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LOCALIZATION OF VASODILATOR DOPAMINE RECEPTORS IN THE CANINE HINDLIMB

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1 Vascular responses to local injection of dopamine and isoprenaline have been compared in the blood-perfused gracilis muscle and hind paw pads of dogs anaesthetized with chloralose.

2 In the paw pads, dopamine (0.5 to 5.0 μg) caused a transient vasoconstriction followed by dose-dependent vasodilatation. α-Adrenoceptor blockade converted this response to pure vasodilatation, which was attenuated or abolished by the dopamine-receptor antagonist, haloperidol (1 to 2 mg i.a.). In the gracilis, dopamine produced only vasoconstriction. Following α-adrenoceptor blockade this was abolished, but only a very small dilator response was revealed.

3 Isoprenaline (0.05 to 0.5 μg) caused dose-dependent dilatation in both beds, which was attenuated by propranolol (0.1 mg/kg i.v.).

4 Glyceryl trinitrate (0.2 to 5.0 μg) was used to assess vascular reactivity. When responses to isoprenaline and dopamine were compared with those to glyceryl trinitrate, both beds had approximately equal reactivity to isoprenaline. In contrast the paw pads were 10 fold more responsive to dopamine than was the gracilis.

5 We conclude that the vessels of the paw pads play an important part in the femoral dilator response to dopamine.

Introduction

It is well documented that the canine vascular system contains specific dopamine receptors, activation of which produces vasodilatation (Eble, 1964; Goldberg, 1972; Higgins, Millard, Braunwald & Vatner, 1973; Bell, Conway & Lang, 1974; Bell, Conway, Lang & Padanyi, 1975). As well, dopamine activates vascular α-adrenoceptors, with resultant vasoconstriction (Hamet, 1931; Allwood, Cobbold & Ginsburg, 1963; Eble, 1964; McNay & Goldberg, 1966). In the renal vasculature the density of the two receptor populations is such that local injection of dopamine in anaesthetized animals causes a net vasodilatation (Eble, 1964; McNay & Goldberg, 1966; Bell *et al.*, 1974). On the other hand, in the femoral vasculature, dopamine receptor-mediated vasodilatation is usually masked by simultaneous α-adrenoceptor activation. (Eble, 1964; Bell *et al.*, 1975).

In contrast, the vasomotor nerve supply to the hind paw contains a population of axons which appear to act by release of dopamine and whose activation produces vasodilatation uncomplicated by vasoconstriction (Bell & Lang, 1974; Lang, Bell, Conway & Padanyi, 1976). Such a difference between the effects of exogenous and neurogenic dopamine on femoral flow could be explained in two ways (Lang *et al.*, 1976). Dopamine receptors adjacent to sites of neural transmitter release might be inaccessible to dopamine within the vessel lumen: alternatively both intraluminal and neurogenic dopamine might be equipotent dilators within a restricted region of the femoral circulation, but this action of intraluminal dopamine might be masked by concomitant α-adrenoceptor activation in other parts of the limb. The present study has been carried out in an attempt to differentiate these possibilities.

Methods

Twelve adult mongrel dogs of either sex weighing between 9 and 30 kg were anaesthetized with α-chloralose (85 mg/kg i.v.) following induction with thiopentone sodium and were artificially respired under positive pressure at 15 breaths/min and with tidal volumes derived from a standard nomogram (Harvard Apparatus). Heparin sodium was administered in an initial dose of 1000 u/kg, followed by an additional 100 u/kg every hour.

Perfusion

The pads of one hind paw were perfused via a polythene cannula (PE 120) in the dorsal pedal artery, and one gracilis muscle was perfused via a polythene cannula (PE 90) in the profunda femoris artery, by means of two Cole-Parmer Masterflex roller pumps. Blood for perfusion was withdrawn through retrograde cannulae passed up one saphenous artery and the contralateral profunda femoris artery into the main femoral arteries. Perfusion pressure was monitored with Statham pressure transducers (P23Db) distal to the roller pumps. Ligatures around the main femoral arteries distal to the openings of the withdrawal cannulae prevented collateral circulation to the perfused tissues, and this was confirmed at the start of each experiment by ascertaining that when the roller pumps were stopped, the perfusion pressure was non-pulsatile and less than 10 mmHg (1 mmHg = 1.333 mbar).

Roller pump speeds were set to produce initial perfusion pressures which approximated systemic blood pressure and were held constant thereafter.

In most cases perfusion and blood pressures could be matched quite closely, but in two animals, steady perfusion pressure could be achieved in the gracilis only by increasing it by up to 50 mmHg above mean blood pressure. With the tubing dimensions and flow rates used, the resistance of the perfusion system itself did not contribute appreciably to the perfusion pressures generated.

