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VASCULAR DOPAMINE RECEPTORS IN THE CANINE HINDLIMB

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1 Increases in femoral blood flow were produced by intra-arterial injections of dopamine (5-50 μg) in some but not all anaesthetized dogs studied, following treatment with the α-adrenoceptor antagonist, phentolamine.

2 The dilator effect of dopamine was not due to inhibition of adrenergic vasomotor tone as it was not affected by pharmacological procedures which completely abolished the activity of vasomotor nerves.

3 Blockade of vascular β-adrenoceptors using propranolol reduced the flow increases produced by dopamine much less than it did those produced by isoprenaline.

4 Responses to dopamine were significantly depressed by intra-arterial administration of ergometrine (0.5 mg). This dose of ergometrine did not reduce femoral dilator responses to acetylcholine, histamine, isoprenaline, bradykinin or 5-hydroxytryptamine.

5 It is concluded that the femoral vascular bed in the dog contains specific vasodilator receptors for dopamine. Ergometrine appears to be a selective antagonist of dopamine at these receptors.

Introduction

Several visceral vascular beds in the dog have been shown to contain dilator receptors for dopamine (DA) which are distinct from β-adrenoceptors (Eble, 1964; Goldberg, 1972). However, McNay & Goldberg (1966) reported that in the canine femoral vasculature, DA produced dilatation only in doses far larger than those which were effective in the visceral beds, and that this femoral response was mediated solely through activation of β-adrenoceptors. In the course of a continuing investigation of the vasodilator innervation of the canine hindlimb we have had occasion to re-examine the effects of DA in this vascular bed. This paper reports evidence for the presence of specific dopamine receptors, together with some further observations which reinforce our previously reported evidence for the usefulness of ergometrine as a selective antagonist of DA at such receptors (Bell, Conway & Lang, 1974).

Methods

Mongrel dogs of either sex weighing 10-20 kg were anaesthetized with α-chloralose (70 mg/kg i.v.) following induction with thiopentone sodium. The left femoral artery was exposed in the thigh and blood flow was recorded using a cuff-type electromagnetic flow probe. Systemic arterial pressure was recorded from a branch of the right femoral artery, and local injections of drugs were made into the aorta via a polythene catheter passed up the right femoral artery. α-Adrenoceptor blockade was maintained by hourly, or more frequent, injections of phentolamine mesylate (Regitine, Ciba) in a dose of 0.5 mg/kg intravenously. β-Adrenoceptor blockade was performed in some experiments by administration of propranolol (Inderal, ICI) in a dose of 0.05 mg/kg intravenously. In some experiments activation of vasomotor reflexes by dilator agonists was prevented by complete ganglion blockade with hexamethonium bromide (Sigma) 10 mg/kg intravenously or by pretreatment with guanethidine sulphate (Ismelin, Ciba) 5 mg/kg subcutaneously 18 and 2 hours before operation, instead of or as well as by α-adrenoceptor blockade.

Other drugs used were: acetylcholine chloride (Roche); atropine methonitrate (Sigma); bradykinin (Sandoz); dopamine hydrochloride (DA; BDH); ergometrine maleate (Burroughs Wellcome); histamine acid phosphate (David Bull); 5-hydroxy-

tryptamine creatine phosphate (5HT; BDH); (±)-isoprenaline hydrochloride (Winthrop) and mepyramine maleate (May & Baker). Doses refer to the above salts. All agonists were freshly diluted with 0.9% w/v NaCl solution (saline) on the day of the experiment from frozen stock solutions, and injected as boluses of between 0.05 and 0.5 ml. Injection of 0.5 ml saline had no effect on femoral flow. Dilutions of DA and isoprenaline contained ascorbic acid 100 μg/ml.

