Opposing Effects of Platelet-Activating Factor and Lyso-Platelet-Activating Factor on Neutrophil and Platelet Activation

Emily J. Welch, Ram P. Naikawadi, Zhenyu Li, Phoebe Lin, Satoshi Ishii, Takao Shimizu, Chinnaswamy Tiruppathi, Xiaoping Du, Papasani V. Subbaiah, and Richard D. Ye

Department of Pharmacology (E.J.W., R.P.N., Z.L., P.L., C.T., X.D., R.D.Y.) and Medicine (P.V.S.), University of Illinois College of Medicine, Chicago, Illinois; and Department of Biochemistry and Molecular Biology, University of Tokyo, Tokyo, Japan (S.I., T.S.)

Received July 31, 2008; accepted October 17, 2008

ABSTRACT

Platelet-activating factor (PAF) is a potent, bioactive phospholipid that acts on multiple cells and tissues through its G protein-coupled receptor (GPCR). PAF is not stored but is rapidly generated via enzymatic acetylation of the precursor 1-O-hexadecyl-2-hydroxy-sn-glycero-3-phosphocholine (lysoPAF). The bioactivity of PAF is effectively and tightly regulated by PAF acetylhydrolases, which convert PAF back to lysoPAF. Previous studies report that lysoPAF is an inactive precursor and metabolite of PAF. However, lysoPAF has not been carefully studied in its own context. Here we report that lysoPAF has an opposing effect of PAF in the activation of neutrophils and platelets. Whereas PAF potentiates neutrophil NADPH oxidase activation, lysoPAF dose-dependently inhibits this function. Inhibition by lysoPAF is not affected by the use of a PAF receptor antagonist or genetic deletion of the PAF receptor gene. The mechanism of lysoPAF-mediated inhibition of neutrophils involves an elevation in the intracellular cAMP level, and pharmacological blockade of adenylyl cyclase completely reverses the inhibitory effect of lysoPAF. In addition, lysoPAF increases intracellular cAMP levels in platelets and inhibits thrombin-induced platelet aggregation, which can be reversed by inhibition of protein kinase A. These findings identify lysoPAF as a bioactive lipid with opposing functions of PAF and suggest a novel and intrinsic regulatory mechanism for balance of the potent activity of PAF.
In this process, phospholipase A₂ cleaves membrane phospholipids at the sn-2 position and yields several lipid products, including lysoPAF (1-O-hexadecyl-2-hydroxy-sn-glycero-3-phosphocholine). The highly specific PAF-acetyltransferase (PAF-AT) then adds an acetyl group to the sn-2 position to yield PAF (Shindou et al., 2007). PAF is subsequently degraded through the removal of the acetyl group by PAF-acetylhydrolases (PAF-AH) (Karasaki et al., 2003). The PAF-AH-mediated conversion to lysoPAF is a primary pathway for PAF inactivation, and there have been efforts in the exploration of PAF-AH as a potential therapeutic agent (Quarck et al., 2001; Arakawa et al., 2005; Gomes et al., 2006). The levels of PAF-AH are inversely correlated with the severity of anaphylaxis (Vadas et al., 2008), and the administration of human recombinant PAF-AH can inhibit several mouse models of anaphylaxis (Fukuda et al., 2000).

Although lysoPAF is believed to lack bioactivity and is often used as a control in experiments exploring PAF function, it has not been carefully studied in its own context. Given that lysoPAF levels are inversely related to those of PAF and that it is a metabolite and a precursor of PAF, there seems to be a need to understand any potential activity that this lipid may have. Furthermore, the potential therapeutic use of recombinant PAF-AH in humans requires a comprehensive understanding of the cellular functions of the lipid produced by this enzyme. In the course of a study of neutrophil activation by bioactive lipids, we found that lysoPAF exhibits an inhibitory effect on neutrophil superoxide production. Further investigation has led to the discovery of lysoPAF-induced intracellular cAMP production as a potential mechanism for its inhibitory effect. Moreover, treatment of platelets with lysoPAF reduces their aggregation after low-dose thrombin stimulation. These data suggest that lysoPAF balances some of the bioactivities of PAF, and the beneficial effects of PAF-AH may be attributed in part to this negative regulatory function of lysoPAF.

