Prostaglandin inactivation in the perfused rat lung: active transport or facilitated diffusion?

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Prostaglandins of the E and F series are efficiently metabolized by lungs *in vivo* or *in vitro* (Piper, Vane & Wyllie, 1970). Since the enzymes responsible for PG metabolism are located in the cytoplasm it has been proposed that uptake of PGs by a transport system is a pre-requisite for their metabolism (Anderson & Eling, 1976; Eling, Hawkins & Anderson, 1977). Nothing, however, is known of the mechanisms whereby PG metabolites are released into the circulation.

We have investigated some of the properties of PG inactivation and the 'uptake' system using the rat isolated lung model.

Ventilated lungs from male Wistar rats were perfused via the pulmonary artery at 7 ml/min with Krebs solution containing 4.5% (w/v) bovine serum albumin (BSA). After exsanguination and equilibration for two minutes the lungs were perfused for 5 min with PG solutions (7–10 ng/ml) containing 0.2 μCi/ml tritium label. The pulmonary venous effluent was collected in 15 s fractions via a cannula inserted into the left ventricle. The PGs were extracted from the effluent and breakdown measured by thin layer radiochromatography.

After perfusion tissue to medium ratios (T/M) were determined by comparing aliquots of the perfusion medium with the total activity present in solubilized lung homogenates. The time course of equilibration of PG within the lung was assessed by plotting the percentage of total radioactivity recovered in each effluent sample and measuring the time taken for 50% to appear (referred to as 'wash-in $T_{\frac{1}{2}}$').

Results are summarized in Table 1. The extent of metabolism was $PGF_{2\alpha} > E_1 > D_2$, 13,14-dihydro-15-keto $PGF_{2\alpha} = 0$, and was inhibited almost totally at 5°C (except PGD_2). Replacement of sodium ions by iso-osmolar sucrose in the perfusate produced a small decrease in metabolism of PGE_1 and PGD_2 but did not affect $PGF_{2\alpha}$. Absence of BSA significantly increased and addition at 10% decreased PGE_1 metabolism but $PGF_{2\alpha}$ was less affected, as expected if binding of PGs to albumin (Gueriguian, 1976) affects pulmonary PG degradation.

Table 1 Inactivation of prostaglandins by the isolated perfused rat lung

	PG and Treatment	*n*[a]	*PG metabolism, %*[b]	*T/M*[c]	*Wash-in* $T_{\frac{1}{2}}$, *s*[d]
	Control (4.5% albumin)	11	55.0 ± 1.3	2.25 ± 0.13	70.0
	Sodium-free	3	46.3 ± 3.7*	1.46 ± 0.17*	17.0
PGD_2	5°C	3	25.7 ± 5.4*	0.35 ± 0.02*	30.0
	Control (4.5% albumin)	8	68.7 ± 1.6	1.22 ± 0.07	33.0
	Sodium-free	3	56.7 ± 3.9	1.31 ± 0.08	30.0
PGE_1	5°C	3	4.6 ± 1.6*	0.60 ± 0.04*	30.0
	Albumin-free	5	78.0 ± 1.9*	1.22 ± 0.06	49.0
	10% albumin	4	33.0 ± 8.8*	0.83 ± 0.07	23.0
	Control (4.5% albumin)	11	90.5 ± 2.1	2.25 ± 0.16	59.0
	Sodium-free	3	89.6 ± 3.8	1.46 ± 0.06*	48.5
$PGF_{2\alpha}$	5°C	3	3.2 ± 0.7*	0.58 ± 0.14*	60.0
	Albumin-free	4	89.8 ± 2.9	1.76 ± 0.06*	58.0
	10% albumin	4	85.3 ± 3.4	1.68 ± 0.09*	54.0
$KH_2F_{2\alpha}$	Control (4.5% albumin)	4	0	0.17 ± 0.01*	21.0
	Albumin-free	4	0	0.31 ± 0.03*	18.0

[a] = number of lungs perfused.
[b] inactivation of PG measured by radiochromatography.
[c] T/M = tissue to medium ratio $\frac{\text{dpm/g lung}}{\text{dpm/ml perfusate}}$.
[d] Approximate value estimated by eye from time course of total radioactivity profile in effluent.
* Difference significant with respect to control (4.5% albumin) treatment. $P < 0.05$–0.001.
$KH_2F_{2\alpha}$ = 13,14-dihydro-15-ketoprostaglandin $F_{2\alpha}$.

Our results suggest that uptake and/or binding to a tissue component are necessary for pulmonary PG breakdown, but do not distinguish which. However, the lack of sodium dependency argues against active transport processes having a major role in PG metabolism.

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The effect of prazosin upon the release of prostaglandins I_2 and E_2 from the perfused mesenteric arterial bed of the rabbit following stimulation of the adrenergic nerves

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Previous studies have shown that the release of prostaglandin (PG) I_2 and PGE_2 from the perfused mesenteric arterial bed of the rabbit increases almost two-fold following stimulation and noradrenaline administration, but remains unchanged following the administration of potassium chloride in a dose which produces comparable vasoconstriction (Horton, Pipili & Poyser, 1980; Pipili & Poyser, 1980).

These results lead to the suggestion that this increase in PG output is due to stimulation of adrenoceptors and not due to vasoconstriction *per se*. To investigate this suggestion further, the effect of the α_1-adrenoceptor blocking drug prazosin upon the release of these prostaglandins has been examined.

Male albino rabbits weighing 2–3 kg were anaesthetized with sodium pentobarbitone (30 mg/kg). The abdomen was opened and the superior mesenteric artery was cannulated. The artery was then removed along with its small resistance vessels as described by McGregor (1965). The isolated vessels were flushed with saline, transferred to a thermostatically controlled box and perfused with McEwen's solution (McEwen, 1956) bubbled with 95% O_2, 5% CO_2 at a constant rate of 5 ml/min. One min samples of the perfusate were collected on a fraction collector. A bipolar platinum electrode was placed around the periarterial nerve plexus and the nerves were stimulated at 10 Hz using supramaximal biphasic rectangular pulses, 1 ms duration for 15 seconds. After an initial 1 h period of equilibration, the tissue was stimulated; 12 min later prazosin (20 μg/ml) was introduced into the perfusing fluid and 90 min later the tissue was again stimulated. Prazosin was very difficult to wash out, therefore each tissue was stimulated only twice, once in the presence and once in the absence of prazosin. PGI_2 (in terms of 6-oxo-$PGF_{1\alpha}$) and PGE_2 content of the perfusate samples were estimated by radioimmunoassay (Poyser & Scott, 1980; Pipili & Poyser, 1980). Results were expressed as output in ng/min and were compared by a paired *t*-test.

PGI_2 (mean ± s.e.mean, $n = 4$) release, in the absence of prazosin, significantly increased from 5.8 ± 2.1 ng/min at rest, to 12.4 ± 4.3 ng/min within 2 min following nerve stimulation ($P < 0.05$). In the presence of prazosin the output (mean ± s.e., $n = 4$) of PGI_2 was similar at rest (6.8 ± 2.0 ng/min) and following nerve stimulation (5.6 ± 1.8 ng/min).

PGE_2 release (mean ± s.e. mean, $n = 4$) significantly increased following nerve stimulation from 0.3 ± 0.06 to 0.7 ± 0.01 ng/min in the absence of prazosin and also from 1.0 ± 0.05 to 1.8 ± 0.6 ng/min in the presence of prazosin.

These results suggest that combination of noradrenaline with α_1-receptors is important for the increase in the release of PGI_2 following nerve stimulation. The increase in the release of PGE_2 in the presence of prazosin suggests that this increase may be due to the stimulation of α_2-receptors, either pre- or post-synaptically, by noradrenaline.

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The effect of prostaglandins on the rabbit and rat vas deferens

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Mating increases the content of prostaglandins (PGs) in rat semen (Marley & Smith, 1975). As these PGs are produced by the vas deferens, the effect of PGs on the rat and rabbit vas deferens *in vivo* has been studied. The tissue was suspended in an organ bath containing Krebs' solution, at 37°C aerated with 95% O_2, 5% CO_2. Changes in isometric tension were recorded.

The rabbit or rat vas deferens did not respond directly to any of the prostanoids tested; viz, PGE_1, PGD_2 and PGI_2 up to 1 μg/ml, PGE_2 and $PGF_{2\alpha}$ up to 20 μg/ml, and 9,11-epoxymethano-PGH_2, 11,9-epoxymethano-PGH_2, ICI 79939 ICI 81008 up to 10 μg/ml.

The rabbit vas deferens responded to noradrenaline (NA) by producing rhythmical contractions. Functional activity (FA) was assessed by summing the increase in tension produced by each contraction during a 5 min period after adding NA. The relationship between FA and log. conc. NA was linear for the dose range 1 to 10 μg/ml (correlation coefficient $r = 0.97$). NA-induced contractions were abolished by phentolamine (10^{-7} M) and were unaffected by hexamethonium (10^{-4} M) or bretylium (10^{-5} M). In a study on 36 rabbits, PGI_2 (10 ng/ml), PGE_2 (200 ng/ml) or $PGF_{2\alpha}$ (200 ng/ml) added 30 s before or after the addition of Na (1–10 μg/ml) to the vas deferens significantly potentiated the responses ($P < 0.05$), although the degree of potentiation was much greater when the PG was added after the NA. This latter effect was studied further and, for an approximately similar shift in the log dose/response curve to the left, PGI_2 was 20 times more potent than either PGE_2 or $PGF_{2\alpha}$. Potentiation of responses to NA by PGs occurred in the presence of bretylium at a concentration (10^{-5} M) which completely inhibited contractions produced by field stimulation. PGE_1, PGE_2, PGI_2 and $PGF_{2\alpha}$ (up to 400 ng/ml) did not affect the log dose/response relationship to NA on the rat vas deferens.

Field stimulation of the rabbit vas deferens was performed at 120 V and 20 Hz, with a pulse duration of 0.8 ms for 5 s every 30 seconds. Contractions were abolished by bretylium (10^{-5} M) or phentolamine (7×10^{-6} M) but were unaltered by hexamethonium (10^{-4} M). PGE_1, PGE_2 and PGI_2 (200 ng/ml) reduced the contractions by 77 ± 4, 78 ± 5 and 58 ± 4% (mean ± s.e. mean, $n = 6$) respectively. $PGF_{2\alpha}$ had no effect. At lower frequencies of stimulation, PGs were more potent in inhibiting the response to field stimulation, with 100% inhibition occurring with 4 ng/ml PGE_1 at a frequency of 3 Hz. However at this frequency, the response was only 10% of maximum. The inhibitory effect of PGs was unaltered by propranolol (10^{-6} M) or atropine (5×10^{-7} M). On the field stimulated rat vas deferens, PGE_1 or PGE_2 (400 ng/ml) inhibited the contractions by 19 ± 2% ($n = 14$). Increasing the dose produced no further inhibition.

On the rabbit vas deferens PGE_2 produced prejunctional inhibitory and post-junctional potentiating effects, in agreement with Hedqvist (1972). PGI_2 had similar actions, but was less potent pre-junctionally and more potent post-junctionally than PGE_2.

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Ulcerative colitis: prostaglandin metabolism and the effect of sulphasalazine, 5 amino salicylic acid and indomethacin in human colonic mucosa

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It has been hypothesized that the therapeutic action of sulphasalazine and its metabolite 5 amino salicylic acid (5 ASA) in the treatment of ulcerative colitis involves an inhibition of colonic mucosal prostaglandin (PG) synthesis or, conversely an inhibition of mucosal PG metabolism (Sharon, Ligumsky, Rachmilewitz & Zor, 1978; Hoult & Moore, 1978). This apparent contradiction is partly due to observations indicating that one property of the PGs is potentially beneficial (e.g. cytoprotection) while under certain circumstances they can be diarrhoeagenic, proinflammatory and cause fluid accumulation in the bowel lumen. Moreover whereas sulphasalazine and 5 ASA inhibit PG synthesis by human colonic mucosa explants in culture, their effect on the colonic mucosal enzymes that metabolise PG in human tissue has not been reported.

Rectal mucosal biopsies (5–20 mg) were collected in liquid nitrogen from six patients with ulcerative colitis, two with Crohns disease and eleven controls (four with irritable bowel syndrome); two larger samples were obtained from hemicolectomies.

Biopsy specimens were homogenized at 4°C in phosphate buffer (0.1 M, pH 7.4) containing EDTA (20 mM) and 20% glycerol; aliquots containing 0.3–0.8 mg protein were incubated with NAD^+ (1 mM) and tritiated PGE_1 (0.0217 nmol, 1 μCi) for 1 h at 37°C. Homogenates were acidified and extracted twice with three volumes of ether. Thin layer chromatography on silica gel plates was carried out in the organic phase of the solvent system ethyl acetate, isooctane, acetic acid, water (11:5:2:10) and the plates scanned radiochromatographically. The radioactivity in zones corresponding to radioactive peaks and authentic standards was counted by standard liquid scintillation techniques; metabolism was calculated as nmoles PGE_1/mg protein.

Larger operative samples were homogenized and different concentrations of drug or control vehicle added to aliquots which were then treated as already described. The percentage substrate metabolized was compared to metabolism in control aliquots always run in parallel.

A major metabolite with an R_F value of 15 oxo PGE_1 or 15 oxo 13,14-dihydro PGE_1 (0.4) was always identified; other unidentified peaks (R_F 0.08 and 0.55) were present in some experiments.

PGE_1 metabolized (mean ± s.e. mean) in all biopsies was 0.02 ± 0.003 nmoles/mg protein. No difference between groups was noted.

Sulphasalazine dose dependently inhibited the formation of the major metabolite of [^{3}H]-PGE_1 when compared to controls. Fifty % inhibition occurred at a concentration of 25 μM and 80% with 62.5 μM; no greater effect was seen up to 500 μM. In contrast up to 2.6 mM 5 ASA was without effect. Indomethacin inhibited metabolism by 50% at 175 μM and a maximum 80% inhibition at 559 μM.

The place of mucosal PGs in ulcerative colitis and their modification by sulphasalazine remains obscure; these data suggest further effort investigating the cytoprotective action of prostaglandins in inflammatory bowel disease would be worthwhile.

