## Acute effects of MPV-295 on the turnover of brain noradrenaline in rats

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MPV-295, [4(5)-2-(2,6 dimethylphenyl) ethyl-imidazole hypotensive is after i.v. injection in rats, but much lower doses are effective if it is injected directly into the third ventricle (Ruskoaho, Puurunen & Kärki, 1981). Since MPV-295 rapidly penetrates into the brain after i.v. injection, it was decided to compare MPV-295 with clonidine, an anti-hypertensive substance whose central effects contribute to its effectiveness.

Male Wistar rats (150–200 g) were injected i.p. 4 or 4.25 h prior to sacrifice, all compounds being dissolved in saline. Brain noradrenaline (NA), dopamine (DA), serotonin and 3-methoxy-4-hydroxyphenylethyleneglycol sulphate (MOPEG-$SO_4$), the principal metabolite of NA in the rat brain, were determined fluorometrically. The turnover of NA and DA in brain was assessed by measuring the decay in their concentrations after administration of α-methyl-*p*-tyrosine methyl ester (α-MPT, 250 mg/kg 4 h before sacrifice). The turnover of brain NA was also indirectly studied through changes in brain MOPEG-$SO_4$ levels.

There was little change in the endogenous concentrations of brain biogenic amines 4 h after administration of MPV-295 (100 μg/kg – 50 mg/kg) or clonidine (30–300 μg/kg). Clonidine slowed the decrease in NA levels after α-MPT at all the doses studied. In contrast, MPV-295 had no inhibitory influence on the α-MPT effect at doses 100 μg/kg – 1 mg/kg. Higher doses (5–50 mg/kg) inhibited the α-MPT response by about 20% which was similar to that obtained with clonidine 100 μg/kg. It was consistently observed that only those doses which inhibited the α-MPT effect caused sedation shortly after injection. Sedation was apparent with all clonidine doses and the very high doses of MPV-295. Neither MPV-295 nor clonidine, at any of the doses studied, were able to inhibit the decrease in brain DA levels induced by α-MPT.

Clonidine caused a dose-dependent decrease in brain MOPEG-$SO_4$ concentrations, a dose of 100 μg/kg causing a 43% decrease which was statistically significant ($P < 0.001$). MPV-295 at doses up to 1 mg/kg, did not alter brain MOPEG-$SO_4$ levels, but at 10 mg/kg a decrease of 40% was observed ($P < 0.001$). MPV-295, at doses which did not themselves alter MOPEG-$SO_4$ levels was incapable of inhibiting the decrease caused by clonidine.

In conclusion, MPV-295 and clonidine differ quantitatively in their effects on brain NA turnover. MPV-295 shows hypotensive effects at doses which do not alter brain NA turnover and this is only inhibited at doses several hundred times greater than therapeutic levels. Sedation, which is a well recognized side effect of clonidine, seemed to correlate well with inhibition of brain NA turnover.

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## Neuroleptic resistant motor change following striatal dopamine denervation

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Interruption of mesolimbic and striatal dopamine (DA) systems in the rodent can modify motor responsiveness to DA agonists. However, effects of such denervation on the interaction between DA agonists and antagonists has not been so closely studied. We report here on a method for selective striatal DA denervation in the rat and the behavioural and biochemical consequences, with particular reference to the development of neuroleptic resistant motor behaviour.

Stereotaxic surgery was carried out on male Sprague-Dawley rats (350 ± 20 g) anaesthetized with chloral hydrate. 6-Hydroxydopamine (6-OHDA) or vehicle was acutely infused in 4 μl, 1 μl/min, at 3 striatal locations (Ant. 8.8, Vert. +1.0, Lat. ±3.0;

Ant. 7.8, Vert. +1.0, Lat. ±3.0; Ant. 6.8, Vert. +2.0, Lat. ±3.0; De Groot, 1959). Three dose regimes of 6-OHDA (2, 4 or 8 μg/μl) were used in control and desmethylimipramine (DMI) plus tranylcypromine pretreated rats, which were then implanted with chronically indwelling bilateral guide cannulae to allow subsequent drug injection at the centre of the striatum. Other groups of rats were subject to unilateral striatal electrolesions (1.5 mA for 15 s) at the above 3 coordinates. The development of spontaneous or drug induced circling (rev/min) and asymmetry was observed over a minimum 6 month period.

6-OHDA (4 μg/μl) injected at the 3 striatal sites was the minimum concentration to cause consistent behavioural changes (single injections were essentially ineffective; the pretreatment with tranylcypromine/DMI enhanced the behavioural and biochemical changes, DA depletions being in excess of 80%). Immediately on recovery from anaesthetic, 6-OHDA treated rats showed ipsilateral asymmetry/circling which was converted to a contralateral asymmetry/circling following the subcutaneous administration of apomorphine (0.0125–0.25 mg/kg), SK & F 38393 (0.625–10 mg/kg) and 2-(N,N-dipropyl)amino-5,6-dihydroxytetralin (0.00063–0.01 mg/kg); maximum responses developed within 8–10 days of denervation. Active circling was exhibited by 67–83% of animals. Rats having unilateral striatal electrolesions responded with ipsilateral asymmetry/circling on challenge with larger doses of apomorphine (0.1–0.5 mg/kg), SK & F 38393 (2.5–10 mg/kg) and the tetralin (0.005–0.01 mg/kg). Intrastriatal DA elicited asymmetry/circling from 6-OHDA denervated and normal striata at minimum doses of 12.5 and 200 μg respectively. Methysergide (5 mg/kg i.p.), atropine (5 mg/kg i.p.), phentolamine (10 mg/kg i.p.) and propranolol (10 mg/kg i.p.) failed to antagonize the asymmetry/circling induced by submaximal doses of apomorphine in electro- of 6-OHDA-lesioned rats. In the electrolesioned rats the apomorphine response was completely antagonized by low doses (0.1 mg/kg i.p.) of a classical neuroleptic such as haloperidol. In contrast, the asymmetry caused by apomorphine in the 6-OHDA-lesioned rats was resistant to neuroleptic antagonism, even when used at cataleptic doses (2.0 mg/kg i.p. haloperidol, 0.5 mg/kg i.p. α-flupenthixol, 1.0 mg/kg i.p. oxiperomide; 10–80 mg/kg i.p. tiapride and 10–40 mg/kg i.p. sulpiride were also ineffective) and the circling was only antagonized at the highest doses used.

Thus, whilst denervation following intrastriatal 6-OHDA in the rat can cause persistent increases in DA agonist action, the most important behavioural correlate being contralateral asymmetry/circling, it can at the same time reduce or abolish the sensitivity of the DA agonist responses to neuroleptic antagonism.

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## Behavioural consequences of discrete, chronic infusion of dopamine into the nucleus accumbens of rat

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Studies on the behavioural and biochemical consequences of chronic drug therapy on central dopamine (DA) mechanisms have utilized a peripheral route for drug administration which, since it allows that drug an indiscriminate access to affect all cerebral DA systems, has precluded interpretations of subsequent changes in a particular behaviour to drug action on a specific DA system. In the present study we attempt a chronic, discrete stimulation of the mesolimbic system to determine effects on spontaneous and drug-induced motor behaviour.

Male Sprague-Dawley rats, selected as low activity responders to (−)-N-n-propylnorapomorphine (0.05 mg/kg s.c., (−)NPA) (see Costall, Hui & Naylor, 1980) were subject to standard stereotaxic surgery for the implantation of chronically indwelling guides for subsequent bilateral intra-accumbens injection. Two weeks after this implantation animals were re-anaesthetized for the subcutaneous (back neck region) implantation of two Alzet osmotic minipumps which were filled and connected via polythene tubing to injection units which were fixed

via the previously implanted guides to terminate at the centre of the nucleus accumbens and, from the time of implantation, delivered to the tips of the injection units 0.50 μl/h DA (4.16 μg/μl) or vehicle for 14 days. Home cages, housing 5 rats, were placed on Automex recorders for continuous measurement of 'general reactivity'. Stereotypy was scored every 8 hours. At specific times animals were removed from home cages for 180 min to gain a further measure of 'locomotor activity' from individual photocell cages. Both spontaneous locomotor activity and the motor responses to (−)NPA (0.05 mg/kg s.c.) were recorded.

The Automex counts for DA treated rats were slightly higher over each 24 h period than those of rats receiving vehicle, although this difference never achieved significance. However, the 3 h daily assessment of locomotor hyperactivity in the photocell cages did show a highly significant difference between the DA and vehicle treated rats. Over the 14 days of infusion, and a subsequent 7 weeks period, vehicle treated rats responded in all situations as untreated controls. However, animals receiving the DA by infusion developed a spontaneous locomotor hyperactivity which was apparent by day 2, persisted for 14 days, but gave peaks of higher intensity on days 3–5 and again on days 10–11. The normal hyperactivity and stereotypy responses to (−)NPA were markedly reduced from the 2nd day of infusion to its termination. Stereotypy responses returned to normal 2–3 weeks after termination of infusion. Hyperactivity returned after some 3 weeks but achieved a significantly higher level than initially recorded; this enhanced response was then maintained to the end of the total 9 week experimentation period.

Thus, the combined use of the osmotic minipumps and chronically indwelling intracerebral cannulae provide a valuable approach to continuous drug administration to a discrete brain area. Continuous DA infusion into rat mesolimbic tissue can modify both spontaneous and drug induced motor behaviour not only during the period of infusion but for several weeks after its termination.

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## Changes in limbic and striatal dopamine in rats treated with morphine for 40 and 60 days

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Earlier findings suggest that in the brains of mice and rats treated chronically with morphine or methadone the synthesis and release of dopamine are reduced but can be activated by acute administration of these drugs (Ahtee, 1974; Rosenman & Smith, 1972). In rats treated for 20 days with morphine, we found that the α-methyl-*p*-tyrosine (α*M*p*T*)-induced dopamine depletion was retarded 1 day after morphine withdrawal in the limbic forebrain but not in the striatum. A single challenge dose of morphine enhanced the α*M*p*T*-induced dopamine depletion similarly in these rats and control rats (Ahtee & Attila, 1980). In the present experiments the chronic morphine treatment lasted 40 or 60 days.

Male Wistar rats were injected s.c. twice daily at 08 h 00 min and at 18 h 00 min for 40 or 60 days with saline (0.9% NaCl solution) or morphine increasing the daily morphine dose gradually from 20 mg/kg to 100 mg/kg or to 130 mg/kg, respectively. After a withdrawal period of 1, 2 or 4 days the rats were given a challenge ('test') injection of either saline or morphine (10 mg/kg s.c.) 2.5 h and α-M*p*T (200 mg/kg i.p.) 2 h before decapitation. Dopamine concentrations were measured by liquid chromatography with electro-chemical detection (Keller *et al.*, 1976).

Chronic morphine treatment for 40 and 60 days decreased the limbic dopamine concentration by about 10% ($P < 0.05$) but did not alter the striatal dopamine concentration. The α*M*p*T*-induced depletion of limbic dopamine was decreased from 54% (controls) to 40% ($P < 0.05$) in the rats treated for 40 days with morphine after 1 day's but not after 2 or

4 days' withdrawal period. In the limbic forebrain of rats treated with morphine for 60 days the αM*p*T-induced depletion of dopamine was decreased after all three withdrawal periods (1, 2 or 4 days) studied. The αM*p*T-induced depletion of striatal dopamine was decreased only in the rats treated with morphine for 60 days and withdrawn from it for 2 days (from 42% to 31%; $P<0.01$).

The test dose of morphine accelerated the αM*p*T-induced depletion of dopamine in the brains of rats treated chronically with morphine for 60 days and withdrawn for 4 days more than that of control rats. The dopamine concentration in the limbic forebrain of control rats treated with αM*p*T and morphine was $1282 \pm 89$ ng/g (mean ± s.e.mean, $n=6$) and that of chronic morphine rats $988 \pm 40$ ng/g ($n=7$; $P<0.05$); the corresponding striatal concentrations were $2816 \pm 130$ ng/g ($n=6$) and $2342 \pm 111$ ng/g ($n=7$; $P<0.05$). In all other chronically with morphine treated rats the test dose of morphine enhanced the depletion of dopamine to about the same degree as in control rats.

The results show that chronic morphine administration weakens the endogenous regulation of cerebral dopamine neurons. The limbic dopamine neurons are more easily affected than the striatal ones. As the neurons still respond to morphine the chronically treated animals' dopamine neurons could well be dependent on morphine for their normal functioning.

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## Supersensitivity of central dopamine autoreceptors following chronic haloperidol treatment in the rabbit

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Postsynaptic striatal dopamine (DA) receptors develop supersensitivity after chronic treatment with antipsychotics (for review see Muller & Seeman, 1978). Release-modulating inhibitory DA receptors in the central nervous system are present on dopaminergic and noradrenergic neurons. In the rabbit these receptors were described respectively in the caudate nucleus (Starke, Reimann, Zumstein & Hertting, 1978; Kamal, Arbilla & Langer, 1981) and hypothalamus (Galzin, Dubocovich & Langer, 1981). The present investigation was aimed at studying the effects of chronic administration of haloperidol on the sensitivity of these release-modulating receptors.

Male rabbits received haloperidol 1 mg/kg (s.c.), either acutely or once daily for 28 days. Slices of caudate nucleus and hypothalamus were labelled with [$^3$H]-DA and [$^3$H]-NA respectively then superfused and electrically stimulated as described by Kamal *et al.* (1981) and Galzin *et al.* (1981). The ratio, $S_2/S_1$, of the percent of total tissue radioactivity released by two consecutive periods of electrical stimulation ($S_1$ and $S_2$), was $1.04 \pm 0.05$ ($n=10$) in controls for [$^3$H]-DA in the caudate nucleus and $0.99 \pm 0.06$ ($n=10$) for [$^3$H]-NA in the hypothalamus. When added before $S_2$ apomorphine (0.1 μM and 1 μM, respectively) significantly inhibited the release of [$^3$H]-DA ($S_2/S_1 = 0.22 \pm 0.02$, $n=8$, $P<0.002$) and [$^3$H]-NA ($S_2/S_1 = 0.47 \pm 0.06$, $n=4$, $P<0.01$). Both inhibitions were antagonized by adding haloperidol to the perfusion medium. Two h after a single injection of haloperidol (1 mg/kg, s.c.), the inhibition by apomorphine 0.1 μM of the overflow of [$^3$H]-DA was partly antagonized ($S_2/S_1 = 0.64 \pm 0.07$, $n=9$, $P<0.01$). This antagonism of the inhibition of [$^3$H]-DA release by apomorphine disappeared 24 h after the acute injection. After 2 days of withdrawal following 28 days of chronic treatment with haloperidol, the inhibition by

apomorphine (1 μM) of the overflow of [$^3$H]-NA remained unaffected. However, treated animals showed a more pronounced inhibition by apomorphine (0.1 μM) of the overflow of [$^3$H]-DA when compared with untreated rabbits ($S_2/S_1 = 0.09 \pm 0.02$, $n = 8$, $P < 0.001$). No significant effects of the chronic treatment of haloperidol were observed for the inhibition by apomorphine of the [$^3$H]-DA or [$^3$H]-NA release after 5 days of withdrawal from the drug.

We conclude, that similarly to the supersensitivity developed by presynaptic DA receptors modulating DA synthesis (Bannon, Zigum, Skirboll & Roth, 1980) the presynaptic DA receptors that modulate DA release from the caudate nucleus may also develop supersensitivity after chronic treatment with haloperidol. However, after the same chronic treatment with haloperidol, the DA receptors modulating NA release did not develop such supersensitivity. Since DA receptors are involved in the physiological modulation of the release of DA (Kamal *et al.*, 1981) but not of NA (Galzin *et al.*, 1981) our results indicate that supersensitivity of DA receptors following chronic blockade with drugs affects only those that are physiologically relevant in modulating transmitter release.

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## Effects of typical and atypical neuroleptics on striatal ($^3$H)dopamine release


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(Introduced by N.S. DOGGETT)

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Many groups have investigated the effects of DA agonists and antagonists on DA release in order to ascertain what presynaptic regulatory mechanisms are present. One of the surprising results found in these studies is the potent direct modification of DA release by neuroleptics. This has been reported by a number of groups, especially Seeman & Lee (1978), who described an extremely good correlation between the abilities of neuroleptics to inhibit stimulus-evoked DA release and their clinical efficacy (which also correlates with affinities for ($^3$H) butyrophenone binding sites).


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Nevertheless in previous studies on the terminals of the corticostriatal tract, we found a blockade of transmitter release by neuroleptics via a non-receptor-mediated mechanism (Mitchell & Doggett, 1980) and considered that effects on DAergic terminals should be re-examined.

