1-O-Alkyl-sn-*glyceryl-3-phosphorylcholines*

A Novel Class of Neutrophil Stimulants

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1-O-Alkyl-2-O-acetyl-*sn*-glyceryl-3-phosphorylcholine aggregates and degranulates platelets and polymorphonuclear neutrophils. Here, the bioactivities of this platelet-activating factor, its 2-O-ethyl, and its 2-lyso derivatives were examined further. Each phospholipid aggregated and degranulated rabbit platelets and neutrophils with relative potencies of about 10,000, 1,000, and 1, respectively. For rabbit neutrophils, the 2-O-acetyl compound was active in nanomolar and lower concentrations; required extracellular calcium and magnesium in order to induce aggregation; and required extracellular calcium and cytochlasin B in order to induce optimal degranulation. Furthermore, the 2-O-acetyl and 2-O-ethyl compounds, in concentrations about tenfold higher than those required for rabbit neutrophils, aggregated and degranulated human neutrophils. With reference to these human neutrophil responses, degranulation required, and aggregation was dramatically enhanced by, cytochalasin B. The lyso analog was unable to induce these responses in the human cells. Thus, these lipids represent a novel class of neutrophil stimulants that closely resemble certain chemotactic factors (eg, C5a and synthetic oligopeptides) in their ability to aggregate and degranulate neutrophils and in the influences which calcium, magnesium, and cytochalasin B have on their bioactions. Because platelet-activating factor circulates in the blood of rabbits and, perhaps, humans during anaphylaxis and is suspected of being involved in other syndromes such as serum sickness, this lipid may have unique biologic significance: it may act to recruit platelets and neutrophils into the lesions of these and similar pathologic syndromes. (Am J Pathol 1981, 103:70–79)

SUBSTANCES that stimulate polymorphonuclear neutrophils (PMNs) to degranulate and aggregate can be classified into complement-related polypeptides (eg, C5a), formylated oligopeptides, bivalent cation ionophores, arachidonic acid and its hydroxylated metabolites, and other substances (eg, phorbol myristate acetate and concanavalin A).[1-6] Here, we report on a new class of compounds related to platelet-activating factor (PAF) (Figure 1) that in nanomolar concentrations induce functional responses in rabbit and human PMNs.

The pathogenesis of inflammatory lesions in rabbits during the course of experimentally induced serum sickness syndromes appears to involve basophil or mast cell degranulation and release of a factor that causes platelets to aggregate and degranulate.[7-11] In acute anaphylactic reactions, a similar substance is found in the blood of rabbits.[12-22] Rabbit or human basophils,[7-13,15-25] mast cells,[11,26] PMNs,[25,27,28] and monocytes[14,25,28] release this substance when exposed to ionophore,[23] C5a,[27] or phagocytosable particles[25,27,28] *in vitro*. Recently, this platelet-activating factor has been characterized as 1-O-alkyl-2-O-acetyl-*sn*-glyceryl-3-phosphorylcholine (PAF).[29-31] PAF aggregates and degranulates platelets (PLs) in subnanomolar concentrations. It is suggested, then, that PAF released *in vivo* may recruit PLs into the lesions of various allergic and autoimmune syndromes. Thus, in serum sickness, antigen may trigger basophils to release PAF, which, in turn, causes PLs to accumulate locally and release substances that alter

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vascular integrity;[7-11] or, alternatively, PAF, again derived from antigenically stimulated basophils, may circulate in the blood during anaphylaxis and induce PLs to aggregate throughout the vasculature, resulting in the clogging of vital capillary networks such as those of the lung.[12-21] It is noted, however, that PMNs appear involved in both of these events: the lesions of serum sickness (eg, coronary arteritis) are characterized by PMN infiltration and do not occur in animals made chronically neutropenic;[7,32] and anaphylactic syndromes induced by PAF or associated with the circulation of PAF are characterized by the abrupt onset of neutropenia as well as thrombocytopenia.[18,19,33,34] It may be, therefore, that PAF has actions on the PMNs which are similar to its actions on the PLs. We have indeed found that PAF can cause PMN aggregation and degranulation.[34] Here we extend these studies to include the 2-O-ethyl analog of PAF (Figure 1), determine the influence of extracellular Ca^{2+}, Mg^{2+} and cytochalasin B on the actions of PAF, and more thoroughly examine the human PMN responses to these lipids. The 2-O-ethyl derivative is of particular interest because it is devoid of a labile C-2 residue. Its mechanism of action and the transient nature of its effects, therefore, should proceed independently of any possible transfer of the C-2 residue.

