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THE EFFECTS OF CATECHOLAMINES AND ADRENOCEPTOR BLOCKING DRUGS ON THE CANINE PERIPHERAL LYMPH FLOW

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1 Blood flow through the femoral artery, lymph flow in a lymphatic vessel in the femoral triangle and metatarsal distal venous pressure were measured simultaneously in a canine moving hind limb.

2 Low intra-arterial doses of adrenaline and noradrenaline increased lymph flow even in the presence of marked arterial vasoconstriction. In contrast, isoprenaline increased arterial blood flow without affecting lymph flow rate.

3 Phenoxybenzamine, dihydroergotoxine, and nicergoline did not inhibit the lymphatic flow increase induced by adrenaline at doses active on arterial or venous vascular alpha-adrenoceptors.

4 Propranolol given intra-arterially into animals pretreated with alpha-adrenoceptor blocking agents restored the vasoconstrictor effect of adrenaline (reversal of adrenaline reversal).

Introduction

Adrenergic amines affect differently central and peripheral lymph flow. Adrenaline always increases *thoracic duct* lymph flow (Gesler, Matsuba & Dragstedt, 1956; Shim, Pollack & Drapanas, 1961; Doemling & Steggerda, 1962). Noradrenaline may decrease flow as a result of an increased thoracic duct contractility (Földi & Zoltàn, 1966). At doses which are hypertensive on arterial blood pressure, thoracic duct lymph flow may be increased (Wernze, Fujii & Sembach, 1965; Fujii & Wernze, 1966).

Renal lymph flow is increased by noradrenaline (Fujimoto & Lockett, 1970). *Hind limb flow* remains mainly unchanged after intra-arterial administration of doses of adrenaline sufficient to affect local venous blood outflow (Lewis & Winsey, 1970).

Whether these actions of catecholamines are direct or indirect is still an open question because many functions susceptible to catecholamines are related to lymph flow regulation.

Our experiments in the canine hind limb were designed to measure lymph flow simultaneously with local arterial blood flow and venous pressure, to study the interrelations between these functions. The effect of blocking the adrenoceptors of the lymphatic system was also investigated using alpha- and beta-adrenoceptor blocking drugs.

Methods

Experiments were carried out on male Beagle dogs anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.). A constant rectal temperature of 37.5° C was maintained and cooling of the limb was prevented.

Blood pressure was recorded from the carotid artery through a polyethylene cannula connected to a Statham strain-gauge.

Venous pressure was measured with a Silastic cannula distally inserted into a small dorsal vein of the paw and connected to a Statham venous pressure transducer.

Femoral blood flow was estimated on the same leg by an electromagnetic blood flow-meter (Nycotron), the flow probe being placed on the femoral artery within the femoral triangle.

Femoral lymph vessels in the femoral triangle on the same side were dissected out and cannulated according to Stürmer (1966), to assess lymph flow.

Evans blue dye (0.5%) was injected i.m. into different areas of the leg to aid visualization of lymphatics. Constant basal lymph flow was assured by a passive mechanical movement of the leg according to Stürmer (1966) and recorded by a photoelectric drop recorder.

Drugs were administered into a systemic vein or into the femoral artery through a tributary artery

arising distal to the flow probe. A Palmer syringe pump was used to infuse drugs or solvents at a constant rate of 0.4 ml/minute. Systemic blood pressure, venous pressure, lymph flow, and femoral blood flow were simultaneously and continually recorded on a galvanometric Honeywell Visicorder.

Vascular resistances were calculated as peripheral resistance units (PRU)

$$PRU = \frac{\text{mean arterial blood pressure}}{\text{mean femoral blood flow}}$$

Drugs

These were, bradykinin triacetate trihydrate (Calbiochem), (±)-isoprenaline hydrochloride (Prodotti Gianni), (−)-adrenaline (Rhône-Poulenc), (−)-noradrenaline (Prodotti Gianni), (±)-propranolol (Prodotti Gianni), phenoxybenzamine HCl (a gift from SKF), nicergoline maleate (Sermion, Farmitalia) and dihydroergotoxine (DHE) as an equal part solution of dihydrocristine, dihydroergocryptine, dihydroergocornine as methansulphonates. The following solution was employed as placebo: NaCl 6 mg, methansulphonic acid 0.0357 mg, ethanol (96%) 2 mg, water up to 1 ml.