The basal efflux of $K^+$-evoked release of [$^3$H]-DA from 0.1 mm striatal prisims (male Wistar rats) was investigated using a continuous superfusion method described in detail previously (Martin & Mitchell, 1979). A series of nine neuroleptics all caused concentration-dependent increases in basal [$^3$H]-DA efflux and inhibitions of stimulus-evoked release. The blockade of evoked release seen with each compound almost exactly parallelled its enhancement of basal efflux suggesting that this effect was apparent due to the elevated baseline efflux. The most potent neuroleptic studied was haloperidol which showed significant effects at 0.3 μM whilst the least potent was sulpiride with significant effects appearing only around 300 μM. Typical neuroleptics such as haloperidol, spiperone, benperidol, fluphenazine and α-flupenthixol all had $IC_{50}$'s on stimulated re-

lease in the range 1–4 μM whilst the atypical benzamide neuroleptics metoclopramide, tiapride and sulpiride had $IC_{50}$'s greater than 400 μM. The β-isomer of flupenthixol (a very poor antagonist of DA receptor response) produced exactly the same alterations of DA release as the active α-isomer, implying lack of involvement of any DA receptor. These results, using the mini prism preparation, are likely to represent direct effects on DA terminals, whereas those of Seeman & Lee (1974) in much larger slices, may involve a postsynaptic DA receptor on an interneurone which forms synaptic contact with DAergic terminals. Clearly, the direct presynaptic effects of neuroleptics seen here do not correlate with their clinical efficacy or ability to block DA receptors but can provide a mechanism for the neuroleptic-induced increase in DA turnover *in vivo*, without the need to postulate either the operation of autoreceptors or any kind of feedback loop. What is notable about these direct presynaptic effects is the extremely low potency of the substituted benzamide drugs (a number of which are reported clinically to show a very low incidence of dyskinetic side effects (see Lin *et al.*, 1980)). The mechanism whereby neuroleptics can directly modify DA release is however unclear at present.

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## Chronic administration (6 months) of L-DOPA to rats: behavioural and neurochemical studies

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It is well reported that the chronic administration of dopamine receptor antagonist (neuroleptic) drugs to laboratory animals induces enhanced behavioural responses to dopamine agonists and increased specific binding of radiolabelled ligands thought to denote dopamine receptor sites (Clow, Jenner, Theodorou & Marsden, 1979; Waddington & Gamble, 1980). Such observations have been interpreted as the induction of supersensitive cerebral dopamine receptors following chronic blockade. To date there are few reports investigating the effects of long-term administration of a dopamine agonist to laboratory animals on dopamine receptor function. Since many Parkinsonian patients receive chronic L-DOPA therapy, we have looked at the behavioural and biochemical effects of continuous administration of 3,4-dihydroxyphenylalanine (L-DOPA) to rats for 6 months.

Female Porton rats (150 ± 10 g at the start of the study) received L-DOPA and carbidopa (methyldopa hydrazine) in a ratio of 10:1, in powdered diet. The drugs were weighed and mixed into set quantities of diet to give an estimated daily intake of 20–30 mg L-DOPA $kg^{-1}$ $day^{-1}$. Age-matched control animals received carbidopa only in powdered diet. After a 6-month period, control and L-DOPA fed rats were challenged with a series of dopamine agonist and antagonist drugs and the behavioural responses observed. Further groups of animals were killed and the specific binding of [$^3$H]-ADTN (6,7-amino-1,2,3,4-tetrahydronaphthalene) and [$^3$H]-spiperone was studied in membrane fractions prepared from the striatum of each group. The radioactive ligands were used in a range of concentrations so that the dissociation constant ($K_D$) and number of binding sites ($B_{max}$) could be obtained from Scatchard analysis.

The behavioural responses (stereotypy and hyperactivity) to amphetamine (1–10 mg/kg) and nomifensine (1–25 mg/kg) were significantly enhanced in L-DOPA animals at all doses. However, it was only the higher doses of apomorphine (2 and 5 mg/kg), L-DOPA (50–200 mg/kg) and piribedil (10–75 mg/kg) which produced significantly enhanced stereotyped responses in L-DOPA treated

rats, this often being associated with production of facial chewing movements. Treatment of chronic L-DOPA rats acutely with the neuroleptics fluphenazine (0.1–5 mg/kg), haloperidol (0.25–5 mg/kg) and thioridazine (5–50 mg/kg) demonstrated a differential action on the cataleptic scores; this behaviour being antagonized in the L-DOPA rats at the lower doses of the neuroleptics used, but being enhanced at the higher doses of neuroleptics.

Biochemically binding studies indicated increased specific binding of both [$^3$H]-spiperone and [$^3$H]-ADTN, being increased 75 and 26% for each ligand respectively ($P<0.05$). The $K_D$ for [$^3$H]-spiperone binding increased from 0.83 nM in control animals to 1.54 nM in L-DOPA fed rats ($P<0.05$).

These behavioural and biochemical studies would suggest that chronic L-DOPA administration in rats leads to an enhancement of dopamine receptor function. These results support the work of others who, after treating rodents with high doses (200–700 mg/kg/day) of L-DOPA for 3–4 weeks also demonstrated enhanced [$^3$H]-spiperone binding in striatum (Suga, 1980; Wilner, Butler, Seifert & Clement-Cormier, 1980) and hypersensitivity to acutely injected L-DOPA (Tang & Cotzias, 1977).

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## Long-term cis-flupenthixol administration produces cerebral dopamine receptor supersensitivity and increases striatal acetylcholine levels

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The continuous administration of neuroleptic drugs to rats for 6 months or longer causes cerebral dopamine receptor supersensitivity (Clow, Theodorou, Jenner & Marsden, 1979a, 1979b). However, enhanced apomorphine-induced stereotyped behaviour and striatal [$^3$H]-spiperone binding do not indicate a spontaneous increase in cerebral dopamine function in intact animals. Striatal post-synaptic dopamine receptors are thought to lie in part on the cell bodies of cholinergic interneurones. We have therefore compared striatal acetylcholine levels in animals receiving *cis*- or *trans*-flupenthixol for 14 months with those in age-matched control animals.

Male Wistar rats (180 ± 10 g at the start of the experiment) received either *cis*-flupenthixol (0.8–1.2 mg/kg/day) or *trans*-flupenthixol (0.9–1.2 mg/kg/day) administered in distilled drinking water for 14 months. Maintained alongside drug-treated animals were an age-matched control group that received distilled water alone.

After 14 months continuous drug intake rats receiving *cis*-flupenthixol (stereotypy score 3.9 ± 0.1) but not those receiving *trans*-flupenthixol (stereotypy score 2 ± 0) showed an enhanced stereotyped response to apomorphine (0.5 mg/kg s.c. 15 min previously) compared to control animals (stereotypy score 2 ± 0). At this time the striatal dopamine content was not different in drug-treated or age-matched control animals. However, in ani-

mals receiving *cis*-flupenthixol, but not *trans*-flupenthixol, the striatal HVA and DOPAC concentrations were lower than those found in control animals. Striatal [$^3$H]-spiperone (0.125–4.0 nM, defined using dopamine $10^{-4}$ M) binding sites (Bmax) were not altered by *cis*-flupenthixol (Bmax $9.2 \pm 0.3$ pmoles g tissue) or *trans*-flupenthixol (Bmax $9.6 \pm 0.6$ pmoles g tissue) treatment compared with control animals (Bmax $10.5 \pm 0.6$ pmoles g tissue) although after a 2 week washout period a 40% increase in Bmax was observed in animals receiving *cis*-flupenthixol. Administration of *cis*-flupenthixol ($K_D$ $0.22 \pm 0.3$ nM) but not *trans*-flupenthixol ($K_D$ $0.13 \pm 0.02$ nM) increased the dissociation constant ($K_D$) for [$^3$H]-spiperone binding compared to control animals ($K_D$ $0.13 \pm 0.02$ nM).

The acetylcholine content in striatum was approximately doubled by *cis*-flupenthixol ($7.4 \pm 0.7$ μg/g) administration, but not *trans*-flupenthixol ($3.8 \pm 0.1$ μg/g), compared to tissue from control animals ($4.1 \pm 0.3$ μg/g).

Acute neuroleptic administration causes a decrease in acetylcholine content (Sethy & van Woert, 1974). We conclude that chronic *cis*-flupenthixol administration results in functional supersensitivity of striatal dopamine receptors since it causes an increase in striatal acetylcholine levels.

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## Hypophysectomy does not prevent the development of striatal dopamine receptor supersensitivity induced by repeated neuroleptic treatment

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The repeated administration of neuroleptic drugs to rodents for a few weeks followed by some days drug free results in the development of dopamine receptor supersensitivity (Muller & Seeman, 1978). Recently Hruska, Ludmer & Silbergeld (1980) have claimed that hypophysectomy prevented the development of increased striatal dopamine receptor numbers after chronic haloperidol treatment, as measured by [$^3$H]-spiperone binding. We have investigated this surprising finding comparing the effects of repeated haloperidol or sulpiride administration to hypophysectomized and sham-operated rats.

Hypophysectomized or sham-operated male Wistar rats ($150 \pm 10$ g at surgery) maintained by free access to glucose supplement and rock salt preparation received haloperidol (0.75 mg daily i.p.), sulpiride (15 mg twice daily i.p.) or 0.9% saline for 17 days and then remained drug free for 3 days. The hypophysectomized animals did not gain weight, their average weight did not change from that at the start of the experiment even if receiving neuroleptic drug administration. Sham-operated animals, irrespective of drug treatment gained an average of 140 g. At the end of the drug free period, plasma prolactin concentrations in sham-operated animals were normal for saline ($26.6 \pm 8.6$ ng/ml) and sulpiride ($22.3 \pm 7.1$ ng/ml) treated rats. However, animals receiving haloperidol had elevated plasma prolactin concentrations ($65.0 \pm 14.6$ ng/ml) suggesting the continued presence of drug despite the withdrawal period. Hypophysectomy reduced plasma prolactin concentrations below the level of detection in the saline and haloperidol groups and the value for sulpride treated animals was less than the limit of sensitivity of the assay procedure ($< 7.5$ ng/ml). Subsequent histological examination revealed that the pituitary was destroyed in animals undergoing full surgery.

Apomorphine (0.5 mg/kg sc 15 min previously) induced stereotyped behaviour was enhanced by haloperidol or sulpiride treatment in hypophysectomized and sham-operated animals compared to their respective saline treated controls. There was no difference before or after drug treatment for the

stereotypy scores of the hypophysectomized animals compared to their respective sham-operated controls.

Specific [$^3$H]-spiperone (0.125–40 nM; defined using (+)-butaclamol $10^{-6}$ M) binding sites (Bmax) in striatal preparations were increased by the administration of either sulpiride or haloperidol pretreatment of hypophysectomized or sham-operated animals compared with saline treated control animals. The dissociation constant ($K_D$) was not altered by either sulpiride or haloperidol administration to hypophysectomized animals. The $K_D$ values for [$^3$H]-spiperone binding to striatal tissue from sham-operated animals receiving haloperidol were increased, but that after sulpiride treatment was not altered.

We conclude that hypophysectomy does not influence the onset of neuroleptic-induced dopamine receptor supersensitivity.

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## Induction of 5-hydroxytryptamine (5HT)-dependent myoclonus in guinea-pigs by indole-containing, but not by piperazine-containing, 5HT-agonists suggests multiple cerebral 5HT receptors

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L-5-Hydroxytryptophan (5HTP)-induced myoclonus in guinea-pigs appears dependent upon stimulation of brainstem 5-hydroxytryptamine (5HT) receptors (Chadwick, Hallett, Jenner & Marsden, 1978). We have reported previously that the differential capacity of 5HT-active compounds to induce, potentiate or antagonize 5HT-dependent myoclonus may indicate multiple cerebral 5HT receptors (Jenner, Luscombe & Marsden, 1980). We have further investigated the ability to induce myoclonus of two structurally distinct groups of 5HT agonists, possessing either an indole nucleus (5HT precursors and agonists) or a piperazine moiety.

Administration of 5HTP (20–200 mg/kg s.c.) to naive female guinea-pigs, or 5.0–120 mg/kg to animals pretreated with the peripheral decarboxylase inhibitor carbidopa (α-methyldopahydrazine, 25 mg/kg i.p. 60 min previously), induced dose-dependent myoclonus. Tryptophan (25–200 mg/kg s.c.) was inactive in naive guinea-pigs but evoked dose-dependent myoclonus in animals pretreated with pargyline (75 mg/kg i.p. 60 min previously). 5HT (1.0–36 mg/kg i.p.) produced no jerking in naive guinea-pigs.

Tryptamine (6–160 mg/kg i.p.) produced dose-dependent jerking only when administered to animals pretreated with pargyline (75 mg/kg i.p. 60 min previously). The following other indole-containing 5HT agonists all evoked a dose-related response in naive guinea-pigs: (+)-LSD (0.25–8.0 mg/kg i.p.), 5-methoxy-N,N-dimethyltryptamine (2.5–20 mg/kg i.p.), psilocin, psilocybin, N,N-dimethyltryptamine (20–160 mg/kg i.p.), bufotenine (80–160 mg/kg i.p.) and RU 24969 (5-methoxy 3-(1,2,3,6-tetrahydro-4-pyridinyl)-IH-indole; 40–120 mg/kg i.p.).

In contrast, 5HT agonists containing a piperazine moiety, quipazine (1.0–200 mg/kg i.p.), 1-(m-trifluoromethylphenyl) piperazine (1.0–160 mg/kg i.p.), m-chlorophenylpiperazine (1.0–160 mg/kg i.p.), p-chlorophenylpiperazine (1.0–160 mg/kg i.p.), 2-(1-piperazinyl)-quinoxaline (1.0–40 mg/kg i.p.) and MK 212 (6-chloro-2-1-piperazinyl pyrazine; 1.0–80 mg/kg i.p.) induced only occasional myoclonus at toxic doses. Jerking was very rarely observed at doses effective in producing other behavioural and biochemical changes indicative of 5HT agonist properties.

The difference in activity between the indole-containing compounds and the piperazine containing 5HT agonists suggests myoclonus is due to activation of an indole-selective brainstem 5HT receptor and provides further evidence for multiple cerebral 5HT receptors.

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## Depressant action of the L-glutamate analogue (+)2-amino-4-phosphonobutyrate

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2-Amino-4-phosphonobutyrate (APB) has been reported to have both excitant (Curtis & Watkins, 1965) and depressant (White, Nadler, Hamberger, Cotman & Cummins, 1977) properties at mammalian central neurones. The present paper reports the properties of the separate optical isomers of APB since it seemed possible to us that the different effects reported above may have reflected different properties of these isomers.

The compounds were tested on hemisected spinal cord preparations from frogs or immature rats. Ventral root responses were measured either to electrical stimulation of dorsal roots (DR-VRPs) or to bath application of agonists in the presence of tetrodotoxin in order to block regenerative activity.

(−)APB (0.25–2 mM) depressed DR-VRPs and depolarizing responses induced by L-aspartate, L-glutamate, kainate, N-methyl-D-aspartate or quisqualate suggesting that the depressant action of this compound may result from antagonism of the postjunctional action of an excitant amino acid transmitter.

(+)APB (5–100 μM) selectively depressed the initial, probably monosynaptic (Otsuka & Konishi, 1974), component of the DR-VRP. At higher concentrations (> 100 μM) (+)APB produced depolarization recorded in ventral roots. Levels of (+)APB which produced marked depression of the monosynaptic component of the DR-VRP had no effect on responses induced by either the above amino acid excitants or substance P. Thus it is unlikely that the depressant effects of (+)APB are mediated through antagonism of transmitter(s) acting at receptors activated by these excitants. The depressant action of (+)APB may represent blockade of some other type of excitatory transmitter receptor or alternatively this compound may act presynaptically to reduce transmitter release. Such a presynaptic action is likely to be selective since (±)APB (1 mM) had no effect on transmission through isolated superior cervical ganglion preparations.

In view of the close structural analogy between (+)APB and L-glutamate we intend to investigate the possibility that (+)APB may be a selective agonist for autoregulatory receptors on excitant amino acid releasing terminals.

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## Amino acid antagonist property of urethane

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Urethane, in contrast to several other anaesthetic agents, produces little or no depression of transmission in autonomic ganglion at anaesthetic levels (Larrabee & Posternak, 1952). Recent evidence suggests that L-glutamate may be the transmitter of baroreceptor afferent fibres (Talman, Perrone & Reis, 1980) and urethane has been shown to selectively depress baroreceptor reflex responses (Downing, 1960). These effects suggest the possibility that the depressant actions of urethane may be mediated through depression of amino acid evoked excitations.

