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Cardiovascular Effects of Fibrinopeptide B

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Abstract—The effect of fibrinopeptide B (FpB) on isolated blood vessels and in the intact rat has been investigated. FpB contracted the rabbit superfused aorta preparation (EC50 = 7·5 nmol) and was some 20 times less potent than noradrenaline. Similarly, FpB (0·2–10 nmol) injected into the rat perfused kidney caused dose related, short-lived increases in perfusion pressure and potentiated the vasoconstrictor effect of injected noradrenaline. These effects were associated with increased efflux of PGE_2 (but not TxB_2) and were reduced but not abolished by indomethacin (10 μM). FpB injected intravenously into the urethane-anaesthetized rat exhibited vasoconstrictor activity (EC50 = 2·5 $\mu g\ kg^{-1}$) but was less potent than noradrenaline (EC50 = 0·9 $\mu g\ kg^{-1}$). The doses of FpB required to contract the rabbit isolated aorta and to constrict the vasculature of the rat kidney occur naturally in the bloodstream of patients with thrombotic disease. FpB released at the site of thrombus formation may play a part in regulating local blood vessel calibre.

Proteolysis of fibrinogen which occurs as a natural consequence of haemostasis and in thrombosis and inflammation results in the thrombin-mediated release of fibrinopeptide A (FpA) and fibrinopeptide B (FpB) (Nossel et al 1983). These peptides, which contain 16 and 18 amino acids, respectively, occur in the blood of normal healthy individuals and are present in even higher concentrations in patients with venous thrombosis, pulmonary embolism or ischaemic heart disease (Nossel et al 1974; Meade et al 1984). Surprisingly, there have been few reports of the pharmacological activity of fibrinopeptides. FpB increases rat blood pressure (Osbahr et al 1967), potentiates contractions of the rat uterus in response to bradykinin (Gladner et al 1963) and is a weak chemoattractant for human neutrophils (Richardson et al 1976). Despite these scattered observations in the literature there have been no systematic attempts to study the biological effects of these naturally occurring peptides. We report here experiments which demonstrate that one of these peptides, FpB, contracts vascular smooth muscle both in-vitro and in-vivo.

Materials and Methods

Rabbits (male, New Zealand White, 2·0–2·8 kg) and rats (male, Sprague-Dawley, 250–300 g) were killed by an intravenous injection of pentobarbitone. The thoracic aorta was removed, cleared of extraneous connective tissue and cut into a spiral. Preparations were placed under an initial resting tension of 2 g and superfused (6 mL min^{-1}) using a Watson-Marlow flow inducer with warmed (37°C), oxygenated (95% O_2/5% CO_2) Krebs solution (composition, mM: NaCl 121, KCl 4·7, $NaHCO_3$ 25, D-glucose 11·1, $CaCl_2.2H_2O$ 2·7, $MgSO_4.7H_2O$ 1·1, K_2HPO_4 1·18). Up to three preparations were superfused in a single experiment. Contractions were measured isometrically using Grass FT03 force transducers connected to a Devices pen recorder. Drugs were injected over the tissues in a volume not exceeding 0·1 mL.

Rats were killed by a blow to the head and exsanguinated. The left kidney was removed, transferred to a heated, water-jacketed chamber and perfused (8 mL min^{-1}) with Krebs solution via a cannula inserted retrogradely into the abdominal aorta. Renal perfusion pressure was measured using a Bell & Howell pressure transducer connected to a Devices pen recorder. Drugs were injected into the Krebs solution via a side arm of the perfusion apparatus in volumes not exceeding 20 μL. For some experiments, indomethacin (10 μM), was added to the Krebs reservoir. Inhibition of renal prostanoid biosynthesis was confirmed by radioimmunoassay of PGE_2 and TxB_2 in perfusate collected over a 2 min period 30 min after starting the experiment. For this purpose, Krebs perfusate (12 mL) was acidified to pH 3 by dropwise addition of concentrated formic acid and extracted using Waters C-18 reverse phase Sep-Pak cartridges as described elsewhere (Berry et al 1986). Extraction efficiencies obtained using radiolabelled prostanoid tracers added to an appropriate volume of normal Krebs solution were PGE_2 87·3 ± 3·9% and TxB_2 89·0 ± 5·9% (both n = 6). Values of PGE_2 and TxB_2 obtained in extracts of renal perfusate have been corrected for extraction efficiency. PGE_2 and TxB_2 were assayed using commercially available kits (Amersham International).

