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Mechanisms of desensitization of vasodilatation induced by platelet-activating factor in hypertensive rats

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Abstract

We found that vasodilator effects of platelet-activating factor (PAF) on the mesenteric arterial bed of the rat were significantly attenuated in spontaneously hypertensive rats (SHR) and renal hypertensive rats (RHR). Perfusion of the mesentery with acetylcholine and PAF caused endothelium-dependent vasodilatation accompanied by an increase in cyclic GMP levels in the mesentery from normotensive Wistar Kyoto rats (WKY). Acetylcholine caused a significant increase in cyclic GMP levels in the effluent in both SHR and RHR, whereas PAF could not increase cyclic GMP levels in SHR and slightly increased cyclic GMP in RHR. Incubating the mesentery with PAF markedly inhibited the vasodilatation induced by PAF, but not acetylcholine or sodium nitroprusside. The cyclic GMP accumulation in the effluent was impaired in the mesenteric arterial bed pretreated with PAF and in that obtained from rats given islet-activating protein (IAP). The PAF-induced vasodilatation was completely reversed by the PAF receptor antagonist, CV-6209 (2-[*N*-acetyl-*N*-(2-methyl-3-octadecylcarbamoyl-oxypropoxycarbonyl)aminomethyl]-1-ethylpyridinium chloride). These results suggest that (1) attenuated vasodilator effects of PAF and decreased cyclic GMP levels in the mesentery from SHR and RHR are due to desensitization but not to impairment of the endothelium; (2) GTP-binding protein, which is IAP-sensitive, may be involved in PAF-induced vasodilatation and cyclic GMP accumulation; (3) desensitization of the mesentery to PAF in SHR and RHR may be due to PAF receptor and GTP-binding protein uncoupling.

Keywords: PAF (platelet-activating factor); Desensitization; Spontaneously hypertensive rat (SHR); Renal hypertensive rat (RHR); GTP-binding protein

1. Introduction

Platelet-activating factor (PAF) is one of the potent mediators produced by stimulated leukocytes, platelets, macrophages and endothelium of various species. The systemic administration of PAF induces powerful and long-lasting hypotension (Lai et al., 1983). The PAF receptor antagonist, CV-3988, raises the blood pressure in spontaneously hypertensive rats (SHR) when administered systemically (Masugi et al., 1985), and in nephrectomy-induced hypertension (Murihead, 1980). The rapid fall in blood pressure seen after unclipping the renal clip hypertensive rats is associated with an increased level of PAF in the blood (McGowan et al., 1988). Changes in circulating PAF correlate significantly and positively with changes in mean arterial blood pressure (Sakaguchi et al., 1991). PAF is one of the most potent naturally occurring endothelium-dependent vasodilators and it causes vasodilatation of the mesenteric arterial bed at extremely low concentrations (Kamata et al., 1989). Recently, we further reported that the endothelium-dependent vasodilator effects of PAF are primarily mediated by endothelium-derived nitric oxide (Kamata et al., 1996). We also reported that the vasodilator effects of PAF on the mesenteric arterial bed are significantly attenuated in SHR (Kamata et al., 1994). In view of these findings, there is substantial evidence to suggest that PAF plays a physiological role in the regulation of vascular tone and blood flow through an endothelium-dependent mechanism. We developed a novel method for simultaneously measuring vasodilatation and changes in the cyclic GMP levels released from the rat mesenteric arterial bed (Abiru et al., 1993). This procedure provides a variety of information about the function of the endothelium in resistance vessels.

The purpose of the present study was to clarify the

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mechanisms by which PAF alters endothelium-dependent vasodilatation in SHR and renal hypertensive rats (RHR). We investigated the PAF-induced vasodilatation of the mesentery and changes in cyclic GMP levels in SHR, RHR, in rats given PAF and treated with islet-activating protein (IAP), using our new method.