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Effects of sulphasalazine on prostaglandin inactivation and synthesis in isolated lungs of guinea-pig, rat and man

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Stability of prostacyclin in plasma and its transformation by platelets to a stable spasmogenic product

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Prostacyclin (PGI_2) is rapidly hydrolyzed to 6-keto-$PGF_{1\alpha}$ in aqueous solutions at physiological temperature and pH. By contrast, no decrease in the anti-aggregatory activity of PGI_2 occurred in human plasma incubated for up to 4 h (Blasko, Nemesanszky, Szabo, Stadler & Palos, 1980). This apparent increased stability in plasma may reflect conversion of prostacyclin to 6-keto-PGE_1 (Gimeno, Stern-Borda, Borda, Lazzari & Gimeno, 1980) which is stable and has similar anti-aggregatory potency (Wong, McGiff, Sun & Lee, 1979). We have studied the fate of prostacyclin and 6-keto-$PGF_{1\alpha}$ in human plasma.

Blood obtained by venepuncture from human volunteers was anti-coagulated with 3.8% trisodium citrate (1:9, v/v). Platelet-rich or platelet-poor plasma (PRP, PPP) were prepared by differential centrifugation. In some experiments PRP was centrifuged for 1 min at 14,000 *g* and the resulting platelet pellet was resuspended in 0.9% saline. All incubations were carried out at 37°C and contained prostacyclin sodium salt (1 μg/ml). Aliquots removed at timed intervals were assayed in two ways: (a) by contraction of the rat isolated stomach strip preparation, bathed in a Krebs solution containing a mixture of antagonists to prevent the action of other spasmogens, and (b) by inhibition of ADP-induced human platelet aggregation.

Prostacyclin was rapidly inactivated in pH 7.4 50 mM Tris buffer ($T_{1/2} = 2.1 \pm 0.2$ min, $n = 4$) and pH 7.6 saline ($T_{1/2} = 3.1 \pm 0.7$ min, $n = 7$) as shown by the simultaneous loss of platelet inhibitory and smooth muscle contracting activity. However, when PGI_2 was incubated in human PRP only a gradual reduction in anti-aggregatory activity was observed ($T_{1/2} = 17.2 \pm 1.9$ min, $n = 9$), coupled with an increase in spasmogenic activity on the rat stomach strip. This peaked after 3–6 min (4.6 ± 1.7 μg PGI_2 equivalents/ml, $n = 14$) and remained in excess of 1 μg PGI_2 equiv/ml for a further 15–30 minutes.

In three experiments, incubations were acidified and extracted into ethyl acetate at the peak of biological activity on the rat stomach strip. After thin layer chromatography in ethyl acetate:2,2,4-trimethylpentane:acetic acid:water (90:50:20:50, v/v) the chromatogram was divided into 1 cm sections and each zone eluted into methanol. PGI_2-like activity (by both assays) was found in the zone corresponding to 6-keto-PGE_1. No activity was detected in samples extracted without incubation or in other regions of the chromatogram.

No increase in spasmogenic activity occurred when PGI_2 was incubated in PPP. Instead a general reduction in both anti-aggregatory ($T_{1/2} = 14.5 \pm 1.1$ min, $n = 4$) and spasmogenic activities ($T_{1/2} = 33.0 \pm 9.4$ min, $n = 4$) was observed. Interestingly, incubation of resuspended platelets with PGI_2 resulted in increased spasmogenic activity (1.4 ± 0.1 μg PGI_2 equivs/ml, $n = 17$, 3 min incubation) and a prolonged anti-aggregatory effect ($T_{1/2} = 9.4 \pm 0.3$ min, $n = 11$; cf. 4.8 ± 0.6 min in saline, $n = 7$, $P < 0.001$). When resuspended platelets were incubated without PGI_2 no inhibition of platelet aggregation or contraction of the rat stomach strip occurred.

No biological activity was observed following incubation of 6-keto-$PGF_{1\alpha}$ (1 μg/ml) with human PRP, PPP or resuspended platelets.

We conclude that human PRP and resuspended platelets (but *not* PPP) convert prostacyclin into a substance with greater biological activity on the rat stomach strip. Preliminary experiments suggest that this substance is 6-keto-PGE_1. 6-keto-$PGF_{1\alpha}$ is not transformed in this way.

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Fibrin, red cell and platelet interactions in an experimental model of thrombosis

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Insertion of a silk thread into an extracorporeal shunt placed between the carotid artery and jugular vein of a lightly heparinized rat anaesthetized with sodium pentobarbitone (50 mg/kg) results in the production of a thrombus on the thread consisting of erythrocytes, fibrin and platelets (Umetsu & Sanai, 1978). We have used a cotton thread, with similar results, and have investigated the role of platelets, major mediators of platelet aggregation, and fibrin in the formation of the thrombus. In all cases wet thrombus weight was determined directly 15 min after insertion of the shunt.

Pretreatment of the rats with rabbit anti-rat platelet anti-sera resulted in a reduction of platelet count to $20 \pm 9.1\%$ of control value and an almost complete inhibition of thrombus formation. The injection of imidazole (10 mg/kg, i.v.) and oral administration of 1-(7-carboxyheptyl)imidazole (100 mg/kg) had no effect but 1-methyl 5-(3-pyridyl)imidazole given orally (100 mg/kg) did produce a significant reduction in thrombus weight in agreement with Umetsu & Sanai (1978). Indomethacin (10 mg/kg, i.v.) and oral diclofenac (30 mg/kg) did not affect thrombus formation. The infusion of β-γ-methylene ATP, a specific inhibitor of ADP-induced primary platelet aggregation (Born & Foulks, 1977) significantly reduced the thrombus weight by $51 \pm 6.2\%$ ($P \leqslant 0.05$) when infused i.v. at 200 µg/ml/min. Ticlopidine (10 mg/kg, i.v.) was also effective leading to a $37 \pm 4.5\%$ ($P \leqslant 0.05$) inhibition of thrombus formation. The results support the notion that ADP is an important mediator for the build up of the thrombus in this model and that PGG_2, PGH_2 and TXA_2 are less important. The possibility that erythrocytes represent one source of the ADP is supported by the finding that chlorpromazine (100 mg/kg) caused a small ($22 \pm 3.6\%$) but significant ($P \leqslant 0.05$) inhibition of thrombus weight when administered orally.

The amount of thrombus decreased with increasing concentrations of circulating heparin thus demonstrating its dependence on thrombin and presumably fibrin formation. In this connection it was of interest to note that pre-soaking the thread in albumin solution (5% w/v) in contrast to a fibrinogen solution, reduced thrombus formation by $60 \pm 6.4\%$ ($P \leqslant 0.05$). Salzman, Merrill, Binder, Wolf, Ashford & Austen (1969) have shown that interaction of platelets with heparin-coated surfaces depends on a protein intermediate. If this mechanism operates in the rat, this protein could have been displaced by the albumin.

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Inhibition of platelet aggregation by AH 19437 A thromboxane receptor blocking drug

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Aggregation of human platelet rich plasma (P.R.P) in response to various stimuli is mediated by thromboxane A_2 (TXA_2) (Hamberg, Svensson & Samuelsson 1975). Antagonism of TXA_2 induced platelet aggregation has been proposed as a possible means of developing treatments for thrombotic disorders (Kam, Portoghese, Dunham & Gerrard, 1979). AH 19437 has recently been described as an antagonist of TXA_2 at receptors mediating smooth muscle contractions (Coleman, Collington, Geisow, Hornby, Humphrey, Kennedy, Levy, Lumley, McCabe & Wallis, 1980). We have therefore studied the effect of AH 19437 (pre-

incubated for 5 min at 37°C) on aggregation of human platelets in response to several stimuli.

Platelet aggregation (Born, 1962) was studied using a Payton aggregometer.

Inhibitory dose response curves for AH 19437 (10^{-7}–10^{-4} mol/l), were prepared against aggregation induced by adenosine diphosphate (ADP, 2×10^{-6} mol/l), adrenaline (4×10^{-6} mol/l), 5-hydroxytryptamine (5-HT, 5×10^{-6} mol/l), collagen (1 μg/ml), arachidonic acid (10^{-3} mol/l), PGH_2 (3×10^{-6} mol/l), TXA_2 (generated from 1.2×10^{-7} mol/l PGH_2) and the TXA_2 mimetic U46619 (3×10^{-6} mol/l) (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980). TXA_2 was prepared by incubation of PGH_2 with platelet microsomes at 0°C for 60 seconds. Aspirin (2.5×10^{-4} mol/l), treated P.R.P was used in those experiments where ADP, 5-HT, adrenaline, U46619, PGH_2 or TXA_2 were used at stimuli. Collagen and ADP induced [^{14}C] 5-HT release was measured using a method based on that of Massini & Lüscher (1971).

AH 19437 (10^{-4} mol/l) was also examined for inhibition of the enzyme fatty acid cyclooxygenase thromboxane synthetase, cAMP phosphodiesterase and prostacyclin synthetase. The effect of AH 19437 on inhibition of ADP (4×10^{-6} mol/l) induced aggregation by prostacyclin (10^{-9}–10^{-8} mol/l) was also studied.

AH 19437, alone, failed to induce shape change, aggregation or release over the concentration range 10^{-7}–10^{-4} mol/l. EC_{50} values (molar concentration of compound causing 50% inhibition of aggregation), for AH 19437 were (mean ± s.e. mean), $2.2 \pm 0.23 \times 10^{-5}$ vs collagen ($n = 13$), $2.9 \pm 0.6 \times 10^{-5}$ vs arachidonic acid ($n = 3$), $5.23 \pm 1.46 \times 10^{-6}$ vs PGH_2 ($n = 4$), $4.65 \pm 2.1 \times 10^{-6}$ vs TXA_2 ($n = 4$), and $8.5 \pm 2.1 \times 10^{-6}$ vs U46619 ($n = 6$). Aggregation induced by ADP, adrenaline or 5-HT was unaffected by AH 19437 (10^{-4} M). Collagen or ADP induced [^{14}C]-5-HT release from human platelets was inhibited in a dose related manner by AH 19437 (10^{-7}–10^{-4} mol/l). Aggregation and release induced by collagen were inhibited in parallel whilst 5-HT release induced by ADP could be totally inhibited by AH 19437 with little effect on simultaneously measured aggregation.

AH 19437 (10^{-4} mol/l) was devoid of significant inhibitory activity against fatty acid cyclooxygenase, platelet thromboxane synthetase, cAMP phosphodiesterase or prostacyclin synthetase. AH 19437 (10^{-4} M), neither antagonized nor potentiated inhibition of ADP induced aggregation by prostacyclin.

The inhibition of platelet aggregation and [^{14}C] 5-HT release by AH 19437 is consistent with the view that the compound is a specific and selective antagonist at platelet TXA_2 receptors.

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Thromboxane receptor antagonism shown by a prostanoid with a *bicyclo* [2,2,1] heptane ring

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The 9,11-ethano analogue of prostaglandin H_2 is a partial agonist on thromboxane-sensitive preparations (Jones & Wilson, 1980). Replacement of the ω-side chain of this compound with certain N-substituted iminomethyl groups results in antagonist activity at thromboxane receptors. The properties of one such analogue, (±)-5-*endo*-(6′-carboxyhex-2′Z-enyl)-6-*exo*[N-(phenylcarbamoyl)hydrazonomethyl]-*bicyclo*[2,2,1] heptane, EP 045, are reported here.

Two spirally-cut strips of either rabbit aorta or dog

saphenous vein were superfused with the effluent from a Krebs-perfused guinea-pig lung. Infusion of arachidonic acid into the pulmonary artery (25–100 μg over 30 s) resulted in uniform release of thromboxane A_2 and contraction of the assay tissues. Exposure of one preparation to EP 045 (10^{-6}–10^{-5} M) abolished its response to thromboxane A_2.

Other experiments were performed with rabbit aorta, dog saphenous vein and guinea-pig trachea preparations in conventional organ baths using the 11,9-epoxymethano PGH_2 analogue, U-46619, (Bundy, 1975) as agonist. Responses to cumulative doses of the agonist were established and then the tissue was exposed to EP 045 for 50 minutes. Responses to suitable doses of the agonist were again established. Parallel shifts of the agonist log concentration-response curves to the right were obtained on all three preparations. Affinity constants derived from Schild plots [log (dose ratio −1) versus log molar antagonist concentration] were 2.0×10^6 for rabbit aorta, 2.2×10^7 for dog saphenous vein, and 3.3×10^7 M^{-1} for guinea-pig trachea. Slopes of the Schild plots were 0.95, 0.98 and 1.00 respectively, consistent in each case with antagonism of a competitive nature. The contractile actions of noradrenaline on the dog saphenous vein and rabbit aorta and of acetylcholine on the guinea-pig trachea were unaffected by EP 045 (2×10^{-5} M).

EP 045 (4×10^{-6}–2×10^{-5} M) inhibited the aggregatory activity of 11,9-epoxymethano PGH_2 on human platelets—$K_B = 0.87 \times 10^6$ M^{-1}, slope of Schild plot = 0.98. Arachidonic acid-induced aggregation was also blocked: analysis of the platelet suspension by solvent extraction and combined gas chromatography-mas spectrometry revealed that thromboxane A_2 synthesis was not inhibited. ADP-induced aggregation was slightly inhibited by EP 045 at 4×10^{-6} M (dose ratio 1.5–1.8)—no greater inhibition was seen at 2×10^{-5}M.

In the anaesthetized guinea-pig EP 045 (0.5–2.5 mg/kg) antagonized the vasopressor and bronchoconstrictor actions of 11,9-epoxymethano PGH_2 injected intravenously. The broncho-constrictor action of histamine was unopposed. Similar results were obtained in the anaesthetized dog.

Further studies relating to the considerable differences in affinity constants for EP 045 on the four *in vitro* preparations are in progress. The problem may be one of differences in drug distribution rather than differences in receptor type.

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Imidazole derivations as inhibitors of thromboxane formation

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Comparison of the effects of thromboxane A_2 on uterine smooth muscle of rat and guinea-pig *in vitro*

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On guinea-pig uterus *in vitro*, E-series prostaglandins (PGs) are more potent than $PGF_{2\alpha}$ in causing contraction, whereas on the rat uterus *in vitro* the converse is true (Whalley & White, 1980a, b). Furthermore the PGH_2 analogue, U-46619, is a potent contractile agonist on guinea-pig but not rat uterus (Whalley & White, 1980a). Since we have suggested that U-46619 mimics the effects of thromboxane A_2 (TxA_2) (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980) we have now examined the effects of TxA_2 on guinea-pig and rat uterus.

Strips of uterine smooth muscle (longitudinal and circular) from rats in oestrus and guinea-pigs in di-oestrus were suspended for cascade superfusion at 10 ml/min with De Jalon's (rat) or Van Dyke & Hastings (guinea-pig) solution at 32°C containing indomethacin (1.4×10^{-6} mol/l) atropine (2×10^{-7} mol/l) and phenoxybenzamine (3.5×10^{-7} mol/l).

