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THE EFFECTS OF PROSTAGLANDINS E_1 AND E_2 ON THE SMOOTH MUSCLE OF THE DOG SPLEEN AND ON ITS RESPONSES TO CATECHOLAMINES, ANGIOTENSIN AND NERVE STIMULATION

BY

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There is evidence that stimulation of the peripheral cut end of the splenic nerve causes the appearance of prostaglandin E_2 in the venous blood draining the isolated perfused spleen of the dog (Davies, Horton & Withrington, 1968).

The present experiments were carried out on the same preparation to compare the actions of prostaglandins E_1 and E_2 and acetylcholine on the splenic vasculature and the smooth muscle of the trabeculae and capsule. Further experiments were made to test the hypothesis that the prostaglandins act on smooth muscle to modify the effects of catecholamines, angiotensin and sympathetic nerve stimulation.

METHODS

Experiments were performed on pairs of dogs weighing 11.4–20.0 kg. The first dog was anaesthetized with intravenous injection of 3–4 ml. of 2.5% methohexitone sodium (Brietal sodium. Lilly) followed by a mixture (5 ml./kg) of 1% chloralose (α-chloralose, Kühlmann, Paris) and 10% urethane (B.D.H.) dissolved in 0.9% NaCl solution. The trachea and an external jugular vein were cannulated. A mid-line abdominal incision was made and the spleen was isolated from surrounding structures, omentum, pancreas and stomach by dividing between ligatures so that at this stage the only connexions were through the splenic artery, vein and postganglionic nerve trunk.

The second dog was premedicated with morphine hydrochloride (1.0 mg/kg intramuscularly) 1 hr before anaesthesia. Subsequently a mixture of 1% chloralose and 10% urethane (5 ml./kg) was administered intravenously. The trachea and an external jugular vein were cannulated and a femoral artery and vein were exposed and cannulated. The spleen of the first dog was then removed, placed in a Perspex plethysmograph, and perfused at constant pressure from the femoral artery of the second dog. Splenic arterial blood flow was measured by means of a Shipley-Wilson rotameter and the arterial perfusion pressure with a Statham pressure transducer (P23Gb). Splenic venous pressure was measured with a saline manometer. The plethysmograph was placed on an electric heating pad and filled with liquid paraffin maintained at 37° C by heating coils. Changes in splenic volume were monitored, by a low pressure Statham transducer (P23Bb), as variations in the height of a liquid paraffin column connected to the plethysmograph. After amplification, the signals were recorded on a Beckman Type R Dynograph.

The postganglionic nerve to the spleen was dissected away from the artery and placed on platinum electrodes with the cathode distal. The electrode leads were connected to a stimulator and pulse counter so that a set number of stimuli (50 V, 0.5 msec) could be delivered at a known rate.

In all experiments coagulation of the blood was prevented by the administration of heparin (Pularin, Evans Medical Ltd.): 500 i.u./kg was administered to dog 1 immediately before excision of the spleen; 1,000 i.u./kg was given to dog 2 immediately before cannulation of the femoral vessels and this dose was supplemented at 2 hr intervals with 500 i.u./kg.

Splenic vascular resistance. At constant arterial perfusion pressure and venous pressure, a change in vascular resistance can be taken as being inversely proportional to the change in blood flow. In the present study, therefore, a change in splenic vascular resistance was expressed as a percentage change in the reciprocal of the blood flow.

Administration of drugs. All drugs were infused or injected into the rubber tubing between the rotameter and the spleen. The splenic venous blood collected during and immediately after the administration of adrenergic blocking drugs was discarded. This restricted their action to the perfused spleen and ensured that no blocking agent entered the systemic circulation of the donor dog.

The following drugs were used: noradrenaline bitartrate (Winthrop), adrenaline bitartrate (Martindale Samoore), acetylcholine chloride (Lematt and Boinot), angiotensin (Hypertensin, Ciba), phenoxybenzamine (Dibenyline, SKF), propranolol (Inderal, ICI).

Prostaglandin E_1 was supplied by Dr. D. A. van Dorp, of Unilever Research Laboratories, Vlaardingen, The Netherlands, and prostaglandin E_2 by Dr. John E. Pike, of the Upjohn Company, Kalamazoo, U.S.A. The stability of these standards was checked during the experiments by thin-layer chromatography and biological assay on the rat fundus.