Results

Basal parameters were initially controlled for 1-2 h and animals with a lymphatic flow of less than 3-4 drops/min were discarded. Mean basal values of femoral blood flow, venous, and arterial blood pressure were respectively 80 ml/min, 10, and 130 mmHg.

Control

Vascular and lymphatic reactivity were subsequently tested by occluding the femoral artery with a pneumatic cuff for at least 1 min, and by injecting bradykinin (0.05-0.1 $\mu g\ kg^{-1}\ min^{-1}$) over 10 minutes. Removal of the arterial occlusion was followed by a marked increase in blood flow (reactive hyperaemia). During arterial occlusion, the lymph flow was stopped completely; a prompt return to control values was observed without any increase of lymph flow. Bradykinin administration increased femoral and lymph flows by 200 and 150% respectively.

The effect of catecholamines and adrenoceptor blocking agents (alone and combined) are described separately.

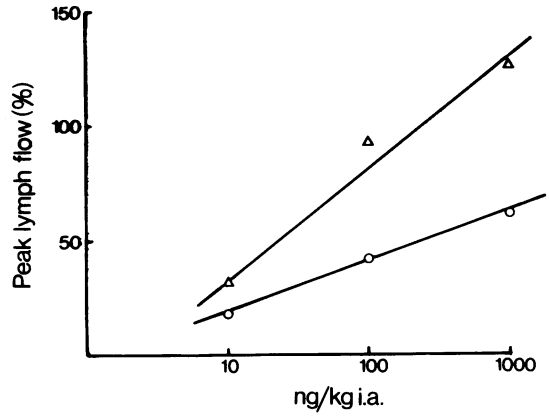

Figure 1 Peak responses of lymph flow in the hind limb to graded doses of adrenaline (○) and noradrenaline (△) given intra-arterially.

Local effects of catecholamines

Intra-arterial injections of adrenaline and noradrenaline increased lymph flow in all dogs (19 animals); the response was satisfactorilly related to the dose in the range, 0.01-1 μg/kg. Noradrenaline was slightly more active than adrenaline (see Figure 1). In contrast, isoprenaline (0.001 to 0.1 μg/kg) did not modify lymph flow. Adrenaline (0.01 μg/kg) produced a marked increased in arterial blood flow (Figure 2). In contrast, higher doses reduced flow down to zero, the effect being preceded by a short initial increase. Venous pressure was slightly decreased by adrenaline (0.01 and 0.1 μg/kg) but was consistently, but variably increased by 1 μg/kg intra-arterially. Increase in lymph flow was generally concomitant with a strong reduction of both blood flow and venous pressure. It preceded venous hypertension when present, i.e. at the dose of 1 μg/kg. Noradrenaline affected the cardiovascular parameters like adrenaline, whereas isoprenaline produced a parallel increase in both blood flow and venous pressure. Systemic arterial blood pressure was not affected by the low and medium doses of the catecholamines: adrenaline (1 μg/kg) produced small negative or positive variations. All the above effects were short-lasting.

Local effects of adrenoceptor blocking drugs

Adrenoceptor blocking drugs (ergotamine, phenoxybenzamine, nicergoline, DHE, and propranolol) were infused intra-arterially for 5-10 min to evaluate their local effects (see Figure 3). Ergota-

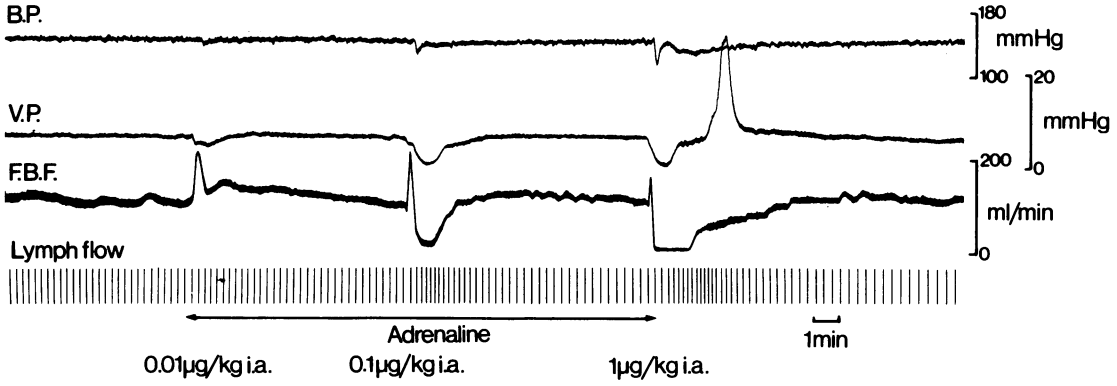

Figure 2 Anaesthetized dog: variations of systemic blood pressure (B.P.), metatarsal venous pressure (V.P.), femoral blood flow (F.B.F.), and lymph flow (recorded as drops) after intra-arterial injections of increasing doses of adrenaline.