Dilator responses were assessed as increases of blood flow (ml min^{-1} kg^{-1} body weight), and the significance of difference in responses before and after antagonist administration was examined using a paired Student's *t*-test. Comparison of the antagonism exerted by propranolol on responses to isoprenaline and dopamine was performed using two submaximal dose levels for each agonist, selected to give approximately equivalent dilator responses. After propranolol administration the doses of each agonist needed to produce responses approximately equal to those obtained under control conditions were obtained. Linear log dose-response curves were constructed for the control data in each experiment and used to calculate the exact dose-ratios necessary for equivalence of responses before and after propranolol. The significance of potency changes was assessed using the Behren-Fisher Statistic (Finney, 1952) on the assumption that no difference existed.

Results

Mean resting blood flow in the left femoral bed was 9.88 ± 1.63 ml min^{-1} kg^{-1} (mean ± s.e. mean). No deterioration in flow was seen over the 4-6 h period of the experiments.

Dilator agonists

The doses of dilator agonists used routinely were: acetylcholine (0.1-1 μg), histamine (5-50 μg), isoprenaline (0.05-0.5 μg), 5-hydroxytryptamine (0.1-50 μg), bradykinin (1-5 μg) and DA (5-50 μg). Acetylcholine, histamine, bradykinin and isoprenaline consistently caused increases in femoral blood flow and falls in systemic blood pressure at all dose levels tested. Responses to 5-hydroxytryptamine (5-HT) were more complex and will be discussed below (see *Specificity of ergometrine*). In 35 out of 77 dogs tested, DA caused femoral dilatation, while in the other animals no response was elicited by the range of doses used. The larger doses of dopamine (20-50 μg) consistently produced falls in blood pressure in those animals where a femoral dilator response was observed, but the smaller doses usually had no effect on blood pressure. Responses to acetylcholine were abolished by atropine (0.4 mg/kg i.v.) and those to histamine were reduced greatly or abolished by mepyramine (2 mg/kg i.a.). Neither of these antagonists affected responses to DA.

Role of ganglionic receptors in mediation of dopamine induced dilatation

Aortic administration of DA has been reported previously to produce femoral dilatation via inhibition of adrenergic vasomotor tone (Eble, 1964; Willems & Bogaert, 1973). This effect appeared to be mediated by an inhibitory action of DA on transmission through sympathetic ganglia. The possibility existed that in our experiments the α-adrenoceptor blockade with phentolamine was incomplete and allowed some residual vasomotor tone to persist. Other procedures were therefore adopted in some experiments to determine whether the DA-induced dilatations seen were due to action at the vascular muscle or at neuronal sites. In three dogs, the dilator effect of 20 μg DA was tested before and after administration of hexamethonium (10 mg/kg i.v.), which is sufficient to cause a sustained fall in blood pressure and abolition of vasomotor responses to preganglionic electrical stimulation. Responses to DA were unaffected by this drug treatment.

In a number of other animals pretreatment with guanethidine was utilized to abolish adrenergic vasomotor tone. The efficacy of this procedure was confirmed by the observation that subsequent administration of hexamethonium (10 mg/kg i.v.) did not cause any fall in blood pressure or increase in femoral blood flow. Dilator responses to DA of similar amplitudes to those seen in normal animals treated with phentolamine were observed in guanethidine-treated animals treated with phentolamine. We concluded that the femoral dilator response to DA which was being investigated was due to an action at sites on the vascular muscle rather than at vasomotor ganglionic synapses.

Role of β-adrenoceptors in the dopamine-induced dilatations

In order to test whether the femoral dilator effect of DA was due to activation of β-adrenoceptors as claimed by McNay & Goldberg (1966), a comparison was made of the antagonism of DA and of isoprenaline by the β-adrenoceptor antagonist propranolol (0.05-0.2 mg/kg i.v.). These doses of propranolol had no appreciable effect on resting femoral flow or on systemic blood

Table 1 Antagonistic effect of propranolol (0.1 mg/kg i.v.) on matched dilator responses to intra-aortic isoprenaline and dopamine on femoral blood flow in anaesthetized dogs treated with phentolamine (0.5 mg/kg i.v.)