RESULTS

Effects of prostaglandins E_1 and E_2 and of acetylcholine on splenic arterial vascular resistance and spleen volume

In three experiments prostaglandin E_1 was infused on nine occasions in doses from 0.5 to 5.0 μg/min and in two experiments prostaglandin E_2 was infused on ten occasions in doses of 0.5–4.0 μg/min. On all occasions the close arterial infusions of both substances caused an immediate increase in splenic arterial blood flow. Because the arterial perfusion pressure was constant, this increase in blood flow indicates a reduction in splenic vascular resistance. Prostaglandin E_1 caused an increase in blood flow of 46–175% (mean 92%) representing a reduction in vascular resistance of 31–64% (mean 46%). With prostaglandin E_2, the blood flow increased 14–75% (mean 41%) corresponding to a reduction in vascular resistance 14–42% (mean 28%).

There were considerable differences in the magnitude and the time-course of the responses to the two substances (Fig. 1). The reduction of splenic vascular resistance during infusions of E_1 was greater than at equivalent infusion rates of E_2 and, at any given rate of infusion, the increased blood flow was maintained throughout the period of infusion. In these experiments the longest period of infusion of E_1 was 36 min at 2.5 μg/min. Prostaglandin E_2, on the other hand, produced a reduction in splenic vascular resistance which did not persist throughout the infusion period. In one experiment for instance, the close arterial infusion of prostaglandin E_2 for 9 min at a rate of 2.5 μg/min resulted in an immediate reduction in splenic vascular resistance which returned to the control level after 2 min.

An increase in spleen volume always accompanied the reduction in splenic vascular resistance during the infusion of prostaglandins E_1 and E_2. The volume increase, however, was always very much slower than the change in vascular resistance.

It has previously been shown (Daly & Scott, 1961) that small doses of acetylcholine injected close arterially to the spleen produced a reduction of splenic vascular resistance.

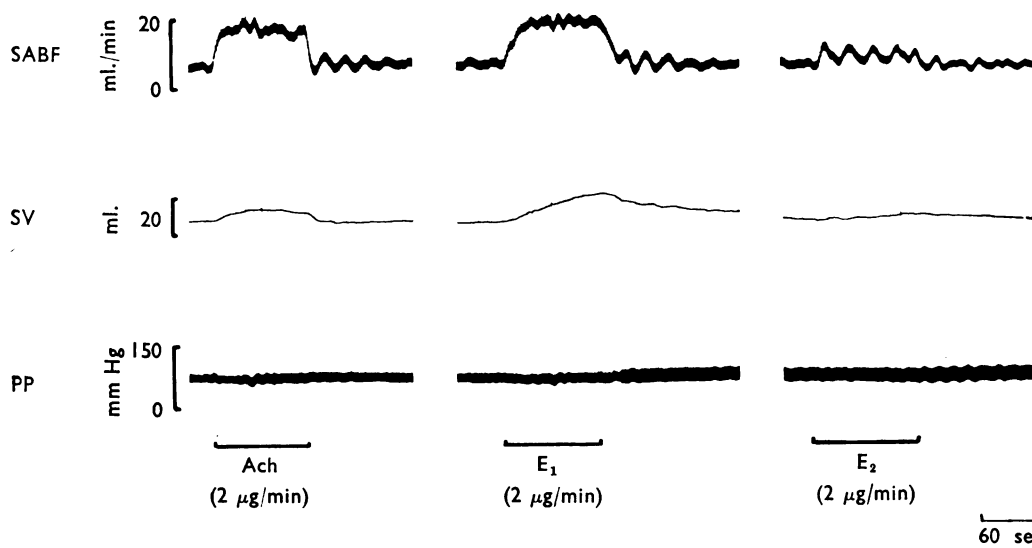

Fig. 1. Dog (11.4 kg). Effect of close arterial infusion of acetylcholine (Ach), prostaglandin E_1 (E_1) and prostaglandin E_2 (E_2) on splenic arterial blood flow (SABF) and spleen volume (SV) at constant perfusion pressure (PP).

