

Platelet-activating factor may act as a second messenger in the release of icosanoids and superoxide anions from leukocytes and endothelial cells

(leukotriene B₄/macrophages/prostacyclin/bradykinin/platelet-activating factor receptor antagonists)

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ABSTRACT Platelet-activating factor (PAF) is generated by endothelial cells, polymorphonuclear leukocytes, and macrophages after activation by appropriate receptor agonists, but much of the PAF remains intracellular. We have investigated whether PAF formation is important for the subsequent generation of icosanoids and superoxide anions by these cells. The generation of prostacyclin and leukotriene B₄ were measured by radioimmunoassay, superoxide anion was measured by reduction of cytochrome *c*, and PAF was measured by bioassay. In each cell type, PAF formation preceded or accompanied icosanoid generation. Bradykinin-induced prostacyclin generation in endothelial cells was markedly reduced by the PAF receptor antagonists WEB 2086 or CV 6209. In guinea pig adherent macrophages *in vitro*, basal prostacyclin generation and that induced by endotoxin and fMet-Leu-Phe were inhibited by either WEB 2086 (1–100 μM) or CV 6209 (0.1–10 μM). In isolated rabbit polymorphonuclear leukocytes, fMet-Leu-Phe stimulated the generation of both leukotriene B₄ and superoxide anion. WEB 2086 and CV 6209 caused concentration-dependent inhibition of both these markers of leukocyte activation. These observations lead us to suggest that PAF may be a second messenger in leukocytes and endothelial cells.

Platelet-activating factor (PAF) is a phospholipid-derived mediator that has been implicated in the pathogenesis of inflammatory diseases (1), cardiovascular shock (2, 3), and thrombosis (4). Participation in these disorders requires the synthesis and release of PAF from immunocompetent cells, including macrophages and neutrophils (5–7). However, in macrophages and neutrophils a significant proportion of the PAF synthesized upon activation remains intracellular (7, 8), but its function remains unknown (9, 10).

Endothelial cells stimulated by a range of compounds including A23187, ATP, and bradykinin (BK) synthesize PAF (11–13), but none is released into the extracellular medium. It has been proposed that PAF is expressed in the outer leaflet of the endothelial cell membrane and causes contacting neutrophils to adhere (13).

We have observed that several PAF receptor antagonists inhibit prostacyclin (PGI₂) production in macrophages (14), and it is well established that PAF mobilizes arachidonic acid in many cell types (1). We now report that specific PAF receptor antagonists, WEB 2086 (15, 16) and CV 6209 (17), inhibit arachidonic acid metabolism and generation of superoxide anions (O₂⁻) initiated by a range of stimuli. Simultaneous production of PAF, arachidonic acid metabolites, and O₂⁻ generation further suggests that PAF may have a second messenger role in endothelial cells, polymorphonuclear leukocytes (PMNs), and macrophages.

MATERIALS AND METHODS

Endothelial Cell Culture. Bovine aortic endothelial cells (BAECs) were obtained from 0.02% collagenase-treated bovine aortae (18, 19) and grown in plastic culture plates until confluent using RPMI 1640 medium containing 20% (vol/vol) fetal calf serum, 15 mM HEPES, penicillin (100 units/ml), and streptomycin (100 μg/ml) (pH 7.4). Experiments were performed using confluent monolayers of BAECs (used between passages 3 and 6) grown in Linbro 6- and 24-well plastic culture dishes. The medium was removed immediately prior to the experiment; the BAECs were washed with RPMI 1640 medium plus 0.25% bovine serum albumin (BSA) and preincubated with PAF receptor antagonists for 15 min before activation. None of the treatments affected the viability of the BAECs (>95%) as assessed by trypan blue exclusion.

Assay of PGI₂ and PAF. PGI₂ was measured by radioimmunoassay of its stable product, 6-oxo-prostaglandin F_{1α} (6-oxo-PGF_{1α}), without prior extraction (20). PAF was measured in the supernatants by bioassay on washed rabbit platelets without prior extraction (14, 21). The cell monolayer was washed twice with RPMI 1640 medium plus 0.25% BSA and extracted with 80% (vol/vol) ethanol (22). Cells were scraped from the plate, the precipitate was removed by centrifugation, and the extract was evaporated to dryness before reconstitution in Tyrode solution plus 0.25% BSA for bioassay. Criteria for identification of PAF bioactivity included antagonism by WEB 2086 (1 μM), resistance to inhibition by indomethacin (2.8 μM), and comigration with authentic [³H]PAF (40 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) on silica gel TLC [chloroform/methanol/H₂O, 65:35:6 (vol/vol)].