Materials and Methods

Reagents and Buffers

Bovine serum albumin (BSA), fibrinogen (human), ethyleneglycol-bis-(β-aminoethyl ether)-N-N′-tetraacetic acid (EGTA) and cytochalasin B were purchased from Sigma Chemical Co. (St. Louis, Mo); ^{14}C-serotonin (specific activity 51.5 mCi/mmol) was purchased from New England Nuclear (Boston, Mass). The modified Hanks' balanced salt solution[2,3] and Tyrode's solution (pH 7.2 buffer)[21] are previously described. Where indicated, $CaCl_2$, $MgCl_2$, or EGTA were added to these buffers.

Lipid Preparations

1-O-Alkyl-*sn*-glyceryl-3-phosphorylcholine (L-PAF, Figure 1) was prepared from beef heart choline phospholipids by the method of Blank et al.[31] This material was acetylated at C2 to obtain PAF by a modification of the procedure reported by Gupta et al.[35] Briefly, L-PAF was slurried in chloroform:pyridine: acetic anhydride (8:2:1; vol:vol:vol) and treated with a twofold molar excess of 4-dimethylaminopyridine. After 45 hours of continuous stirring at 25 C, the material was adjusted to pH 3 with 0.5 N HCl in methanol:water (2:1), diluted further with 1 volume of water, and extracted three times with chloroform. The pooled extracts were washed with 0.25 N HCl, dried over anhydrous sodium sulfate, and evaporated. Preparative thin-layer chromatography (chloroform:methanol:acetic acid:water, 75:60:15:5) of the residue gave chromatographically homogeneous material with R_f values similar to those previously reported.[29,31] Furthermore, gas-liquid chromatography and mass spectroscopy (performed by Dr. W. Niehaus, Virginia Polytechnic Institute and State University, Blacksburg, Va) of the saponified, trimethylsilyl derivatives confirmed the structure of L-PAF and PAF and indicated that the C-1 alkyl position contained 65% hexadecyl, 14% octadecyl, and 21% other unidentified ethers (the majority of which was tetradecyl ether).

E-PAF (Figure 1) was synthesized organically from chimyl alcohol (Western Chemical Industries Ltd., Vancouver, Canada). Gas-liquid chromatographic analysis of this alcohol revealed that it contained 82% 1-0-hexadecyl-glycerol, 15% 1-0-octadecyl-glycerol, and 3% 1-0-tetradecyl-glycerol. It was converted into 1-0-alkyl-2-0-ethylglycerol by a three-step process described by Bauman and Mangold.[36] Briefly, chimyl alcohol was tritylated at the C3 oxygen, ethylated at the C2 oxygen using ethyl methanesulfonate, and then detritylated at room temperature with ethanol:ether:concentrated HCl (4:1:1). Infrared and nuclear magnetic resonance spectroscopy established the product as 1-0-alkyl-2-0-ethyl-glycerol. Conversion of this product to E-PAF was accomplished by exposure to phosphoryl chloride and choline tosylate as described by Brockerhoff and Ayengar.[37] The product was purified on Silca Gel chromatographic columns by sequential elution with increasing proportions of methanol:chloroform and was homogeneous on thin-layer chromatography (run as described above). Its structure was confirmed as 1-O-alkyl-2-O-ethyl-*sn*-glyceryl-3-phosphorylcholine by field desorption-mass spectroscopy performed by Dr. M. M. Bursey, University of North Carolina, Chapel Hill, and by nuclear magnetic resonance spectroscopy. Residues at C1 in E-PAF were essentially the same as the chimyl alcohol precursor (as evidenced by gas chromatography of the reduced product). Each of the product lipids was stored in chloroform:methanol (2:1) at −20 C at a concentration of 2–4 mg/ml as determined by lipid phosphorous.[38] Small portions of the solution were evaporated under a stream of nitrogen and taken up in the appropriate buffer containing 2.5 mg/ml BSA. This solution was further diluted with the same BSA-containing buffer. The appropriate amount of stimulus was added to cells in a ratio

of 5–50 μl of the stimulus (in BSA buffer) to 1 ml of the cell suspension.

