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Characteristics of vasodilatation induced by acetylcholine and platelet-activating factor in the rat mesenteric arterial bed

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Abstract

We examined the nature of the endothelium-dependent vasodilator effects of acetylcholine and platelet-activating factor (PAF) on the perfused mesenteric arterial bed of the rat. Acetylcholine-induced concentration-dependent vasodilatation of the mesentery was not affected by pretreatment with 10^{-4} M N^G-monomethyl-L-arginine (L-NMMA), indomethacin, ouabain, or glibenclamide, whereas pretreatment with 10^{-5} M oxyhemoglobin, 10^{-5} M methylene blue, or 10 mM tetraethylammonium shifted the concentration-response curves to the right. PAF-induced concentration-dependent vasodilatation of the mesentery was inhibited by pretreatment with L-NMMA, oxyhemoglobin, or methylene blue, and slightly but significantly inhibited by tetraethylammonium, whereas indomethacin, glibenclamide, and ouabain had no inhibitory effects. PAF-induced vasodilatation of the mesentery was more sensitive to nitric oxide-cyclic GMP pathway inhibitors (a combined application of L-NMMA, oxyhemoglobin, and methylene blue) than was the vasodilatation induced by acetylcholine. Perfusion of the mesentery preparations with acetylcholine or PAF increased the levels of cyclic GMP in the effluent. These effects were completely inhibited by L-NMMA or oxyhemoglobin. These results suggest that the endothelium-dependent vasodilator effects of PAF are primarily mediated by endothelium-derived nitric oxide (NO) and those of acetylcholine are mediated by both NO and endothelium-derived hyperpolarizing factor (EDHF).

Keywords: Acetylcholine; PAF (platelet-activating factor); Nitric oxide (NO); EDHF (endothelium-derived hyperpolarizing factor)

1. Introduction

Platelet-activating factor (PAF, acetyl glyceryl ether phosphorylcholine) is a unique lipid mediator released by stimulated leukocytes, platelets, macrophages, and vascular endothelial cells of various species (Shaw et al., 1981; Hartung, 1983; Camussi et al., 1983). The systemic administration of PAF induces strong and long-lasting hypotension (Lai et al., 1983). One antagonist of PAF, CV-3988 ((*R*,*S*)-2-methoxy-3-(octadecylcarbomoyloxy)propyl-2-(3-thiazolio)ethyl phosphate), raises the blood pressure in spontaneously hypertensive rats (Masugi et al., 1985) and in cases of nephrectomy-induced hypertension (Murihead, 1980). A recent report indicates that changes in circulating PAF correlate significantly and positively with changes in mean arterial blood pressure (Sakaguchi et al., 1991), and the vasodilator effects of PAF on the mesenteric arterial bed are significantly attenuated in spontaneously hypertensive rats (Kamata et al., 1994). We also have reported that PAF is one of the most potent naturally occurring endothelium-dependent vasodilators and has been shown to cause vasodilatation of the mesenteric arterial bed at extremely low concentrations (Kamata et al., 1989). In view of these studies, there is substantial evidence to suggest that PAF may have a physiological role in the regulation of vascular tone and blood flow by an endothelium-dependent mechanism.

It has been well documented that acetylcholine causes endothelium-dependent relaxation by releasing endothelium-derived relaxing factor (EDRF) in various blood vessels (Furchgott, 1984). Evidence that nitric oxide (NO) is a candidate for EDRF (Palmer et al., 1987) and is formed from L-arginine has been presented by several investigators (Palmer et al., 1988; Moncada et al., 1989). There are data suggesting the existence of another EDRF that evokes hyperpolarization of smooth muscle and which has been called endothelium-dependent hyperpolarizing factor (EDHF) (Bolton et al., 1984; Komori and Suzuki, 1987; Feletou and Vanhoutte, 1988; Dohi et al., 1990; Garland and McPherson, 1992; Fujii et al., 1992; Waldron and

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Garland, 1994; Schulz and Triggle, 1994; Plane et al., 1995). Moreover, we recently showed that there are differences between the degree of vasodilatation and the changes in cyclic GMP levels in response to various concentrations of acetylcholine in the mesenteric arterial bed (Abiru et al., 1993). It appears, therefore, that endothelium-dependent vasodilatation in response to vasoactive agents may be involved in the release of EDHF as well as NO in the mesenteric arterial bed of the rat. In the present study, we examined the involvement of NO and EDHF in acetylcholine- and PAF-induced vasodilatation of the perfused mesenteric arterial bed of the rat. Furthermore, we were especially interested in determining which of the endothelium-dependent factors is primarily responsible for the acetylcholine- and PAF-induced vasodilatation of the mesentery.