Bioassay of Endothelium-Derived Relaxing Factor (EDRF). BAECs were grown to confluence on Cytodex microcarrier beads (Pharmacia) and packed into a column perfused with Krebs solution containing 2.8 μM indomethacin (19). The effluent superfused three strips of rabbit aorta in series that were used to detect the release of EDRF. WEB 2086, the PAF antagonist, was infused directly over the bioassay tissues and then through the cells to investigate whether it affected EDRF release from the cells (19).

Leukocyte Isolation. Resident peritoneal macrophages were obtained from healthy male guinea pigs (0.6–1.0 kg) by lavage using 50 ml of isotonic phosphate-buffered saline containing heparin at 50 units/ml (14). More than 98% of cells showed nonspecific esterase staining. None of the treatments altered the cell viability, which was >95%. The cells were

Abbreviations: BAEC, bovine aortic endothelial cell; BSA, bovine serum albumin; BK, bradykinin; EDRF, endothelium-derived relaxing factor; fMLP, fMet-Leu-Phe; PAF, platelet-activating factor; PMN, polymorphonuclear leukocyte; PGI₂, prostacyclin; 6-oxo-PGF_{1α}, 6-oxo-prostaglandin F_{1α}; O₂⁻, superoxide anion; LT, leukotriene.

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allowed to adhere to Linbro 24-well plastic culture dishes in RPMI 1640 medium plus 20% fetal calf serum for 2 hr. At the end of the adherence period the nonadherent cells (0–20% of total) were removed by washing with RPMI 1640 medium plus 0.25% BSA. The cells were pretreated with the antagonist for 15 min and exposed to stimuli for 60 min after which the supernatants were harvested to assay 6-oxo-PGF_{1α} and PAF.

Rabbit PMNs were prepared as described in detail (14, 23) and resuspended in Tyrode buffer containing 1.8 mM CaCl₂, 0.25% BSA, and 2.8 μM indomethacin at 2–3 × 10⁶ cells per ml (viability was >85%). After antagonist pretreatment (15 min) and stimulation (60 min), leukotriene B₄ (LTB₄) levels in the supernatant were determined by specific RIA (24) without prior extraction. PAF was measured in supernatants and cell extracts (22) by platelet bioassay (14, 21).

O₂⁻ Measurement. O₂⁻ generation was measured by reduction of cytochrome *c* (100 μM; horse heart, type III, Sigma), followed by monitoring the change in absorbance at 550 nm (Pye Unicam spectrophotometer). The superoxide dismutase (30 units/ml; Sigma)-sensitive reduction of cytochrome *c* defined O₂⁻ generation, calculated as nmol of O₂⁻ formed using the extinction coefficient of 21 × 10³ M⁻¹cm⁻¹ (10, 25). Although the responses to fMet-Leu-Phe (fMLP) were complete within 15 min, the A23187-induced increase in LTB₄ levels continued for 20 min. A 60-min incubation period with the stimuli (fMLP or A23187) was chosen to ensure that all responses were complete and to enable a larger number of assays to be carried out simultaneously.

Materials. Hexadecyl PAF was supplied by Nova Biochem (Laufelfinger, Switzerland), 6-oxo-[³H]PGF_{1α} and [³H]LTB₄ were from Amersham, RPMI 1640 medium and fetal calf serum were from Commonwealth Serum Laboratories (Melbourne, Australia) and Flow Laboratories, respectively. Antisera for 6-oxo-PGF_{1α} and LTB₄ RIAs were generous gifts from J. Salmon (Wellcome Research Laboratories). WEB 2086 and CV 6209 were gifts from N. Latt (Boehringer Ingelheim, Australia) and Y. Oka (Takeda, Osaka, Japan), respectively. LTB₄ and LTC₄ were gifts from J. Rokach (Merck Frosst Laboratories, Pointe Claire, Canada). All other chemicals were purchased from Sigma.