Materials and Methods

Materials. Synthetic lysoPAF (16:0, 18:0) and PAF (16:0) were obtained from Avanti Polar Lipids (Alabaster, AL). The lipids were dissolved in 50% (v/v) EtOH/H₂O. Percoll was purchased from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). The protein kinase A (PKA) inhibitors H-89 and KT5720 and the adenylyl cyclase inhibitor SQ22536 were purchased from Calbiochem (San Diego, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, NJ). Isoluminol, C₅a, fMLF, and cholera toxin were obtained from Invitrogen (Carlsbad, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanofi-aventis, Bridgewater, CA). The PAF receptor (PAFR) antagonist SR27417 was a generous gift from Dr. J. M. Herbert (sanof...
became more evident (a 6.92-fold increase) after fMLF stimulation ($p < 0.01$). LysoPAF (1 $\mu$M) caused a 57% reduction of the fMLF-induced superoxide production compared with buffer-treated neutrophils under the same experimental conditions (Fig. 1, A and B). These results suggest that lysoPAF is bioactive and produces an inhibitory effect on fMLF-induced neutrophil superoxide production.

The alkyl group in PAF and lysoPAF is connected through an ether linkage at the C1 position to a carbon chain of variable lengths. In the above study, 16:0 PAF and lysoPAF was used. To determine whether the length of the carbon chain affects the bioactivity of lysoPAF, we examined 18:0 PAF and lysoPAF from the same source and found it to be equally effective in the inhibition of fMLF-induced superoxide production (Fig. 1C). Next, we determined the potency of PAF and lysoPAF in superoxide production assays. Both PAF and lysoPAF exhibited bioactivity at concentrations as low as 10 nM. The opposing effects of PAF and lysoPAF on superoxide production continue to increase up to the concentration of 1 $\mu$M (Fig. 1D).

Neutrophil activation can lead to microendothelial injury, manifested as increased endothelial permeability and loss of barrier function. These changes are seen during Gram-negative bacterial infection and in numerous inflammatory disorders such as adult respiratory distress syndrome (Lee and Downey, 2001). In addition, pulmonary edema is one of the hallmarks of anaphylaxis. We determined the effects of PAF and lysoPAF on neutrophil-dependent changes of endothelial permeability by measuring transendothelial electrical resistance (TER), a well established in vitro assay of endothelial barrier function (Furie et al., 1984; Tiruppathi et al., 1992). In this assay, changes in TER reflect the integrity of the endothelial monolayer, which is compromised in the presence of reactive oxygen species from activated neutrophils. As shown in Fig. 2A, fMLF stimulation of neutrophils caused a

![Fig. 1](image1.png)

**Fig. 1.** PAF and lysoPAF differentially regulate fMLF-induced neutrophil NADPH oxidase activation. A, detection of fMLF-induced superoxide generation as a function of time. CPS, counts per second of chemiluminescent light emitted. Isolated human neutrophils ($5 \times 10^6$/sample) were preincubated with indicated lipid (1 $\mu$M) or vehicle for 5 min before stimulation with 1 $\mu$M fMLF. Shown is a set of representative tracings from one of the three experiments that produced similar results. B, data from all three experiments were quantified based on integrated areas under curve (AUC) and then expressed as a percentage of fMLF-induced response using the condition without lipid pretreatment as 100%. C, comparison of the effects of lysoPAF pretreatment using two lysoPAF species with different chain lengths (16:0, 18:0) on fMLF-induced superoxide production in neutrophils from different donors ($n = 3$). D, dose-response of lysoPAF and PAF in fMLF-induced superoxide generation. Shown is a representative set of data, as mean $\pm$ S.E.M., from one of the three experiments that produced similar results.