2. Materials and methods

2.1. Preparation of the mesenteric arterial bed

Male Wistar rats, weighing from 250 to 350 g, were anesthetized with ether and then given an intravenous injection of 1000 units/kg of heparin. Following the injection, a midline incision was made, and the mesenteric arterial bed was rapidly dissected and placed into ice-cold modified Krebs-Henseleit solution (KHS) consisting of 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 11.0 mM dextrose. The mesenteric artery and vein were tied off near the caecum, and the remaining intestine was separated from the arterial bed along the intestinal wall. The mesenteric arterial bed was then perfused as described by McGregor (1965), with various modifications by us (Kamata et al., 1989; Abiru et al., 1993). Briefly, warm (37°C), oxygenated (95% O_2-5% CO_2) KHS was pumped into the mesenteric arterial bed, using a peristaltic pump operating at a rate of 5 ml/min, through a cannula inserted into the superior mesenteric artery. Vascular responses were detected as changes in perfusion pressure, which was monitored continuously with a pressure transducer (Nihon Kohden, Model AP2001, Tokyo, Japan) and recorded on a pen recorder (Yokogawa, Model 3021, Tokyo, Japan). Following a 60-min equilibration period, the perfusion circuit was transformed into a closed system by collecting the perfusion solution in a second bath and recirculating it through the mesenteric arterial bed. The total volume of the closed system was 50 ml, and agents were administered via the bath. After equilibration, the mesentery preparation was contracted by perfusion of a solution containing 5×10^{-6} M to 4×10^{-5} M methoxamine, which resulted in a perfusion pressure of approximately 113–129 mmHg, and was then maximally relaxed with a perfusion solution containing 10^{-6} M acetylcholine, to confirm the integrity of the endothelium. Acetylcholine- and PAF-induced relaxations were expressed as a percentage of the increase in perfusion pressure induced by methoxamine (5×10^{-6} M to 4×10^{-5}M). After contraction was induced with methoxamine, each agent was administered cumulatively. Each preparation received a different agent, and the relaxation response was determined.

2.2. Measurement of cyclic GMP

The content of cyclic GMP in the perfusate was assayed as previously described by Abiru et al. (1993). In the present study, the mesentery was perfused with KHS containing 3-isobutyl-methylxanthine (IBMX, 10^{-4} M) to inhibit phosphodiesterase activity. For the cyclic GMP determinations, the perfusate was collected over a 30 s period during which the vasodilator response was stabilized. Since the maximal release of cyclic GMP into the perfusate in response to acetylcholine or PAF occurred after 5 min, samples were collected between 5 and 5.5 min. The samples were stored at −20°C. The concentration of cyclic GMP was determined by radioimmunoassay, using com-

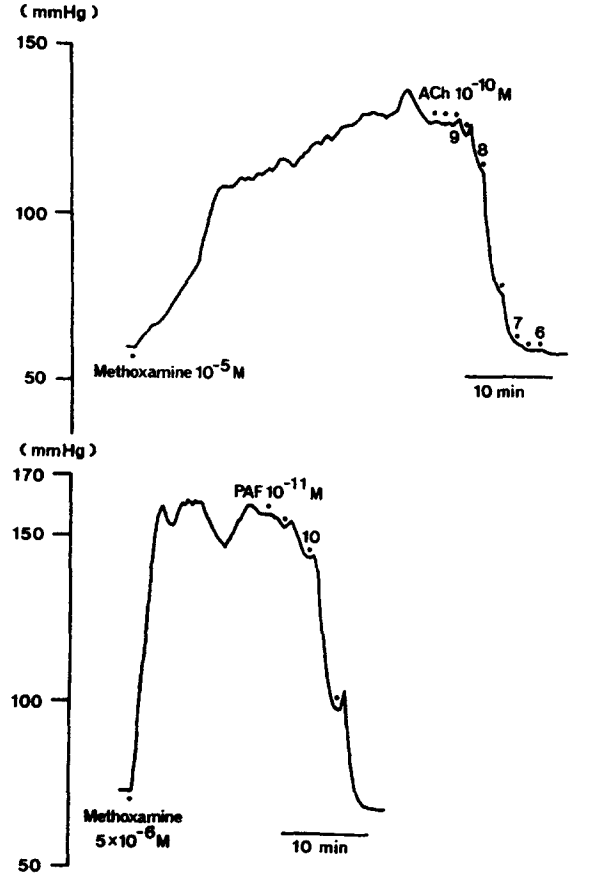

Fig. 1. Typical records showing the effects of increasing concentration of acetylcholine (10^{-10}–10^{-6} M, upper panel) and PAF (10^{-11}–3×10^{-10} M, lower panel) on perfusion pressure of the mesenteric arterial bed precontracted with methoxamine (5×10^{-6}–2×10^{-5} M). The dots indicate when drug was added. The single numerals paired with dots indicate increasing concentrations of drug, e.g., 9 refers to 10^{-9} M.

mercially available kits (Yamasa Cyclic GMP Assay Kit, Yamasa Corp., Choshi, Japan). The release of cyclic GMP induced by vasodilators is expressed as the change from the basal level.