RESULTS

Generation and Release of PAF by BAECs, Macrophages, and PMNs. The ionophore A23187 stimulated PAF generation in all cell types tested, as did other cell-specific stimuli (Table 1). In BAECs, PAF was synthesized in response to ATP (10 μM), A23187 (10 μM), or BK (0.01 μM), but none was released into the extracellular medium. The generation of PAF in macrophages stimulated by fMLP peaked at 5 min and declined to a low level at 60 min. Endotoxin also stimulated PAF generation in macrophages but the time course was different: PAF levels were undetectable at 5 min but accumulated in cells and medium after 60 min. In PMNs, fMLP also stimulated PAF generation, which was detectable in supernatants and cell extracts after 60 min (Table 1).

Effects of PAF and PAF Receptor Antagonists on PGI₂ Generation by BAECs. Exogenous PAF (0.1 μM) did not stimulate PGI₂ formation in BAECs. However, BK (0.01–10 nM) stimulated PGI₂ production by confluent BAECs in a concentration-dependent manner (Fig. 1).

The competitive PAF receptor antagonist WEB 2086 (0.1–1.0 μM) caused a substantial rightward shift (≈1.5–2.0 log₁₀ units, *P* < 0.01, analysis of variance) in the BK concentration–response curve, whereas a 10-fold higher concentration of WEB 2086 (10 μM) caused no further displacement. Similar shifts were observed with the more potent PAF receptor antagonist CV 6209 (0.01–0.1 μM, data not shown).

Table 1. PAF generation in BAECs, PMNs, and macrophages in response to BK, A23187, ATP, fMLP, and endotoxin

Stimulus	PAF, pmol per 10 ⁶ cells			
	5 min		60 min	
	Cell associated	Super-natant	Cell associated	Super-natant
BAECs				
Basal	ND	ND (8)	ND	ND
BK (10 nM)	0.76 ± 0.21	ND (8)	0.24 ± 0.17	ND
A23187 (10 μM)	3.42 ± 0.79	ND (8)	2.69 ± 0.66	ND
ATP (10 μM)	0.59 ± 0.31	ND (4)	—	—
Macrophages				
Basal	ND	ND (4)	0.05 ± 0.03	ND
fMLP (1 μM)	0.67 ± 0.05	ND (4)	0.06 ± 0.05	ND
ETX (100 μg/ml)	ND	ND (4)	0.09 ± 0.02	0.09 ± 0.05
A23187 (10 μM)	—	—	1.43 ± 0.61	1.77 ± 0.55
PMNs				
Basal	—	—	ND	ND
fMLP (10 nM)	—	—	0.18 ± 0.07	0.07 ± 0.04
A23187 (1 μM)	—	—	2.46 ± 1.06	0.66 ± 0.22

—, Not tested; ND, not detectable; ETX, endotoxin. Numbers in parentheses are *n*. *n* equals 8 for all 60-min values. Data are presented as mean ± SEM.

A wide range of stimuli for PGI₂ generation by BAECs was examined including histamine, substance P, calcitonin gene-related peptide, [Arg⁸]vasopressin, ATP, BK, LTC₄, A23187, angiotensin II, and U46619. Of these stimuli, only BK, ATP, and A23187 consistently caused generation of 6-oxo-PGF_{1α} (Fig. 2).

The generation of 6-oxo-PGF_{1α} by equiactive submaximal concentration of BK (0.1 nM), A23187 (3 μM), and ATP (10 μM) was inhibited by WEB 2086 (1 and 10 μM) and CV 6209 (0.1 and 1.0 μM). The extent of inhibition was dependent on the stimulus (Fig. 2). Basal generation of 6-oxo-PGF_{1α} was unaffected by the PAF receptor antagonists.

Effects of PAF and PAF Receptor Antagonists on EDRF Release. PAF (0.1–100 nM, 1-min infusion) did not elicit the release of EDRF from perfused BAECs. BK (1–100 nM and 1-min infusion) caused a concentration-dependent release of EDRF from BAECs that showed a characteristically short half-life, as detected on superfused rabbit aortic strips precontracted with phenylephrine (1 μM). The PAF receptor

