Evidence That Hydrolysis of Ethanolamine Plasmalogen Triggers Synthesis of Platelet-activating Factor via a Transacylation Reaction*

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Addition of 1-O-alk-1’-enyl-2-lyso-sn-glycero-3-phosphoethanolamine (alkenyl-lyso-GPE) to human neutrophil membrane preparations containing 1-O-[3H]alkyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (1-O-[3H]alkyl-2-arachidonoyl-GPC) resulted in rapid deacylation of the 1-O-[3H]alkyl-2-arachidonoyl-GPC to 1-O-[3H]alkyl-2-arachidonoyl-GPC (lyso-platelet-activating factor, lyso-PAF). When acetyl-CoA was included in the incubation mixture, the [3H] lyso-PAF was converted to [3H]PAF. Studies of [3H] arachidonate-labeled neutrophils permeabilized with Staphylococcus aureus α-toxin revealed a major shift of labeled [3H]arachidonate from the choline to the ethanolamine-containing phosphoglycerides upon addition of alkyl-lyso-GPE. The studies indicated that lyso-PAF is formed in the system by the transfer of arachidonate from 1-O-alkyl-2-arachidonoyl-GPC to the alkyl-lyso-GPE by a CoA-independent transacylase reaction. Mass measurements revealed a rapid loss of arachidonate from 1-radyl-2-acyl-GPE and a concomitant increase in alkyl-lyso-GPE upon stimulation of the neutrophils by ionophore A23187.

Based on these and other findings, a pathway is proposed that may play a significant, if not obligatory, role in the synthesis of PAF in intact stimulated neutrophils. It has been widely accepted that phospholipase A2 acts directly on 1-O-alkyl-2-arachidonoyl-GPC as the first step in the synthesis of PAF via formation of lyso-PAF. In the proposed scheme, phospholipase A2, upon stimulation, acts rapidly on ethanolamine plasmalogen selectively releasing arachidonic acid and generating lyso-PAF, which is then acetylated to form PAF. The interactions outlined can account for the synthesis of 1-acyl-2-acetyl-GPC, 1-O-alkyl-1’-enyl-2-acetyl-GPE, and eicosanoids, in parallel with PAF.

It is well established that 1-O-alkyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (1-O-alkyl-2-arachidonoyl-GPC) can serve as a precursor of platelet-activating factor (PAF); 1-O-alkyl-2-acetyl-GPC) in stimulated neutrophils (1–7) and other cells (8, 9). It has been presumed that the first step in the synthesis of PAF from the endogenous precursor is catalyzed by a phospholipase A2 (1–15) which acts directly on 1-O-alkyl-2-acetyl-GPC. However, we recently demonstrated a very active phospholipase A-independent deacylation of 1-O-[3H]alkyl-2-arachidonoyl-GPC to 1-O-[3H]alkyl-2-arachidonoyl-GPC (lyso-PAF) in a membrane system obtained from prelabeled human neutrophils (16). The accumulation of [3H]lyso-PAF required the presence of either 1-O-alkyl-2-lyso-GPC or 1-acyl-2-lyso-GPC and was attributed to the CoA-independent transacylase present in neutrophils.