In the present experiments it was found that urethane reversibly depressed ventral root potentials evoked by electrical stimulation of corresponding dorsal roots (DR-VRP) of frog spinal cord preparations ($ED_{50}$ 50 mM). In the presence of sufficient tetrodotoxin to abolish DR-VRPs urethane (50 mM) reversibly antagonized submaximal depolarizations induced by N-methyl-D-aspartate, kainate, quisqualate or L-glutamate, giving close ratios of approximately two at this level. Depolarizations evoked by substance P or eledoisin-related-peptide recorded in ventral roots of immature rat spinal cord preparations were not depressed by urethane (50 mM).

Urethane is one of several depressants reported to reverse the antagonist action of picrotoxin (Bowery & Dray, 1976) suggesting that urethane can potentiate the action of GABA. Picrotoxin (25 μM) had little or no effect on depressant responses produced by urethane, in contrast, similar depressant responses produced by amylobarbitone were reduced considerably in the presence of this concentration of picrotoxin.

This combination of observations supports the suggestion that urethane may produce its depressant action by antagonizing the postjunctional effects of excitant amino acid transmitter(s).

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## Relationships between behavioural and *in vivo* voltammetric responses to dopamine and 5-hydroxytryptamine releasers

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(+)-Amphetamine and *p*-chloroamphetamine induce various behaviours primarily due to dopamine (DA) and 5-hydroxytryptamine (5HT) release respectively, although high doses of either drug cause behaviours reflecting release of both transmitters (Fernando & Curzon, 1981). Individual behaviours have different dependences on DA and 5HT (Andrews, Fernando & Curzon, 1981) and characteristic time courses. With the advent of *in vivo* voltammetry (Huff, Adams & Rutledge, 1979) DA and 5HT release may be repetitively monitored and related to behaviour (Marsden, Conti, Strope, Curzon & Adams, 1979; Curzon & Hutson, 1981). In the present study, time courses of behavioural effects of the above drugs are compared with time courses of striatal voltammetric responses.

Male Sprague-Dawley rats (190–210 g) were used with electrodes implanted in the anterior striatum. Voltammetric procedures were as described by Curzon and Hutson (1981) with recordings at 0.2 h intervals. Behavioural components were scored every 0.25 h for 1 minute.

Voltammetric peaks occurred at about 120 mV and 260 mV. Preliminary experiments indicate that they reflect release of DA and 5HT respectively although other compounds probably also contribute. (+)-Amphetamine (2 mg/kg i.p., $n = 7$) increased

the DA peak but only increased the 5HT peak marginally. Behaviour was largely classically DA dependent (forward locomotion, rearing, sniffing) with time courses largely preceding that of the voltammetric signal. *p*-Chloro-amphetamine (10 mg/kg i.p., $n = 11$) increased the 5HT peak most prominently at 1 h, but only increased the DA peak marginally. 5HT dependent behaviours (hind limb abduction, head weaving, forepaw treading) were prominent initially but then declined; DA behaviours (forward locomotion, then sniffing) became prominent. (+)-Amphetamine (25 mg/kg, i.p., $n = 8$) increased both peaks and their time courses largely paralleled appropriate behaviours. Thus in the first hour backward locomotion and the above 5HT behaviours were prominent. Concurrently, the 5HT peak rose markedly and the DA peak slightly. In the second hour 5HT remained steady and the DA peak increased markedly; 5HT behaviours remained prominent except for limb abduction. Lesion experiments (Andrews *et al.*, 1981) indicate this is inhibited by DA. From 2–4 h, the other 5HT-dependent behaviours and the 5HT peak declined with the DA peak remaining steady. From 4–6 h headbobbing (which had been prominent) decreased and behaviours typical of amphetamine at low dosage (sniffing, then some rearing) appeared; the DA peak declined somewhat.

In these experiments amine changes were monitored at one striatal site. Other loci are probably more relevant to certain behaviours e.g. DA dependent locomotion and rearing. However, findings indicate considerable correspondence between voltammetric and behavioural effects of amine releasers and illustrate the potential value of repetitive automatic *in vivo* voltammetry in behavioural studies.

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## Synaptic targets of drug action in the basal ganglia: morphological studies on monosynaptic pathways in neuronal networks

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Although the sites of action of drugs in the basal ganglia may be pharmacologically characterized, little is known about the morphology and connections of neurones which are targets of the drugs. Using a new morphological procedure that combines, in one experimental animal, the retrograde transport of horseradish peroxidase (HRP), Golgi-staining and anterograde synaptic degeneration (Somogyi, Hodgson & Smith, 1979) we have studied some of the monosynaptic pathways in the basal ganglia of the rat.

Following the injection of HRP into the substantia nigra, a high proportion of striatal neurones were retrogradely labelled. These were of two morphologically distinct types. One of them was identified as the medium-size densely spiny type by the Golgi stain and had local axon collaterals. Ultrastructural examinations of these identified striatonigral neurones revealed that they receive a direct synaptic input from the cerebral cortex (Somogyi, Bolam & Smith,

1981). The second type of striatonigral neurone was found, by indirect correlation with Golgi-stained neurones, to fall into a class of Golgi neurone that has long, predominantly smooth dendrites which extend as far as 700 μm from the cell soma.

Neurones in the substantia nigra that project to the neostriatum, identified by the retrograde transport of HRP, receive a direct synaptic input from neurones in the same region of the neostriatum to which they project and also from neurones in the nucleus accumbens/ventral striatum region. Additionally, neurones in the substantia nigra, identified as nigrothalamic neurones by the retrograde transport of HRP, receive monosynaptic input from neurones in the neostriatum (Somogyi *et al.*, 1979).

Studies are in progress to identify the putative neurotransmitters used by neurones in these characterized monosynaptic pathways in the basal ganglia.

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## The effects of basal ganglia lesions on tremorine-induced rigidity in rats

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Several different types of drugs cause rigid immobility in rats, including opiates, reserpine and tremorine. The areas of the CNS responsible for the generation of drug-induced rigidity have not been positively identified. Morphine caused eletromyographically recorded rigidity when injected into the neostriatum and bilateral, kainic acid-induced striatal lesions abolished the EMG activation produced by morphine administered systemically (Havemann, Winkler, Genc & Kuschinsky, 1980). Reserpine-induced rigidity has been attributed to a reduction in striatal dopamine function (Anden & Johnels, 1977). The very high density of muscarinic receptors in the striatum (Kobayashi, Palkovits, Hruska, Rothschild & Yamamura, 1978) suggests that it could be partly responsible for the pharmacological actions of tremorine. We report the effects of lesioning the striatum and some anatomically related areas, as well as the cerebral cortex, on tremorine-induced limb rigidity.

The muscle tone of conscious, lightly restrained female Sprague Dawley rats (180–220 g) was recorded by measuring the resistance of the hindlimb to a partial flexion. A mechanical device (Dickinson, Slater & Longman, 1981), was used to flex the limb and record the force (g). The technique was similar to that described by Johnels, Steg & Ungerstedt (1978). Frequent measurements were made 10 min before and 35 min after the administration of tremorine dihydrochloride (20 mg/kg i.p.) to rats pretreated (15 min) with atropine methylnitrate (1 mg/kg).

The force needed to flex one leg of normal rats was $48.1 \pm 2.6$ g ($n = 25$). Tremorine increased the force required to flex the leg; the peak increase ($+20.8 \pm 3.2$ g) was recorded at 20 min. The two hindlimbs always provided identical measurements. Kainic acid (1 μg in 1 μl) was injected stereotaxically in the left striatum (A 8.5, L 2.6, H −1.0; König & Klippel, 1963). The resistance to flexion of the ipsilateral (IL) and contralateral (CL) legs was measured after 10 days. The striatal lesion did not affect limb tone (IL $43.5 \pm 4.7$ g, $n = 6$; CL $40.7 \pm 2.7$ g) but al-

most entirely prevented tremorine-induced rigidity in both legs (IL + 1.8 ± 3.1 g, $P < 0.05$; CL + 5.6 ± 2.4 g, $P < 0.05$). A unilateral electrolesion of the globus pallidus (A 6.6, L 2.5, H − 1.1) reduced the CL tone (21.4 ± 7.2 g, $n = 5$, $P < 0.05$). Tremorine rigidity was reduced in IL (+ 8.8 ± 4.6 g, $P < 0.05$) but increased in CL (+ 40.2 ± 5.8 g, $P < 0.05$). A unilateral electrolesion of the entopeduncular nucleus (A 4.7, L 2.2, H − 2.0) reduced the resistance to flexion in both legs (IL 31.9 ± 3.6 g, $n = 6$, $P < 0.05$; CL 26.6 ± 4.2 g, $P < 0.05$) but had no effect on tremorine-induced rigidity. Hindlimb tone and tremorine rigidity were not affected by unilateral electrolesions in the subthalamic (A 3.4, L 2.0, H − 2.3) and accumbens (A 9.7, L 1.3, H − 1.3) nuclei or by suction removal of one cerebral cortex. This preliminary data demonstrates that tremorine rigidity is subcortical in origin and suggests that it may be mediated in part by descending striatal and pallidal efferent pathways.

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## Tracheal relaxant effects of prostanoids are dependent on their contractile effects

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Gardiner & Collier (1980) obtained evidence that the contractile and relaxant effects of prostanoids in airway smooth muscle are due to actions at different receptors. However, Coleman, Collington, Geisow, Hornby, Humphrey, Kennedy, Levy, Lumley, McCabe & Wallis (1981), have proposed that there are at least four different types of prostanoid receptor which can mediate contraction in smooth muscle. We have attempted to identify which, if any, of these receptors mediate contractile effects of prostanoids in airway smooth muscle.

Contractile responses were measured in guinea-pig tracheal muscle as described by Gardiner & Collier (1980) except that indomethacin (2.8 μM) was included in the bathing Kreb's solution. Concentration-effect curves were obtained cumulatively to $PGF_{2\alpha}$ and one other prostanoid in each preparation, and except for $PGE_1$ were very consistent. Potency (EC50) values were determined as described by Coleman & Kennedy (1980) using tracheas from six animals for each prostaroid. The rank order for contractile efficacy ($PGF_{2\alpha}$ = 1) was U46619 (1.1) > $PGF_{2\alpha}$ (1.0) > $PGD_2$ (0.83) > $PGE_2$ (0.54) > $PGE_1$ (0.12). $PGE_2$, $PGD_2$ and $PGF_{2\alpha}$ all caused relaxation at high concentrations, their concentration effect curves being biphasic in shape. The order of potency (values in μM) was different to that for maximum effectiveness: $PGE_2$ (0.013) > U46619 (0.08) > $PGF_{2\alpha}$ (0.55) > $PGD_2$ (0.7). A potency value for $PGE_1$ could not be obtained due to the extreme flatness of the concentration-effect curve. The contractile responses to $PGE_2$ and $PGF_{2\alpha}$ were strongly antagonized by SC-19220 (3–30 μM) whereas those to U46619 were unaffected. These results suggest that there are at least two types of prostanoid receptor mediating contraction in the guinea-pig trachea: group I receptors activated potently by $PGE_2$ and inhibited by SC-19220 (Coleman, Kennedy & Levy, 1980) and probably group III receptors (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980) since the trachea is potently activated by U46619 and also $TXA_2$ (Svensson, Strandberg, Tuvemo & Hamberg, 1977).

We have also used the zero-tone trachea to ex-

amine for the presence of any contractile effects of bronchodilator prostanoid analogues. Some prostanoids such as TR4161 ($PGE_1$-alcohol) cause contractile effects similar to $PGE_1$ while others, TR4752 (15-deoxy, 16-hydroxy, 17-dimethyl-$PGE_1$-alcohol) and TR4979 (15-deoxy, 16-hydroxy, 17-cyclobutyl-$PGE_1$-methyl-ester) caused no contractile effects over a wide range of concentrations (Copas, Gardiner & Wilson, unpublished observations). Since the latter analogues are the most effective relaxants that we have studied in the trachea, we considered that the existence of contractile effects in prostanoids may reduce their relaxant efficacy. This possibility was strongly supported by the fact that when the contractile effects of $PGE_2$ and TR4161 were inhibited by SC-19220 (300 $\mu$M) their relaxant efficacy (measured against histamine or acetylcholine-induced tone) was greatly increased.

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## The selectivity of TR4979 for 'ψ' prostanoid receptors


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Three classes of prostanoid receptors have been identified in the airways and classified according to agonist action i.e. 'χ' for contraction; 'ψ' for relaxation/inhibitory and 'ω' for irritancy/sensitization (Gardiner & Collier, 1980). TR4979 has already been described as a selective stimulant for 'ψ' receptors in the airways (Copas, Gardiner & Wilson, this meeting). We have extended these studies to the prostanoid receptors outside the airways to determine the therapeutic potential of TR4979 as a selective bronchodilator agent.

The following isolated tissues contain a number of 'χ' contractant prostanoid receptors similar to types I–IV previously described by Coleman, Humphrey, Kennedy, Levy & Lumley (1980), and Coleman, Kennedy, Levy & Penning (1980):– human, guinea-pig and rat pregnant uteri, rat stomach strip, rat colon, chick rectum. A wide range of prostanoids $PGE_1$, $PGE_2$, $PGF_{2\alpha}$, ICI 81008, U46619, $PGA_1$, $PGI_2$, TR4161 ($PGE_1$ carbinol) were tested on these tissues and were found to contract one or more of these, albeit to varying degrees. In contrast TR4979 and TR4752 (15-deoxy, 16-hydroxy, 17-dimethyl-$PGE_1$-alcohol) had no activity on any of these tissues over the concentration range $10^{-8}-10^{-5}$ g/ml$^{-1}$.

The 'ω' irritant/sensitization effects of prostanoids were evaluated in the following systems designed to detect increases in respiratory rate in the conscious cat and anaesthetized guinea-pig, and tracheobronchial irritancy in the conscious cat (Gardiner, Copas, Elliott & Collier, 1978). TR4979 was found to have little or no activity in any of these systems whereas all of the prostanoids tested were effective, but to varying degrees, as shown in the following rank orders of potency for cat cough; $PGF_{2\alpha} > TR4752 > PGF_{2\beta} > PGE_2 = PGA_1 = PGE_1$; cat respiratory rate $PGE_1 > TR4161 > PGE_2 > TR4752 > PGA_1 > TR4979$; $PGF_{2\alpha}$ and $PGD_2$ reduced respiratory rate; and guinea-pig respiratory rate, $PGE_1 > TR4161 > TR4752 > PGD_2 > PGE_2 > PGF_{2\alpha} > PGA_1 > TR4979$.

TR4979 and a wide range of prostanoids have also been evaluated in the following systems; inhibition of human platelet aggregation induced by arachidonic acid, vasodilatation of the carotid artery in the anaesthetized guinea-pig and inhibition of antigen-induced degranulation of rat peritoneal mast cells. In

the latter system, however, none of the prostanoids except $PGE_1$ showed any inhibitory actions. In contrast the following two rank orders of potency were determined in the platelet aggregation test and vasodilatation respectively:– $PGI_2 \gg PGD_2 = PGE_1 \gg PGF_{2\beta} > PGF_{2\alpha} = PGE_2 = TR4979 > TR4161 > TR4752$; and $PGE_1 > PGE_2 > TR4161 > PGA_1 > TR4979 > TR4752$, $PGF_{2\alpha}$ and $PGD_2$ acted as vasoconstrictors in this test system. The differences in the latter two rank orders of potency and that for bronchodilatation (Gardiner & Collier, 1980) may suggest the presence of subclasses of the relaxant/inhibitory '$\psi$' receptors as has been shown for '$\chi$' receptors.

TR4979 appears to have no significant activity in any of the test models in which '$\chi$' or '$\omega$' receptors are present, suggesting that it is a very selective '$\psi$' agonist.

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## A study of the prostanoid receptors mediating bronchoconstriction in the anaesthetized guinea-pig and dog

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We have recently defined three prostanoid receptors mediating smooth muscle contraction (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980) at which respectively $PGE_2$, $PGF_{2\alpha}$ and the $TXA_2$-like agonist U-46619 (Coleman *et al.*, 1981) are potent selective agonists. We now describe an analysis of prostanoid-induced bronchonconstriction in the guinea-pig and dog in terms of these receptors. Guinea-pigs were anaesthetized with urethane (2 g/kg i.p.), dogs were anaesthetized with thiopentone (25 mg/kg i.v.) and chloralose (80 mg/kg i.v.) for measurement of pulmonary resistance and com-

**Table 1** Comparison of the potencies of U-46619, $PGF_{2\alpha}$, $PGD_2$, ICI 80996 and ICI 81008 in the anaesthetized dog, anaesthetized guinea-pig, guinea-pig lung strip and dog iris sphincter muscle.

| | *Molar equipotent concentration/dose* | | | | |
|---|---|---|---|---|---|
| *Preparation* | *U-46619* | *$PGF_{2\alpha}$* | *$PGD_2$* | *ICI 80996* | *ICI 81008* |
| Guinea-pig* lung strip | 1 (0.4–2.2) | 1111 | 155 (78–322) | 1370 (630–3000) | 32700 (7700–134000) |
| Anaesthetized† guinea-pig | 1 | 223 (115–432) | 1032 (698–1527) | 240 (168–344) | inactive |
| Dog iris* sphincter muscle | 131 (52–333) | 1 | 13 (8–20) | 0.37 (0.15–0.89) | 0.65 (0.24–1.78) |
| Anaesthetized† dog | 0.12 (0.08–0.18) | 1 | $n=2$ 2.6, 3.3 | 0.05 (0.02–0.13) | 0.05 (0.03–0.09) |

Each value is the mean of at least 4 determinations (95% confidence limits) except for $PGD_2$ in the anaesthetized dog. In each experiment at least 2 concentration/dose-effect curves were obtained for the standard agonist followed by another for one other compound. For each preparation the value without limits corresponds to the standard agonist.