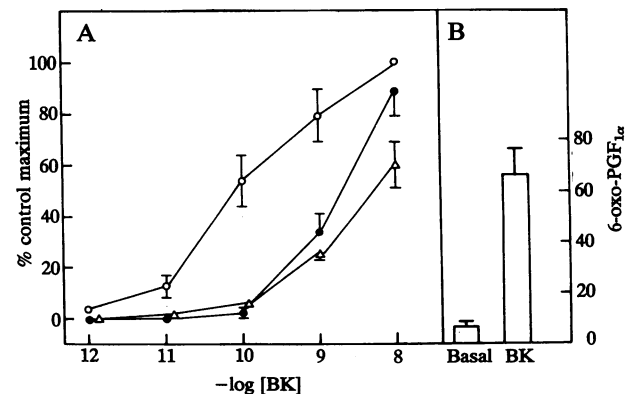


FIG. 1. (A) BK-induced stimulation of BAEC PGI₂ generation and its inhibition by WEB 2086 (0.1 and 1.0 μM). PGI₂ levels, measured as 6-oxo-PGF_{1α} (pmol per 10⁶ cells per hr), are presented as a percentage of the BK-induced increase in generation in the absence of antagonist pretreatment. The data represent the mean and SEM of observations from BAEC cultures from nine bovine aortae. ○, Control; ●, 0.1 μM WEB 2086; △, 1.0 μM WEB 2086. (B) Absolute basal and 10 nM BK-stimulated levels of 6-oxo-PGF_{1α} in the absence of antagonist treatment (control).

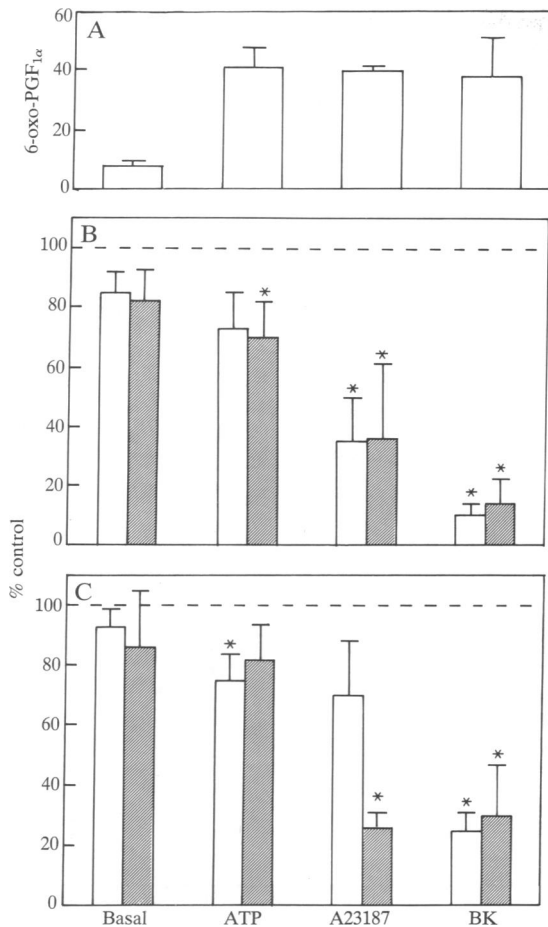


FIG. 2. Comparison of the sensitivity of equieffective concentrations of A23187 (3 μ M), ATP (10 μ M), and BK (0.1 nM) to the PAF receptor antagonists, WEB 2086 (1 μ M and 10 μ M) (B) and CV 6209 (0.1 μ M and 1 μ M) (C). The amount of 6-oxo-PGF_{1 α} (pmol per 10⁶ cells per hr) produced is expressed as percentage of that in the absence of antagonist treatment (control) (A). Data are the mean and SEM of five to nine observations. *, *P* < 0.05, paired *t* test. (B) Bars: open, 1 μ M WEB 2086; hatched, 10 μ M WEB 2086. (C) Bars: open, 0.1 μ M CV 6209; hatched, 1.0 μ M CV 6209.

antagonist WEB 2086 (0.1–1 μ M) did not inhibit EDRF release (*n* = 6, data not shown).

Effects of PAF and PAF Receptor Antagonists on Macrophages. Adherent macrophages generated increased amounts of 6-oxo-PGF_{1 α} in response to stimulation by PAF (0.1 μ M), endotoxin (serotype O11 B4, 100 μ g/ml), and fMLP (1 μ M) over a 60-min period (Fig. 3). Preincubation of macrophages with CV 6209 (0.1–10 μ M) or WEB 2086 (10 and 100 μ M) for 15 min resulted in concentration-dependent inhibition of each of the stimuli for 6-oxo-PGF_{1 α} generation and also markedly reduced the basal generation. The extent of the inhibition was complete for PAF, with maximal inhibitory effects on fMLP and endotoxin of 30–50%, and these were similar for each antagonist. In contrast, PGI₂ generation induced by A23187 (10 μ M) was not affected by either of the PAF antagonists.