Platelet Isolation and Bioassays

Platelet-rich plasma (PRP) was isolated from rabbit whole blood anticoagulated with 1/9 volume of acid-citrate-dextrose by centrifugation (200*g* for 10 minutes).[21] For studies on the release reaction, the PRP was incubated with 0.5 μCi/ml ^{14}C-serotonin at 37 C for 20 minutes. PLs were isolated from PRP by centrifugation (800*g* for 10 minutes) and washed twice in calcium-free Tyrode's buffer containing 0.1 mM EGTA. Subsequent to washing, the PLs were suspended at 250,000/μl in Tyrode's buffer containing 4.0 mM calcium and incubated at 37 C for 20 minutes. PL release was carried out by adding 200 μl of this suspension to 10 μl of the stimulus. After 1 minute, 20 μl of formaldehyde (1.5 M) was added and the PLs placed on ice. Shortly thereafter the aliquots were centrifuged to obtain supernatant fluid, which was assayed for radiolabel with a Tracor Analytic Model 1185 Auto Gamma Counter (Tracor Corp., Atlanta, Ga). Stimulated release was always compared with that found in suspensions exposed to 10 μl of BSA-buffer (for backround release) and to suspensions exposed to 0.1% Triton X-100 (for total cell content).

For PL aggregation, PLs were isolated from PRP, washed as described above, suspended in Tyrode's buffer containing 4.0 mM calcium and 1 mg/ml fibrinogen, and incubated briefly at 37 C. Aggregation was evaluated by the turbidometric technique with a Chronolog Aggregometer (Chronolog Corp., Havertown, Pa) at a PL concentration of 250,000/μl.

PMN Isolation and Bioassays

Rabbit peritoneal PMNs were obtained 3–5 hours after the intraperitoneal instillation of 0.1% shellfish glycogen.[2] Human PMNs were isolated by centrifugation of normal donor blood through Ficoll-Hypaque discontinuous gradients.[2,3] These preparations were briefly exposed to hypotonic media to lyse contaminating erythrocytes. The final preparations contained fewer than 5 PLs per 100 PMNs and no erythrocytes. For aggregation studies, human PMNs were suspended (4500 cells/μl) in Hanks' buffer, incubated for 4 minutes at 37 C, treated with 1.4 mM $CaCl_2$ and 0.7 mM $MgCl_2$, and 1 minute thereafter exposed to a stimulus. Where indicated, cytochalasin B (0.5 μg/ml) was added at the onset of the incubation period. For rabbit cells, this procedure led to aggregation when the bivalent cations were added. We obviated this result by including the cations in the washing fluid and suspending buffer before the incubation period. The rabbit cells (4500/μl) were then incubated at 37 C for 5 minutes and exposed to a stimulus. Just before and at ¼, ½, 1, 2, 4, 8, 11, and 15 minutes after this exposure (in rabbit or human PMN suspensions), 20-μl samples were taken from the suspension, diluted in 10 ml of 37 C Isoton (Coulter Electronics, Hialeah, Fla), and immediately analyzed for total and large particle concentrations with a Model ZBI Coulter Counter (Coulter Electronics, Hialeah, Fla) as described previously.[2,3,39] Large particles were defined as particles greater than 1.8 times the (volume) size of unaggregated PMNs. The results are reported as the large particle percentage (LPP) or the maximal change in the LPP (MLPP). The LPP is 100 times the large particle concentration divided by the total particle concentration. The MLPP is the largest LPP found at ¼, ½, 1, 2, 4, 8, 11, or 15 minutes after treatment with a stimulus minus the LPP found just before this treatment. Rises in the LPP and MLPP indicate the formation of PMN aggregates.[2,3,39] We have previously determined that standard PL aggregating agents (eg, thrombin, adenosine diphosphate, and epinephrine) do not aggregate the PMN suspensions.[3] This, plus the paucity of PL contamination, effectively excludes a role for PLs in the PMN response, as measured here.