![Fig. 2](image2.png)

**Fig. 2.** PAF- and lysoPAF-treated neutrophils affect endothelial barrier function. A, HPMECs were grown to confluence on a gold electrode. Human neutrophils ($4 \times 10^6$) were preincubated with 1 $\mu$M concentration of the indicated lipid or vehicle control for 10 min, added to the electrode well, and stimulated with 1 $\mu$M fMLF. The changes in TER were recorded over time. Data shown are from one representative experiment chosen from a total of four similar experiments. No significant changes in TER observed with PAF or lysoPAF in the absence of PMNs (data not shown). B, the peak values at the 4-h time point of the above experiments are shown as mean $\pm$ S.E.M. based on four separate experiments; ns, not significant, *, $p < 0.05$; ***, $p < 0.001$. 
decrease in TER over time, and this change was further enhanced by pretreatment of neutrophils with PAF (1 μM). In contrast, lysoPAF used at the same concentration significantly reduced the fMLF-stimulated decrease in TER but had no significant effect on TER when applied alone (Fig. 2B). These results suggest a potential function of lysoPAF in preventing neutrophil-mediated endothelial injury.

LysoPAF Does Not Block the fMLF Receptor. Inhibition of fMLF-stimulated superoxide generation could occur at multiple steps. To identify the related mechanisms, we first examined whether lysoPAF could block fMLF interaction with the formyl peptide receptor (FPR). One of the proximal signaling events downstream of the activated FPR is mobilization of intracellular Ca²⁺. Neutrophils were loaded with Indo-1/AM, then treated with either lysoPAF or vehicle control before stimulation with fMLF (Fig. 3A). The results indicate that lysoPAF did not induce Ca²⁺ mobilization in neutrophils, and treatment of neutrophils with lysoPAF had no effect on the fMLF-induced Ca²⁺ mobilization. These results preclude that lysoPAF blocks the fMLF interaction to FPR.

To rule out the possibility that the inhibitory effect of lysoPAF is specifically targeted at FPR signaling pathways, we investigated whether lysoPAF affects C5a signaling. Stimulation of neutrophils with C5a caused superoxide production (Fig. 3B, ■). In cells treated with PAF, the C5a-induced superoxide production was markedly increased (○). In contrast, lysoPAF treatment markedly decreased the C5a-induced superoxide production (△). Therefore, the inhibitory effect of lysoPAF is not confined to one chemoattractant receptor.

The Inhibitory Effect of lysoPAF Is Independent of the PAF Receptor. Because of the structural similarity between PAF and lysoPAF, we investigated whether the inhibitory effects of lysoPAF are mediated through the PAF receptor. When neutrophils were exposed to both PAF and lysoPAF at equal molar concentrations, the effect of the individual lipids was negated (Fig. 4A). To determine whether lysoPAF directly affects PAFR-mediated signaling, we conducted a Ca²⁺ mobilization assay in which neutrophils were treated with lysoPAF before measurement of PAF-induced increase in intracellular Ca²⁺ concentration. As shown in Fig. 4B, lysoPAF did not induce Ca²⁺ mobilization, and it produced no effect on PAF-induced Ca²⁺ mobilization. We also used a PAF receptor antagonist, SR27417 (Herbert et al., 1991), to determine whether blocking PAFR could alter the effects of PAF and lysoPAF on fMLF-induced superoxide production. Neutrophils were incubated in the presence or absence of SR27417 (10 nM) and either PAF or lysoPAF and then stimulated with fMLF. As shown in Fig. 3C, SR27417 abrogated PAF priming in the neutrophils but did not change

![Fig. 3. The effect of lysoPAF is not mediated through inhibition of the fMLF receptor or confined to fMLF stimulation. A, real-time measurement of Ca²⁺ mobilization in human neutrophils stimulated with 1 μM fMLF in the presence or absence of 1 μM lysoPAF. B, detection of the effects of lysoPAF and PAF on C5a-induced superoxide generation. CPS, counts per second of chemiluminescent light emitted. Isolated human neutrophils (5 × 10⁶/sample) were preincubated with the indicated lipid (1 μM) or vehicle (same concentration of ethanol, 0.025% v/v) for 5 min before stimulation with 100 nM C5a. Shown is a representative set of tracings from one of the three independent experiments, and each produced similar results.]