2.3. Chemical agents

Methoxamine, N^G-monomethyl-L-arginine, methylene blue, hemoglobin, indomethacin, ouabain, tetraethylammonium, glibenclamide, and 3-isobutyl-1-methylxanthine were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetylcholine chloride was purchased from Daiichi (Tokyo, Japan). PAF (1-*O*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) was purchased from Bachem (Switzerland). PAF was dissolved in ethanol and stored at −20°C. On the day of use, the ethanol was evaporated under a stream of nitrogen gas and the vasodilator was dissolved in KHS containing 0.25% bovine serum albumin (Fraction V, Sigma). Sonication for 30 s ensured that the agents were completely dissolved. A stock solution of oxyhemoglobin was prepared as previously reported (Martin et al., 1985).

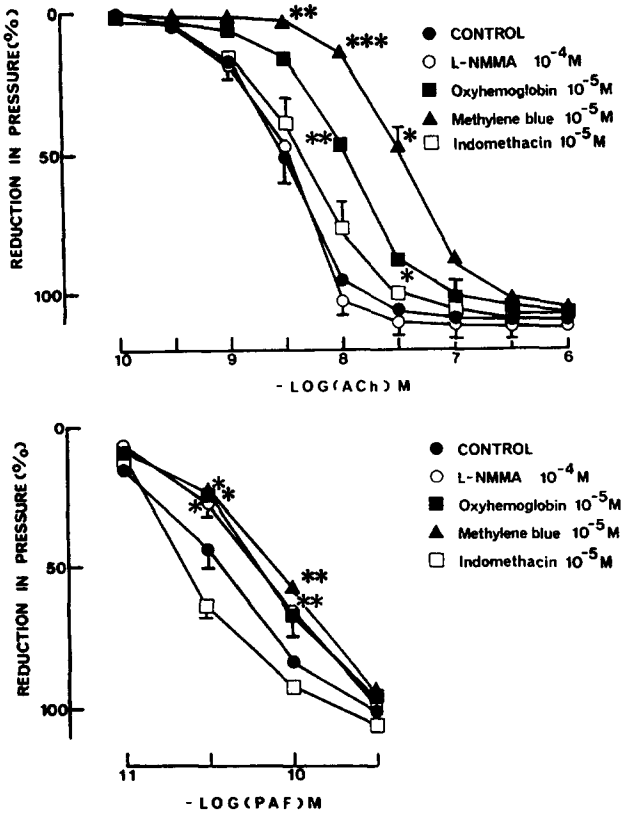

Fig. 2. Concentration-response curves for the vasodilatation induced by acetylcholine (upper panel) and PAF (lower panel) in the methoxamine-precontracted mesenteric arterial bed. The mesenteric arterial beds were precontracted with methoxamine (5×10^{-6}–4×10^{-5} M), then incubated for 15 min with either 10^{-4} M L-NMMA, 10^{-5} M oxyhemoglobin, 10^{-5} M methylene blue, or 10^{-5} M indomethacin. Acetylcholine or PAF was added to the mesenteric perfusion bath cumulatively. The amount of vasodilatation induced is expressed as a percentage of the methoxamine-induced increase in perfusion pressure. Each point is the mean of four to six determinations, and vertical bars represent the S.E. from four to six determinations. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