For degranulation studies, 0.5 ml of a rabbit PMN suspension (2600 cells/μl) was incubated with 1.4 mM calcium and 0.7 mM magnesium for 20 minutes at 37 C and then exposed to 5 μg/ml cytochalasin B plus a test stimulus. For human PMNs, cells were first incubated with cytochalasin B for 2–4 minutes before exposure to the stimulus; for rabbit PMNs, cells were exposed to a stimulus and cytochalasin B simultaneously. After 5 minutes of incubation with a stimulus, the cells were placed on ice and, shortly thereafter, centrifuged (800*g* for 3 minutes) so that we could obtain supernatant fluid, which was assayed for lysozyme, B-glucuronidase, and lactic acid dehydrogenase, using previously described methods.[39] Release was always compared with that occurring in cells exposed to BSA plus cytochalasin B (for background release) and with cells exposed to 0.1% Triton X-100 (for total cellular enzyme content).

Cellular Toxicity

The stimuli and conditions used here did not cause PMNs to release significant amounts of the cytosolic enzyme lactic acid dehydrogenase.

Results

Rabbit Platelets

Rabbit PLs suspended in Tyrode's buffer containing calcium and fibrinogen promptly aggregated after exposure to PAF, E-PAF, and L-PAF. At all but the highest doses of these lipids, the responses rapidly reversed. About 60 pM PAF induced a half-maximal response (ED_{50}). This value closely agrees with that reported by others.[29,30] The ED_{50} values for E-PAF and L-PAF were 1.7 nM and 5.0 μM, respectively. PAF and E-PAF (L-PAF was not studied in this fashion) also induced serotonin release; ED_{50} values for these responses were 200 pM and 9 nM, respectively. (Degranulation of PLs in conjunction with serotonin release was verified by electron microscopy). Thus, the relative potencies of PAF, E-PAF, and L-PAF for activating PLs were about 4×10^4, 10^3, and 1. To insure that the activity for E-PAF was not due to contamination with PAF, PAF and E-PAF were saponified by exposure to mild alkaline conditions as described.[34,40] Under these conditions the aggregating action of PAF was reduced 1000-fold; the action of E-PAF was virtually uninfluenced by this treatment (data not shown). Taken together, these data indicate that the test lipids are platelet-activating factors and that the bioaction of E-PAF is not due to PAF contamination.

Rabbit PMNs

Figure 2 shows that PAF and E-PAF also aggregated rabbit PMNs; at all effective doses of each lipid, the response rapidly reversed. These responses were performed in cells suspended in Hanks' buffer containing calcium and magnesium. In the absence of these cations (ie, either calcium, magnesium, or both), the response did not occur (Table 1). Each lipid was optimally active between 20 and 200 nM; higher doses induced submaximal responses (not shown); incremental decreases in dosage produced progressively less of a response. However, the optimal response to E-PAF was not as prominent as the response to PAF. Furthermore, only PAF was active at 2 and 0.2 nM (Figure 2, upper two panels). L-PAF (Figure 2, lower panel) was virtually inactive: at 200 and 600 nM it induced only slowly developing, comparatively small amounts of aggregation; at 20 nM or lower doses, it induced no response.

The lipids also stimulated rabbit cells to degranulate. PAF and E-PAF were active at very low concen-

PLATELET ACTIVATING FACTOR AND ITS ANALOGUES

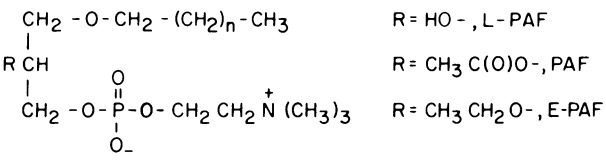

Figure 1—The structure of PAF, E-PAF, and L-PAF.

