration.

In contrast to the ophthalmological changes seen in hypertension patients, no sign of slow, progressive damage to the blood vessels of the retina were observed in dogs with marked and sustained hypertension. These findings suggest that ophthalmology is not an appropriate means of estimating the progression and prognosis of hypertension in the dog.

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Effect of various prostanoids on the human basilar artery *in vitro*: relevance to aetiology and treatment of cerebral arterial spasm

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Various substances have been implicated in the aetiology of cerebral vasospasm (CAS) which results from subarachoid haemorrhage. These include 5-hydroxytryptamine (5-HT), angiotensin II (Ang II), noradrenaline (NA) and various prostanoids (see Boullin, 1980) in particular thromboxane A_2 (TxA_2) (Hagen, White & Robertson, 1979). This study investigates the effect of a wide range of prostanoids on the human *in vitro* basilar artery in an attempt to classify the prostanoid receptor mediating contraction. In addition, prostacyclin (PGI_2) is assessed for its ability to reverse the effects of various contractile agonists on the human *in vitro* basilar artery.

Human basilar arteries were prepared as described previously (Forster, Whalley, Mohan & Dutton, 1980). Initially agonist concentration-effect curves were constructed to PGE_1, PGE_2, $PGF_{2\alpha}$, U-46619, U-44069, PGI_2, 5-HT, Ang II, NA and potassium chloride (KCl). All compounds tested (except PGI_2) produced concentration-dependent contractions on the human basilar artery. By comparing the geometric mean EC_{50} of each prostanoid a molar potency ratio was determined and a rank order of contractile potency (potency ratio shown in parentheses, $PGF_{2\alpha}$ being assigned a value = 1) was U-46619 (1,340) $\gg$ U-44069 (68.6) $\geq$ PGE_1 (43.5) $\gg$ PGE_2 (2.28) $\geq$ $PGF_{2\alpha}$ (1). PGI_2 produced a biphasic response, 10^{-9}–10^{-6} M producing concentration-related relaxations and 10^{-5} M resulting in a contractile response.

Secondly, TxA_2 was generated by agitation of chopped guinea-pig lung and, utilising a superfusion

technique as described by Coleman, Kennedy & Sheldrick (1980), was examined on human basilar arteries. The response of the human basilar artery to the product of agitation was not antagonized by phentolamine (10^{-6} M) or methysergide (5×10^{-7} M). Both imidazole (4.4×10^{-3} M) and indomethacin (2.8×10^{-6} M) perfused through the chopped lung abolished the response to the product of agitation. In contrast imidazole and indomethacin were ineffective when allowed to perfuse over the human basilar arteries only.

The ability of PGI_2 to antagonize the contractile effect of cerebrospinal fluid from vasospastic patients (VS.CSF) and various substances implicated in the aetiology of CAS was investigated. EC_{50} and concentrations producing maximum responses on the human basilar artery were chosen for U-46619, $PGF_{2\alpha}$, 5-HT, Ang 11, NA, KCl and VS.CSF. PGI_2 produced concentration-dependent relaxation of maximal contraction produced by each agonist. The maximum percentage relaxation obtained for each agonist was as follows: U-46619 (31.7 ± 5.6); 5-HT (117.4 ± 14.8); $PGF_{2\alpha}$ (62.2 ± 11.1); NA (73.0 ± 13.4); Ang 11 (13.5 ± 6.5); KCl (106.5 ± 5.9) and VS.CSF (81.0 ± 16.1). PGI_2 at 10^{-5} M always produced a contraction. Against EC_{50} of each contractile agent PGI_2 (10^{-10}–10^{-6} M) produced relaxations, maximal % relaxations being in excess of 100% in all cases. PGI_2 (10^{-5} M) produced a contraction.

PGI_2 applied over the cascade tissues contracted maximally by constant agitation of the chopped lung produced concentration-related relaxations of the human basilar artery.

These results suggest that the prostanoid receptor mediating contraction of the human basilar artery may be similar to that described recently by Coleman, Humphrey, Kennedy, Levy & Lumley (1980) being a TxA_2 receptor. In addition PGI_2 is a potent physiological antagonist of a wide range of agents which contract the human basilar artery, however, the extent of the antagonism appears to depend upon the degree of tone imposed on the tissue by certain agents.

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Predilective relaxation by the calcium antagonist nimodipine (BAY e 9736) of isolated cerebral blood vessels, contracted with autologous blood

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Recent reports have shown that some calcium antagonists relax cerebral blood vessels contracted by various agonists more powerfully than peripheral vessels (Allen & Banghard, 1979; Shimizu, Ohta & Toda, 1980), and it has been suggested that such drugs may therefore provide a rational therapy for cerebral vasospasm (Edvinsson *et al.*, 1979). The new calcium antagonist nimodipine (BAY e 9736) has been shown to prevent cerebral postischaemic impaired reperfusion (Kazda *et al.*, 1979), and has also been shown to have more powerful dilator actions on cerebral than peripheral vessels (Towart & Kazda, 1980; Towart, 1981). It was the purpose of this investigation to compare its effects on isolated cerebral and peripheral vessels constricted with autologous blood.

Ring segments (3–4 mm) of Chinchilla rabbit isolated basilar and saphenous arteries were suspended in oxygenated (95% O_2 and 5% CO_2) Krebs-Henseleit solution at 37°C, and isometric tension measured with a strain gauge (Towart & Kazda, 1980). Contractions were induced by the addition to the organ bath of 0.2 ml autologous blood (i.e. final bath concentration 1%). This had been removed at sacrifice, allowed to clot, and was later homogenized and kept on ice. This procedure produced reproducible contractions of the basilar and saphenous arteries which averaged 63% ± 7 (N = 10) and 49% ± 5 (n = 10) respectively of the control contractions induced by KCl (55.6 mM).

Results are shown in Figure 1, and indicate a dose-

dependent reduction by nimodipine of the blood-induced contractions of both vessels. Nimodipine was around 30 times more potent an inhibitor of the basilar artery ($ID_{50} = 1.2 \times 10^{-8}$ M) than the saphenous artery ($ID_{50} = 4.3 \times 10^{-7}$ M).

The precise mechanism of this predilective effect of nimodipine on blood-induced contractions of cerebral vessels is unknown. The contractions of cerebral vessels induced by a range of agonists, including serotonin, catecholamines, and prostaglandins are selectively inhibited (Towart & Kazda, 1980; Shimizu, Ohta & Toda, 1980). Many such agents undoubtedly contribute to the contractions induced *in vitro* in this study and also probably to the pathological constriction after subarachnoid haemorrhage (see Boullin, 1980). Thus the predilective effects of nimodipine on blood-induced contraction of cerebral vessels served in this study support the view that calcium antagonists may be of value in the clinical management of cerebral vasospasm.

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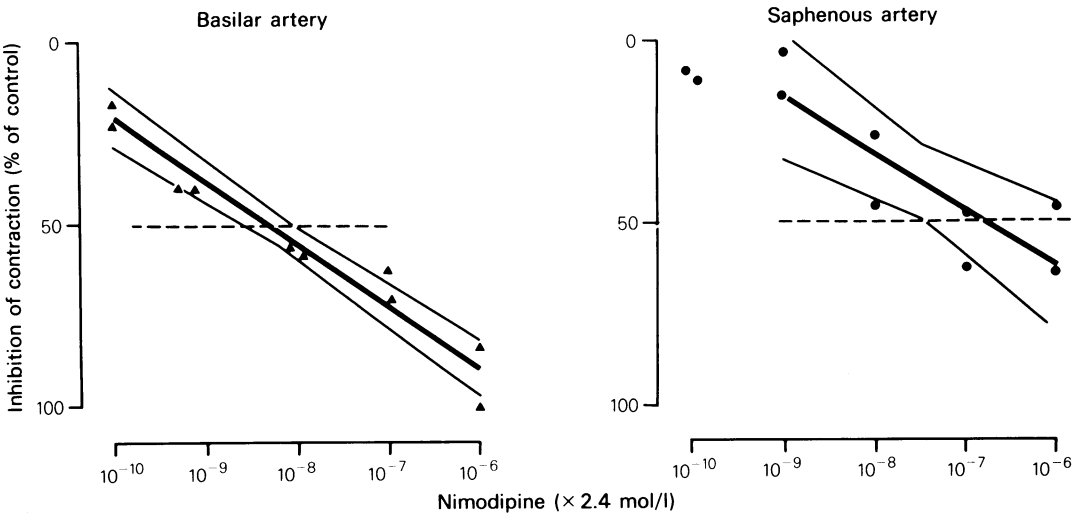

Figure 1 Percentage inhibition of the blood-induced contractions of the basilar (left) and saphenous (right) arteries by nimodipine. Each symbol represents a separate vessel segment; 5 animals were used in all.

Effects of magnesium ions on responses of mesenteric arteries from normotensive and spontaneously hypertensive rats

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Changes in extracellular magnesium ion concentration ($[Mg^{2+}]$) have been reported to affect contractile responses of isolated vascular muscle and to be involved in the regulation of vascular tone (Altura & Altura, 1978). In previous studies on rabbit ear artery withdrawal of magnesium ions from the perfusate caused differential changes in responsiveness to various agonists; responses to noradrenaline were unaffected while those to histamine and ATP were significantly potentiated at all dose levels. Increase in $[Mg^{2+}]$ caused a small reduction in responsiveness to higher doses of all three agonists (Asmawi, Irving & McCurrie, 1980).

In the present experiments the effects of changes in $[Mg^{2+}]$ on perfused rat mesentery were studied. The isolated mesentery of male Sprague-Dawley (CD) rats (280–480 g) was perfused with oxygenated Krebs solution at 37°C (McGregor, 1965) at a flow rate of 3.5–4 ml/min. Only one modification of the ionic content of the perfusion fluid was made and one agonist tested on each preparation ($n = 6$ for each procedure).

The responses of the mesenteric vessels to noradrenaline (12–6,400 ng) and ATP ($1–10^4$ μg) were both significantly potentiated over most of the dose response curve by withdrawal of magnesium ions ($P < 0.05$ to $P < 0.001$, correlated 't' test). However, the response maxima were not affected by the absence of magnesium. A fourfold increase in $[Mg^{2+}]$ in the perfusate caused little change in responses to ATP at any dose level but significantly reduced responses to high doses of noradrenaline (3–5 μg) ($P < 0.01$, correlated 't' test).

It has been proposed that changes in response to agonists caused by alterations in extracellular $[Mg^{2+}]$ may be due to altered binding, membrane permeability or distribution of calcium within vascular muscle (Altura & Altura, 1978). Changes in calcium binding and transport and in extracellular calcium dependence have also been implicated in the development of hypertension (Bohr & Berecek, 1976; Johansson, 1978; Lederballe Pedersen, 1979; Mendlowitz, 1979). Experiments were, therefore, carried out on mesenteries from spontaneously hypertensive rats (SHR) which might be expected to show marked changes in response to alterations of $[Mg^{2+}]$ if the latter involves changes in calcium metabolism.

The experimental protocol was as described previously. Male Okamoto, SHR rats weighing 280–380 g were used. Blood pressures were measured by the tail cuff method; animals with systolic pressures above 175 mmHg were used. The effects of alterations in $[Mg^{2+}]$ on responses to noradrenaline and ATP in SHR mesenteries were not significantly different from those observed in normotensives. Furthermore, preliminary experiments showed no difference between the two preparations in their dependence on extracellular calcium. Responses to noradrenaline and ATP (ED_{50}) were tested at intervals following withdrawal of calcium from the perfusing fluid. The rate at which responses to noradrenaline and ATP declined and returned when perfused with normal Krebs solution were similar in both normotensive and SHR mesenteries. The present experiments, therefore, provide no evidence for altered calcium or magnesium dependence in vascular muscle from spontaneously hypertensive animals.

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Studies in the mechanism of the bradycardia produced by pergolide, a dopamine receptor agonist, in the anaesthetized normotensive rat

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Pergolide, a synthetic ergoline derivative with potent dopamine (DA) receptor agonist properties (Fuller, Clemens, Kornfeld, Snoddy, Smalstig & Bach, 1979), produced blood pressure and heart rate lowering effects in conscious normotensive and spontaneously hypertensive rats which were blocked by haloperidol (Yen, Stamm & Clemens, 1979). Recently, we reported that in pentobarbitone anaesthetized rats and in conscious spontaneously hypertensive rats N,N-di-n-propyldopamine, a stimulant of DA receptors (Kohli, Goldberg, Volkman & Cannon, 1978), produced bradycardia which was antagonized by either sulpiride or halperidol (Cavero, 1980; Lefèvre-Borg & Cavero, 1980). This effect was found to result from an action on the autonomic nervous system consisting of a centrally-mediated inhibition of sympathetic drive accompanied by an increased efferent parasympathetic activity.

The aim of this communication is to present a series of experiments designed to clarify the mechanism of the heart rate lowering effects of pergolide in the anaesthetized normotensive rat.

Male rats (Sprague Dawley, C. River) weighing 200–300 g were anaesthetized with pentobarbitone sodium (60 mg/kg, i.p.) and placed under artificial respiration. Blood pressure was taken at the level of left carotid or tail artery. Heart rate was measured with a cardiotachometer. The effects of pergolide (10.0 μg/kg, i.v., infused over 10 min) were studied in intact, methylatropine (0.3 mg/kg, i.v.), sulpiride (0.3 mg/kg, i.v.) or phentolamine (1.0 mg/kg, i.v.) pretreated rats. Studies were also carried out in control and phentolamine pretreated pithed rats in which a sustained tachycardia (50–100 beats/min) was evoked by electrical stimulation (0.2–0.4 Hz, 0.5 ms, 40 V) of the thoracic spinal cord or by an infusion of noradrenaline (NA) (0.25 μg kg^{-1} min^{-1}). Before starting electrical stimulation the animals were given atropine (1.0 mg/kg, i.v.) and alcuronium (2.5 mg/kg, i.v.). In a group of pithed rats, the peripheral end of the right vagus nerve was electrically stimulated (3–9 Hz, 5 ms, 10 V) before and after administration of pergolide.

In intact pentobarbitone anaesthetized rats heart rate was decreased by 68 ± 6 beats/min (resting value 430 ± 8, n = 8) 30 min after administration of pergolide. In methylatropine pretreated rats, the fall in heart rate was 36 ± 6 beats/min (resting value 448 ± 7, n = 5). Phentolamine and sulpiride inhibited, respectively, by 40 and 66% the bradycardia produced by pergolide which was entirely prevented by the association of these antagonists.

In the pithed rat pergolide changed neither the baseline heart rate nor the tachycardia produced by NA nor the bradycardia evoked by electrical stimulation of the peripheral end of the right vagus nerve.

Pergolide decreased by approximately 33% the 100 beats/min increase in heart rate evoked by electrical stimulation of the spinal cord in the pithed rat. This effect was 80% less in phentolamine pretreated rats and not modified by sulpiride.

These results indicate that in the intact rat pergolide exerts a cardiac negative chronotropic effect by enhancing vagal drive and reducing sympathetic tone to the cardiac pacemaker. Activation of DA receptors and cardiac presynaptic α-adrenoceptors appears to mediate this bradycardia since it was blocked by phentolamine plus sulpiride. Pergolide, similarly to certain aminotetraline derivatives (Hicks & Cannon, 1979), stimulated cardiac presynaptic α-adrenoceptors in the rat. However, in the spinal dog with a sustained neural tachycardia pergolide stimulated only cardiac presynaptic DA receptors (Cavero, 1981). These findings confirm our previous suggestion that DA receptors are probably not present (at least in a relevant concentration to mediate relevant functional effects when pharmacologically activated) on the nerve terminals conveying sympathetic tone to the sinus node area of the rat. Finally, the DA receptors stimulated by pergolide to reduce heart rate in the intact rat are tentatively suggested to be located in the central nerve system since no peripheral site could be unveiled for them.