2. Materials and methods

2.1. Preparation of the mesenteric arterial bed

Male Wistar rats, SHR, Wistar Kyoto rats (WKY) were purchased from Sankyo Labo Service (Tokyo, Japan). Blood pressure in the unanesthetized animals was measured using a tail-cuff method before the experiment began. The rats were anesthetized with ether, then given an i.v. injection of 1000 units/kg of heparin. The rats were killed by decapitation, then the mesenteric arterial bed was rapidly dissected through a midline incision and placed in 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 11.9 mM dextrose. This study was conducted in accordance with the Guide for Care and Use of Laboratory Animals adopted by the Committee on Care and Use of Laboratory Animals of Hoshi University which is accredited by the Ministry of Education, Science and Culture, Japan. The mesenteric artery and vein were tied off close to 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 (Kamata et al., 1989; Abiru et al., 1993). Briefly, the mesenteric arterial bed was perfused with warm (37°C) and oxygenated (95% O_2-5% CO_2) KHS by means of a peristaltic pump (SJ-1220, Atto, Japan) at a rate of 5 ml/min via a cannula inserted into the superior mesenteric artery. Vascular responses were shown 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. The perfusate was collected in a second bath and re-circulated into 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 with a solution containing 5×10^{-6} M to 4×10^{-5} M methoxamine, which resulted in a perfusion pressure of about 113–129 mm Hg. It was then relaxed by perfusion with a solution containing 10^{-6} M acetylcholine, to confirm the integrity of the endothelium. The mesenteric arterial bed was completely relaxed after exposure to 10^{-6} M acetylcholine. Drug-induced relaxation was 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, all drugs were administered cumulatively. Each preparation received a different drug, and the relaxation response was determined.

2.2. Preparation of the renal hypertensive rat

To induce two-kidney, one-clip (2K1C) hypertension, male Wistar rats (7-weeks-old) were anesthetized with sodium pentobarbital (30 mg/kg, i.p.). After an incision of the left flank, the left renal artery was separated from the renal vein and surrounding tissue. A U-shaped solid silver clip with an opening of 0.1 mm was applied to the exposed renal artery, while the right renal artery was left intact. The systolic blood pressure was measured by the tail-cuff method in the unanesthetized animals before experiments.

2.3. Treatment with islet-activating protein (IAP)

Wistar rats were treated with IAP for 3 days prior to experiments. The rats were anesthetized with an i.p. injection of sodium pentobarbital (50 mg/kg) and were then injected intravenously with 10 μg/kg IAP in 0.0125 M Tris buffer, containing 25% glycerin and 0.25 M NaCl, or with the buffer alone, as a control.

2.4. Measurement of cyclic GMP

The content of cyclic GMP in the perfusate was assayed as described by Abiru et al. (1993). In the present study, we perfused the mesentery with KHS containing 3-isobutyl-1-methylxanthine (IBMX: 10^{-4} M) to inhibit phosphodiesterase activity. The perfusate was collected for 30 s, when each vasodilator response reached a plateau. Since the release of cyclic GMP in the perfusate in response to acetylcholine or PAF was maximal at 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 commercially available kits (Yamasa Cyclic GMP assay Kit, Yamasa Co., Choshi, Japan). The release of cyclic GMP induced by vasodilators is expressed as the change from the basal level.

2.5. Drugs

Methoxamine, sodium nitroprusside and 3-isobutyl-1-methyl-xanthine were purchased from Sigma Chemical Co. (St. Louis, MO). Acetylcholine chloride was purchased from Daiichi Pharmaceutical Co. (Tokyo, Japan). PAF (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phospho-rylcholine) was purchased from Bachem (Switzerland). PAF and CV-6209 (2-[*N*-acetyl-*N*-(2-methyl-3-octadecylcarbamoyl-oxy-propoxy-carbonyl) aminomethyl]-1-ethylpyridinium chloride; Takeda, Osaka, Japan) were dissolved in ethanol and stored at −20°C. On the day of use, the ethanol was dried

off under a stream of nitrogen gas, and the drug was sonicated for 30 s in KHS containing 0.25% bovine serum albumin (Fraction V, Sigma).

2.6. Statistical analysis

The data are expressed as the means ± S.E. Statistical differences were measured using 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 according to Fleming et al. (1972).

3. Results

3.1. Relaxation in response to acetylcholine and PAF

The values of systolic blood pressure in SHR and WKY were 231.2 ± 6.3 mm Hg and 126.5 ± 4.3 mm Hg ($P < 0.01$, $n = 15$), and those in RHR and control rats were 201.5 ± 2.3 mm Hg and 129.3 ± 4.9 mm Hg, respectively ($P < 0.01$, $n = 15$).