TxA_2 was prepared by incubation of PGH_2 with human platelet microsomes (Coleman *et al.*, 1980). PGH_2 was prepared by the method of Gorman, Sun, Miller & Johnson (1977).

In the first series of experiments the effects of PGE_2, $PGF_{2\alpha}$ and U-46619 were compared. By comparison of the geometric mean of the ED_{50} values of the individual dose response curves, taking ED_{50} as 50% of the achieved maximal response, on guinea-pig uterus PGE_2 (10–10,000 ng) was about 4 times more potent than U-46619 and about 30 times more potent than $PGF_{2\alpha}$ in causing contraction. In contrast, on rat uterus $PGF_{2\alpha}$ (100–30,000 ng) was 2–3 times more potent than PGE_2 and at least 16 times more potent than U-46619. In each species the orders of potency of these PGs were similar on longitudinal and circular muscle. These results are similar to those previously obtained (Whalley & White, 1980a, b). In the second series of experiments the effects of PGH_2 and TxA_2 were examined. On guinea-pig uterus PGH_2 (15–1500 ng) had about half the contractile potency of PGE_2, and TxA_2 (15–1500 ng) was slightly less potent than PGH_2. TxA_2-induced contractile activity disappeared following 2 mins incubation at 37°C, whereas that of PGH_2 was little altered under these conditions. This excludes the possibility that the activity was due to contamination of TxA_2 with unconverted PGH_2. PGH_2 and TxA_2 had little or no effect on rat uterus. Again, in each species similar results were obtained on longitudinal and circular muscle.

In conclusion, uterine smooth muscle of the guinea-pig but not the rat is contracted by TxA_2. In this respect guinea-pig uterus resembles human uterus (Wilhelmson, Lindblom, Hamberger, Samsioe, Hammarstrom, Wiqvist & Samuelsson, 1979).

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Endometrial uptake and esterification of arachidonic acid during the oestrous cycle of the guinea-pig

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Arachidonic acid constitutes the major source of prostaglandin precursor in the uterus (Leaver & Poyser, 1980). The lipids that incorporate exogenous arachidonic acid were investigated by using tissue culture, and the influence of the stage of the oestrous cycle on this distribution was studied. This was compared with the distribution of endogenous arachidonic acid in the guinea-pig uterus.

Guinea-pigs (600–900 g) were killed on days 7 and 15 of the oestrous cycle, the days of low and highest uterine prostaglandin synthesis respectively (Poyser, 1972; Blatchley *et al.*, 1972). Endometrium was cultured as described previously (Baker & Neal, 1969) for 24 h at 37°C in Medium 199 + Earles Salts (Flow) with glutamate (1%), amphotericin (2.5 μg/ml), kanamycin (30 μg/ml, Flow) and [^{3}H]-arachidonate (80 μCi/g uterus, New England Nuclear). Tritiated and unlabelled arachidonic acid (Sigma, 99% pure) were purified by t.l.c. and converted into the sodium salt (Tannenbaum, Splawinski, Oates & Nies, 1975). After the uptake incubation, tissue was washed twice with unlabelled culture medium, blotted, weighed and homogenized in 5 ml methanol. Neutral lipids and phospholipids in the homogenate were separated by silicic acid (Unisil) column chromatography using diethyl ether (150 ml) and methanol (150 ml) respectively. Column fractions were resuspended in 5 ml petroleum spirit and 100 μl withdrawn for radioactive counting. Of the [^{3}H]-radioactivity 90% was recovered after silicic acid chromatography. The remaining lipid was applied to preparative t.l.c. plates (Merck). Standard lipids were run simultaneously on each plate. Neutral lipids were eluted with chloroform/methanol/acetic acid (18:1:1), and phospholipids with chloroform/methanol/NH_3 (13:7:1). After t.l.c., lipids were detected with I_2 vapour. Radioactivity in areas corresponding to the R_F values of standard lipids was detected by scintillation counting.

After 24 h incubation of [^{3}H]-arachidonate with endometrium at 37°C, approx. 10% of radioactivity was present in the tissue. Unlabelled arachidonate decreased the tissue uptake of [^{3}H] to 80 ± 5% and 75 ± 7% at concentrations of sodium arachidonate, 50 μg/ml and 100 μg ml respectively (mean ± s.e. mean, $n = 7$). Over 95% of [^{3}H]-arachidonate taken up by the tissue was esterified to endometrial lipids. The pattern of esterification varied during the oestrous cycle. On day 7, 48 ± 4% of [^{3}H]-arachidonate was incorporated into phospholipids, compared with 67 ± 4% on day 15 (mean ± s.e. mean, $n = 13$). This represented a significant ($P > 0.001$) increase in incorporation of [^{3}H] arachidonic acid into the phospholipids on day 15, using a paired '*t*' test. [^{3}H]-arachidonate was taken up principally into two lipid classes in the endometrium, the phospholipids and the triacylglycerols. These lipids contain over 95% of endogenous uterine arachidonic acid (Leaver & Poyser, 1980), probably representing a value close to the endometrial distribution, (endometrial tissue constitutes approx. 80% of guinea-pig uterus). Of the endogenous uterine arachidonic acid 90% is esterified to phospholipids, compared with uptake of only 48% and 67% of arachidonate into the phospholipids of day 7 and day 15 endometrium. Thus there is a relatively high rate of esterification into the neutral-lipid classes, particularly triacylglycerol which contained 20–30% exogenous arachidonate, whereas esterification with cholesterol was low. The enhanced esterification of arachidonate into endometrial phospholipids on day 15 could increase the amount of substrate available for phospholipase activity at the time of highest prostaglandin synthesis by the uterus.

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Effect of various prostaglandins on the *in vitro* activation of kallikrein in rat plasma

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It has been suggested that the kallikrein-kinin system participates in a wide range of pathophysiological processes that involves the release of other substances such as the prostaglandins (PG's) which may contribute to the amplification or the attenuation of the actions of kinins (see Terragno & Terragno, 1979). There is, however, little evidence to suggest that PG's are involved in the activation of the kallikrein-kinin system. The present study reports the effects of various PG's on the *in vitro* conversion of rat plasma pre-kallikrein to kallikrein.

Blood (9 vol) was obtained by cardiac puncture from ether anaesthetized male rats (Sprague-Dawley, 200–250 g) mixed with sodium citrate (0.1 mol/l, 1 vol) and centrifuged at 2000 g for 15 min at room temperature. The synthetic chromogenic substrate H-D-Proline-Phenylalanine-Arginine-paranitroanilide (S2302, Kabi, Sweden) was used for determination of plasma kallikrein using a slightly modified manufacturers instructions (Kabi Diagnostica, 1978). 0.89 ml buffer pH 7.8 (0.05 M Tris-hydrochloride) was mixed with 10 µl plasma (determined from preliminary experiments) at 37°C in the plastic cuvette of a Beckmann Spectrophotometer (Model 25) for 120 seconds. Following this 0.1 ml of substrate was added and the rate of release of paranitroaniline read at 405 nm. In control samples incubation was carried out with drug addition. In test samples the relevant drugs added to the Tris buffer. The results are shown in Table 1.

It can be seen that all PG's used caused an increased kallikrein activity the most significant effects being with the endoperoxide analogue's U-46619 and U-44069, PGE_2 and PGI_2. Acetylcholine and 5-hydroxytryptamine were without effect. U-46619 has been demonstrated to have an identical profile to g activity to $T \times A_2$ (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980) being a potent vasoconstrictor, as is U-44069. In contrast PGE_2 and PGI_2 are vasodilators. Soya Bean Trypsin Inhibitor added to the incubate prevented the kallikrein reaction occurring suggesting that the conversion of prekallikrein to kallikrein was a specific effect. The significance of these observations is unclear at present, since generation of free kinin as a consequence of this observed increase in plasma kallikrein activity has not yet been demonstrated.

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Table 1 The effect of various prostaglandins, acetylcholine (Ach) and 5-hydroxytryptamine (5-HT) on *in vitro* plasma kallikrein activity. (Δ absorbance/min × 10^4)

		Molar concentration of drug					
Drug	*Control*	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}
U-46619	47.6 ± 2.7	54.1 ± 2.3	68.1 ± 2.0*	68.8 ± 1.2*	59.4 ± 2.4*	—	57.3 ± 2.7
U-44069	56.5 ± 4.8	69.7 ± 3.7*	82.3 ± 9.6*	118.7 ± 5.2*	87.2 ± 5.2*	85.9 ± 3.9*	80.3 ± 15.3
$PGF_{2\alpha}$	47.6 ± 2.7	—	50.7 ± 3.7	51.4 ± 1.8	57.8 ± 2.7	52.2 ± 3.1	56.7 ± 1.1*
PGE_1	47.6 ± 2.7	53.4 ± 1.8	59.0 ± 1.1*	53.3 ± 3.6	51.2 ± 3.4	—	47.3 ± 1.8
PGE_2	53.4 ± 3.3	60.2 ± 5.2	69.8 ± 1.6*	68.7 ± 3.6*	71.6 ± 1.8*	75.8 ± 3.7*	63.2 ± 5.6
PGA_2	47.6 ± 2.7	62.0 ± 1.2*	57.4 ± 1.5*	55.0 ± 3.7	56.1 ± 3.4	—	52.4 ± 1.7
PGI_2	50.4 ± 3.4	60.3 ± 1.2*	63.3 ± 2.3*	—	76.1 ± 3.1*	74.5 ± 5.3*	62.3 ± 2.4*
Ach	50.2 ± 4.4	—	—	52.9 ± 3.5	58.5 ± 5.1	53.8 ± 2.3	52.6 ± 2.7
5-HT	35.9 ± 9.5	33.0 ± 2.1	—	40.5 ± 2.3	34.6 ± 2.7	31.9 ± 3.1	40.7 ± 5.3

Results are expressed as Mean ± s.e. mean.
* $P < 0.05$ (Students t-test).
$n = 3$–7.

M&B 28,767, a potent anti-ulcer and anti-secretory analogue of 11-deoxy-prostaglandin E_1

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When administered orally to rats, several analogues of 11-deoxy-prostaglandin E_1 have been shown to reduce gastric acid secretion and to inhibit the ulceration induced by indomethacin. We have previously reported (1978) that a number of these analogues were less potent than PGE_2 in our tests. There was low selectivity between doses causing the desired effects and those causing adverse responses such as increased gastrointestinal motility. We have studied the pharmacological profile of a new analogue, M&B 28,767 [(±)11 deoxy-15 (*nat*)-16-phenoxy-ω-tetranor PGE_1] and have compared its spectrum of activity with that of 16,16'-dimethyl-PGE_2 methylester (DMPG).

Gastric acid secretion (GAS) from anaesthetized rats (Ghosh & Schild, 1958) was stimulated by a continuous i.v. infusion of pentagastrin (30 μg/kg/h). M&B 28,767 (3–15 μg/kg/h) and DMPG (3–60 μg/kg/h) were perfused through the stomach for 1 hour. They reduced GAS in a dose-related manner, the ED_{50} values being 4.3 and 15 μg/kg/h respectively. In conscious rats possessing indwelling gastric cannulae, oral doses of M&B 28,767 (0.025–0.100 μg/kg) and DMPG (0.25–1.00 μg/kg) caused a prolonged inhibition of pentagastrin-stimulated GAS. M&B 28,767 was 17 times more potent than DMPG; the respective ED_{50} values were 0.036 and 0.60 μg/kg. After a s.c. administration of M&B 28,767 (0.075–7.5 μg/kg), there was no consistent pattern of secretory response.

Gastric lesions in the rat were induced by indomethacin administered s.c. (Lee, Mollison & Cheng, 1970). 30 min later, a PG analogue or placebo was administered orally. The examination of stomachs 5.5 h after administration of indomethacin showed that M&B 28,767 (1–30 μg/kg) and DMPG (0.1–3.0 μg/kg) reduced the extent of gastric damage. M&B 28,767 was about 4 times less potent than DMPG, the respective ED_{50} values being 3.0 and 0.8 μg/kg.

The gastrointestinal side effects of these analogues were investigated in mice and dogs. Oral doses of M&B 28,767 (1000–3000 μg/kg) and DMPG (100–300 μg/kg) caused diarrhoea in mice, M&B 28,767 being about 1/10th as potent as DMPG. In another test, mice received sufficient morphine to reduce gastrointestinal motility (Christmas, 1979). M&B 28,767 (500–4000 μg/kg) and DMPG (10–40 μg/kg) overcame the constipating effect of morphine, the respective ED_{50} values being 900–1400 and 20–40 μg/kg. After their administration to Beagle dogs, M&B 28,767 (5–20 μg/kg) and DMPG (1–5 μg/kg) caused emesis and, at the higher dose, DMPG also caused diarrhoea.

M&B 28,767, a 16-phenoxy analogue of 11-deoxy PGE_1, is thus one of the most potent and selective orally active anti-secretory and anti-ulcer agents reported to date. In addition, this compound shows greater selectivity than DMPG in animal tests.

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Comparison of the gastric ulcerogenic activity of new non-steroid anti-inflammatory drugs in stressed rats

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A procedure was developed to enhance the sensitivity of the gastric mucosa to ulcerogenic NSAI drugs by exposing the animals to a short period of physical stress (i.e. cold or restraint) concurrent with drug administration (Rainsford, 1975, 1977). The stress treatment was of such short duration as to be insufficient to cause gastric mucosal damage alone, but it does interact synergistically with ulcerogenic NSAI drugs (Rainsford, 1975). This assay has now been employed to evaluate the ulcerogenic activities of over 20 new NSAI drugs which have been recently introduced for clinical use.

Gastric ulcer assays were performed in 24 h fasted Hooded rats (180–240 g body weight) of both sexes. Following oral administration of 1 ml of the aqueous drug suspensions, the animals were stressed by exposure to cold (−15°C for 35 min) (Rainsford, 1975). The animals were placed in all-steel mesh cages with 10 rats per 0.15 m^2 to ensure equal cold exposure. A standard group of rats dosed with 150 mg/kg aspirin was employed in each experiment in order to obtain reproducible data. The animals were killed 2 h after drug administration and the number and severity of gastric mucosal lesions determined as described (Rainsford, 1975).

The results (Table 1) when compared on a logarithmic basis with data on anti-oedemic activity and the clinically accepted dose ranges shows that six drugs (azapropazone, benoxaprofen, meclofenamic acid, oxaprozin, proquazone and sulindac) do not show the general trend of NSAI drugs in which gastric ulcerogenic activity is directly related to anti-inflammatory activity. These results were consistent when the data was analyzed on a mg/kg or mmol/kg dosage basis.