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Studies on the pharmacological effects of ifenprodil on the cardiovascular system of the rat

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Ifenprodil [DL-erythro-2-(4-benzylpiperidino)-1-(4'-hydroxyphenyl)-1-propanol L-(+)-tartrate monohydrate] was the most active vasodilator (dog hind limb) in a series of 2-piperidino alkanol derivatives (Carron, Jullien & Bucher, 1971). This effect would appear to be due to α-adrenoceptor blockade and to a direct vascular myorelaxation of undertermined mechanism. *In vitro* studies demonstrated that ifenprodil blocks both α_1- and α_2-adrenoceptors (Casadamont, Cathala, Langer, Massingham & Moret, 1981).

Normotensive rats (Sprague Dawley, C. River France), weighing 250–300 g were anaesthetized with pentobarbitone sodium (50 mg/kg, i.p.) and artificially respired. Blood pressure was measured from the carotid artery and the heart rate counted with a cardiotachometer triggered by the pressure pulse. The femoral vein was used for intravenous injections. Most experiments were conducted in rats with no sympathetic tone to the cardiovascular system since they were pithed or pretreated with syrosingopine (5 mg/kg, i.p., 18 h and 0.5 mg/kg, i.p. 1 h before anaesthesia). In addition, a group of freely moving spontaneously hypertensive rats (SHR) was prepared for continuous blood pressure and heart rate recording (Lefèvre-Borg & Cavero, 1980). Initially, the cardiovascular effects of ifenprodil were studied in conscious SHR and in anaesthetized normotensive rats. The α-adrenoceptor blocking activity of ifenprodil was assessed on dose-pressor response curves to noradrenaline (NA), phenylephine (PE) and electrical stimulation (ES) of the entire spinal cord in adrenalectomized pithed rats given propranolol (0.75 mg/kg, i.v.). The possible direct vasodilator action and the blockade of neuronal uptake by ifenprodil were studied in syrosingopine pretreated animals in which the low baseline blood pressure and/or heart rate was elevated by infusing NA (0.25 μg kg^{-1} min^{-1}, i.v.), vasopressin (0.012 μkg^{-1} min^{-1}, i.v.) or isoprenaline (0.01 μg/kg/min, i.v.). Finally, the directly and/or indirectly mediated effects of ifenprodil on cardiac chronotropism were evaluated in pithed adrenalectomized or syrosingopine pretreated rats receiving in addition desipramine (0.25 mg/kg, i.v.) alone or plus propranolol.

Ifenprodil (1.0 mg/kg, i.a., infused over 10 min or 30.0 mg/kg, p.o.), reduced mean tail blood pressure by 27% (initial blood pressure: 221 ± 9 mmHg, n = 10) and did not significantly change heart rate in freely moving SHR. This effect did not occur in animals pretreated with syrosingopine. In intact pentobarbitone anaesthetized normotensive rats, ifenprodil (3.0 mg/kg, i.v.) produced a moderate decrease in mean carotid blood pressure (−33 ± 1 mmHg, 2 min after the end of administration; initial blood pressure: 116 ± 2 mmHg) accompanied by a slight initial increase followed by a decrease in heart rate.

In adrenalectomized and propranolol pretreated pithed normotensive rats, ifenprodil (3.0 mg/kg, i.v.) shifted the control dose-pressor response curve to NA, PE and ES of the spinal cord to the right in a parallel manner with no change in the maximum. This dose of ifenprodil inhibited by approximately 50% the effects of either noradrenaline or electrical stimulation of the spinal cord. Phenylephrine responses were 2–3 times more susceptible to ifenprodil blockade than those elicited by noradrenaline or electrical stimulation of the spinal cord.

Ifenprodil did not modify the blood pressure raised with vasopressin in syrosingopine pretreated rats, whilst it lowered the blood pressure increased with NA (3.0 mg/kg, i.v.)..

Ifenprodil produced tachycardia in adrenalectomized pithed rats and a negative chronotropic effect in syrosingopine pretreated animals or in pithed rats given propranolol. In the pithed rat, ifenprodil potentiated the tachycardia evoked by NA without affecting the heart rate elevated with isoprenaline. Desipramine prevented the NA potentiating effect of ifenprodil.

These results demonstrate a complex mechanism of action for ifenprodil on the cardiovascular system of the rat. Ifenprodil behaves as a competitive postsynaptic α-adrenoceptor antagonist with no relevant direct vasodilator action. Furthermore, it inhibits

neuronal uptake of NA. The bradycardia produced by ifenprodil in propranolol pretreated pithed rats appears to be the result of a direct depressant action on the sinus node firing whilst the increase in heart rate elicited by this compound in adrenalectomized pithed rats was due to the release of neuronal noradrenaline.

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The effect of ethanol on ^{14}C-GABA release from mouse cortical slices

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Ethanol may decrease (Subramanian, Schinzel, Mitznegg & Estler, 1980), or have no effect on (Sunahara & Kalant, 1980), potassium-evoked transmitter release; whereas electrically stimulated release is decreased by ethanol (Carmichael & Israel, 1975; Sunahara & Kalant, 1980). Using slice and synaptosomal preparations, many reports have documented an increase in basal release of various transmitters by ethanol (Carmichael & Israel, 1975; Seeman & Lee, 1974). This effect may be related to the inhibitory effect of ethanol on Na^+, K^+-adenosine triphosphatases (ATP-ases).

In this study we have investigated the effect of ouabain and ethanol in combination, on [^{14}C]-GABA efflux to further elucidate the releasing effect of ethanol.

Slices of mouse (TO and C57BL/6J strains) cerebral cortex, cut to a thickness of 350 μ, were incubated in oxygenated medium at 37°C, for a period of 30 minutes.

A second incubation was carried out in the presence of 1 μ Ci (10^{-6} M [^{14}C]-GABA (226 mCi/mmol). The slices were then perfused at the rate of 0.5 ml/minute. After a 30 min wash out period, 2 min samples were

Table 1 Values for release of [^{14}C]-GABA from mouse cortical slices expressed as ratio of peak height to basal (mean ± 1 s.e. mean)

Drug	*Concentration*	*N*	*Peak/Basal*	*Significance (t test)*
Ethanol	250 mM	8	1.43 ± 0.04	—
Ethanol	500 mM	8	1.93 ± 0.04	—
Ethanol + Ca^{2+}-free EGTA	500 mM	8	1.57 ± 0.12	$P < 0.01$
Ethanol + Ca^{2+}-free/Verapamil	500 mM	8	1.66 ± 0.05	$P < 0.001$
Ethanol (C57 BL/6J)	250 mM	4	1.38 ± 0.08	N.S
Ethanol (C57 BL/6J)	500 mM	89	1.50 ± 0.05	$P < 0.001$
Ouabain	0.01 mM	12	10.47 ± 1.03	—
Ouabain (C57 BL/6J)	0.01 mM	8	7.66 ± 0.44	$P < 0.05$
Ouabain	0.1 mM	8	45.57 ± 4.06	—
Ouabain + Ethanol	0.01 mM 250 mM	8	17.40 ± 2.08	
Ouabain + Ethanol	0.01 mM 500 mM	8	28.84 ± 1.42	
Ouabain + Ethanol	0.1 mM 500 mM	8	44.68 ± 2.20	

collected and [^{14}C]-content estimated by liquid scintillation spectrometry.

Ethanol (250 and 500 mM) caused a dose dependent increase over basal efflux (Table 1). The effect was partially reduced in calcium-free, EGTA-containing medium, and in the presence of verapamil. Ouabain also produced a dose dependent increase over basal efflux. Cortical slices from C57 BL/6J mice (ethanol preferring) were less sensitive to the effects of ouabain and ethanol.

Potassium-free solutions greatly potentiated the effect of ouabain and ethanol. Ouabain (0.01 mM) and ethanol (250 and 500 mM) in combination produced a potentiated effect on efflux, yet there was no potentiation at higher doses (Ouabain 0.1 mM and ethanol 500 mM; Ouabain 1 mM and ethanol 500 mM). This may suggest that ethanol is causing release by inhibition of ouabain-sensitive Na^+, K^+-ATP-ase.

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Ethylenediamine acts upon GABA receptors and uptake sites

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Ethylenediamine (NH_2-CH_2-CH_2 NH_2) is usually considered to be a pharmacologically inert compound, and is widely used for its ability to form a soluble complex with theophylline, as aminophylline ([theophylline]$_2$ ethylenediamine).

We now report the following evidence of actions of ethylenediamine at GABA recognition sites.

(i) Slices of mouse cerebral cortex were cut to a thickness of 350–400 μ and incubated in oxygenated medium at 37°C for 30 minutes. A second incubation followed, in the presence of 1 μCi[^{14}C]-GABA (226 mCi/mmol). Slices were then perfused at a rate of approximately 0.5 ml/min and after a 30 min washout period, 2 min samples of perfusate were collected, and their [^{14}C] content estimated by liquid scintillation spectrometry. Ethylenediamine at 1 mM evoked an apparent release of 9.22 ± 1.0 (± s.e. mean, n = 8) times the basal efflux. Nipecotic acid (1 mM) caused an apparent release of 14.36 ± 1.33 (8), and a combination of ethylenediamine and nipecotic acid released 17.17 ± 1.31 (4) times basal efflux. When perfused with Na^+-free (choline substituted) medium, ethylenediamine caused no increase of GABA efflux.

(ii) using slices of rat cerebral cortex, ethylenediamine inhibited the uptake of [^{14}C]-GABA from a solution containing 4 μM [^{14}C]-GABA. At 1 mM, uptake was reduced by 23%, and at 0.1 mM by 18%. Nipecotic acid at 1 mM reduced GABA uptake by 70%.

(iii) In microiontophoretic experiments on cells in the cerebral cortex of rats anaesthetized with urethane (1.3 g/kg) GABA depressed the firing of 26 of 27 cells tested, glycine depressed 11 of 12, and ethylenediamine depressed 35 of 37. The potency and rapid time course of response were similar for ethylenediamine and GABA. Picrotoxin blocked ethylenediamine on 7 of 9 cells, and GABA on 4 of 5 cells. Bicuculline blocked both ethylenediamine and GABA in parallel on all of 13 cells, while glycine was unaffected on 5 of 8 of these cells.

In view of the less than additive effect of ethylenediamine and nipecotic acid on GABA release, it is possible that the apparent release produced by these compounds is a result of their respective inhibitory actions on uptake processes.

Although many studies of structure—activity relationships have been performed on compounds related to GABA, it is possible that neither hydroxyl nor carboxyl groups are essential for interaction with GABA recognition sites involved in uptake or neuronal depression.

Supported by The MRC, Wellcome Trust and Action Research.

On the motor inhibitory effects of dopamine agonists in the mouse

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Motor inhibition in the mouse has been studied using apomorphine, and has generally been explained in terms of activation of presynaptic dopamine receptors (Strombom, 1975, 1979). The nature of this purported dopamine mechanism is more accurately assessed using a wider range of dopamine agonists.

Male, BKW mice (25–40 g) injected with dopamine agonist or vehicle were placed, after 10 min, in individual cages measuring 10 × 14 × 24 cm each fitted with two photocell units. A summation of light beam interruptions over 20 min gave a measure of spontaneous locomotor activity. Apomorphine (0.002–0.064 mg/kg s.c.), bromocriptine (0.031–2.0 mg/kg s.c.), N-n-propyl-3-(3-hydroxyphenyl)-piperidine (3-PPP, 0.318–2.5 mg/kg s.c.), (3,4-dihydroxyphenylamino)-2-imidazoline (DPI, 0.065–0.4 mg/kg s.c.), N,N-dipropyl-dopamine (0.25–4.0 mg/kg s.c.), 2-di-n-propylamino-5,6-dihydroxytetralin (0.0002–0.0016 mg/kg s.c.) and 1-aza-6,7-dihydroxy-1-propyl, 1, 2, 3, 4, 5, 5a, 10, 10a-octahydrobenzo(g)quinoline (0.000125–0.004 mg/kg s.c.), but *not* SK & F38393 (0.001–10.0 mg/kg s.c.), each caused dose-dependent reductions in spontaneous locomotor activity. Further studies used 2-di-n-propylamino-5,6-dihydroxytetralin following pretreatment with antagonist drugs at doses which did not inhibit locomotor activity *per se*. Prazosin (0.031–0.156 mg/kg i.p.), methysergide (0.125–0.5 mg/kg i.p.), yohimbine (0.625–1.25 mg/kg i.p.), (±)propranolol (0.625–2.5 mg/kg i.p.), atropine (1.25–10.0 mg/kg i.p.) and SK & F 38393 (0.25–0.5 mg/kg i.p.) failed to reverse the motor inhibitory effects of the tetralin. Spiperone (0.004–0.031 mg/kg i.p.), haloperidol (0.025–0.2 mg/kg i.p.) and (−)sulpiride (1.25–10.0 mg/kg i.p.) caused some reversal of the motor inhibition, although this could not be shown for fluphenazine (0.05–0.1 mg/kg i.p.) or thioridazine (1.25–5.0 mg/kg i.p.). The antagonistic effect of spiperone (0.05 mg/kg i.p.) applied to the motor inhibitory effects of all dopamine agonists except DPI. Prazosin (0.125 mg/kg i.p.) and yohimbine (1.25 mg/kg i.p.) failed to modify the dopamine agonist effects, although yohimbine reversed the DPI response.

The data show that a neuroleptic sensitive site is involved in the mediation of the motor inhibition caused by the various purported dopamine agonists (the ineffectiveness of fluphenazine and other neuroleptics may be more apparent than real indicating an inhibition of motor activity *per se* prior to the antagonism of the motor inhibitory effects). This motor inhibitory site cannot be reliably distinguished by antagonists from that mediating motor facilitation (as measured by stereotypy induction), but agonist action reveals some differences. Thus, although the tetralin derivative, the (g) benzoquinoline, N,N-dipropyldopamine and apomorphine can both induce stereotypy (see review by Costall & Naylor, 1981) and cause motor inhibition, whilst SK & F 38393 has neither action (Setler, Sarau, Zirkle & Saunders, 1978), the inhibitory mechanism can be distinguished by its extreme sensitivity to the actions of the tetralin and (g) benzoquinoline derivatives, and because bromocriptine and 3-PPP, whilst lacking motor facilitatory effects (see also Hjorth, Carlsson, Lindberg, Sanchez, Wikström, Arvidsson, Hacksell, Nilsson & Svensson, 1980), can activate that mechanism which causes inhibition of spontaneous locomotion.

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Behavioural effects of muscimol, amphetamine and chlorpromazine on ethanol tolerant mice

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For some years the central GABAergic system has been implicated in the development of tolerance to and dependence on various drugs, particularly ethanol (Rix & Davidson, 1977). Ticku & Burch (1980) have shown changes in GABA binding in mouse brain after acute injections of ethanol and we have observed a loss of high affinity binding sites after chronic ethanol treatment (Unwin & Taberner, 1980). The present experiments were designed to investigate the behavioural changes which might result from this action of ethanol on the GABA system.

Male and female LACG mice from inbred colonies were made tolerant to ethanol by a previously described method (Unwin & Taberner, 1980) in which they were given a schedule of increasing concentrations of ethanol in tap water as their sole drinking fluid (up to 20% w/v over a period of a week). They were then kept at this concentration for at least 2 weeks before experimentation. Controls were age and weight matched. Ethanol tolerance was manifested by a significantly decreased duration of loss of righting reflex after an acute dose of ethanol (3.5 g/kg, i.p.)

Control and ethanol tolerant mice were injected with muscimol, a relatively specific, long-lasting GABA agonist which crosses the blood-brain barrier (Naik, Guidotti & Costa, 1976) (2.5 mg/kg, i.p.). After 30 min behaviour was scored by a trained observer on a single blind basis as either: no effect, decreased spontaneous activity or immobility. Both male and female ethanol tolerant mice were found to be significantly less affected by muscimol ($P < 0.05$ using Fisher's exact probability test).

Since ethanol tolerance appears to be associated with tolerance within other transmitter systems in the central nervous system (Liljequist (1978) for example, has shown that ethanol tolerant rats were also tolerant to increased activity produced by dopamine injected into the nucleus accumbens), we have examined the effects of a sympathomimetic agonist in tolerant mice. Female tolerant and control mice were injected with (+)-amphetamine sulphate (5 mg/kg, s.c.) and their locomotor activity measured for 5 min every 0.5 h using an ultrasonic activity meter (Morris & Taberner, 1980). Although there was no difference between saline injected ethanol tolerant and control mice, the increase in activity of ethanol tolerant mice 1 h and 1.5 h later amphetamine was significantly less than that of controls ($P < 0.01$ using Student's t-test).

