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Actions of prostaglandin $F_{2\alpha}$ on the splenic vascular and capsular smooth muscle in the dog

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Summary

1. The actions of prostaglandin $F_{2\alpha}$, which is known to be released by the spleen on sympathetic nerve stimulation, have been investigated in the isolated blood perfused spleen of the dog.

2. Low arterial concentrations of $F_{2\alpha}$ (less than 10 μg/100 ml) caused marked reductions in splenic vascular resistance.

3. High arterial concentrations of $F_{2\alpha}$ (above 10 μg/100 ml) caused increases in splenic vascular resistance.

4. Little action on splenic volume was observed by any arterial concentration of $F_{2\alpha}$.

5. The reductions in splenic vascular resistance caused by low arterial concentrations of $F_{2\alpha}$ were reversed by blocking doses of phenoxybenzamine.

6. No appreciable interaction between $F_{2\alpha}$ and the splenic responses to sympathetic nerve stimulation, adrenaline and noradrenaline was observed.

7. The role of the prostaglandins released by the spleen is discussed.

Introduction

The prostaglandins are a family of acidic lipids widely distributed in animal tissues. Two prostaglandins are released from the spleen into the circulation, prostaglandin E_2 and a prostaglandin of the F series having been identified in the splenic venous blood of dogs following splenic nerve stimulation (Davies, Horton & Withrington, 1968). Gilmore, Vane & Wyllie (1968) confirmed the presence of E_2 and identified $F_{2\alpha}$ in the effluent of the dog's spleen perfused with Krebs solution. Furthermore they showed that these substances were released from the spleen following the injection of adrenaline or noradrenaline.

The actions of prostaglandins E_1 and E_2 on splenic smooth muscle were subsequently analysed (Davies & Withrington, 1968). The present series of experiments were designed to examine the actions of $F_{2\alpha}$ on splenic vascular and capsular smooth muscle and its interaction with sympathetic nerve stimulation, and the catecholamines adrenaline and noradrenaline. Some of the observations have already been communicated (Davies & Withrington, 1969).

Methods

Experiments were performed on sixteen pairs of dogs weighing between 9·5 and 18·0 kg. Both dogs were 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, Kuhlmann, Paris) and 10% urethane (B.D.H.) dissolved in 0·9% NaCl by warming. The solution was filtered before injection.

Preparation of the spleen for perfusion in the femoral circulation of the second dog was exactly as described in detail by Davies & Withrington (1968). Mean splenic arterial blood flow was measured by means of a Shipley-Wilson rotameter calibrated with blood at the end of each experiment. The arterial perfusion pressure (1 mmHg≡1.333 mbar) was measured with a Statham high pressure transducer (P23Gb) and splenic venous pressure with a Statham low pressure transducer (P23Bb). The plethysmograph was placed on an electric heating pad and filled with liquid paraffin maintained at 37° C by heating coils. Changes in spleen 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 splenic vascular resistance was calculated as splenic arterial perfusion pressure/splenic arterial blood flow.

Administration of drugs

All drugs were administered through the rubber tubing just proximal to the cannulated splenic artery. The action of blocking drugs was restricted to the spleen by collecting the splenic venous blood during and immediately after administration of the drug; the collected blood was discarded. The blood concentrations of $F_{2\alpha}$ stated in the text were obtained by dividing the rate of drug infusion (μg/min) by the mean splenic arterial blood flow (ml/min).

The following drugs were used: adrenaline bitartrate (Martindale Samoore), noradrenaline bitartrate (Winthrop), phenoxybenzamine (Dibenyline, SKF) and propranolol (Inderal, I.C.I.).

Prostaglandin $F_{2\alpha}$ was kindly supplied by Dr. John E. Pike of the Upjohn Company, Kalamazoo, U.S.A.

Results

Effects of infusions of $F_{2\alpha}$ on splenic vascular resistance and spleen volume

Prostaglandin $F_{2\alpha}$ (0·5–50 μg/min) was infused by the close arterial route into the dog's spleen for 1–2 min on sixty-eight occasions in sixteen experiments. The response of the splenic vascular bed depended on the splenic arterial blood flow and the rate of drug infusion. In the experiment illustrated in Fig. 1 the splenic arterial blood flow increased from 63 to 78 ml/min as the result of an infusion of

TABLE 1. *Response of the splenic vascular bed to varying concentrations of prostaglandin $F_{2\alpha}$*

Concentration of $F_{2\alpha}$ (μg/100 ml of blood)	0·5–1·9	2·0–3·9	4·0–5·9	6·0–9·9	10–19·9	20–49·9	50–
Number of tests	10	13	14	8	8	11	4
Vasodilatation	6	7	8	4	3	1	0
No change in flow	4	3	0	1	0	0	0
Vasoconstriction	0	3	6	3	5	10	4
Mean % change in splenic vascular resistance from control	−5·3	−3·7	−2·1	−2·3	+11·8	+36	+75·3
S.E.M.	2·25	2·38	3·44	4·96	10·0	6·66	27·1