The correlation between gastric ulcerogenicity and anti-inflammatory activity of NSAI drugs has been suggested as being due to the effects of these drugs in inhibiting prostaglandin biosynthesis (Vane, 1971). It is possible that the above-named drugs with low gastric ulcerogenicity do so because of weak activity on prostaglandin synthesis and/or pattern of uptake of active drug into gastric mucosal cells.

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Table 1 Comparison of the gastric ulcerogenic activity of non-steroid anti-inflammatory drugs

Drug	**Anti-oedemic Activity ED_{35} (mg/kg)	Gastric Mucosal Lesions ED_{10} mg/kg	mmol/kg	Drug	**Anti-oedemic Activity ED_{35} (mg/kg)	Gastric Mucosal Lesions ED_{10} mg/kg	mmol/kg
Alclofenac	279	9.5	0.042	Isoxicam	97	217	0.65
***Aspirin	160	5.04	0.028	*Meclofenamic acid	2.0	~3000	10.0
*Azapropazone	316	240.3	0.80	Mefenamic acid	13.0	101	0.42
*Benoxaprofen	4.5	90.5	0.30	Naproxen	4.5	1.6	0.007
Bumadizon	—	35.9	0.11	Niflumic acid	68.0	93.1	0.33
Carprofen	3.0	16.2	0.077	*Oxaprozin	200	851	2.9
Diclofenac	3.5	1.02	0.0032	Phenylbutazone	52.0	25.0	0.081
Diflunisal	42.5	212	0.85	*Piroxicam	4.5	0.10	0.0003
Fenbufen	50	165	0.65	Proquazone	89.2	834	3.0
Fenclofenac	255	184	0.62	Salicylic acid	360	60.7	0.44
Fenoprofen	718	120	0.40	Sudoxicam	2.9	0.72	0.0022
Flufenamic acid	14.5	20.5	0.073	*Sulindac	5.6	70.5	0.198
Flubiprofen	0.8	2.2	0.009	Sulphinpyrazone	—	77.6	0.200
Ibuprofen	38	14.4	0.07	Suprofen		0.68	0.0026
Indomethacin	2.8	2.7	0.0076	Tolmetin	64	8.1	0.029

ED_{10} is the dose required to produce 10 gastric mucosal lesions and is derived from log dose-response curves from at least 5 animals per dosage group over ≥ 5 doses.

* denotes drugs showing departure from the relationship between ulcerogenic and anti-inflammatory activities.

** Carrageenan-oedema, 3.5 h.

*** The range of lesion numbers in rats dosed with aspirin (150 mg/kg, as standard) was 37.5–46.0.

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The effect of histamine antagonists on gastric mucosal haemorrhage in the rat

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Cimetidine has been shown to produce a dose-related reduction in gastric haemorrhage caused by reinfusion of blood during gastric ischaemia in the rat (Owen *et al.*, 1979). Since this effect could be shown in the presence of exogenous acid the mechanism of action of cimetidine was proposed to be independent of its antisecretory action. We have further investigated the underlying mechanism of this form of gastric mucosal damage and the effect of histamine antagonists on haemorrhage.

The method was essentially that described by Owen *et al.* (1979) using an *ex vivo* gastric chamber technique. Withdrawal of blood for 15 min while the

Table 1 Effect of cimetidine and mepyramine on gastric mucosal haemorrhage. Values shown are mean ± standard error n = number of observations

	Hydrogen ion back diffusion (μeq/15 min)	*Transmucosal p.d. (mV)*
	$n = 5$	$n = 5$
Control	−44 ± 2.9	−47 ± 2.5
Ischaemia	−46 ± 2.6	−40 ± 2.7
Haemorrhage	−41 ± 6.9	−33 ± 4.1
+ Cimetidine (10 μmole kg^{-1} min^{-1})		
	$n = 6$	$n = 6$
Control	−43 ± 4.2	−43 ± 1.5
Ischaemia	−46 ± 2.9	−30 ± 2.6
Haemorrhage	−53 ± 3.3	−30 ± 2.4

	Blood loss (ml)	*Protein loss (ml)*	*Volume of mucosal bathing fluid (ml)*
	$n = 7$	$n = 7$	$n = 7$
Control	0.0015 ± 0.0006	0.065 ± 0.006	3.25 ± 0.04
Ischaemia	0.0007 ± 0.0002	0.028 ± 0.004	3.04 ± 0.03
Haemorrhage	0.1987 ± 0.0354	0.670 ± 0.090	4.37 ± 0.18
+ Cimetidine (10 μmole kg^{-1} min^{-1})			
	$n = 6$	$n = 6$	$n = 6$
Control	0.0019 ± 0.0001	0.082 ± 0.0039	3.14 ± 0.06
Ischaemia	0.0007 ± 0.0002	0.044 ± 0.023	3.03 ± 0.03
Haemorrhage	0.0723 ± 0.0142	0.267 ± 0.064	3.38 ± 0.09
+ Mepyramine (0.1 μmole kg^{-1} min^{-1})			
	$n = 7$	$n = 7$	$n = 7$
Control	0.0007 ± 0.0003	0.058 ± 0.016	3.16 ± 0.06
Ischaemia	0.0007 ± 0.0002	0.024 ± 0.004	3.05 ± 0.04
Haemorrhage	0.0782 ± 0.0096	0.337 ± 0.043	3.74 ± 0.07

mucosa was exposed to HCl (100 mM) led to haemorrhage which was measured by blood loss into the mucosal bathing fluid using [^{51}Cr]-labelled erythrocytes. Samples of mucosal bathing fluid were collected after three 15 min periods comprising a control period, 15 min ischaemia and 15 min haemorrhage and analysed for H^+ content to determine rate of H^+ back diffusion. Sample volumes were recorded and transmucosal p.d. was monitored throughout the experiment using an electrode placed in the bathing fluid. The appearance of [^{125}I]-labelled HSA injected simultaneously with the [^{51}Cr]-labelled erythrocytes was also measured since a marked increase in the volume of mucosal bathing fluid was observed during haemorrhage.

The results are shown in Table 1. Gastric mucosal damage caused by ischaemia and acid was not accompanied by an increase in H^+ back diffusion either in the absence or presence of cimetidine. The fall in p.d. was small compared to that accompanying other forms of gastric damage. Cimetidine (10 μmole kg^{-1} min^{-1} i.v.) reduced haemorrhage as previously reported ($P < 0.01$) (Student's t-test), and inhibited the marked increase in mucosal bathing fluid volume ($P < 0.001$) and protein appearance ($P < 0.01$) seen during haemorrhage.

Mepyramine (0.1 μmole kg^{-1} min^{-1} i.v.) also reduced mucosal haemorrhage ($P < 0.01$), an effect which was accompanied by a reduction in appearance of [^{125}I]-labelled HSA in the bathing fluid ($P < 0.01$). Mepyramine produced a smaller reduction in the volume of the mucosal bething fluid during haemorrhage, although this was significant ($P < 0.01$). Possible mechanisms of action of histamine antagonists on haemorrhage will be discussed.

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How do benzodiazepines reduce formation of gastric stress ulcers?

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With acute treatment, benzodiazepines reduce the formation of gastric stress ulcers (Bonfils & Dubrasquet, 1969) and produce marked sedation (File, 1980). These experiments were designed to distinguish between sedative, anxiolytic and anti-ulcer actions. Acute effects of chlordiazepoxide and lorazepam were investigated on ulcers induced by cold restraint. Chronic treatment with chlordiazepoxide was included to study tolerance to anti-ulcer and sedative effects, the latter measured by locomotor activity in a holeboard (File & Wardill, 1975).

Male hooded rats (250–350 g) were starved overnight but allowed water. Immediately after injection (i.p.) each rat was placed in a restraint cage for 2 h at 4°C. The number and severity (scale 1–5) or gastric erosions were scored by two independent observers.

Single doses of chlordiazepoxide reduced ulcer formation and caused sedation (Table 1), but the magnitude of these effects was unrelated. With chronic treatment there was marked tolerance to the sedative effects, but much less to the anti-ulcer action. All doses of lorazepam caused significant sedation, but ulcers were only reduced at the highest dose.

Benzodiazepines reduce stress ulcer formation at doses that are considerably higher than required for anxiolytic activity (Cook & Sepinwall, 1975; File, 1980) or to block the pituitary-adrenal stress response (File & Peet, 1980); furthermore instead of increasing with chronic treatment, as these other effects do, some tolerance developed. Their anti-ulcer activity therefore seems unrelated to these other actions and cannot be explained purely by sedation. The possibility of a direct action of benzodiazepines on gastric secretion, so reducing ulcer formation, was investigated using the rat isolated gastric mucosa (Main & Pearce, 1978) where their effects were compared with doses of

Table 1 Effects of benzodiazepines on stress ulcers and locomotor activity. Mann-Whitney U (ulcer) and Student's *t*-test (activity): $P < 0.05$ * $P < 0.005$ ** $P < 0.001$ ***

Treatment (*n*) (*mg/kg*)	*Cold-restraint ulcers:* *Number:* ($\bar{x} \pm$ *s.e. mean*)	*Severity:* ($\bar{x} \pm$ *s.e. mean*)	*Locomotor activity:* (*% of control,* $\bar{x}$, $n = 8$)
Chlordiazepoxide:			
Control (37)	16.0 ± 2.5	25.9 ± 4.2	100
Acute: 5 (12)	24.0 ± 5.8	34.6 ± 9.6	72**
10 (12)	10.7 ± 3.3**	15.7 ± 5.6**	58**
50 (12)	4.0 ± 0.8***	5.1 ± 1.0***	6***
Chronic:			
5 days: 10 (10)	11.9 ± 4.0	21.3 ± 7.1	89
50 (10)	2.3 ± 0.8***	3.1 ± 1.0***	51**
10 days: 50 (10)	1.5 ± 0.7***	3.0 ± 1.6***	45***
Lorazepam:			
Control (21)	17.2 ± 3.5	26.6 ± 5.4	100
Acute: 0.25 (10)	14.4 ± 4.1	23.4 ± 7.4	62*
1.25 (11)	16.4 ± 3.8	23.7 ± 5.3	25***
2.50 (10)	7.5 ± 2.3*	12.6 ± 4.3*	32***

cimetidine found to be as effective against stress ulcer formation. No effect of chlordiazepoxide on responses to pentagastrin, methacholine or histamine, was observed below 2×10^{-4} M.

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Pentobarbitone and phenobarbitone interact differently with the γ-aminobutyric acid antagonists bicuculline and picrotoxin

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In view of the well-known therapeutic distinction between pentobarbitone as an anaesthetic and phenobarbitone as an anticonvulsant, a corresponding difference in mechanisms of action might be expected. As regards the interaction with responses to γ-aminobutyric acid (GABA), it is generally found that 5–20 times more phenobarbitone than pentobarbitone is required for equal effects, whether in potentiating the actions of GABA (Nicoll & Wojtowicz, 1980) or reversing GABA antagonism by bicuculline (Bowery & Dray, 1978). Such a difference correlates with the relative anaesthetic potencies of these barbiturates. MacDonald & Barker (1978), however, have shown that paroxysmal depolarizing events induced by picrotoxin in cultured mammalian neurones were abolished by phenobarbitone at concentrations which did not depress spontaneous activity, whereas pentobarbitone depressed all activity. They suggested that this

distinction could explain the anticonvulsant properties of phenobarbitone at sub-anaesthetic doses. The present experiments explore whether a similar distinction can be seen in the interactions of pentobarbitone and phenobarbitone with bicuculline and picrotoxin-sensitive responses to GABA.

Experiments were made on slices of rat cuneate nucleus from which were recorded depolarizing responses of the afferent nerve fibres to the GABA analogue muscimol (Simmonds, 1980a). Pentobarbitone (10^{-5} M) shifted the muscimol log dose/response curve to the left in a parallel fashion by 0.221 ± 0.020 (mean $\pm$ s.e. mean) log unit. A similar degree of potentiation (0.190 ± 0.016 log unit) was caused by phenobarbitone (10^{-4} M). With the barbiturate still present, the potencies of the GABA antagonists (+) bicuculline and picrotoxin were determined. pA_5 values were calculated from Schild plots for each antagonist and compared with the corresponding pA_5 values in the absence of barbiturates (Simmonds, 1980a). Neither pentobarbitone (10^{-5} M) nor phenobarbitone (10^{-4} M) caused any significant change in the potency of the GABA receptor antagonist bicuculline. The potency of picrotoxin, which acts on the GABA response mechanism rather than the GABA receptor (Simmonds, 1980a), was also not changed by pentobarbitone (10^{-5} M) but was significantly reduced by 0.240 ± 0.048 log unit in the presence of phenobarbitone (10^{-4} M). To obtain a similar reduction in picrotoxin potency with pentobarbitone, a concentration of 10^{-4} M was required and this also caused a substantial potentiation of muscimol by 0.837 ± 0.036 log unit and a significant reduction in the potency of bicuculline.

The selective reduction in the potency of picrotoxin by phenobarbitone (10^{-4} M), a concentration similar to the serum levels in anti-epileptic therapy (Ahmad, Clarke, Hewett & Richens, 1976) resembles the effect of flurazepam on this system (Simmonds, 1980b). It is conceivable, therefore, that the use of both phenobarbitone and the benzodiazepines as anticonvulsants may depend on an action at the picrotoxin site in the GABA response mechanism without a concomitant large potentiation of GABA.

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Repeated electroconvulsive shock attenuates clonidine-induced hypoactivity in both mice and rats

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Repeated but not a single electroconvulsive shock (ECS) potentiates the behavioural responses to noradrenaline (Modigh, 1975), dopamine and 5-hydroxytryptamine (Evans, Grahame-Smith, Green & Tordoff, 1976) and recently Green & Deakin (1980) have shown that normal noradrenergic function is essential for the development of the enhanced responses to the latter two monoamines.

Injection of clonidine to mice or rats produces behavioural hypoactivity, presumably by stimulation of central α_2-adrenoceptors (Drew, Gower & Marriott, 1979; Gower & Marriott, 1980) and this is attenuated by acute administration of various antidepressant drugs (Gower & Marriott, 1980). Because of this study and the known effects of ECS on brain noradrenaline systems, we have studied the effect of repeated ECS on clonidine-induced hypoactivity in mice and rats.

Experiments were performed on either adult male C57-B1-6 OLA mice (20–30 g) or male Sprague–Dawley derived rats (50–150 g). ECS (90 v and 120 v, respectively, for 1 s) was given under halothane anaesthesia via ear-clip electrodes. Clonidine-induced hypoactivity was assessed on a scale of 0–3 using 5 behavioural parameters for mice and 4 for rats, based on the method of Drew *et al.* (1979).