![Fig. 4. The inhibitory effect of lysoPAF is not mediated through the PAF receptor. A, human neutrophils (5 × 10⁶/sample) were incubated with the indicated lipid(s) (1 μM each) for 5 min before stimulation with 1 μM fMLF. The changes in superoxide generation, expressed as integrated AUC, are shown, with fMLF-induced response in the absence of lipid set as 100%. Data shown are mean ± S.E.M. from three independent experiments. B, real-time measurement of calcium mobilization in human neutrophils loaded with Indo-1/AM and stimulated with 1 μM PAF or with 1 μM lysoPAF and then PAF. A representative set of tracings, from a total of three experiments, is shown. C, superoxide generation was assessed in neutrophils treated for 5 min with the PAFR antagonist SR27417 (10 nM) before the addition of PAF or lysoPAF (1 μM) or vehicle. Cells were then stimulated with fMLF (1 μM) and production of superoxide was measured over time. Data procession and presentation are similar to that in A. D, mouse neutrophils (1 × 10⁶/sample) isolated from PAFR−/− or PAFR−/− mice were incubated with indicated lipid for 5 min before stimulation with fMLF (1 μM). Integrated AUC was calculated for each mouse and data (mean ± S.E.M.) expressed as a percentage of fMLF stimulation for each experiment (n = 3).]
the inhibitory effect of lysoPAF. This result argues against the notion that lysoPAF competes with PAF in using PAFR, although it does not rule out the possibility that lysoPAF may interact with the receptor at a site different from the PAF binding site. This latter possibility was tested using neutrophils derived from PAFR knockout mice (Ishii et al., 1998). As shown in Fig. 4D, genetic deletion of the PAFR gene abolished the priming effect of PAF but did not alter the inhibitory effect of lysoPAF on fMLF-induced superoxide production. Taken together, results from these experiments indicate that inhibition by lysoPAF is independent of PAFR.

LysoPAF Activates Adenylyl Cyclase and Increases Intracellular cAMP Concentration in Neutrophils. Molecular characterization of PAFR has led to the identification of its signaling pathways that include functional coupling to the Gq class of G proteins, activation of phospholipase Cβ, and induction of second messengers inositol (1,4,5)-triphosphate and diacyl glycerol (Honda et al., 2002). PAFR also couples to the Gi class of G proteins that mediate the chemotactic and cross-regulatory signals and also contribute to the activation of phospholipase Cβ and exocytosis in transfected RBL-2H3 cells with the G proteins fused to the PAFR (Brown et al., 2006). As a result, PAF stimulation leads to Ca2+ mobilization in neutrophils. We have shown that stimulation of neutrophils with lysoPAF does not cause an increase in intracellular Ca2+ concentration (Fig. 4B), suggesting differences between PAF and lysoPAF in the activation mechanism and the use of downstream effectors. Because several anti-inflammatory molecules stimulate the adenylyl cyclase-cAMP signaling pathway (Rivkin and Becker, 1976; Fantone et al., 1983, 1984), we tested whether lysoPAF could activate this pathway and increase cAMP concentration in human neutrophils. As shown in Fig. 5A, stimulation of neutrophils with lysoPAF resulted in dose-dependent increases in intracellular cAMP concentrations that reached up to 2-fold above baseline. It is possible that lysoPAF activates a Gq-coupled receptor, leading to cAMP production. This is suggested by the observation that choleratoxin, which ADP ribosylates and activates Gq, increased cAMP level to the same extent as in lysoPAF-stimulated cells (Fig. 5B). Likewise, choleratoxin dose-dependently inhibited fMLF-stimulated superoxide production (Fig. 5C). Pertussis toxin, which ADP ribosylates the Gi proteins and interferes with Gi-receptor interaction, had no effect on lysoPAF-induced elevation of cAMP concentration.

The involvement of an adenylyl cyclase is suggested by the results from experiments using the adenylyl cyclase inhibitor SQ22536 (Graber and Hawiger, 1982), which dose-dependently reversed the inhibitory effect of lysoPAF on the production of superoxide (Fig. 5D). PKA, a key kinase activated by cAMP, also plays a role in the inhibition by lysoPAF. Using the PKA inhibitor H-89, we observed a partial restoration (p < 0.05) of superoxide production in fMLF-stimulated neutrophils that were pretreated with lysoPAF (Fig. 5E). This result is confirmed in a parallel experiment using another PKA inhibitor, KT5720, which produced a similar effect in reversal of the effect of lysoPAF. These results indicate that lysoPAF signaling involves adenylyl cyclase-mediated generation of cAMP, and that PKA contributes to the inhibitory effect of lysoPAF.