*Results from Coleman *et al.* (1980) except for $PGD_2$ and ICI 80996.

†Both pulmonary compliance and resistance were measured, values quoted are for compliance.

pliance (Daly, Farmer & Levy, 1971). Drugs were administered intravenously. Agonists used were $PGD_2$, $PGF_2\alpha$, U-46619 and two $PGF_2\alpha$ analogues, ICI 80996 and ICI 81008 (Dukes, Russell & Walpole, 1974).

In the guinea-pig, U-46619 (0.03–1 μg/kg) was the most potent bronchoconstrictor, the other prostanoids (10–200 μg/kg) being much less active. The order of agonist potency was similar to that obtained on guinea-pig lung strip, a preparation containing U-46619/$TXA_2$-sensitive receptors (Table 1).

In the dog, $PGF_2\alpha$, $PGD_2$ (0.1–10 μg/kg) and the two ICI compounds (0.03–1 μg/kg) were all potent bronchoconstrictors. These prostanoids were also potent agonists on dog iris, a preparation containing $PGF_2\alpha$-sensitive receptors (Table 1). Interestingly while U-46619 (0.03–1 μg/kg) was a potent bronchoconstrictor it had little activity on dog iris.

These results can be accounted for if both $PGF_2\alpha$-sensitive and U-46619/$TXA_2$-sensitive receptors mediate bronchoconstriction in the dog. The following results support this hypothesis; in the dog both ICI compounds induced specific desensitization; thus following administration of either, the potency of $PGF_2\alpha$ and $PGD_2$, but not U-46619, was reduced by up to 20 fold. In conclusion, this study suggests that prostanoid bronchoconstriction in the guinea-pig is mediated by U-46619/$TXA_2$-sensitive receptors, whilst in the dog both U-46619/$TXA_2$-sensitive and $PGF_2$ α-sensitive receptors are involved.

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## Prevention by prostaglandin of an indomethacin-caused decrease in hepatic microsomal drug metabolizing enzymes

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The nonsteroidal anti-inflammatory agent indomethacin (IND) is well known to produce gastrointestinal lesions (Katz, Pearson & Kennedy, 1965). It is less widely realized that IND is also hepatotoxic and that it causes a decrease in hepatic microsomal drug metabolizing enzymes (Vukoson, Greiner, Kramer & Colby, 1978). As IND inhibits the synthesis of prostaglandins, we have investigated whether this hepatic effect of IND is modified by the concomitant administration of a prostaglandin.

Adult (220–270 g) male Sprague-Dawley rats were injected with either IND (8.5 mg/kg i.p.), 16,16-dimethylprostaglandin $F_{2\alpha}$ (PG, 0.5 mg/kg i.v.) or IND + PG in close succession, once daily for 3 days. Untreated and vehicle-treated rats served as controls. On the 4th day, liver microsomes were prepared and their cytochrome P-450 contents and aminopyrine N-demethylase (AD) specific activities measured.

Three separate series of experiments (controls and tests) were completed. In series I (Table 1) the vehicle for IND and PG (0.1 M phosphate buffer, pH 7.6) decreased AD by 40% but not P-450. In a second series phosphate buffer unexplainedly did not affect AD or P-450. Due to this inconsistency a further series of experiments (3 rats per group) were run in which the vehicle was changed to sodium bicarbonate (for IND) and isotonic saline (for PG) and again there was no effect of the vehicle. The results of the second (phosphate and third ($NaHCO_3$-NaCl) series of experiments are combined in the Table as series II. In both series (I and II) IND caused a 55% loss in AD (average of 61%, series I and 50%, series II) and a 48% loss in P-450 (average of 54% and 42%) relative to the appropriate vehicle-treated control. Concomitant administration of PG with IND protected the liver against these losses, which were now only 13% in AD (average of 14% and 12%) and 9% in P-450 (average of 7% and 11%). PG itself did not affect either AD or P-450 relative to untreated controls.

**Table 1** Effects of indomethacin (IND), 16,16-dimethylprostaglandin $F_{2\alpha}$ or IND + PG concomitantly, *in vivo* on rat hepatic microsomal aminopyrine N-demethylase and cytochrome P-450.

| Treatment | *Aminopyrine N-demethylase* (*nmol HCHO* $min^{-1}$ *mg protein*$^{-1}$) | | | | *Cytochrome P-450* (*nmol/mg protein*) | | | |
|---|---|---|---|---|---|---|---|---|
| | Series I | *n* | Series II | *n* | Series I | *n* | Series II | *n* |
| Untreated | 9.8 ± 1.1 | 12 | 10.1 ± 0.7 | 13 | 1.09 ± 0.24 | 12 | 0.93 ± 0.08 | 13 |
| Vehicle i.p. | 5.9 ± 0.9*† | 11 | 9.0 ± 1.0† | 12 | 0.87 ± 0.07† | 11 | 0.83 ± 0.05*† | 12 |
| Indomethacin | 2.3 ± 0.9* | 9 | 4.5 ± 0.7 | 11 | 0.40 ± 0.06 | 9 | 0.48 ± 0.06 | 11 |
| IND + PG | 5.1 ± 0.9† | 7 | 7.9 ± 0.5† | 13 | 0.81 ± 0.07† | 7 | 0.74 ± 0.10† | 13 |
| PG | 9.1 ± 0.8 | 6 | 9.7 ± 0.8 | 5 | 1.05 ± 0.19 | 6 | 0.89 ± 0.03 | 5 |
| Vehicle i.v. | — | | 9.4 ± 1.2 | 3 | — | | 0.85 ± 0.04 | 3 |

Data are means ± s.d. for 2 series of experiments (I and II) with n = number of rats individually measured. *Significantly different from untreated group, $P < 0.001$. †Significantly different from indomethacin (alone) treated group, $P < 0.001$. (Student's *t*-test for non-paired samples.)

We are grateful to Merck Sharp and Dohme for indomethacin and to Dr J.E. Pike of the Upjohn Company, Kalamazoo, for 16,16-dimethylprostaglandin $F_{2\alpha}$.

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## Evidence that platelet arachidonate metabolites play only a minor role in collagen-induced thrombocytopenia in mice

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The lethal effect of collagen administered intravenously to mice is platelet-dependent and prevented by aspirin at doses of 1.67–2.22 mmol/kg p.o. (Nishizawa, Wynalda, Suydam, Sawa & Schultz, 1972). As aspirin inhibits platelet production of prostaglandins $G_2$ and $H_2$ and thromboxane $A_2$ (Hamberg, Svensson & Samuelsson, 1974), the protective effect of aspirin may suggest that such platelet arachidonate metabolites are important mediators of the thrombocytopenic and lethal effect of collagen. However, blockade of mouse platelet cyclo-oxygenase with aspirin *in vitro* has only a weak effect on collagen-induced aggregation (Nunn, 1981). The present experiments were carried out to investigate this apparent inconsistency.

First, the minimum dose of aspirin required totally to block platelet cyclo-oxygenase was determined. Two h after aspirin or 1% (w/v) methyl cellulose, male mice (28–42 g) were placed in $CO_2$ until respiration ceased. Blood (0.5 ml) drawn from the inferior vena cava was mixed with 55 μ trisodium citrate (102 mM) and 0.7 ml $Ca^{++}$-free Tyrode solution and then centrifuged twice on a Thrombo-Fuge (Coulter Electronics Ltd) to sediment erythrocytes. Responsiveness of the supernatant platelet-rich plasma (PRP) to arachidonate was measured photometrically as previously described (Nunn, 1981). Aspirin caused total inhibition at a dose of 0.6 mmol/kg (Figure 1a).

The effect of this dose of aspirin on collagen-induced thrombocytopenia was then examined. Mice were injected intravenously with collagen (Hormon-Chemie, Munich) and exactly 30 s later placed in $CO_2$ until respiration ceased. Platelet count was determined (Ultra-Flo 100 whole blood platelet counter, Clay Adams) in blood samples (3 μl) taken from the inferior vena cava. Each count was expressed as a per cent of that obtained in a tail blood sample immediately before injection of collagen. Aspirin shifted the dose-response curve for collagen to the right (Figure 1b). The dose-ratio of approximately

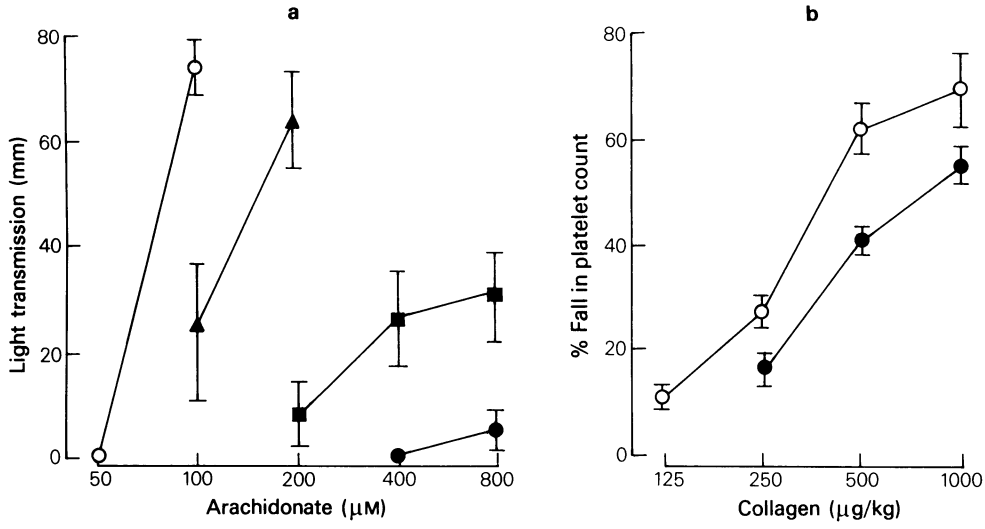


**Figure 1** Effect of aspirin on responsiveness of mouse platelets to (a) arachidonate *ex vivo* and (b) collagen *in vivo*. 2 h after methyl cellulose p.o. (○) or aspirin, 0.15 mmol/kg (▲), 0.3 mmol/kg (■) or 0.6 mmol/kg (●), mice were either (a) bled, PRP prepared and responsiveness to arachidonate assessed photometrically or (b) given intravenous collagen, pH 6.3 and per cent fall in blood platelet count determined as described in the text. Points are mean ± s.e.mean, $n = 10–12$ (a) or 6–9 (b).

1.5 is similar to that found *in vitro* (1.4, Nunn, 1981) and supports the conclusion that platelet arachidonate metabolites play only a minor role in collagen-induced mouse platelet aggregation. This dose of aspirin had no effect on thrombocytopenia associated with lethal doses of collagen (> 4 mg/kg, data not shown). The protective activity of high doses of aspirin reported by Nishizawa *et al.* (1972) is presumably due to a mechanism unrelated to inhibition of platelet cyclo-oxygenase.

I would like to thank Mr. P.D. Chamberlain for excellent technical assistance.

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## Platelet-activating factor (PAF), a secretory product of polymorphonuclear leucocytes, increases vascular permeability in rabbit skin

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Leucotactic substances, such as C5a, increase microvascular permeability in rabbit skin by a mechanism which requires a rapid interaction between polymorphonuclear (PMN) leucocytes and vascular endothelial cells (Wedmore & Williams, 1981). Oedema formation by these substances is dependent on synergism with a vasodilator substance such as prostaglandin (PG) $E_2$ or $I_2$ (Williams, 1979). We have explored the possibility that C5a triggers the PMN leucocyte to release another mediator which acts on the endothelial cell. One of the substances investigated for this role was PAF (see Benveniste,

1980). We considered that four primary criteria should be fulfilled. C5a should stimulate secretion of PAF by PMN leucocytes. Like C5a, oedema induced by PAF should be independent of histamine release, but dependent on the presence of a vasodilator prostaglandin. Finally, unlike C5a, the action of PAF should be independent of PMN leucocytes.

PAF is secreted by PMN leucocytes in response to C5a, *in vitro* (Camussi, Tetta, Bussolino, Caligaris Cappio, Coda, Masera & Segolini, 1981) and we report here that PAF fulfills the other criteria stated above.

Test substances (0.1 ml volumes) were injected into rabbit dorsal skin and plasma exudation measured as the 30 min accumulation of intravenously-injected [$^{125}$I]-albumin (Williams, 1979). Synthetic PAF induced little exudation alone but induced large responses when mixed with $PGE_2$; exudation induced by PAF ($9 \times 10^{-10}$ mol/0.1 ml) = $14.8 \pm 1.7$ µl, $PGE_2$ ($2.8 \times 10^{-10}$ mol/0.1 ml) = $9.2 \pm 4.8$ µl, PAF + $PGE_2$ = $83.3 \pm 12.6$ µl, buffered saline control value of $18.5 \pm 3.7$ µl subtracted from all results, $n = 5$ rabbits. PAF showed activity at doses down to $10^{-11}$ mol. Responses to PAF + $PGE_2$ were unaffected by the antihistamine, mepyramine.

Rabbits were depleted of circulating PMN leucocytes by two different techniques. Acute depletion was achieved by i.v. infusion of zymosan-activated plasma (1 mg zymosan/ml plasma, 37°C for 30 min; plasma infused at 3 ml/0.5 min initially and then 1 ml/min for 30 min); the skin was tested before and during infusion. Chronic depletion was obtained using nitrogen mustard (1.75 mg/kg) as previously described (Wedmore & Williams, 1981). Skin responses to PAF ± $PGE_2$ could be elicited in rabbits acutely or chronically depleted of circulating PMN leucocytes. In this respect the responses were like those to bradykinin, but unlike those to C5a which were abolished by depletion (Wedmore & Williams, 1981).

We do not interpret these results to mean that platelets are involved in increasing permeability in response to C5a, although this is a possibility. Further, we have no *direct* evidence that the release of PAF from PMN leucocytes is involved in skin responses to leucotactic substances, such as C5a; in fact, high doses of C5a are necessary to induce PAF release *in vitro* (Camussi *et al.*, 1981). Nevertheless, these results suggest that there is a possible link between C5a, PMN leucocytes and PAF in our model. The potent action of PAF on the microvasculature makes this substance of considerable interest in its own right.

We thank Dr J. Benveniste, INSERM, Paris, for synthetic PAF-acether.

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## A method for studying lipolysis in cultures of human adipocytes

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Lipolysis of white adipose tissue from man and various animals has been extensively studied during short term *in vitro* incubations. In such studies human adipose tissue is both less sensitive and less responsive to lipolytic agents than fat from animal species (James, Burns & Chase, 1969). For example, we find that rat epididymal adipocytes give approximately a two-fold greater lipolytic response (101 µg glycerol $2 \times 10^5$ cells) to isoprenaline ($10^{-7}$ M), than the same number of human abdominal subcutaneous adipocytes (52 µg glycerol $2 \times 10^5$ cells) when incubated under similar conditions for 2 h.

Therefore we have developed a method that enables lipolysis of human fat to be monitored more consistently by a longer 48 h incubation of isolated

adipocytes. Using aseptic techniques we isolated adipocytes from chopped fat by incubation with collagenase ($1.25 \times 10^3$ μu/ml for 1 h at 37°C) using a method first described by Rodbell (1964). Washed isolated adipocytes were then incubated in medium 199 containing antibiotics (50 u/ml penicillin and 50 μg/ml streptomycin) and supplemented with 4% w/v fatty acid deficient bovine serum albumin at 37°C in an atmosphere of 5% carbon dioxide in humidified air for 48 h. Histological studies using trypan blue uptake showed that $68 \pm 6\%$ ($n = 4$) cells remained intact during incubation. Smith (1970) showed that adipocytes could remain morphologically intact for periods up to 30 weeks in culture medium. Metabolic function as assessed by lipolysis (measured in terms of glycerol release using a Boerhringer Mannheim Kit No. 124966) was found to be similar when stimulated by isoprenaline ($10^{-6}$ M) added to adipocytes at zero time ($72 \pm 11$ μg/24 h) and after 48 h preincubation of the cells ($65 \pm 15$ μg/24 h).