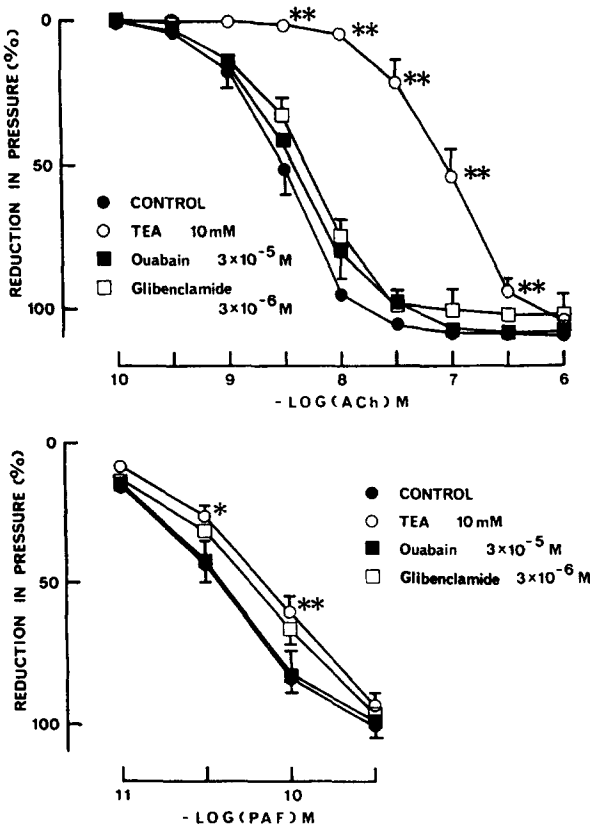

Fig. 3. Concentration-response curves for the vasodilatation induced by acetylcholine (upper panel) and PAF (lower panel) in the methoxamine-precontracted mesenteric arterial beds. The mesenteric arterial beds were precontracted with methoxamine (5×10^{-6}–4×10^{-5} M), then incubated for 15 min with either 10 mM tetraethylammonium, 3×10^{-5} M ouabain, or 3×10^{-6} M glibenclamide. Acetylcholine or PAF was added to the mesentery perfusion bath cumulatively. Each point is the mean of four to six determinations and vertical bars represent the S.E. from four to six determinations. * $P < 0.05$; ** $P < 0.01$.

2.4. Statistical analysis

Data are expressed as the means ± S.E.M. Statistical differences were measured using the Student's *t*-test for unpaired observations, following a one-way analysis of variance. The level of significance was $P < 0.05$. The EC_{50} values were determined using the method of Fleming et al. (1972).

3. Results

3.1. Relaxation of the mesenteric arterial bed by acetylcholine and PAF

The basal perfusion pressure of the rat mesenteric arterial bed was 63.3 ± 1.7 mmHg ($n = 20$), and perfusion

with methoxamine (5×10^{-6} to 4×10^{-5} M) increased the perfusion pressure to 120.1 ± 2.1 mm Hg ($n = 20$). In perfused mesenteric arterial beds precontracted with methoxamine, infusion of cumulative concentrations of either acetylcholine (10^{-10}–10^{-6} M) or PAF (10^{-11}–3×10^{-10} M) caused concentration-dependent vasodilatation (Fig. 1). Relaxation of the mesentery was induced at lower concentrations of PAF than of acetylcholine. The maximal acetylcholine-induced relaxation was 109.4% (EC_{50} for acetylcholine: $2.9 \pm 0.4 \times 10^{-9}$ M, $n = 6$) while the maximal PAF-induced relaxation was 100.7% (EC_{50} for PAF: $3.6 \pm 0.5 \times 10^{-11}$ M, $n = 10$).

3.2. Effects of NO-cyclic GMP pathway inhibitors on acetylcholine- and PAF-induced vasodilatation

The effects of L-NMMA, oxyhemoglobin, methylene blue, and indomethacin on the vasodilatation induced by acetylcholine and PAF were examined (Fig. 2). None of

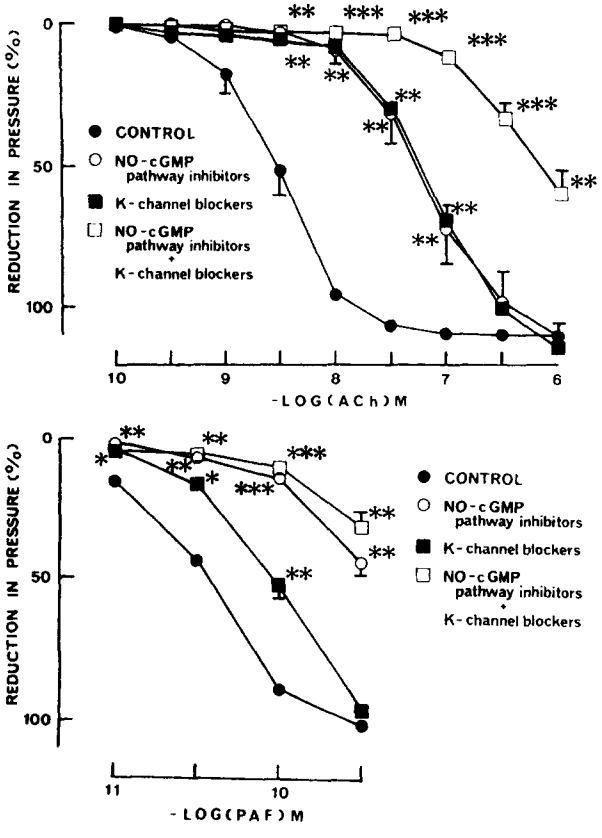

Fig. 4. Concentration-response curves for the relaxation induced by acetylcholine (upper panel) and PAF (lower panel) in the methoxamine-precontracted mesenteric arterial beds. The mesenteric arterial beds were precontracted with methoxamine (5×10^{-6}–4×10^{-5} M), then incubated for 15 min with either NO-cyclic GMP pathway inhibitors (10^{-4} M L-NMMA, 10^{-5} M oxyhemoglobin and 10^{-5} M methylene blue), K^+-channel blockers (10 mM tetraethylammonium, 3×10^{-5} M ouabain, and 3×10^{-6} M glibenclamide) or NO-cyclic GMP pathway inhibitors plus K^+-channel blockers. Acetylcholine or PAF was added to the mesentery perfusion bath cumulatively. Each point is the mean of four to six determinations and vertical bars represent the S.E. from four to six determinations. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