Both the dopaminergic (Creese & Iversen, 1966) and serotonergic (Breese, Cooper & Mueller, 1974) systems have been implicated in amphetamine induced locomotor activity. In order to examine the possible role of the dopaminergic system, mice were injected with chlorpromazine (4.2 mg/kg, s.c.). After 3 h their behaviour was scored for decreased activity and catalepsy and their body temperature measured. No significant differences between ethanol tolerant and control animals were observed.

From these results we conclude that tolerance to ethanol is correlated with changes in the GABA and catecholamine systems, but probably not the dopamine system.

J.W. Unwin is supported by an M.R.C. Research Studentship.

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Social interaction in male rats after septal administration of ACTH[4-10] and ORG 2766

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Administration of ACTH fragments to pairs of male rats reduces the time spent in active social interaction, without reduction in motor activity (File & Vellucci, 1978; File, 1979). This action is centrally-mediated and independent of corticosteroid release (File, 1979; File & Clarke, 1980). In contrast, a trisubstituted ACTH[4–9] analogue (ORG 2766) increases social interaction (File, 1980a).

The septum is rich in binding sites for ORG 2766 (Verhoef, Palkovits & Witter, 1977; Verhoef, Witter & de Wied, 1977), and in this experiment we investigated the effects of septal administration of ACTH[4–10] and ORG 2766.

Male hooded rats were anaesthetized with halothane, and permanent bilateral cannulae implanted into the septum (co-ordinates w.r.t. ear bar: A 6.8, L ± 1.1, V 4.2); they were allowed two weeks recovery.

Rats were isolated for five days, then pairs of rats were scored for social interaction on two consecutive days, in a low light familiar arena, after the administration of peptide or vehicle (half tested with peptide first). ACTH[4–10] (100–500 ng) and ORG 2766 (100 and 250 pg) were injected bilaterally in 0.5 μl of an artificial CSF. The behaviours scored as active social interaction included: sniffing, following, grooming, nipping, wrestling, boxing, mounting and crawling over or under the partner (for a fuller account of this test, see File, 1980b). At the end of each test, boluses were counted and removed, and the arena wiped with detergent then dried.

Time spent in social interaction was decreased by ACTH[4–10] (250 and 500 ng) and increased by ORG 2766 (250 pg) (Figure 1), but neither peptide significantly affected motor activity. Thus the septum is a site of action of these peptides, but their actions appear to differ.

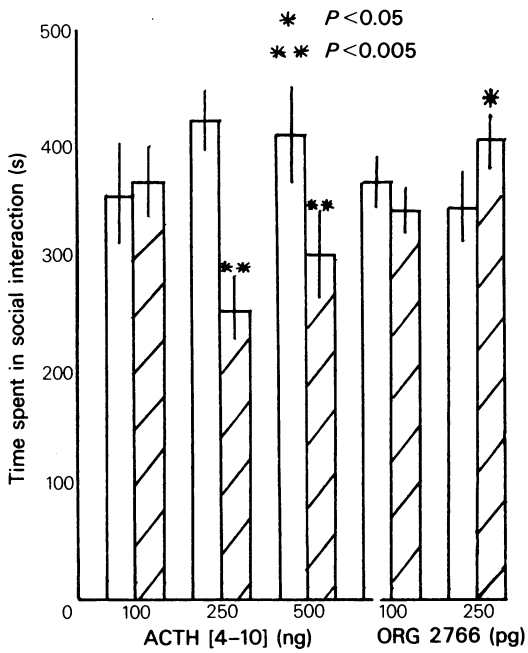

Figure 1 The time spent in active social interaction by pairs of male rats, after septal administration of vehicle (0.5 μl), ACTH[4–10] (100–500 ng) or ORG 2766 (100 and 250 pg). The open bars are the control responses, whilst the hatched bars are the responses after peptide administration. Results are expressed as the mean (± s.e. mean), and were analysed with paired *t*-tests (one tailed).

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Differential effects of dopamine antagonists on nomifensine hyperalgesia in a modified tail immersion test

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In animal nociceptive tests, the dopamine receptor stimulants apomorphine and (L-DOPA) produce an increased sensitivity to noxious stimuli, or a state of hyperalgesia (Tulunay, Sparber & Takemori, 1975). In our laboratory, using a modified tail immersion test, employing a lowered stimulus temperature of 45°C, we have been able to measure attenuated response latencies associated with hyperalgesic treatments in mice (Gonzalez, Sewell & Spencer, 1980). Nomifensine, a powerful inhibitor of dopamine and noradrenaline reuptake (Hunt, Kannengiesser & Raynauld, 1974; Schacht and Heptner, 1974), in doses from 0.1 to 10.0 mg/kg i.p., produced graded reductions in nociceptive response latencies which were significantly lower than those of naïve controls ($P < 0.001$), indicating pronounced dose-related hyperalgesia. In a previous study we have shown that subcutaneous doses of either phentolamine, phenoxybenzamine or propranolol did not significantly modify the hyperalgesic activity of concurrently administered nomifensine (Gonzalez, Sewell & Spencer, 1981). In addition, co-administration of pimozide (0.5 mg/kg s.c.) which alone did not modify control reaction latencies, totally blocks the hyperalgesic effect of nomifensine (10 mg/kg i.p.) ($P < 0.001$). Conversely domperidone, a peripheral dopamine blocker (Van Neuten, 1980), over a range of doses up to 50 mg/kg s.c., failed to modify nomifensine hyperalgesia ($P > 0.05$). However, when administered intra-cerebroventricularly (10 μg/animal) domperidone abolished nomifensine hyperalgesia ($P < 0.001$) without modifying control latencies on its own. Sulpiride (1.0–30 mg/kg s.c.) which itself was marginally hyperalgesic, did not antagonize nomifensine hyperalgesia ($P > 0.05$), but by way of contrast metoclopramide at 1.0–5.0 mg/kg s.c. produced blockade ($P < 0.01$).

In summary and conclusion it may be postulated that nomifensine induced hyperalgesia involves a central dopaminergic mechanism to the exclusion of an adrenergic component. Moreover, this hyperalgesia appears to differentiate between the actions of metoclopramide and sulpiride and this might be related to the respective predominantly pre- and postsynaptic actions of these drugs in the CNS (Alander, Anden & Grabowska-Anden, 1980) though this requires further study, particularly in relation to their own effects in this modified tail immersion test.

This work is generously financed by Ciba Geigy Ltd, Horsham, Sussex. The authors acknowledge gifts of pimozide and domperidone (Janssen) and nomifensine (Hoechst).

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The interaction of naloxone and calcitonin in the production of analgesia in the mouse

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Calcitonin produces analgesia when injected centrally (Pecile, Ferri, Braga & Olgiati, 1975). Similarities have been observed between opiate and calcitonin-induced analgesia, with regard to their interactions with calcium ions (Bates, Buckley, Eglen & Strettle, 1980a). However, two differences have been reported. Repeated central administration of calcitonin does not induce tolerance (Braga, Ferri, Santagostino, Olgiati & Pecile, 1978) and Braga *et al.*, (1978) have shown that calcitonin is not antagonized by the opiate antagonist, naloxone. This has also been shown using levallorphan (Yamamoto, Kumagai, Tachikawa & Maeno, 1979). We have reinvestigated the interactions between naloxone and calcitonin-induced analgesia using several doses of each agent.

Abdominal constrictions were induced by i.p. injection of acetic acid (1% solution in 0.154 mol/l NaCl) in CFLP mice (♂ and ♀, 30 g) after the method of Collier, Dinneen, Johnson & Schneider (1968) as modified by Bates *et al.* (1980b). The number of abdominal constrictions was counted between the 10th and 14th min inclusive after the injection of acetic acid. Ten μl of salmon calcitonin (SCT) was administered by intracerebroventricular (ICV) injection (Haley & McCormick, 1957) 10 min prior to the injection of acetic acid. SCT was dissolved in tris-saline, pH 7.35. Control animals received tris-saline alone. Naloxone hydrochloride (0.01–10 mg/kg) was injected sub-cutaneously in 0.154 mol/l NaCl both alone or simultaneously with ICV SCT. Twenty-four animals were used in the control group and ten used in each test group. Statistical significance was assessed using the Mann-Whitney U test.

The frequency of abdominal constrictions induced by acetic acid in the control animals was 3.36 ± 0.11 per min (mean ± s.e. mean). SCT (0.04–10 MRC u/kg) produced a dose related inhibition of abdominal constrictions (Bates *et al.*, 1980a).

The lower naloxone doses of 0.01 and 0.1 mg/kg had no significant effect upon the frequency of abdominal constrictions, but higher doses of naloxone (1 and 10 mg/kg) induced a significant ($P < 0.001$) increase in the frequency of abdominal constrictions (5.3 ± 0.1 and 5.2 ± 0.11 per min respectively, mean ± s.e. mean $n = 10$).

The lowest dose of naloxone (0.01 mg/kg) did not affect the analgesia induced by SCT. However, doses of 0.1 and 1.0 mg/kg, naloxone totally reversed the analgesic effect of doses of SCT up to and including 2 MRC u/kg. The dose of 10 mg/kg naloxone was required to reverse the analgesic effects of 10 MRC u/kg SCT.

In summary, the effects of naloxone upon SCT-induced analgesia, in this preparation, are more complex than has been previously reported (Braga *et al.*, 1978). The analgesic effects of SCT can be reversed by naloxone but only using doses 10–1000 fold greater than that required to reverse the effects of morphine in the mouse (AMD 50 = 0.01 mg/kg; Collier & Schneider, 1972). The reversal of SCT induced analgesia is not entirely due to the hyperalgesic effect of naloxone, since doses which are not hyperalgesic (0.1 mg/kg) antagonize the analgesic effect of SCT.

The salmon calcitonin was generously donated by Drs J.W. Bastian and J.D. Aldred, Armour Pharmaceuticals Corp., Kankakee, Ill., USA. Naloxone hydrochloride was generously donated by Endo Laboratories Inc., New York, U.S.A.

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Hyperalgesia induced by chronic subcutaneous injection of calcitonin

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Single injections of calcitonin into the cerebral ventricles induce a transient analgesic response (Pecile, Ferri, Braga & Olgiati, 1975; Yamamoto, Kumagai & Tachikawa, 1979). Daily intracerebroventricular injection of calcitonin does not induce tolerance to the analgesic properties of calcitonin (Braga, Ferri, Santagostino, Olgiati & Pecile, 1978). Single subcutaneous injections of calcitonin do not induce analgesia (Bates, Buckley, Eglen & Strettle, 1980). In the present work, we have studied the effect of repeated subcutaneous injections of calcitonin.

Groups of 8–10 CFLP mice (♂ and ♀, 30 g) were given subcutaneous injections of salmon calcitonin (SCT, 10 MRC u/kg in 0.154 mol/l NaCl plus 1 g/l BSA) on alternate days for periods up to 14 days. Control animals received vehicle alone. The animals were allowed access to food and water *ad libitum* and the amount of food and water consumed was recorded daily. There was a statistically significant decrease in food intake during the 24 h after each SCT injection ($P < 0.02$). Water intake and weight gain were not different from the controls.

The sensitivity to peritoneal irritation by acetic acid was assessed at varying times following the final injection of SCT or vehicle using the method of Collier, Dineen, Johnson & Schneider (1968) as modified by Bates *et al.* (1980). The statistical significances of the results were assessed using the Mann-Whitney *U* test.

Control animals, pretreated with vehicle gave 3.30–3.78 abdominal constrictions per min between the 10th–14th min inclusive after the injection of the acetic acid. Mice receiving either 1, 2 or 3 injections of SCT and tested with acetic acid 48 h after the final injection showed no change in the frequency of abdominal constrictions. In contrast, animals receiving four injections of SCT exhibited a gradual increase in the sensitivity to acetic acid. The frequency of abdominal constriction was not significantly different from the controls at 6 h, but at 12, 24 and 48 h after the final SCT injection the frequency of constrictions had increased by 27% ($P < 0.001$), 45% ($P < 0.001$) and 46% ($P < 0.001$) respectively.

After 8 injections of SCT the frequency of abdominal constrictions was again not significantly altered at 10 min after the final injection but it had increased by 37% ($P < 0.001$) 48 h after the final injection.

Animals pretreated with SCT showed a decrease of 0.28 m mol/l in the plasma calcium concentration 1 h after the fourth injection but the plasma calcium concentration was restored to control values after 12 hours.

It is concluded that withdrawal of SCT after 4 or more subcutaneous injections at 48 h intervals produces a long-lasting hyperalgesia in mice which is not coincident with the changes in plasma calcium concentration.

The salmon calcitonin was generously donated by Drs J.W. Bastian and J.P. Aldred, Armour Pharmaceuticals Corp, Kankakee, Ill., USA.

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Stimulation-produced analgesia is dependent upon the influence of both circadian and circannual rhythms

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It is known that many physiological and pharmacological effects of endogenous substances are subject to circadian control and in many instances to rhythmic influences of greater duration (Menaka, Takahashi & Eskin, 1978). Electrical stimulation-produced analgesia of short duration in mice (Buckett, 1979) may represent an endogenous pain control system (Buckett, 1981) utilized in the face of noxious stimuli to allow aversive action to be taken during antinociception. It has now been demonstrated that such analgesia is susceptible to both daily and annual rhythmic influences.

Stimulation-produced analgesia was induced by caudal stimulation of lightly restrained mice (female CD1 albino, 20 to 25 g bodyweight) with a surface electrode and stimulator (Grass S48). Rectangular wave pulses of 15 ms pulse width at 20 Hz were passed for 30 s at threshold voltage for vocalization. Analgesia was immediately determined by recording the escape latency of the animals from a 52° hot plate. Each mouse was only used once.

Results obtained over a 24 h period indicated that the stimulation-produced analgesia fluctuated markedly and rhythmically, with peak effect during the afternoon (Table 1a). Retrospective analysis of afternoon results obtained over a year showed that the analgesia altered annually with maximal effects during winter months (Table 1b). These findings may account for some of the variability obtained with various types of stimulation-produced analgesia in both animals and man (Akil, Watson, Holman & Barchas, 1978). Since some of the characteristics of this analgesia in mice are similar to those of morphine, for example antagonism by naloxone and modulation by serotoninergic manipulation (Buckett, 1979; 1981), it is of interest to note the close parallel between this circadian rhythm and that of morphine (Frederickson, Burgis & Edwards, 1977).

The circannual rhythm reported here is the first indication of this type of control in an antinociceptive system and the pharmacological influences on it remain to be explored.

Table 1 Escape latencies of mice on a 52°C hot plate after the induction of stimulation-produced analgesia. The latencies in seconds (mean ± s.e. mean) are shown (a) against times of day in hours (mean data of two experiments carried out in February; $n = 20$), and (b) against times of year in months (mean monthly data from afternoon experiments over one year; $n = 20$ to 60)

(a) *Time (h)*	*Escape latency (s, mean ± s.e. mean)*	*(b)* *Month*	*Escape latency (s, mean ± s.e. mean)*
0800	152 ± 17	01	221 ± 9
1100	190 ± 15	03	188 ± 8
1230	207 ± 10	04	168 ± 12
1400	213 ± 22	05	159 ± 8
1700	204 ± 11	07	146 ± 9
2000	179 ± 12	09	158 ± 14
2359	153 ± 9	11	192 ± 5
		12	210 ± 8

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Naloxone-precipitated withdrawal symptoms in rats chronically treated with small concentrations of morphine in drinking water

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In studies of morphine dependence, the drug is administered to animals by various routes, including the oral one (for references, see Fuentes, Hunt & Crossland, 1978). When administered in drinking water, some investigators do not attempt to mask the taste of morphine, whereas others do so by using saccharin. The above authors, however, reported that their rats refused to drink such drug solution, but that intake was achieved by the use of a 45% sucrose syrup. Whereas combination with sucrose may be an effective means of oral morphine administration, the effects of this sugar on brain 5-hydroxytryptamine synthesis (Badawy, Punjani & Evans, 1980) resemble those of morphine (Badawy, Evans & Punjani, 1981), and may therefore influence the interpretation of data concerning the effects of morphine on this indolylamine, particularly in relation to tolerance and dependence.