The basal perfusion pressures of SHR and WKY mesenteric arterial beds were 69.1 ± 6.7 mm Hg ($n = 18$) and 67.2 ± 7.0 mm Hg ($n = 18$), respectively. Perfusion with methoxamine (5 ± 10^{-6} M to 4 ± 10^{-5} M) increased the perfusion pressures to 124.4 ± 3.4 mm Hg ($n = 18$) and 128.6 ± 5.8 mm Hg ($n = 18$), respectively. Consistent with reported results (Kamata et al., 1994), acetylcholine (10^{-10} to 3×10^{-8} M) induced a concentration-dependent vasodilatation of mesenteric arterial beds that were contracted with methoxamine, which did not differ significantly between SHR and WKY (data not shown). The EC_{50} values for acetylcholine were $3.3 \pm 0.5 \times 10^{-9}$ M (SHR, $n = 6$) and $2.3 \pm 0.4 \times 10^{-9}$ M (WKY, $n = 6$). As shown in Fig. 1, the acetylcholine-induced vasodilatation also did not differ significantly between control rats and RHR. The EC_{50} values for acetylcholine were $4.9 \pm 1.8 \times 10^{-9}$ M (RHR, $n = 6$) and $2.9 \pm 0.4 \times 10^{-9}$ M (control rat, $n = 6$). PAF caused concentration-dependent vasodilatation of the methoxamine-contracted mesenteric arterial beds from SHR and WKY. Consistent with reported results (Kamata et al., 1994), the concentration-response curve for PAF-induced vasodilatation displayed a marked shift to the right in SHR (data not shown). The EC_{50} values for PAF were 3.1 ± 0.8

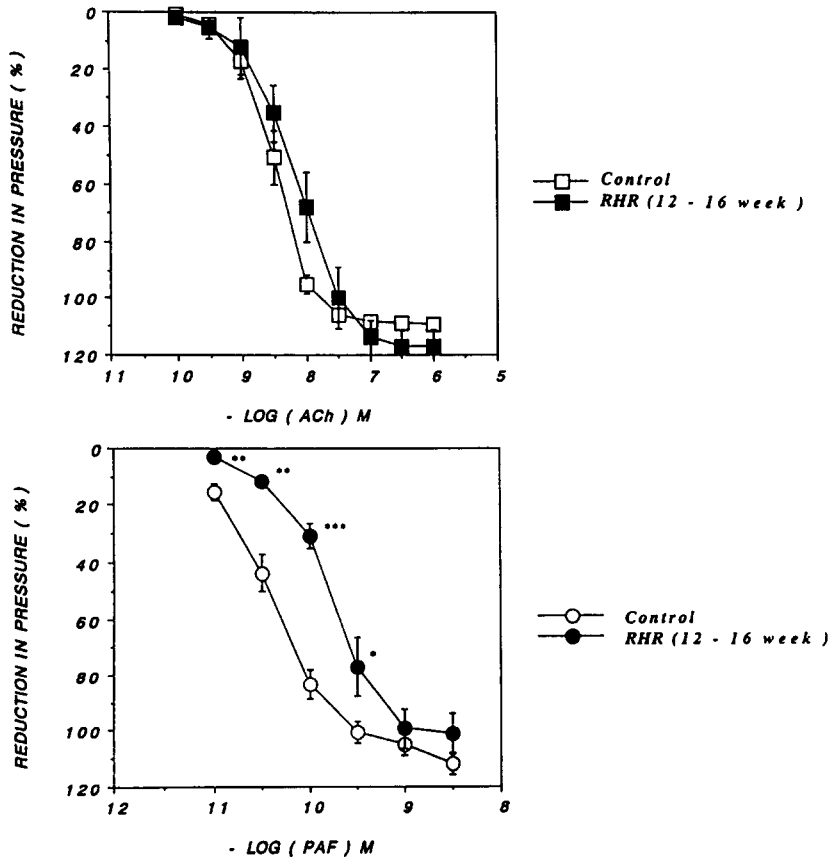

Fig. 1. Concentration-response curves for the relaxation induced by acetylcholine (upper panel) and PAF (lower panel) in methoxamine-precontracted mesenteric arterial beds obtained from control rats (opened symbol) and RHR (solid symbol). The amount of relaxation induced by the drugs is expressed as a percentage of the methoxamine-induced increase in perfusion pressure. Each point is the mean of six determinations, and vertical bars represent the S.E.M. from six determinations. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