In one experiment a comparison was made between the activities of prostaglandins E_1 and E_2 and acetylcholine in reducing splenic vascular resistance. The results are shown in Fig. 2 and indicate that on a molar basis prostaglandin E_1 is slightly less potent in reducing splenic arterial vascular resistance than acetylcholine and that prostaglandin E_2 is considerably less effective than either.

Effects of adrenergic blocking drugs on the response of the spleen to adrenaline and prostaglandins E_1 and E_2

It was found that the reduction in splenic vascular resistance produced by the close arterial infusion of prostaglandins E_1 and E_2 was unaffected by the close arterial administration of 10 mg phenoxybenzamine, an adrenergic α-receptor blocking agent. This dose of phenoxybenzamine was sufficient to abolish the excitatory actions of adrenaline on both the splenic vascular and capsular smooth muscle.

After the administration of phenoxybenzamine, restricted to the perfused spleen (see METHODS), the close arterial injection of adrenaline produced a decrease in splenic vascular resistance in contrast to the increase observed before the blocking agent was given. Furthermore sufficient adrenaline then entered the systemic circulation of the dog to produce a fall in arterial blood pressure probably because the uptake of exogenous catecholamines by the sympathetic nerve terminals in the spleen had been arrested by phenoxybenzamine (Gillespie & Kirpekar, 1965). It is interesting to note that at this stage the intra-arterial infusion of prostaglandin E_1 or E_2 was accompanied by a fall in arterial blood pressure which suggested that phenoxybenzamine had affected mechanisms concerned with the removal of prostaglandins entering the spleen.

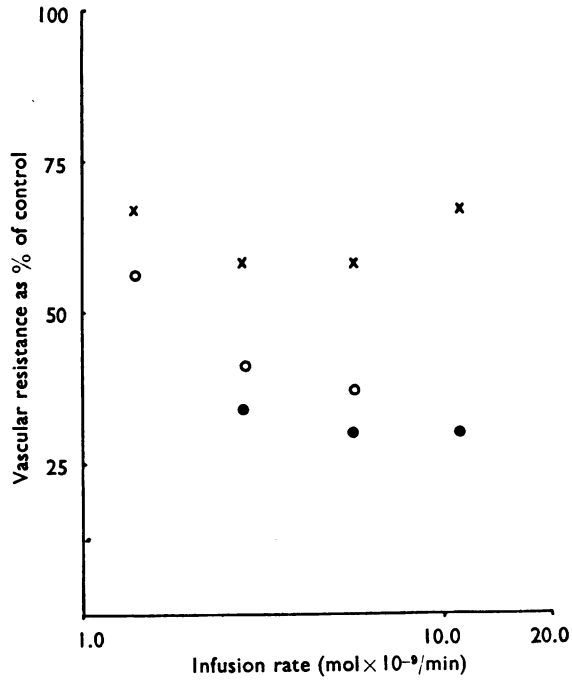

Fig. 2. Relationship between the dose of acetylcholine (●), prostaglandin E_1 (○) and prostaglandin E_2 (×) and splenic vascular resistance at constant perfusion pressure. Ordinate: splenic arterial vascular resistance as percentage of pre-infusion control value. Abscissa: infusion rate in mol × 10^{-9}/min on logarithmic scale.

The subsequent close arterial administration of a β-receptor adrenergic blocking agent, propranolol, in a dose (5 mg) sufficient to abolish the reduction in the splenic arterial resistance produced by adrenaline, did not alter the pattern of response to either of the prostaglandins (Fig. 3).

The effects of prostaglandin E_1 on the splenic responses to sympathetic nerve stimulation, adrenaline, noradrenaline and angiotensin

Many authors (Daly & Scott, 1961; Haefely, Hürlimann & Thoenen, 1965; Hertting & Suko, 1966) using a variety of preparations of the spleen have shown that adrenaline, noradrenaline, angiotensin and sympathetic nerve stimulation produce contraction of the splenic capsule and increase in the splenic vascular resistance. In agreement with these authors we have found that in the present preparation adrenaline and noradrenaline injected close arterially to the spleen stimulate both vascular and capsular smooth muscle, adrenaline being the more potent; angiotensin has a predominant effect on vascular smooth muscle inducing an increase in splenic vascular resistance with a small decrease in spleen volume; postganglionic sympathetic nerve stimulation at 3/sec causes a marked splenic contraction but at this frequency only a slight increase in splenic vascular resistance (Fig. 4).