In separate experiments rats were anaesthetized with urethane (1 g kg^{-1}) and the carotid artery and femoral vein cannulated for measurement of blood pressure and injection of drugs, respectively, as described elsewhere (Moore et al 1980).

All drugs used were purchased from Sigma and dissolved in saline. Results are mean ± s.e. with the number of observations shown in parentheses. Statistical significance of differences between groups was determined using unpaired Student's *t*-test. A probability (*P*) value of 0·05 or less was taken to indicate statistical significance.

Results

Human FpB produced dose-related contractions of the rabbit superfused aorta. The maximal attainable contraction to FpA was 3·2 ± 0·3 g (n = 6). The amount of FpB required to produce 50% of the maximal contraction (EC50) was

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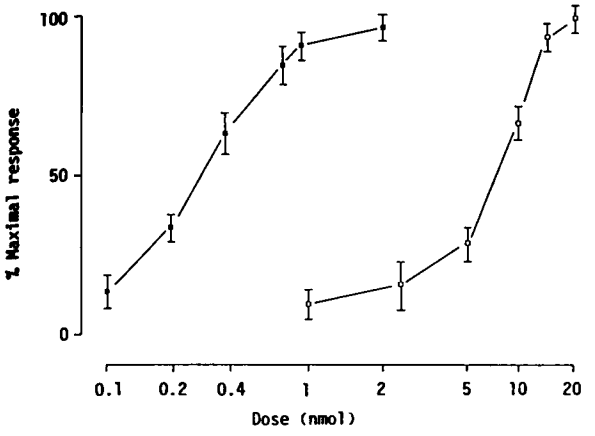

FIG. 1. Contraction of the rabbit isolated, superfused aorta preparation by noradrenaline (■) and FpB (□). Results show % of the maximum response to each agonist and are mean ± s.e., n = 6.

7·5 ± 0·05 nmol (n = 6). The corresponding EC50 value and maximal increase in tension for noradrenaline in this preparation were 0·3 ± 0·04 nmol and 3·5 ± 0·8 g (both n = 6). Dose-effect curves for FpB and noradrenaline on the rabbit superfused aorta are shown in Fig. 1. Contractions of the rabbit aorta in response to FpB were unaffected by indomethacin (10 μM), propranolol (10 μM), atropine (7 μM), hexamethonium (12 μM), mepyramine (10 μM) or methysergide (10 μM). Phentolamine (10 μM), at a concentration which prevented the contractions due to noradrenaline, was also without effect on the response to FpB.

In separate experiments the rat isolated perfused kidney maintained a steady basal perfusion pressure of 80·3 ± 5·2 mm Hg (n = 15) for periods of up to 4 h. Injection of FpB (0·2–10 nmol) produced a short-lived and dose-related increase in perfusion pressure (Fig. 2). At the highest dose of FpB used in this study (10 nmol), a maximal increase in perfusion pressure of 53·3 ± 6·0 mm Hg (n = 8) was observed. Noradrenaline (0·01–10 nmol) also caused vasoconstriction

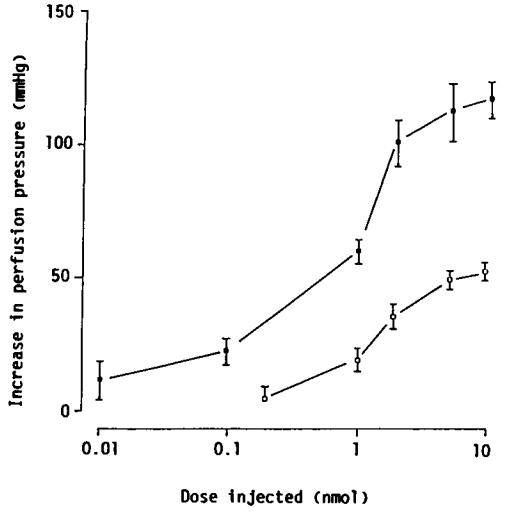

FIG. 2. Vasoconstrictor effect of noradrenaline (■) and FpB (□) in the rat isolated, perfused kidney. Results show increase in renal perfusion pressure in mm Hg and are mean ± s.e., n = 8.