The metabolism of PAF and arachidonic acid are closely interrelated in human neutrophils (1–7, 17). Of the total arachidonate in human neutrophil phospholipids, approximately 70% is present in the ethanolamine-containing fraction, where 70–80% is found in the plasmalogen subclass (17, 18). Thus, the major store of arachidonate in human neutrophils is the ethanolamine plasmalogen, 1-O-alk-1’-enyl-2-acyl-sn-glycero-3-phosphoethanolamine (alkenyl-lyso-GPE). Chilton and Connell (7) demonstrated that, based on mass, a much greater amount of arachidonate is released from ethanolamine-linked phosphoglycerides (PE) than from the choline-linked phosphoglycerides (PC) in stimulated neutrophils. We recently confirmed these findings using an independent approach and found that arachidonate-containing species of PE are hydrolyzed selectively upon treatment with ionophore A23187 (19); our studies revealed that most of the arachidonate was derived from 1-O-alkyl-2-arachidonoyl-GPE. A striking accumulation of 1-O-alk-1’-enyl-2-lyso-sn-glycero-3-phosphoethanolamine (alkenyl-lyso-GPE) also was observed upon stimulation of the cells (19). These findings led us to examine the effects of alkenyl-lyso-GPE on the hydrolysis of membrane-bound 1-O-[3H]alkyl-2-arachidonoyl-GPE. We demonstrate here that alkenyl-lyso-GPE is highly effective in eliciting the accumulation of [3H]lyso-PAF which is converted to [3H]PAF in the cell-free system when acetyl-CoA is present. We propose a scheme that can account for the observations in the cell-free system and may be operative in intact neutrophils.

The abbreviations used are: alkenyl-lyso-GPE, 1-O-alk-1’-enyl-2-lyso-sn-glycero-3-phosphoethanolamine; EGTA, [ethylenebis(oxyethylenenitrilo)]tetracetic acid; GPE, sn-glycero-3-phosphorylcholine; GPC, sn-glycero-3-phosphoethanolamine; HPLC, high performance liquid chromatography; HSA, essentially lipid-free human serum albumin; lyso-PAF, lyso platelet-activating factor (1-O-alkyl-2-lyso-GPC); PAF, platelet-activating factor, PAF (1-O-alkyl-2-acetyl-GPC); PC, choline-containing phosphoglycerides; PE, ethanolamine-containing phosphoglycerides; TLC, thin-layer chromatography; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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EXPERIMENTAL PROCEDURES

MATERIALS—1-0-[9,10-3H]Hexadecyl-2-lyso-GPC (56 Ci/mmole) was synthesized as described previously (20) and purified before use by repeated precipitation in ethylene glycol and centrifugation (21, 22). Preparations labeled in this manner incorporated the fatty acid methyl ester standards. P. F. Harshman (Serdary) by treatment with monomethylamine for 1.5 h at 53 °C (22). The final product was purified by TLC using chloroform/methanol/acetic acid/water (50:25:8:4 v/v) as the solvent system and eluting from the silica gel as described by Christiansen for eluting from glass (23). The product was quantitated by measuring the lipid phosphorus (24). The preparation of alkenyl-lyso-GPC, and 1-O-hexadecyl-2-lyso-GPC were obtained from Avanti Polar Lipids (Birmingham, AL). Other phospholipid standards were from Merck (Darmstadt), Sigma, and Sonicates—human neutrophils were isolated as described previously (25) from heparinized blood collected from healthy, medication-free donors. To obtain preparations labeled with 1-O-[3H]alkyl-2-lyso-GPE (Serdary), intact cells suspended in PBS (3.5×10^7 cells/ml) with [3H]lyso-PAF formation and were found to be comparable to native ionophore A_23,87 (final concentration 5 μM) added in dimethyl sulfoxide (0.5 μM incubation mixture). After shaking at times varying from 0 to 6 min as indicated, aliquots of the mixture (1 ml; 3.5×10^7 cells/ml) were removed and the lipids extracted immediately by the procedure of Bligh and Dyer (26). The extraction mixture was acidified with formic acid. Two internal phospholipid standards were added to aid in quantitation of arachidonate, 13.6 μg of dipentadecanoyl-GPE (di-17:0 PC) and 13.7 μg of dipentadecanoyl-GPE (di-15:0 PE). The upper phase was extracted a second time with chloroform. The phospholipids were separated by TLC in a solvent system that comprised chloroform/methanol/acetic acid/water (50:25:8:2 v/v). Phospholipids were visualized with primulin (30); bands corresponding to PE, PE, and 1-acyl-2-lyso-GPE were scraped off plates and the phospholipids eluted from the silica gel by the method described by Christiansen for elution of lipids on the basis of their lipid phosphorus content (24). The arachidonate content of the PE and PC was measured as described in detail previously (19).