	Isoprenaline				*Dopamine*			
Expt	*Response to isoprenaline, 0.05 μg (ml min^{-1} kg^{-1})*	*Propranolol dose-ratio*	*Response to isoprenaline, 0.1 μg (ml min^{-1} kg^{-1})*	*Propranolol dose-ratio*	*Response to dopamine, 10 μg (ml min^{-1} kg^{-1})*	*Propranolol dose-ratio*	*Response to dopamine, 20 μg (ml min^{-1} kg^{-1})*	*Propranolol dose-ratio*
1	1.25	8.0	1.38	2.5	1.18	1.1	1.59	1.1
2	1.0	50.0	1.07	2.0	1.0	1.5	1.21	1.7
3	1.09	5.0	1.87	5.0	1.21	1.4	1.45	1.0
4	0.68	3.6	1.13	5.6	0.49	0.8	0.83	1.1
5	0.85	10.0	2.20	20.0	0.85	1.0	1.70	0.7
6	0.30	3.1	0.46	4.2	0.23	0.5	0.30	0.4
7	0.33	4.0	0.85	5.0	0.80	3.3	0.95	2.9
Mean ± s.e. mean	0.79 ± 0.14	13.3**	1.28 ± 0.23	6.3*	0.82 ± 0.14	1.4	1.15 ± 0.19	1.3

* $P < 0.05$. ** $P < 0.01$.

Propranolol caused a considerable increase in the doses of isoprenaline necessary to match the control responses. The effect of propranolol on the potency of dopamine was much less pronounced. Results are expressed in terms of the initial increments in femoral flow produced by each agonist and the increase in dose necessary to produce responses of comparable magnitude following administration of propranolol, for seven separate experiments.

pressure. Propranolol appeared to depress responses to DA to a lesser and more variable extent than those to isoprenaline. A series of experiments was therefore performed to quantify the dose-ratio changes induced for submaximal doses of each agonist. Under control conditions isoprenaline (0.05 and 0.1 μg) gave dilator responses which approximated in size to those produced by 10 and 20 μg DA respectively (Table 1). In 7 experiments, administration of propranolol 0.1 mg/kg intravenously increased the doses of isoprenaline needed to produce responses of similar size by 13.3 and 6.3 fold respectively, while those of DA were increased by only 1.4 and 1.3 fold (Table 1). The control doses of isoprenaline and of DA used in this series of experiments produced only small decreases in arterial blood pressure. Thus isoprenaline (0.05 and 0.1 μg) lowered blood pressure by 2.1 ± 0.8 and 4.6 ± 0.8 mm Hg (mean ± s.e. mean) respectively and DA (10 μg and 20 μg) lowered blood pressure by 1.7 ± 0.6 and 4.0 ± 0.6 mm Hg (mean ± s.e. mean) respectively. Doses of each agonist needed to produce comparable flow responses after administration of propranolol also produced comparable depressor effects. Thus after propranolol isoprenaline (0.05 and 0.1 μg) lowered blood pressure by 1.3 ± 0.5 and 2.6 ± 0.8 mm Hg (mean ± s.e. mean) respectively and DA (10 μg and 20 μg) lowered blood pressure by 2.4 ± 0.5 and 3.9 ± 0.5 mm Hg (mean ± s.e. mean) respectively. We therefore felt justified in regarding the dilator activity of DA at least in the most part as due to activation of a receptor population distinct from β-adrenoceptors.

Effect of ergometrine on dopamine-induced dilatations

We have recently shown that ergometrine appears to act as a specific antagonist of DA at dopamine receptors in the canine renal vasculature (Bell *et al.*, 1974). It was therefore logical to test ergometrine as an antagonist of DA in the hind limb. Intra-aortic injection of ergometrine (0.5 mg) produced an immediate reduction in femoral blood flow which generally returned to the control resting level (±10%) within 1-2 minutes. Ergometrine also produced a slight increase in diastolic and a greater increase in systolic blood pressure, as described previously (Bell *et al.*, 1974). When femoral blood flow and blood pressure had stabilized following ergometrine injection, its effect on drug-induced dilator responses was investigated. In a series of 7 dogs, dose-response curves were obtained to DA, acetylcholine and histamine before and after intra-aortic injection of ergometrine. This procedure caused significant depression of responses to DA at all dose levels tested, but had no antagonistic effect on responses to the other agonists (Table 2).