Basal lipolysis ($36 \pm 5$ μg glycerol/48 h) was seen when $6 \times 10^4$ adipocytes were incubated in 1 ml culture medium. Under these conditions catecholamines ($10^{-8}$–$10^{-5}$ M) stimulated lipolysis in a dose-dependent manner with a rank order of potency of isoprenaline > noradrenaline > adrenaline. The order of potency is similar to that seen by incubating human adipose tissue in short term studies (Fain, 1973) and was inhibited by the β-adrenoceptor antagonist propranolol ($10^{-6}$ M). Time-course studies showed that lipolysis in response to stimulation was more rapid in the first 24 h but continued throughout the incubation.

The method utilizes fewer cells/sample than short-term incubation which allows a more economical use of available tissue than was previously possible. This method of studying human adipocyte function has proved effective in furthering studies on prostaglandin biosynthesis by adipose tissue during lipolysis and has potential for the evaluation of the longer term effects of pharmacological agents on adipocyte function.

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## Auto-fluorescence associated with fatty-acid peroxides

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Prostaglandin biosynthesis from polyunsaturated fatty-acids takes place predominantly by enzymatic reactions. Lipid autoxidation, on the other hand, is essentially a non-enzymatic auto-catalytic process initiated and sustained by free-radicals. This type of oxidation has been studied in detail for a much longer period of time, particularly in the food industry where fat rancidity poses many problems. These two apparently dissimilar pathways have recently been shown to share common intermediates such as the cyclic endoperoxides and possibly free radicals as well (Pryor, Stanley & Blair, 1976; Hemler & Lands, 1976). In both fields of study reactivity with 2-thiobarbituric acid (TBA) has been successfully applied as a measure of oxidative fatty-acid conversion (Smith, Ingerman & Silver, 1976; Kwon & Olcott, 1966). When lipid oxidation products are heated with TBA a chromogenic adduct is formed between malondialdehyde and the TBA. Most of the malondialdehyde so detected is formed during the TBA-test by acid/thermal decomposition of peroxidic precursors (Pryor, Stanley & Blair, 1976; Gutteridge, Stocks & Dormandy, 1974).

Fluorescence has been shown to be a sensitive measure of polyunsaturated fatty-acid autoxidation when secondary carbonyls, such as malondialdehyde, complex with amino groups to form fluorescent Schiff bases (Tappel, 1975). Recently, however, auto-fluorescence has been shown to accompany the auto-oxidation of polyunsaturated fatty-acids in the

absence of amino groups (Gutteridge, Lunec & Heys, 1978). The fatty-acids linoleic, linolenic, arachidonic and docosahexaenoic when oxidized in an aqueous milieu by slow autoxidation over several days or by enzymic incubation with plant lipoxidase subsequently showed quite different fluorescent properties. Lipoxidase catalyzed oxidation is known to produce specific hydroperoxides whereas autoxidation gives rise to a more complex mixture of peroxides which includes the endoperoxides. When examined spectrofluorimetrically, only the products from fatty-acids with three-or-more double bonds derived from autoxidation showed fluorescence properties. Incubation of the autoxidised samples with horseradish peroxidase greatly reduced the observed fluorescence, whilst incubation of the lipoxidase-derived samples with 100% oxygen for three days brought about the formation of fluorescent components. Our present studies suggest that certain endoperoxides may have auto-fluorescent properties which could be used to detect their presence in biological systems.

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## 15-lipoxygenase: a rapid, sensitive assay for lipoxygenase inhibitors

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Arachidonic acid is a substrate for cyclo-oxygenase and lipoxygenase enzymes. Nugteren (1975) demonstrated the presence of an arachidonic lipoxygenase in platelets which produces 12-HETE. Polymorphonuclear leucocytes contain a 5-lipoxygenase which can give rise to a group of compounds containing a triene structure named leucotrienes (Murphy, Hammerstrom & Samuelsson, 1979). Hamberg & Samuelsson (1967) showed the presence of a 15-lipoxygenase in soybean which stereospecifically produces 15L-hydroperoxyeicosatetraenoic acid from arachidonic acid.

It has been suggested that dual inhibitors of cyclo-oxygenase and lipoxygenase are likely to be effective anti-inflammatory agents (Higgs, Moncada & Vane, 1980). Dual inhibition has been shown for the anti-inflammatory drugs benoxaprofen (Walker & Dawson, 1979), and BW 755C (Higgs, Flower & Vane, 1979). The preparation and assay of 5- and 12-lipoxygenase is time-consuming. It was the purpose of the investigation to study 15-lipoxygenase from soybean and investigate the actions of a number of drugs which have been reported to inhibit the 5- and 12-lipoxygenase enzymes. The drugs used were benoxaprofen, BW 755C, 1-phenyl-3-pyrazolidone (1-P-3-P) and nordihydroguaiaretic acid (NGA).

15-Lipoxygenase activity was measured by two enzyme assays, a polarographic and a radiochemical assay. The polarographic assay was based on the assay of Saeed, Drew & Collier (1980). Incubations were performed at 37°C in 100 mM phosphate buffer pH 7.5 containing arachidonic acid (240 μM) and soybean lipoxygenase (80 μg, Sigma). Enzyme activity was determined with an oxygen electrode. The change in oxygen saturation of the mixture was recorded for 3 min. For the radiochemical assay soybean lipoxygenase (80 μg) was incubated in phosphate buffer (100 μM, pH 7.5) at 37°C for 5 min with

unlabelled (240 μM) and labelled (0.1 μCi, 1-[$^{14}$C]) arachidonic acid. Reactions were terminated by acidification to pH 3 followed by acid-lipid extraction and thin layer chromatography. The radioactive zones were quantitated by liquid scintillation counting.

All four drugs tested produced a concentration-dependent inhibition of lipoxygenase activity. Similar results were obtained for both assay methods. The $IC_{50}$ values obtained were: NGA, 2.4 μM; BW 755C, 8.2 μM; 1-P-3-P, 15 μM and benoxaprofen 5.3 mM. These results compare favourably with published values for inhibition of the 5 and 12-lipoxygenase enzymes. Hamberg (1976) observed 90% inhibition of 12-lipoxygenase with 0.5 mM NGA. The $IC_{50}$ for 1-P-3-P on 12-lipoxygenase was 0.31 mM (Blackwell & Flower, 1978). The $IC_{50}$ values of benoxaprofen and BW 755C respectively have been reported to be 82 μM (Walker & Dawson, 1979) and 6.4 μM (Higgs, Flower & Vane, 1979).

15-Lipoxygenase may thus prove a useful assay for lipoxygenase inhibitors and offers a number of advantages: firstly, the enzyme is commercially available; secondly, the assay is sensitive and specific; thirdly, the polarographic assay is rapid and inexpensive.

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## The lipoxygenase inhibitor, BW 755C, stimulates prostacyclin production from the rat gastric mucosa *in vitro*


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Aspirin, indomethacin, naproxen and flurbiprofen, in doses which reduce prostaglandin levels in inflammatory exudates also reduce prostacyclin formation in the gastric mucosa and lead to gastric erosions in the rat (Whittle, Higgs, Eakins, Moncada & Vane, 1980). In contrast, the dual cyclo-oxygenase-lipoxygenase inhibitor BW 755C (Higgs, Flower & Vane, 1979) in doses which reduce prostaglandin levels in inflammatory exudates, fails to inhibit gastric prostacyclin formation or produce gastric damage. To investigate further this selectivity of action, we have now compared the actions of BW 755C to other non-steroid anti-inflammatory agents on prostacyclin formation from rat gastro-intestinal tissue *in vitro*.

Strips of rat gastric mucosa and segments of the muscular forestomach or small-intestine were weighed, washed in Tris buffer (50 nM; pH 7.4 at 4°C), homogenized in the buffer (0.2 g/ml) and stored on ice. Aliquots (0.5 ml) of the homogenate were incubated with 0.5 ml of Tris buffer containing $Ca^{++}$ (5 mM) or the same buffer with the drug under investigation. After incubation (10 min at 22°C), the suspension was rapidly centrifuged (20 s at 9000 × g) in an Eppendorf bench centrifuge, resuspended and re-centrifuged. The pellet was again resuspended in the $Ca^{++}$ buffer or drug solution and vortexed for 30 s. After a further rapid centrifugation (30 s) the supernatant was immediately tested for its ability to inhibit human platelet aggregation, and this prostacyclin-like activity, characterized as before (Whittle *et al.*, 1980) was assayed against authentic prostacyclin.

Non-steroid anti-inflammatory drugs dose-dependently inhibited prostacyclin formation in the

homogenate of rat gastric mucosa. The dose causing 50% inhibition ($ID_{50}$) of prostacyclin formation was, for indomethacin (3.9 μM), ketoprofen (5.3 μM), naproxen (3.9 μM), flurbiprofen (0.5 μM) and meclofenamate (11.8 μM). Similar $ID_{50}$ values for these compounds were found for prostacyclin generation in homogenates of rat forestomach.

In contrast, BW 755C (0.5–80 μM) caused significant stimulation of prostacyclin generation from mucosal homogenates; BW 755C (40 μM) increased prostacyclin production to 261 ± 31% of control ($n = 10$; $P < 0.001$). At higher concentrations BW 755C (100–1000 μM) inhibited prostacyclin generation ($ID_{50}$ 380 μM). In forestomach homogenates, BW 755C (2 μM) stimulated prostacyclin formation (178 ± 8% of control, $n = 3$; $P < 0.05$) but inhibition occurred at much lower doses than in mucosal homogenates ($ID_{50}$ 26 μM).

The formation of prostacyclin from homogenates of rat ileum was also inhibited by incubation with anti-inflammatory drugs, with $ID_{50}$ values for indomethacin (5.6 μM), flurbiprofen (0.5 μM) and naproxen (6.5 μM). Stimulation of prostacyclin formation was again observed with BW 755C (159 ± 17% of control with 4 μM, $n = 4$; $P < 0.05$), with inhibition at higher doses ($ID_{50}$ 400 μM).

This study demonstrates that following a suitable preparative, incubation and washing procedure, homogenates of gastro-intestinal tissue can be provoked into reproducibly generating prostacyclin from its endogenous substrate. This technique thus provides a rapid and convenient method for the study of drugs on prostaglandin formation from gastro-intestinal and perhaps other tissues *in vitro*. The concentration of indomethacin and the other anti-inflammatory agents which inhibit prostacyclin production from the rat gastric mucosa were similar to those inhibiting cyclo-oxygenase activity in other preparations (Vane, 1971; Flower, 1974). However, BW 755C inhibited prostacyclin production from gastric mucosa and ileum only at high concentrations. This contrasts with the comparable potency of BW 755C to indomethacin as an inhibitor of cyclo-oxygenase in platelets (Higgs *et al.*, 1979), and its potency as an inhibitor of prostacyclin generation from the muscular forestomach. Thus, as in the previous studies *in vivo*, BW 755C clearly shows selectivity of action against different tissue cyclo-oxygenase *in vitro*. The ability of BW 755C to enhance substantially the prostacyclin generation in the rat gastric mucosa *in vitro* may reflect inhibition of the lipoxygenase pathway, since hydroperoxy arachidonic acid products inhibit prostacyclin synthetase (Gryglewski, Bunting, Moncada & Vane, 1976). In addition, it may reflect diversion of substrate to the cyclo-oxygenase pathway.

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## Is increased vascular permeability induced by C5a dependent on the generation of lipoxygenase products?

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Intradermal injection of C5a or C5a des Arg results in increased vascular permeability in rabbit skin (Williams & Jose, 1981). Oedema formation by these substances is dependent on synergism with a vasodilator substance such as $PGE_2$ or $PGI_2$ (Williams, 1979). A lipoxygenase product of arachidonic acid (AA) derived from polymorphonuclear leucocytes, leukotriene $B_4$ ($LTB_4$), has also been shown to induce oedema formation when in the presence of a vasodilator substance (Wedmore & Williams, 1981; Bray, Cunningham, Ford-Hutchinson & Smith, 1981).

Experiments in some systems have suggested that the effects of C5a may be mediated by lipoxygenase products (Stimler, Brocklehurst, Bloor & Hugli, 1980; Regal & Pickering, 1981). For this reason we have investigated the possibility that C5a may be acting, at least partly, by releasing $LTB_4$ in the skin.

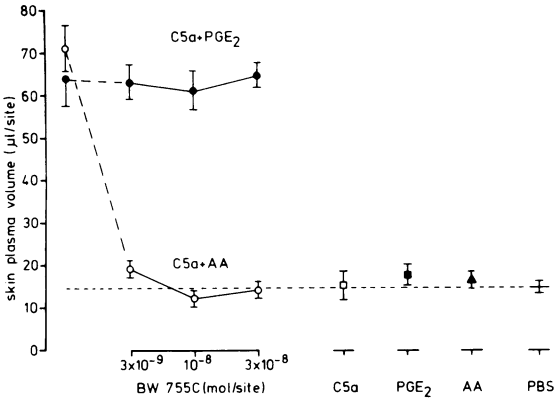


**Figure 1** Potentiation of the effects of C5a des Arg on oedema formation by $PGE_2$ and AA. Oedema to C5a des Arg + AA was inhibited by BW 755C at all doses used.

Agonists in combination with the cyclo-oxygenase/lipoxygenase inhibitor BW 755C (Higgs, Flower & Vane, 1979) were injected (0.1 ml volumes) into rabbit dorsal skin and plasma exudation measured as the 30 min accumulation of intravenously-injected [$^{125}$I]-albumin (Williams, 1979). As shown in figure 1, C5a des Arg ($6 \times 10^{-11}$ mol), $PGE_2$ ($3 \times 10^{-10}$ mol) and AA ($3 \times 10^{-9}$ mol) alone induced little exudation when compared to the phosphate-buffered saline (PBS) control value. Both $PGE_2$ and AA potentiated responses to C5a des Arg. Responses to C5a des Arg + AA were clearly inhibited by BW 755C at all the doses used; a phenomenon similar to the effects of indomethacin on responses to bradykinin + AA (Williams, 1979). This shows that BW 755C inhibits prostaglandin formation from substrate, by inhibiting cyclo-oxygenase. However, BW 755C had no effect on oedema induced by C5a des Arg mixed with exogenous $PGE_2$, suggesting that C5a des Arg does not release $LTB_4$ in order to increase permeability. In a series of experiments, the top dose of BW 755C used ($3 \times 10^{-8}$ mol) produced a $73.4 \pm 8.1\%$ ($n = 5$) inhibition of responses to C5a des Arg + AA, but no significant effect on responses to C5a des Arg + $PGE_2$, i.e. $6.2 \pm 8.7\%$ ($n = 6$).

These results suggest that increased vascular permeability induced by C5a des Arg is not due to endogenous $LTB_4$ production in the skin.

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## Generation of a Leukotriene (LT) $B_4$-like Material from rat basophilic leukaemia (RBL-1) cells, and its actions in guinea-pig lung *in vitro*

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Leukotriene $B_4$ has potent chemokinetic and aggregating properties in polymorphonuclear leukocytes (PMNs) (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980) and was found to be an active bronchoconstrictor in guinea-pig lung *in vitro* (Sirois, Borgeat, Jeanson, Roy & Girard, 1980). Using a modification of the method for preparation of $LTD_4$ from RBL-1 cells (Morris, Taylor, Piper, Samhoun & Tippins, 1980) we have generated a potent $LTB_4$-like material. Cells ($1.0 \times 10^7$ cells/ml) were incubated with arachidonic acid (25 µg/ml), calcium ionophore A 23187 (10 µg/ml) for 4 min at 37°C.

Following extraction, samples were purified by reverse phase high pressure liquid chromatography (HPLC) (micro Bondapak $C_{18}$) in the n-propanol:acetic acid system (Morris *et al.*, 1980). The column eluate was monitored by ultraviolet (u.v.) absorbance ($A_{280}$, $A_{254}$) and bioassay on guinea-pig parenchymal strips (GPP) and guinea-pig ileum smooth muscle (GPISM). Naturally-occurring $LTB_4$ obtained from PMNs (a gift from Dr A.W. Ford-Hutchinson) and used as a standard, was chromatographed immediately after the RBL-material. Biological activity on GPP only was associated with a u.v. absorbing material ($MeOH_{max}$ 270 nm) eluting in the same position as the standard $LTB_4$. This was not fully resolved from the major u.v. absorbing species eluting earlier ($MeOH_{max}$ 268 nm), presumably the trans isomer of $LTB_4$ (Morris *et al.*, 1980). A material with $LTC_4$-like properties on GPP and GPISM, eluted prior to $LTB_4$.