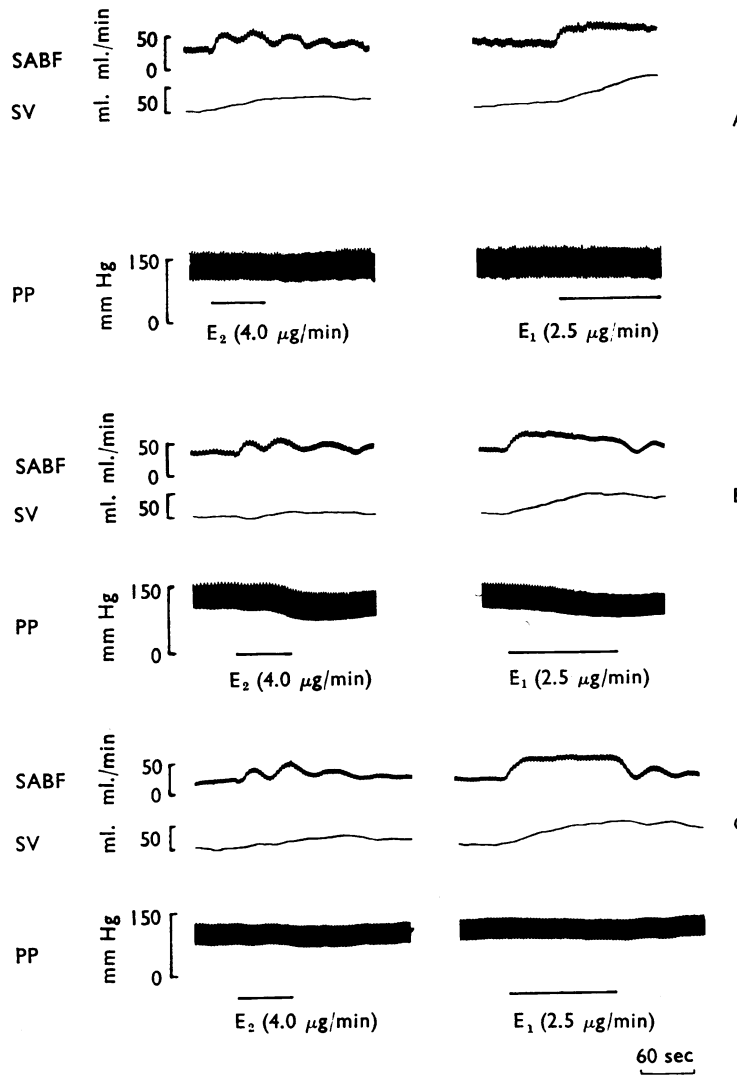

Fig. 3. Dog (11.5 kg). Effects of phenoxybenzamine and propranolol on the responses of the dog's spleen to prostaglandin E_1 (E_1) and prostaglandin E_2 (E_2). The sets of experimental tracings (A, B, C) each consist of a record of splenic arterial blood flow (SABF), splenic volume (SV) and perfusion pressure (PP). Phenoxybenzamine (10 mg) was administered by close arterial injection to the spleen between A and B, and propranolol (5 mg) between B and C. The responses to the close arterial infusions of prostaglandins E_1 (2.5 μg/min) and E_2 (4.0 μg/min) are illustrated.

In two preparations these tests were applied before and during an intra-arterial infusion of prostaglandin E_1 in a dose (2.5 μg/min) which produced a marked reduction in splenic arterial vascular resistance. The results of one experiment (Fig. 4) show that the actions of adrenaline, noradrenaline, angiotensin and sympathetic nerve stimulation at 3/sec are unaffected by the simultaneous infusion of prostaglandin E_1.