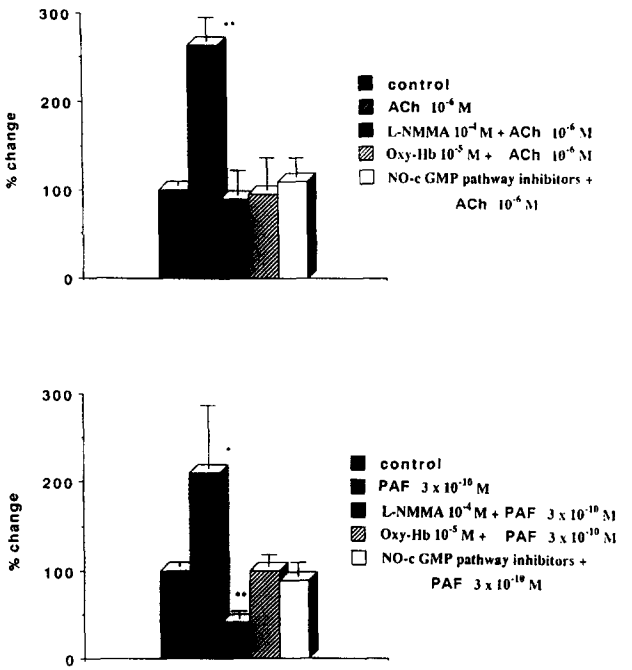

Fig. 5. Effects of 10^{-4} M L-NMMA, 10^{-5} M oxyhemoglobin, and NO-cyclic GMP pathway inhibitors (10^{-4} M L-NMMA, 10^{-5} M oxyhemoglobin, and 10^{-5} M methylene blue) on the increase in cyclic GMP production induced by acetylcholine (upper panel) and PAF (lower panel). Changes in cyclic GMP levels in the effluents are expressed as a percentage of the control level. Each point is the mean of four determinations and vertical bars represent the S.E. from those determinations. * $P < 0.05$; ** $P < 0.01$.

these agents affected the basal perfusion pressure. Preincubation with L-NMMA (10^{-4} M) had no significant effect on the acetylcholine-induced relaxation (EC_{50} for acetylcholine: $4.1 \pm 1.9 \times 10^{-9}$ M, $n = 4$). Indomethacin (10^{-5} M) also had no effect on the relaxation induced by acetylcholine (EC_{50} for acetylcholine: $4.1 \pm 1.3 \times 10^{-9}$ M, $n = 4$). However, acetylcholine-induced vasodilatation of the mesentery were significantly inhibited by 10^{-5} M oxyhemoglobin (EC_{50} for acetylcholine: $4.1 \pm 1.2 \times 10^{-8}$ M, $n = 4$) and 10^{-5} M methylene blue (EC_{50} for acetylcholine: $3.1 \pm 3.3 \times 10^{-8}$ M, $n = 5$).

Preincubation with L-NMMA (10^{-4} M) significantly inhibited PAF-induced vasodilatation of the mesenteric arterial bed (EC_{50} for PAF: $6.2 \pm 1.3 \times 10^{-11}$ M, $n = 7$). Similarly, PAF-induced vasodilatation was attenuated by 10^{-5} M oxyhemoglobin (EC_{50} for PAF: $7.4 \pm 0.4 \times 10^{-11}$ M, $n = 6$) and 10^{-5} M methylene blue (EC_{50} for PAF: $7.5 \pm 0.8 \times 10^{-11}$ M, $n = 5$). Indomethacin (10^{-5} M) did not affect the PAF-induced relaxation (EC_{50} for PAF: $2.8 \pm 0.5 \times 10^{-11}$ M, $n = 4$).

3.3. Effects of K^+ channel-blocking agents on acetylcholine- and PAF-induced vasodilatation

The effects of tetraethylammonium, ouabain, and glibenclamide on vasodilatation induced by acetylcholine and PAF were examined (Fig. 3). None of these agents

affected the basal perfusion pressure of the mesenteric arterial bed. In the presence of 10 mM tetraethylammonium, the acetylcholine-induced relaxation was significantly attenuated (EC_{50} for acetylcholine: $4.5 \pm 0.4 \times 10^{-8}$ M, $n = 4$). The vasodilatation induced by acetylcholine were not affected by 3×10^{-6} M glibenclamide (EC_{50} for acetylcholine: $5.3 \pm 1.0 \times 10^{-9}$ M, $n = 4$) or 3×10^{-5} M ouabain ($4.1 \pm 1.4 \times 10^{-9}$ M, $n = 4$).

