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Endothelium-dependent vasodilator effects of platelet activating factor on rat resistance vessels

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1 To elucidate the mechanisms of the powerful and long-lasting hypotension produced by platelet activating factor (PAF), its effects on perfusion pressure in the perfused mesenteric arterial bed of the rat were examined.

2 Infusion of PAF (10^{-11} to 3×10^{-10} M; $EC_{50} = 4.0 \times 10^{-11}$ M; 95%CL = $1.6 \times 10^{-11} - 9.4 \times 10^{-11}$ M) and acetylcholine (ACh) (10^{-10} to 10^{-6} M; $EC_{50} = 3.0 \pm 0.1 \times 10^{-9}$ M) produced marked concentration-dependent vasodilatations which were significantly inhibited by treatment with detergents (0.1% Triton X-100 for 30 s or 0.3% CHAPS for 90 s).

3 Pretreatment with CV-6209, a PAF antagonist, inhibited PAF- but not ACh-induced vasodilatation.

4 Treatment with indomethacin (10^{-6} M) had no effect on PAF- or ACh-induced vasodilatation.

5 These results demonstrate that extremely low concentrations of PAF produce vasodilatation of resistance vessels through the release of endothelium-derived relaxing factor (EDRF). This may account for the strong hypotension produced by PAF *in vivo*.

Introduction

Platelet activating factor (PAF, acetyl glyceryl ether phosphorylcholine) has been shown to produce strong and long-lasting hypotension in various animal species, e.g. normotensive and spontaneously hypertensive rats, rabbits, guinea-pigs, and dogs (Tanaka *et al.*, 1983). This action of PAF is thought to be endothelium-dependent (Kamitani *et al.*, 1984; Kasuya *et al.*, 1984a,b; Shigenobu *et al.*, 1985; 1987). In a previous study (Shigenobu *et al.*, 1987), we found that relatively low concentrations of PAF (10^{-9}–10^{-7} M) produced endothelium-dependent relaxation of the rat aorta in the presence of bovine serum albumin. This vasodilator action of PAF at low concentrations might be the cause of its hypotensive action *in vivo*. While the aorta will offer a resistance to flow, it is obvious that the contribution of vessels of smaller diameter to peripheral vascular resistance is much greater. In this regard, the mesenteric circulation of the rat receives approximately one-fifth of the cardiac output (Nichols *et al.*, 1985) and, thus, regulation of this bed may make a significant contribution towards systemic blood pressure and circulating blood volume. In the present study, therefore, we examined the effect of PAF on the resistance vessels of the rat mesenteric vascular bed and found that extremely low concentrations (10^{-11} to 3×10^{-10} M) can produce endothelium-dependent vasodilatation.

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Methods

Perfusion of the mesenteric arterial bed

Male Wistar rats (190–360 g) were killed by decapitation. The mesenteric arterial bed was removed and perfused according to the method described by McGregor (1965), with various modifications. Briefly, the abdominal cavity was opened by midline incision, and the entire mesenteric arterial bed and associated intestinal tract was removed from the rat. The mesenteric artery and vein were tied off close to the caecum, and the remaining intestine was separated from the vascular bed along the intestinal wall. A cannula of PE90 tubing was inserted approximately 1.5 cm into the superior mesenteric artery from its junction with the aorta and tied firmly. The isolated

preparation was placed in an organ bath and perfused through the cannula with Krebs-Henseleit solution by a peristaltic pump (Tokyo Rikakikai, Model MP-3) at a rate of 5 ml min^{-1}. The Krebs-Henseleit solution was composed (in mM) of NaCl 118.0, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.2, $NaHCO_3$ 25, dextrose 11.1 and 0.25% bovine serum albumin. The perfusate was maintained at 37°C and gassed constantly with 95% O_2 and 5% CO_2. Changes in perfusion pressure were monitored with a pressure transducer (Nihon Kohden, Model AP-2001) and recorded on a pen recorder (Yokogawa, Model 3021). After an equilibration period of 60 min, the perfusion circuit was changed to a closed system, i.e., the perfusion solution was collected in a second bath and recirculated into the mesenteric arterial bed. The total volume of the closed system was 50 ml, and agents were administered via the second bath. The mesenteric arterial bed was precontracted with 10^{-5} M methoxamine. Acetylcholine (ACh) was administered cumulatively, but PAF was infused in single doses to avoid tachyphylaxis.