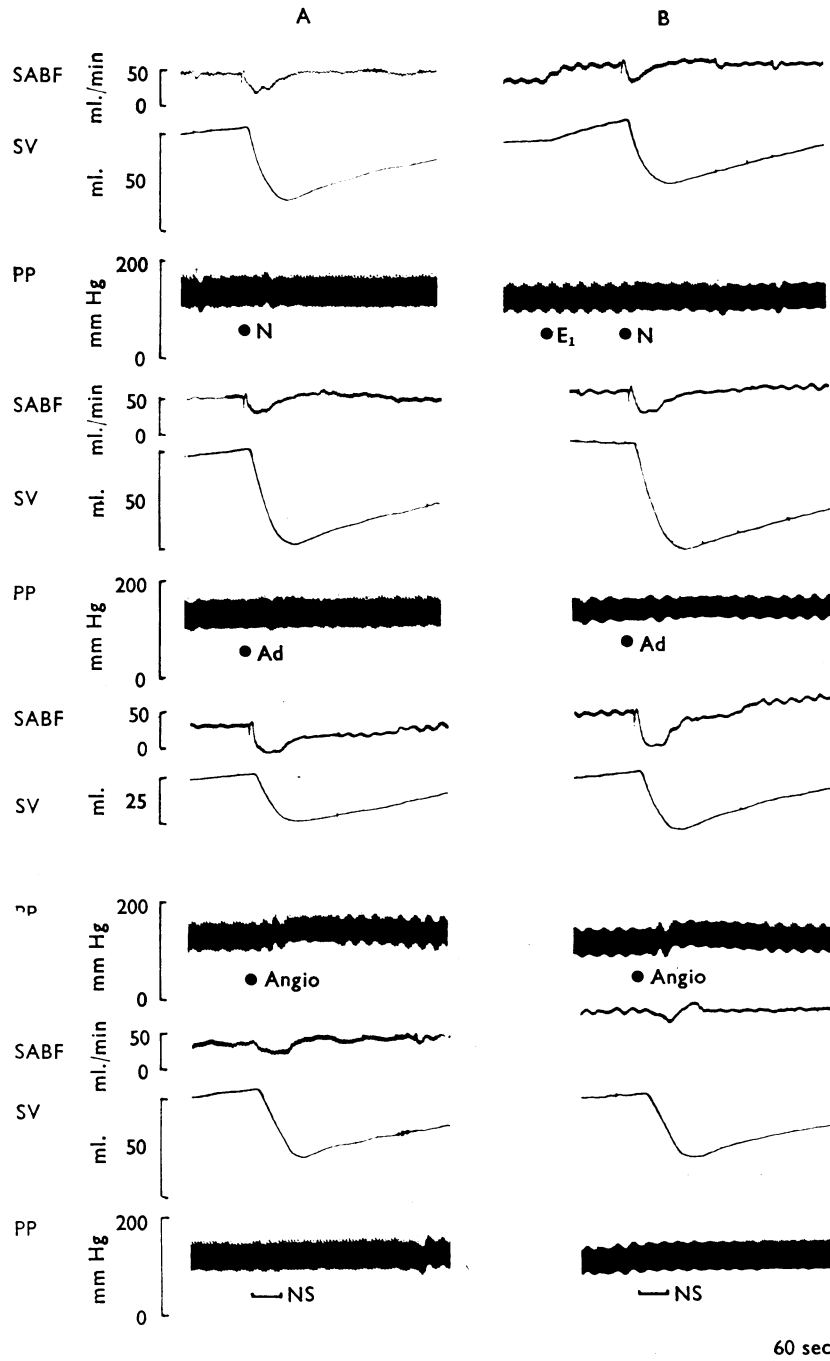

Fig. 4. Dog (15 kg). Effects of noradrenaline (N), adrenaline (Ad), angiotensin (Angio) and sympathetic nerve stimulation (NS) on splenic arterial blood flow (SABF) and splenic volume (SV) before (A) and during (B) an infusion (2.5μg/min) of prostaglandin E_1. The infusion commenced at E_1 and continued for 36 min. 3 μg noradrenaline, 3 μg adrenaline and 3 μg angiotensin were administered by close arterial injection. The splenic nerve was stimulated for 30 sec at 3/sec.

The same tests applied after the cessation of the prostaglandin infusion produced responses which were the same as the control responses.