Tetraethylammonium (10 mM) slightly but significantly inhibited PAF-induced vasodilatation (EC_{50} for PAF: $6.5 \pm 0.8 \times 10^{-11}$ M, $n = 7$). The inhibitory effects of tetraethylammonium on PAF-induced vasodilatation were significantly less than those on acetylcholine-induced vasodilatation. PAF-induced vasodilatation of the mesentery was not inhibited by 3×10^{-6} M glibenclamide (EC_{50} for PAF: $5.9 \pm 1.2 \times 10^{-11}$ M, $n = 4$), or 3×10^{-5} M ouabain (EC_{50} for PAF: $3.4 \pm 0.8 \times 10^{-11}$ M, $n = 4$).

3.4. Effects of the combined application of NO-cyclic GMP pathway inhibitors and K^+ channel-blocking agents on acetylcholine- and PAF-induced vasodilatation

The effects of the combined application of NO-cyclic GMP pathway inhibitors and/or K^+ channel-blocking agents were examined (Fig. 4). The combined application of three NO-cyclic GMP pathway inhibitors (10^{-4} M L-NMMA, 10^{-5} M methylene blue, and 10^{-5} M oxyhemoglobin) greatly inhibited the relaxation induced by acetylcholine (EC_{50} for acetylcholine: $4.6 \pm 8.9 \times 10^{-8}$ M, $n = 4$) but did not affect the maximal relaxation. The combined application of three K^+ channel-blocking agents (10 mM tetraethylammonium, 3×10^{-5} M ouabain and 3×10^{-6} M glibenclamide) significantly attenuated the relaxation induced by acetylcholine (EC_{50} for acetylcholine: $6.3 \pm 0.5 \times 10^{-8}$ M, $n = 4$). The simultaneous application of the three NO-cyclic GMP pathway inhibitors and the three K^+ channel blockers significantly reduced the mesenteric arterial bed vasodilatation induced by acetylcholine. The combined application of the three NO-cyclic GMP pathway inhibitors markedly inhibited the relaxation induced by PAF, and in contrast to the case of acetylcholine, significantly decreased the maximal relaxation. The combined application of the three K^+ channel blockers significantly attenuated the PAF-induced vasodilatation of the mesentery (EC_{50} for PAF: $9.7 \pm 0.9 \times 10^{-11}$ M, $n = 4$). The simultaneous application of these six agents inhibited PAF-induced vasodilatation only slightly more than did the treatment with NO-cyclic GMP pathway inhibitors alone.

3.5. Effects of acetylcholine and PAF on cyclic GMP levels in the perfusate

The basal level of cyclic GMP in effluents collected from the mesentery was 39.8 ± 1.9 fmol/ml. The production of cyclic GMP increased by 163.4% ($n = 4$) in response to 10^{-6} M acetylcholine and by 110% ($n = 4$) in response to 3×10^{-10} M PAF (Fig. 5). Treatment of the mesenteric arterial bed for 20 min with L-NMMA (10^{-4} M) or oxyhemoglobin (10^{-5} M) significantly decreased both the acetylcholine- and PAF-induced increase in cyclic GMP production. Incubation of the mesenteric arterial bed for 20 min with three NO-cyclic GMP pathway inhibitors (10^{-4} M L-NMMA, 10^{-5} M oxyhemoglobin, and 10^{-5} M methylene blue) also inhibited the increase of cyclic GMP levels induced by both acetylcholine and PAF.