Removal of endothelium by detergents

The endothelium was removed by intraluminal perfusion with Krebs-Henseleit solution containing detergents (0.1% Triton X-100 for 30 s or 0.3% CHAPS for 90 s). After a re-equilibration period of 30 min, responses to ACh or PAF were examined.

Statistics

Since ACh was administered cumulatively and PAF was infused in single doses, EC_{50} data for ACh are presented as the mean ± s.e.mean and EC_{50} data for PAF and CV-6209 are presented as 95% confidence limits (CL) of EC_{50} according to the method of Litchfield & Wilcoxon (1949). Significance of differences was determined with Student's non-paired *t* test.

Drugs

PAF (1-O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine; Bachem, Switzerland) and CV-6209 (2-[N-acetyl-N-(2-methoxy-3-octadecylcarbamoyloxypropoxycarbonyl)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 under a stream of nitrogen gas and the drugs dissolved in Krebs-Henseleit solution containing 0.25% bovine serum albumin (Fraction V, Sigma, St. Louis, U.S.A.). Sonication for 30 s ensured that the drugs were completely dissolved. Methoxamine hydrochloride, indomethacin, 3-[(cholamidopropyl)-dimethyl-ammonio]-1-propanesulphonate-(CHAPS), Triton X-100 (Sigma) and acetylcholine hydrochloride (Daiichi, Tokyo, Japan) were dissolved in 0.9% saline.

Results

Perfused mesenteric arterial bed of rat

The basal perfusion pressure of the rat mesenteric arterial bed was 51.4 ± 1.2 mmHg ($n = 10$). Perfusion with methoxamine (10^{-5} M) increased the perfusion pressure to 118.4 ± 6.1 mmHg ($n = 10$).

Relaxation in response to ACh

In the perfused mesenteric arterial bed preconstricted with methoxamine (10^{-5} M), infusion of increasing

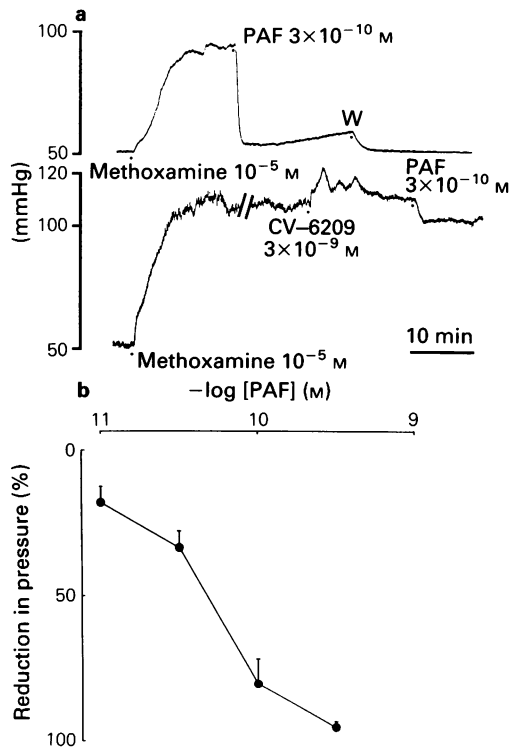

Figure 1 Effects of PAF on the perfusion pressure of the methoxamine (10^{-5} M)-constricted mesenteric vascular bed. (a) Upper panel: relaxation induced by PAF (3×10^{-10} M). Lower panel: effects of the PAF-antagonist, CV-6209 (3×10^{-9} M), on the relaxation induced by PAF (3×10^{-10} M). (b) Concentration-response curve for the relaxation produced by PAF (10^{-11} to 3×10^{-10} M) in the methoxamine (10^{-5} M)-constricted mesenteric vascular bed. Each point is the mean and vertical bars represent the s.e.mean from 5 experiments.

concentrations of ACh (10^{-10} to 10^{-6} M) produced concentration-dependent vasodilatations. The EC_{50} concentration was $3.0 \pm 0.1 \times 10^{-9}$ M ($n = 6$) and the maximal relaxation induced was $95.5 \pm 8.6\%$.