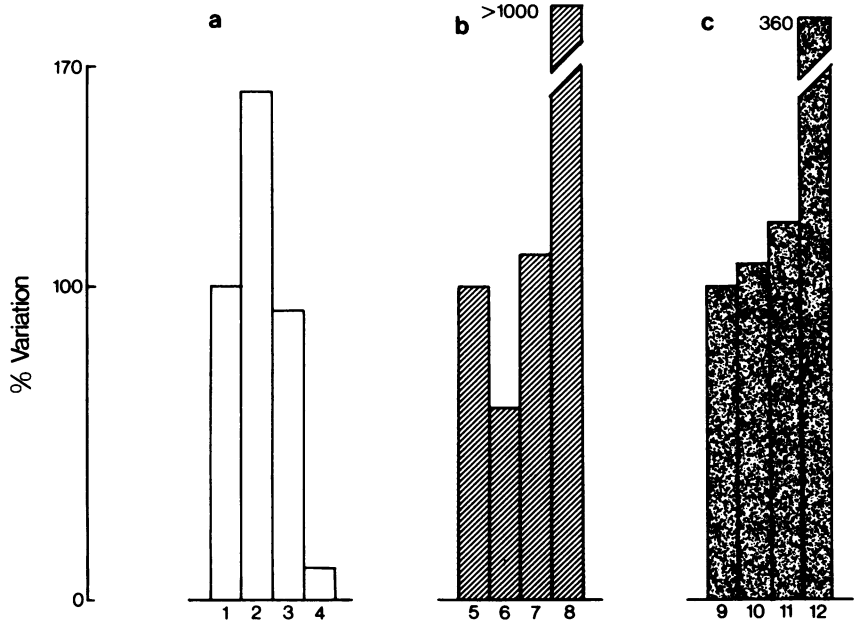

Figure 3 Mean percentage variations of femoral blood flow, vascular femoral resistance and venous pressure (from a metatarsal vein) during intra-arterial administration of nicergoline, dihydroergotoxine, and ergotamine. Mean percentage variations of (a) femoral blood flow, (b) vascular femoral resistance and (c) venous pressure (from a metatarsal vein) during intra-arterial administration of nicergoline (1 $\mu g\ kg^{-1}\ min^{-1}$; 2-6-10), dihydroergotoxine (1 $\mu g\ kg^{-1}\ min^{-1}$; 3-7-11) and ergotamine (1 $\mu g\ kg^{-1}\ min^{-1}$; 4-8-12), control values 1-5-9.

mine decreased femoral blood flow and increased venous pressure without arterial blood pressure modifications; these effects, present at 0.2 $\mu g\ kg^{-1}\ min^{-1}$, became marked at 1 $\mu g\ kg^{-1}\ min^{-1}$. Lymph flow was increased the first minutes of infusion and then decreased for a long period of time (over 1 hour).

DHE administered at 1 $\mu g\ kg^{-1}\ min^{-1}$, was completely ineffective, while at 5 $\mu g\ kg^{-1}\ min^{-1}$ it decreased femoral blood flow by 40% and increased venous pressure by 50% without modification of lymph flow. All these effects were long-lasting. The long-lasting DHE vasoconstrictor effect was sometimes preceded by a short-lasting