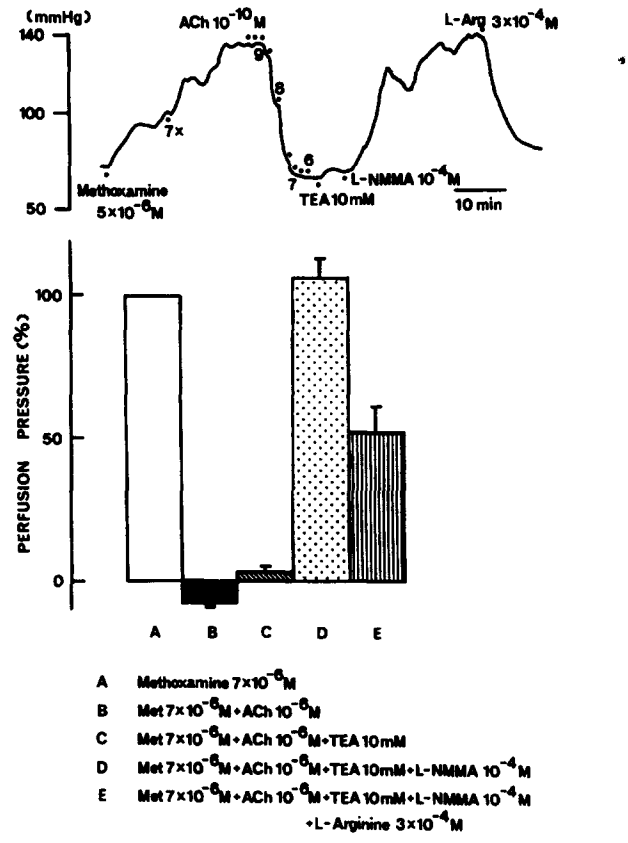

Fig. 6. Typical record of the effects of 10 mM tetraethylammonium, 10^{-4} M L-NMMA, and 3×10^{-4} M L-arginine on acetylcholine-induced vasodilatation of the mesenteric arterial bed precontracted with 7×10^{-6} M methoxamine (Met; upper panel). When acetylcholine-induced concentration-dependent relaxation was maximal, tetraethylammonium, then L-NMMA, then L-arginine were added to the mesentery perfusion bath. These results are summarized in the lower panel. Each point is the mean of four to six determinations and vertical bars represent the S.E. from four to six determinations.

3.6. Reversal of acetylcholine- and PAF-induced vasodilatation by L-NMMA

Treatment with 10 mM tetraethylammonium slightly inhibited acetylcholine- and PAF-induced vasodilatation of the mesenteric arterial bed. However, further treatment with 10^{-4} M L-NMMA greatly inhibited both acetylcholine-induced vasodilatation (Fig. 6) and PAF-induced

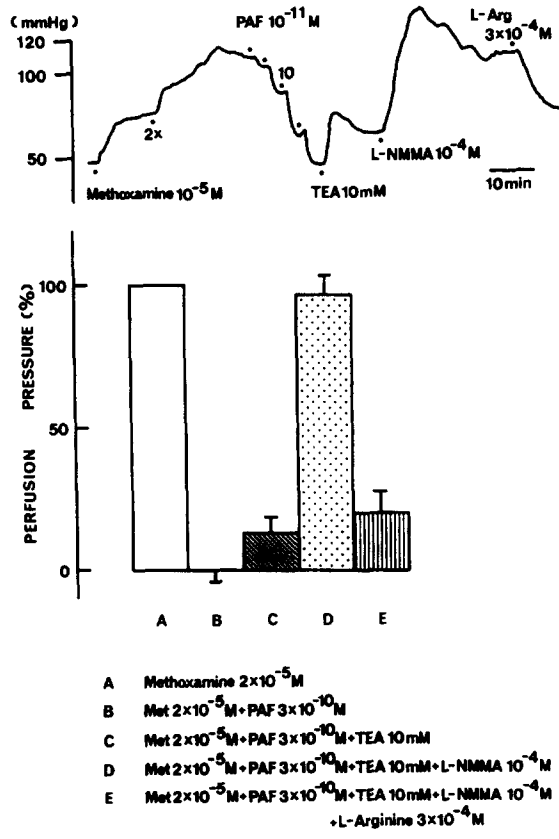

Fig. 7. Typical record of the effects of 10 mM tetraethylammonium, 10^{-4} M L-NMMA, and 3×10^{-4} M L-arginine on PAF-induced vasodilatation of the mesenteric arterial bed precontracted with 2×10^{-5} M methoxamine (upper panel). When the PAF-induced concentration-dependent relaxation was maximal, tetraethylammonium, L-NMMA, then L-arginine were added to the mesentery perfusion bath. These results are summarized in the lower panel. Each point is the mean of four to six determinations and vertical bars represent the S.E. from four to six determinations.

vasodilatation (Fig. 7). These inhibitors were both counteracted by treatment with 3×10^{-4} M L-arginine.

4. Discussion

The main conclusion from the present study is that the contributions of NO and EDHF to acetylcholine-induced vasodilatation of the mesenteric arterial bed are nearly equal, while the contribution of NO to PAF-induced vasodilatation is greater than that of EDHF.

It has been well demonstrated that in many blood vessels, the NO synthesized from L-arginine has strong vasodilator effects accompanied by the accumulation of cyclic GMP (Furchgott and Zawadzki, 1980; Rapoport and Murad, 1983; Ignarro et al., 1987; Moncada et al., 1991). In the present study, acetylcholine- and PAF-induced vasodilatation were decreased by treatment with oxyhemoglobin, which is a scavenger of nitric oxide (NO), and methylene blue, which is an inhibitor of soluble guanylate cyclase. Pretreatment with L-NMMA, which is a competitive inhibitor of NO synthase (Palmer and Moncada, 1989; Mayer et al., 1989), inhibited the vasodilatation induced by PAF in the mesenteric arterial bed. Pretreatment with L-NMMA had no effect on acetylcholine-induced vasodilatation. Although the combined application of three NO-cyclic GMP pathway inhibitors (L-NMMA, oxyhemoglobin, and methylene blue) significantly reduced the relaxation responses induced by both PAF and acetylcholine, the combined effects of these inhibitors on PAF-induced relaxation were more potent than their combined effects on acetylcholine-induced relaxation. These results strongly suggest that the NO-cyclic GMP pathway may be involved in the vasodilatation responses of the mesenteric arterial bed to both acetylcholine and PAF, but there may be differences in the relaxation mechanisms of PAF and acetylcholine. It is likely that while the vasodilatation induced by PAF may be primarily mediated by the NO-cyclic GMP pathway, not only NO but also EDHF may have important roles in the acetylcholine-induced vasodilatation of the mesentery.