Briefly, the fatty acid methyl esters and dimethyl acetates of the phosphoglyceride-derived aldehydes were prepared by heating the samples with anhydrous 7% HCl in CH₃OH at 100 °C for 12 min. The methyl esters and dimethyl acetates were isolated and their composition determined (19) by gas-liquid chromatography using methyl pentadecanoate and methyl heptadecanoate derived from the added phospholipid standards as internal standards.

Neutrophil Isolation and Preparation of Labeled Cells, Membranes, and Sonicates—human neutrophils were isolated as described previously (28) from heparinized blood collected from healthy, medication-free donors. To obtain preparations labeled with 1-O-[3H]hexadecyl-2-lyso-GPC containing predominantly the sn-2-arachidonoyl species (19), intact cells suspended in PBS (3.5×10^7 cells/ml) were incubated at 37 °C for 45 min with 1-O-[3H]alkyl-2-lyso-GPE added as an albumin complex (5×10^6 dpm; 1 nmol added in 50 μl of 2.5 mg/ml HSA). To remove unincorporated labeled precursor, the cells were pelleted (275×g for 10 min) and washed once by resuspending and pelleting in PBS. Preparations labeled in this manner incorporated the fatty acid methyl ester standards. P. F. Harshman (Serdary) by treatment with monomethylamine for 1.5 h at 53 °C (22). The final product was purified by TLC using chloroform/methanol/acetic acid/water (50:25:8:4 v/v) as the solvent system and eluting from the silica gel as described by Christiansen for eluting from glass (23). The product was quantitated by measuring the lipid phosphorus (24). The preparation of alkenyl-lyso-GPC, and 1-O-hexadecyl-2-lyso-GPC were obtained from Avanti Polar Lipids (Birmingham, AL). Other phospholipid standards were from Merck (Darmstadt), Sigma, and Sonicates—human neutrophils were isolated as described previously (25) from heparinized blood collected from healthy, medication-free donors. To obtain preparations labeled with 1-O-[3H]alkyl-2-lyso-GPE (Serdary), intact cells suspended in PBS (3.5×10^7 cells/ml) with [3H]lyso-PAF formation and were found to be comparable to native ionophore A_23,87 (final concentration 5 μM) added in dimethyl sulfoxide (0.5 μM incubation mixture). After shaking at times varying from 0 to 6 min as indicated, aliquots of the mixture (1 ml; 3.5×10^7 cells/ml) were removed and the lipids extracted immediately by the procedure of Bligh and Dyer (26). The extraction mixture was acidified with formic acid. Two internal phospholipid standards were added to aid in quantitation of arachidonate, 13.6 μg of dipentadecanoyl-GPE (di-17:0 PC) and 13.7 μg of dipentadecanoyl-GPE (di-15:0 PE). The upper phase was extracted a second time with chloroform. The phospholipids were separated by TLC in a solvent system that comprised chloroform/methanol/acetic acid/water (50:25:8:2 v/v). Phospholipids were visualized with primulin (30); bands corresponding to PE, PE, and 1-acyl-2-lyso-GPE were scraped off plates and the phospholipids eluted from the silica gel by the method described by Christiansen for elution of lipids on the basis of their lipid phosphorus content (24). The arachidonate content of the PE and PC was measured as described in detail previously (19).

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RESULTS

1-0-Alk-1'-enyl-2-lyso-GPE Elicits Deacylation of 1-0-[3H]Alkyl-2-arachidonoyl-GPC to [3H]Lyso-PAF in Prelabelled Neutrophil Membranes—Neutrophil membranes containing 1-0-[3H]alkyl-2-arachidonoyl-GPC were incubated in the presence of unlabeled alkyl-lyso-GPE (Fig. 1). Addition of the exogenous alkyl-lyso-GPE promoted the deacylation of 1-0-[3H]alkyl-2-arachidonoyl-GPC to [3H]lyso-PAF in a concentration-dependent manner. Half-maximal conversion to [3H]lyso-PAF was observed at approximately 5 μM alkyl-lyso-GPE. In further experiments, lipids containing 15 μM alkyl-lyso-GPE and alkyl-lyso-GPE (15 μM) were compared as inducers of [3H]lyso-PAF formation and were found to be comparable (Fig. 2).