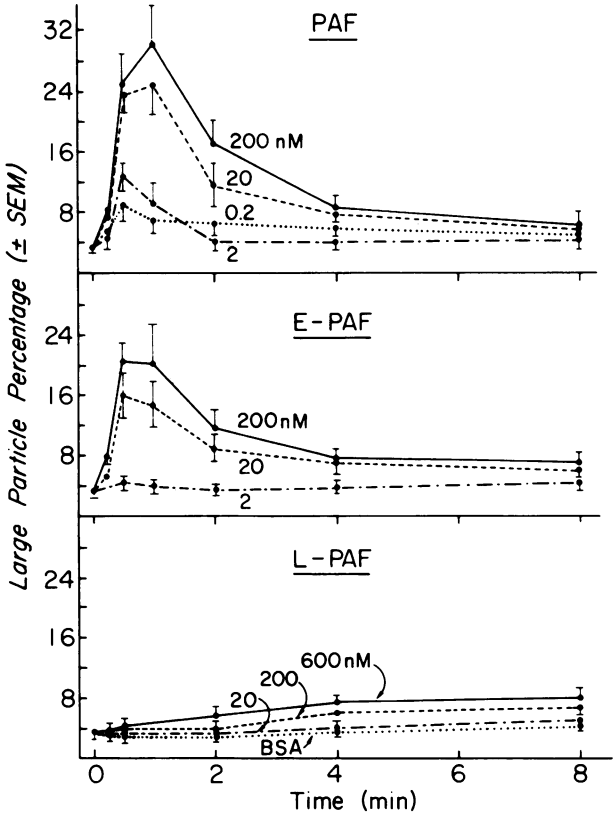

Figure 2—Large particle percentage of rabbit neutrophil suspensions after exposure to the indicated concentration of PAF, E-PAF, L-PAF, or bovine serum albumin. Each curve gives mean values for six experiments.

Table 1—The Influence of Extracellular Calcium and Magnesium on the Action of PAF Upon Rabbit Neutrophils*

Cation present	Aggregation†	Degranulation‡ Lysozyme	Degranulation‡ β-glucuronidase
Ca^{2+}, Mg^{2+}	10.4 ± 1.6§	49.8 ± 5.9	39.7 ± 2.7
Ca^{2+}	0.7 ± 0.3	51.5 ± 4.0	55.4 ± 9.1
Mg^{2+}	0.8 ± 0.2	17.6 ± 6.6	19.0 ± 1.3
None	0.3 ± 0.2	25.0 ± 6.6	17.2 ± 5.1

* Calcium and magnesium were added in a final concentration of 1.4 and 0.7 mM, respectively. The cells were then exposed to 200 nM PAF.

† Expressed as the maximal change in the large particle percentage found after stimulation.

‡ Expressed as percentage of total cellular enzyme released minus the release found in unstimulated cells.

§ Each value is the mean of five or more experiments ± SEM.

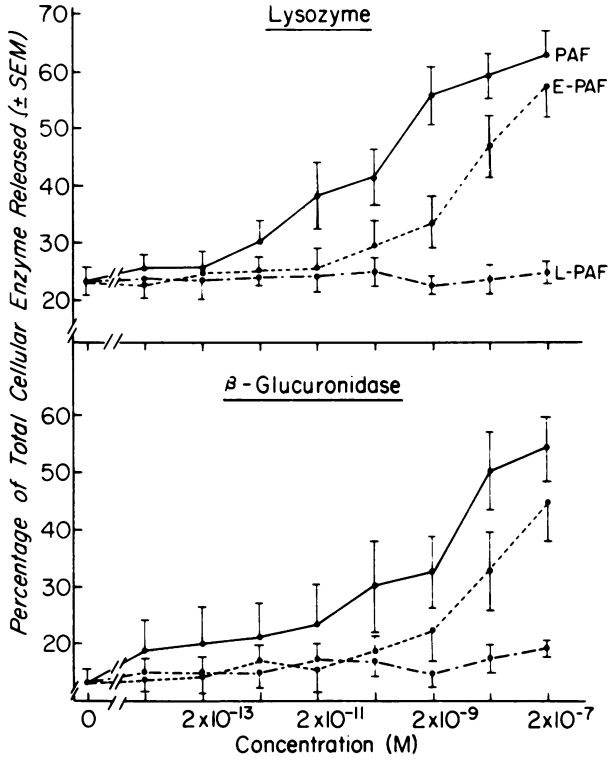

Figure 3—Release of lysozyme and β-glucuronidase by rabbit neutrophils exposed to varying concentrations of PAF, E-PAF, or L-PAF. Each point is the mean of at least five experiments. Cells exposed to 0 concentration of stimulus were exposed to bovine serum albumin.

trations (ie, 0.02–2 nM); L-PAF required concentrations greater than 0.2 μM to effect even partial release of lysozyme and B-glucuronidase (Figure 3). For release of either enzyme, PAF was about 20–50 times more active than E-PAF and greater than 1000-fold more active than L-PAF. At all concentrations, however, the lipids did not cause PMNs to release the cytosolic enzyme, lactic acid dehydrogenase. It is emphasized that degranulation required the presence of cytochalasin B. In the absence of this lysosomal labilizing agent,[41] for instance, even 2 μM PAF or L-PAF did not degranulate the cells.