Rats accept morphine in the absence of taste-masking chemicals. The results presented by Badawy, Evans & Punjani (1981) were obtained in rats chronically treated with morphine as described by Badawy & Evans (1975). The drug was then provided in increasing concentrations (48 h apart) of 0.1, 0.15, 0.2, 0.3 and finally 0.4 mg/ml of drinking water, and the animals were then maintained on the latter concentration for the remainder of the experimental period (3 weeks or longer). In a typical 3-week experiment, the starting and final body weights (in g, means ± s.e. for each group of 20 rats) were as follows: control rats (146 ± 1 and 275 ± 1 respectively); morphine-treated rats (153 ± 1 and 261 ± 1 respectively). The latter rats therefore gained weight, but at a rate 19% lower than that of controls. This weight lag and the observation of a drastic loss of weight at 24 h after withdrawal confirm previous reports (Fuentes *et al.*, 1978 and references cited therein).

Average daily water consumption by a control rat was 30 ml, whereas the corresponding volume of the 0.4 mg/ml solution of morphine sulphate was 28 ml. Daily drug intake per rat therefore varied between 45 and 55 mg/kg body weight.

Naloxone (1 mg/kg) produced withdrawal symptoms in chronic morphine-treated rats. The following symptoms appeared significantly (P = 0.02–0.001): body shakes, chewing, diarrhoea, irritability to handling, paw tremor, ptosis, teeth chattering and writhing.

These results and those of Badawy, Evans & Punjani (1981) therefore suggest that rats receiving morphine in drinking water in small doses become dependent on, and exhibit the many biochemical changes caused by, the drug. The possibility that such rats also develop tolerance to morphine is currently under investigation.

C.M.E. is a Medical Council on Alcoholism Research Fellow.

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The effects of coping behaviour on the analgesic response to acute footshock stimulation in rats

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Exposure of individual rats of footshock elicits a wide variety of physiological consequences ranging from activation of the pituitary-adrenocortical axis, through weight loss to the production of gastric lesions (Weiss, 1971). Significantly, these effects are dramatically attenuated if the animals are shocked in pairs, thereby eliciting fighting behaviour (coping response) (Conner *et al.*, 1971; Williams & Eichelman, 1971). Since it has recently been reported that individual exposure to footshock also elicits analgesia (e.g. Lewis *et al.*, 1980), it was of interest to determine whether this effect might similarly be attenuated by shocking the animals in pairs.

In the first experiment, adult male Sprague-Dawley rats were randomly allocated to one of three experimental conditions: no shock (n = 5, NS), shock alone (n = 5, S) or shock fight (n of pairs = 5, SF). Prior to treatment, baseline tail-flick latencies (5 trials, inter-trial interval = 120s) were determined for all animals on the tail immersion test (55°C ± 0.5°C). Animals in the shock conditions were then exposed to electric footshock (2 mA intensity, 0.5 s duration, 6/min) for 5 min, whilst animals in the no-shock control group were kept in holding cages. Immediately following treatment, tail-flick latencies were again determined.

Analysis of variance revealed an overall significant effect of test condition ($F(2, 12) = 16.86, P < 0.01$), time($F(1, 12) = 24.50, P < 0.01$) and condition × time interaction ($F(1, 12) = 27.25, P < 0.01$). Further analysis indicated that these effects were due to an increase in tail-flick latencies in the S group ($t(\mathrm{df} = 12) = 6.23, P < 0.001$). No significant changes in tail-flick latencies were observed in either NS or SF groups. These data clearly demonstrate that engaging in fighting behaviour prevents the analgesic response characteristically observed in individually-shocked rats. Since exposure to footshock results in five-six fold increases in both plasma ACTH and beta-endorphin (Rossier *et al.*, 1977), presumably reflecting their concomitant release under stress (Guillemin *et al.*, 1977), and since the ACTH response is attenuated by fighting (Conner *et al.*, 1971), the observed differences between S and SF groups may relate to differential output of endorphin. To test this possibility, a second study was performed to examine the effects of naloxone on both the analgesic response to footshock and its prevention by coping behaviour, i.e. fighting.

As in Experiment 1, animals were randomly allocated to one of three experimental conditions (NS = 10, S = 10, SF = 10 pairs). Within each condition, animals were further assigned to either naloxone hydrochloride (5 mg/kg) or vehicle control (0.9% saline) groups. Injections were made intraperitoneally in a volume of 1 ml/kg, after the determination of baseline tail-flick latencies, and 10 min before experimental treatment. Following shock/no shock treatment, tail-flick latencies were again determined.

Analysis of variance revealed an overall significant effect of test condition ($F(2, 24) = 13.74, P < 0.01$), time($F(1, 24) = 37.00, P < 0.01$) and condition × time interaction ($F(1, 24) = 36.75, P < 0.01$). These results are vitually identical to those obtained in the first study, indicating that only animals in the S condition displayed analgesia. However, no significant drug effect was obtained ($F(1, 24) = 0.35$, ns), demonstrating that naloxone treatment failed to alter latencies in the NS and SF groups and did not prevent the analgesic response in the S group.

In conclusion, these data confirm that exposure of individual rats to acute electric footshock elicits analgesia, indicate that this response is not antagonized by naloxone and demonstrate that engaging in fighting behaviour (coping responses) prevents the development of analgesia. Furthermore, the data suggest that either naloxone-insensitive opiate receptors or non-opioid mechanisms are involved in acute footshock analgesia and its prevention by coping behaviour.

The author wishes to thank Dr M.J. Ferster (Endo Laboratories, Brussels) for the kind gift of naloxone hydrochloride. This work was supported by the Medical Research Council (grant number G977/473/N).

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Antinociceptive activity of some μ- and κ-opiate agonists determined by a combined nociceptive testing method.

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Differences in the relative antinociceptive potencies of μ- to κ-opiate agonists have been described in various types of nociceptive tests in different animals (Martin, Eades, Thompson, Huppler & Gilbert, 1976; Tyers, 1980). In the following study we compare the effects of μ- (morphine, levorphanol and heroin) and κ-(ethylketocyclazocine, ketocyclazocine and MR 2033) agonists in a combined tail immersion plus tail clip nociceptive testing method performed concurrently on the same animals.

Nociceptive sensitivity of untreated (control) and drug-treated male albino mice (GB1 variants of an ICI-derived strain weighing 20–22 g) in randomly assigned minimum groups of ten was evaluated firstly after tail immersion at 48°C (Sewell & Spencer, 1976) and secondly after application of an artery clip to the tail base 1 min later, the end-point being similar to that employed by Bianchi & Franceschini (1954). Nociceptive reaction latencies in seconds were determined immediately before and at successive 20 min intervals after drug administration for a total period of 100 minutes. To assess the antinociceptive effect (AE) given by each agent, the area (or integral) derived from the graph of mean nociceptive reaction latency against experiment duration was calculated and expressed as a percentage increase over the control integral (Sewell & Spencer, 1976). Dose-response regression lines were the constructed and AE_{200} values (arbitrarily defined as the dose of test drug capable of elevating control integrals by 200%) determined.

Dose-response regression lines for all μ- and κ-agonists tested were found to be steep and essentially parallel. However, the ratios of AE_{200} values for tail clip/tail immersion revealed a separation between μ- and κ-agonists in the two tests. Thus potency ratios were found to fall within the range 0.36–0.51 for κ-agonists and 1.32–2.20 for μ-agonists (see Table 1). Hence in the same animals κ-agonists proved to be more potent against pressure nociception than against heat nociception, the converse being true for μ-agonists. We therefore conclude that the combined nociceptive test described here enabled differentiation between those putative μ- and κ-agonists which produced steep dose-response lines in both tests.

This work was generously supported by C.H. Boehringer Sohn, Ingelheim and the authors also wish to thank Dr A.E. Soria of Sterling Winthrop N.Y. for his gifts of ethylketocyclazocine and ketocyclazocine.

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Table 1 Antinociceptive potencies (AE_{200}) and relative potency ratios of subcutaneously administered μ- and κ-agonists in the mouse tail immersion plus tail clip test determined in the same animals

Drug	AE_{200} (mg/kg) Tail clip test (TC)	AE_{200} (mg/kg) Tail immersion test (TI)	Potency ratio $\frac{TC}{TI}$
Morphine	3.70 ± 0.55	2.80 ± 0.52	1.32
Levorphanol	0.94 ± 0.04	0.43 ± 0.05	2.20
Heroin	1.05 ∓ 0.09	0.78 ± 0.04	1.35
Ethylketocyclazocine	0.20 ± 0.01	0.39 ± 0.06	0.51
Ketocyclazocine	0.64 ± 0.04	1.80 ± 0.14	0.36
MR 2033	0.41 ± 0.06	1.00 ± 0.08	0.41

MR 2033 is (±)-α-5,9-dimethyl-2-(L-tetrahydrofurfuryl)-2'-hydroxy-6,7-benzomorphan (courtesy of Dr H. Merz, C.H. Boehringer Sohn, Ingelheim).

A comparative study of the actions of opioids on single neurones in different brain regions

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From the results of experiments with opiate drugs on chronic spinal dogs Martin *et al.* (1976) proposed the existence of three different types of opiate receptors in the CNS. Further evidence for three types of opiate receptors has come from binding studies (Lord *et al.*, 1976; Gillan *et al.*, 1980; Kosterlitz & Francis, 1978) and peripherally, differential sensitivity to μ - and δ-agonists has been demonstrated in different bioassay preparations (guinea-pig ileum and mouse vas deferens) (Lord *et al.*, 1976).

Using the technique of microiontophoresis, a comparative study of the effects of a number of opioids on single neurones in different brain regions has been carried out. The experiments were performed on urethane-anaesthetized male Sprague Dawley rats as described previously (Bradley & Dray, 1974). Two of the drugs, FK33-824 (D-Ala^2, MePhe^4, Met(O)^5-ol-enkephalin, Roemer *et al.*, 1977) and ethylketocyclazocine, have not previously been studied by microiontophoresis. The drugs used are those thought to have a predominant action at a particular opiate receptor type: μ-agonists: morphine hydrochloride (25 mM, pH 4.5), normorphine base (30 mM, acidified to pH 4.5) and FK33-824 (15 mM, pH 5.0); the δ-agonists: BW180C (15 mM, pH 5.7) and leu-enkephalin (15 mM, pH 4.5); and the κ-agonist, ethylketocyclazocine methane sulphonate (100 mM, pH 4.0). Naloxone hydrochloride (20 mM, pH 5.3) was used as an antagonist. All neurones studied were spontaneously active, except those in the caudate nucleus which were activated by iontophoretic application of monosodium glutamate (500 mM, pH 8.0, 0 to 5 nA).

In the brain stem reticular formation all the opioid agonists, except morphine, consistently depressed neuronal firing and this effect was antagonized by naloxone. Morphine was found to depress some neurones (approx. 45%) and excite others (approx. 45%) but only the inhibitory response was antagonized by naloxone. This is consistent with previous findings with morphine (Bradley & Dray, 1974; Bradley & Bramwell, 1977). Neurones in the caudate nucleus were depressed by all the opioids tested, these effects being antagonized by naloxone. In contrast, in the hippocampus, all the opioids caused excitation of neuronal firing, with the exception of ethylketocyclazocine, which produced depression. Both the excitation and depression were antagonized by naloxone, however.

Thus, whereas the excitatory effects of morphine on brain stem neurones may be non-specific (Bradley & Bramwell, 1977), the different effect of ethylketocyclazocine, compared to the other opioids, in the hippocampus appears to be related to its opioid activity.

A.B. is an SRC CASE Student.

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Tryptamine-induced myoclonus in the guinea-pig suggests involvement of pre- and post-synaptic indoleamine mechanisms

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The presence of functionally distinct 5-hydroxytryptamine (5HT) receptors in guinea-pig brain is suggested by differential effects of 5HT antagonists to inhibit and 5HT re-uptake blockers to potentiate L-5-hydroxytryptophan (5HTP)-induced myoclonus (Jenner, Luscombe & Marsden, 1980). However, distinct receptors for tryptamine and 5HT may exist as suggested by opposing pharmacological actions (Cox & Lee, 1980; Marley & Nistico, 1975) and by differential inhibition of comparable tryptamine and 5HT-induced behavious (Clineschmidt & Lotti, 1974). We now report the characteristics of tryptamine-induced (plus a monoamine oxidase inhibitor) myoclonus in guinea-pigs in comparison to the 5HTP-induced behaviour.

Tryptamine hydrochloride (5–320 mg/kg ip) evoked only weak myoclonic jerking in guinea-pigs. However, following pre-treatment with pargyline hydrochloride (75 mg/kg ip 1 h prior) tryptamine hydrochloride (10–160 mg/kg ip) induced dose-dependent myoclonic jerking of 5–10 min onset, maximal after 30 min and lasting approximately 1.0–2.5 hours. Using tryptamine hydrochloride (40 mg/kg ip plus pargyline pre-treatment) as a dose producing a consistent myoclonic response a range of 5HT antagonists were examined for ability to inhibit jerking. The order of potency was methergoline (5 mg/kg ip, 45 min previously) ($P < 0.05$) >methysergide (10 mg/kg ip 15 min following tryptamine administration) ($P < 0.05$), mianserin (10 mg/kg ip 45 min previously) ($P < 0.05$) >cyproheptadine (10 mg/kg ip 60 min previously) ($P < 0.05$), cinanserin (10 mg/kg ip 45 min previously) ($P < 0.05$). Excepting methergoline, which is most potent in inhibiting both 5HTP- and tryptamine-induced myoclonus, this ranking differs markedly from that for inhibition of 5HTP-induced myoclonus (Jenner, Luscombe & Marsden, 1980) and suggests involvement of different post-synaptic mechanisms for induction of 5HTP- and tryptamine-induced myoclonus.

5HT reuptake blockers (chlorimipramine, paroxetine, femoxetine, fluoxetine, Org 6582 and desmethylimipramine 2.5–20 mg/kg ip 10 min prior to tryptamine) did not consistently inhibit or potentiate the myoclonus induced by a threshold dose of tryptamine (10 mg/kg ip plus pargyline pre-treatment) or by 40 mg/kg ip tryptamine (plus pargyline pre-treatment). This suggests presynaptic release of 5HT is not involved in tryptamine-induced myoclonus although such re-uptake blockers may prevent entry of tryptamine into presynaptic 5HT sites. However, pre-loading of indoleamine stores using a non-myoclonic dose of L-tryptophan (15 mg/kg ip 60 min previously) potentiated the action of a threshold dose of tryptamine (6 mg/kg ip) in pargyline (75 mg/kg ip 2 h prior)—pretreated animals. The importance of normal presynaptic indoleamine function was confirmed by the total inhibition of myoclonus to tryptamine (40 mg/kg ip plus pargyline pretreatment) following prior administration of p-chlorophenylalanine (150 mg/kg ip 48 h, 24 h and 2 h previously).

Administration of tryptamine (40 mg/kg ip 30 min previously) to pargyline (75 mg/kg ip) pre-treated animals caused a large increase in whole brain tryptamine content (3786% of control values) and a smaller increase in pons 5HT levels (150%) compared to animals receiving pargyline alone. However, administration of 5HTP (80 mg/kg sc) to carbidopa (25 mg/kg ip 60 min prior) pre-treated guinea-pigs caused a more marked increase in pons 5HT content (518%) but decreased whole brain tryptamine levels (55%) compared to animals receiving carbidopa alone.

The data suggests that tryptamine does not produce myoclonus in the guinea-pig only by activation of post-synaptic 5HT receptors but that both pre- and post-synaptic indoleamine mechanisms are involved and that these may differ from those involved in the 5HTP-mediated behaviour.