Relaxation in response to PAF

PAF (10^{-11} to 3×10^{-10} M) produced concentration-dependent, long-lasting relaxation of the methoxamine (10^{-5} M)-constricted mesenteric arterial bed (Figure 1). The EC_{50} value for PAF was 4.0×10^{-11} M (95% CL = 1.6×10^{-11}–9.4×10^{-11} M, $n = 5$) and the maximal relaxation induced was $95.5 \pm 8.6\%$. CV-6209 (3×10^{-11}–3×10^{-9} M), a PAF antagonist, inhibited PAF (3×10^{-10} M)-induced vasodilatation in a dose-dependent manner. The IC_{50} ($n = 5$) value for CV-6209 was 1.0×10^{-10} M (95% CL = 3.1×10^{-10}–2.8×10^{-9} M, $n = 5$). CV-6209 (3×10^{-9} M) had no effect on ACh-induced relaxation (data not shown). Infusion of lysoPAF (3×10^{-10} M) had no effect on the perfusion pressure of the mesentery (data not shown).

Effects of detergents on responses to ACh

Perfusion of the mesentery with detergents (0.1% Triton X-100, $n = 5$; 0.3% CHAPS, $n = 3$) produced a slight but significant increase in methoxamine (10^{-5} M)-induced vasoconstriction (data not shown). Both detergents significantly suppressed the vasorelaxant response to ACh (10^{-7}–10^{-4} M) (Figure 2). Pretreatment with indomethacin (10^{-6} M) did not affect ACh-induced relaxation (data not shown).

Effects of detergents on responses to PAF

PAF (3×10^{-10} M)-induced vasodilatation was significantly inhibited by pretreatment of mesentery with Triton X-100 (0.1%, $n = 5$) or CHAPS (0.3%, $n = 3$). In contrast, pretreatment with indomethacin (10^{-6} M) had no effect on PAF-induced vasodilatation (Figure 3).

Discussion

Since Furchgott & Zawadzki (1980) demonstrated the obligatory role of endothelium in vascular relaxation by ACh, many studies have suggested that endothelium-derived relaxing factor (EDRF) is released from endothelial cells in response to a large number of agonists (Furchgott, 1984). In the present study with perfused resistance vessels, ACh produced vasodilatation in a concentration-dependent manner and the vasorelaxant responses were significantly suppressed by perfusion with detergents such as CHAPS or Triton X-100. Since the detergents are

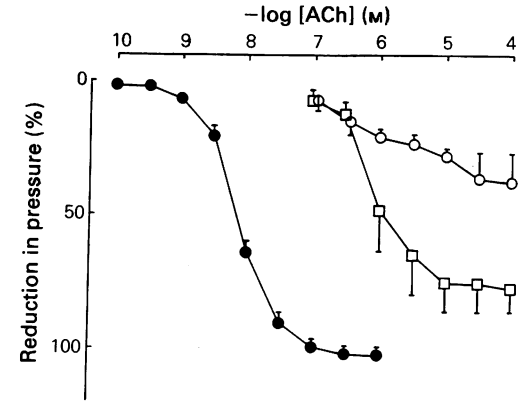

Figure 2 Effects of detergents on acetylcholine (ACh)-induced relaxation of the methoxamine (10^{-5} M)-constricted mesenteric vascular bed. Concentration-response curves are shown for ACh-induced vasodilatation before (●) and after treatment with 0.3% CHAPS (□) or 0.1% Triton X-100 (○). Each point is the mean and vertical bars represent the s.e.mean from 5 experiments.

thought to destroy the endothelial cells only, these results confirmed that ACh-induced vasodilatation was mediated through release of EDRF. Moreover, in agreement with recent findings by Randal & Hiley (1988) and Tesfamariam & Halpern (1988), these detergents enhanced the ability of the vascular smooth muscle to contract in response to methoxamine, an α-adrenoceptor agonist.