Synthesis of PAF from 1-0-[3H]Alkyl-2-arachidonoyl-GPC Triggered by 1-0-alk-1'-enyl-2-lyso-GPE—Sonicates of neutrophils prelabelled with 1-0-[3H]alkyl-2-arachidonoyl-GPC were incubated in the presence of alkyl-lyso-GPE and acetyl-CoA, which is required for the synthesis of PAF from lyso-PAF (10, 15). The formation of [3H]lyso-PAF was
observed upon addition of alkenyl-lyso-GPE alone as observed above. When acetyl-CoA was also added, a marked increase in [3H]PAF synthesis was observed (Fig. 3). These results demonstrated that in the cell-free system, alkenyl-lyso-GPE elicits the synthesis of [3H]PAF via deacylation of 1-O-[3H]alkyl-2-arachidonoyl-GPC to [3H]lyso-PAF and subsequent acetylation by the acetyl-CoA:lyso-PAF acetyltransferase.

Transfer of [3H]Arachidonate from 1-Radyl-2-[3H]Arachidonoyl-GPC to PE in the Presence of 1-O-Alk-1'-enyl-2-lyso-GPE—The generation of lyso-PAF from 1-O-alkyl-2-arachidonoyl-GPC by the CoA-independent transacylase reaction elicited by alkenyl-lyso-GPE should result in transfer of arachidonate from the PAF precursor into the PE fraction. We searched for such a transfer in two different systems.

In the first system, neutrophils were prelabeled with [3H] arachidonic acid, washed, and disrupted by sonication. The 1000 x g supernatant containing the [3H]arachidonate-labeled lipids was then incubated in the presence or absence of alkenyl-lyso-GPE. At the end of the incubation period (15 min), the distribution of label in the PC and PE fractions was measured. A 3-4% transfer of [3H]arachidonate from PC to PE was observed consistently in the presence of the alkenyl-lyso-GPE (data not shown). No significant changes in other labeled lipids, phosphatidyl ethanolamine and triglyceride, were noted. In intact neutrophils, exogenous-labeled arachidonate is incorporated first into the diacyl-GPC component of PC and then transferred to 1-O-alkyl-2-acyl-GPC (17). Because the distribution of label within the PC fraction might influence its transfer to PE, we examined the influence of different preincubation times before disruption of the cells. Although no major effect of the different preincubation times was observed, the optimal transfer to PE was observed after 15 min.
min of labeling by [$^3H$]arachidonate (data not shown). A much larger shift of [$^3H$]arachidonate was observed in the second system. In this system, untreated neutrophils were prelabeled with [$^3H$]arachidonate, washed, and then permeabilized with S. aureus $\alpha$-toxin (28) as described under "Experimental Procedures." The amounts of labeled arachidonate in the PC and PE fractions were then measured in the presence or absence of varying concentrations of exogenous alkenyl-lyso-GPE (2-20 $\mu$M). In this system, the added exogenous acceptor effected a major shift of [$^3H$]arachidonate from PC to PE (Table I). After incubation for 10 min in the absence of alkenyl-GPE, 27% of the label in phospholipids was in PC and 15.4% in PE, whereas in the presence of 20 $\mu$M alkenyl-lyso-GPE 17.5% was in PC and 30.7% in PE. Thus, 40% of the [$^3H$]arachidonate in PC was lost, whereas the label in PE increased by 88% in the presence of alkenyl-lyso-GPE. There was a 3.3% decrease in label of phosphatidylserine/phosphatidylglycerol in the presence of 20 $\mu$M alkenyl-lyso-GPE, but no significant change in the neutral lipids. In the absence of the alkenyl-lyso-GPE, the distribution was changed little during the incubation. It is clear from these results that the presence of alkenyl-lyso-GPE resulted in a major shift of [$^3H$]arachidonate from PC to PE in the permeabilized neutrophils.