Reflex changes in local vascular tone due to systemic effects of the dilator agonists used were prevented by administration of the ganglion blocking drug, hexamethonium bromide (10 mg/kg i.v.)

All parameters were recorded on a Grass model 7B polygraph. In order to obtain mean pressure records the half-amplitude frequency response of the writing oscillographs were limited to 0.1 Hz. Although this restricted the amplitude of fast transient changes in perfusion pressure, the time-courses of dilator responses to the drugs studied were such that no appreciable (less than 10%) attenuation occurred.

Drugs

Dilator agonists used were dopamine hydrochloride (Sigma), glyceryl trinitrate (Anginine; Wellcome) and isoprenaline hydrochloride (Sigma). The amines were diluted on the day of the experiment from frozen acidic stock solutions into 0.9% w/v NaCl solution (saline) containing 100 μg/ml ascorbic acid and the glyceryl trinitrate solution was freshly prepared from sublingual tablets. Injections into the perfusion streams were made through thick rubber tubing proximal to the roller pumps in volumes of 0.05 ml or less. In each experiment injections of equal volumes of saline were also made to determine any effect of the injection itself on perfusion pressure. Such effects were allowed for when measuring the amplitudes of drug-induced responses. Ascorbic acid in the concentration used had no vasoactive effect of itself.

α-Adrenoceptor blockade was, when necessary, produced by phentolamine mesylate (Regitine, Ciba-Geigy) 0.5 mg/kg intravenously, followed by 0.5 mg kg^{-1} h^{-1} intravenously. Haloperidol (Serenace, Searle) 1 to 2 mg injected into the perfusion stream and propranolol hydrochloride (Inderal, ICIANZ) 0.1 mg/kg intravenously were used for blockade of dopamine receptors and β-adrenoceptors respectively. Neither of these antagonists altered significantly resting perfusion pressures or vasodilator responses to glyceryl trinitrate.

Statistical analysis

The significance of differences of mean responses to the dilator agonists before and after antagonist drugs were assessed by paired, two-tailed Student's *t* tests, on the assumption that no difference existed.

Results

Control values

The mean ± s.e. mean systemic blood pressure at the beginning of the experiments, for all dogs used, was 98 ± 4.9 mmHg. Following hexamethonium, this fell to 64 ± 4.0 mmHg. The perfusion pressure in the paw was 99 ± 4.8 mmHg, achieved with a perfusion rate of 34 ± 4.0 ml/min (range 10–48 ml/min), while that in the gracilis was 123 ± 8.8 mmHg, achieved with a perfusion rate of 10 ± 1.2 ml/min (range 7 to 15 ml/min). No substantial variation in perfusion pressures occurred over the 3 to 5 h period of the experiments.

Doses of agonists used

The doses used were: dopamine 0.5, 2 and 5 μg; isoprenaline 0.05, 0.2 and 0.5 μg; glyceryl trinitrate 0.2, 1 and 5 μg.

Responses to dopamine

Typical responses of each vascular bed to dopamine are shown in Figure 1. In the paw (10 dogs), dopamine produced a transient vasoconstriction which was followed by a dose-dependent vasodilatation. After α-adrenoceptor blockade, the constrictor component of the response was abolished and the ampli-

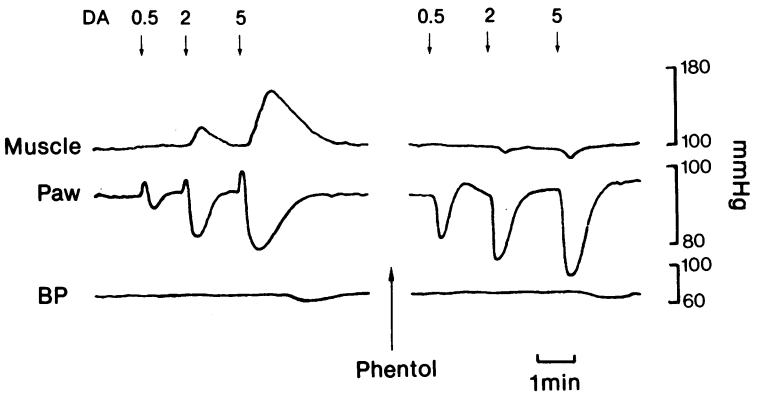

Figure 1 Responses of the blood-perfused gracilis muscle (perfusion rate 11 ml/min) and hind paw (perfusion rate 46 ml/min) of a ganglion-blocked anaesthetized dog to intra-arterial injections of dopamine 0.5, 2 and 5 μg; before and after α-adrenoceptor blockade with phentolamine (Phentol, 0.5 mg/kg i.v.). Note that dopamine (DA) produces predominantly α-adrenoceptor mediated constriction in the gracilis and predominantly dopamine-receptor mediated dilatation in the paw. The bottom trace represents systemic blood pressure.

tude of the dilator component was increased. The largest dilator response observed to 5 μg of dopamine was 33 mmHg. Dilator responses of the paw to dopamine were not affected by β-adrenoceptor blockade. However, administration of the dopamine-receptor antagonist, haloperidol, caused attenuation of responses which was significant ($P < 0.001$) at all dose-levels of dopamine (Figure 2).