$F_{2\alpha}$ at 1·0 μg/min (equivalent to 1·6 μg $F_{2\alpha}$/100 ml blood; see **Methods**), while the flow decreased from 64 to 38 ml/min when the infusion rate of $F_{2\alpha}$ was subsequently increased to 10 μg/min (equivalent to 15·6 μg $F_{2\alpha}$/100 ml blood). Since the splenic perfusion pressure was constant, these alterations in flow reflect changes in splenic vascular resistance and indicate vasodilatation at the low blood concentration of $F_{2\alpha}$ and vasoconstriction at the higher concentration.

The results of all experiments are shown in Table 1. The response of the splenic vascular bed to the lower blood concentrations of $F_{2\alpha}$ (less than 10 μg/100 ml blood) was vasodilatation in twenty-five of the forty-five tests, while no change in splenic vascular resistance was observed on eight occasions. At the higher blood levels of $F_{2\alpha}$ (above 10 μg/100 ml blood) the response was, in nineteen of the twenty-three tests, vasoconstriction. These effects were small in individual experiments and the variations between animals were large, so that when all the results from the sixteen experiments are considered together the mean changes in splenic vascular resistance are only significantly different from the control at the lowest (0·5–1·9 μg/100 ml blood) and highest (20–49·9 μg/100 ml and above 50 μg/100 ml blood) concentrations of $F_{2\alpha}$ in the blood (Table 1).

The response of the splenic capsule to infusions of prostaglandin $F_{2\alpha}$ was usually small and most frequently was an increase in volume with a time course which suggested a passive change in size secondary to alterations in splenic arterial blood flow. However, in fourteen of the twenty-nine infusions of $F_{2\alpha}$ which caused vaso-

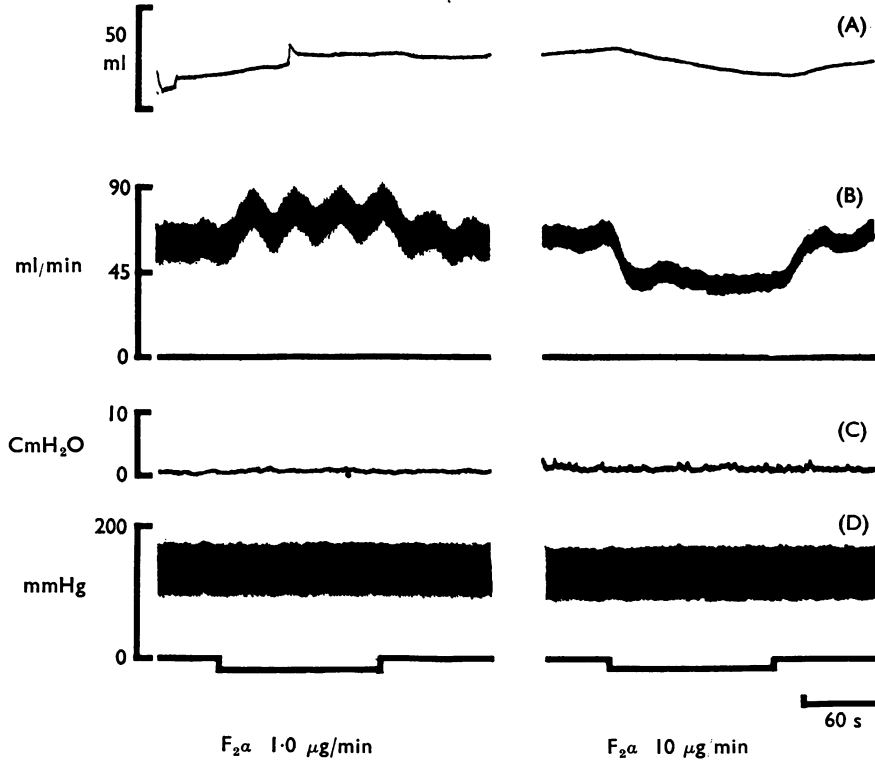

FIG. 1. Changes in (A) spleen volume, (B) splenic arterial blood flow, (C) splenic venous pressure and (D) perfusion pressure in response to infusions of prostaglandin $F_{2\alpha}$ at 1·0 μg/min and 10 μg/minute.

dilatation there was a concomitant decrease in spleen volume. This reduction in volume (mean 7·9 ml) was small and not statistically significant.

