

## ERGOMETRINE AND APOMORPHINE AS SELECTIVE ANTAGONISTS OF DOPAMINE IN THE CANINE RENAL VASCULATURE

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- 1 Increases in renal blood flow were produced by intra-arterial injections of dopamine (5-50  $\mu$ g) in anaesthetized dogs pretreated with  $\alpha$ - and  $\beta$ -adrenoceptor antagonists.
- 2 Intra-arterial administration of ergometrine (0.5 mg) or apomorphine (1 mg) produced a depression of the renal dilator responses to dopamine, without affecting renal dilatations in response to intra-arterial acetylcholine (0.1-1  $\mu$ g) or histamine (2-50  $\mu$ g).
- 3 The depression of dopamine responses lasted 10-15 min, and was greater with ergometrine than with apomorphine.
- 4 It is concluded that both ergometrine and apomorphine can be used as specific blocking agents at vascular dopamine receptors. Ergometrine is the preferred drug for this purpose.

### Introduction

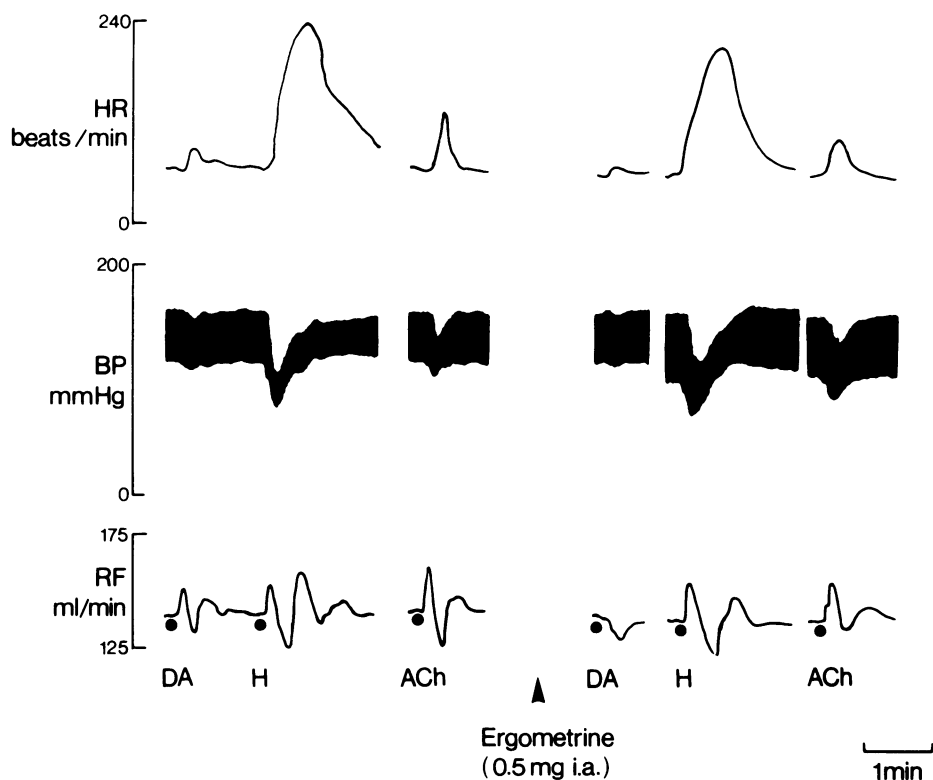
It is now established that certain vascular beds, including that supplying the kidney, possess vasodilator receptors which are specific for dopamine (see Goldberg, 1972). Although dopamine increases blood flow in both the inner and the outer cortical zones there is an overall shift in renal blood flow from the outer two-thirds to the inner one-third of the cortex (Hardaker & Wechsler, 1973) suggesting that these dopamine receptors may be present in greater numbers in the vasculature of the inner cortex. The best documented antagonist of dopamine at these receptors is the butyrophenone, haloperidol (Rossum, 1966; Yeh, McNay & Goldberg, 1969; Schuelke, Mark, Schmid & Eckstein, 1971), but this drug has the disadvantage of being an effective antagonist for only about 2 min after intra-arterial administration (Yeh *et al.*, 1969; Schuelke *et al.*, 1971), which makes its use difficult in some situations. We are currently concerned with analysis of the intra-renal actions of dopamine (Bell & Lang, 1973) and it is therefore desirable to find other antagonists which are less evanescent in effect. This paper reports a comparative study of ergometrine, which has been shown to be an effective antagonist of dopamine in *Helix* ganglia (Walker, Woodruff, Glaizner, Sedden & Kerkut, 1968), and apomorphine, which has been briefly reported as producing antagonism of dopamine in both dog