We have investigated the actions of the $LTB_4$-like substance produced from RBL-1 cells using GPP and GPISM and compared them with those of standard $LTB_4$, and synthetic $LTD_4$. The $LTB_4$-like substance (quantified using u.v. absorbance) contracted GPP ($7.5 \times 10^{-12}$ mol – $7.5 \times 10^{-11}$ mol), but had no effect on the GPISM, while $LTD_4$ ($2 \times 10^{-12}$ mol – $2 \times 10^{-11}$ mol) contracted both tissues. The standard $LTB_4$ produced similar results to those obtained with the $LTB_4$-like material. Imidazole (200 μg/ml) reduced the contractions of GPP induced by $LTD_4$, standard $LTB_4$ and the $LTB_4$-like material by 65–100%. FPL 55712 (1 μg/ml) completely antagonized contractions on GPP, GPISM induced by $LTD_4$ and partially reduced those induced by standard $LTB_4$ and $LTB_4$-like material.

The $LTB_4$-like material generated from RBL-1 cells, like $LTC_4$, $LTD_4$, is a potent constrictor of GPP, but may be distinguished from these by its lack of activity on GPISM. The effects of imidazole, suggest that, as with $LTC_4$, $LTD_4$, the action of $LTB_4$-like material on GPP is mediated via thromboxane $A_2$ (Piper & Samhoun, 1981).

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## Biological activities of leukotriene $B_4$ and the cytoskeleton

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Cytotaxins such as N-formylmethionylleucylphenylalanine (FMLP), C5a and leukotriene $B_4$ isomer III ($LTB_4$) stimulate the aggregation and chemokinesis of polymorphonuclear leucocytes (PMNs) and the release of lysosomal enzymes from cytochalasin B pretreated PMns (Wilkinson & Lackie, 1979; Hoffstein, 1980; Smith, 1981). Microtubules may be involved in the above phenomena, and FMLP and C5a have been shown to promote microtubular assembly in PMNs (Hoffstein, 1980).

We have examined the effects of the microtubular disruptive drugs, colchicine and vinblastine on $LTB_4$ stimulated aggregation of rat PMNs, the chemokinesis of human PMNs and the release of lysosomal enzymes from human PMNs pretreated with cytochalasin B. The involvement of microfilaments in aggregation and chemokinesis has also been investigated using cytochalasin B.

Preincubation of rat PMNs for 30 min with colchicine and vinblastine caused a dose related inhibition of $LTB_4$ stimulated aggregation. The release of β glucuronidase and lysozyme from human PMNs was also inhibited by preincubation of the cells for 60 min with colchicine. Chemokinesis was inhibited in a dose dependent manner by colchicine and vinblastine. High concentrations of both drugs also inhibited random migration. $LTB_4$ stimulated chemokinesis and random migration was inhibited by cytochalasin B. Aggregation was enhanced by concentrations of cytochalasin B which inhibited cell movement.

The results suggest that both microtubules and microfilaments are involved in the responses of PMNs to $LTB_4$. It will be necessary for this to be demonstrated by more direct methods such as electron microscopy.

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## The effects of leukotriene $B_4$ on isolated smooth muscle preparations

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Leukotrienes $C_4$ and $D_4$, formed from arachidonic acid via an unstable epoxide intermediate (leukotriene $A_4$) cause slow contractions of isolated smooth muscle preparations (Hedquist, Dahlen, Gustafsson, Hammarström & Samuelsson, 1980). Leukotriene $B_4$ (5,12,dihydroxyeicosatetraenoic acid, $LTB_4$), also derived from leukotriene $A_4$, has been shown to affect both vascular and cellular aspects of inflammation (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980; Bray, Cunningham, Ford-Hutchinson & Smith, 1981). We have studied the effects of $LTB_4$ on smooth muscle preparations that have been used to assay such mediators of inflammation as histamine, bradykinin and the prostaglandins. Standard agonists were used as positive controls.

The guinea-pig vas deferens and tracheal spiral, the rat fundic strip and the rabbit aorta did not contract in response to concentrations of $LTB_4$ giving maximal effects on leucocyte aggregation and movement (0.1–20 ng/ml). An increase in spontaneous activity of the guinea-pig ileum was observed after addition of these concentrations of $LTB_4$, and a contraction was also observed in some ileal preparations. The responses to other agonists such as acetylcholine and histamine were increased after exposure of the ileum to $LTB_4$. A similar effect was observed with $LTC_4$ and $LTD_4$.

It has been reported that $LTB_4$ in microgram amounts can cause contraction of pulmonary muscle *in vitro* (Sirois, Borgeat, Jeanson, Roy & Girard, 1980). The results obtained in this study suggest that $LTB_4$, in concentrations found in inflammatory exudates (Klickstein, Shapleigh & Goetzl, 1980), does not act directly on smooth muscle but may potentiate the effects of other agonists which may be present.

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# Neutrophil aggregation induced by PAF-acether and leukotriene $B_4$

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Platelet activating factor (PAF-acether) is a phospholipid mediator recently identified as 1-0-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (Demopoulos, Pinkard & Hanahan, 1979; Benveniste, Tence, Varenne, Bidault, Boullet & Polonsky, 1979). This compound aggregates platelets and releases their granule constituents (Vargaftig, Chignard & Benveniste, 1981). PAF-acether and leukotriene $B_4$ ($LTB_4$) are both produced by polymorphonuclear leucocytes (PMNs) and induce the aggregation of PMNs (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980; Camussi, Tetta, Bussolino, Cappio, Coda, Masera & Segolini, 1981). It is possible that either agent may induce the aggregation of PMNs indirectly by releasing the other agent.

Both $LTB_4$ and PAF-acether were tested for their ability to aggregate washed rabbit platelets suspended at a concentration of $3 \times 10^8$/ml in Tris-Tyrode solution containing 0.25% (w/v) bovine serum albumin. $LTB_4$ over the concentration range 100 pg/ml to 100 ng/ml caused no platelet aggregation. PAF-acether induced platelet aggregation at concentrations as low as 300 pg/ml ($ED_{50}$ $1.2 \times 10^{-9}$ M). Intravenous administration of $LTB_4$ into rabbits causes a profound neutropenia with no effect on platelet numbers (Bray, Ford-Hutchinson & Smith). These results demonstrate first that $LTB_4$ has different properties to PAF-acether and secondly that $LTB_4$ does not release PAF-acether *in vivo*.

PMN aggregation was assessed using rat peritoneal PMNs as previously described (Cunningham, Shipley & Smith, 1980). Both $LTB_4$ and PAF-acether caused partially reversible PMN aggregation at concentrations as low as 100 pg/ml ($ED_{50}$ $1.2 \times 10^{-9}$ M) for $LTB_4$ and 300 pg/ml ($ED_{50}$ $3.3 \times 10^{-9}$ M) for PAF-acether. In contrast, the non-acetylated analogue of PAF-acether (Lyso-PAF-acether) caused a small neutrophil aggregation response only at 1 $\mu$g/ml. The PMN aggregation response to 10 ng/ml PAF-acether was unaffected by pretreatment of the cells with either indomethacin ($10^{-4}$ M) or nordihydroguaiaretic acid ($10^{-5}$ M). Desensitization studies were carried out in which the response to either PAF-acether (10 ng/ml) or $LTB_4$ (1 ng/ml) was measured 4 min after an aggregation response to the same or a different aggregating agent. There was a 71% reduction in the response to $LTB_4$, added 4 min after $LTB_4$, and a 74% reduction in the second response to PAF-acether in a similar experiment. In contrast the responses to PAF-acether after addition of $LTB_4$ and $LTB_4$ after PAF-acether were reduced by only 11 and 32% respectively. These results clearly demonstrate that PAF-acether does not induce PMN aggregation by releasing $LTB_4$ and suggest that both agents have separate receptor sites on the neutrophil cell surface.

I would like to thank Dr J. Benveniste for the supply of synthetic PAF-acether.

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## Action of Leukotrienes C & D on the motility of the guinea-pig stomach *in vivo* and *in vitro*

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On previous occasions we have reported to the society (Chadwick, Francis & Goadby, 1981, Francis & Goadby, 1981) that anaphylaxis in the guinea-pig stomach *in vivo* and *in vitro* leads to increases in motility mediated, at least in part; by SRS-A. As SRS-A has now been identified as comprising leukotrienes C and D (Sammuelsson, 1980, Morris *et al.*, 1980) the availability of small quantities of these compounds allowed a comparison of their effects on gastric motility with those of anaphylaxis.

Changes in motility were studied as previously described (Chadwick, Francis & Goadby, 1981) where drugs were injected directly into the gastric vasculature via a retrograde cannula in the coeliac artery *in vivo*. *In vitro* the coeliac artery was cannulated and the drugs injected into the vasculature as described by Spedding (1977). Stimulation of the attached vagus nerves, in the isolated stomach, was used to obtain an indication of the maximal response (MVR) of the stomach (designated 100%) and provided a response with which responses to other mediators could be compared.

Injections of LTC (160 ng or 320 ng) *in vivo* caused a large sustained contraction of the stomach which did not return to pre-injection levels within 30 minutes. However although LTD (80 ng or 160 ng) caused initial large contractions of a similar size to those provoked by LTC, the baseline motility was re-established within 5 minutes.

When compared with the effects of exogenous SRS-A (50–200u Fisons) the contraction produced by the leukotrienes were considerably greater, but of the same approximate size and duration to the response to antigen challenge in the case of LTC. The slow decline in systemic blood pressure observed after antigen challenge was not observed with either LTC or LTD.

In the isolated stomach LTD (83 ng) caused a mean displacement of 68% MVR the contractions being of short duration. LTC at higher doses (160 and 320 ng) gave responses of a mean 55 and 80% MVR respectively. These responses could be compared with the mean responses produced by injection of antigen (1 mg egg albumin) to stomachs of actively sensitized guinea-pigs $75 \pm 7\%$ MVR (mean $\pm$ s.e.mean $n = 11$) and the maximal responses produced by $PGE_2$ (100 ng) $58 \pm 5\%$ MVR ($n = 5$) and SRS-A (100U Fisons) $63 \pm 9\%$ MVR ($n = 9$).

Previous work (Francis & Goadby, 1981) has shown that the principal mediators of gastric motility changes induced by anaphylaxis are prostaglandins and/or SRS-A. The current experiments show that leukotrienes are capable of producing contractions of the stomach of a similar order to those produced by anaphylaxis. When samples of leukotrienes become readily available it will be possible to assess their importance in the mediation of other changes in visceral organs induced by immunological reactions.

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## Differential effects of 'calcium-antagonists' on heart rate and atrio-ventricular node conduction in pithed rats

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'Calcium-antagonists' show varying abilities to cause bradycardia and second degree atrioventricular (2°AV) block in man (Leonetti, Sala, Bianchini, Terzoli & Zanchetti, 1980; Opie, 1980). Some of the differences have been attributed to reflex sympathetic nerve activity counteracting the effects of the drugs (e.g. Leonetti *et al.*, 1980). In this study, I have compared the effects of a number of 'calcium-

antagonists' on cardiac rate and AV node conduction in pithed rats, a model devoid of autonomic reflex activity.

Male Sprague Dawley rats (250–300 g) were anaesthetized with sodium pentobarbitone (40 mg/kg, i.p.), pithed and respired with oxygen. Body temperature was maintained between 35.0 and 36.5°C. Blood pressure was measured from the left carotid artery and the antagonists were administered cumulatively at 15–30 min intervals via the left jugular vein. Heart rate was calculated from the ECG and averaged 280 ± 4 beats/min, $n = 68$, after 30 min.

(±)-Verapamil (0.1–3 μmol/kg) lowered blood pressure and prolonged P-P and P-R intervals. Heart rate at the onset of 2°AV block (as indicated by the first dropped beat) was 263 ± 14 beats/min ($n = 5$) and occurred at 1 μmol/kg. Pretreatment with atropine (1 mg/kg, i.v.), (±)-propranolol (1 mg/kg, i.v.) or reserpine (5 mg/kg, i.p., 18 h previously) did not change initial P-P or P-R intervals or the responses to (±)-verapamil. Diltiazem (0.1–10 μmole/kg) resembled verapamil in causing 2°AV block at a heart rate of 254 ± 10 beats/min, $n = 6$.

However, whereas cinnarizine (0.1–10 μmol/kg), pimozide (0.1–10 μmol/kg) and fendiline (0.1–10 μmol/kg) reduced blood pressure and slowly (10–30 min) prolonged P-P and P-R intervals, 2°AV block either did not occur (cinnarizine, 10 μmol/kg, heart rate 196 ± 14 beats/min, $n = 6$; fendiline 10 μmol/kg, < 200 beats/min, in 3 of 6 experiments) or occurred at low heart rates (pimozide 10 μmol/kg, heart rate 192 ± 3 beats/min, $n = 6$; fendiline 10 μmol/kg, heart rates 242, 230, 218 beats/min, 3 of 6 experiments). These latter drugs were dissolved in 30% polyethylene glycol 300 in saline. The solvent did not affect P-P or P-R intervals.

Nifedipine (0.03–1 μmol/kg) did not significantly change P-P or P-R intervals although all the doses reduced blood pressure, confirming the selectivity of this drug for vascular smooth muscle (Taira & Narimatsu, 1975).

These findings indicate that 'calcium-antagonists' may affect the sinus and AV nodes to different extents, in that only some of the drugs caused appreciable cardiac slowing prior to the onset of 2°AV block. Further, these differences are not secondary to reflex activation of the autonomic nervous system. It remains to be seen whether the bradycardia caused by some of the drugs results from a specific inhibition of $Ca^{++}$ influx.

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## Differential effects of dopamine receptor and $\alpha_2$-adrenoceptor agonists on pressor responses to sympathetic nerve stimulation in pithed rats

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Presynaptic $\alpha_2$-adrenoceptors present in peripheral sympathetic neurons when stimulated by appropriate agonists reduce the output of noradrenaline released during nerve stimulation (Langer, 1980). On the basis of this observation, the idea was advanced that a peripherally acting $\alpha_2$-adrenoceptor agonist might be a potentially interesting antihypertensive drug (Langer, Massingham & Cavero, 1980). However, this working hypothesis is not yet supported by experimental results obtained in intact animal preparations. Recently, we presented experimental evidence that neuronal dopamine receptors are responsible for the blood pressure lowering effects of dopamine agonists and may be considered a potential site of action for novel peripherally acting antihypertensive agents (Lefèvre-Borg & Cavero, 1980; Cavero & Lefèvre-Borg, 1981a; Cavero, Lefèvre-Borg & Gomeni, 1981).

The relative effectiveness of pergolide, a dopamine receptor agonist (Cavero & Lefèvre-Borg, 1981b) and clonidine to inhibit sympathetic nerve function was assessed in the vascular bed of pithed rats.

Normotensive male rats (Sprague Dawley, Charles River France) weighing 220–250 g were anaesthetized with pentobarbitone, pithed and treated with atropine (1.0 mg/kg, i.v.) plus (+)-tubocurarine (5.0 mg/kg, i.v.). Mean carotid artery blood pressure and heart rate were measured as described by Cavero *et al.* (1981).

Initially, we determined the doses of clonidine and pergolide decreasing by 50% ($ED_{50}$) the tachycardia (70–85 beats/min) evoked by electrical stimulation (0.1–0.3 Hz, 0.5 ms, 50 V) of the thoracic spinal cord.

The entire sympathetic ouflow was stimulated for 20 s (0.5–8.0 Hz, 0.5 ms, 60 V) in propranolol (1.5 mg/kg, i.v.) pretreated adrenalectomized rats which were injected either saline or sulpiride followed 10 min later by clonidine or pergolide. Electrical stimulation was performed when the pressor effect of both compounds had disappeared (10–15 min after administration).

The $ED_{50}$'s of clonidine and pergolide were $1.4 \pm 0.1$ (mean ± s.d.) and $9.0 \pm 0.6$ μg/kg, i.v. respectively. They were not significantly affected by sulpiride (0.3 mg/kg, i.v.) pretreatment but were increased by approximately 4–5 fold by yohimbine (0.1 mg/kg, i.v.).

When the entire spinal cord was stimulated, clonidine (10.0 μg/kg, i.v.) produced a small but non-significant reduction of peak blood pressure responses evoked by frequencies below 1.0 Hz. In contrast, pergolide (10.0 μg/kg, i.v.) significantly ($P < 0.05$: unpaired *t*-test) inhibited the pressor response to each frequency used. For instance, under control conditions 1.0 and 8.0 Hz resulted in $40.7 \pm 3.3$ and $91.7 \pm 9.6$ mmHg (mean ± s.d., $n = 6$) increase in blood pressure, respectively. After pergolide these responses were $22.2 \pm 1.8$ and $55.2 \pm 5.2$ mmHg ($n = 5$). Whilst sulpiride (0.3 mg/kg, i.v.) entirely antagonized this effect it did not by itself modify the blood pressure frequency-response curve.