Infusions of extremely low concentrations of PAF (10^{-11} to 3.1×10^{-10} M) produced a marked and long-lasting vasodilatation which was significantly suppressed by treatment with detergents (CHAPS or

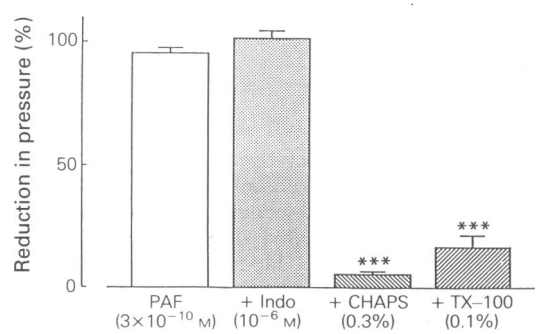

Figure 3 Effects of indomethacin (Indo, 10^{-6} M, $n = 4$) and detergents (0.3% CHAPS, $n = 3$; 0.1% Triton X-100, $n = 5$) on the relaxation induced by PAF (3×10^{-10} M) in the methoxamine (10^{-5} M)-constricted mesentery. Each column is the mean and vertical bars represent the s.e.mean. *** $P < 0.001$.

Triton X-100). These data strongly suggest the possible involvement of the endothelium in the relaxation induced by PAF.

CV-6209, a PAF antagonist, inhibited PAF-induced but not ACh-induced vasodilatation in a concentration-dependent manner. Specific antagonism by CV-6209 has already been obtained with respect to PAF-induced hypotension or platelet aggregation (Terashita *et al.*, 1987). An accumulating body of evidence suggests that 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 & Benveniste, 1979; Camussi *et al.*, 1983) and platelets (Chingard *et al.*, 1979). Indeed, PAF antagonists can reverse established endotoxin-induced hypotension (Terashita *et al.*, 1985; Handley *et al.*, 1985a,b). From the above data and the results of the present study, one possible explanation for endotoxin-induced hypotension may be that the release of PAF occurs, which then binds to its receptors located on the endothelial cells, stimulating production of EDRF.

Prostaglandin I_2 a powerful vasodilator is synthesized by the endothelial cells (Moncada *et al.*, 1977). However, Furchgott's group showed that ACh-induced, endothelium-mediated vasodilatation was not modified by the cyclo-oxygenase inhibitor, indomethacin. We found that the endothelium-dependent vasodilatation induced by PAF was not inhibited by indomethacin, ruling out the involvement of vasodilator prostanoids. Furthermore, PAF at a low concentration showed neither chronotropic nor inotropic effects on the guinea-pig isolated right and left atria, respectively (Tanaka *et al.*, 1983; Shigenobu *et al.*, 1989), excluding the possible involvement of cardiac depression in the PAF-induced hypotension.

In conclusion, we demonstrated that extremely low concentrations of PAF produce long-lasting vasodilatation in a resistance vessel of the mesenteric vasculature. Moreover, we showed that this PAF-induced vasodilatation is mediated by a vasodilator substance released from endothelial cells (EDRF) which is not a prostaglandin. Since the PAF-induced endothelium-dependent relaxation observed in the present study was elicited at low concentrations and was long-lasting, it may be the main mechanism by which PAF induces hypotension *in vivo*.

References

CAMUSSI, G., AGLIETTA, M., MALAVASI, F., TETTA, C., PIACIBELLO, W., SANOVIO, F. & BUSSOLINO, F. (1983). The release of platelet-activating factor from human endothelial cells in culture. *J. Immunol.*, **131**, 2397–2403.

CHINGARD, M., LECOUEDIC, J.P., TENCE, M., VARGAFTIG, B.B. & BENVENISTE, J. (1979). The role of platelet-activating factor in platelet aggregation. *Nature*, **279**, 799–800.

DEMOPOULES, C.A., PINCKARD, R.N. & HANAHAN, D.J. (1979). Platelet-activating factor: evidence for 1-O-alkyl-2-acetyl-*sn*-glycerol-3-phosphoryl-choline as the active component (a new class of lipid chemical mediators). *J. Biol. Chem.*, **254**, 9355–9358.

FURCHGOTT, R.F. (1984). The role of endothelium in the response of vascular smooth muscle to drugs. *Ann. Rev. Pharmacol. Toxicol.*, **24**, 175–197.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

HANDLEY, D.A., TOMESCH, J.D. & SAUNDERS, R.N. (1986a). Inhibition of PAF-induced responses in the rat, guinea pig, dog and primate by the receptor antagonist, SRI 63-441. *Thromb. Haemostasis*, **56**, 40–44.