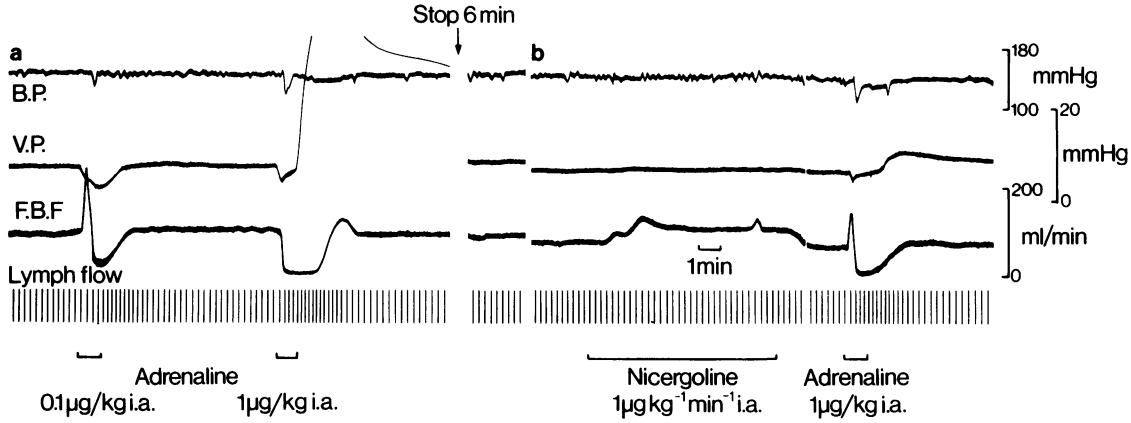

Figure 4 Anaesthetized dog: variations of systemic blood pressure (B.P.), metatarsal venous pressure (V.P.), femoral blood flow (F.B.F.), and lymph flow (recorded as drops). (a): Effect induced by adrenaline, 0.1 and 1 µg/kg. (b): Effect of adrenaline (1 µg/kg) after intra-arterial infusion of nicergoline.

vasodilatation which was also observed after administering its solvent alone.

At a constant infusion rate of 1-5 $\mu g\ kg^{-1}\ min^{-1}$ nicergoline (Arcari, Dorigotti, Fregnan & Glässer, 1968) produced marked increases (60 and 115% respectively) in femoral blood flow, which lasted throughout the administration time, returning to control level as soon as the infusion was completed. Arterial blood pressure and lymph flow were not modified. A slight (<10%) and brief increase in venous pressure was observed after administration of 5 $\mu g\ kg^{-1}$ minute^{-1}. Phenoxybenzamine at a constant infusion rate of 5 and 10 $\mu g\ kg^{-1}\ min^{-1}$ did not affect arterial or venous pressure or lymph flow, while it had a weak action on peripheral blood flow, which increased during the administration period with a quick return to near control values when the infusion was over. Intra-arterial administration of propranolol (10, 100 and 1000 µg/kg) did not modify the above parameters.

Adrenaline: effects on animals pretreated with alpha- and beta-adrenoceptor blocking drugs

In this set of experiments a standard dose of adrenaline (1 µg/kg) was injected intra-arterially 2, 5 and 30 min after the administration of phenoxybenzamine, DHE, nicergoline, and propranolol. Combined pretreatments of phenoxybenzamine-propranolol, and DHE-propranolol were also tested. Ergotamine was not used as an alpha-adrenoceptor blocking drug because of its marked and long-lasting intrinsic effects on the above functions. Neither DHE, (1-5 $\mu g\ kg^{-1}\ min^{-1}$) nicergoline, (1-5 $\mu g\ kg^{-1}\ min^{-1}$) (Figure 4), phenoxybenzamine (5 and 10 $\mu g\ kg^{-1}\ min^{-1}$) nor propranolol (10, 100 and 1000 µg/kg) modified the increase in lymph flow to adrenaline. The venous hypotensive phase induced by adrenaline remained practically unchanged in the animals treated by alpha-adrenoceptor blocking drugs. In contrast, venous hypertension was abolished, or at least reduced up to 80%, by DHE, phenoxybenzamine, and nicergoline. In addition, DHE and phenoxybenzamine converted the reduction in arterial blood flow into a clear-cut increase (adrenaline reversal). Nicergoline reduced the intensity and duration of adrenaline vasoconstriction. In control experiments, the highest intra-arterial dose of propranolol reduced the increase in arterial flow to isoprenaline by at least 60%. The increase of blood flow induced by adrenaline in animals pretreated with DHE and phenoxybenzamine was converted into a small decrease of flow by administering propranolol, 10 or 100 µg/kg intra-arterially (Figure 5); also the adrenaline hypertensive effect on venous pressure was restored (reversal of adrenaline reversal).