Effect of prostaglandin E_2 on the splenic responses to adrenaline and noradrenaline

The supply of prostaglandin E_2 was limited and therefore only two experiments could be performed to test its effect on the responses to close-arterially injected adrenaline and noradrenaline.

The results of each experiment were, however, the same and clearly indicated that close arterial infusion of prostaglandin E_2 did not alter the response of either the capsule or the vascular smooth muscle to adrenaline or noradrenaline.

DISCUSSION

Davies *et al.* (1968) showed that a number of acidic lipids were released into the splenic venous blood after electrical stimulation of the postganglionic sympathetic nerves. Chromatographic analyses and bioassay procedures indicated that prostaglandin E_2 was a major constituent and that the appearance of this substance in the splenic venous blood was abolished after the administration of the adrenergic blocking drug phenoxybenzamine.

In the present study an attempt has been made to elucidate a possible role for the prostaglandins in the spleen. Close arterial infusions of prostaglandins E_1 and E_2 always caused an immediate reduction in splenic vascular resistance at constant perfusion pressure. Although this change in vascular resistance was always accompanied by an increase in spleen volume, there was not a constant relationship between the two parameters. We therefore interpret the reduction in splenic vascular resistance as the result of vasodilatation. Prostaglandin E_1 has been shown to cause vasodilatation in other vascular territories such as the skin and gastrocnemius muscle of the cat (Horton & Main, 1963). More recently, Nakarno & McCurdy (1967) showed that prostaglandin E_1 decreased the vascular resistance in coronary, brachial, carotid and renal arteries.

Paradoxically E_2, the prostaglandin released from the spleen (Davies *et al.*, 1968), has considerably less vasodilator activity than the closely related substance E_1 which we have shown to be intrinsically only slightly less active as a vasodilator of the splenic vasculature than acetylcholine. The question remains whether E_1, the more active form when infused into the spleen, is produced by structures within the spleen and that after exerting its action it is degraded to the less active form E_2. This differential sensitivity of the blood vessels of the spleen to prostaglandin E_1 compared with E_2 is to be contrasted with the findings of Horton & Main (1963) that prostaglandins E_1 and E_2 were equiactive on the blood vessels of the cats gastrocnemius muscle and skin.

There have been several reports that the actions of catecholamines, angiotensin and other vasoactive drugs are inhibited by prostaglandin E_1 (Holmes, Horton & Main, 1963; Steinberg, Vaughan, Nestel, Strand & Bergström, 1964; Bergström, Carlsson & Orö, 1964). It has therefore been suggested that a function of the prostaglandins may be to modify the sympathetic nerve-transmitter-smooth muscle system. The results in the present paper do not lend support to the application of this hypothesis to the vasculature of the dog spleen. Similarly, in the dog, Nakano & McCurdy (1967) showed that prostaglandin

E_1 modified neither the pressor, positive inotropic nor chronotropic responses to intravenous noradrenaline.

It is possible that the effects of intra-arterially infused prostaglandin have no bearing on the function of prostaglandin E_2 which is released from the spleen on nerve stimulation. The function of the prostaglandin may be concerned with intracellular events at the post-synaptic membrane which cannot be mimicked by injecting prostaglandin. Alternatively, the target site for the prostaglandin E_2 released from the spleen may be the liver, an organ known to concentrate prostaglandin from the blood (Samuelsson, 1965).

SUMMARY

1. The close arterial infusion of prostaglandins E_1 and E_2 into the isolated blood-perfused dog spleen produced a reduction in splenic vascular resistance and a slight increase in spleen volume.

2. Because the perfusion pressure and the venous pressure were constant and because there was no apparent relationship between the volume change and the reduction in vascular resistance, we have interpreted the increase in blood flow as the result of vasodilatation.

3. Prostaglandin E_1 was a more potent vasodilator of splenic blood vessels than prostaglandin E_2 but slightly less active than acetylcholine.

4. The vasodilatation produced in the spleen by the prostaglandins was not affected by α- or β-adrenergic blocking agents.

5. The splenic responses to adrenaline, noradrenaline, angiotensin and sympathetic nerve stimulation were not modified by the simultaneous close arterial infusion of prostaglandin E_1.

6. The effects of adrenaline and noradrenaline on the spleen were unaffected by close arterial infusions of prostaglandin E_2.

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