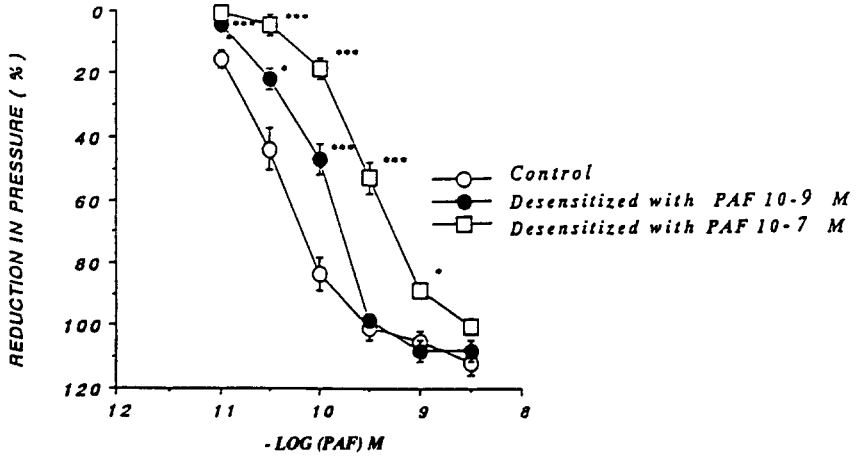

Fig. 2. Concentration-response curves for the relaxation induced by PAF in methoxamine-precontracted mesenteric arterial beds that had been incubated with 10^{-9} M or 10^{-7} M PAF for 1 min. The amount of relaxation induced by the drugs is expressed as a percentage of the methoxamine-induced increase in perfusion pressure. Each point is the mean of six determinations, and vertical bars represent the S.E.M. from six determinations. * $P < 0.05$; *** $P < 0.001$.

$\times 10^{-10}$ M and $2.7 \pm 0.3 \times 10^{-11}$ M ($P < 0.001$, $n = 12$) in SHR and WKY, respectively. The PAF-induced vasodilatation was significantly attenuated in RHR as shown in Fig. 1. The EC_{50} values for PAF were $1.8 \pm 0.4 \times 10^{-10}$ M and $3.6 \pm 0.5 \times 10^{-11}$ M ($P < 0.05$, $n = 12$) in RHR and control rats, respectively.

3.2. Effects of preincubation with PAF on the vasodilatation induced by PAF, acetylcholine and sodium nitroprusside

After the mesenteric arterial beds were incubated with 10^{-9} M or 10^{-7} M PAF for 1-min infusion, we examined

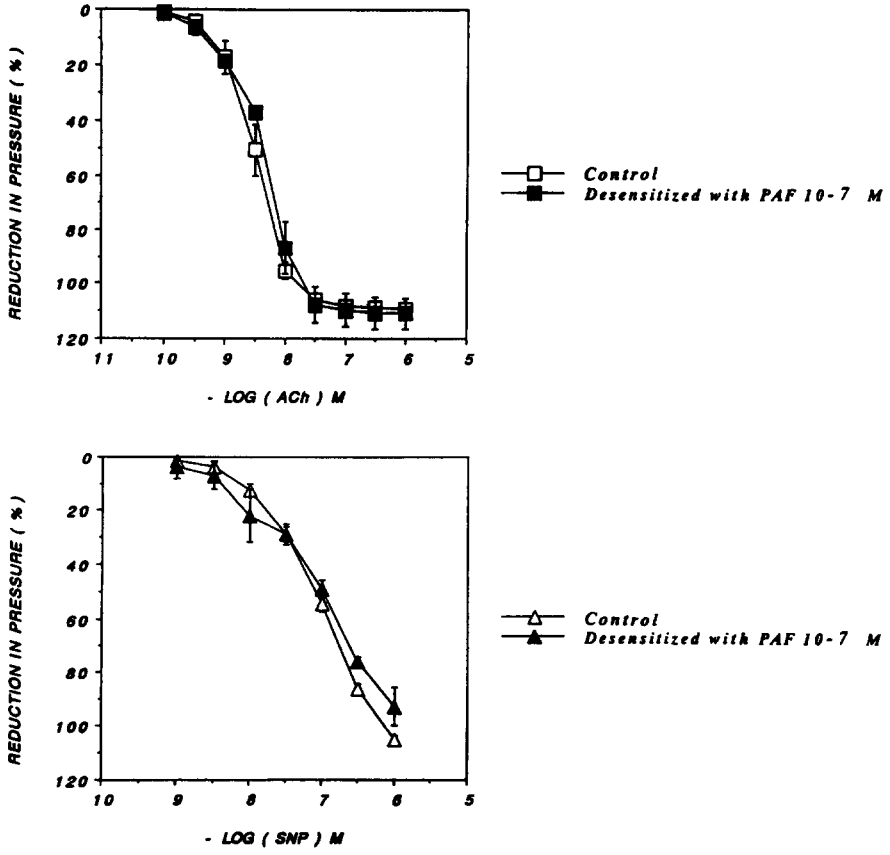

Fig. 3. Concentration-response curves for the relaxation induced by acetylcholine (upper panel) and sodium nitroprusside (lower panel) in methoxamine-precontracted mesenteric arterial beds which had been incubated with 10^{-7} M PAF for 1 min. The amount of relaxation induced by the drugs is expressed as a percentage of the methoxamine-induced increase in perfusion pressure. Each point is the mean of six determinations, and vertical bars represent the S.E.M. from six determinations.