Effect of phenoxybenzamine

Since a range of blood concentrations of $F_{2\alpha}$ induces either predominantly splenic vasodilatation or vasoconstriction it was necessary to investigate the association of these responses with the classical α-β system of receptors in the adrenergic innervation to the spleen (Ahlquist, 1948 ; Davies, Robinson & Withrington, 1969). After a series of control responses to $F_{2\alpha}$, the adrenergic α-receptor antagonist phenoxybenzamine was administered in a dose which blocked the effects of sympathetic nerve stimulation and close-arterially injected catecholamines. The infusions of $F_{2\alpha}$ were then repeated. These results are shown in Fig. 2. In eleven tests, the initial response to $F_{2\alpha}$ (less than 10 μg/100 ml blood) was a decrease in splenic vascular resistance or no change (mean reduction 8·2% S.E. 2·06). In six of the eleven tests after phenoxybenzamine, there was a vasoconstriction, while in four tests the vasodilatation was abolished. The response to $F_{2\alpha}$ in the remaining test was unchanged. The mean increase in splenic vascular resistance due to $F_{2\alpha}$ after phenoxybenzamine in all eleven experiments was 29·6%, S.E. 13·2.

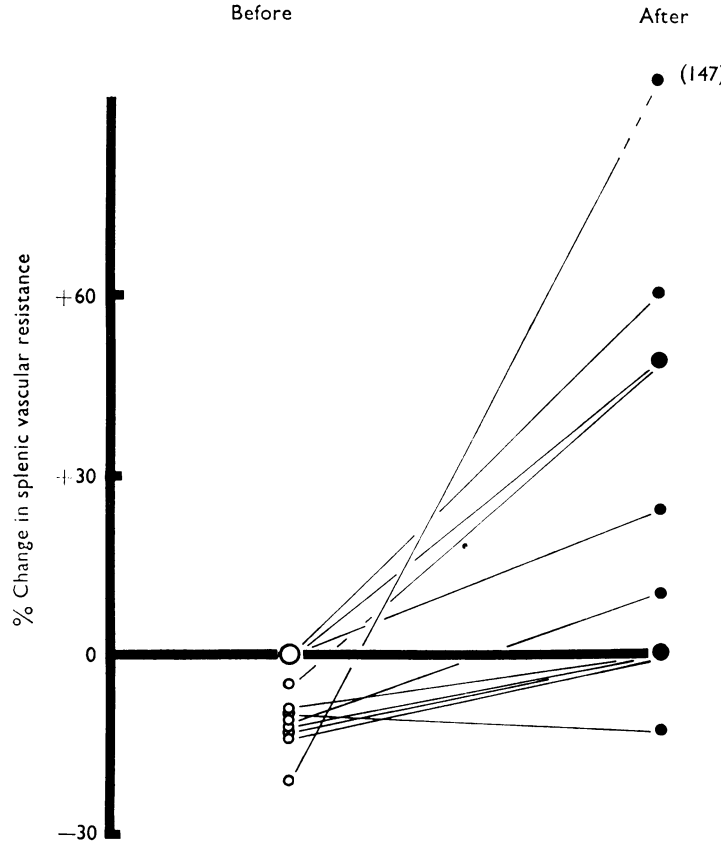

FIG. 2. Changes in splenic vascular resistance produced by low arterial concentrations of prostaglandin $F_{2\alpha}$ before (○) and after (●) blocking doses of phenoxybenzamine (3–5 mg). Eleven tests.

Effect of $F_{2\alpha}$ on the splenic responses to sympathetic nerve stimulation and catecholamines

Control submaximal responses of the splenic capsular and vascular smooth muscle to sympathetic nerve stimulation (1 and 3 Hz) and close-arterial injections of adrenaline (1 and 3 μg) and noradrenaline (1 and 3 μg) were obtained. $F_{2\alpha}$ was then infused at a rate (1–5 μg/min) which produced a slight vasodilatation. The test stimuli were repeated during the infusion and again subsequently when the infusion of $F_{2\alpha}$ was stopped and the splenic volume and arterial blood flow had returned to the control values. This procedure was adopted on eleven occasions in five experiments.

The effect of $F_{2\alpha}$ on the responses to the different test stimuli was very small and difficult to distinguish from the normal variation that we have observed in the responses of this preparation. Changes could only be considered valid if the test responses before and after the infusion of $F_{2\alpha}$ were identical. This resulted in 'no change' being registered a number of times when, because of a small change in the 'background' level of arteriolar and capsular tone of the preparation, the post infusion test responses were not identical with the control responses before infusion.

The results can be summarized briefly as follows:

1. Sympathetic nerve stimulation (1 and 3 Hz). In ten tests, the capsular contraction was increased on five occasions by $F_{2\alpha}$. 'No change' was recorded in the other five. The vasoconstrictor response was potentiated in eight of the tests, the remaining two showing 'no change'.