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Absence of sodium ions in dopamine stimulated striatal adenylate cyclase preparations does not explain lack of inhibition by sulpiride

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Failure of the neuroleptic sulpiride to inhibit *in vitro* dopamine stimulation of striatal adenylate cyclase has suggested the compound is a specific antagonist of dopamine receptors not linked to this enzyme (Elliott, Jenner, Huizing, Marsden & Miller, 1977). However, we demonstrated recently that specific binding of [^{3}H]-sulpiride to striatal dopamine receptors is critically dependent on the presence of sodium ions (Theodorou, Hall, Jenner & Marsden, 1980). Since no sodium ions are present in the standard adenylate cyclase incubation buffer, we have examined whether the failure of sulpiride to associate with its receptor might explain lack of inhibition of dopamine stimulated cyclic AMP formation.

Striatal tissue from male Wistar rates (150 ± 10 g) was pooled and basal and dopamine (1–1000 μM) stimulated adenylate cyclase activity assayed according to Miller, Horn & Iversen (1974). Comparison was made of cyclic AMP formation in standard sodium-free buffer or in the same buffer containing sodium chloride (10–200 mM), with or without the addition of the active isomer (−)-sulpiride or inactive isomer (+)-sulpiride (10^{-7}–10^{-4} M).

In the absence of sodium ions, cyclic AMP formation was increased by incorporation of dopamine (1–1000 μM). A sub-maximal concentration (100 μM) was used to produce an approximate doubling of cyclic AMP formation (basal activity 50.5 ± 3.0 pmoles cyclic AMP/2.5 min/2 mg tissue; dopamine 100 μM 104.8 ± 4.9 pmoles cyclic AMP/2.5 min/2 mg tissue). The isomers of sulpiride (10^{-7}–10^{-4} M) were without effect on either basal or dopamine stimulated activity ($P < 0.05$).

Inclusion of sodium chloride (10–200 mM) alone increased cyclic AMP formation. A sub-maximal physiological concentration (120 mM) caused an approximate two-fold increase in cyclic AMP formation (basal activity 50.5 ± 3.0 pmoles cyclic AMP/2.5 mins/2 mg tissue; sodium chloride 120 mM 89.3 ± 5.0 pmoles cyclic AMP/2.5 mins/2 mg tissue). While (−)-sulpiride (10^{-7}–10^{-4} M) had no effect on sodium-activated cyclic AMP formation, the incorporation of (+)-sulpiride (10^{-7}–10^{-4} M) tended to enhance cyclic AMP formation ($P < 0.05$), but this effect was not concentration dependent.

A combination of dopamine (100 μM) and sodium chloride (120 mM) caused a small increase in cyclic AMP formation compared to dopamine (100 μM) or sodium chloride (120 mM) alone but this effect was not additive. The incorporation of (−)-sulpiride (10^{-7}–10^{-4} M) had no effect on cyclic AMP formation in the presence of dopamine (100 μM) plus sodium chloride (120 mM). Again (+)-sulpiride (10^{-7}–10^{-4} M) tended to increase in cyclic AMP formation over that produced by dopamine plus sodium chloride, but this effect was not concentration dependent.

These data confirm that sulpiride does not antagonize dopamine stimulation of adenylate cyclase, even in the presence of a physiological concentration of sodium ions which ensure association of sulpiride with its receptor. Sulpiride would therefore appear to act selectively at dopamine receptors not linked to adenylate cyclase.

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Hypophysectomy does not prevent increased cerebral dopamine turnover following sulpiride administration

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Portaleone, Di Carlo, Grispino & Genazzani (1978) claim that hypophysectomy prevents the increased dopamine turnover caused by sulpiride. They suggest central effects of sulpiride originate at the hypophyseal level, a finding consistent with poor penetration of sulpiride into brain (Benakis & Rey, 1976). However, Portaleone and colleagues measured striatal 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations 30 min following sulpiride administration although peak effects are only observed after approximately 3 hours. We have therefore re-investigated the effect of hypophysectomy on the time course of cerebral dopamine turnover following sulpiride administration.

Male Wistar rats (150 ± 10 g) underwent hypophysectomy or sham-surgery 1 week or 1 month prior to biochemical analysis. Removal of the pituitary was confirmed histologically and serum prolactin levels were below the detection limit of assay (ca < 7.5 mg/ml). Subsequently normal, sham-operated or hypophysectomized rats received either (+)-sulpiride (50 mg/kg ip) or 0.9% saline and were killed over the following 24 h period. Homovanillic acid (HVA) and DOPAC levels were measured in the striatum, nucleus accumbens and tuberculum olfactorium. Surgery did not consistently alter basal HVA or DOPAC concentrations in these areas compared to normal animals.

Sulpiride (50 mg/kg ip) 30 min previously caused inconsistent changes in dopamine turnover. Thus, DOPAC concentrations were elevated in nucleus accumbens and striatum of both control animals or animal hypophysectomized 1 week previously. HVA levels were raised in the nucleus accumbens of control animals and in the nucleus accumbens and striatum of animals hypophysectomized 1 month earlier but not elsewhere.

Administration of sulpiride (50 mg/kg ip) caused maximal changes in HVA or DOPAC in the three brain areas between 2–8 h following drug administration in all groups of rats. At the time of peak effect HVA and DOPAC concentrations were markedly elevated in the tuberculum olfactorium, nucleus accumbens and striatum of both control animals and animals hypophysectomized 1 week or 1 month previously (Table 1).

We therefore find hypophysectomy to have no effect on the ability of sulpiride to increase forebrain dopamine turnover and conclude that this is a direct effect of the drug on cerebral dopamine systems.

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Table 1 Cerebral HVA and DOPAC concentrations in control and hypophysectomized rats following administration sulpiride (50 mg/kg ip)

Time after sulpiride administration	*Pre-treatment*	*% of values for saline treated control animals*					
		HVA			*DOPAC*		
		TUO	*NA*	*ST*	*TUO*	*NA*	*ST*
30 min	None	160 ± 31	203 ± 31*	153 ± 21	183 ± 20*	190 ± 20*	161 ± 20*
	Hypophysectomy (1 week)	117 ± 16	110 ± 31	121 ± 9	165 ± 16*	154 ± 9*	157 ± 8*
	Hypophysectomy (1 month)	148 ± 19	362 ± 98*	188 ± 37	130 ± 16	173 ± 22*	146 ± 19*
Maximal effect (2–8 h)	None	266 ± 49*	249 ± 49*	276 ± 52*	271 ± 28*	226 ± 43*	271 ± 34*
	Hypophysectomy (1 week)	207 ± 24*	295 ± 46*	503 ± 31*	207 ± 15*	269 ± 20*	440 ± 20*
	Hypophysectomy (1 month)	230 ± 22*	448 ± 67*	486 ± 78*	194 ± 13*	236 ± 13*	400 ± 15*

Results are expressed as the mean (± s.e. mean) of at least 6 determinations.
*$P < 0.05$ compared to saline treated animals.
TUO = tuberculum olfactorium; NA = nucleus accumbens; ST = striatum.

On the binding characteristics of [^{3}H]-ADTN to rat striatal tissue

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In studies where [^{3}H]-ADTN has been used to label dopamine receptors for an assessment of their locations in rat brain the conditions of assay have generally been similar to those used for human or calf tissue. However, it is not clear whether such conditions represent the optimum for binding to rat striatal tissue, particularly since in such assays one requires very high concentrations of cold dopamine agonist to displace the tritiated ligand (Davis, Poat & Woodruff, 1980). Hence, we have carried out detailed studies to determine the conditions for [^{3}H]-ADTN binding to rat striatal tissue.

Following tissue homogenization (Polytron setting 5, 10 s in Tris HCl buffer, pH 7.4 at 25°C) and centrifugation of the suspension (50,000 g for 10 min), the supernatant was discarded and the pellet resuspended in the above buffer and recentrifuged. The pellet thus obtained was resuspended in Tris HCl buffer, pH 7.4 at 37°C and incubated at 37°C for 10 min: the tissue was then centrifuged and washed twice and used immediately. The binding of [^{3}H]-ADTN (40 Ci/mM, Radiochemical Centre, Amersham, and 35 Ci/mM, New England Nuclear), 0.0625–64 mM, and the displacement afforded by cold ADTN, apomorphine, dopamine, bromocriptine, fluphenazine, α-fluphenthixol, haloperidol, spiroperidol, (+) and (−)butaclamol was assessed under conditions of varied temperature, 25–37°C, pH, 7.2–8.0, ascorbic acid content, 10^{-8}–10^{-3} M, Na_2EDTA content, 10^{-8}–10^{-3} M, and in conditions of various ion concentrations, paying particular attention to Mn^{++}, Mg^{++}, Ca^{++} and Na^{+}.

The major findings of the study are (i) concentrations of ascorbic acid routinely employed by other workers markedly reduce binding (up to 71%, see also Kayaalp & Neff, 1980), (ii) ascorbic acid also reduces the ability of cold dopamine agonists to displace [^{3}H]-ADTN, (iii) Na_2EDTA reduces binding (40% reduction at 1 mM), (iv) Mn^{++} elevates binding by some 190–330% dependent on the concentration of [^{3}H]-ADTN, (v) cold ADTN and apomorphine could displace up to 80% of [^{3}H]-ADTN binding whilst bromocriptine displaced a maximum of 27%. None of these dopamine agonists, even at 10^{-5} M, could be used to adequately demonstrate a saturable specific binding for [^{3}H]-ADTN whether Mn^{++}, Na_2EDTA or ascorbic acid was present or not, (vi) the neuroleptic agents used in the present study all caused weak and inconsistent displacement of [^{3}H]-ADTN.

Using two sources of [^{3}H]-ADTN and two strains of rats, the present studies would confirm that exceptionally large doses of cold dopamine agonists may be required to displace [^{3}H]-ADTN, and that this may to some degree reflect the inclusion of ascorbic acid in the incubation media. The difficulties of demonstrating a saturable specific binding with the cold dopamine agonists may result from additional actions of such compounds to displace from non-specific binding sites. We would thus emphasize a need for caution in an interpretation of data from [^{3}H]-ADTN binding studies in the rat: if this ligand is to be used in meaningful dopamine receptor binding studies, it is of critical importance that authors uniquivocally demonstrate the precise nature of the characteristics of the binding obtained.

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5-hydroxytryptamine receptors in the raphe nucleus of the rat

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We have previously demonstrated the presence of inhibitory 5-hydroxytryptamine (5-HT) receptors located on dopamine nerve terminals in the striatum (Cox, Ennis & Kemp, 1981). Antagonist studies indicate that these receptors are different from those located on 5-HT neuroterminals (Cerrito & Raiteri, 1979). However, since the latter study used only single dose determinations we have characterized the inhibitory receptors on 5-hydroxytryptamine neurones using a more quantitative approach.

The method used was essentially that described previously (Ennis, Kemp & Cox, 1981) except that the slices were prepared from tissue samples taken from the raphe nuclei of the rat and preloaded with tritiated 5-HT.

The 5-HT agonists, 5-methoxytryptamine, tryptamine and LSD all inhibited K^+-evoked tritium release from raphe-slices with pIC_{25} values of: 6.72 ± 0.39, 6.00 ± 0.24 and 6.00 ± 0.18 respectively. 5-Hydroxytryptamine also inhibited release in low concentrations but homoexchange prevented estimation of a pIC_{25}. The pIC_{25} values for 5-methoxytrypamine and tryptamine were not significantly different from those obtained for the inhibition of dopamine release (Ennis, Kemp & Cox, 1981). However, LSD had no effect on K^+-evoked DA release whilst it was equipotent with the other agonists in inhibiting 5-HT release.

The potency of the 5-HT antagonists to reverse the inhibition of 5-HT release produced by 5-methoxytryptamine is shown in Table 1. The pA_{10} values were obtained by the rapid method of Schild (1947). Methiothepin was the most potent compound tested whilst methysergide was a hundred times less effective. Cyproheptadine and cinanserin were not active. This order of potency is different from that for the antagonism of 5-methoxytryptamine induced inhibition of K^+-evoked dopamine release, where methysergide was ten times more potent than methiothepin and metergoline.

These results provide quantitative evidence in support of the suggestion proposed by Haigler & Aghajanian (1977) on the basis of iontophoretic studies that the 5-hydroxytryptamine receptors located on the cell bodies in the raphe are different from those located in areas innervated by 5-hydroxytryptamine neurones.

Table 1 Effect of 5-HT antagonists on the release of [^{3}H]-5-hydroxytryptamine from rat raphe nucleus slices

Antagonist	*pA_{10} (± s.e. mean)*	*n*
Methiothepin	7.26 ± 0.22	(4)
Methysergide	5.40 ± 0.23	(4)
Cinanserin	No antagonism	(4)
Cyproheptadine	No antagonism	(4)

n refers to the number of determinations.

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Inhibition of TRH-induced behavioural activity by histidyl-proline diketopiperazine

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Thyrotrophin-releasing hormone (TRH) produces locomotor activity and behavioural excitation when injected into the rat n. accumbens, NA (Morley, 1979); the mechanism involves release of dopamine (Heal & Green, 1979; Kerwin & Pycock, 1979). A metabolite of TRH, histidyl-proline diketopiperazine (cyclo-His-Pro), has CNS actions which may either resemble or antagonize the effects of TRH (Peterkofsky, 1980). The interactions between cyclo-His-Pro, TRH and (+)-amphetamine in the NA have studied; the degradation of TRH by the NA has also been investigated.

Female Sprague Dawley rats (160–180 g) were fitted with bilateral cannulae for microinjections (1 μl) into both NA. Locomotor activity was measured by single beam photocell cages and general activity by LKB Animex activity meters.

The animals were allowed 30 min to acclimatize; recordings were made 5–35 min after injection. The post-injection behaviour was monitored by an observer. TRH inactivation *in vitro* was studied by incubating TRH with soluble and particulate fractions prepared from rat NA; degradation products were identified by TLC (Griffiths, Kelly, White & Jeffcoate, 1980).

TRH (2 × 10 μg) in NA increased both motor activity scores (Table 1). The behaviour consisted of rearing, climbing, and increased locomotion (Miyamoto & Nagawa, 1977; Heal & Green, 1979). In contrast, cyclo-His-Pro (2 × 10 μg) in NA had no effect on photocell counts but greatly increased the Animex score. The behaviour consisted mainly of stereotyped rearing, climbing and sniffing movements but very little locomotion. Cyclo-His-Pro in NA entirely prevented the TRH-induced movements and locomotor stimulation (Table 1). Cyclo-His-Pro, given 5 min before TRH, produced photocell cage and Animex counts that were less than those recorded with control rats. The treated rats were seen to remain motionless for periods of several minutes. (+)-Amphetamine (2 × 10 μg) in NA caused an intense, prolonged stimulation of locomotor activity; prior administration of cyclo-His-Pro had no effect on this behaviour.

Inactivation studies showed that the products of TRH degradation produced by the subcellular fractions of NA *in vitro* were cyclo-His-Pro, deamidated TRH and the constituent amino acids.

These preliminary data show that cyclo-His-Pro, a degradation product of TRH, resembles TRH, in causing some stereotyped movements in NA, but differs from TRH in having no locomotor stimulant action. Furthermore, cyclo-His-Pro completely antagonized all TRH-induced motor stimulation; the mechanism of this interaction is uncertain. Cyclo-His-Pro had no effect on (+)-amphetamine-induced hyperactivity and is unlikely therefore to prevent dopamine release directly. The actions of TRH in NA might be regulated by its conversion to cyclo-His-Pro.