Pergolide did not affect pressor responses produced by intravenous administration of noradrenaline in pithed rats nor decreased arterial pressure in this preparation even when the latter parameter was elevated to pre-pithing levels by infusing vasopressin. This excludes the possibility that pergolide possesses α-adrenoceptor antagonist properties or that it produces active vasodilatation.

These results indicate that pergolide (10 μg/kg, i.v.) inhibits neurally induced pressor responses and tachycardia in pithed rats. The latter effect is due to stimulation of $\alpha_2$-adrenoceptors (Cavero & Lefèvre-Borg, 1981b), whilst the former is compatible with stimulation of peripheral neuronal dopamine receptors since it was blocked by sulpiride. Conversely, a dose of clonidine seven times greater than the $ED_{50}$ of pergolide at cardiac presynaptic $\alpha_2$-adrenoceptors did not significantly modify the pressor responses to electrical excitation of sympathetic nerves. Thus, sympathetic tone to the vascular bed may be more easily reduced by pharmacological activation of presynaptic dopamine receptors than $\alpha_2$-adrenoceptors present on peripheral sympathetic neurons.

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## The effect of clonidine on the physostigmine-induced vasopressor response in anaesthetized rats

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In rats, the intravenous injection of physostigmine causes a vasopressor response that is mediated via an increased sympathetic outflow; this effect stems from stimulation of central muscarinic receptors mainly by an increased release of acetylcholine (Stamenovic & Varagic, 1970). The possible central sites of action include the posterior hypothalamus (P.H.) and brain stem (Brezenoff & Rusin, 1974; Buccafusco & Brezenoff, 1979). Clonidine reduces the pressor response to physostigmine (Buccafusco, Finberg & Spector, 1980). We have examined the interaction between clonidine and physostigmine and attempted to characterize the receptors involved.

Female Lister Hooded (L.H.) rats (180–250 g) were anaesthetized with urethane (1.25 g/kg, i.p.) and pretreated with methylatropine (1 mg/kg, i.v.) to block peripheral muscarinic receptors. Physostigmine (100 μg/kg, i.v.) caused reproducible increases in blood pressure of 28–36 mmHg; peak effect was seen within 3 min and the response lasted approximately 30 min.

The pressor response to physostigmine was unaffected by pretreatment (20 min) with saline (10 μl intracerebroventricularly; i.c.v.), procaine (53 μg, i.c.v.), or clonidine (3 μg, i.c.v.) but higher doses of clonidine (10–30 μg, i.c.v.) reduced the responses. In contrast, clonidine (10 μg, i.c.v.) did not reduce the pressor response produced by arecoline (100 μg/kg, i.v.). Thus clonidine (10 μg, i.c.v.) probably reduces the response to physostigmine by stimulating central presynaptic receptors and inhibiting acetylcholine release.

Yohimbine (10–30 μg, i.c.v.) and prazosin (0.5–10 μg, i.c.v.) reduced resting blood pressure by 14–28% and 25–43%, respectively, but did not reduce the peak pressor response to physostigmine. In separate experiments, the clonidine-induced reduction in the pressor response to physostigmine was inhibited by pretreatment (20 min) with yohimbine (30 μg, i.c.v.) but not by prazosin (10 μg, i.c.v.). These results suggest an effect of clonidine at $\alpha_2$-adrenoceptors but higher doses of the antagonists could not be studied because they, themselves, reduced the pressor response to physostigmine.

To avoid the complicating hypotensive effects of the α-adrenoceptor antagonists given i.c.v., an attempt was made to localize the brain region in which physostigmine and clonidine interacted. Physostigmine (10–30 μg) injected bilaterally into the P.H. increased blood pressure, and atropine (30 μg bilateral P.H.) prevented the response. However, neither atropine (30 μg bilateral P.H.) nor clonidine (5 μg bilateral P.H.) reduced the pressor response to *intravenously* injected physostigmine.

These results suggest that clonidine inhibits the vasopressor response to physostigmine by stimulating presynaptic $\alpha_2$-adrenoceptors on central cholinergic nerves but that the site of action is not the posterior hypothalamus.

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# A comparison of the cardiovascular effects of meptazinol, morphine and naloxone in haemorrhagic shock in rats

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Meptazinol, m-(3-ethyl-1-methyl-hexahydro-1H-azepin-3-yl) phenol hydrochloride, possesses potent analgesic activity in man and animals with no significant cardiovascular effects (Goode & White, 1971; Oosterlinck & De Sy, 1975; Paymaster, 1976; Rashid & Waterfall, 1979). Morphine produces hypotension particularly in patients with a depleted blood volume whereas naloxone, a specific opiate antagonist, facilitates recuperation of arterial pressure in various shock states (Drew, Dripps & Comroe, 1946; Faden & Holaday, 1979; Peters *et al.*, 1981).

Since meptazinol is an opiate partial agonist this study examines the effect of this agent on blood pressure and heart rate in conscious rats subjected to a 20% haemorrhage.

Female Sprague Dawley rats were prepared, under anaesthesia, with indwelling left carotid cannulae. Two hours later 20% haemorrhage was produced by removal of 5% aliquots of the blood volume at 5 min intervals. After 20 min the rats received placebo, morphine or meptazinol i.m., or naloxone i.v. Equianalgesic doses of meptazinol and morphine were administered.

Administration of morphine produced an immediate hypotension whereas meptazinol elicited a rapid increase in arterial pressure which returned to pre-haemorrhage levels within 5 min after administration. Naloxone produced an increase in arterial pressure which was significant 15, 30 and 60 min after treatment.

These results confirm previous observations that morphine evokes an immediate hypotensive effect and naloxone reverses haemorrhagic hypotension. Meptazinol produced an effect similar to the opiate antagonist but which was faster in onset. Meptazinol appears, therefore, to combine its analgesic properties with cardiovascular actions similar to naloxone.

Naloxone may produce pressor effects in haemorrhagic and endotoxic shock by antagonizing the cardiodepressant actions of endorphins released by shock (Faden & Holaday, 1979; Holaday & Faden, 1979). Meptazinol may produce a similar effect as an antagonist within the cardiovascular system whilst retaining analgesic activity.

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**Table 1** Cardiovascular effects of meptazinol, naloxone and morphine in hypovolaemic rats

| *Variable* | *Treatment* | *Dose (mg/kg)* | *Control before haemorrhage* | *20 min post haemorrhage* | *Time after treatment 5* | *15* | *60* |
|---|---|---|---|---|---|---|---|
| Mean BP (mmHg) | Placebo | Vol | 133 ± 4.08 | 112.0 ± 6.07 | 114.0 ± 4.85 | 122 ± 4.32 | 121 ± 3.81 |
| | Meptazinol | 17.4 | 142 ± 7.70 | 117.0 ± 10.20 | 149.0 ± 8.15** | 153 ± 4.75** | 150 ± 6.23** |
| | Naloxone | 10.0 | 128 ± 4.64 | 99.4 ± 6.84 | 108.0 ± 4.64 | 117 ± 7.59* | 115 ± 8.98* |
| | Morphine | 3.4 | 125 ± 3.84 | 101.0 ± 8.47 | 91.4 ± 7.13 | 115 ± 6.98* | 122 ± 3.64* |
| Heart rate (beats/min) | Placebo | Vol | 402 ± 10.80 | 413.0 ± 23.30 | 417.0 ± 28.00 | 433 ± 20.50 | 426 ± 19.80 |
| | Meptazinol | 17.4 | 437 ± 14.60 | 403.0 ± 34.40 | 379.0 ± 21.20 | 432 ± 22.00 | 413 ± 21.30 |
| | Naloxone | 10.0 | 397 ± 29.70 | 348.0 ± 40.00 | 345.0 ± 33.50 | 377 ± 40.10 | 393 ± 24.00 |
| | Morphine | 3.4 | 467 ± 11.70 | 421.0 ± 36.20 | 372.0 ± 31.70 | 472 ± 31.70 | 494 ± 12.60 |

Statistical analysis of the results was performed by analysis of variance.
The readings in the column headed 20 min post-haemorrhage were obtained immediately before drug administration.
In all groups the fall in mean arterial pressure was statistically significant ($P < 0.01$).
Statistical analysis after drug administration was made within groups with respect to post-haemorrhage values.
*$P < 0.05$, **$P < 0.001$.

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## Binding and effects of MPV-295, a novel antihypertensive compound, at $\alpha_2$-adrenoceptors in the rat

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MPV-295 is a novel imidazole derivative, which lowers the blood pressure of normotensive and spontaneous hypertensive rats (Ruskoaho, Puurunen & Kärki, 1981). The mechanism of action is still unclear, in this study we compare the effects of MPV-295 and clonidine at $\alpha_2$-adrenoceptors, since these have been implicated in the mode of action of clonidine.

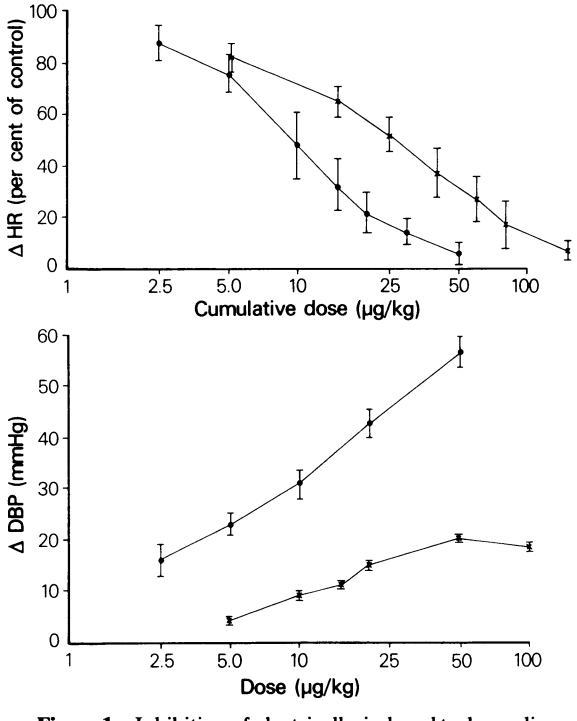


**Figure 1** Inhibition of electrically-induced tachycardia (upper trace) and the immediate hypertensive effect (lower trace) after i.v. injection of MPV-295 (-x-) or clonidine (- · -) in pithed rats.

MPV-295 showed strong affinity for clonidine binding sites in rat brain membranes (normally considered to be $\alpha_2$-receptors). The $K_i$ for the displacement of [$^3$H]-clonidine high affinity binding was $3.9 \pm 0.6$ nmol ($\pm$ s.e.mean) ($n = 5$) compared to $1.4 \pm 0.3$ nmol for clonidine itself. The affinity to $\alpha_1$ binding sites as measured by [$^3$H]-prazosin displacement was much weaker, $K_i$ for MPV-295 was $520 \pm 50$ nmol compared to $1210 \pm 230$ nmol for clonidine. The $K_i$ for prazosin itself was $0.2 \pm 0.1$ nmol.

The effects of MPV-295 at $\alpha$-receptors were examined in the pithed rat preparation. Female F-344 rats (normotensive) weighing 200 g were pithed under ether anaesthesia and respired with air. Blood pressure was recorded from the carotid artery and drugs injected via the external jugular vein. Heart rate was extracted from the pressure curve. The heart rate was increased by stimulation of the cardiac acceleratory nerve with parameters selected to cause a sub-maximal tachycardia of between 50–70 beats per minute.

MPV-295 caused a dose dependent inhibition of the electrically-induced tachycardia (Figure 1), 50% inhibition being obtained at $33.7 \pm 8.4$ μg/kg ($n = 7$). Clonidine caused a similar inhibition at $10.8 \pm 2.2$ μg/kg ($n = 5$). This effect is considered to be mediated via presynaptic $\alpha_2$-receptors (Drew, 1976) and in all cases at the end of the experiment when the cardiac stimulatory effect was completely inhibited, the original response could be regained by administration of yohimbine (0.5 mg/kg) which is considered to be an $\alpha_2$-blocking drug.

Unlike clonidine, MPV-295 had only a slight immediate hypertensive effect in the pithed rat (Figure 1). However doses greater than 200 μg/kg were able to cause reversal of the hypertensive effect of adrenaline suggesting blockade of $\alpha$-receptors.

It is concluded that MPV-295 is an agonist at

peripheral $\alpha_2$-receptors approximately one third as potent as clonidine. A similar potency range was found in binding studies to rat brain membranes and the hypotensive effect is also found at doses approximately three times more than with clonidine. The weaker $\alpha_1$-adrenoceptor effects of MPV-295 compared to clonidine contrast with the stronger binding affinity for prazosin binding sites. This may be due to the fact that MPV-295 is a partial agonist at these sites.

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## Effect of propranolol on blood pressure and vascular responsiveness of conscious normotensive and hypertensive rats

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A previous report by Burkan & Leach (1981) demonstrated that in addition to the antihypertensive effect of propranolol in spontaneously hypertensive rats changes also occur in the organ responsiveness to agonists and sympathetic nerve stimulation. Therefore, we have decided to study the effect of one week propranolol treatment on blood pressure and isolated organ responsiveness of spontaneously, DOCA saline and renal artery-clip hypertensive rats as well as normotensive and genetic non-hypertensive rats.

Female groups of DOCA-saline unilaterally nephrectomized (Stanton & White, 1965), renal artery-clip unilaterally nephrectomized (Goldblatt, Lynch, Hanzal & Summerville, 1934) and spontaneously hypertensive (SHR) rats, as well as normotensive and non-hypertensive rats from the spontaneously hypertensive colony, aged from 10–12 weeks, were used in the studies. Each group was divided into two sub-groups, one group received propranolol ($8\,mg\,kg^{-1}\,day^{-1}$ i.p.) for 7 days whilst the other group was injected with n. saline (0.5 ml/day i.p.). Systolic blood pressures were measured by the tail cuff method prior to and after one week of treatment. At the end of the treatment period each animal was anaesthetized with sodium pentobar-

**Table 1** Effect of propranolol ($8\,mg\,kg^{-1}\,day^{-1}$ i.p.) on conscious systolic blood pressure and responsiveness of isolated mesenteric artery and hepatic portal vein preparations from hypertensive and non-hypertensive groups of rats.

| | | | *Mesenteric artery* *Increase in resting perfusion pressure* (mmHg ± s.e.mean) | | *Hepatic portal vein* *Increase in resting tension* (g ± s.e.mean) | |
|---|---|---|---|---|---|---|
| | *Treatment* | *Conscious systolic blood pressure* (mmHg ± s.e.mean) | NA (0.4 μg) | Stimulation 12(Hz) | NA (0.064 μg/ml) | Stimulation 12(Hz) |
| DOCA-saline | Treated | 140 ± 12*** | 40 ± 7.5*** | 5 ± 1.9 | 0.8 ± 0.05* | 0.78 ± 0.08 |
| hypertensive ($n=10$) | Untreated | 215 ± 8 | 92 ± 10 | 9.7 ± 2.9 | 0.95 ± 0.04 | 0.87 ± 0.05 |
| Renal clip | Treated | 144 ± 8** | 32 ± 6*** | 6.0 ± 2** | 0.81 ± 0.05* | 0.78 ± 0.06 |
| hypertensive ($n=8$) | Untreated | 180 ± 5 | 80 ± 10 | 16 ± 3 | 1.0 ± 0.04 | 0.9 ± 0.05 |
| Spontaneously | Treated | 145 ± 5** | 50 ± 8*** | 4.6 ± 1.4* | 0.99 ± 0.06* | 0.76 ± 0.02* |
| hypertensive ($n=6$) | Untreated | 175 ± 5 | 82 ± 5 | 10 ± 2 | 1.21 ± 0.05 | 0.9 ± 0.06 |
| Non-hypertensive | Treated | 122 ± 5 | 66 ± 6 | 8.5 ± 1.5 | 1.12 ± 0.1 | 1.03 ± 0.08 |
| ($n=8$) | Untreated | 125 ± 5 | 69 ± 6 | 10 ± 2 | 1.0 ± 0.06 | 0.88 ± 0.05 |
| Normotensive | Treated | 120 ± 6 | 62 ± 8 | 13.5 ± 2 | 0.9 ± 0.1 | 0.85 ± 0.08* |
| ($n=8$) | Untreated | 118 ± 7 | 58 ± 10 | 11 ± 1.5 | 0.81 ± 0.08 | 0.64 ± 0.06 |

*** $P = < 0.001$; ** $P = < 0.02$; * $P = < 0.05$.

bitone (60 mg/kg i.p.) and the mesenteric artery (McGregor, 1965) and hepatic portal vein (Axelsson, Wahlström, Johansson & Jonsson, 1967) preparations were isolated and their responsiveness to noradrenaline (NA) (0.1–0.8 μg) and (0.008–0.256 μg/ml) respectively were assessed as well as the effect of peri-arterial/field stimulation of the sympathetic nerves (0.3 ms, 40 V, 3–50 Hz).

