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Role of eicosanoids but not nitric oxide in the platelet-activating factor-induced increase in vascular permeability in mouse skin

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Abstract

We investigated the role of endogenous eicosanoids and nitric oxide (NO) in the platelet-activating factor (PAF)-induced increase in vascular permeability in mouse skin. Subcutaneous injection of PAF (45–180 pmol/site) induced a dose-related increase in vascular permeability at the injection site. The vascular permeability induced by PAF (180 pmol/site) was significantly inhibited by pretreatment with an intraperitoneal injection of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phospho (*N*,*N*,*N*-trimethyl) hexanolamine (PAF receptor antagonist) (5 and 25 mg/kg) and indomethacin (cyclooxygenase inhibitor) (10 mg/kg), whereas it was not affected by concurrent intravenous administration of NO synthase inhibitors N^G-nitro-L-arginine methyl ester (10 mg/kg) or methylene blue (100 μg/kg) nor by topical injection of N^G-nitro-L-arginine methyl ester. The inhibitory effect of indomethacin was partially reversed by topical administration of prostaglandin E_2. These results suggest that PAF increases venular permeability by activating PAF receptors and that plasma extravasation is potentiated by the release of prostanoids which cause arteriolar dilatation. However, NO is not involved in the effect of PAF in mouse skin.

Keywords: Vascular permeability; PAF (platelet-activating factor); Nitric oxide (NO); Indomethacin; (Skin); (Mouse)

1. Introduction

Some endogenous mediators of inflammation, including 5-hydroxytryptamine (5-HT), bradykinin, histamine, platelet-activating factor (PAF) and substance P, are known to increase vascular permeability (Maling et al., 1974; Humphrey et al., 1982; Hughes et al., 1990), probably through the transient, reversible formation of junctional gaps between endothelial cells in postcapillary venules (Majno and Palade, 1961; Schachter, 1963; Arfors et al., 1979; Fujii et al., 1994b). Williams and Peck (1977) proposed the two-mediator hypothesis that the quantity of plasma protein leaking from the microvascular bed is dependent on both the extent of venular permeability and the magnitude of arteriolar vasodilatation, based on their finding that bradykinin and histamine mainly increase venular permeability, whereas prostaglandins of the E-type mediate arteriolar vasodilation and potentiate the exudation elicited by other mediators, but have no potency to induce plasma leakage by themselves.

PAF is one of the most potent lipid mediators known, and has been considered to be a component of the inflammatory response (Snyder, 1990). The PAF-induced increase in vascular permeability appears to result from a direct action on vascular endothelial cells, because it is unaffected by depletion of circulating neutrophils or platelets (Wedmore and Williams, 1981; Pirotzky et al., 1984). Actually, Dewar et al. (1983) and Bjork and Smedegard (1983) reported that PAF acts on the vascular endothelium, produces dysjunction of endothelial cells of postcapillary venules and increases vascular permeability in guinea-pig skin and hamster cheek pouch. PAF acts synergistically with vasodilator prostaglandins (E-type and prostacyclin) to induce local oedema formation, one of the features of the cutaneous inflammatory response (Wedmore and Williams, 1981; Morley et al., 1983; Archer et al., 1984; Hellewell and Williams, 1986; Teixeira et al., 1993a,b). However, it is not clear whether the effect of PAF on plasma leakage is mediated by endogenous prostanoids.

The vascular endothelium can exert an important

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modulatory role on blood vessel tone by releasing prostacyclin (Moncada and Vane, 1979), endothelium-derived relaxing factor (EDRF)/nitric oxide (NO) and endothelin-1 (Palmer et al., 1987; Yanagisawa et al., 1988). The in vivo role of NO in inflammation and other pathophysiological states remains poorly understood. Hughes et al. (1990) reported that substance P-induced extravasation in rat skin was reduced by pretreatment with the NO synthase inhibitor N^G-nitro-L-arginine methyl ester, and they suggested that endogenous NO has a modulatory role in oedema formation induced by substance P by increasing microvascular permeability. Recently, we found that endogenous NO is involved in the effect of 5-HT to increase vascular permeability, whereas involvement of NO was not shown in the effect of histamine in mouse skin (Fujii et al., 1994a,b). In order to investigate the possible role of endogenous prostanoids and NO in the PAF-induced increase in vascular permeability, we studied the effects of inhibitors of cyclooxygenase and NO synthase on the PAF response in the mouse skin.

2. Materials and methods

Male ddY strain mice (Sankyo Laboratory Service, Tokyo, Japan), weighing about 35 g were used. They were housed in an air-conditioned room (temperature 22 ± 2°C, humidity 55 ± 5%) with a controlled light-dark cycle (light on 06:00–20:00 h). Food and water were freely available.