Table 1 Effects of TRH, cyclo-His-Pro and d-amphetamine sulphate in n. accumbens on motor activity in rats

Compound	*Dose*	*Animex counts*[1]	*Photocell counts*[1]
Saline (control)	1 μl	152 ± 40	61 ± 17
TRH	10 μg	736 ± 205**	127 ± 42*
Cyclo-His-Pro	10 μg	563 ± 142**	74 ± 21
Cyclo-His-Pro[2] + TRH	10 μg 10 μg	47 ± 24*	25 ± 11*
Cyclo-His-Pro[3] + TRH	10 μg 10 μg	170 ± 47	66 ± 27
(+)-Amphetamine	10 μg	—	471 ± 78*
Cyclo-His-Pro[2] + d-amphetamine	10 μg 10 μg	—	623 ± 118*

[1]Results (Mean ± s.e., n = 6–8) are total counts recorded over a 30 min period after bilateral intra-accumbens injection in a total volume of 1 μl, using Animex meters (sensitivity and tuning 40 μA) and photocell activity cages.
Cyclo-His-Pro was given 5 min[2] or 10 min[3] before TRH/(+)-amphetamine. Significance of difference from control $*P < 0.05$, $**P < 0.01$, using a Student's unpaired t-test.

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Electrochemistry of neuropeptides: a novel method for assay and *in vivo* detection

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Sensitive electrochemical techniques for the measurement of brain catechol and indoleamines have been developed incorporating either high performance liquid chromatography (HPLC) with electrochemical detection (Mefford, 1980) or the use of implanted electrochemical electrodes to determine amine release and metabolism *in vivo* (Conti, Strope, Adams & Marsden, 1978; Gonon, Buda, Cespuglio, Jouvet & Pujol, 1980). We have carried out experiments which indicate that similar procedures may be applicable for measurement of certain neuropeptides.

Electrochemical techniques depend on the oxidation of electroactive substances to produce current at specific applied potentials. The electroactivity of a series of neuropeptides and amino acids was tested in 5 ml buffered solutions (either 0.1 M phosphate, pH 7.4 or 0.15 M citrate/acetate pH 4.6), at concentrations of 10^{-4} to 10^{-6} M using differential pulse voltammetry (174A Princeton Instruments) attached to an X-Y plotter and a micro-carbon paste working electrode (Marsden, Bennett, Brazell, Sharp & Stolz, 1981).

The table shows that at pH 7.4 electroactive peptides and amino acids possessed peak oxidation potentials (i.e. potentials at which maximum current is generated) between +0.60 V and +0.80 V. This contrasts with amine peaks which occur between +0.20 V and +0.60 V. The amino acids tyrosine, tryptophan and cysteine showed single oxidation peaks (0.66, 0.74 and 0.71 V respectively) while all other amino acids tested (including phenylalanine, glutamate, glutamine, methionine, proline and histidine) were inactive. The electroactivity of the peptides appeared to relate to their content of either tyrosine or tryptophan (Table 1) although the presence of other amino acids, particularly cysteine, influenced the peak positions. At pH 4.6 the peptide oxidation peaks were shifted (~0.15 V) to a higher potential and only single peaks were observed for those peptides with double peaks at pH 7.4. Peptides tested which do not contain tyrosine, tryptophan or cysteine were not electroactive at either pH (e.g. thyrotrophin releasing hormone (TRH), Substance P and $ACTH_{25-39}$).

These results indicate that electrochemical detection, incorporated with reverse phase HPLC techniques to purify and separate the neuropeptides (Morris, Etienne, Dell & Albuquerque, 1980), would provide a neuropeptide assay method equal in sensitivity to that available for the amines and would avoid the antibody specificity problems associated with immunological assays. Developments *in vivo* may include monitoring of iontophoresed neuropeptide in addition to endogenous extra-cellular peptide measurements. Preliminary differential pulse scans with an electrode in the striatum of the anaesthetized rat showed peaks between +0.6 V and +0.8 V but these peaks remain to be identified.

We thank the Wellcome Trust for financial support and Dr J.S. Morley, ICI Pharmaceutical Division, Dr P.A. Desaulles, CIBA-GEIGY, Basel, and Dr R. de Castiglione, Farmitalia Carlo Erba, Milan, for their generous gifts of peptides.

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Table 1 Peak oxidation potentials (V) of various neuropeptides and tyrosine and tryptophan at pH 7.4

Single peak between +0.62–+0.70 V	*Single peak between* +0.72–0.80 V	*Double peak between* +0.62–+0.80 V
Tyrosine	Tryptophan	Tyrosine and Tryptophan
Neurotensin	Cholecystokinin (CCK-4)	Cholecystokinin (CCK-8)
Leu- and Met-enkephalin	Caerulein	Gonadotrophin releasing hormone (LH–RH)
Oxytocin		
$ACTH_{17-39}$ (CLIP)	Somatostatin	α-melanocyte stimulating hormone (α MSH)
β-endorphin		

The neuropeptides and amino acids were dissolved in 5 ml 0.1 M phosphate buffer pH 7.4 at concentrations of 10^{-4} to 10^{-6} M.

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Depolarizing actions of Substance P 1-9 amide on the isolated spinal cord of the neonatal rat

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In a previous communication to the society (Growcott *et al.*, 1980) it was reported that Substance P 1-9 amide (SP 1-9) was approximately equipotent with Substance P (SP) when applied iontophoretically to rat substantia nigra neurones, or when microinjected into the ventral tegmentum. However, SP 1-9 was devoid of activity when tested on the guinea-pig ileum *in vitro*, and in salivation and hypotension models. These results suggested the possibility of a difference between central and peripheral Substance P receptors.

The effects of SP 1-9 have now been studied on the rat isolated hemisected spinal cord preparation (Otsuka & Konishi, 1974). In contrast to the potent effects seen after microinjection or iontophoretic application, SP 1-9 produced small and inconsistent depolarizations of the ventral roots in comparison with Substance P (potency ratio 0.001). However, there appeared to be a correlation between the potency of SP 1-9 and the prior application of SP to the cord. It was also noticed that SP 1-9 always produced a significant response on preparations which had previously been exposed to SP plus an extract of *Peltiphyllum peltatum* (Briggs *et al.*, 1981). The most likely explanation for this enhanced effect seemed to be the displacement of SP from the plant extract by SP 1-9.

Such a displacement of SP from its binding site on the plant extract was subsequently shown to occur, the usually inactive SP 1-9 producing contractions of the guinea-pig ileum in the presence of the plant extract plus SP.

Could SP 1-9 similarly displace SP from binding sites on biological tissue? This might explain the previously reported wide variation in potency of SP 1-9 (Growcott *et al.*, 1980).

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Binding of Substance P and other peptides by a constituent of an extract of Peltiphyllum peltatum

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In initial pharmacological studies with a methanol extract of a saxifrage, *Peltiphyllum peltatum,* there appeared to be a potent and selective antagonism of Substance P-induced contractions of the guinea-pig ileum *in vitro*. After a 30 min contact time, 10 μg/ml of extract caused a shift to the right of the Substance P dose-response curve (EC_{50} dose ratio = 8.0; n = 3). Under similar conditions the dose ratios for other agonists were: acetylcholine 1.9, histamine 0, serotonin 0, barium ions 0.

Studies with a range of peptide agonists produced an interesting spectrum of activity: in concentrations giving 100% antagonism of Substance P, the only other peptide to be blocked was bradykinin. Responses to eledoisin, physalaemin and Substance P 6-11 hexapeptide fragment were not affected.

The rat isolated hemisected spinal cord was also used to study the selectivity of the antagonism. Depolarizations of the ventral roots by Substance P and bradykinin were again blocked, whereas responses to eledoisin, Substance P 6-11 hexapeptide, glutamate, glycine, carbachol, noradrenaline and 5-hydroxytryptamine on ventral or dorsal roots were not affected.

Dialysis and ultrafiltration analysis of the extract indicated that the active constituent was large, or was associated with other material of molecular weight >10,000. It binds avidly to most chromatographic supports, and remains at the origin. The unusual spectrum of selective antagonism led us to suspect that the apparent antagonism was not occurring at the receptor sites for Substance P and bradykinin. Further experiments showed that the extract appeared to gain potency if it was preincubated for short periods with Substance P before adding the mixture to the ileum bath, suggesting an interaction between the extract and Substance P itself, rather than a receptor blockade. Chromatographic studies supported this idea, since the movement of Substance P with the solvent was prevented in the presence of the plant extract.

Further pharmacological evidence in support of this novel type of interaction between peptides and plant constituents will be presented at the meeting.

Characterization of the effect of 5-hydroxytryptamine on N1E-115 neuroblastoma cells

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N1E-115 neuroblastoma cells (Amano, Richelson & Nirenberg, 1972) maintained in 2% (vol/vol) dimethyl sulphoxide developed into non-dividing, morphologically differentiated neurons (resting potential ca. 48 mV; input resistance ca. 52 MΩ) (Kimhi, Palfrey, Spector, Barak & Littauer, 1976). The voltage current curves of non-differentiated and all stages of differentiated cells showed anomalous and delayed rectification.

Only a small fraction (ca. 5%) of undifferentiated cells responded to either bath-applied or iontophoretically applied 5-hydroxytryptamine (5-HT). The number of differentiated cells responding to this drug increased with the time in dimethyl sulphoxide to reach a value of about 60% by 24 days. 90% of responding cells were depolarized by iontophoretic and bath-applied 5-HT; the magnitude of the response depended upon the concentration of the drug and the stage of differentiation of the cells. A biphasic change of membrane potential was recorded in 7% of the differentiated cells, comprising a brief depolarization followed by a longer hyperpolarization. A minority of cells were hyperpolarized by 5-HT in the bath application studies but during 5-HT iontophoresis, all responding cells were depolarized by this drug. The reversal value for the depolarization was ca. +5 mV whereas that for the hyperpolarization was close to the chloride equilibrium potential (−55 to −60 mV). Addition of Co^{++} (2 mM) to the bathing medium reversibly abolished the depolarization induced by bath-applied and iontophoretically applied 5-HT within 30 min, indicating the involvement of Ca^{++} in the generation of this response.

Chlorpromazine and mianserin hydrochloride in μM concentrations reversibly blocked the 5-HT induced depolarization (50% down at 10^{-6} M and 8×10^{-7} M respectively). Haloperidol and apomorphine also blocked this response but at higher concentrations (50% down at 2×10^{-5} M and 10^{-4}M respectively). Chlorpromazine and apomorphine depolarized the cells by ca. 5 mV.

We thank Dr. P. Strange, Dept. of Biochemistry, Nottingham University, for the neuroblastoma line.

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The effects of nandrolone decanoate (ND) on the toxicity and anti-tumour action of 1-(2-chloroethyl)-3 cyclohexyl-1-nitrosourea (CCNU) and 5 fluorouracil (5FU) in experimental murine colon tumours

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There are conflicting reports in the literature on the value of anabolic steroids in the management of patients undergoing cancer chemotherapy. Most reports describe simultaneous treatment with low doses of anabolics which appear to be of no value. The Bradford group have maintained that in order to achieve clinical benefits it is necessary to pretreat the patients with high doses of anabolics (Hancock *et al.*, 1979). Clinically ND appears to reduce the toxicity of various cytotoxic agents. The present study is an attempt to transfer a clinical impression to numerical data using an experimental model system. The spectrum of chemotherapeutic sensitivity to standard agents of the mouse adenocarcinoma of the colon (MAC) system has been shown to be similar to human large bowel cancer (Double & Ball, 1979). Therapeutic indices are low and responses are only seen close to maximum tolerated dose.

In normal 6–8 week pure strain NMRI mice the LC_{50} values for single i.p. injections of CCNU and 5FU were 80 and 180 mg/kg respectively. There was no sex difference in LD_{50} values in mice of this age group. Groups of 8 mice were injected with ND (50 mg/kg) on day 0 and received cytotoxic drugs at various intervals afterwards. In the case of CCNU the LD_{50} value increased to 120 mg/kg by day 7 and that of 5FU to 330 mg/kg on day 10. These represent an increase of 50% and 83% respectively.

Simultaneous administration of ND had no effect on the toxicity and anti-tumour action of either agent. Seven-day pretreatment with ND did not alter the anti-tumour activity of CCNU against MAC 13 but increased the LD_{50} value of CCNU from 77 mg/kg to 110 mg/kg, an increase of 43%. Similar effects are seen with 5FU against a different tumour line (MAC 26). Ten-day pretreatment with ND raises the LD_{50} value of 5FU from 180 to 295 mg/kg, an increase of 79%, without altering the anti-tumour action.

In both these experiments, ND has no significant effect on tumour growth.

Administration of ND results in a sharp rise in peripheral white cell counts which is maintained for at least 30 days. CCNU treatment on day 7 results in a fall in white cell count both in the non ND group and in the ND pretreated group. Control values remain stable for the duration of the experiment. Both CCNU groups show similar falls in white cell counts but the group pretreated with ND does not fall below control levels.

CCNU and 5FU are widely used in the management of metastatic cancer. If these observations could be repeated in the clinic they would represent a significant improvement in cancer chemotherapy.

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4-aminopyridine and quinidine effects on adenosine inhibition of vas deferens contractions

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It has been suggested that 4-aminopyridine (4AP) can block the response of neurones in the central nervous system to microiontophoretically applied adenosine (Perkins & Stone, 1980). Although responses to GABA were unaffected, adenosine has potent inhibitory actions on transmitter release (Stone, 1981) while GABA is primarily a postsynaptic agonist. To determine the relevance of this difference the interaction of 4-AP and three compounds known to depress transmitter release: adenosine, morphine and noradrenaline have been studied on the isolated vas deferens.

Immediately after death vasa were dissected from mice. Individual vasa were suspended in a Mg^{++}-free Krebs solution at 37°C under a resting tension of 0.5–1 g, and perfused continuously at a rate of 3 ml/min. Field stimulation of the vas was achieved by a pair of platinum wire electrodes, using single pulses (0.1 Hz) or pairs of pulses at 10 Hz delivered every 10 s, of duration 1 ms, 80 V amplitude (supramaximal). Drugs were added to the bath in a volume of 0.1 or 0.2 ml and the concentrations quoted are the estimated bath concentration.

Concentration response curves were constructed for the depression of twitch height produced by adenosine, morphine and noradrenaline, giving ID_{50} values of 5.0 μM, 0.85 μM and 24 μM respectively. 4-AP (0.1 mM) in the perfusion medium increased twitch height by 172% and reduced the depressant effect of these agonists (ID_{50} values 41 μM, 1.62 μM and 52 μM), as measured both by the percentage depression of twitch height and by the absolute level of tension developed.

The apparent antagonist action of 4-AP towards adenosine is thus not unique, but probably reflects an increased calcium influx into presynaptic terminals resulting from the blockade of potassium channels by 4-AP. This would be a comparable situation to that encountered with trains of pulses at high frequencies where the effectiveness of presynaptic inhibitory compounds is also reduced.

Contrasting with the effects of 4-AP it has been found that quinidine (50 μM) also increases twitch size (126%) but *increases* the depressant effects of adenosine, morphine and noradrenaline (ID_{50} values 3.5 μM, 0.88 μM and 5.9 μM). Part of the enhancement of noradrenaline inhibition is probably due to the blockade of the postsynaptic contractile effects of the amine. The explanation for the increased inhibitory responses in the presence of quinidine is unclear, but these observations may suggest that the reduction of responses in the presence of 4-AP is not simply related to the increased twitch height.

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The effects on contractions of the vas deferens of nifedipine and verapamil in relation to their local anaesthetic activity

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High concentrations of verapamil are required to inhibit noradrenaline contractions in rat and guinea-pig aorta (Bilek, Laven, Peiper & Regnat, 1974; Golenhofen & Hermstein, 1975), peristatic contractions in the guinea-pig ureter (Golenhofen & Lammel, 1972) and acetylcholine contractions in the dog trachea (Farley & Miles, 1978). As verapamil has local anaesthetic activity in high concentrations (Haeusler, 1972; Singh & Vaughan Williams, 1972), it was suggested that inhibition of methoxamine-induced rythmic contractions in the rat vas deferens might be due to this (Hay & Wadsworth, 1980). We have investigated this possibility.

Vasa deferentia from Wistar rats (250–450 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 36–38°C and contractions were recorded isometrically. For assessment of local anaesthetic activity, isometric twitches of rat hemidiaphragms were recorded on stimulation of their

phrenic nerves. A double organ bath was used, drugs being added cumulatively to the nerve chamber.