kidney (Goldberg & Musgrave, 1971) and rat vas deferens (Simon & Van Maanen, 1971).

### Methods

Mongrel dogs of either sex weighing 10-17 kg were anaesthetized with  $\alpha$ -chloralose (70 mg/kg i.v.) following induction with thiopentone sodium. The main left renal artery was exposed and blood flow was recorded with a cuff-type electro-magnetic flow probe. In some experiments flow in the left femoral artery was also recorded. Systemic blood pressure was recorded from a branch of the right femoral artery. Injections of drugs were made into the aorta just proximal to the origin of the left renal artery via a polythene catheter passed up the right femoral artery. The catheter was positioned by advancing it up the aorta until responses to injected dilator agonists were obtained in the left renal vascular bed.  $\alpha$ -Adrenoceptor blockade was maintained by hourly injections of phentolamine mesylate (Regitine, Ciba) in a dose of 0.5 mg/kg intravenously. Propranolol (Inderal, ICI) was administered in a dose of 0.05-0.1 mg/kg intravenously in order to abolish responses to  $\beta$ -adrenoceptor activation.

Other drugs used were: acetylcholine chloride (Roche), apomorphine hydrochloride (Parke Davis



**Fig. 1** Recordings of heart rate (HR), systemic arterial blood pressure (BP) and left renal blood flow (RF) in an anaesthetized dog. Intra-arterial injections of dopamine (DA), histamine (H) or acetylcholine (ACh) caused increases in renal blood flow. Because of autoregulation in the kidney only the initial increments in renal blood flow were considered in assessing the responses. Following intra-arterial administration of ergometrine (0.5 mg), the response to dopamine was abolished while those to histamine and to acetylcholine were unchanged.

& Co.), atropine methonitrate (Sigma Chemical Co.), dopamine hydrochloride (BDH), ergometrine maleate (Burroughs Wellcome), histamine acid phosphate (David Bull), isoprenaline hydrochloride (Winthrop) and mepyramine maleate (May & Baker). Doses refer to the above salts. Apomorphine was made up freshly in 0.9% w/v NaCl solution (saline) on the day of the experiment. The other drugs were freshly diluted in saline from frozen stock solutions. Agonists were administered as bolus injections of volumes between 0.05 and 0.5 ml. Injections of 0.5 ml saline had no effect on renal blood flow. Dilutions of dopamine and isoprenaline solutions contained ascorbic acid 100 µg/ml. The significance of difference of means was assessed with Student's *t*-test.

## Results

Average resting blood flow in the main renal artery of 13 dogs was  $10.96 \pm 1.19$  ml min<sup>-1</sup> kg body

wt<sup>-1</sup> (mean  $\pm$  s.e. mean). These flows were well maintained over the 4-6 h period of the experiment.