ECS was given to mice once daily for 10 days. Twenty four h after the final shock the hypoactivity produced by clonidine (0.1, 0.5 and 1.0 mg/kg i.p.) was markedly attenuated compared with halothane-treated controls. When individual behavioural parameters were examined, ECS significantly ($P < 0.05$ or better) attenuated passivity, tactile responsiveness, posture and gait but not body sag. Repeated ECS also inhibited the hypoactivity induced by clonidine (0.1 mg/kg) in rats.

When mice were injected with clonidine (0.1 mg/kg) either 24 h after a single electroconvulsive shock, or a single subconvulsive shock given once daily for 10 days, there were no differences between the control and experimental groups. Similarly there were no differences in the responses to clonidine (0.1 mg/kg) between mice given halothane once daily for 10 days and handled controls, when tested 24 h after the final treatment.

We therefore conclude that ECS given once daily for 10 days but not a single ECS attenuates clonidine-induced behaviour in both mice and rats and that production of the seizure is essential, since 10 subconvulsvie shocks had no effect.

The behavioural attenuation observed may be due to subsensitivity of presynaptic α_2-adrenoceptors in the brain. On the other hand repeated ECS may inhibit clonidine hypoactivity by increasing the function of post-synaptic adrenoceptors (Modigh, 1975) or altering the modulatory role of other neurotransmitter systems.

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Relation between plasma concentration of valproic acid and its anti-convulsant and behavioural effects in rats

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Recently, it has been demonstrated in experimental animals that both the anticonvulsant and neurotoxic effects of the major anti-grand mal drugs are more closely correlated with their plasma drug concentrations than with the dosages administered (Masuda, Utsai, Shiraishi, Karasawa, Yoshida & Shimuzu, 1979). This approach, relating pharmacological effect to plasma drug concentration, has been used in the present study to provide a detailed profile of sodium valproate, an anticonvulsant with a fairly broad spectrum of activity in the treatment of epilepsy.

Plasma concentrations of valproic acid were measured in rats subjected to one of four tests commonly used in the evaluation of potential anticonvulsant drugs: the maximal electroshock seizure test (MES: 150 V, 0.4 ms pulses, 0.3 s duration—applied by corneal electrodes); the maximal metrazol (leptazol) seizure test (MMS: leptazol, 80 mg/kg i.v., after Desmedt, Niemegeers, Lewi & Janssen 1976); kindled amygdaloid seizures (KAS) (Salt, Tulloch & Walter, 1980); the rotarod test (RR: 16 rpm, 30 s trial). Visual observation of ataxia (ATA) or loss of righting reflex (LRR) was also made. Sprague–Dawley rats were

used in all the tests: male 90–110 g, fasted 20 h, in the MES test; female, 90–110 g, in the MMS test; male, 350–500 g, in KAS; male, 90–110 g, fasted 20 h, in the RR test. Sodium valproate was dissolved in dist. H_2O and administered either p.o. (MES, MMS, KAS and RR) or i.p. (RR); the dosage time was 1 h and the dosage range was 50–1600 mg/kg p.o., or 100–800 mg/kg i.p. Blood was sampled by cardiac puncture or by tail bleeding immediately after each test procedure, and placed in a tube containing heparin-saline (1000 iu/ml, 50 µl/ml blood). Valproic acid was extracted from an acidified (50 µl, $2MH_2SO_4$) mixture of rat plasma (20 µl) and dist. H_2O (50 µl) into ethyl acetate (100 µl) containing 0.2 mg/ml of caprilic acid as internal standard. Assays were performed by gas liquid chromatography: 1 µl injections of extract were made onto a 1 m × 2 mm column of 3% Poly A103 on Diatomite CQ, 80–100 mesh, run at 150°C, using N_2 carrier gas. The flame ionisation detector was maintained at 250°C.

Plasma concentrations of valproic acid were measured in individual rats and compared directly with the observed behavioural effects. This permitted the determination of the critical plasma concentrations for abolition of each of the convulsive components or for the appearance of neurotoxic symptoms. In the MES test, tonic hindlimb extension was always blocked at plasma valproic acid concentrations $\geqslant$224 µg/ml while tonic forelimb extension was blocked at $\geqslant$530 µg/ml. Similar plasma concentrations were associated with a blockade of leptazol-induced hindlimb tonus (225 µg/ml) and forelimb tonus (510 µg/ml). In fully kindled rats, two seizure components were investigated: (1) amygdaloid afterdischarge duration, which was reduced by 35–82% in the concentration range 235–577 µg/ml; (2) stage 5 seizures (rearing and falling over), which were always blocked at concentrations $\geqslant$276 µg/ml. With plasma concentrations of 450–530 µg/ml the rats showed an inability to negotiate the rotarod; above 530 µg/ml the rats were markedly ataxic and always fell off the rotarod. LRR occurred at a plasma concentration of approx. 1200 µg/ml.

These results demonstrate that sodium valproate produces marked anticonvulsant and antiepileptic effects at plasma concentrations which do not induce overt neurotoxic symptoms. The protective index of sodium valproate in the rat, expressed as the ratio of the neurotoxic plasma drug concentration to the anticonvulsant plasma concentration, is approximately 2.

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The broad anticonvulsant spectrum of GABAmimetic drugs: relevance to antiepileptic drug research

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Investigations of animal models for epilepsy and of epileptic states in man suggest a central role of γ-aminobutyric acid (GABA) in these phenomena (Lloyd, Munari, Worms, Bossi, Bancaud, Talairach & Morselli, 1981). Furthermore, direct or indirect GABAmimetic drugs such as muscimol, progabide, dipropylacetate (DPA) and the benzodiazepines have been shown to be effective anticonvulsants in different models of epilepsy (Worms, Depoortere & Lloyd, 1979; Lloyd, Worms, Depoortere & Bartholoni, 1979; Pinder, Brogden, Speight & Avery, 1977; Reinhard and Reinhard, 1977).

In order to extend these observations and further to characterize the anticonvulsant spectrum of GABAmimetic drugs, we studied the activities of direct (muscimol; progabide (SL 76002); SL 75 102, the acidic metabolite of progabide), or indirect (aminooxyacetic acid, AOAA; DPA; DPAmide; gabaculine; chlordiazepoxide, CDX) GABAmimetic drugs in seven animal models of epilepsy: bicuculline-, picrotoxinin-, metrazol-, electrically- (MES) or sound-induced convulsions in mice; bicuculline- and strychnine-induced lethality in mice. From these models,

Table 1

Test drugs (Pretreatment time in h)		ED_{50}'s (mg/kg i.p.)						
		Bicuculline		*Picrotox.*[a]	*Metrazol*	*Strychn.*[a]	*MES*[a]	*Audiog.*[a]
		Clon.[b]	*Death*	*Clon.*[b]	*Clon.*[b]	*Death*	*Ton.*[c]	*Clon.*[b]
Muscimol	(0.5)	0.25	>3	1.5	1.0	0.9	>3	0.35
Progabide	(0.5)	20	65	105	30	75	75	50
SL 75 102	(0.5)	15	70	80	8	30	80	30
AOAA	(2)	8.0	20	20	9	20	>25	15
GABAculine	(2)	15	50	37	15	35	—	50
DPA	(0.5)	40	130	240	42	160	190	55
DPAmide	(0.5)	11	50	80	20	35	50	35
CDX	(0.5)	0.75	2.0	3.0	1.0	3.8	3.5	1.2

[a] Picrotox. = Picrotoxinin; Strychn. = Strychnine; Audiog. = Audiogenic Seizures; MES = Maximal Electroshock convulsions.
[b] Clon. = Clonic seizures – [c] Ton. = Tonic seizures.

bicuculline, picrotoxinin and metrazol probably act through GABAergic mechanisms, whereas this does not seem to be the case for strychnine. The mechanism of action is unknown for MES and audiogenic seizures (for review, see Woodbury, 1980).

Male mice (CD1, Charles River, France) weighing 16 to 20 g were used for most experiments. Sound-sensitive mice (DBA_2, Iffa Credo, France) weighing 10 to 12 g were used for audiogenic seizures. The methods used were described by Worms *et al* (1979), except for audiogenic seizures where mice were treated i.p. with test compounds 30 min. or 2 h (AOAA, Gabaculine) before challenge by a sound (10 KHz; 100 Db) during 30 s; mice were then observed for the occurrence of clonic seizures.

As shown in Table 1, the GABAmimetic drugs tested exhibited anticonvulsant properties in all of the models utilized, except muscimol which was inactive in the bicuculline-lethality and the MES tests.

These results contrast with those obtained with the classical antiepileptic drugs, diphenylhydantoin (DPH) and ethosuximide (ETX), which were inactive or weakly active at subtoxic doses in the picrotoxinin and metrazol tests (DPH) or, in the bicuculline-lethality and MES tests (ETX). In addition, the activities of the GABAmimetic drugs in the bicuculline, picrotoxinin, metrazol and strychnine tests are highly correlated ($r = 0.86 - 0.94$; $P < 0.001$). This suggests that: (i) a broad spectrum of activity for a given drug in all of these models is likely to be predictive of a GABAergic mechanism, (ii) a mechanism involving GABA may, in fact, be involved in the convulsant effect of metrazol and (iii) GABA mimetic drugs reverse some of the effects of glycine (strychnine sensitive)-receptor blockade.

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The effect of catechol on amino acid transmitter release from thalamic slices

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Low doses of catechol induce in rats a convulsive state in which non specific sensory stimulation gives rise to brief myoclonic jerks; at higher doses this pattern gives way to one of spontaneous convulsive activity. Whilst the latter may be due to an action on the brain-stem reticulo-spinal system (Angel & Lemon, 1973), there is evidence that the sensory myoclonic state caused by catechol involves increased transmission in the dorsal column sensory pathway at the level of the ventro-basal thalamus, which in turn may be caused by an increased excitatory inflow and a decreased inhibitory inflow to this area from cells outside it (Angel, 1969). The experiments described here were designed to establish whether these findings could be explained by a presynaptic action of catechol on neurotransmitter release.

The thalamus was dissected from frontal slices of rat brain and chopped into small (0.1 × 0.1 × 2.0 mm) slices. These were incubated with [^{3}H]-D-aspartate and [^{14}C]-GABA, collected by filtration on to glass fibre filters and superfused with warm, oxygenated Krebs-phosphate solution, containing 10 μM amino oxyacetic acid (0.5 ml/min). Fractions were collected every 3 min and the radioactivity estimated by liquid scintillation spectrometry.

Exposing the slices to 40 mM K^+ for 9 min resulted in a 95 ± 8% (s.e. mean, $n = 19$) increase in the fractional efflux rate constant for D-aspartate and a 602 ± 34% (s.e. mean, $n = 19$) increase for GABA. When Ca^{2+} was omitted from the superfusing medium and the Mg^{2+} concentration raised to 10 mM, the K^+-stimulated release of D-aspartate was reduced to 43 ± 11% (s.e. mean, $n = 5$) of control and that of GABA to 44 ± 6% (s.e. mean, $n = 5$) of control.

The effect of catechol on K^+-stimulated release of D-aspartate and GABA is shown in Table 1. The large increase in evoked D-aspartate efflux, compared to that of GABA, caused by low concentrations of catechol suggests a possible mechanism for increased excitability within the thalamus.

Table 1 The effect of catechol on K^+-stimulated D-aspartate and GABA efflux

Catechol (μM)	*K^+-stimulated efflux, % of control* *D-aspartate*	*GABA*
1	94 ± 19 (6)	90 ± 17 (6)
10	205 ± 16 (4)	122 ± 14 (4)
100	139 ± 20 (4)	104 ± 7 (4)
500	103 ± 7 (4)	89 ± 7 (4)

Slices were exposed to catechol for 6 min before and during depolarization by 40 mM K^+. Each value is the mean ± s.e. mean, with the number of experiments shown in brackets.

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Ontogeny of neurochemical markers for glutamatergic neurons in cerebellum: implications for development of kainate neurotoxicity

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Injection of nanomole quantities of kainate into the adult rat cerebellum causes a rapid loss of neurochemical markers for GABAergic neurons intrinsic to the tissue (Foster & Roberts, 1980). The granule cells, which may use glutamate as their transmitter, and project to all other cerebellar cell types, are relatively spared (Herndon & Coyle, 1977; 1980). Since kainate neurotoxicity may require the presence of a functional glutamatergic system (McGeer, McGeer & Singh, 1978), we have examined the relationship between the development of toxicity and the glutamate system in the cerebellum.

Female Wistar rats (aged 4–25 days) were anaesthetized with halothane and injected with kainate (2 μg in 1 μl 50 mM Tris-citrate buffer, pH 7.4) at a depth of 3 mm below the vermal surface. After 2 days, animals were killed either by perfusion with Susa-Heidenhain fixative and the tissues processed for light microscopy, or by decapitation, and cerebellar glutamate decarboxylase (GAD) activity determined. No loss or damage to intrinsic neurones was observed in rats younger than 10–12 days. However, after this time there was a progressive loss of inhibitory cells, accompanied by a 36% reduction in GAD activity at 10 days old, as compared with litter mate controls.

We have examined the development of both presynaptic (glutamate tissue concentrations, uptake, and release) and postsynaptic (glutamate binding to synaptic membranes, and activation of cyclic GMP) neurochemical parameters of glutamatergic innervation. The concentration of glutamate in cerebella of 4–8 day old animals was low (approx. 3.0 μmol/g wet tissue). Between ages 8 and 12 days however, there was a rapid increase to levels of 10 μmol/g, which did not differ from adult values. The uptake of L-[^{3}H]-glutamate also increased rapidly until approx. 10 days, after which there was a steady decline to adult levels. This could be attributed to a decrease in the overall capacity of the transport system since at 5 days, $K_m = 11.39 \pm 2.3$ μM and $B_{max} = 2141 \pm 243$ pmol/mg protein, while at 23 days, $K_m = 6.00 \pm 1.91$ μM, $B_{max} = 487 \pm 77$ pmol/mg protein. This decline in capacity however, contrasts with the development of the striatal glutamate uptake system (Campochiaro & Coyle, 1978). The development of the calcium-dependent endogenous glutamate release was particularly striking. In cerebellar slices from rats less than 12 days old, elevated K^+ failed to increase glutamate release above basal levels (0.07 nmol/mg tissue). By day 18 however, K^+ stimulation produced a 250% enhancement in release, and by day 21 it was further increased to 450%.