2. Adrenaline (1 and 3 μg). In eight tests, the capsular response was increased on three occasions and the vasoconstrictor response on five. The remaining tests were recorded as 'no change'.

3. Noradrenaline (1 and 3 μg). In eight tests, the splenic capsule contraction was potentiated in two and reduced in two experiments. On four other occasions 'no change' was observed. The vasoconstrictor response to noradrenaline was increased on three occasions and showed 'no change' in the other five.

The most consistent observation therefore was the increase in responses to sympathetic nerve stimulation during infusions of $F_{2\alpha}$. However, considering all the test stimuli, it was apparent that the responses of the splenic vascular smooth muscle to test stimuli were more often increased by $F_{2\alpha}$ than were the responses of the smooth muscle of the spleen capsule.

Discussion

The appearance of prostaglandins in the splenic venous effluent of the dog is closely related to contraction of the splenic capsule (Davies *et al.*, 1968; Gilmore, Vane & Wyllie, 1968).

The actions of prostaglandin E_2 on the vascular and capsular smooth muscle of the dog's spleen were analysed by Davies & Withrington (1968) and it was suggested on the basis of the results that E_2, in this organ, could not function as a transmitter nor as a modulator of sympathetic function as had been suggested by Coceani, Pace-Asciak, Volta & Wolfe, (1967) and Horton (1969).

The experiments described in the present paper were designed to investigate the effects of prostaglandin $F_{2\alpha}$, also released by the spleen, on splenic capsular and

vascular smooth muscle. The compound was infused into the splenic artery at varying rates to produce blood concentrations of 0–50 μg/100 ml. Low concentrations produced splenic vasodilatation and higher concentrations, vasoconstriction. Changes in spleen volume were usually small and passively related to alterations in arterial inflow. On several occasions splenic vasodilatation was accompanied by a slight reduction in spleen volume which we have previously suggested (Davies & Withrington, 1969) is due to venoconstriction.

The vasoconstrictor responses to $F_{2\alpha}$ after the administration of phenoxybenzamine in doses sufficient to block the responses to nerve stimulation, adrenaline and noradrenaline, indicate that the action of $F_{2\alpha}$ on vascular smooth muscle in no way resembles that produced by the sympathetic innervation. They also demonstrate that the interaction of prostaglandin $F_{2\alpha}$ and the vascular smooth muscle cells does not involve the classical receptors of the adrenergic system. It is therefore improbable that prostaglandin $F_{2\alpha}$ functions as a transmitter of sympathetic activity in this tissue.

In the tests designed to investigate any interaction between sympathetic nerve stimulation, adrenaline and noradrenaline, and prostaglandin $F_{2\alpha}$, the most consistent observation was a potentiation of the vasoconstrictor response produced by sympathetic nerve stimulation. No appreciable alteration in either the vascular or capsular responses of the spleen to adrenaline or noradrenaline were observed during infusions of $F_{2\alpha}$. In twenty-six tests involving sympathetic nerve stimulation and injections of catecholamines, a reduction of response was observed on only two occasions during the simultaneous infusions of $F_{2\alpha}$. The evidence shows that in the dog's spleen prostaglandin $F_{2\alpha}$, released during sympathetic nerve stimulation, does not act as a means of limiting sympathetic activity.

Ferreira & Vane (1967) have shown that the liver and lungs provide an efficient protective mechanism for the removal of almost all the prostaglandin $F_{2\alpha}$ released by the spleen before it reaches the systemic circulation. Their observations suggest that if the prostaglandins have a physiological action, it is likely to be at the site of release, within the spleen.

The appearance of prostaglandins $F_{2\alpha}$ and E_2 in the splenic venous blood is clearly associated with contraction of the capsular smooth muscle rather than the vascular since Gilmore *et al.* (1968) have shown that vasopressin, a potent vasoconstrictor with little action on splenic capsular smooth muscle, is not associated with the release of any prostaglandins. Yet the main site of action of E_2 and $F_{2\alpha}$ when infused into the spleen close-arterially is the splenic vascular smooth muscle. The possibility exists that the prostaglandins are released by the capsular smooth muscle to have actions on the splenic vascular bed and therefore alter the distribution of blood within the spleen when the organ contracts. Davies, Gamble & Withrington (1968a) have shown that stimulation of the splenic sympathetic nerves at low rates of 0·1–1 Hz leads to an almost maximal contraction of the capsular smooth muscle with no changes in splenic vascular resistance, a pattern mimicked by the close arterial administration of adrenaline and noradrenaline (Davies, Gamble & Withrington, 1968b). Under these circumstances it is possible that the prostaglandins released by the contracted capsular smooth muscle are causing vasodilatation of that fraction of the splenic vascular bed which remains perfused, thereby maintaining overall splenic vascular resistance at the control value. Perhaps the prostaglandins have a role in autoregulation of splenic blood flow.

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