2.1. Assessment of vascular permeability induced by PAF

Vascular permeability was quantified by the extravasation of pontamine sky blue. The pontamine sky blue concentration can be regarded as a measure of plasma leakage (Udaka et al., 1970). Five minutes after intravenous (i.v.) injection of pontamine sky blue (50 mg/kg), PAF (45–180 pmol/site) or saline was administered subcutaneously (s.c., 0.1 ml/site) into the back. One site of PAF injection was studied per animal unless otherwise stated. Sixty minutes later, the mice were killed by cervical dislocation and the stained area of the back skin was cut out. The dye accumulated in the skin was extracted with acetone-Na_2SO_4 solution (acetone:0.5% (w/v) Na_2SO_4 = 14:6 v/v) and the concentration determined colorimetrically at 590 nm.

2.2. Time course of PAF-induced vascular permeability

Five minutes after i.v. injection of pontamine sky blue, PAF and saline were injected into the right or the left side of the back (two sites per animal), and the dye accumulation in the skin was then determined at 5, 30 and 60 min.

2.3. Effects of inhibitors of NO synthase, PAF receptor antagonist and prostaglandin E_2 on stimulation of vascular permeability by PAF

Inhibitors of NO synthase (N^G-nitro-L-arginine methyl ester and methylene blue) or the ineffective isomer N^G-nitro-D-arginine methyl ester were administered i.v. 5 min before the s.c. injection of PAF or 5-HT. In one experiment, N^G-nitro-L-arginine methyl ester mixed with PAF was coadministered s.c. 1-*O*-Hexadecyl-2-acetyl-*sn*-glycero-3-phospho (*N*,*N*,*N*-trimethyl) hexanolamine (hexanolamine PAF) or indomethacin was administered intraperitoneally (i.p.) 35 min before the s.c. injection of PAF. To study the reversal by prostaglandin E_2 of the inhibitory effect of indomethacin, prostaglandin E_2 alone or prostaglandin E_2 (0.3–30 nmol/site) mixed with PAF (180 pmol/site) was injected into the skin 35 min after indomethacin. The dye accumulation was then determined 60 min after s.c. injection of prostaglandin E_2 with or without PAF.

2.4. Drugs

The following drugs were used: 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (C_{16}-PAF), 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phospho (*N*,*N*,*N*-trimethyl) hexanolamine, N^G-nitro-D-arginine methyl ester HCl (Nova Biochem, Switzerland); 5-hydroxytryptamine creatinine sulphate (Daiichi Pure Chemical, Tokyo, Japan); N^G-nitro-L-arginine methyl ester HCl, indomethacin, prostaglandin E_2 (Sigma Chemical, Mo, USA); methylene blue (Kanto Chemical, Tokyo, Japan); pontamine sky blue 6B (Tokyo Kasei Kogyo, Tokyo, Japan). Indomethacin was dissolved in a small volume of absolute ethanol and diluted with 50% propylene glycol. Other drugs were dissolved in physiological saline. All doses refer to the salt forms of the drugs.

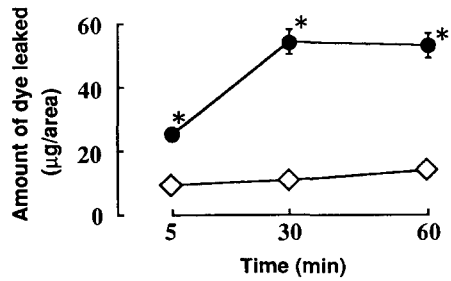

Fig. 1. Time course of the effect of PAF on the dye leakage in mouse skin. Five minutes after i.v. injection of pontamine sky blue (50 mg/kg), PAF (180 pmol/site, •) or saline (0.1 ml/site) (◇) was administered to the back of the mice. At the indicated times after PAF or saline (controls) injection, dye accumulated in the skin was determined colorimetrically. Values represent the means ± S.E.M. of five experiments. * $P < 0.01$ vs. saline.

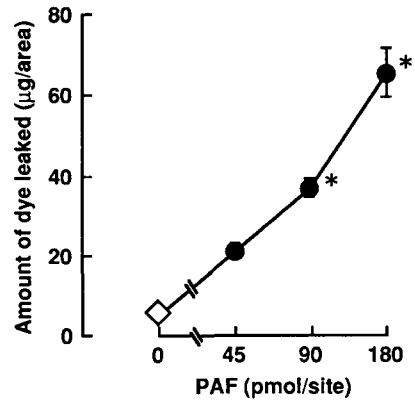

Fig. 2. Effects of increasing doses of PAF on dye leakage in mouse skin. Dye leakage induced by PAF (●) and saline (◇) was assessed after 60 min. Values represent the means ± S.E.M. of five experiments. * $P < 0.01$ vs. saline.