With regard to the development of postsynaptic sensitivity to glutamate, a similar pattern emerged. At ages up to 8 days, [^{3}H]-glutamate binding of <0.4 pmol/mg protein was detected. By day 16 however, this had increased to 2.5 pmol/mg protein. The stimulation of cyclic GMP levels by L-glutamate alse increased rapidly during development, with a maximal effect at days 8–12, after which the stimulation declined.

In conclusion, we have demonstrated that the development of sensitivity of the cerebellum to kainate, correlates with the glutamatergic innervation of the sensitive neurones.

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Intrastriatal injection of apomorphine and climbing behaviour in chronically cannulated mice

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Apomorphine, a dopamine agonist (Ernst & Smelik, 1966) given peripherally produces a stereotyped climbing behaviour in mice which is dose related (Protais, Costentin & Schwartz, 1976). This climbing activity is antagonized by peripheral injection of haloperidol (Puech, Simon & Boisser, 1978). The striatum and the mesolimbic system are thought to be involved in the stereotyped behaviour induced by apomorphine (Costall, Naylor & Nohria, 1979). We have chronically cannulated the striatal mesolimbic region in the mouse to examine the central biochemical events mediating apomorphine-induced climbing. This report documents the climbing response to apomorphine given centrally.

Cannulae (21 gauge) were stereotaxically implanted into the striatum of male albino mice (Manchester Medical School strain, weight 30–40 g). The mice were allowed to recover for 1 week before being used in experiments. Throughout the experimental period the behaviour of the mice appeared normal. Centrally cannulated mice climbed in the same way as non-cannulated mice in response to apomorphine injected s.c. (0.3 and 0.6 mg/kg). Each mouse received a total of 5 micro-injections; 4 different doses of apomorphine and 1 injection of vehicle (0.1% ascorbic acid in water for injection). The time between each treatment was not less than 48 h.

Apomorphine (5–50 μg) injected centrally caused a dose-dependent climbing behaviour (Table 1) measured by an automated procedure (Farrant, Thompson & Schnieden, 1977). The time of onset was less than one minute and the duration of climbing was approximately 30–40 min. Haloperidol (0.2–5 μg) given into the striatum antagonised the climbing response to apomorphine injected both peripherally (0.6 mg/kg s.c.) and into the striatum (50 μg in 2 μl). Changes in mouse behaviour, other than climbing, after intrastriatal apomorphine appeared the same as those induced by peripheral apomorphine in non-cannulated mice when assessed by the method of Irwin (1968).

Table 1 Intrastriatal apomorphine and climbing behaviour in mice

Drug	*Dose* (μg)	*Volume* (μl)	*Climbing Index* (*mean ± s.e. mean*)	*n*
Vehicle	—	2	89 ± 7	6
Apomorphine	5	1	93 ± 16	6
Apomorphine	12.5	1	249 ± 80*	6
Apomorphine	25	1	400 ± 86**	6
Apomorphine	50	2	456 ± 59**	12

Climbing Index is the integral of the force exerted by the mouse climbing measured for 30 min after intrastriatal injection. (Farrant, Thompson & Schnieden, 1977).
Significance of difference compared with vehicle * $P < 0.05$ ** $P < 0.005$.

G.G. Foote is a SRC CASE award student.

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Inhibition by apomorphine of the amphetamine-induced changes in a dopaminergic system

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Small doses of the dopamine receptor agonist, apomorphine which do not cause stereotypies, decrease the rate of dopamine synthesis and induce sedation. Both effects of the drug are believed to be mediated via direct stimulation of presynaptic dopamine receptors (autoreceptors) which have been postulated to regulate dopamine synthesis and release (Carlsson, 1975). The mechanism whereby the occupancy of these receptors affects tyrosine hydroxylation—which regulates dopamine synthesis is still controversial (Morgenroth, Walters & Roth, 1976; Zivkovic, Guidotti & Costa, 1975). In these experiments we studied the effect of small doses of apomorphine on the cytoplasmic pool of dopamine which controls the activity of tyrosine hydroxylase and which is easily releasable by amphetamine. The influence of apomorphine on this dopamine pool was assessed indirectly by measuring the effects of amphetamine on dihydroxyphenylalanine (DOPA) synthesis and tyrosine hydroxylase activity in striata of rats, and on motor activity in mice.

Male Sprague–Dawley rats (150–175 g) and mice (20–22 g) (Charles River, France) were used. Drugs were injected i.p. Motor activity in mice was measured during 60 min following the drug treatment, in activity cages equipped with photo cells. DOPA accumulation and tyrosine hydroxylase activity in rat striata were measured by the methods of Carlsson, Davis, Kehr, Lindqvist & Atack (1972) and Zivkovic, Guidotti & Costa (1974), respectively.

In mice apomorphine decreased motor activity in a dose-dependent manner ($ED_{50} \sim 0.1$ mg/kg i.p.). Apomorphine, given 5 min before amphetamine (2 mg/kg), also antagonized the increase in motor activity induced by the dopamine releasing agent ($ED_{50} \sim 0.15$ mg/kg). In rat striata, amphetamine (2.5 mg/kg) increased the rate of DOPA synthesis and antagonized haloperidol (0.25 mg/kg)-induced activation of tyrosine hydroxylase. Apomorphine (0.2 mg/kg) given 5 min before amphetamine, counteracted both the amphetamine-induced increase in DOPA synthesis and the blockade of the activation of tyrosine hydroxylase induced by haloperidol.

The results demonstrated that substereotypic doses of apomorphine effectively antagonize the behavioural and biochemical effects of amphetamine. Since the amphetamine-induced effects are mediated indirectly by the enhancement of dopamine release, these data strongly suggest that apomorphine influences the size and/or the availability of the free cytoplasmic pool of dopamine which is releasable by amphetamine. However, as apomorphine antagonized the effects of amphetamine even in the presence of haloperidol, the present data cannot be explained simply by the presynaptic dopamine receptor mechanism unless it is postulated that these receptors possess a higher affinity for apomorphine than for haloperidol. An alternative mechanism whereby apomorphine affects the cytoplasmic pool of dopamine may be direct inhibition of tyrosine hydroxylase by the drug.

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Apocodeine as a dopamine antagonist in the rabbit

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The concept of dopaminergic involvment in stereotyped movements is generally accepted; as far as the locomotor behaviour is concerned the mechanism is complicated, dopaminergic and noradrenergic pathways being both concerned (Lloyd & Hornykiewicz, 1975). The problem is even more complex because three elements combine: results are conditioned by dosage, time and mode of administration (periodicity which avoids subsensitivity) and the animal individual response. Under these conditions the apparent advantages of using behaviour tests for estimating catecholaminergic activity of a drug were lessened. Strict rules were not previously determined and applied. We have tried to find out which are the best experimental conditions: animal of choice, drug, dosage and recovery time between two administrations (see Mignot & Savini, poster communication this meeting).

Apomorphine 5 μmol/kg (1.33 mg/kg) was given i.m. to three batches of 10 female fulvous Burgundy rabbits. Methamphetamine 50 μmol/kg (7.46 mg/kg) was given by i.v. route. The antagonists were: pimozide 1 μmol/kg (0.46 mg/kg), sulpiride 25 μmol/kg (8.54 mg/kg) and apocodeine 35 μmol/kg (9.8 mg/kg), all given by i.m. route respectively, 4 h, 1.5 h and 0.25 h before agonist administration, thus corresponding to the maximal effect of each drug (Niemegeers & Janssen, 1979). At least 5 days later agonist + antagonist were injected. After another 5 days period the sensitivity of animals was tested again by injecting the same dose of agonist.

As shown in the table (Table 1) apomorphine predominately produced stereotyped responses while methamphetamine increased turning behaviour and only moderately increased stereotypy. During the second part of the assay the antagonist was administered beforehand in order to obtain a partial block of agonist effect. Stereotyped movements after apomorphine were almost completely blocked by pimozide, moderately by sulpiride and apocodeine. Turning behaviour was negligible after apomorphine and cannot be recommended as a test. Methamphetamine induced fewer stereotypies in the rabbit. Much more important was the decrease of turning behaviour by apocodeine > sulpiride > pimozide. Thus, apocodeine was the most active anti-methamphetamine drug at the dosage of 35 μmol/kg; at lower doses (0.17 μmol/kg) apocodeine had an opposite action i.e. stimulated the dopaminergic system in the rat (Baraldi *et al.*, 1979).

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Table 1 Effects of apomorphine, methamphetamine and antagonists (score in 60 min)

	Dose	*Stereotypies*	*Turning*
Apomorphine	5 μmol/kg	80.67 ± 10.05	0.86 ± 0.23
Apomorphine + Pimozide	5 μmol/kg 1 μmol/kg	2.06 ± 0.26	0
Apomorphine + Sulpiride	5 μmol/kg 25 μmol/kg	29.86 ± 0.81	0.42 ± 0.18
Apomorphine + Apocodeine	5 μmol/kg 35 μmol/kg	24.64 ± 3.1	0.24 ± 0.15
Metamphetamine	50 μmol/kg	18.13 ± 2.97	138.33 ± 16.01
Metamphetamine + Pimozide	50 μmol/kg 1 μmol/kg	14.83 ± 2.20	54.36 ± 3.12
Metamphetamine + Sulpiride	50 μmol/kg 25 μmol/kg	19.25 ± 4.38*	34.76 ± 19.80
Metamphetamine + Apocodeine	50 μmol/kg 35 μmol/kg	6.51 ± 0.51	17.96 ± 7.77

* Results frequently variable.

Yohimbine blocks dopaminergic transmission in the rat striatum

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The indole alkaloid, yohimbine, increases cerebral noradrenaline (NA) and striatal dopamine (DA) turnover in the rat, both effects thought to occur via blockade of central α_2-adrenoceptors (Andén & Grabowska, 1976). However, since yohimbine antagonizes amphetamine- and apomorphine-induced stereotyped behaviour (Boissier, Simon & Giudicelli, 1968), it might also block postsynaptic DA receptors, thus, decreasing dopaminergic transmission. This possibility was studied by investigating the effects of yohimbine on DA neurons and their target cholinergic cells, in comparison with those elicited in NA neurones.

All drugs were administered i.p. to male Sprague–Dawley rats (Charles River, France) weighing 140–160 g, at doses which refer to the free bases. The following parameters were measured: acetylcholine (ACh) (Scatton & Worms, 1979); [^{3}H]-ACh release *in vitro* (Stoof, Den Breejen & Mulder, 1979); dihydroxyphenylalanine (DOPA) (Kehr, Carlsson & Lindqvist, 1972); tyrosine hydroxylase (TH) (Zivkovic, Guidotti & Costa, 1974); 3-methoxy-4-hydroxyphenylethyleneglycol sulphate (MOPEG) (Meek & Neff, 1972); DA, NA, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) (Westerink & Korf, 1977); DA-sensitive adenylyl cyclase (Clément-Cormier, Parrish, Petzold, Kebabian & Greengard, 1975).

Yohimbine (1–10 mg/kg) increased striatal levels of HVA (ED_{50} = 2.6 mg/kg, 2 h) and DOPAC, TH activity (ED_{50} = 2.8 mg/kg, 1 h), DOPA accumulation *in vivo* following DOPA decarboxylase inhibition by NSD 1015 (ED_{50} = 2.1 mg/kg, 1 h), and DA utilization rate after TH inhibition by α-methyl-*p*-tyrosine. These effects, which reflect enhanced DA turnover, are probably not triggered by blockade of α_2-adrenoceptors as (i) clonidine (0.3 mg/kg) did not antagonize the yohimbine (3 mg/kg)-induced increase in striatal HVA levels (in contrast to its antagonism toward the enhancement of cerebral NA turnover caused by the alkaloid); (ii) the α-adrenoceptor blocking agents tolazoline (20 mg/kg), phentolamine (30 mg/kg) and prazosin (20 mg/kg) failed to increase striatal HVA concentrations at doses which enhanced cerebral MOPEG levels maximally.

It is more likely than the increase in DA turnover induced by yohimbine is due to blockade of postsynaptic DA receptors. Yohimbine (1 mg/kg) and haloperidol (0.5 mg/kg) reduced striatal ACh levels (78 and 54% of saline-treated controls, respectively), while phenoxybenzamine (10 mg/kg) and prazosin (20 mg/kg) had no effect. Yohimbine also antagonized ($IC_{50} = 10^{-6}$ M) the DA (10^{-5} M)-induced inhibition of the potassium-evoked release of [^{3}H]-ACh from striatal slices. At a dose of 3 mg/kg, yohimbine did not increase further the striatal HVA levels of rats treated with a single, maximally effective dose of haloperidol (0.5 mg/kg) (ng/g: saline 720 ± 53, haloperidol 3035 ± 149, haloperidol + yohimbine 3132 ± 121; $n = 10$). Also, the increase in HVA (+226%) and decrease of ACh levels (−33%) in striatum induced by a single injection of yohimbine (10 mg/kg) were attenuated in rats pretreated with haloperidol for 10 days (1 mg/kg/day) (+16 and −11%, respectively, $P < 0.001$, $n = 12$), indicating cross-tolerance between the two compounds. However, yohimbine (up to 10^{-4} M) did not antagonize the DA (10^{-4} M)-induced activation of striatal adenylyl cyclase, suggesting that the compound blocks D_2 receptors.

In conclusion, yohimbine, in addition to its property of blocking α_2-adrenoceptors, appears to block postsynaptic DA receptors, probably of the D_2-type.

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Six months phenothiazine treatment differentially influences distinct dopamine-mediated behaviours

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Neuroleptic treatment for prolonged periods of a rat's adult life best reflects the clinical use of such compounds, and thus has the potential to indicate mechanisms both of their enduring antipsychotic action and concurrent side effects (Clow *et al.*, 1979, Waddington & Gamble, 1980). We here describe the behavioural sequelae of 6 months administration of the phenothiazine neuroleptic trifluoperazine HCl (TPZ) to rats.

Male Sprague–Dawley rats were given TPZ via drinking water for 1 week or 6 months (2.8–4.8 mg/kg/day). Spontaneous activity was assessed using an Animex monitor, while stereotypy was assessed by rating scale before and after challenge with apomorphine (APOM, 0.15 or 1.0 mg/kg s.c.) (Waddington & Gamble, 1980). All measures were made while animals continued to receive the drug.

After 1 week of TPZ treatment Animex activity was depressed by 50% ($P < 0.01$); this was reflected in reduced ratings of behaviour before APOM challenge ($P < 0.01$). Following APOM (1 mg/kg), stereotypy responses were attenuated in TPZ animals ($P < 0.01$).