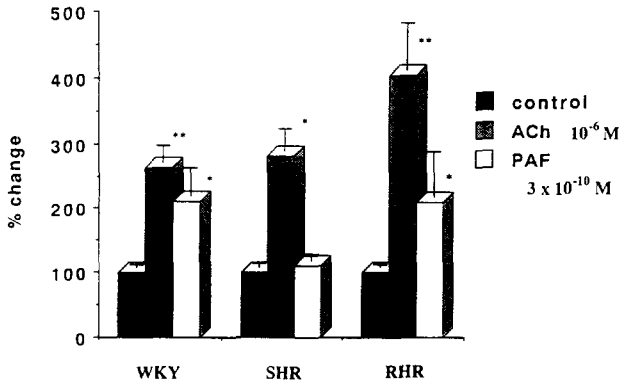

Fig. 4. Effects of 10^{-6} M acetylcholine and 3×10^{-10} M PAF on the levels of cyclic GMP in the effluent from perfused rat mesenteric arterial beds obtained from WKY, SHR and RHR. Each effluent was collected for 30 s. Changes in cyclic GMP are expressed as percentages of control levels. Each column represents the mean ± S.E. for four preparations. * $P < 0.05$; ** $P < 0.01$.

the PAF-induced vasodilatation. As shown in Fig. 2, the PAF-induced dose-dependent vasodilatation of the mesentery was markedly shifted to the right after incubation with 10^{-9} M and 10^{-7} M PAF for 1 min. The EC_{50} values for PAF were $3.6 \pm 0.5 \times 10^{-11}$ M (control, $n = 10$), $1.6 \pm 0.5 \times 10^{-10}$ M (preincubated with 10^{-9} M PAF, $P < 0.05$, $n = 5$) and $2.9 \pm 0.3 \times 10^{-10}$ M (preincubated with 10^{-7} M PAF, $P < 0.01$, $n = 5$), respectively. On the other hand, acetylcholine- and sodium nitroprusside-induced vasodilatation was not affected by incubation with 10^{-7} M PAF as shown in Fig. 3.

3.3. Effects of acetylcholine and PAF on cyclic GMP levels in the effluents

The basal levels of cyclic GMP in effluent collected from the mesentery in WKY, SHR and RHR were 23.5 ± 3.1 fmol/min ($n = 6$), 15.6 ± 1.4 fmol/min ($n = 6$) and 26.6 5.3 fmol/min ($n = 6$), respectively. Acetylcholine (10^{-6} M) caused a significant increase in cyclic GMP levels in WKY, SHR and RHR, respectively (Fig. 4). PAF (3×10^{-10} M) markedly increased cyclic GMP levels in WKY, but failed to increase the cyclic GMP level in SHR. In RHR, a significant increase in cyclic GMP levels was induced by PAF, but the increase was less than that with acetylcholine.

3.4. Effects of PAF and IAP on PAF-induced cyclic GMP accumulation

After the mesenteric arterial beds were incubated with 10^{-9} M or 10^{-7} M PAF for 1 min, we examined the PAF-induced cyclic GMP accumulation. As shown in Fig. 5, the 3×10^{-10} M PAF-induced accumulation of cyclic GMP was significantly decreased by 10^{-7} M PAF. The PAF-induced accumulation of cyclic GMP was significantly inhibited when the rats received IAP beforehand.

3.5. Effects of CV-6209 on PAF-induced vasodilatation

Perfusion of the mesentery preparation with increasing concentrations of PAF relaxed the methoxamine-induced contraction as shown in Fig. 6, and this relaxation was completely reversed by the perfusion of 3×10^{-9} M CV-6209 (2-[*N*-acetyl-*N*-(2-methyl-3-octadecylcarbamoyloxypropoxycarbonyl)-aminomethyl]-1-ethylpyridinium chloride). This effect was the same whether CV-6209 was applied to the mesentery immediately, or 30 min after PAF-induced vasodilatation ($n = 4$). The basal perfusion