Methoxamine (2 μg/ml) or barium (1 mM) induced rhythmic contractions having a frequency of 1.3–4.8/min and amplitude of 0.02–2.38 g. The amplitude of these contractions was inhibited by nifedipine (0.5–2 μg/ml) or verapamil (5–50 μg/ml). Responses to KCl were inhibited by lower concentrations (50% inhibition of the tonic component occurred with verapamil 0.35 μg/ml or nifedipine 0.01 μg/ml). Verapamil had local anaesthetic activity (threshold concentration = 10 μg/ml) but nifedipine was inactive (up to 20 μg/ml). The threshold concentration for the local anaesthetic activity of lignocaine was 50 μg/ml.

In contrast to the calcium antagonists, lignocaine (30–300 μg/ml) increased the frequency of methoxamine or barium-induced rhythmic contractions. A slight reduction in amplitude was seen with 100 μg/ml, but to abolish contractions higher concentrations (300–1000 μg/ml) were required. Both phases of the KCl contraction were reduced by lignocaine (30–300 μg/ml). In unstimulated vasa deferentia, lignocaine (100 μg/ml) alone induced rhythmic contractions. Lignocaine (300 μg/ml) caused a slight rise in baseline tension associated with high frequency rhythmic contractions. These contractions were not reduced by phentolamine (5 μg/ml). The stimulant action of lignocaine has also been reported by Cliff (1968) and Vohra (1970).

In order to inhibit rhythmic contractions in the rat vas deferens with verapamil, it is necessary to use concentrations that have local anaesthetic activity. However, the effect on rhythmic contractions is unlikely to be due to this since (a) nifedipine inhibits rhythmic contractions although devoid of local anaesthetic activity and (b) lignocaine has a stimulant action both alone and in the presence of methoxamine or barium.

D.W.P.H. is supported by an M.R.C. scholarship. We thank Bayer, Knoll, Macfarlan Smith and Wellcome for gifts of their drugs.

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Age-related changes in properties of acetylcholine receptors in singly and multiply innervated skeletal muscles of the chicken

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We investigated the possibility that the type of innervation of skeletal muscle influences development of acetylcholine receptors. We studied receptor properties in chicken posterior latissimus dorsi (PLD) muscles, which contain mainly singly innervated fibres, and anterior latissimus dorsi (ALD) muscles, in which the fibres are multiply innervated (Ginsborg, 1960 a & b). We used muscles from 17–19 day chick embryos and from chicks aged 1–100 days. Spontaneous miniature endplate currents (mepcs) were recorded in voltage clamped fibres and peak amplitudes (Ip) and decay time constants (τ_D) were calculated. Acetylcholine-induced current fluctuations ('noise') were recorded during iontophoretic application of acetylcholine to endplates and conductances (γ) and average lifetimes (τ_N) of endplate channels were calculated.

Table 1 Mean time constant of decay (τ_D) and mean amplitude (Ip) of mepcs, and mean channel lifetime (τ_N) and mean single channel conductance (γ) in PLD and ALD muscles from chickens of different ages (at −40 mV)

	τ_D (ms)		Ip(nA)		τ_N (ms)		γ (pS)	
Age	*PLD*	*ALD*	*PLD*	*ALD*	*PLD*	*ALD*	*PLD*	*ALD*
1–4 day	6.6 ± 0.8 (16)	6.7 ± 0.5 (12)	1.2 ± 0.1 (16)	1.1 ± 0.1 (12)	5.1 ± 1.1 (4)	4.6 ± 0.3 (7)	16 ± 6 (4)	20 ± 2 (5)
4 week	5.1 ± 0.5 (16)	6.7 ± 0.5 (16)	1.1 ± 0.1 (16)	1.2 ± 0.1 (16)	5.6 ± 0.9 (4)	4.6 ± 0.4 (14)	29 ± 4 (4)	26 ± 2 (14)
8 week	5.7 ± 0.3 (16)	5.7 ± 0.5 (6)	2.2 ± 0.1 (16)	2.2 ± 0.1 (6)	4.2 ± 0.3 (6)	4.2 ± 0.5 (5)	39 ± 1 (6)	35 ± 3 (5)
14 week	4.6 ± 0.4 (8)	5.0 ± 0.2 (12)	2.0 ± 0.1 (8)	2.1 ± 0.1 (12)	4.2 ± 0.6 (5)	5.0 ± 0.4 (7)	37 ± 3 (4)	35 ± 1 (5)

Values are expressed as means ± s.e. mean.
Figures in brackets are numbers of fibres used.

At all ages, mepcs could be recorded from only one area on PLD fibres but from any location on ALD fibres. Mepcs in ALD fibres had a much wider distribution of growth times than those in PLD fibres. In both muscles at all ages, mepc decay was exponential, and there was little difference between PLD and ALD values (Table 1). τ_D decreased and Ip increased with age (Table 1).

Throughout development, noise spectra were well fitted by a single Lorentzian function. Values for γ and τ_N did not differ between PLD and ALD (Table 1). There was no difference in acetylcholine null potential (about −2 mV). There was little change in τ_N with age although γ almost doubled between 1 and 8 weeks (Table 1).

Thus, the type of innervation does not alter the properties of acetylcholine receptors of chicken muscle during development. The age-related changes occur in parallel in multiply and singly innervated muscles. No evidence was obtained for the existence of two populations of receptors, one with longer channel lifetime than the other, as found in developing rat muscle (Sakmann & Brenner, 1978).

Supported by grants from the Medical Research Council and the Clive and Vera Ramaciotti Foundations.

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Pre- and postjunctional blocking actions of clindamycin and lincomycin at the snake neuromuscular junction

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It has previously been shown that the lincosamide antibiotics lincomycin and clindamycin alter the decay constant (τ) of miniature endplate current (mepcs) at the snake neuromuscular junction (Fiekers, Marshall & Parsons, 1979). This effect has been ascribed to a blocking action of the compounds on the open form of the acetylcholine activated receptor-ion channel complex (Fiekers *et al.*, 1979). As these antibiotics also possess prejunctional blocking actions (Singh, Marshall & Harvey, 1979), the present study was designed to assess the concentration dependence of pre- and postjunctional actions at the same motor endplate.

To study evoked transmitter release by intracellular

recording in the absence of any drugs other than the antibiotic under study, experiments were performed on the cut costocutaneous nerve-muscle preparation of the garter snake. Preparations were voltage clamped at −55 mV and endplate currents (epcs) and mepcs were digitized and averaged by a DEC PDP 8 computer. At least 100 epcs and 60 mepcs were recorded from the same endplate before and 15 min after drug. Epc quantal content was calculated by the direct (epc amplitude/mepc amplitude) and variance methods. In some experiments, the effect of the antibiotics on epc time course was studied over a range of membrane potentials.

Initial experiments were designed to ascertain if the endplate channel behaviour, as measured from epcs and mepcs in cut muscle, was similar to that previously measured from mepcs in normal snake muscle (Dionne & Parsons, 1978; Fiekers *et al.*, 1979). Values of τ_o and of the voltage dependence of τ were found to be similar to those found in normal muscle. In addition lincomycin and clindamycin produced similar qualitative and quantitative effects to those seen in normal muscle (Fiekers *et al.*, 1979). Clindamycin reduced τ and the voltage sensitivity of τ and lincomycim caused the currents to decay in a double exponential manner. Thus channel activity in cut snake muscle appeared similar to that in normal muscle.

Lincomycin (5×10^{-5}–2×10^{-3} M) and clindamycin (2×10^{-5}–5×10^{-4} M) were studied on epc quantal content and on epc and mepc decay. Low concentrations of lincomycin (5×10^{-5}–5×10^{-4} M) had no effect on quantal content but produced changes in τ. Larger concentrations (10^{-3}–2×10^{-3} M) reduced quantal content and produced greater effects on τ. The minimum effective concentration of clindamycin was 2×10^{-4} M. This reduced both quantal content and τ simultaneously.

The results indicate that lincomycin can block endplate channels at lower concentrations than those required to reduce transmitter release. However, clindamycin exerts both pre- and postjunctional blocking actions in the same concentration range.

Supported by grants from N.A.T.O., Muscular Dystrophy Association of America, Carnegie Trust for the Universities of Scotland, Wellcome Trust, Scottish International Educational Trust, The Dale Fund of the Physiological Society, The Churchill University Scholarship Trust.
F.H. is supported by an M.R.C. Scholarship.

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Studies on pangamic acid—a nutritional and a therapeutic agent

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Pangamic acid (Greek: pan means universal, gamic means seed) was assigned 15th position in the Vitamin B series by its discoverers (Krebs, Krebs, Beard, Malin, Harris & Bartlett, 1951) on the basis of its presence in foodstuffs and the role it plays in a broad spectrum of physiological functions.

The pangamic acid isolated, synthesized and subjected to investigations by us conforms in all respects (C, H analysis, infra-red and NMR spectrum) with the one reported by its discoverers (Krebs *et al.*, 1951; Bukin & Garkins, 1977).

Both the naturally occurring and synthetic samples of pangamic acid have been used in our investigations. In the study on anti-hyperlipidaemic activity, pangamic acid showed a dose related decrease of 23–69% and 46–68% in serum cholesterol and triglyceride respectively in different species of animals made hyperlipidaemic by different experimental models. In the study of its anti-atherosclerotic activity in rabbits fed on 2% cholesterol and 5% saturated fat supplemented diet, pangamic acid in 50 and 100 mg/kg administered orally (p.o.) showed dose dependent prevention of atheroma development in

the aorta and deposition of fat in the different regions of the body as compared to controls.

The compound also showed anti-stress activity as evidenced by an increase of eosinophil count by only 27 and 52% and 6 and 28% with 50 and 100 mg/kg p.o. treatment in rats for 12 days, at 2 and 4 h after heat stress (immersion of one hind limb in water at 70°C for 1 min) as compared to an increase of 114 and 136% respectively in controls. In the physical performance tests, mice showed an increase of 56 and 91% in the swimming time after 14 days treatment with 50 and 100 mg/kg p.o. daily doses and rats an increase of 22 and 88% in their retention time on a rotating rod after treatment with pangamic acid, 100 and 200 mg/kg p.o. for 10 days. The rise in the lactic acid level after exercise stress was 36–52% less in the treated group (100 mg/kg p.o. single treatment 3 hrs before) as compared to controls. The P values in all the foregoing findings were less than 0.01 to 0.001. Six months chronic toxicity study completed in rats have shown pangamic acid to be free from any undesirable effect.

These results are explicable on the basis of the reported biochemical effects of pangamic acid causing stimulation of the transmethylation reaction, tissue O_2 uptake and inhibition of fatty liver formation. The foregoing findings on vitamin B_{15} support the nutritional and therapeutic status which the compound enjoys in East European countries.

Some controversial reports have appeared regarding its chemical identity and its role as a food factor or therapeutic agent are misleading as these are based on the analysis of a different chemical compound bearing the same name. It is also evidenced by the incorrect and different structural/molecular formulae recorded for pangamic acid in the 8th and 9th editions of Merck Index. It is hoped that a detailed communication of our work on this product will attract the attention of other investigators.

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The effects of the non-polar solvent, N,N-dimethylformamide, on the coaxially stimulated ileum and the taenia caecum preparations of guinea-pig

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Many novel chemical structures synthesized for pharmacological evaluation are not soluble in water, and for this reason organic solvents are employed as the vehicle in preliminary investigations. Although there is a substantial literature on the effects of non-polar solvents in intact animals (see, for example, Budden, Kühl & Bahlsen, 1979), less has been published on isolated organ experiments. As we have previously used N,N-dimethylformamide (DMF) to administer isatogens to tissues (Foster, Hooper, Spedding, Sweetman & Weetman, 1978), we now report the effects of this solvent on two isolated preparations.

Coaxially stimulated guinea-pig ileum preparations (0.1 Hz, 0.3 ms pulses, maximal voltage) were suspended in Krebs's solution at 37°C, which was gassed with 95% O_2 and 5% CO_2, and the tension in the muscle was monitored with a Dynamometer UF1 force-displacement transducer and Washington 400 MD2C recorder. Periodically, the stimulator was switched off and acetylcholine was added in a concentration selected to match the response to electrical stimulation. Taenia were dissected from the guinea-pig caecum and suspended in isolated organ baths containing McEwen's solution at 35°C and gassed with 95% O_2 and 5% CO_2. Isotonic relaxations of the taenia were recorded on a smoked drum. In all experiments, full concentration-response curves to agonist drugs were obtained cumulatively before DMF was added to the bath, and again after 30 min contact with the solvent.

DMF (16.3–130 mM, i.e. 0.125–1.0% v/v) initially increased the response of the ileum to coaxial stimulation (n = 8), but the enhanced response gradually declined towards the original height 5–30 min after the application of the solvent. In contrast, these concentrations of DMF reduced the response to exogenous acetylcholine in a concentration-related manner. The initial enhancement of the twitch

response of the ileum to electrical stimulation produced by DMF was reversible on washing, and was not tachyphylactic. A higher concentration of DMF (260 mM) reduced the contraction of the ileum to electrical stimulation by 15% and virutally abolished the response to acetylcholine (n = 8). The reduction of the twitch response of the ileum to coaxial stimulation by the addition of either noradrenaline (3 nM–7 μM) or adenosine (100 nM–100 μM) was not modified by up to 130 mM DMF (n = 7).

DMF (65 mM) relaxed 5 of 12 taenia (< 30% maximal relaxation relative to noradrenaline in those preparations that responded), whereas at higher concentrations (130 mM–1.3 M) all preparations relaxed (n = 9). There was no change in the sensitivity of the taenia to the relaxant effects of noradrenaline with DMF (65–130 mM, n = 18), but ATP-induced relaxations were increased between two and threefold (n = 11).

The results indicate that the overt effects of the non-polar solvent on the coaxially stimulated ileum and taenia represent a more sensitive indication of an action of DMF than does the modification in the response to control drugs. In general, a concentration of 65 mM DMF (0.5% v/v) did not adversely affect the preparations, although adjacent pairs of ileal segments should be employed, one for assessment of water insoluble drugs, the other to determine the effects of the vehicle.

This work was supported by a grant from the Science Research Council.

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Degradation of enkephalin by the guinea-pig ileum

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It has been reported that enkephalins are rapidly degraded *in vivo* (Dupont, Cusan, Garon, Alvarado-Urbina & Labrie, 1977). Although enkephalin can act as a substrate for a number of non-specific peptidases recent reports have suggested the existence of a specific dicarboxypeptidase 'enkephalinase' (Malfroy, Swerts, Guyon, Rogues & Schwartz, 1978). The aim of this study was to investigate the breakdown of enkephalin by the guinea-pig ileum—a tissue known to contain high concentrations of enkephalin (Hughes, Smith, Morgan & Fothergill, 1975).

Guinea-pig myenteric plexus—longitudinal muscle strips were prepared as described by Paton & Zar (1968) and mounted in 5 ml organ baths containing Krebs solution and gassed with 95% O_2–5% CO_2. The tissues were stimulated through ring electrodes at supramaximal voltage with 1 ms pulses at a frequency of 0.1 Hz. Contractions were recorded isotonically under a tension of 0.3 g. Addition to the bath of leucine enkephalin at concentrations of 10 nM–4 μM produced a dose-dependent inhibition of contraction size. If enkephalin at a concentration of 40 nM was left in contact with the tissue, the contraction size returned to original height with a $T½$ of 3.7 ± 0.7 (s.e. mean) min (n = 6). When doses of enkephalin were repeatedly added to the bath allowing time for recovery between each dose, no tachyphylaxis was observed. This suggests that the recovery is due to enzymic degradation of the enkephalin.

To study the substrate specificity of the enzymes involved a number of enkephalin analogues were examined. A standard dose of 40 nM of each enkephalin analogue was added to the bath, and the $T½$ for the contraction size to return to normal was determined. From the results it appears that D-Ala^2-leucine enkephalin ($T½$ = 13.3 ± 3 min) is more stable than leu-enkephalin ($T½$ = 3.7 ± 0.7 min). Since this analogue has been shown to be stable to cleavage of the Tyr-Gly bond (Pert & Pert, 1976) this suggests that aminopeptidase contributes to enkephalin degradation in this tissue. However, a further increase in stability was observed with D-Ala^2-D-Leu^5-enkephalin ($T½$ = 32 ± 5.9 min), suggesting the presence of a second enzyme acting at the carboxyl end of the molecule.