Several investigators have reported that endothelium-dependent vasorelaxants, such as acetylcholine, release EDHF from the endothelium, which induces vasorelaxation via membrane hyperpolarization (Bolton et al., 1984; Komori and Suzuki, 1987; Feletou and Vanhoutte, 1988; Dohi et al., 1990; Garland and McPherson, 1992; Schulz and Triggle, 1994; Plane et al., 1995). Moreover, we showed recently that the degree of vasodilatation is greater than the change in cyclic GMP levels, in response to various concentrations of acetylcholine in the mesenteric arterial bed (Abiru et al., 1993). It appears, therefore, that acetylcholine-induced vasodilatation may be mediated by the release of NO and also by hyperpolarization of the mesenteric arterial bed. Although the chemical nature of EDHF has not been defined, it has been suggested that EDHF relaxes vascular smooth muscle cells through hyperpolarization via opening of K^+ channels. In the present study, therefore, we used potent K^+ channel-blocking agents to determine if EDHF is involved in acetylcholine- and PAF-induced vasodilatation of the mesentery. Glibenclamide, an ATP-sensitive K^+ channel blocker, and ouabain, a Na^+, K^+-ATPase inhibitor, had no significant effect on the relaxations induced by either PAF or acetylcholine. In preliminary experiments, apamin, an inhibitor of Ca^{2+}-dependent K^+ channels, also had no effects on either PAF- or acetylcholine-induced vasodilatation (data not shown). However, tetraethylammonium, a non-selective K^+-channel blocker, markedly reduced the acetylcholine-induced vasodilatation and slightly reduced the PAF-induced vasodilatation. Thus, it appears that acetylcholine may open K^+ channels, which is tetraethylammonium-sensitive, and thus may hyperpolarize the mesentery. Although tetraethylammonium has a non-selective action on K^+ channels, the release of EDHF in the mesentery in response to acetylcholine may not be due to

the increased activity of Ca^{2+} dependent K^+ channels or ATP-sensitive K^+ channels.

Our results contradict other studies with regard to the involvement of ATP-sensitive K^+ channels in endothelium-dependent hyperpolarization. In rabbit arteries, the hyperpolarization in response to acetylcholine was blocked by glibenclamide (Standen et al., 1989; Brayden, 1990), whereas in the present study the responses in the mesenteric arterial bed were resistant to glibenclamide. A recent study involving the rat small mesenteric artery (Brayden, 1990; McPherson and Angus, 1991) also showed that glibenclamide did not affect acetylcholine-induced hyperpolarization, even though glibenclamide-sensitive K^+ channels are present in this blood vessel.

The present investigation showed that both acetylcholine and PAF increased the level of cyclic GMP in the effluents from the mesenteric arterial bed. The increased production of cyclic GMP in response to acetylcholine and PAF was inhibited by treatment with L-NMMA, oxyhemoglobin, and three NO-cyclic GMP pathway inhibitors combined (L-NMMA, oxyhemoglobin, and methylene blue). Although pretreatment with these agents completely inhibited the acetylcholine-induced increase in the level of cyclic GMP in the effluents, the vasodilatation induced by acetylcholine was not completely inhibited, indicating that EDHF may be important for the acetylcholine-induced vasodilatation of the mesentery. This conclusion is supported by the finding that the acetylcholine-induced vasodilatation was effectively antagonized by pretreatment with tetraethylammonium. While pretreatment with NO-cyclic GMP pathway inhibitors completely inhibited the PAF-induced increase in the level of cyclic GMP in the effluents, the vasodilatation induced by PAF was also markedly but not completely inhibited by these agents, indicating that NO is more important for the PAF-induced vasodilatation of the mesentery than is EDHF.

Pretreatment with L-NMMA had no effect on the acetylcholine-induced vasodilatation, whereas the acetylcholine-induced vasodilatation was completely reversed by L-NMMA. L-NMMA is reported to depress vascular responses non-specifically by some mechanism other than NO synthase inhibition (Thomas et al., 1989). This was not the case in the present study, however, because the reversal of acetylcholine-induced relaxation by L-NMMA was counteracted by L-arginine. Similar effects of L-NMMA were observed on the PAF-induced vasodilatation. Thus, we propose that NO synthase stimulated by agonists may be more sensitive to L-NMMA than resting NO synthase.

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