2.5. Statistical analysis

Results are expressed as means ± S.E.M. Comparisons among multiple groups were evaluated non-parametrically by the Kruskal-Wallis method followed by the Wilcoxon rank sum test. For the time course study, Student's *t*-test was used.

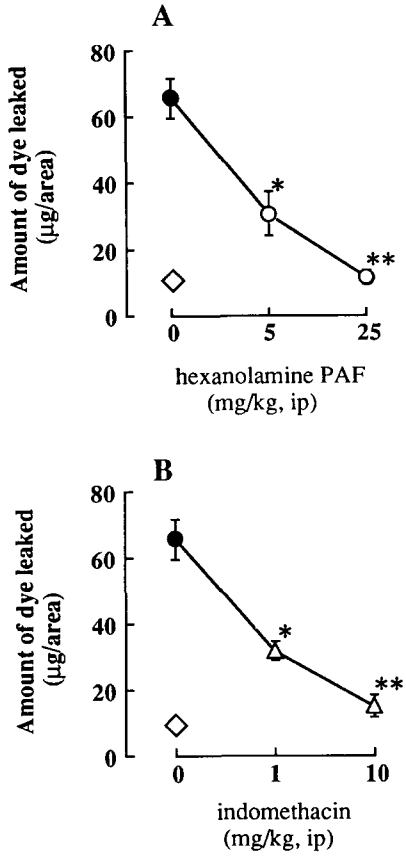

Fig. 3. Effects of hexanolamine PAF (A) and indomethacin (B) on PAF-induced dye leakage in mouse skin. Mice were treated with hexanolamine PAF (○) or indomethacin (△) i.p. 35 min before PAF (180 pmol/site, s.c.). Some mice were injected s.c. with PAF (180 pmol/site) (●) or saline (◇) alone. The dye accumulation was assessed 60 min after the s.c. injection of PAF or saline. Values represent means ± S.E.M. of five experiments. * $P < 0.05$, ** $P < 0.01$ vs. PAF alone.

3. Results

3.1. Effect of PAF on vascular permeability

The time course study revealed that the dye leakage induced by PAF (180 pmol/site) occurred rapidly in the first 5 min and reached a plateau after 30 min (Fig. 1). Therefore we studied the dye leakage induced by PAF at 60 min in further studies. PAF (45–180 pmol/site) produced dose-related increases in vascular permeability (Fig. 2). To confirm the involvement of PAF receptors, we investigated whether or not a PAF receptor antagonist inhibits the cutaneous extravasation. Hexanolamine PAF (5 and 25 mg/kg) significantly inhibited the PAF (180 pmol/site)-induced dye leakage in mouse skin, indicating that the effect of PAF is mediated by PAF receptors (Fig. 3A).

3.2. Effects of eicosanoids on stimulation of vascular permeability by PAF

To examine the role of eicosanoids, we investigated the effect of indomethacin on the PAF-induced increase in vascular permeability. Indomethacin (1 and 10 mg/kg) significantly inhibited the PAF (180

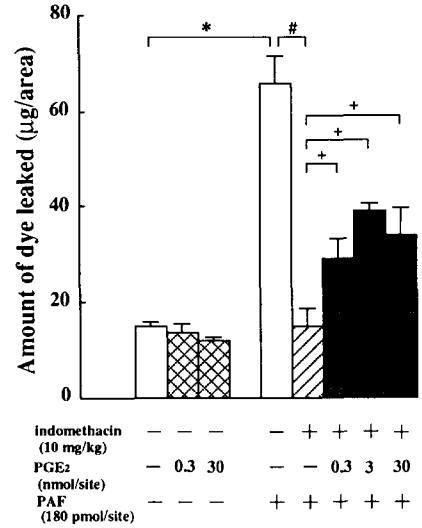

Fig. 4. Reversal by topical prostaglandin E_2 of the inhibitory effect of indomethacin on PAF-induced dye leakage in mouse skin. Indomethacin was administered i.p. 30 min before i.v. injection of pontamine sky blue. Five minutes after pontamine sky blue, prostaglandin E_2 alone or prostaglandin E_2 mixed with PAF (180 pmol/site) was administered to the skin. The dye accumulation was assessed 60 min after the s.c. injection of prostaglandin E_2 with or without PAF. Columns and bars represent the mean ± S.E.M. of five experiments. * $P < 0.001$, # $P < 0.01$, + $P < 0.05$.