LysoPAF Induces cAMP Elevation in Platelets and Inhibits Platelet Aggregation. In addition to neutrophils,
other blood cells, including platelets, are involved in inflammatory responses. PAF was originally named for its activating effect on platelets (Benveniste et al., 1972). However, a bioactivity of lysoPAF on platelets has not been reported. We conducted experiments to determine whether lysoPAF could affect the intracellular cAMP levels in platelets and whether treatment of platelets with lysoPAF would affect its activation. In washed human platelets, lysoPAF induced a significant increase in cAMP levels (p < 0.01) (Fig. 6A), whereas PAF does not have an effect on the intracellular cAMP levels in platelets under the same experimental conditions. Similar to neutrophils, cAMP inhibits many activation processes in platelets, including platelet aggregation (Salzman et al., 1972). We therefore tested whether lysoPAF-treated platelets display changes in agonist-induced platelet aggregation. When washed, platelets were preincubated with lysoPAF and then stimulated with α-thrombin (0.03 U/ml), a lysosPAF dose-dependent inhibition in platelet aggregation was observed (Fig. 6B). Also similar to neutrophils, PKA is known to be involved in downstream signaling of cAMP. The inhibitory effect of lysoPAF on thrombin-induced platelet aggregation is partially reversed by the PKA inhibitors H-89 and KT5720 (Fig. 6C).

Discussion

Results from this study demonstrate that lysoPAF is bioactive and produces an effect opposite that of PAF in neutrophils and platelets. LysoPAF displayed PAF-like activities in stimulating DNA synthesis in smooth muscle cells (Chai et al., 2000) and microvascular leakage when administered to guinea pigs by inhalation (Sakamoto et al., 1993). These PAF-like activities, including the ability to induce Ca++ mobilization, were blocked by PAFR antagonists, indicating that they are mediated by the identified PAFR (Sakamoto et al., 1993). Marathe and colleagues (2001) have shown that treatment of the bioactive lysoPAF and lysoPC with PAF-AH or with saponification abolishes the activities of these lysophospholipids, indicating that these activities come from contaminating phospholipids. Before chemically synthesized PAF became widely available, many studies were conducted using PAF isolated from crude methanol extracts of cells or egg yolk, and those studies were prone to contamination with PAF-like phospholipids. The same methods apparently contributed to the identification of lipids migrating with a similar Rf values as PAF on thin-layer chromatography and containing both platelet-activating and neutrophil-inhibiting properties (ODonnell et al., 1981). To eliminate contaminations with other phospholipids, in this study, we used synthetic lysoPAF and PAF from the same source and prepared stock solutions for both lipids using exactly the same method. Experiments that compared lysoPAF and PAF were strictly carried out in parallel. Our study has shown dose-dependent inhibition of neutrophil NADPH oxidase activation by lysoPAF, with minimal active concentrations as low as 10 nM. Under the same experimental conditions, the potentiation effect of PAF was detectable.

The exact mechanism by which lysoPAF exerts its bioactivity is still unknown. As the first step toward an answer to this question, we determined whether elevation of intracellular cAMP level contributes to the inhibitory effects of lysoPAF. Our results confirmed that lysoPAF, but not PAF, was able to stimulate an increase in cAMP levels in both neutrophils and platelets. Moreover, pharmacological inhibition of PKA, an effector of cAMP, partially reversed the inhibitory effect of lysoPAF, indicating that the cAMP-PKA pathway is involved, but PKA-independent mechanism may...
also exist. We also found that treating neutrophils with SQ22536, an inhibitor of adenyl cyclase, dose-dependently reversed the inhibitory effect of lysoPAF on superoxide generation. These results confirm that adenyl cyclase-mediated production of cAMP is key to the inhibitory activity of lysoPAF. These effects are not likely to be the result of nonspecific actions of the inhibitors on other kinases, most of which are important for neutrophil NADPH oxidase activation and platelet aggregation.