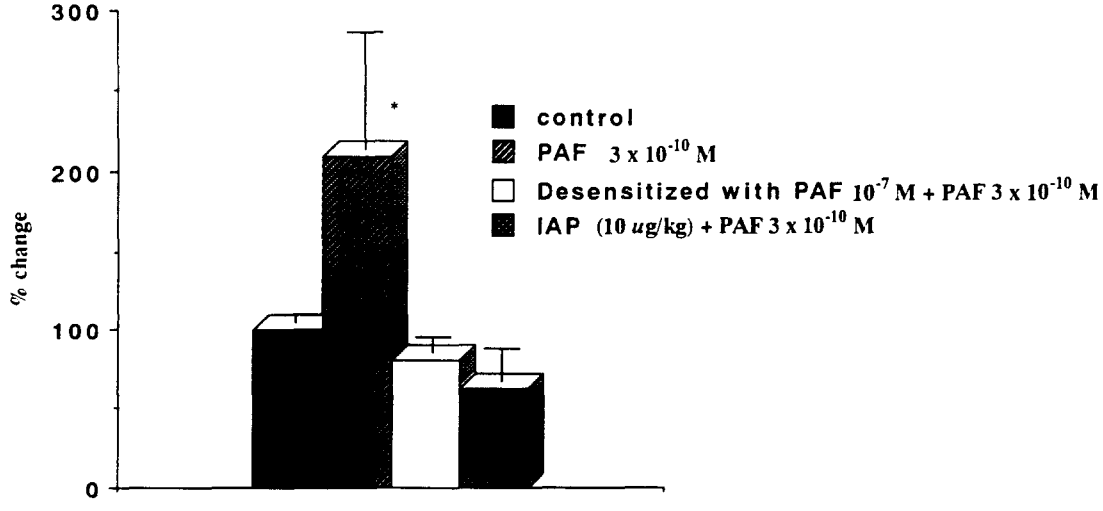

Fig. 5. Effects of 3×10^{-10} M PAF on the levels of cyclic GMP in the effluent from perfused rat mesenteric arterial beds. The mesentery preparation was incubated with 10^{-7} M PAF for 1 min, and the effect of PAF was evaluated. After the rats were injected intravenously with 10 μg/kg IAP, the cyclic GMP levels in the effluent were changed. Each effluent was collected for 30 s. Changes in cyclic GMP are expressed as percentages of control levels. Each column represents the mean ± S.E. for four preparations. * $P < 0.05$.

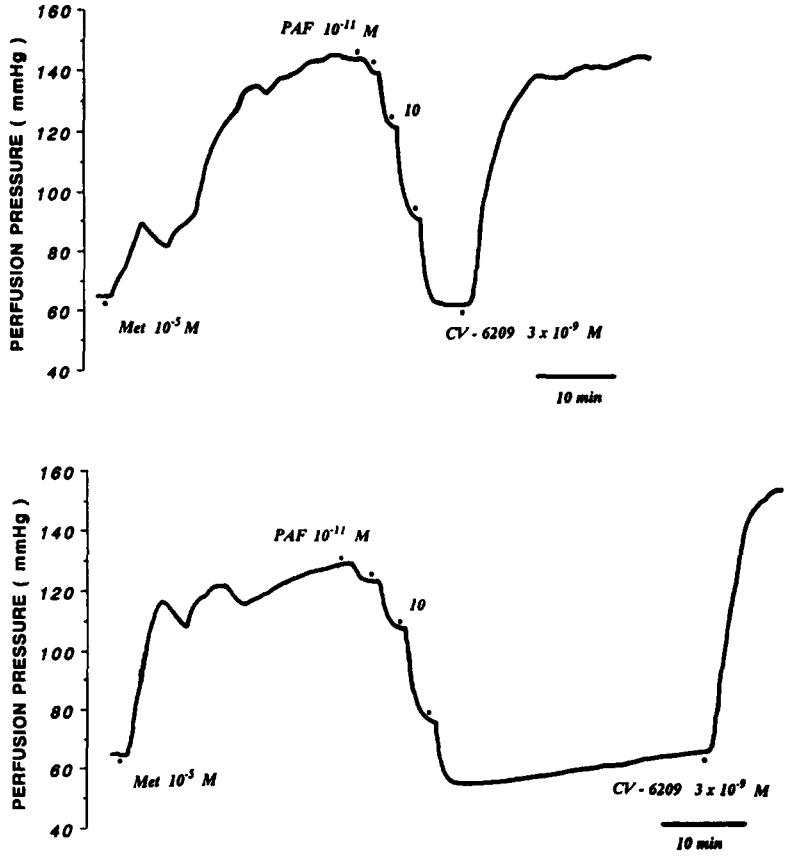

Fig. 6. Effects of CV-6209 (3×10^{-9} M) on PAF-induced vasodilatation of the mesenteric arterial bed. CV-6209 was applied to the mesentery immediately or 30 min after PAF-induced vasodilatation.

pressure of the mesenteric arterial beds was not affected by CV-6209 (3×10^{-9} M) itself.