The agonists were injected intra-aortically and the doses used were: acetylcholine 0.1-1 µg, dopamine 5-50 µg and histamine 2-50 µg. Over the dose ranges selected all agonists produced increases in blood flow in the renal vascular bed. Acetylcholine and histamine produced systemic vasodepressor effects at all dose levels. The larger doses of dopamine (20-50 µg) also produced a fall in arterial blood pressure, while smaller doses had no effect. Responses to acetylcholine were abolished by intravenous administration of atropine methonitrate (0.4 mg/kg) and those to histamine were abolished or greatly reduced by intra-aortic administration of mepyramine maleate (2 mg/kg), indicating that they were due to activation of specific acetylcholine and histamine receptors respectively. Dilator responses to dopamine were unaffected by propranolol, although this completely abolished the femoral dilator response to

intra-aortic injection of 0.5  $\mu$ g isoprenaline. Thus the dopamine-induced dilatation could be attributed to activation of specific dopamine receptors. For all agonists reproducibility of responses was taken as an indication of adequate aortic mixing.

#### *Effect of ergometrine on renal responses*

Intra-aortic injection of ergometrine 0.5 mg produced an immediate fall in renal blood flow which returned to the control resting level after 1-2 minutes. After a stable resting flow was re-established, the dilator responses to dopamine were depressed significantly at all dose levels. In contrast responses to acetylcholine and histamine were not reduced at any dose level tested (Table 1, Figure 1). The antagonism of responses to dopamine produced by this dose of ergometrine lasted about 15 min, and the antagonist effect could be restored by injection of a further 0.5 mg of the drug.

In some experiments the effects of other doses of ergometrine were examined. Appreciable antagonism of dilator responses to dopamine could be elicited with doses as low as 0.1 mg, but the effects of such doses were short-lived. No further increase in degree or time course of antagonism was noted when the dose of ergometrine was increased above 0.5 mg: with doses of more than 2 mg, non-selective depression of all dilator stimuli was seen.

Ergometrine also produced a slight increase in

diastolic and a greater increase in systolic blood pressure. These effects persisted throughout the experiment after the antagonism to dopamine had worn off.

#### *Effect of apomorphine on renal responses*

Intra-aortic injection of apomorphine 1 mg caused a transient increase in blood flow in the renal vascular bed, with no effect on blood pressure. When renal blood flow had returned to the control resting level, the recorded dilator responses to dopamine at all dose levels were depressed. However the depression was statistically significant only with the highest dose of dopamine used. When dose-response curves were constructed from the pooled data using the least squares regression method, *t*-test application indicated that a significant ( $P < 0.01$ ) shift of the dose-response curve to the right was produced by apomorphine. Responses to acetylcholine and histamine were not depressed by apomorphine (Table 1). The degree of antagonism of dopamine produced by apomorphine was less than that produced by ergometrine (Table 1) and its effect was not maintained for more than about 10 minutes. Unlike the situation with ergometrine, further doses of apomorphine given at this time did not consistently produce further periods of dopamine antagonism. Administration of doses of 2 mg apomorphine caused vomiting and non-specific depression of all dilator stimuli.

**Table 1** Increments in renal arterial blood flow ( $\text{ml min}^{-1} \text{ kg body wt}^{-1}$ ) produced by intra-aortic injections of dopamine, acetylcholine and histamine before and after intra-aortic administration of ergometrine (0.5 mg) or apomorphine (1 mg).