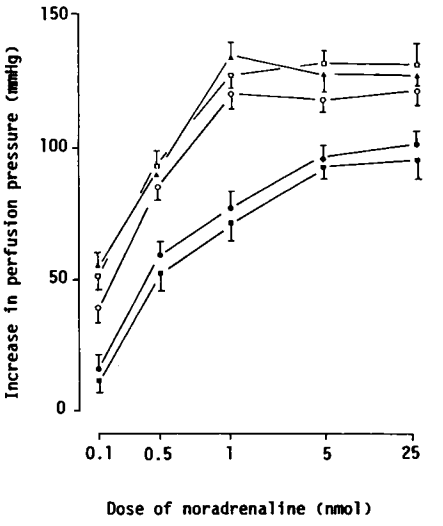

FIG. 3. Vasoconstrictor effect of noradrenaline in the isolated perfused rat kidney in the absence (■) and presence of 0·1 nM FpB (○) or 7 nM PGE_2 (□). Responses to noradrenaline in the presence of indomethacin (10 μM) and either 0·1 nM FpB (●) or 7 nM PGE_2 (▲) are also shown. Results indicate increase in renal perfusion pressure in mm Hg and are mean ± s.e., n = 6. $^*P < 0·05$, $^{**}P < 0·025$.

of the perfused kidney. As can be seen from Fig. 2, noradrenaline exhibited both a lower threshold and a greater maximum vasoconstriction of the perfused kidney and thus it was not possible to compare the potency of FpB and noradrenaline in this preparation. Addition to the perfusing Krebs solution of either FpB (0·1 nM) or PGE_2 (7 nM), at concentrations which did not affect renal perfusion pressure directly, did potentiate the vasoconstrictor response to injected noradrenaline (Fig. 3).

The possibility that FpB might owe some of its vasoconstrictor activity in this preparation to stimulation of renal prostanoid biosynthesis has also been investigated. Bolus injection of a maximally effective dose of FpB (10 nmol) stimulated renal efflux of PGE_2 (9·8 ± 1·2 ng released over the following 2 min collection period, cf. 3·2 ± 0·4 ng both n = 6, $P < 0·05$) but not TxB_2 (1·9 ± 0·3 ng 2 min^{-1} collection period, cf. 1·4 ± 0·6 ng, both n = 6). Furthermore, indomethacin (10 μM) added to the perfusing Krebs solution significantly reduced ($P < 0·05$) the renal vasoconstrictor effect of FpB (10 nmol) from 53·3 ± 6·0 mm Hg (n = 8) to 39·4 ± 3·1 mmHg (n = 6) and, at the same time, not only prevented the associated rise in PGE_2 efflux, but also considerably reduced basal release of this prostanoid (0·8 ± 0·5 ng 2 min^{-1} collection period, n = 6). Indomethacin pretreatment also abolished the ability of FpB (0·1 nM) but not PGE_2 (7 nM) to potentiate the vasoconstrictor effect of noradrenaline (Fig. 3). Vasoconstriction due to FpB in the perfused rat kidney was unaffected by addition to the Krebs reservoir of phentolamine (10 μM) at a concentration which prevented the vasoconstrictor response to noradrenaline. Furthermore, neither hexamethonium (7 μM), atropine (10 μM), mepyramine (10 μM) nor methysergide (10 μM) influenced the response to FpB.

The effect of FpB and noradrenaline on the blood pressure of urethane-anaesthetized rats was also studied. The initial

mean arterial blood pressure (MABP) of these animals was 120 ± 5 mm Hg (n = 12). Bolus intravenous injection of FpB resulted in a dose related increase in MABP with an EC50 of $2{\cdot}5 \pm 0{\cdot}4$ $\mu g\ kg^{-1}$ (n = 8). The maximum increase in MABP ($25{\cdot}6 \pm 1{\cdot}3$ mm Hg, n = 7) was obtained following administration of 5 $\mu g\ kg^{-1}$ FpB. Noradrenaline (0·05–4·0 $\mu g\ kg^{-1}$) was a more potent vasoconstrictor than FpB in the anaesthetized rat. The EC50 value for noradrenaline in this preparation was $0{\cdot}9 \pm 0{\cdot}1$ $\mu g\ kg^{-1}$ (n = 5). However, intravenous injection of a large dose of noradrenaline (2·5 $\mu g\ kg^{-1}$) produced a greater maximal increase in MABP ($61{\cdot}0 \pm 7{\cdot}6$ mm Hg, n = 6) than FpB. Thus, a direct comparison of the potency of FpB and noradrenaline cannot be made. Unlike experiments with the rat isolated perfused kidney, indomethacin (8 mg kg^{-1}) injected intravenously at the start of the experiment and at 30 min intervals thereafter did not influence the potency or maximal effect of FpB or noradrenaline in the anaesthetized rat.