In the gracilis (8 dogs), dopamine produced only a dose-dependent vasoconstriction under control conditions. This was abolished by α-adrenoceptor blockade, revealing a variable, small dilator response (less than 4 mmHg in response to 5 μg dopamine in 5 of 8 dogs). The amplitude of these dilator responses was not affected by β-adrenoceptor blockade, but was attenuated by haloperidol ($P < 0.05$) (Figure 3).

Responses to isoprenaline

In the paw (10 dogs) isoprenaline produced dose-dependent dilator responses similar in amplitude to those elicited by dopamine (Figure 2). Blockade of β-adrenoceptors caused attenuation of responses to all dose-levels of isoprenaline ($P < 0.001$) but subsequent blockade of dopamine-receptors had, in 8 dogs, no consistent effect on the residual response (Figure 2). In a further series of 4 dogs, dopamine-receptor blockade in the absence of prior β-adrenoceptor blockade also had no effect on responses to isoprenaline 0.05 μg (control 6.0 ± 0.97 mmHg; haloperidol 5.0 ± 0.96 mmHg), but caused some depression of responses to isoprenaline 0.2 μg (control 9.9 ± 1.7 mmHg; haloperidol 7.5 ± 1.4 mmHg, $0.02 < P > 0.05$). In the gracilis (8 dogs) isoprenaline produced dose-dependent dilator responses considerably greater in amplitude than those elicited by dopamine (Figure 3). As in the paw, these responses were attenuated by β-adrenoceptor blockade ($P < 0.001$). In 4 dogs, subsequent administration of haloperidol caused further reduction of responses to isoprenaline (Figure 3). However, the small number of experiments did not allow any statistical significance of this effect to be demonstrated.

Assessment of relative responsiveness to dopamine and isoprenaline of the paw and gracilis

The amplitude of responses to glyceryl trinitrate could be taken as a gauge of the capacity of each bed to dilate in response to injected agonists. It was judged that both isoprenaline and dopamine were sufficiently similar to glyceryl trinitrate in molecular weight (±16%) that responsiveness to them could be assessed in terms of the ratio of amplitudes of responses to each amine and to an equivalent dose of glyceryl trinitrate. By this method of assessment both beds exhibited similar degrees of responsiveness to isoprenaline (0.2 μg isoprenaline: 0.2 μg glyceryl trinitrate; paw 2.7, gracilis 3.6). In contrast, the responsiveness to dopamine (5 μg dopamine: 5 μg glyceryl trinitrate) of the paw (1.1) was far greater than that of the gracilis (0.1) (Figures 2 & 3).

Discussion

Dilator responses to the paw and gracilis muscle to intra-arterial injections of dopamine, isoprenaline and

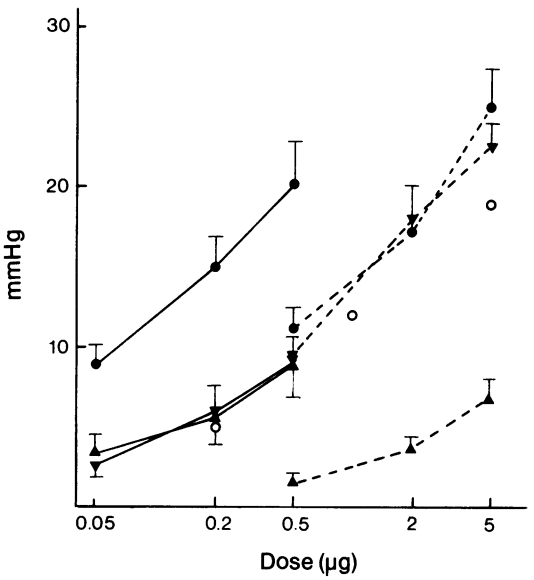

Figure 2 Dose-response curves of the blood perfused hind paw to isoprenaline (complete lines) and to dopamine (dashed lines) under control conditions (●), following β-adrenoceptor blockade (▼) and following subsequent dopamine-receptor blockade (▲). The vertical lines represent s.e. means. The open circles (○) represent responses of the bed to glyceryl trinitrate.