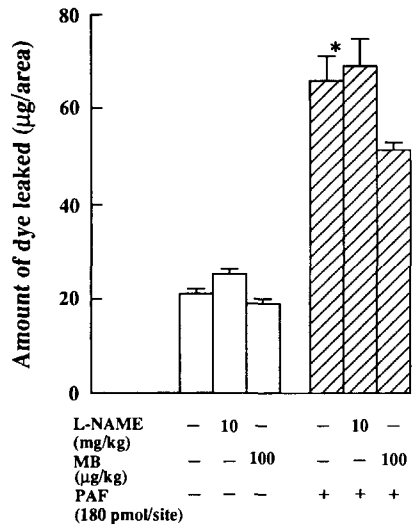

Fig. 5. Effects of N^G-nitro-L-arginine methyl ester and methylene blue (MB) on PAF-induced dye leakage in mouse skin. Saline, N^G-nitro-L-arginine methyl ester or MB was administered i.v. immediately before pontamine sky blue, followed by PAF (180 pmol/site, s.c.) 5 min later. Dye leakage induced by PAF was assessed 60 min after PAF administration. Open columns indicate saline-treated mice, hatched columns PAF-treated mice. Values represent the means ± S.E.M. of five experiments. * $P < 0.001$ vs. saline alone.

pmol/site)-induced dye leakage in mouse skin, indicating that prostanoids are involved in the effect of PAF (Fig. 3B). While prostaglandin E_2 by itself did not alter the vascular permeability, the coadministration of prostaglandin E_2 (0.3–30 nmol/site) mixed with PAF (180 pmol/site) partially reversed the inhibitory effect of indomethacin on the PAF-induced increase in vascular permeability (Fig. 4).

3.3. Effects of inhibitors of NO synthase on stimulation of vascular permeability by PAF

To examine the role of NO, we investigated the effects of inhibitors of NO synthase on the PAF-induced increase in vascular permeability. Neither the basal dye leakage elicited by topical injection of saline nor the increase in vascular permeability induced by PAF (180 pmol/site) was affected by i.v. injection of

Table 1
Effect of topical administration of N^G-nitro-L-arginine methyl ester (L-NAME) on PAF-induced vascular permeability in mouse skin

	Vascular permeability (μg/area)	
	Control	L-NAME
Saline	10.8 ± 1.53	13.1 ± 1.66
PAF	65.6 ± 5.91 [a]	62.5 ± 3.28 [a]

PAF (0.18 nmol/site) was mixed with L-NAME (1 μmol/site) before their s.c. injection and vascular permeability induced by PAF was assessed 60 min later. Values represent the means ± S.E.M. of five experiments. [a] $P < 0.01$ vs. corresponding saline.

Table 2
Effects of systemic N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-D-arginine methyl ester (D-NAME) on PAF- and 5-HT-induced vascular permeability in mouse skin

	Vascular permeability (μg/area)	
	5-HT	PAF
Saline	57.4 ± 7.9	54.7 ± 5.7
L-NAME	37.2 ± 2.2 [a]	58.3 ± 8.7
D-NAME	62.7 ± 10.8	61.5 ± 4.7

Saline, L-NAME or D-NAME was administered i.v. immediately before pontamine sky blue, followed by 5-HT (240 pmol/site, s.c.) or PAF (180 pmol/site, s.c.) 5 min later (two sites per animal). 5-HT and PAF were injected at different sites on the back of the same animal. Vascular permeability induced by 5-HT and PAF was assessed 60 min later. Values represent the means ± S.E.M. of five experiments. [a] $P < 0.05$ vs. corresponding saline.

N^G-nitro-L-arginine methyl ester (10 mg/kg) or methylene blue (100 μg/kg) nor by s.c. coinjection of N^G-nitro-L-arginine methyl ester (1 μmol/site) (Fig. 5; Table 1). When PAF and 5-HT were injected at different sites in the same mouse, systemic N^G-nitro-L-arginine methyl ester stereo-specifically inhibited the cutaneous extravasation elicited by 5-HT, confirming our previous report (Fujii et al., 1994b), but had no effect on that induced by PAF (Table 2).