	Controls	After ergometrine (0.5 mg).	After apomorphine (1 mg)
Dopamine			
5 $\mu$ g	0.55 $\pm$ 0.10 (6)	0.00 $\pm$ 0.00 (3)*	0.49 $\pm$ 0.21 (4)
10 $\mu$ g	1.09 $\pm$ 0.20 (7)	0.07 $\pm$ 0.07 (7)***	0.71 $\pm$ 0.08 (5)
20 $\mu$ g	1.07 $\pm$ 0.16 (9)	0.32 $\pm$ 0.13 (8)**	0.65 $\pm$ 0.15 (6)
50 $\mu$ g	1.61 $\pm$ 0.18 (9)	0.76 $\pm$ 0.16 (7)**	1.04 $\pm$ 0.18 (7)*
Acetylcholine			
0.1 $\mu$ g	1.75 $\pm$ 0.78 (4)	0.89 $\pm$ 0.12 (5)	1.71 $\pm$ 0.44 (4)
0.2 $\mu$ g	1.71 $\pm$ 0.46 (6)	1.48 $\pm$ 0.45 (3)	1.54 $\pm$ 0.30 (5)
0.5 $\mu$ g	1.82 $\pm$ 0.36 (7)	1.85 $\pm$ 0.51 (4)	2.08 $\pm$ 0.65 (5)
1.0 $\mu$ g	2.42 $\pm$ 0.45 (8)	1.89 $\pm$ 0.43 (7)	2.30 $\pm$ 0.59 (5)
Histamine			
2 $\mu$ g	0.35 $\pm$ 0.14 (5)	—	0.55 $\pm$ 0.10 (4)
5 $\mu$ g	0.78 $\pm$ 0.19 (6)	0.59 $\pm$ 0.12 (4)	0.94 $\pm$ 0.45 (5)
20 $\mu$ g	1.44 $\pm$ 0.30 (5)	2.32 $\pm$ 0.84 (3)	1.61 $\pm$ 0.39 (5)
50 $\mu$ g	2.18 $\pm$ 0.33 (4)	2.74 $\pm$ 0.84 (3)	2.03 $\pm$ 0.16 (5)

The number of experiments at each dose level are shown in parentheses. Values are means with s.e. mean.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.005$ ; † Dose-response curve shifted significantly ( $P < 0.01$ ) to the right.

## Discussion

Both ergometrine and apomorphine when administered intra-arterially produced abolition or attenuation of renal dilator responses to intra-arterial dopamine in the anaesthetized dog, while not reducing renal dilator responses to acetylcholine or histamine. Since acetylcholine has been shown to produce renal vasodilatation and a redistribution of intrarenal blood flow similar to that occurring with dopamine (McNay & Abe, 1971; Hardaker & Wechsler, 1973) these results suggest that ergometrine and apomorphine are acting as specific antagonists at dopamine dilator receptors. The antagonism of responses to dopamine lasted for about 10 min with apomorphine and for rather longer with ergometrine. Thus both drugs appear to offer useful alternatives to haloperidol and other butyrophenones, whose anti-dopamine effect in the vasculature lasts for only about 2 minutes. Of the two, ergometrine was the more satisfactory antagonist. Not only was its duration of action longer than that of apomorphine but the absolute degree of dopamine antagonism produced was greater. Furthermore, increases in the dose administered above that producing adequate dopamine antagonism caused less non-selective depression of dilator responses with ergometrine than with apomorphine.

The observation that both apomorphine and L-DOPA produce compulsive gnawing in rats led Ernst (1967) to suggest that apomorphine acts as an agonists at central dopamine receptors, while Goldberg, Sonnevile & McNay (1968) proposed that the renal vasodilator effect of apomorphine in the dog might also be attributable to direct

activation of dopamine receptors. However both Goldberg & Musgrave (1971) using the renal vasculature, and Simon & Van Maanen (1971) using the rat vas deferens noted antagonism by apomorphine of the effects of dopamine itself. It seems likely that the present observations on the relative ineffectiveness of apomorphine in antagonizing dopamine in the renal vasculature might be related to its admixture of agonist with antagonist properties.

Ergometrine was originally shown to be a specific antagonist of dopamine at the neuronal dopamine receptors in the ganglia of *Helix aspersa* (Walker *et al.*, 1968). The present results provide evidence for its also behaving as a specific dopamine antagonist in the canine renal vasculature. In addition we have recently demonstrated the presence in the canine femoral vasculature of dopamine receptors: ergometrine acts as a specific antagonist of dopamine in this situation as well (Bell, Conway & Lang, unpublished observations). Thus at least in their susceptibility to ergometrine, the dopamine receptors in the mammalian vasculature seem to be similar to those in the *Helix* nervous system. Whether those present in the mammalian central nervous system are also similar remains to be seen.

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