As shown in the results Table I, the propranolol blood pressure lowering effect was demonstrated in all three hypertensive groups of animals whilst in the normotensive and genetic non-hypertensive rats no significant blood pressure changes were seen. The responses of the mesenteric artery and hepatic portal vein preparations to NA (0.4 μg) and (0.064 μg/ml) respectively are summarized in the table as well as the responses to sympathetic nerve stimulation (12 Hz).

It can be seen that the anti-hypertensive effect of propranolol as well as the inhibitory actions on vascular responsiveness were only demonstrated in hypertensive rates and not in normotensive animals. The results may suggest that the inhibition of the vascular responsiveness caused by propranolol treatment may contribute to its antihypertensive effect.

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## The interaction of yohimbine, rauwolscine and corynanthine on the central cardiovascular effects of clonidine in conscious SHR

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Clonidine is an antihypertensive agent that acts on central α-adrenoceptors (Kobinger, 1978). We have previously reported that yohimbine and its diastereoisomers rauwolscine and corynanthine, exhibit no preferential antagonism of the central cardiovascular effects of clonidine in the conscious cat (Beckett & Finch, 1981); now we have extended these studies to the conscious spontaneously hypertensive rat (SHR), in an attempt to determine the central $\alpha_1$- or $\alpha_2$-adrenoceptor subtype on which clonidine acts in these species.

Male Okamoto SHR weighing between 350–420 g were used. Drugs were administered intracerebroventricularly (i.c.v.) into a lateral brain ventricle and blood pressure and heart rate recorded via a polythene catheter chronically implanted in the aortic arch (Borkowski & Finch, 1978). In antagonism experiments the antagonists were given 30 min prior to clonidine.

Clonidine (1–8 μg i.c.v.) caused pronounced falls in both blood pressure and heart rate, the maximal effect occurring 30–40 min after administration. Sedation accompanied these effects.

Neither antagonist (25–100 μg i.c.v.) significantly altered resting blood pressure and heart rate 30 min after dosing. Each antagonist was capable of antagonizing the falls in blood pressure and heart rate caused by a submaximal dose of clonidine (4 μg), the rank order potency being rauwolscine > yohimbine > corynanthine (Table 1). According to Weitzell, Tanaka & Starke (1979) this ranking would suggest a central $\alpha_2$-adrenoceptor to be involved in the action of clonidine in SHR. The sedation appeared to be similarly antagonized and this agrees with the results of Timmermans, Schoop, Kwa and Van Zwieten (1981). Contrary to Rockhold & Gross (1981), the antagonists did not cause a rise in blood pressure and heart rate, but this may reflect a difference in the strain of rat used.

**Table 1** The interaction of clonidine with yohimbine and its diastereoisomers in the conscious SHR

| *Dose given i.c.v.* | *Max % Δ from resting levels* B.P. ± s.e.mean | H.R. ± s.e.mean | *n* |
|---|---|---|---|
| Clonidine (1 μg) | −14.2 ± 5.2 | −8.2 ± 4.1 | 6 |
| Clonidine (2 μg) + vehicle | −20.4 ± 5.1 | −14.5 ± 3.3 | 6 |
| Clonidine (4 μg) + vehicle | −33.7 ± 4.2 | −23.7 ± 4.2 | 6 |
| Clonidine (8 μg) | −40.0 ± 1.9 | −35.4 ± 3.3 | 6 |
| Clonidine (4 μg) | | | |
| + corynanthine (25 μg) | −30.1 ± 4.1 | −23.2 ± 4.1 | 6 |
| corynanthine (50 μg) | −23.2 ± 2.8 | −19.2 ± 4.3 | 6 |
| corynanthine (100 μg) | −14.8 ± 1.8** | −7.8 ± 6.7 | 6 |
| + yohimbine (25 μg) | −21.2 ± 2.1* | −14.5 ± 2.4 | 6 |
| yohimbine (50 μg) | −17.5 ± 4.3* | −9.1 ± 4.2* | 6 |
| yohimbine (100 μg) | −7.8 ± 1.8** | −3.8 ± 1.9** | 6 |
| + rauwolscine (25 μg) | −15.4 ± 1.6** | −11.7 ± 2.7* | 6 |
| rauwolscine (50 μg) | −6.3 ± 2.0** | −4.4 ± 3.2** | 6 |
| rauwolscine (100 μg) | −2.6 ± 3.8** | +3.6 ± 4.1** | 6 |

*Significantly different from controls $P < 0.05$, unpaired students *t*-test.
**Significantly different from controls $P < 0.01$, unpaired students *t*-test.
Resting M.A.P. and H.R. = 168.9 ± 10.3 mmHg and 379.3 ± 16.3 beats/min respectively (Mean ± s.e.mean, $n = 15$).

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## The effect of β-blockade on the cardiovascular responses to centrally-administered adrenaline in the rat

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Although a considerable body of circumstantial evidence points to a central component in the antihypertensive action of β-blocking drugs, the results of experiments designed to test this hypothesis are equivocal. For example, intracerebroventricular (i.c.v.) injection of propranolol in the rat was reported by Wepierre, Lindenbaum, Porquet & Cohen (1978) to cause a fall in blood pressure, whereas Sweet, Scriabine, Wenger, Ludden & Stone (1976) reported a transient rise. Similar experiments with other species have yielded equally ambivalent results. In an attempt to delineate further the central

actions of β-blocking drugs on the cardiovascular system, we have used i.c.v. adrenaline as an agonist capable of stimulating both α and β receptors, and possibly even specific adrenaline-receptors, and have investigated the ability of centrally-administered β-blocking drugs to modify the cardiovascular responses to i.c.v. adrenaline.

Male Wistar rats (Alderley Park strain) weighing 220–270 g were anaesthetized with thiobutobarbitone sodium ('Inactin', BYK Ltd) i.p. at a dose of 100 mg/kg. Blood pressure was recorded from a carotid artery and heart rate was derived from the blood pressure pulse. All drugs were injected through a 30 gauge stainless steel cannula inserted by means of a David Kopf stereotaxic instrument into the left lateral cerebral ventricle (co-ordinates A 3.29, L 4.4, H-O. 4 mm; König & Klippel, 1963). Adrenaline hydrogen tartrate (BDH), freshly dissolved in artificial CSF at a concentration of 4 mg/ml, was injected at a rate of 2 μl/min. The total dose of 20 μg was thus contained in 5 μl. The β-blockers (±-propranolol HCl, ±-atenolol; I.C.I. Ltd), or +-propranolol HCl (I.C.I. Ltd) were administered by the same route in a volume of 10 μl, the injections beginning 15 min and ending 10 min before the adrenaline injection.

Administered in this way 20 μg adrenaline alone was without effect on mean arterial pressure (MAP) but reduced heart rate by some 20–30 beats per min. When preceded by 30 μg ±-propranolol, however, the same dose of adrenaline produced a sustained rise in MAP of 32 ± 3 mmHg ($n = 7$). This pressor response was dependent on the dose of propranolol within the range 10–100 μg; these doses of propranolol did not themselves alter MAP. A similar dose-relationship was observed with atenolol pretreatment; after atenolol (100 μg), adrenaline (20 μg) increased MAP by 45 ± 8 mmHg ($n = 7$), an effect comparable in magnitude to that seen after the same dose of propranolol, though of shorter duration.

That the ability of propranolol to unmask the pressor response to adrenaline was dependent on blockade of β-receptors was shown by the lack of response to adrenaline following 30 μg d-propranolol.

That the effect of the β-blockers is mediated centrally was shown by comparing the effect of atenolol, which does not readily cross the blood-brain barrier (Day, Hemsworth & Street, 1977), administered intravenously and i.c.v.; centrally injected adrenaline produced no pressor response after atenolol (100 μg i.v.).

Assuming, therefore, a central locus of action for both adrenaline and the β-blocking drugs, these results suggest that adrenaline may exert both an inhibitory and an excitatory action on MAP. The inhibitory effect appears to be mediated via an action on β-receptors and when this effect is prevented by central β-receptor blockade, the pressor response is revealed. The identity and location of the receptors responsible for this pressor response are currently being investigated.

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## The relationship between *in vivo* pressor responses to alpha adrenoceptor agonists and *in vitro* receptor binding after phenoxybenzamine

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Decreases in α adrenoceptor number which may be related to altered *in vivo* responses have been observed under a variety of conditions (Williams & Lefkowitz, 1977; 1979; Elliot, Peters & Grahame-Smith, 1980).

We have compared the changes observed *in vitro* in radioligand binding and *in vivo* in responses to α adrenoceptor agonists in male White New Zealand

**Table 1** Changes in *in vivo* responses to α adrenoceptor agonists and *in vitro* in the maximum number of binding sites in rabbits pretreated with phenoxybenzamine (mean ± s.d.: $n = 4-6$)

| | *Increase in mean arterial pressure (mmHg)* | | *Maximum number binding sites (fM/mg protein)* | | | |
|---|---|---|---|---|---|---|
| | | | *Spleen* | | *Forebrain* | |
| *Phenoxybenzamine* (mg/kg) | *Phenylephrine* (40 μg/kg) | *Guanabenz* (100 μg/kg) | *Prazosin* | *Clonidine* | *Prazosin* | *Clonidine* |
| — | 75 ± 6 | 26 ± 4 | 163 ± 10 | 202 ± 8 | 133 ± 8 | 189 ± 7 |
| 0.001 | 69 ± 10 | — | $^{**}$57 ± 18 | — | $^{**}$76 ± 15 | — |
| 0.01 | $^{**}$52 ± 8 | — | $^{**}$42 ± 7 | — | $^{**}$61 ± 14 | — |
| 0.05 | $^{**}$35 ± 10 | 27 ± 6 | — | — | — | — |
| 0.1 | $^{**}$20 ± 4 | 24 ± 4 | $^{**}$49 ± 8 | 168 ± 27 | $^{**}$65 ± 9 | — |
| 0.5 | $^{**}$12 ± 3 | $^{*}$17 ± 3 | $^{**}$41 ± 15 | $^{**}$129 ± 20 | $^{**}$51 ± 10 | $^{*}$134 ± 38 |
| 1.0 | $^{**}$ < 10 | $^{*}$17 ± 3 | — | — | — | — |
| 2.5 | **undetectable | $^{**}$13 ± 4 | — | — | — | — |
| 5.0 | **undetectable | $^{**}$6 ± 3 | **undetectable | < 30 | **undetectable | $^{**}$93 ± 42 |

$^{*}P < 0.05$, $^{**}P < 0.01$.

rabbits treated with the irreversible $\alpha_1$ selective adrenoceptor antagonist phenoxybenzamine (Harvey & Nickerson, 1954). Catheters were placed in the central artery and vein of the ear for measurement of mean arterial pressure and injection of drugs. The animals received phenoxybenzamine (0.001–5.0 mg/kg, i.v.) 30 min before pressor responses to the $\alpha_1$ and $\alpha_2$ selective adrenoceptor agonists phenylephrine and guanabenz were studied. For the radioligand binding studies animals were sacrificed 30 min after receiving phenoxybenzamine. [$^3$H]-prazosin and [$^3$H]-clonidine were used as specific ligands (Hamilton & Reid, 1981) and the maximum number of binding sites ($B_{max}$) and their dissociation constant ($K_D$) were calculated by Scatchard analysis.

Phenoxybenzamine (0.01–5.0 mg/kg) caused dose related decreases in phenylephrine pressor responses (Table 1). Much larger doses of phenoxybenzamine were required before pressor responses to guanabenz were significantly reduced.

In the binding studies the reduction in the maximum number of clonidine binding sites closely followed the reduction in *in vivo* guanabenz pressor responses. In contrast, although high doses of phenoxybenzamine produced large reductions in *in vivo* phenylephrine pressor responses and in specific prazosin binding, lower doses caused a greater reduction in binding than in pressor responses. No consistent differences in the dissociation constant ($K_D$) for either ligand were observed.

A similar discrepancy between *in vivo* pressor responses and *in vitro* binding data was observed for $\alpha_1$ adrenoceptors when recovery from a single dose of phenoxybenzamine (5 mg/kg) was followed for eight days.

The discrepancy between the *in vivo* responses to the $\alpha_1$ agonist and the binding data could be accounted for by spare receptor theory (Stephenson, 1956) while the apparent absence of 'spare' $\alpha_2$ adrenoceptors could result from guanabenz being a weak or partial agonist or could reflect differences between postsynaptic $\alpha_1$ and $\alpha_2$ adrenoceptors in coupling receptor activation to smooth muscle contraction.

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## A comparison of histamine receptor distribution in atria from rats and guinea-pigs

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The positive inotropic and chronotropic actions of histamine on guinea-pig atria involve both $H_1$- and $H_2$-receptors: in left atria inotropic effects are mediated by $H_1$-receptors, while the chronotropic effects in right atria involve $H_2$-receptors (Steinberg & Holland, 1975; Inoue, McNeill, Puill & Tenner, 1979). Part of the positive inotropic effect on right atria may be due to a positive frequency-force relationship, (Reinhardt, Wagner & Schümann, 1974). This study compares the distribution and function of histamine receptors in rat atria with those in guinea-pig atria.

Separated left and right atria from rats and guinea-pigs were suspended in Locke Ringer at 32°C, gassed with oxygen. Right atria were always allowed to beat spontaneously: all left atria were driven at 0.5 Hz. All right atria and some left atria were partially depolarized in Locke solution containing 15 mM KCl, which made the right atria quiescent, and the left atria unresponsive to electrical pacing.

Table 1 shows the inotropic effects of histamine ($1 \times 10^{-5}$ M, Hist), 4-methylhistamine ($1 \times 10^{-5}$ M, 4MH), an $H_2$-agonist, and pyridylethylamine ($1 \times 10^{-5}$ M, PEA), an $H_1$-agonist: inotropic effects in depolarized atria are expressed as the percentage restoration of control contraction size, while in the non-depolarized atria inotropic effects are expressed as the percentage increase over control contractions. Table 1 also shows the effectiveness of the $H_1$- and $H_2$-antagonists, mepyramine and metiamide respectively. In other experiments, histamine increased the spontaneous rate of guinea-pig right atria ($41 \pm 2\%$ increase at $1 \times 10^{-6}$ M): this effect was antagonized ($3 \pm 1\%$) by metiamide ($1 \times 10^{-5}$ M), but not by mepyramine ($1 \times 10^{-5}$ M). Histamine, up to $1 \times 10^{-3}$ M had no chronotropic effect on rat right atria.

These results show that histamine receptors are absent from rat left atria, but rat right atria contain $H_1$-receptors mediating the positive inotropic response to histamine. In guinea-pig left atria, only $H_1$-receptors are present, while guinea-pig right atria contain $H_1$- and $H_2$-receptors, both of which mediate inotropic responses to histamine: the $H_2$-receptors also mediate the positive chronotropic response.

### References

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**Table 1** Inotropic actions of histamine (Hist), 4-methylhistamine (4MH) and pyridylethylamine (PEA) on isolated atria from rats and guinea-pigs in the presence of mepyramine (MEP) and metiamide (MET).

| | | *RAT* | | | *GUINEA-PIG* | | |
|---|---|---|---|---|---|---|---|
| | | *% restoration* | *% block of contractions by* MEP $5 \times 10^{-6}$ M | MET $3 \times 10^{-6}$ M | *% restoration or* potentiation | *% block of contractions by* MEP $5 \times 10^{-6}$ M | MET $5 \times 10^{-6}$ M |
| Right Atria (depolarized) | Hist | $77 \pm 20$ | 100 | 0 | $81 \pm 10$ | $47 \pm 6$ | $30 \pm 12$ |
| | 4MH | *78(1/3) | 100 (1/1) | 0 | $50 \pm 5$ | $13 \pm 13$ | $28 \pm 10$ |
| | PEA | $101 \pm 15$ | 100 | 0 | $77 \pm 7$ | $75 \pm 12$ | $5 \pm 4$ |
| Left Atria normal | Hist | 0 | – | – | $122 \pm 27$ | $44 \pm 10$ | $3 \pm 2$ |
| | 4MH | 0 | – | – | $62 \pm 15$ | $15 \pm 5$ | 0 |
| | PEA | 0 | – | – | $55 \pm 10$ | $68 \pm 14$ | 0 |
| Depolarized | Hist | 0 | – | – | $160 \pm 18$ | 100 | 0 |
| | 4MH | 0 | – | – | †$34 \pm 17$(4/8) | 100 (4/4) | 0 |
| | PEA | 0 | – | – | $115 \pm 13$ | 100 | 0 |

All results are the means ± s.e.mean of at least 5 tissues except where indicated.

*In only one of three tissues did 4MH cause a restoration: this was antagonized by mepyramine, but not by metiamide.

†In four of eight experiments 4MH caused restoration which was antagonized by mepyramine, but not by metiamide.