Effects of PAF and PAF Receptor Antagonists on PMNs. Exogenous PAF (0.1 μ M) failed to stimulate LTB₄ generation above the low level detected in unstimulated PMNs and elicited only a small increase in O₂⁻ (0.3 \pm 0.1 nmol per 10⁶ PMNs per hr) that was completely prevented by WEB 2086 (10 μ M) or CV 6209 (1 μ M). However, fMLP (10 nM) not only stimulated PAF formation (Table 1) but also induced the generation of significant amounts of LTB₄ and O₂⁻ during a 60-min incubation period with rabbit PMNs (Fig. 4). CV 6209 (0.1–10 μ M) and WEB 2086 (1–100 μ M) reduced both the

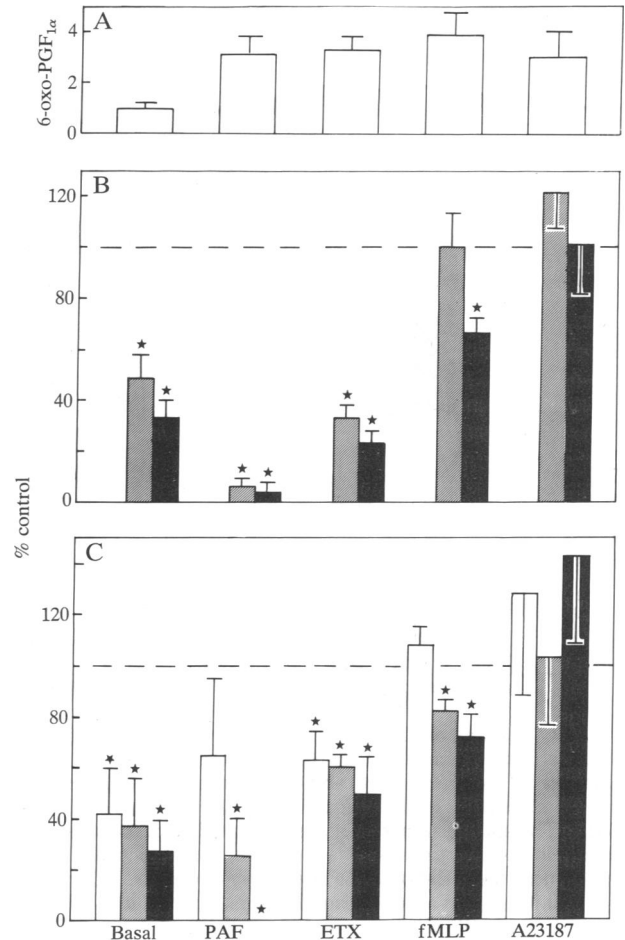


FIG. 3. Inhibition of PGI₂ production in basal, PAF (0.1 μ M), endotoxin (100 μ g/ml, ETX), fMLP (1 μ M), and A23187 (10 μ M) stimulated guinea pig macrophages by PAF receptor antagonists, WEB 2086 (10 μ M and 100 μ M) (B) and CV 6209 (0.1 μ M–10 μ M) (C). (A) Absolute levels of 6-oxo-PGF_{1 α} in the absence of antagonist treatment are shown. The data represent the mean and SEM of eight observations. *, *P* < 0.05, paired *t* test. (B) Bars: hatched, 10 μ M WEB 2086; solid, 100 μ M WEB 2086. (C) Bars: open, 0.1 μ M CV 6209; hatched, 1.0 μ M CV 6209; solid, 10 μ M CV 6209.

LTB₄ and the O₂⁻ generation in response to fMLP. A23187 (10 μ M) also stimulated LTB₄ and O₂⁻ generation by PMNs, but neither response was inhibited by WEB 2086 or CV 6209, except for O₂⁻ at the highest concentration of CV 6209 (Fig. 4). Neither WEB 2086 nor CV 6209 at the highest concentrations used in this study inhibited O₂⁻ generation by a hypoxanthine (100 μ M)/xanthine oxidase (25 milliunits/ml)-generating system (control, 5.9 nmol of O₂⁻; WEB 2086, 6.3 nmol of O₂⁻; CV 6209, 6.5 nmol of O₂⁻).