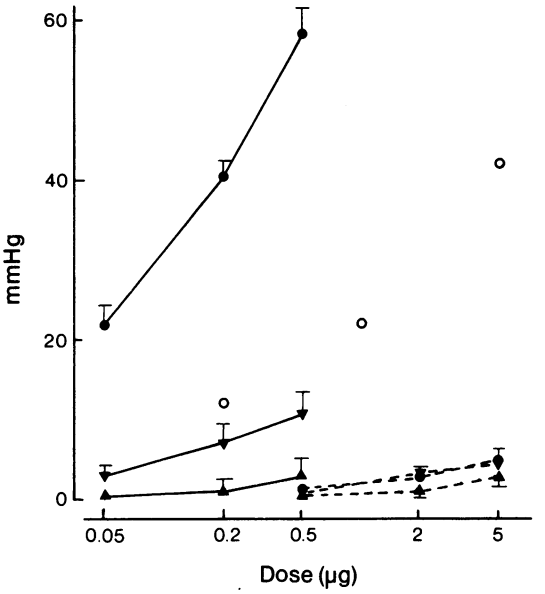

Figure 3 Dose-response curves of the blood perfused gracilis muscle to isoprenaline (complete lines) and to dopamine (dashed lines) under control conditions (●), following β-adrenoceptor blockade (▼) and following subsequent dopamine-receptor blockade (▲). The vertical lines represent s.e. means. The open circles (○) represent responses of the bed to glyceryl trinitrate.

glyceryl trinitrate were obtained under conditions of constant perfusion rate and in the presence of systemic ganglionic blockade. The responses seen can therefore be regarded as representing direct effects of each agonist on the vascular musculature of the perfused beds without interference from neurally-mediated changes in vascular tone secondary to drug-induced changes in blood pressure.

Although the dorsal pedal artery gives off branches to the lumbrical and interosseus muscles, it supplies primarily the pads of the hind paws (Miller, Christensen & Evans, 1964). Despite an early report that the vascular bed of the hairy skin of the dog leg responded to isoprenaline almost as much as did that of skeletal muscle (Walters, Cooper, Denison & Green, 1955), it is widely assumed that β-adrenoceptors are rare or absent in the cutaneous circulation (see, for instance, Furchgott, 1955; Skinner & Whelan, 1962; Allwood *et al.*, 1963; Mellander & Johansson, 1968). We were therefore interested to observe that the responsiveness of the paw circulation to isoprenaline, as judged by the ratio of isoprenaline and glyceryl trinitrate responses, was similar to that of the gracilis muscle. It may be that, at least in certain areas of the cutaneous vascular bed, β-adrenoceptors are more involved in control of regional resistance than is generally recognized.

In contrast, we observed using the same criteria of responsiveness that dopamine produced a 10-fold greater dilator effect in the paw than in the gracilis, suggesting that the vessels of the paw play an important part in the femoral dilator response to injected dopamine. This explains why McNay & Goldberg (1966) were unable to demonstrate the existence of dopamine-receptors using hind limbs with the paw circulation occluded. It also indicates that the difficulty encountered in producing femoral vasodilatation with dopamine unless α-adrenoceptor blockade has been produced (Eble, 1964; Bell *et al.*, 1975) is due to concomitant dilatation and constriction in different parts of the limb. Nevertheless, even in the paw some α-adrenoceptor activation by dopamine was apparent, although stimulation of the putatively dopaminergic nerve supply to the paw produces only dilatation (Lang *et al.*, 1976). This could be due to different relative densities of α-adrenoceptor and dopamine-receptors on the luminal and adventitial surfaces of the paw resistance vessels: neurogenic transmitter probably acts solely on the latter population (Bell, 1969), while injected agonists must act pri-

marily on the former. However, a simpler explanation is that the bed we perfused through the dorsal pedal artery, although more restricted than the whole femoral vasculature, is still larger than that in which neurogenic dilatation occurs. Recent histochemical and biochemical observations suggest that this response is restricted to the arteriovenous shunts of the paw pads (Bell, Lang & Laska, 1978).

The lack of reduction in dilator responses to dopamine following propranolol indicated that in the doses used, dopamine does not produce appreciable β-adrenoceptor activation. This is in agreement with previous reports of the low potency of dopamine as a β-adrenoceptor agonist in several canine vascular beds (McNay & Goldberg, 1966; Schuelke, Mark, Schmid & Eckstein, 1971; Bell *et al.*, 1975; Bell & Mya, 1977). On the other hand we did observe some reduction by haloperidol of isoprenaline-induced dilator responses in the paw, suggesting that this amine may have the capacity to activate dopamine-receptors. This is consistent with a previous report that the dilator response to isoprenaline in the dog kidney is considerably reduced by blockade of either dopamine-receptors or β-adrenoceptors (Bell & Mya, 1977).

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