Discussion

The experiments were performed to study the effects of catecholamines and adrenoceptor blocking agents (alone and combined) on the lymphatic system of the hind limb of the dog. Our results clearly show that only adrenaline and noradrenaline increase lymph flow when injected intra-arterially at doses inactive on systemic arterial blood pressure.

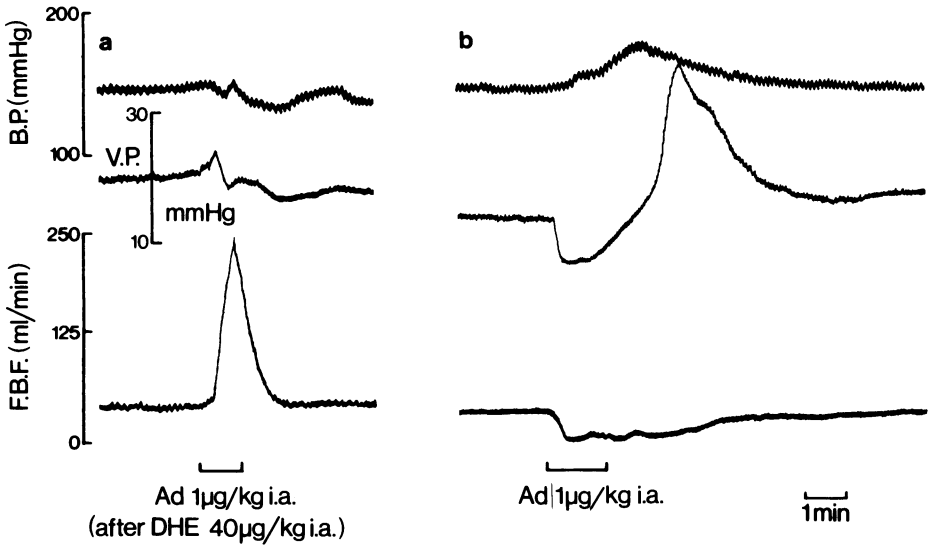

Figure 5 Anaesthetized dog: variations of systemic blood pressure (B.P.), metatarsal venous pressure (V.P.), and femoral blood flow (F.B.F.). (a): Adrenaline (Ad) femoral vasodilatation in dihydroergotoxine (DHE) pretreated animal (adrenaline reversal). (b): Return to the previous vascular effects of adrenaline following propranolol, 10 μg/kg, i.a. (reversal of adrenaline reversal) 30 min previously.

These effects might be due to a direct action of adrenaline and noradrenaline on lymphatic vessels; in fact, they are independent of blood flow changes and of venous hypertension. Lymph flow is also increased by adrenaline when its vasoconstrictor effect is abolished by alpha-adrenoceptor blocking agents. An increase in blood flow induced in various ways (isoprenaline, reactive hyperaemia or nicergoline) does not influence lymph flow *per se*. On the other hand, a reduction of arterial blood flow does not produce an increase in lymph flow; occlusion of the femoral artery immediately stops lymph movement.

Venous hypertension might be excluded as a causal factor of the lymph flow increase since α-adrenoceptor blocking agents abolish venous hypertension without influencing the increase in lymph flow induced by adrenaline and noradrenaline.

The stimulatory activity of adrenaline and noradrenaline together with the lack of effect of isoprenaline, a pure beta-adrenoceptor stimulant, suggest that α-adrenoceptors are present in the lymphatic vessels of the canine hind limb.

In accordance with this hypothesis, noradrenaline was more active than adrenaline. Contrary to our expectation, alpha-adrenoceptor blocking agents did not antagonize the effects of adrenaline and noradrenaline even when given locally at doses sufficient to invert the adrenaline vasoconstriction to a clear-cut vasodilation (adrenaline reversal).

The observations provide further information about the interaction of alpha- and beta-adrenoceptor blocking agents. Smith & Nash (1969) showed that some beta-blocking drugs may restore to near normal values, the systemic blood pressure response to noradrenaline in phenoxybenzamine-treated animals. The results clearly show that a similar phenomenon can be observed also locally in the canine hind limb vasculature. Propranolol converted the vasodilator effect of adrenaline (in DHE and phenoxybenzamine-treated animals) to a vasoconstriction similar to the one displayed before alpha-adrenoceptor blockade.

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