Time-Course of PAF and Icosanoid Generation. After stimulation of BAECs with BK (10 nM), PAF levels increased after a 30-s exposure and peaked at 2 min, whereas an increase in 6-oxo-PGF_{1 α} was first detected after 60 s and continued to increase up to 5 min (Fig. 5). Thereafter, 6-oxo-PGF_{1 α} levels reached a plateau and those of PAF decreased. A time-course study of PAF and LTB₄ generation by fMLP-stimulated PMNs indicated that these products were synthesized in parallel, whereas in guinea pig macrophages, fMLP-stimulated PAF levels increased before those of PGI₂ (Fig. 5).

DISCUSSION

In BAECs, macrophages, and PMNs, activation with the ionophore A23187 or with stimuli acting on specific receptors

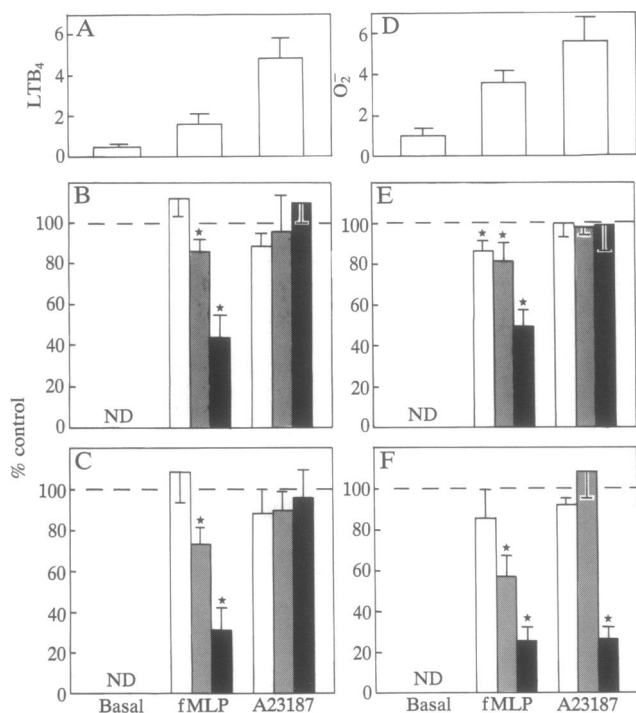


FIG. 4. Effects of PAF receptor antagonists WEB 2086 (1 μM –100 μM) (B and C) and CV 6209 (0.1 μM –10 μM) (C and F) on LTB_4 (pmol per 10^6 cells per hr) (A) and O_2^- (nmol per 10^6 cells per hr) (D) generation in rabbit PMNs induced by fMLP (10 nM) and A23187 (10 μM). LTB_4 and O_2^- generation are presented as percentages of the amount in nonpretreated PMNs (controls, shown in A and D). Data are the mean and SEM of seven or eight observations. ND, not done; *, $P < 0.05$, paired t test. (B and E) Bars: open, 1 μM WEB 2086; hatched, 10 μM WEB 2086; solid, 100 μM WEB 2086. (C and F) Bars: open, 0.1 μM CV 6209; hatched, 1.0 μM CV 6209; solid, 10 μM CV 6209.

caused generation of PAF that remained largely or entirely cell-associated. PAF synthesis appeared to precede PGI_2 generation in BAECs and macrophages, whereas synthesis of PAF and LTB_4 was coincident in PMNs. Competitive PAF receptor antagonists, WEB 2086 and CV 6209, inhibited icosanoid synthesis by each cell type and the generation of O_2^- by PMNs. These observations suggest an involvement of cell-associated PAF in the signal transduction process.