After 6 months TPZ administration, Animex activity was no longer depressed while ratings of spontaneous behaviour immediately before APOM challenge showed elevated activity in the TPZ group ($P < 0.05$). Stereotypy responses to APOM (0.15 mg/kg) were attenuated ($P < 0.01$), while responsivity to APOM (1 mg/kg) was influenced in a complex manner: APOM-induced sniffing was attenuated ($P < 0.02$) over a 50 min period after challenge, during which TPZ animals exhibited an elevated incidence ($P < 0.05$) of a paradoxical behavioural composite of gnawing while locomoting. At 2 h after APOM challenge stereotypy was elevated in the TPZ group. The overall incidence of APOM-induced gnawing was 44% in control and 80% in TPZ animals.

These results indicate: (i) spontaneous behaviour is markedly depressed by 1 week of TPZ treatment but after 6 months is increased *above* control levels. (ii) some features of APOM stereotypy are enduringly antagonised during 6 months TPZ administration while others, particularly perioral responses, are facilitated despite continuing drug intake. The results are consistent with the functional heterogeneity of multiple dopamine receptor mechanisms (Kebabian & Calne, 1979; Waddington *et al.*, 1979a) and their differential responsiveness to prolonged phenothiazine treatment. They indirectly support the contention (Kebabian & Calne, 1979; Waddington *et al.*, 1979b) that one distinct class of dopamine receptor may be involved in the pathophysiology of schizophrenia and the antipsychotic action of neuroleptic drugs.

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Differential dopamine receptor supersensitivity after chronic phenothiazine treatment in rats

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Cerebral dopamine receptors have been characterized into two groups; one type being linked to adenylate cyclase (D-1 receptor) and the other which is not (D-2 receptor) (Kebabian & Calne, 1979). It is now well established that chronic blockade of dopamine receptors by administration of neuroleptic drugs induces increased sensitivity of these receptors as measured by behavioural and biochemical tests (Clow, Jenner, Theodorou & Marsden, 1979). However, there is no indication as to whether only one type or both types of dopamine receptor change in sensitivity. The

results of this work suggest that only D-1 receptors become supersensitive after chronic blockade.

Female Porton rats were administered trifluoperazine hydrochloride in their drinking water for 4–6 months, to give a calculated intake of 0.8–1 mg/kg/day. After this time rats were treated acutely with a series of dopamine agonists, and stereotyped behaviour assessed against age-matched control animals who had received no drug in their drinking water. Drugs used were apomorphine (0.5–5 mg/kg), (+)-amphetamine (1–8 mg/kg), nomifensine (1–25 mg/kg), bromocriptine (2–20 mg/kg), lisuride (1–10 mg/kg), piribedil (10–75 mg/kg) and lergotrile (1–10 mg/kg). Neuroleptic administration was on-going at the time of the behavioural studies.

The binding of [^{3}H]-dopamine was studied in crude membrane fractions prepared from striata of trifluoperazine-treated and control rats. Dopamine, in a wide concentration range (0.1–40 nM) will bind to both D-1 and D-2 receptors. Addition of guanine nucleotides is believed to selectively couple to the adenylate cyclase linked receptor and thus distinguish the two dopamine binding sites (Creese, Usdin & Snyder, 1979). Conversely, the butyrophenone domperidone is believed to bind to D-2 receptors (Watling, Dowling & Iversen, 1979).

The behavioural responses to apomorphine, amphetamine and nomifensine were all significantly enhanced in neuroleptic-treated rats at all doses ($P < 0.05$, 0.01, Mann-Whitney U test). None of the other dopamine agonists tested caused any such potentiation, and in general, neuroleptic-treated rats were indistinguishable from control animals. The percentage increase in specific binding of [^{3}H]-dopamine (as judged in the presence of 1 μM ADTN) in striatal membranes from neuroleptic rats was considerably reduced in the presence of guanosine triphosphate (50 μM, GTP), although a significant increase in binding of [^{3}H]-dopamine was still observed in the presence of domperidone (1 μM).

The ergot drugs (lisuride, lergotrile, bromocriptine) are believed to exert most agonist activity at D-2 receptors, while apomorphine acts at both types of dopamine receptor (Kebabian & Calne, 1979). In this model only apomorphine and drugs dependent on presynaptic terminals significantly enhanced stereotyped behaviour after neuroleptic treatment; the ergot drugs usually produced similar effects in control and trifluoperazine-treated rats. In binding studies an enhancement of [^{3}H]-dopamine binding was still observed in neuroleptic-treated rats in the presence of domperidone, while a similar amount of [^{3}H]-dopamine binding was observed in the presence of GTP in both groups. These results, together with the reported enhancement of dopamine-sensitive adenylate cyclase activity in trifluoperazine-treated rats (Clow *et al.*, 1979), suggest that the D-1 receptors may predominantly become supersensitive after chronic neuroleptic therapy.

DD is a student of the Parkinson's Disease Society.

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Effect of chronic antidepressant administration on rat brain α_2-adrenoceptor sensitivity

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A number of adaptive changes occur in rat brain noradrenergic systems during long-term antidepressant treatment (Sulser, Vetulani & Mobley, 1978). The chronic administration of desipramine has been demonstrated neurochemically to be associated with the development of subsensitive α_2-adrenoceptors in rat brain (McMillen, Warnack, German & Shore, 1980; Sugrue, 1981). In both investigations the approach adopted was to study the effect of chronic antidepressant administration on the ability of a low dose of clonidine to lower rat brain content of 3-methoxy-4-hydroxyphenylethyleneglycol sulphate

(MPHG-SO_4). Low doses of clonidine have been shown to selectively activate α_2-adrenoceptors in rat brain (Andén, Grabowska & Strömbom, 1976). The clonidine-induced response was attenuated following the chronic administration of desipramine. The objective of this study was to determine if this property of desipramine was shared by other established and putative antidepressants possessing markedly different acute pharmacological profiles.

Adult male Sprague-Dawley rats were used. The following drugs were injected i.p. every 12 h for 14 days: desipramine (10 mg/kg), mianserin (10 mg/kg), iprindole (10 mg/kg), nisoxetine (10 and 20 mg/kg), trazodone (10 mg/kg) and salbutamol (5 mg/kg). Clonidine (25 μg/kg, i.p.) was injected 12 h after cessation of drug administration and the rats were killed 3 h later for the spectrophotofluorometric determination of MHPG-SO_4 (Meek & Neff, 1972). Rat brain MHPG-SP_4 levels are approx. 75% of control 3 h after this dose of clonidine.

Basal levels of MHPG-SO_4 in the brains of rats killed 12–15 h after the last injection of antidepressant were elevated by mianserin and nisoxetine (10 mg/kg) and unaltered by the other administration schedules. Of the drugs investigated, only desipramine attenuated the clonidine-induced reduction in rat brain MHPG-SO_4 content. The ability of desipramine to block the response to clonidine was dependent upon a number of factors. These included the dose of clonidine and the frequency and duration of desipramine administration. For example, the twice daily administration of desipramine (10 mg/kg) for 14 days failed to block the ability of a larger dose of clonidine (100 μg/kg, i.p.) to lower MHPG-SO_4 levels. Moreover, the response to clonidine (25 μg/kg) was unaltered by the once daily administration of desipramine (10 mg/kg) for 14 days. The ability of twice daily desipramine to attenuate the diminution in brain MHPG-SO_4 levels elicited by clonidine (25 μg/kg) was an adaptive response as indicated by the observation that, whereas treatment with desipramine for 5 days failed to modify the clonidine-induced reduction, the response to the latter was blocked by administering the antidepressant for 9 days.

In summary, of the six established and putative antidepressants investigated only desipramine, under the experimental conditions employed, elicited a change in the sensitivity of rat brain α_2-adrenoceptors.

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Drug holidays do not prevent the development of cerebral dopamine receptor supersensitivity during chronic neuroleptic administration

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Discontinuous neuroleptic therapy (drug holidays) has been suggested to be less likely than conventional continuous therapy to induce tardive dyskinesias in schizophrenic patients. We now report that the continuous chronic administration of trifluoperazine or *cis*-flupenthixol for 9 months, or the use of a drug holiday regime over the same period, both induce an equal degree of dopamine receptor supersensitivity.

Male Wistar rats received either continuous administration of trifluoperazine dihydrochloride (4.5–5.6 mg/kg/day) or *cis*-flupenthixol (0.8–1.2 mg/kg/day) in drinking water for 9 months. Other age-matched animals received a regime in which drug was withdrawn for months 2, 5 and 8 during this period. To ensure total drug intake was approximately the same for

both groups, drug holiday animals received 6.7–7.9 mg/kg/day trifluoperazine dihydrochloride or 1.3–1.6 mg/kg/day *cis*-flupenthixol in months 1, 3, 4, 6, 7 and 9. Control animals receiving normal drinking water were maintained alongside the drug treated groups. At 3, 6 and 9 months after the start of drug administration (when all animals were receiving drugs), specific striatal [^{3}H]-spiperone (0.125–4.0 nM) binding as defined using dopamine (10^{-4} M) was determined in all groups. At the same time other animals were assessed for stereotyped behaviour in response to apomorphine hydrochloride (0.5 mg/kg; s.c. 15 min previously).

At 3 months and thereafter continuous trifluoperazine administration caused an increase in the number of [^{3}H]-spiperone binding sites (B_{max}), and an increase in the dissociation constant (K_D). After 3 months drug holiday regime B_{max} was unchanged but K_D was increased. After 6 and 9 months of the drug holiday regime, both B_{max} and K_D were elevated compared to contr ! animals. After 9 months drug intake B_{max} and K_D values were 152% and 248% of control values respectively for the continuous group and 128% and 196% respectively for drug holiday animals. In both the continuous and drug holiday regimes the initial inhibition of apomorphine-induced stereotyped behaviour was reversed by 3 months and after 9 months animals receiving continuous therapy exhibited an enhanced response to apomorphine.

Continuous administration of *cis*-flupenthixol for 3 and 6 months caused an apparent decrease in B_{max} but no change in K_D. After 9 months drug intake both B_{max} (115%) and K_D (175%) were elevated compared to control animals. Similarly after 3 months drug holiday regime B_{max} was depressed and K_D unaltered, but at 6 and 9 months both parameters were increased. At 9 months B_{max} was 120% and K_D 213% of values for control animals. Stereotyped behaviour to apomorphine was almost normal in the continuous *cis*-flupenthixol group after 3 months but remained markedly inhibited in drug holiday animals. However apomorphine-induced stereotyped behaviour was enhanced after 6 and 9 months drug intake in both groups.

The data suggests that as we have previously demonstrated for continuous neuroleptic administration (Clow, Theodorou, Jenner & Marsden, 1980a, b & c), drug holiday neuroleptic regimes also induce dopamine receptor supersensitivity. The behavioural expression of the enhanced receptor function, however, depends on the level of neuroleptic administration.

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Comparison of the effect of haloperidol on excitatory responses of cortical neurones to dopamine, noradrenaline and phenylephrine

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Cortical neurones can respond both with excitation and depression to noradrenaline and dopamine applied by microelectrophoresis; on the other hand, phenylephrine evokes only excitatory responses (Bevan, Bradshaw & Szabadi, 1977; Bevan, Bradshaw, Pun, Slater & Szabadi, 1980). At least three different receptors seem to be involved in mediating these responses: the excitatory responses to the catecholamines may be mediated by both α-adrenoceptors and excitatory dopamine receptors, whereas the depressant responses to noradrenaline may be mediated by β-adrenoceptors (Szabadi, 1979). This model is supported by the observation that the neuroleptic haloperidol, a dopamine-receptor blocking agent (Goldberg, 1975), can discriminate between the excitatory responses to dopamine and noradrenaline, the response to dopamine being more susceptible to antagonism by haloperidol than the response to noradrenaline (Bevan *et al.*, 1978). We report here some further experiments in which we compared the effects of haloperidol on excitatory responses to noradrenaline and phenylephrine, and on excitatory responses to dopamine and phenylephrine.

Spontaneously active single neurones were studied in the somatosensory cortex of the halothane-anaesthetized rat. All the drugs were applied by microelec-

trophoresis. Our techniques, and our criteria for discrimination by antagonists between responses to agonists have been described elsewhere (Bevan *et al.*, 1977; 1978).

The effect of haloperidol on responses to noradrenaline and phenylephrine was compared on 14 cells. Acetylcholine was used as a control agonist. On 12 cells, the response to phenylephrine was reduced to a greater extent than the response to noradrenaline; on 2 cells there was no discrimination between the responses. Responses to acetylcholine were not affected.

The effect of haloperidol on responses to dopamine and phenylephrine was studied on 10 cells. On 8 cells, the response to dopamine was reduced more than the response to phenylephrine; on 2 cells haloperidol did not discriminate between the responses. Responses to acetylcholine were not antagonised.

The present results show that haloperidol can discriminate between excitatory neuronal responses to the three catecholamines, the order of susceptibility to the antagonistic effect of haloperidol being dopamine > phenylephrine > noradrenaline. This observation suggests that there are three pharmacologically distinct excitatory receptors on cortical neurones. The preferential effect of haloperidol on responses to dopamine is consistent with an action of these drugs at the excitatory dopamine receptor. It is an intriguing possibility that the differential effect of haloperidol on responses to phenylephrine and noradrenaline reflects a discrimination between α_1- and α_2-adrenoceptors. There is evidence that both α_1- and α_2-adrenoceptors occur in brain tissue, and that phenylephrine is a selective α_1-adrenoceptor stimulant, whereas noradrenaline has an equal affinity for both types of α-adrenoceptor (Langer, 1980). Our suggestion implies that haloperidol should have a greater affinity for α_1- than α_2-adrenoceptors; further experiments are needed to test this hypothesis.

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6(±)-Fluorotryptophan: Sleep and wakefulness in the rat

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A full paper describing this work is to appear in *Neuropharmacology*.

Microiontophoretic study of the interaction between β-adrenoceptor antagonists and 5-hydroxytryptamine in the rat brain stem

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There is evidence that propranolol antagonizes certain responses to 5-hydroxytryptamine (5-HT) at peripheral sites (Weinstock & Schechter, 1975) and it is generally assumed that some central effects of propranolol are due to 5-HT antagonism. However, the evidence for this is largely indirect (Green & Grahame-Smith, 1976) and the only direct evidence comes from receptor binding studies (Middlemiss, Blakeborough & Leather, 1977).