Specificity of ergometrine

As an additional check of the proposal that DA

Table 2 Increments in femoral arterial blood flow (ml min^{-1} kg^{-1}) produced by intra-aortic injections of DA, acetylcholine, and histamine before and after intra-aortic administration of ergometrine (0.5 mg)

Agonist	*Controls*	*In presence of ergometrine (0.5 mg)*
Dopamine		
5 μg	0.52 ± 0.16 (5)	0.08 ± 0.08 (5)*
10 μg	0.96 ± 0.10 (6)	0.33 ± 0.11 (6)**
20 μg	1.19 ± 0.08 (6)	0.72 ± 0.08 (6)**
50 μg	2.70 ± 0.23 (7)	1.00 ± 0.26 (7)***
Acetylcholine		
0.1 μg	2.29 ± 0.74 (5)	2.27 ± 0.71 (5)
0.2 μg	2.46 ± 0.71 (3)	2.12 ± 0.71 (3)
0.5 μg	3.66 ± 0.93 (4)	4.58 ± 1.34 (4)
1.0 μg	4.16 ± 0.89 (7)	6.46 ± 0.91 (7)
Histamine		
2 μg	1.05 ± 0.31 (3)	1.36 ± 0.97 (3)
5 μg	2.67 ± 0.75 (4)	2.89 ± 1.17 (4)
20 μg	4.87 ± 1.27 (4)	5.01 ± 1.26 (4)
50 μg	4.91 ± 0.50 (3)	6.03 ± 1.56 (3)

* $P < 0.05$. ** $P < 0.005$. *** $P < 0.001$.
The number of experiments at each dose level are shown in parentheses. Values are means with s.e. mean.

was acting to produce femoral dilatation through receptors other than β-adrenoceptors, we examined the effect of ergometrine (0.5 mg intra-aortically) on responses to isoprenaline. In 6 experiments, there was slight potentiation of isoprenaline by ergometrine (Table 3). Although the absence of a depressant effect on responses to acetylcholine, isoprenaline and histamine indicated that ergometrine has no non-specific inhibitory effect on dilator responses in the dose used, its antagonism of DA could be explained through mechanisms other than blockade of a specific vascular DA receptor if one proposes that DA might act indirectly through local liberation of 5-HT or of a kinin and that ergometrine antagonizes the vasodilator effect of this substance. To exclude this possibility we tested the effect of ergometrine (0.5 mg intra-aortically) on femoral dilator responses to bradykinin and 5-HT. In 5 experiments, ergometrine caused potentiation of responses to 1-5 μg of bradykinin (Table 3). The predominant effect of 5-HT was to produce femoral constriction, and consistent increases in flow could be elicited only in some dogs tested, and then usually over only a narrow dose range which varied between individual animals. However, in 6 experiments of this type, ergometrine had no appreciable effect on the flow increases produced by 5-HT (Table 4).

Discussion

McNay & Goldberg (1966) reported that, in anaesthetized dogs, femoral dilator responses were elicited only with doses of DA ten or more times as high as those which produced renal vasodilatation. Furthermore, while the renal responses to DA were unaffected by β-adrenoceptor blockade, the femoral responses were abolished, suggesting the absence of specific DA receptors from the femoral bed.