HANDLEY, D.A., VAN VALEN, R.G., MELDEN, M.K., FLURY, S., LEE, M.L. & SAUNDERS, R.N. (1986b). Inhibition and reversal of endotoxin-, aggregated IgG- and paf-induced hypotension in the rat by SRI 63-072, a paf receptor antagonist. *Immunopharmacology*, **12**, 11–17.

KAMITANI, T., KATAMOTO, M., TATSUMI, M., KATSUTA, T., ONO, H., KIKUCHI, H. & KUMADA, S. (1984). Mechanism(s) of the hypotensive effect of synthetic 1-O-octadecyl-2-O-acetyl-glycero-3-phosphorylcholine. *Eur. J. Pharmacol.*, **98**, 357–366.

KASUYA, Y., MASUDA, Y. & SHIGENOBU, K. (1984a). Hypotensive actions of some analogues of platelet-activating factor (PAF) with higher potencies than natural PAF. *Can. J. Physiol. Pharmacol.*, **62**, 457–459.

KASUYA, Y., MASUDA, Y. & SHIGENOBU, K. (1984b). Possible role of endothelium in the vasodilator response of rat thoracic aorta to platelet activating factor (PAF). *J. Pharmacobio-Dyn.*, **7**, 138–142.

LITCHFIELD, J.T. & WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.*, **96**, 99–113.

McGREGOR, D.D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J. Physiol.*, **177**, 21–30.

MENCIA-HUERTA, J.M. & BENVENISTE, J. (1979). Platelet-activating factor and macrophages. I. Evidence for the release from rat and mouse-peritoneal macrophages and not from mastocytes. *Eur. J. Immunol.*, **9**, 409–415.

MONCADA, S., HERMAN, A.G., HIGGS, E.A. & VANE, J.R. (1977). Differential formation of prostacyclin (PGX or PGI_2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of the vascular endothelium. *Thromb. Res.*, **11**, 323–344.

NICHOLS, A.J., WILSON, A.C. & HILEY, C.R. (1985). Effects of sympathectomy with 6-hydroxydopamine on cardiac output and its distribution in the rat. *Eur. J. Pharmacol.*, **109**, 263–268.

RANDALL, M.D. & HILEY, C.R. (1988). Detergent and methylene blue affect endothelium-dependent vasorelax-

ation and pressure/flow relations in rat blood perfused mesenteric arterial bed. *Br. J. Pharmacol.*, **95**, 1081–1088.

SHIGENOBU, K., MASUDA, Y., TANAKA, Y. & KASUYA, Y. (1985). Platelet activating factor analogues: lack of correlation between their activities to produce hypotension and endothelium-mediated vasodilation. *J. Pharmacobio-Dyn.*, **8**, 128–133.

SHIGENOBU, K., TANAKA, Y., MAEDA, T. & KASUYA, Y. (1987). Potentiation by bovine serum albumin (BSA) of endothelium-dependent vasodilator response to acetyl glyceryl ether phosphorylcholine (AGEPC). *J. Pharmacobio-Dyn.*, **10**, 220–228.

SHIGENOBU, K., MORI, T., KAMATA, K. & KASUYA, Y. (1989). Platelet activating factor: lack of direct action on guinea pig myocardium and possible transmitter release from cardiac sympathetic nerve endings at high concentrations. *Can. J. Physiol. Pharmacol.*, **67**, 669–674.

TANAKA, S., KASUYA, Y., MASUDA, Y. & SHIGENOBU, K. (1983). Studies on the hypotensive effects of platelet activating factor (PAF, 1-O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) in rats, guinea pigs, rabbits, and dogs. *J. Pharmacobio-Dyn.*, **6**, 866–873.

TERASHITA, Z., IMURA, Y. & NISHIKAWA, K. (1985). Inhibition by CV-3988 of the binding of [^{3}H]-platelet activating factor (PAF) to the platelet. *Biochem. Pharmacol.*, **43**, 1491–1495.

TERASHITA, Z., IMURA, Y., TAKATANI, M., TSUSHIMA, S. & NISHIKAWA, K. (1987). CV-6209, a highly potent antagonist of platelet activating factor in vitro and in vivo. *J. Pharmacol. Exp. Ther.*, **242**, 263–268.

TESFAMARIAM, B. & HALPERN, W. (1988). Endothelium-dependent and endothelium-independent vasodilation in resistance arteries from hypertensive rats. *Hypertension*, **11**, 440–444.

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