We hypothesize that lysoPAF activates adenyl cyclase through either one or both of the following pathways. First, lysoPAF may bind to a receptor that couples to the Gβ class of G proteins. Because Goα activates adenyl cyclase, lysoPAF binding to this receptor leads to increased cAMP production. This is likely given the emergence of an increasing number of GPCRs for bioactive lipids (Im, 2004). Our study using cholera toxin, which activates Goα, produced a similar effect in the elevation of cAMP levels and inhibition of superoxide generation as seen in lysoPAF-stimulated cells. To determine which receptor(s) is activated by lysoPAF, we have conducted an exhaustive search of the existing GPCR database and identified 28 receptors that are related in sequence to known lipid receptors. These receptors were individually analyzed in transfected cells for their abilities to mediate lysoPAF-induced increase in intracellular cAMP, cAMP response element-binding protein-driven luciferase reporter expression, and calcium mobilization. G2A has been shown to mediate the bioactivity of lysoPC (Lin et al., 2005; Wang et al., 2005), although it lacks certain pharmacological properties of a receptor such as direct binding of lysoPC (Kabarowski et al., 2001; Witte et al., 2005). When tested in our assays, G2A and structurally similar receptors such as GPR4, TDAG8, and OGR1, did not respond significantly to lysoPAF stimulation (Supplemental Table 1). Other GPCRs tested also failed to respond to lysoPAF in these functional assays. Therefore, screening of additional GPCRs is necessary to further test the hypothesis. We noticed that fMLF, which activates a Gβγ-coupled receptor FPR, is reported to enhance intracellular cAMP levels through a pertussis toxin-sensitive mechanism (Ali et al., 1998). However, fMLF also stimulates Ca2+ flux, whereas lysoPAF does not, indicating that lysoPAF does not activate the same signaling pathway that is induced by fMLF. Nevertheless, expanding the search to other GPCRs may be beneficial to understanding how lysoPAF induces cAMP elevation. The second possible mechanism for the inhibitory effect of lysoPAF involves direct activation of adenyl cyclase. To date, 10 isoforms of transmembrane adenyl cyclase have been identified (Hurley, 1999). Adenyl cyclase isoform 1 expression is restricted to the brain. Isoforms 6 and 7 are ubiquitously expressed in cells and tissues. We have tested several other cell types, including HPMECs, which were unable to respond to lysoPAF with increased cAMP levels (data not shown). Therefore, it is unlikely that the candidate is a ubiquitously expressed isoform of adenyl cyclase. Store-operated calcium channels are known to activate adenyl cyclase isoforms 3 and 8; however, we observed no changes in calcium levels in cells treated with lysoPAF. A further analysis is required to more carefully examine these cyclase isoforms.

In summary, results from the current study identify lysoPAF as a bioactive lipid with inhibitory functions in neutrophil NADPH oxidase activation and platelet aggregation. This novel finding may create an opportunity to investigate the biological functions of PAF-AHs in regulating the activities of PAF. Because lysoPAF is a precursor and a metabolite of PAF, its tissue concentration is regulated by the presence of enzymes in the PAF remodeling pathway. Recent cloning and characterization of acyl-CoA:lyso-PAF acetyltransferase (Shindou et al., 2007) and clinical correlation between lowered PAF-AH concentration and severity of anaphylaxis (Vadas et al., 2008) are expected to promote continued exploration of PAF-AH and related pathways. The discovery of lysoPAF as having opposite activities of PAF in the functional assays performed in this study may contribute to a better understanding of these phospholipids for their functions in human physiology.

Acknowledgments

We thank Dr. Jean-Marc Herbert for the gift of SR27417, Dr. Guy Le Breton and Dr. Tohru Kozasa for suggestions on cAMP assays, Dr. Feng Qian for assistance with blood cell preparation, Fumie Hamano for technical assistance in receptor screening assays, and members of the Ye Laboratory for helpful discussions.

References


Address correspondence to: Dr. Richard D. Ye, University of Illinois College of Medicine, 835 South Wolcott Avenue, M/C 868, Chicago, IL 60612.

E-mail: yer@uic.edu