Degranulation induced by PAF was complete within 2 minutes of exposure of the cells to the stimulus (not shown). Thus, this response developed about as rapidly as the aggregation response (Figure 2). Degranulation required extracellular calcium: cells preincubated in Hanks' buffer free of added calcium (with or without 1 mM EGTA) and containing cytochalasin B exhibited less than 50% of the response of cells suspended with calcium at all active concentrations of drug (Table 1).

Human PMNs

PAF and E-PAF also caused human cells to aggregate (Figure 4). This response was similar to that seen in rabbit cells in that it was transient, induced by submicromolar concentrations of lipid, and blunted at higher (eg, 2 μM) doses of stimulus. However, compared with the response of rabbit PMNs, the magnitude of this response was small at all concentrations of the stimulus and required about tenfold greater concentrations of the compounds. Cytochalasin B (0.5 μg/ml) dramatically enhanced the magnitude and duration of the human PMN response (Figure 4, upper panel). L-PAF, again, was virtually ineffective in stimulating this response in the absence or presence of cytochalasin B (Figure 4).

PAF and E-PAF also degranulated cytochalasin B-pretreated human PMNs (Figure 5). The magnitude of this response was lower than the response of rabbit cells; furthermore, human cells required a tenfold greater amount of the stimulus in order to give appreciable responses (compare Figures 3 and 5). Again, cytochalasin B was required for degranulation. In its

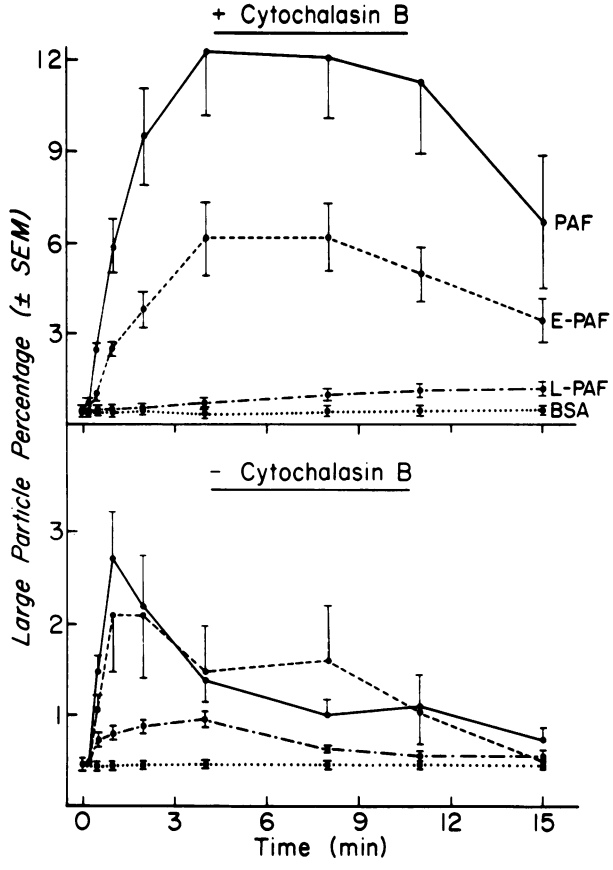

Figure 4—Large particle percentage of human neutrophil suspensions after exposure to 200 nM PAF, 200 nM E-PAF, or 600 nM L-PAF. Cells were pretreated with (*upper panel*) or without (*lower panel*) 0.5 μg/ml cytochalasin B for 5 minutes before exposure to the stimulus. The lowest curve in each panel gives the effects of bovine serum albumin. Each curve gives mean values for at least eight experiments.