It is likely that the PAF receptor antagonists were acting by antagonism of PAF. Nonspecific actions are unlikely for several reasons: (i) They are structurally distinct: CV 6209 is a potent and highly selective phospholipid analogue antagonist of PAF (17); WEB 2086 is a triazolodiazepine of lesser potency but similar selectivity (15, 16). (ii) CV 6209 has been reported to have no inhibitory effects on PGI_2 synthetase or lipoxygenase (17) over the same concentration range as used in the present study. Moreover, the inhibition of PGI_2 generation by BAECs was dependent on the stimulus used and the failure of both CV 6209 and WEB 2086 to inhibit basal PGI_2 generation (in BAECs) and A23187-stimulated icosanoid generation (in macrophages and PMNs) also suggests that they do not inhibit directly the enzymes of arachidonic acid metabolism. The difference in the extents of inhibition by the PAF antagonists may be related to the distinct mechanisms by which the stimuli activate endothelial cells: BK stimulation involves phospholipase C (26); A23187 elicits an elevation of intracellular Ca^{2+} (27); whereas ATP stimulates the Na^+/H^+ exchanger (28). The lack of effect of PAF antagonists on A23187-induced activation of leukocytes could be considered inconsistent with a role for PAF since A23187 caused the generation of large amounts of PAF. However, A23187 causes a large and persistent increase in

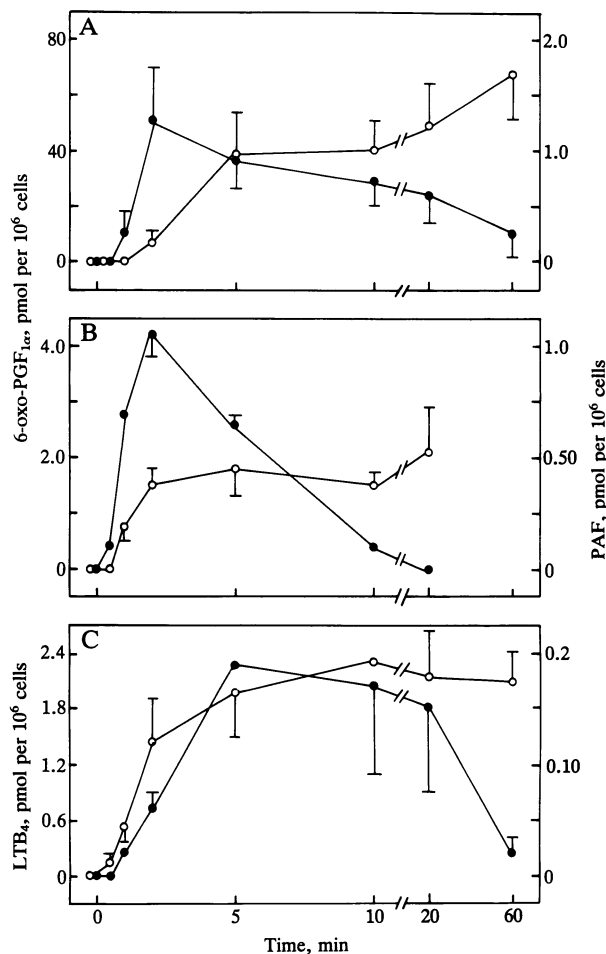


FIG. 5. Relationship between PAF and PGI_2 generation in BAECs (A) stimulated by 10 nM BK and in macrophages (B) and PMNs (C) stimulated by 1 μM fMLP. All the PAF measured in BAECs and macrophages was cell-associated, whereas in PMNs a proportion was released into the supernatant ($n = 4-8$). (A and B) \circ , 6-oxo- $\text{PGF}_{1\alpha}$; \bullet , PAF. (C) \circ , LTB_4 ; \bullet , PAF.

Ca^{2+} that may activate directly O_2^- generation and arachidonic acid metabolism independently of intracellular PAF. Finally, there was no evidence of a direct free-radical scavenging action since these compounds did not affect O_2^- generation by A23187-stimulated PMNs or by a cell-free system (hypoxanthine and xanthine oxidase).