Discussion

FpB contracts the rabbit superfused aorta in-vitro, increases perfusion pressure in the rat isolated kidney ex-vivo and also increases blood pressure in the urethane-anaethetized rat in-vivo. Compared with noradrenaline, FpB was at least one order of magnitude less potent in each case.

Indomethacin pretreatment of the isolated perfused kidney reduced both the direct vasoconstrictor effect of FpB and the ability of this peptide to potentiate vasoconstriction due to noradrenaline, suggesting that part of the mechanism of action of FpB in this preparation involves the release of vasoconstrictor prostanoids. The identity of the prostanoids involved in the renal vasoconstrictor response to FpB has been studied. Numerous prostanoids including PGE_2, prostaglandin endoperoxides (PGG_2 and PGH_2) and thromboxane A_2 are vasoconstrictor in the rat perfused kidney in the experimental conditions employed in this study (Flamembaum & Kleinman 1977; Pace-Asciak & Rosenthale 1982). However, since FpB injection increased renal efflux of PGE_2 but not TxB_2, the most likely mediator of the vasoconstrictor effect of this peptide in the kidney would appear to be PGE_2. It should be made clear that the present data do not exclude the involvement of prostaglandin endoperoxides. The manner in which FpB promotes prostanoid biosynthesis in the rat perfused kidney is not known. Since FpB does not affect seminal vesicle cyclo-oxygenase activity (Hussaini & Moore, unpublished) it seems likely that this peptide stimulates membrane phospholipase enzyme activity thereby liberating arachidonic acid for conversion into PGE_2 and/or prostaglandin endoperoxides.

However, a considerable part of the vasoconstrictor reponse to FpB, in the kidney as well as the contractile effect of this peptide in the rabbit aorta and its pressor effect in the anaesthetized rat presumably results from a mechanism which is independent of arachidonic acid metabolites. This conclusion is based upon the fact that indomethacin pretreatment, although abolishing the increase in PGE_2 release by FpB in the perfused kidney produced only a small inhibition (<25%) of the associated vasoconstriction. Furthermore, indomethacin pretreatment did not affect the vasopressor activity of FpB in the intact rat or the contractile effect of this peptide on the rabbit superfused aorta. It may be suggested that the dose of indomethacin administered to anaesthetized rats was insufficient to inhibit prostanoid biosynthesis in the whole animal. Although we did not investigate directly, we believe that the dose of indomethacin used in these experiments is likely to result in substantial inhibition of prostanoid biosynthesis. Mallarkey & Smith (1985), for example, have previously reported that indomethacin, at the dose and route of administration used by us, reduced the concentration of 6-oxo-prostaglandin $F_1\alpha$ and TxB_2 in rat plasma to levels below the limit of detection of the radioimmunoassay procedures employed (i.e. 200 pg mL^{-1} and 300 pg mL^{-1}, respectively).

The nature of the prostanoid-independent component of the vasoconstrictor effect of FpB has yet to be elucidated. Clearly the mechanism of action does not involve the release of noradrenaline, acetylcholine, histamine or 5-hydroxytryptamine since phentolamine, atropine, hexamethonium, mepyramine and methysergide did not antagonize the effect of FpB in any of the preparations studied.

Whatever the precise mechanism of action the vasoconstrictor effect of FpB may be important for the control of blood vessel calibre at the site of thrombus formation. Although the basal concentration of fibrinopeptides (both FpA and FpB) is low in plasma of healthy human individuals (approximately 0·33 nmol L^{-1} according to Nossel et al 1974) the concentration of these peptides increases dramatically in patients suffering from thrombotic disease of one form or another. For example, FpA concentrations as high as 650 nmol L^{-1} occur in blood collected after the sudden death of patients suffering from ischaemic heart disease (Meade et al 1984). In the present experiments the threshold dose of FpB required to contract the rabbit aorta and increase renal perfusion pressure in the rat was approximately 1 nmol. Additionally, the half life of FpB in the circulation is only 3–5 min suggesting that this peptide is likely to be cleared very quickly from the site of formation presumably by proteolytic enzymes. Clearly, the concentration of fibrinopeptides produced locally at the site of thrombus formation, which cannot be measured directly, may be considerably greater than the concentration detected in withdrawn blood.

These results highlight an interesting and hitherto largely unknown pharmacological effect of FpB. It remains to be seen whether FpB has a physiological role to play in constricting blood vessels in the vicinity of a developing thrombus.

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