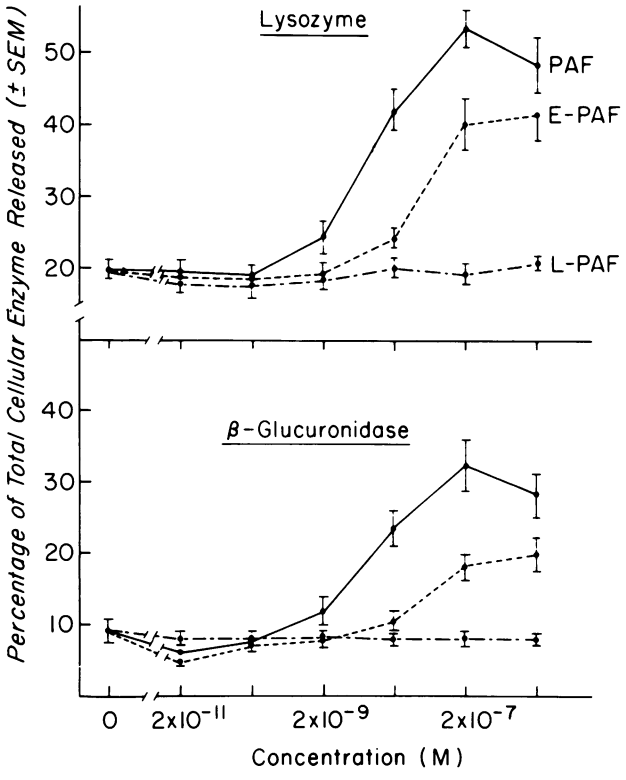

Figure 5—Release of lysozyme and B-glucuronidase by human neutrophils exposed to varying concentrations of PAF, E-PAF, or L-PAF. Each point is the mean of at least eight experiments. Cells exposed to 0 concentration of stimulus were exposed to bovine serum albumin.

absence, neither lipid degranulated the cells (not shown). L-PAF, in concentrations as high as 1 μM, did not degranulate cytochalasin B-pretreated PMNs (Figure 5).

Discussion

PAF and E-PAF stimulate PMN aggregation and degranulation in a manner resembling that of certain chemotactic factors (eg, C5a and formylated oligopeptides) and arachidonic acid. These chemotactins, arachidonate, and PAF degranulate PMNs optimally only in the presence of extracellular calcium and cytochalasin B[5,41,42] (Table 1); aggregate the cells only in the presence of extracellular calcium and magnesium[43-45] (Table 1); induce a rapid burst of degranulation, which is completed within 2 minutes;[5,42,46] and cause transient aggregation responses, the magnitude and duration of which are dramatically enhanced by cytochalasin B[43,44] (Figures 2, 4, and 5). In contrast, ionophores (eg, A23187), phorbol myristate acetate, and concanavalin A produce appreciable degranulation in the absence of cytochalasin B,[41,46-49] degranulation which progresses over at least 15 minutes,[41,46-49] and irreversible aggregation.[42,44,50] Furthermore, phorbol myristate acetate stimulates prominent degranulation and aggregation in the absence of extracellular calcium[49,50] and causes an aggregation response that is virtually uninfluenced by cytochalasin B;[50] and concanavalin A aggregates PMNs in the absence of extracellular calcium and magnesium.[42] As a final point distinguishing these stimuli, arachidonic acid does not degranulate human PMNs.[39] The lipids studied here, therefore, appear to activate human and rabbit PMNs by mechanisms that, at least on the basis of these initial investigations, are similar to those employed by C5a and synthetic oligopeptides, through pathways dependent upon the presence of extracellular calcium and greatly influenced by cytochalasin B.[1]