4. Discussion

In the present study, we confirmed that there are diminished relaxation responses of the mesenteric arterial beds to PAF in both SHR and RHR. In contrast, relaxation responses to acetylcholine were unchanged in SHR and RHR. The PAF-stimulated cyclic GMP accumulation was markedly blunted in both SHR and RHR, whereas there were no changes in the ability of acetylcholine to stimulate cyclic GMP accumulation in SHR and RHR. Furthermore, we found that incubating the mesentery preparation with PAF led to insensitivity of the tissue to PAF-induced vasodilatation. There was no change in the maximal responsiveness of the mesentery, nor was there any change in the sensitivity of the mesenteric arterial bed to acetylcholine or sodium nitroprusside. The desensitization of the mesentery preparation to PAF was associated with a decrease in cyclic GMP accumulation.

Consistent with our earlier results (Kamata et al., 1994), the vasodilator effect of PAF on the mesentery was significantly attenuated in SHR and RHR, in sharp contrast to their acetylcholine responses. Accumulating evidence suggests that circulating PAF contributes to the regulation of blood pressure. The rapid fall in blood pressure seen after unclipping the renal clip hypertensive rats is associated with an increased level of PAF in the blood (McGowan et al., 1988). An increase in blood pressure during high dietary salt intake induces PAF biosynthesis (Sakaguchi et al., 1991). An antagonist of PAF, CV-3988, raises the blood pressure of SHR when administered systemically (Masugi et al., 1985), and in nephrectomy-induced hypertension (Murihead, 1980). Hypotension resulting from endotoxin challenge is due to the endogenous release of PAF from endothelial cells (Camussi et al., 1983), leukocytes (Demopoules et al., 1979), macrophages (Mencia-Huerta and Benveniste, 1979; Camussi et al., 1983) and platelets (Chingard et al., 1979). Indeed, PAF receptor antagonists can reverse established endotoxin-induced hypotension (Terashita et al., 1985; Handley et al., 1986). An extremely low concentration of PAF produces endothelium-dependent relaxation in the mesenteric arterial bed (Kamata et al., 1989). Recently, we further reported that the endothelium-dependent vasodilator effects of PAF are primarily mediated by endothelium-derived nitric oxide (Kamata et al., 1996). Regulation of the mesenteric arterial bed may significantly affect systemic blood pressure, since the

mesenteric circulation of the rat receives about one-fifth of the cardiac output (Nichols et al., 1985). These results indicate that circulating PAF regulates the blood pressure. It is likely, therefore, that chronically increased blood pressure in SHR or RHR may cause overproduction of PAF in order to regulate the blood pressure. Indeed, it has been reported that changes in circulating PAF correlate significantly and positively with changes in mean arterial blood pressure (Sakaguchi et al., 1991). Attenuated vasodilator effects of PAF in the SHR and RHR mesentery may result from desensitization rather than impairment of the endothelium, because there were no differences in acetylcholine-induced endothelium-dependent vasodilatation in the mesentery between SHR and WKY. It is likely, therefore, that a chronically increased level of PAF in the blood induces desensitization of the mesentery to PAF.

Most investigators have focused on the β-adrenoceptor, through which the stimulatory actions of catecholamines on the enzyme (adenylate cyclase) are mediated by the β-adrenoceptor (Sibley and Lefkowitz, 1985). In general, ligand-induced desensitization can be divided into two general categories: agonist-specific or 'homologous' desensitization, and agonist-nonspecific or 'heterologous' desensitization. Homologous refers to that form of desensitization in which only the subsequent response to the desensitizing hormone or drug is attenuated, while the efficacy of other activators is unimpaired. Conversely, heterologous indicates that exposing a cell to an agonist attenuates the response to multiple agonists operating through distinct receptors (Sibley et al., 1987). The PAF-induced desensitization of the relaxation response in the mesentery was of the homologous type, because the acetylcholine- or sodium nitroprusside-induced vasodilatation was not affected by PAF. Furthermore, desensitization of the endothelium-dependent vasodilatation induced by PAF was also not due to a decreased activity of guanylate cyclase, because incubation of the mesentery with PAF did not affect the vasodilatation induced by sodium nitroprusside which is an activator of soluble guanylate cyclase.