Our results show a considerable divergence from those of McNay & Goldberg (1966). In about half of the dogs studied we could demonstrate femoral dilatation in response to doses of DA in the same range as are needed to produce renal vasodilatation (Eble, 1964; McNay & Goldberg, 1966; Bell *et al.*, 1974). These responses were much less affected by β-adrenoceptor blockade than were those to isoprenaline, indicating that they were at least primarily not attributable to activation of β-adrenoceptors. However, our results do not preclude the possibility of appreciable β-adrenoceptor stimulation by DA in higher doses. Although Eble (1964) and Willems & Bogaert (1973) have demonstrated that DA can produce peripheral dilatation by inhibiting transmission through tonically active vasomotor ganglia, such a mechanism of action could not be evoked in our study since complete abolition of vasomotor tone by ganglion blockade or by guanethidine treatment did not prevent the dilator action of DA. We must conclude therefore that this effect of DA is due at least in the main to a direct action on the smooth muscle of the femoral vascular bed and not to activation of adrenoceptors. Support for a direct, dilator effect

Table 4 Increments in femoral arterial blood flow (ml min^{-1} kg^{-1}) produced by intra-aortic injections of 5-hydroxytryptamine (5-HT) before and after intra-aortic administration of ergometrine (0.5 mg)

Expt.	*5-HT (μg)*	*Control*	*After ergometrine*
1	1	0.96	0.96
2	5	1.10	1.08
	10	1.55	1.55
	40	2.17	1.86
3	40	1.17	1.10
4	10	0.34	0.34
5	1	0.45	0.70
6	1	0.25	0.25

Table 3 Increments in femoral arterial flow (ml min^{-1} kg^{-1}) produced by intra-aortic injections of isoprenaline and bradykinin before and after intra-aortic administration of ergometrine (0.5 mg)

	Control	*After ergometrine*
Isoprenaline		
0.1 μg	1.38 ± 0.37 (6)	1.63 ± 0.51 (6)
0.2 μg	2.21 ± 0.78 (5)	2.75 ± 0.66 (5)
0.5 μg	4.04 ± 1.06 (5)	4.25 ± 0.64 (5)
Bradykinin		
1 μg	1.25 ± 0.3 (4)	1.45 ± 0.48 (4)
2 μg	2.76 ± 0.65 (5)	2.79 ± 0.65 (5)
5 μg	2.89 ± 0.57 (3)	3.58 ± 1.01 (3)

of DA in the femoral bed has also come from the observations that DA-induced dilatation in the hindlimbs of conscious dogs is unaffected by α- and β-adrenoceptor blockade or by atropine (Higgins, Millard, Braunwald & Vatner, 1973).

Ergometrine has been reported to be an effective antagonist of DA in *Helix* ganglion (Walker, Woodruff, Glaizner, Sedden & Kerkut, 1968), and we have recently demonstrated that intra-arterial administration of ergometrine causes profound depression of dilator responses of DA in the dog renal vasculature without depressing responses to acetylcholine or histamine (Bell *et al.*, 1974). The present results have shown that intra-arterial injection of ergometrine produced profound depression of femoral dilator responses to DA while being devoid of antagonistic activity towards the dilator effect not only of acetylcholine and histamine but also of isoprenaline, bradykinin and 5-HT. They therefore strengthen further the position of ergometrine as a specific antagonist of DA in the mammalian vasculature.

The difference between our results and those of McNay & Goldberg (1966) may be due to several factors. McNay & Goldberg used animals in which the paw circulation was occluded, in order to study a more homogeneous vascular bed. Experiments in which we have examined femoral dilator responses with and without paw occlusion have indicated that an appreciable component of the dilator activity of DA is due to an effect within the vasculature of the paw. In view of the fact that we were unable to demonstrate a dilator response to DA in all animals tested, individual or strain differences between the receptor populations of different dogs may also be involved. A further possible factor is the use of barbiturate anaesthesia by McNay & Goldberg and of chloralose by ourselves. In this context, however, it may be noted that Eble (1964), using intact hindlimb preparations in barbiturate-anaesthetized dogs, observed femoral dilatation after α-adrenoceptor blockade in response to doses of DA similar to those necessary for the production of direct dilatation in the renal and mesenteric beds.

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