Also similar to those of C5a and synthetic oligopeptides,[1] the bioactions of these lipids have a high degree of structural specificity: slight alterations (eg, removal [L-PAF] or reduction [E-PAF] of the C-2 acetyl residue) in PAF profoundly influence its potency. It has been suggested that PAF donates its C-2 acetyl group to a cellular element which, when acetylated, triggers PL function.[51] Alternatively, cells rapidly deacylate PAF[52] and, conceivably, could use this as a mechanism to limit or reverse their response to the lipid. Deacylation, then, may underlie the transiency of the PMN and PL aggregation response. Neither the acylation nor deacylation mechanisms, however, explains the actions of E-PAF (which contains a relatively nonlabile ethyl ether at C-2 [Figure 1]). These data are, however, compatible with the hypothesis that this stimulation is mediated by a single receptor for PAF and E-PAF. Indeed, in preliminary studies we find that PAF and E-PAF can desensitize PMNs and PL to subsequent challenge with PAF or E-PAF but not with other stimuli. This selective desensitization (which was performed as described by Demopoulos et al[29] and O'Flaherty et al[53]) suggests that stimulation may proceed through receptor activation.[53] The varying potency of the three lipids, then, may reflect their varying affinities for a cell-associated receptor; the similar potency profiles of the lipids in stimulating PMN and PL responses may indicate that both cell types possess this receptor; and the common influences which extracellular calcium, magnesium, and cytochalasin B have on the PMN responses to chemotactic factors and PAF, together with the similar kinetics of the responses induced by these stimuli, may indicate that similar intercellular response pathways are activated by chemotactic and phosphorylcholine receptors. Whether or not receptor-mediated, one important difference between the bioactions of PAF and chemotactic factors exists: C5a and the formylated oligopeptides do not stimu-

late PL function.[3,42] A unique pathophysiologic significance for PAF may be attributable to this difference.

Stimuli that aggregate and degranulate PMNs or PLs *in vitro* frequently possess a well-defined set of *in vivo* actions: PMN stimuli cause neutropenia, stasis of PMNs in lung capillaries, and mild pulmonary dysfunction when injected into rabbits;[1,53-57] PL stimuli produce an analogous syndrome of thrombocytopenia, PL stasis in lung capillaries, and severe pulmonary dysfunction.[58-60] The toxicity of these stimuli may reflect their respective abilities to stimulate PMNs or PLs *in vivo*; ie, they may cause circulating blood cells to aggregate, settle within, and occlude lung blood vessels, and release deleterious substances.[1,53-61] Ventilation-perfusion abnormalities, vascular damage to lung, and pulmonary dysfunction may ensue. (However, chemotactic substances have yet to be shown to cause histologically identifiable tissue injury in our animal models). PAF circulates in the blood of rabbits[12-22] and, perhaps, man[9] during anaphylaxis; on the basis of *in vitro* bioactions of PAF upon (rabbit and human) PMNs and PLs, the suggestion is made that this circulating PAF may be responsible for the thrombocytopenia, neutropenia, and pulmonary dysfunction of anaphylactic and other acute allergic reactions. The ability of PAF to produce these cytopenias and an anaphylactic syndrome when injected into rabbits[33,34] along with a similar ability of phorbal myristate acetate, a drug that also aggregates and degranulates PMNs and PLs *in vitro* to produce these cytopenias and pulmonary dysfunction, support this possibility. There is, then, a fairly consistent association between the *in vitro* actions and *in vivo* toxicities of a series of aggregating agents that suggests that circulation of naturally occurring PMN or PL stimuli (eg, C5a or PAF) may be injurious. Furthermore, in subacute allergic reactions such as serum sickness, PAF released by stimulated leukocytes[7-28] may cause PMNs and PLs to accumulate locally and contribute to vascular lesions.[7-11] In a diversity of syndromes, then, which associate cytopenia with unexplained lung dysfunction as well as in autoallergic inflammatory conditions PAF may recruit PMNs and PLs into the pathologic lesions.

However, the bioactions of PAF, as described here, complicate as well as enrich our study of these various clinical and experimental syndromes. Recent reports indicate that PMN aggregating activity, presumably C5a, circulates freely in the blood of patients during the progress of various shocklike and immunologic syndromes.[62,63] This aggregating activity was detected by adding patients' plasma to cytochalasin B-pretreated normal human PMNs. As seen in Figure 4 (upper panel), such aggregating activity could be due to PAF rather than to C5a. Although PAF is rapidly inactivated by serum[21] and therefore may not be responsible for this effect, further studies to exclude a role for PAF (or other aggregating agents) and to determine whether this activity influences PL appear necessary. Indeed, because C5a can induce PMNs to release PAF,[27] the possibility that PAF mediates the PMN response to C5a and, potentially, other stimuli, must be considered. We are currently investigating this possibility. Our evidence to date suggest that this may not be the case: animals given injections of C5a or other chemotactic factors develop profound neutropenia with no appreciable change in the circulating PL count. Nevertheless, the role of PAF in the various experimental and clinical syndromes discussed needs further evaluation.

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