In many blood vessels, the endothelium-derived nitric oxide 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). We developed a novel method for simultaneously measuring vasodilatation and changes in cyclic nucleotide levels released from the rat mesenteric arterial bed (Abiru et al., 1993). In WKY, both acetylcholine and PAF caused a marked increase in the cyclic GMP levels of the mesenteric arterial bed. Acetylcholine increased cyclic GMP levels in SHR and RHR, whereas PAF had no increasing effects on cyclic GMP levels in SHR and slightly increased them in RHR. Since the acetylcholine-induced increase in cyclic GMP was not changed in SHR and RHR, the attenuation of the increasing effects of PAF on cyclic GMP levels in SHR and RHR was not due to impairment of the endothelium of the SHR and RHR mesentery. Consistent with the relaxation response to PAF, incubating the mesentery preparation with PAF rendered it insensitive to PAF-induced cyclic GMP accumulation, suggesting desensitization to PAF.

The activation of regulatory G-proteins is associated with agonist stimulation of most receptors (Rodbell, 1985; Dohlman et al., 1987; Dolphin, 1987). When an agonist binds to its receptor, GTP binds to an α-subunit of the protein, then G-protein is activated. The G-protein then dissociates from the receptor, causing the affinity of the receptor for the agonist to be reduced, and the α-subunit is released (Kurose et al., 1983). The distinct α-subunits derived from the different G-proteins can activate or inhibit a number of intracellular processes (Dolphin, 1987). IAP ADP ribosylates and inactivates the G_i-protein, which mediates the inhibitory effects of receptors on adenylate cyclase (Kurose et al., 1983; Dolphin, 1987). IAP interferes with the release of the endothelium-derived relaxing factor evoked by the α_2-adrenoceptor agonist, 5-hydroxytryptamine, thrombin and by aggregating platelets, and some but not all, endothelial activators (Flavahan et al., 1989). Here, we found that the PAF-stimulated cyclic GMP accumulation was also blunted in rats given IAP, suggesting that endothelial PAF receptors are closely coupled to G_i-protein. It is most likely, therefore, that desensitization of the mesentery to PAF in SHR and RHR or after incubating mesentery preparations with PAF is, at least in part, due to the uncoupling of PAF receptors from IAP-sensitive G-protein. This process may reduce the affinity of the receptor for PAF. Impairment of G_i-protein function can occur under physiological conditions, following endogenous ADP ribosylation of the protein (Ui et al., 1985) and also during pathological states (Gawler et al., 1987). It is unknown at present whether such alterations in G_i-protein function occurs in SHR and RHR. Further investigations are required to clarify this point.

PAF-induced vasodilatation of the mesenteric arterial bed was reversed by CV-6209, a PAF receptor antagonist (Terashita et al., 1987). This reversal was induced by CV-6209 to the same degree whether it was applied to the mesentery immediately or 30 min after PAF-induced vasodilatation. These results suggest that desensitization of the mesentery to PAF is not due to the internalization of PAF receptors. There should be the same number of PAF receptors on the endothelium immediately or 30 min after PAF-induced vasodilatation, because the reversal effects of CV-6209 on PAF-induced vasodilatation were of the same size.

In conclusion, the vasodilatation effect of PAF was markedly attenuated in SHR and RHR, whereas the relaxation response to acetylcholine was unchanged in both. The PAF-stimulated cyclic GMP accumulation was markedly blunted in both SHR and RHR, whereas there were no changes in the ability of acetylcholine to stimulate cyclic GMP accumulation in SHR and RHR. Furthermore,

we found that incubating the mesentery preparation with PAF led to insensitivity of the tissue to PAF-induced vasodilatation. There was no change in the maximal responsiveness of the mesentery, nor was there any change in the sensitivity of the mesenteric arterial bed to acetylcholine or sodium nitroprusside. The desensitization of the mesentery preparation to PAF was associated with a decrease in cyclic GMP accumulation. Therefore, PAF-induced desensitization in SHR and RHR can be explained as follows: high blood pressure may cause overproduction of PAF in order to regulate blood pressure; overproduced PAF binds chronically to its receptors; chronic exposure of PAF receptors to PAF may cause uncoupling of PAF receptors from IAP-sensitive G-protein; which then induces desensitization of the mesentery to PAF.

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