We have attempted to obtain direct evidence for this antagonism by studying the interaction between iontophoretically applied β-blockers and 5-HT on single neurones in the rat brain stem. Experiments were performed on urethane-anaesthetized male Wistar rats, prepared as described previously (Bradley & Dray, 1974). Five barrelled micropipettes were used, the central recording barrel containing 4 M NaCl, while the other barrels contained a selection of the following: acetylcholine chloride (0.15 M), monosodium L-glutamate (0.06 M), L-noradrenaline HCl (0.15 M), 5-hydroxytryptamine bimaleate (0.15 M), methysergide bimaleate (0.14 M), (±)-propranolol HCl (0.12 and 0.19 M), (−)-propranolol HCl (0.019 and 0.038 M), pindolol (0.02 M) and sotalol HCl (0.02 M). All drugs were used at pH 4–5, except L-glutamate which was used at pH 8.0. Only spontaneously active cells were studied.

(−)-Propranolol, (±)-propranolol and pindolol had no effect on excitatory responses of neurones to 5-HT, except where there was also a reduction in the response to a control agonist, eg acetylcholine, L-glutamate or noradrenaline, or there was a reduction in spike amplitude. However, excitatory responses to 5-HT were readily antagonized by iontophoretic application of methysergide. Neither the excitation nor inhibition produced by noradrenaline was influenced by propranolol. Sotalol was ineffective in these experiments.

Thus, no evidence could be found for a *selective* antagonism of 5-HT responses by β-adrenoceptor antagonists and the results of this study do not support the concept that they are 5-HT antagonists, at least insofar as neurones in the rat brain stem are concerned. However, the results do not exclude the possibility that an antagonism might exist at other sites in the brain or of other 5-HT responses.

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Site of interaction between morphine and 5-hydroxytryptamine-containing neurones in the rat brain

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Acute administration of morphine causes an increase in the release and turnover of 5-hydroxytryptamine (5-HT) in the central nervous system (Yarbrough, Buxbaum & Sanders-Bush, 1973; Yaksh & Tyce, 1979). Whether the interaction between morphine and 5-HT neurones occurs at cell bodies or nerve terminals is unknown. We attempted to answer this question by injecting morphine locally in different regions of the brain and measuring changes in 5-HT turnover.

Male albino rats weighing 250–350 g were anaesthetized with pentobarbitone sodium (40 mg/kg) plus ketamine (20 mg/kg), and a metal guide cannula (length 4 mm, diameter 0.9 mm) was implated into the skull at the following stereotaxic co-ordinates: for striatum: A1.5 mm and L2.5 mm, for aqueduct P4.4 mm and L 0 mm, using bregma as AP zero. After the operation, the rats were housed individually for 4–7 days in controlled environmental conditions. For all experiments, rats received an intraperitoneal injection of probenecid (200 mg/kg) at 120 min, an injection into the cannula of either morphine HCl or sodium chlor-

ide (all solutions were made isotonic) at 90 min, and a further injection into the cannula of bromphenol blue (0.2 μl) in artificial cerebrospinal fluid immediately before decapitation. The morphine or saline were injected in a volume of 0.5 μl given over a period of 4 minutes. After decapitation, the brains were rapidly excised, dissected on ice, weighed, and homogenized in 0.1 N HCl containing ascorbic acid. 5-Hydroxytryptamine and 5-hydroxyindol-3-yl acetic acid (5-HIAA) were extracted and assayed by fluorimetry (Ahtee, Sharman & Vogt, 1970).

Injection of morphine (10 μg) into the right striatum caused a significant increase in the content of 5-HIAA (1371 ± 46 ng/g) as compared to saline injection (1074 ± 38 ng/g). Since there was no change in the 5-HT content, the increase in 5-HIAA implies an increase in 5-HT turnover. No significant change in 5-HT or 5-HIAA content was observed in the left striatum (non-injected) or the anterior medulla (region of dorsal and median raphe nuclei).

Systemic administration of naloxone (1 mg/kg i.p.) 15 min before the morphine injection antagonized the effect on 5-HT turnover.

Local injection of morphine into the aqueduct caused an increase ($P < 0.01$) in 5-HT turnover in the anterior medulla. The 5-HIAA content increased from 1329 ± 82 to 1825 ± 93 ng/g. The 5-HT content was unchanged as were the 5-HT and 5-HIAA levels in striatum and spinal cord. In all regions discussed above, systemic administration of morphine (7 mg/kg s.c.) resulted in a significant increase in 5-HT turnover.

The data support the concept that morphine increases 5-HT turnover at certain sites by actions at the nerve terminals rather than at the cell bodies. Whether this action is direct or *via* other neurones is not known.

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Hypothalamo-pituitary-adrenocorticotrophic activity in the pentobarbitone and morphine-treated rat

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It is generally agreed that hypothalamo-pituitary-adrenocortical activity is impaired in rats treated with pentobarbitone and morphine (Briggs & Munson, 1955). In an attempt to determine the mechanism whereby the drug combination produces its effects experiments were done in which the concentrations of corticotrophin (ACTH) (Alaghband-Zadeh, Daly, Bitensky & Chayen, 1974) and corticotrophin releasing factor (CRF) (Buckingham & Hodges, 1977a) in the hypothalamus were determined and the functional capacities of the adenohypophysis (Buckingham & Hodges, 1977a) and hypothalamus (Buckingham & Hodges, 1977b) assessed in male rats treated with pentobarbitone and morphine.

A single injection of morphine (2 mg/100 g, i.p.) increased markedly the concentrations of ACTH in both the plasma and the adenohypophysis and the CRF content of the hypothalamus. However, when the opioid was given to rats treated 10 min previously with sodium pentobarbitone (4 mg/100 g, i.p.) its effects were substantially reduced and the hypothalamo-pituitary-adrenocorticotrophic response to stress (adrenaline, 10 μg/100 g, i.p.) was abolished. Pituitary glands removed from the drug-treated rats responded normally *in vitro*, with respect to ACTH secretion, when hypothalamic extracts prepared from control animals were added to the incubation medium. Similarly, the functional activity *in vitro* of pituitary tissue removed from control rats was not affected by the addition to the incubation medium of pentobarbitone (10^{-6} M) and/or morphine (10^{-5} M). On the other hand, hypothalami removed from pentobarbitone/morphine treated rats showed a marked reduction in their ability to secrete CRF *in vitro* in response to acetylcholine (10^{-10}–10^{-9} M) or 5-hydroxytryptamine (10^{-8}–10^{-9} M). Additionally the responsiveness of hypothalami removed from control animals to

trophic stimuli was also impaired when the organs were pre-incubated with either pentobarbitone (10^{-6}–10^{-5} M) or morphine (10^{-8} M).

The results suggest that the hypothalmus may be an important site of action of pentobarbitone and morphine in modulating the functional activity of the hypothalamo-pituitary-adrenocortical system.

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Tyrosine hydroxylase activation: a possible mechanism

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The activity of tyrosine hydroxylase in the striatum of the rat is increased 30 min after the administration of haloperidol (Zivkovic, Guidotti & Costa, 1975). This is a result of a decrease in the K_m for the pterin substrate. Using tetrahydrobiopterin in the assay medium described by Mann (1980) we have observed that the K_m decreased from 303 ± 123 to 122 ± 41 μM when striata from rats receiving haloperidol (3 mg/kg) were homogenized in pH 6.0, 0.1 M dimethyl glutarate (DMG) buffer. Using subsaturating concentrations of tyrosine (40 μM) K_s values of 782 ± 61 μM were obtained for controls and 331 ± 64 μM for haloperidol treated rats. There was no significant difference between the values for V_{max} at saturating or subsaturating levels of substrate.

In attempting to determine the effect of preparing homogenates at differing values of pH, homogenates in a weak DMG solution (20–50 mM) at pH 8.5 were incubated at pH 6.0. It was found that under subsaturating substrate conditions (40 μM tyrosine, 253 μM tetrahydrobiopterin) the observed enzyme reaction velocity decreased from 0.418 ± 0.029 to 0.309 ± 0.018 nmol mg^{-1} h^{-1} in control animals and from 0.471 ± 0.016 to 0.301 ± 0.018 nmol mg^{-1} h^{-1} in haloperidol treated animals. The K_s for tetrahydrobiopterin in striatal homogenates prepared from haloperidol treated animals increased from 414 ± 90 μM to 1580 ± 543 μM when the homogenates were prepared at pH 8.5. Similarly, in controls the K_s increased from 554 ± 184 μM to a value in the region of 1900 μM. In all cases the values for V_{max} remained unaltered indicating that when the homogenate is prepared at pH 8.5 there is no difference in the amount of enzyme obtained from control and haloperidol treated rats.

It has been shown that the methylation of proteins which occurs in the brain is reversed at pH 8.0 and above since the carboxymethyl esters hydrolyse spontaneously (Dilberto & Axelrod, 1976). It is possible that the kinetic changes caused by administration of haloperidol are a result of a methylation of the enzyme which is reversed at pH 8.5. The results also indicate that the normal (control) enzyme exists, partially at least, in the activated state. We have however been unable to rule out the possibility that we are looking at a phosphorylation rather than a methylation of the enzyme protein.

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Substance P in the developing pig

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The undecapeptide substance P is widely distributed throughout both the central and the peripheral mammalian nervous system and there is considerable evidence that substance P is a neurotransmitter in certain neuronal pathways (Nicoll, Schenker & Leeman, 1980). In the rat, substance P-containing neurones appear during embryonic life, but adult levels of substance P are not reached until 3 to 4 weeks after birth (Gilbert & Emson, 1979) when the growth spurt of the rat brain has been completed (Davison & Dobbing, 1968). Unlike the rat brain, but like the human, the brain of the pig undergoes this growth spurt mainly prenatally (Davison & Dobbing, 1968). This led us to investigate the substance P content in different tissues of the domestic pig at birth and during its postnatal development.

The tissues were taken from 'Large White' pigs and extracted with boiling acetic acid (1 M) and their content of substance P was determined by radioimmunoassay using an antiserum directed against the C-terminal pentapeptide of substance P (Lee, Emson & Iversen, 1980). Gel permeation chromatography on Sephadex G-15 of an extract of the medulla oblongata of a 24 days old piglet showed that all the immunoreactivity ran at the same position as synthetic substance P.

It was found that, in general, the tissue levels of substance P in the newborn piglets are similar to those in 3 weeks old piglets, but about twice as high as the levels in approximately 7 months old pigs (Table 1). However, two exceptions from this pattern were seen. Firstly, the substance P concentration of the duodenum had fallen already in the first three postnatal weeks to the adult level. Since in the intestine substance P is contained within neurones as well as endocrine cells, this rapid decline of the substance P content cannot be interpreted without additional histochemical data. Secondly, the substance P content of the caudate nucleus increased considerably in the first three postnatal weeks and did not fall thereafter. It would appear that the substance P-containing neurones in the caudate nucleus mature later than the other substance P-containing neurones in the brain and spinal cord.

The findings indicate that the substance P-containing neurones of the pig central nervous system are already highly developed at birth which is in keeping with the data on the morphological development of the pig brain (Davison & Dobbing, 1968). The decline of the substance P levels during postnatal development is at present not understood and requires further investigation.

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Table 1 Concentrations of substance P (pmol/g tissue) in different tissues of the developing pig

Tissue	*Age* 0–5 days	16–24 days	180–220 days
Duodenum	35.6 ± 5.9 (7)	11.6 ± 1.9 (11)	9.6 ± 1.0 (7)
Saphenous nerve	246 ± 20.0 (8)	225 ± 21.5 (6)	89.8 ± 8.2 (6)
Dorsal root ganglia	113 ± 17.8 (7)	120 ± 19.3 (7)	57.1 ± 10.4 (5)
dorsal half	819 ± 49.7 (8)	973 ± 70.5 (6)	553 ± 48.2 (6)
Lumbar spinal cord ventral half	155 ± 17.1 (8)	117 ± 9.6 (6)	37.1 ± 4.5 (6)
Medulla oblongata	401 ± 38.6 (7)	341 ± 62.3 (5)	175 ± 8.9 (5)
Midbrain	461 ± 27.4 (6)	425 ± 21.6 (4)	242 ± 14.1 (4)
Caudate nucleus	226 ± 17.8 (7)	300 ± 24.5 (5)	274 ± 26.0 (6)
Frontal cerebral cortex	21.5 ± 2.2 (7)	19.3 ± 2.4 (6)	14.8 ± 2.6 (6)

All values are given as means ± s.e. mean with the number of determinations in parentheses.

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The effect of flupenthixol isomers on [^{3}H]-5-HT uptake into blood platelets

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Cis α flupenthixol is a potent anti-psychotic agent and dopamine receptor blocker while the trans β isomer is inactive in both respects (Crow & Johnstone, 1978). The drug is administered as a 1:1 mixture of the two isomers and as such is an effective antischizophrenic and antidepressant compound (Johnson, 1979). We have compared the effects of the two isomers separately with the effects of chlorimipramine on active 5-HT uptake into human platelets to establish if the flupenthixol isomers are similar to tricyclic antidepressants in terms of inhibition of amine uptake and also to determine the relative potency of the flupenthixol isomers. Platelet rich plasma was prepared from 20 ml human venous blood collected into 2 mls tri sodium citrate. (The same two volunteers were used throughout the study). The active uptake characteristics of [^{3}H]-5HT into human platelets were studied over 0–20 s using the method of Gordon & Olverman (1978). [^{3}H]-5-HT uptake was expressed as pmol 5-HT/10^8 cells. For studies of uptake inhibition, platelets were incubated with [^{3}H]-5-HT (0.5 μM–0.58 Ci/mmol) for 10 s as active uptake using this final incubation concentration was linear with respect to time over at least a 10 s incubation time. The uptake of [^{3}H]-5-HT was then determined in the presence or absence of (a) chlorimipramine (1×10^{-9}–1×10^{-6} M) (b) cis flupenthixol (1×10^{-8}–1×10^{-4} M) or (c) transflupenthixol (1×10^{-8}–1×10^{-4} M). The concentration of each compound producing a 50% inhibition of 5-HT uptake ($1C_{50}$) was determined by examination of the respective percentage inhibition $\log_{10}$ drug concentration curves. The most potent inhibitor of uptake was chlorimipramine ($1C_{50}$ 8×10^{-7} M) while cis and trans flupenthixol were approximately equipotent with $1C_{50}$'s of 5×10^{-4} M and 4×10^{-4} M respectively.

It is concluded that though flupenthixol is a potent antidepressant (Johnson, 1979) each of its isomers is only a weak inhibitor of platelet [^{3}H]-5-HT uptake.

Flupenthixol may exert an inhibitory effect on the uptake of other amines or may be like a small number of new antidepressant compounds which have little effect on amine re-uptake mechanisms.

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