4. Discussion

We observed that PAF increased dye leakage in mouse skin in a dose-dependent manner, confirming the previous observation that intradermal injection of PAF increases vascular permeability in other species of animals (Humphrey et al., 1982; Teixeira et al., 1993b). We showed that the PAF-induced increase in vascular permeability was significantly inhibited by indomethacin, indicating that cyclooxygenase products play an important role in the PAF-induced increase in vascular permeability in mouse skin. Indomethacin is believed to suppress inflammation by preventing the production of a vasodilator substance, such as prostaglandins, by inhibiting the cyclooxygenase pathway (Vane, 1971). Our finding that the coinjection of prostaglandin E_2 with PAF partially reversed the inhibitory effect of indomethacin would support the involvement of E-type prostaglandins in the effect of PAF.

The role of eicosanoids (prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostacyclin and thromboxane A_2) in mediating the vascular permeability response to PAF has already been documented in shock states (Lefer, 1989; Feuerstein and Hallenbeck, 1987; Filep et al., 1991). In contrast, Teixeira et al. (1993b) suggested that acute inflammatory reactions induced by PAF are not mediated by prostaglandins, based on the lack of any effect

of ibuprofen in guinea-pig skin. The cause of the discrepancy between our findings and those of Teixeira et al. (1993b) on the inhibitory effect of inhibitors of cyclooxygenase on PAF-induced vascular permeability is not clear at present. However, we would point out that there are differences in the experimental conditions used, such as the route of PAF administration (s.c. vs. i.d.), cyclooxygenase inhibitor (indomethacin vs. ibuprofen), and species of animals (mice vs. guinea-pigs).

However, in our study of mouse skin, neither N^G-nitro-L-arginine methyl ester nor methylene blue inhibited the PAF-induced vascular permeability, indicating that NO plays no role in the PAF-induced increase in vascular permeability. Methylene blue is widely used to inhibit soluble guanylate cyclase (Martin et al., 1985). However, it has been found to act as a direct inhibitor of NO synthase by generating superoxide anion and to be a weak and incomplete inhibitor of guanylyl cyclase (Marczin et al., 1992; Mayer et al., 1993). The failure to cause inhibition by the two NO synthase inhibitors in this study could not be due to an insufficient dose, because the doses of N^G-nitro-L-arginine methyl ester and methylene blue used in this study inhibited the 5-HT-induced increase in vascular permeability in mouse skin (Table 2; Fujii et al., 1994b). This result was again in contrast to the observation of Teixeira et al. (1993b) that acute inflammatory reactions induced by PAF are inhibited by local administration of N^G-nitro-L-arginine methyl ester. The cause of the discrepancy is not clear, but might be attributable to species differences, because it is well known that there is a marked species difference in the pharmacological effects of PAF (Braquet et al., 1987). Tomeo and Durán (1991) reported that PAF induces both arteriolar constriction and postcapillary venular permeability responses in hamster cheek pouch. This is in contrast to our hypothesis that PAF dilates arterioles and promotes plasma extravasation from the venular leaky sites. Further research is needed to check whether or not PAF dilates precapillary arterioles in mouse skin.

Filep and Földes-Filep (1993) reported that systemic administration of PAF significantly enhanced albumin extravasation in the various vascular beds (e.g. large airways, stomach and duodenum), but had no effect in the skin of the rat. N^G-Nitro-L-arginine methyl ester treatment markedly pontentiated the PAF-induced albumin extravasation in these tissues, whereas it did not modify the skin response to PAF. These results suggest that NO inhibits the plasma leakage elicited by PAF. The lack of a PAF effect in the skin may be due to the fact that the skin PAF concentration did not rise sufficiently.

The existence of different PAF receptor subtypes on various cells (Hwang, 1988,1990; Kroegel et al., 1989; Growley et al., 1991; Honda et al., 1991; Nakamura et al., 1991) and on arterioles and venules (Tomeo and Durán, 1991) has been reported. To demonstrate that the vascular effect of PAF is specific for a PAF receptor, we arbitrarily chose hexanolamine PAF as a PAF receptor antagonist, because it is a close structural analogue of C_{16}-PAF. Although hexanolamine PAF is a partial agonist at PAF receptors in macrophages and platelets (Grigoriadis and Stewart, 1991), we found that hexanolamine PAF inhibited dose dependently the increase in vascular permeability induced by PAF, indicating that this effect of PAF is mediated by specific PAF receptors. However, the design of the present study does not allow us to specify the subtype of PAF receptor and the type of cells involved in the plasma leakage.

In conclusion, it is suggested that PAF increases vascular permeability through acting on specific PAF receptors. Similarly to bradykinin and histamine, we speculate that PAF increases plasma leakage by increasing postcapillary venular permeability, an effect which is potentiated by arteriolar dilatation through the production of prostanoids. The mechanism of PAF-induced plasma extravasation in mouse skin is different from that of the guinea-pig in that endogenous eicosanoids but not NO play a role in dye leakage in mouse skin.

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