Exogenous PAF stimulated PGI_2 generation by peritoneal macrophages and was a weak stimulus for O_2^- generation by rabbit PMNs, as reported for human PMNs (10). In contrast, exogenous PAF did not activate PGI_2 metabolism in BAECs (29, 30). Nevertheless, exogenous PAF does appear to elicit functional responses in cultured endothelial cells, including morphological changes (31) and a transient increase in intracellular Ca^{2+} (31–33). PAF may act on intracellular receptors, for it has been found that platelet PAF receptors are internalized after binding of PAF (34) and in PMNs, PAF binding sites have been identified in membranes associated with intracellular granules (35). Moreover, external and internal PAF receptors may be linked to distinct cell effector systems. The finding that PAF receptor antagonists inhibit BK-induced PGI_2 generation whereas exogenous PAF fails to stimulate PGI_2 generation is consistent with our hypothesis, if exogenous PAF has restricted access to the intracellular compartment. This may be due to rapid transmembrane metabolism (13). An intracellular PAF receptor may also explain why higher concentrations of the PAF antagonists are required to inhibit intracellular PAF in BAECs and leuko-

cytes than are required to inhibit PAF-induced platelet activation. Alternatively, actions at a PAF receptor subtype may explain these differences (14, 35). The potency of the antagonists not only reflects their biophase concentrations (likely to be lower than the extracellular concentrations) but also is dependent on the biophase concentration of PAF.

In BAECs, the generation of PAF appeared to precede generation of PGI₂, and the peak of PAF formation clearly preceded the peak of PGI₂ formation. In macrophages, fMLP stimulated a short-lived PAF generation with levels falling by 50% after 5–10 min. None of this PAF was released into the extracellular medium, in agreement with other studies (11–13). These observations suggest that PAF is not an intercellular mediator under these conditions. In contrast, in PMNs the intracellular PAF content increased transiently after stimulation with fMLP, but some was released into the supernatant. Thus, in each cell type, intracellular PAF generation precedes or accompanies the response.

PAF appears to be involved in intracellular control mechanisms only in activated cells. However, in adherent macrophages the basal level of PGI₂ was markedly reduced by PAF antagonists, confirming our earlier observations (14). In these adherent cells a low level of cell-associated PAF was detected in the absence of any additional stimulus. These observations suggest that macrophages may be partly activated during the adherence process.

In a recent study (10) of O₂⁻ generation by PMNs, it was proposed that endotoxin induced the synthesis of a low level of cell-associated PAF that amplified fMLP-induced O₂⁻ generation. It could be argued that the inhibitory effects of the PAF antagonists may have resulted from inadvertent exposure to endotoxin during the isolation procedure. However, this seems unlikely in our experiments since no cell-associated PAF was detected in unstimulated PMNs (limit of detection, ≈0.02 pmol per 10⁶ cells). Furthermore, inhibition of O₂⁻ generation by PAF antagonists has been observed in PMNs prepared in endotoxin-tested reagents and these PMNs are sensitive to priming by endotoxin at 1–10 ng/ml (A.G.S. and T.H., unpublished observations). Indeed, our observations suggest that the role of cell-associated PAF may extend beyond priming cells for enhanced O₂⁻ generation to a more integral role in the fMLP-response coupling.

The site of action of intracellular PAF and its interaction with other second messenger systems have not been addressed in the present study. However, it is apparent from studies in a number of cell types that PAF receptors are closely linked to phospholipase C activation by a guanine nucleotide-binding regulatory protein (1). The recognition of the importance of phospholipase C for BK-stimulated PGI₂ production (26, 36) and EDRF release (36) suggests that intracellular PAF may be linked to phospholipase C activation. However, WEB 2086 did not inhibit EDRF release, a finding that may indicate that different isozymes of phospholipase C are involved in the pathways for PGI₂ and EDRF biosynthesis. The production of these vasodilator and antiaggregatory mediators also shows differences in sensitivity to an intracellular Ca²⁺ channel-blocking agent and to modification of external Ca²⁺ levels (37). Phospholipase C activation in PMNs causes formation of the second messengers, inositol triphosphate and diacylglycerol, which are important transducers of O₂⁻ generation (38). Inhibition of PMN responses that are dependent on arachidonic acid metabolism (LTB₄) as well as those that are independent of arachidonic acid metabolism (O₂⁻, ref. 38) suggests that intracellular PAF may be acting at an earlier stage of cell activation.

The present study identifies cellular responses to receptor agonists that (i) are associated with significant intracellular PAF generation, (ii) show a time course that parallels, and in macrophages and endothelial cells follows, that of PAF

generation, and (iii) are sensitive to inhibition by specific PAF receptor antagonists. The fine control apparent in the synthesis and metabolism of PAF is consistent with a second messenger role, as is the amplification from low PAF levels to high levels of both arachidonic acid metabolites and O₂⁻. We interpret these data as indicating that PAF may have a role as a second messenger within these cells.

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