Della Bella, Carenzi, Frigeni & Santini (1979) have reported that the carboxypeptidase A inhibitors D-phenylalanine and hydrocinnamic acid potentiate

the effect of enkephalin on the myenteric plexus longitudinal muscle. We could find no evidence for such as effect, nor did these inhibitors at a concentration of 10^{-4} M prolong the recovery time following the addition of a standard concentration of leu-enkephalin to the bath. Enkephalin could also act as a substrate for angiotensin converting enzyme (ACE). However, the ACE inhibitors SQ14225 and SQ20881 at concentrations up to 10^{-4} M failed to increase the recovery time.

We thus conclude that aminopeptidase accounts for the major breakdown of enkephalin. However,there appears to be a second enzyme involved, the nature of which has not been determined. This additional enzyme is unlikely to be either carboxypeptidase A, or angiotensin converting enzyme, but may be similar to the specific enzyme 'enkephalinase' found in brain.

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Inhibition or induction of drug-metabolism in relation to dextropropoxyphene-induced glutathione depletion in isolated hepatocytes

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It has been suggested that hepatic glutathione plays a protective role against drug-induced liver injury (Gillette, Mitchell & Brodie, 1974) and depletion of this compound is associated with numerous toxic effects (Weddle, Hornbrook & McCay, 1976; Anundi, Hogberg & Howard, 1979), particularly those activated within the cells by drug-metabolizing enzymes. Glutathione depletion has been measured in rat isolated hepatocytes as an indicator of the possible metabolic pathway for the production of the toxic metabolite of dextropropoxyphene.

Standardized isolated cell preparations were obtained from livers of unstarved male rats (CFE), perfused *in situ*, under anaesthesia, with EGTA-collagenase in Krebs-Henseleit solution at 37°C, according to a modified method of Berry & Friend (1969). For perfusion and incubation the buffer was supplemented by glucose, serine (2.0 mM) and methionine (2.0 mM). Washed hepatocytes were quantified in terms wet cell weight and % viability was assessed by the trypan-blue exclusion test. Isolated cells were suspended in supplemented Krebs-Henseleit solution with calf serum (10% v/v) oxygenated and incubated at 37°C. Glutathione levels were measured by the method of Ellman (1959).

Glutathione levels were markedly depleted by incubation of isolated hepatocytes for 2 h with paracetamol (1–10 mM) or dextropropoxyphene (0.1–1 mM), the extent of depletion being dose-related. In hepatocytes from rats pretreated with phenobarbitone the depletion caused by paracetamol was potentiated, whereas cobalt chloride and SKF525A pretreatments inhibited the depletion. These observations were consistent with effects expected on the basis that toxicity was activated by the cytochrome P450 mediated mixed-function oxidase system. Such pretreatments did not affect the marked depletion exerted by dextropropoxyphene, nor its toxic effects on the cells. Pretreatment with diazinon (2 daily doses, 30 mg/kg) inhibited both toxicity and glutathione depletion. This suggested the production of a toxic metabolite, probably a benzylic alcohol derivative by ester hydrolysis. A similar toxic effect of a metabolite with a benzyl alcohol group was suggested by Miller & Hulbert, 1976.

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Inhibition of mitochondrial calcium transport by the nonsteroidal anti-inflammatory agent, diflunisal

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The movement of ions across the mitochondrial membrane is important in the control of cellular reactions; for example, mitochondria play a role in the maintenance of calcium levels in the cytoplasm of many cell types (Borle, 1973). The uptake of calcium, and its subsequent retention by the mitochondrion, is dependent on the presence of an intact oxidative phosphorylation system. Since diflunisal has been found to inhibit mitochondrial ATP synthesis by uncoupling the oxidation of substrates from the phosphorylation of ADP (McDougall, Markham & Sweetman, 1980), we have examined the effect of this compound on the uptake and release of calcium by mitochondria isolated from rat liver.

Calcium movements were followed using a calcium-specific electrode coupled to a Bryans 2800 pen recorder. For uptake measurements, the reaction chamber contained 0.25 mM sucrose, 10 mM Tris-HCl buffer, pH 7.4, 3.3 mM sodium succinate, 3.3 mM phosphate buffer, pH 7.4 and rat liver mitochondria (14–22 mg protein). The reaction was initiated by the addition of calcium chloride (2 μmol), to give a final volume of 10 ml. The temperature was 30°. Drugs were added 2 min before the addition of calcium chloride. The same reaction conditions were used to study calcium release, except that the mitochondria were allowed to accumulate calcium for 2 min before the addition of drugs.

Control experiments showed that when rat isolated liver mitochondria were exposed to 2 μmol calcium chloride, all the available calcium was sequestered by the mitochondria within 60 seconds. This calcium uptake was specifically inhibited by the mitochondrial calcium transport inhibitor, ruthenium red (IC_{50} = 0.12 ± 0.02 μM; n = 5), thus establishing the carrier mediated nature of the process.

Preincubation of the mitochondria with diflunisal resulted in a concentration-dependent inhibition of calcium uptake (IC_{50} = 2.5 ± 0.8 μM; n = 5). Similar results were obtained with the anti-inflammatory agent, flufenamic acid (IC_{50} = 1.2 ± 0.15; n = 5) and the uncoupling agent, 2,4-dinitrophenol (IC_{50} = 4.5 ± 1.1 μM; n = 5). In addition to this inhibition of calcium uptake, the compounds were found to promote the release of calcium from mitochondria preloaded with the cation; EC_{50} values were, diflusinal 1.9 ± 0.25 μM; n = 5, flufenamic acid 0.8 ± 0.15 μM; n = 5 and 2,4-dinitrophenol 4.2 ± 0.8 μM; n = 5.

The amount of diflunisal required to inhibit mitochondrial calcium accumulation was significantly less than that needed to block mitochondrial ATP synthesis (McDougall, Markham & Sweetman, 1980). The greater susceptibility of calcium transport may be an important factor in the mode of action of diflunisal as an anti-inflammatory agent.

P.McD. is an S.R.C. student.

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Dopamine-induced natriuresis—an effect mediated via the adrenal gland?

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Carey, Thorner & Ortt (1979) have suggested that in man dopaminergic neurones exert a maximal tonic inhibition on the production and release of aldosterone. We have investigated the effects of dopamine (DA)-antagonists on DA-induced diuresis and natriuresis in the normal and adrenalectomized rat, with the aim of differentiating between renal and adrenal actions of DA.

Urine was collected for 6 h periods from male Wistar rats (University of Bath strain) as previously described (Akpaffiong, Redfern & Woodward, 1980). The DA-antagonists, flupenthixol and sulpiride were injected s.c. 2 h before the administration of s.c. DA, which marked the beginning of the 6 h collection period. As has previously been reported (Akpaffiong *et al.*, 1980) in the normal rat DA (1–30 mg/kg) produced a dose-dependent diuresis and natriuresis, accompanied by a decrease in K^+ excretion. The same doses of DA in adrenalectomized rats also produced significant diuresis and a fall in K^+ excretion, but there was no increase in Na^+ excretion above the already-elevated levels.

Administered alone to normal rats, both flupenthixol (0.25–5 mg/kg) and sulpiride (1–10 mg/kg) reduced Na^+ and K^+ excretion without affecting urine volume e.g. (mmol kg^{-1} 6 h^{-1}) control: Na^+ 0.225 ± 0.01; K^+ 0.65 ± 0.06; flupenthixol 0.5 mg/kg; Na^+ 0.101 ± 0.01; K^+ 0.33 ± 0.08; sulpiride 5 mg/kg; Na^+ 0.096 ± 0.001; K^+ 0.338 ± 0.05; all differences from controls statistically significant. In the adrenalectomized rat, only the decrease in K^+ excretion was observed.

In normal rats the diuresis induced by DA 10 mg/kg was not affected by either the DA-antagonists. On the other hand Na^+ excretion after 10 mg/kg^{-1} DA (1.23 ± 0.07 mmol kg^{-1} 6 h^{-1}) was reduced to 0.34 ± 0.02 mmol kg^{-1} 6 h^{-1} in the presence of 0.5 mg/kg flupenthixol and to 0.52 ± 0.05 mmol kg^{-1} 6 h^{-1} in the presence of sulpiride 10 mg/kg. In the adrenalectomized rat, the diuresis induced by DA was again unaffected by the antagonists, and Na^+ excretion also remained unchanged. It is interesting to note that both DA and the DA-antogonists significantly decreased K^+ excretion, an apparently additive effect in both normal and adrenalectomized rats.

These results show that it is possible to distinguish between the diuretic and natriuretic effects of DA, in that the former persists after adrenalectomy and is unaffected by DA-antagonists. The failure of the antagonists to reduce natriuresis after adrenalectomy would appear to support the hypothesis of a tonic inhibitory role for Da on aldosterone release. However, these results must be interpreted cautiously in view of the high basal level of Na^+ excretion in these animals.

We are grateful to Lundbeck & Co, A/S for the gift of flupenthixol, and to SESIF for sulpiride.

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The effect of cortisol on the response of the depolarized, calcium free mouse uterus to calcium in different stages of the oestrous cycle

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Several of the stimulatory effects of oestrogens on uterine metabolism can be inhibited by anti-inflammatory corticoids (Szego & Davies, 1969). These steroids can also affect the response of the potassium-depolarized, calcium-free uterus to calcium, when they are added directly to the tissue bath (Henry, Jackson & Knifton, 1973). Anti-inflammatory steroids disrupt the oestrous cycle of the mouse possibly by altering the sex-steroid status (Ghaffar & Jackson, unpublished data).

Vaginal smears were taken daily from virgin, Porton mice (20 g), so that the stage of the oestrous cycle could be estimated. Animals were either injected with 0.1 ml arachis oil i.p. (vehicle-only controls) or

cortisol in 0.1 ml arachis oil i.p. (1.0 mg/100 g) for 5 days. On the fifth day vaginal smears were taken and the animals were killed if they were either in oestrus or dioestrus. Cumulative log dose-response curves (DRC) to calcium were obtained on the mouse uterus with the technique described by Simonis, Ariens & Van den Broeke (1971). Contractions were recorded isotonically with a Washington transducer and MD400 pen recorder.

Some DRCs were plateau-shaped. The mean DRC for the dioestrous control group ($n = 10$) was significantly different from the oestrous control group ($P < 0.05$—tested by analysis of variance) and was shifted to left.

Cortisol made no significant difference to either DRCs obtained at oestrus or dioestrus. This is a different effect from that reported earlier (Ghaffar & Jackson, 1979), in which it was reported that cortisol given together with oestradiol produced a significantly different DRC from oestradiol alone. Perhaps this indicates that the uterus of the ovariectomized, oestrogen-treated mouse behaves differently from the mouse uterus at oestrus and may be related to the duration of oestrogen treatment.

Kimura, Kimura & Mackawa (1978) observed a plateau-like DRC in similar experiments on rat uterus, which they attributed to an interaction between extracellular and membrane bound calcium. Since we observed this type of DRC in some groups, we support their claim that this is not an artifact.

The author wishes to thank Dr J. Jackson for his advice.

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The inhibitory actions of 2O-ethyl-tyrosine analogues of oxytocin and lysine vasopressin on human myometrium *in vitro*

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2-O-alkyltyrosine analogues of oxytocin (OT) and lysine vasopressin (LVP) are antagonists of oxytocin on the non-pregnant rat uterus *in vitro* (Rudinger & Krejci, 1962; Zaoral & Sorm, 1965; Vogel & Hergott, 1963). More recent studies by Melin, Akerlund & Vilhardt (1979) have shown that the 2-O-ethyl-tyrosine analogues of oxytocin (ethyl-OT) and lysine vasopressin (ethyl-LVP) antagonize the effects of OT and LVP on human myometrial preparations *in vitro* and the effect of OT on the intra-uterine pressure of anaesthetized rats.

Human myometrial tissues were obtained at hysterectomy. Strips were prepared from the area of the utero-tubal junction and were suspended in Krebs' solution at 36.5°C, gassed with 95% O_2, 5% CO_2. When regular spontaneous contractions were recorded, dose response curves were obtained for OT and LVP both alone and in the presence of the analogues. After each administration the contractile activity of the strip was recorded for at least 10 minutes. Maximal responses were obtained at 10 μM concentration for OT and 10 pM for LVP. Both analogues showed competitive antagonism for both OT and LVP. The pA_2 values were calculated by the method of Arunlakshana & Schild (1959) and were for ethyl-LVP 6.2 ± 0.8 (OT) and 6.3 ± 0.7 (LVP) and for ethyl OT 7.3 ± 0.4 (OT) and 7.2 ± 0.5 (LVP) (ten estimations were made for each pA_2 value).

Experiments with analogues alone showed that at concentrations, which antogonized OT and LVP, they inhibited the spontaneous contractions of the myometrium in a dose-related manner. This inhibition was both reversible on washing and repeatable. This observation may support the hypothesis that the antagonism produced by the analogues may be associated both with the loss of functionality at the second amino acid in the hormone molecules and with steric hindrance. This suggests that some process essential for activity but not for binding takes place in the proximity of this site in the hormone-receptor

complex (Zhuze *et al.*, 1964; Rudinger, 1965; Cort *et al.*, 1966).

The author thanks C.W.P. Anderson for his skilful technical assistance and Dr P. Melin for the gift of the peptides.

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Effect of animals maturity on histamine receptor activity in the rat uterus

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Histamine relaxes the rat isolated uterus by stimulation of H_2-receptors (Black, Duncan, Durant, Ganellin & Parsons, 1972). The preparation is thus frequently employed in the characterization of histamine H_2-receptor agonists and antagonists (Bertaccini, Molina, Vitali & Zappia, 1979). In the experiments of Ash & Schild (1966), it was observed that the inhibitory effects of histamine were enhanced by mepyramine pretreatment. Mepyramine could potentiate the inhibitory effects of histamine in the rat uterus if histamine also stimulated excitatory H_1-receptors. The present experiments were designed to investigate the occurence of H_1-receptors in the rat uterus and the influence of animal age on the relative activities of the two functionally opposite receptor types.

Mature female albino rats (> six months old) weighing 250–300 g and immature rats (< four weeks old) weighing 35–70 g were used. Animals were given stilboestrol (100 μg/kg) subcutaneously, 24 h previously. Two uterine horns were set up in 10 ml organ baths containing de Jalon solution gassed with air at 34°C. Inhibitory action of histamine was determined by its reduction in the size of carbachol-induced contractions (Ash & Schild, 1966). Uterine contractions were recorded isotonically on smoker paper or isometrically with Bell & Howell Transducers on a Devices M19 recorder from a baseline tension of 0.5 g (mature) and 0.2 g (immature rats). Histamine (10^{-6} M) inhibited carbachol contractions in the mature uterus by 28.1 ± 1.2% (n = 8). Mepyramine (6×10^{-9} M) significantly increased this response to 55.1 ± 2.1% ($P < 0.001$). Inhibitory responses by histamine at high doses or by dimaprit, a specific H_2-receptor agonist (Parsons, Owen, Durant & Ganellin, 1977) were not enhanced by mepyramine. All inhibitory responses by histamine were abolished by metiamide (5×10^{-5} M).

In immature uteri histamine (up to 10^{-4} M) did not inhibit carbachol contractions in doses which caused near maximum inhibition of submaximal contractions in mature uteri. Indeed, histamine, (10^{-6} M) increased carbachol contractions from 53.1 ± 2.5% of maximum to 67.3 ± 3.7% (n = 6) ($P < 0.025$). The enhancement of carbachol contractions was abolished by mepyramine, (6×10^{-9} M) suggesting that the enhancement was due to excitatory H_1-receptor stimulation. Asano & Hidaka (1980) have shown that low concentrations of contractile agonists acting at different receptors can potentiate acetylcholine contractile responses in rabbit aortic strips. These experiments demonstrate the presence of H_1-(excitatory) and H_2-(inhibitory) receptors in the rat uterus. In mature rats, H_2-receptor activity predominates.

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