**Examination of the Early Release of Endogenous Arachidonate from PC and PE in Stimulated Neutrophils**—If accumulation of alkenyl-lyso-GPE plays a role in initiating PAF synthesis in intact stimulated cells, one would expect a rapid hydrolysis of PE. Our earlier studies (19) indicated that stimulation of neutrophils by ionophore A$_{23187}$ elicits a selective hydrolysis of arachidonate-containing species of PE accompanied by the formation of alkenyl-lyso-GPE. In the present study we measured the release of arachidonate from PC and PE from 0.5 to 6 min after stimulation (Fig. 4). The results indicate that the hydrolysis of arachidonate from PE occurs rapidly. In five separate experiments, an initial rapid loss of arachidonate from PE was observed, then within 1 min, partial restoration of the pool occurred followed by a further depletion. A transfer of arachidonate from PC, and possibly other lipids, to PE would replenish some of the arachidonate lost from PE at the early times and may account for the transient increase in the arachidonate content of PE.

**Accumulation of 1-Radyl-2-lyso-GPE as a Function of Time in Stimulated Neutrophils**—In the same experiments in which the release of arachidonate from PE and PC was measured, we also examined the amount of 1-radyl-2-lyso-GPE formed (Fig. 5). After a possible slight initial decrease, there was a time-dependent accumulation of 1-radyl-2-lyso-GPE (approximately 1 nmol/min per 3.5 x 10$^5$ cells). In studies reported earlier (19), we examined the accumulation of 1-radyl-2-lyso-GPE at 5 min and found that 80% of the 1-radyl-2-lyso-GPE formed by the neutrophils is 1-O-alk-1'-enyl linked. The amount of lyso-PE found in the earlier studies at 5 min (approximately 17 nmol/6 x 10$^6$ cells) was somewhat higher than observed here; this may be a result of the higher concentrations of cells used in the earlier studies (2 x 10$^6$ cells/ml). There are also variations among donors. Together these studies indicate that 1-radyl-2-lyso-GPE, composed largely of alkenyl-lyso-GPE, accumulates in a time-dependent manner in stimulated neutrophils immediately following stimulation of the cells.

**Comparison of 1-0-$^3H$Alk-1'-enyl-2-lyso-GPE and 1-O-$^3H$Alkyl-2-lyso-GPE as CoA-independent Transacylase Substrates**—Our evidence indicated that the hydrolysis of 1-O-alkyl-2-alkenyl-lyso-GPC is catalyzed by the CoA-independent transacylase. Since both alkyl-lyso-GPC and alkenyl-lyso-GPE were shown in the above experiments to elicit hydrolysis, we compared the two lysophospholipids as acyl acceptors in the transacylase reaction (Fig. 6). The lysophospholipids were tested as mixtures at concentrations of 2 and 20 $\mu$M each. Little difference in acylation was observed at 2 $\mu$M, but at 20 $\mu$M the [H]alkenyl-lyso-GPE was acylated to a significantly greater extent than the [H]alkyl-lyso-GPE. These findings, along with our other observations, indicate that alkenyl-lyso-GPE can compete strongly with alkyl-2-lyso-GPC as an acceptor substrate in the transacylase reaction.

![Figure 4: Time course of changes in arachidonate content in PC and PE of stimulated neutrophils.](image-url)
Comparison of the Decacylation of 1-O-['H]Alkyl-2-arachidonoyl-GPC and 1-O-['H]Alk-1'-enyl-2-arachidonoyl-GPE in Response to Unlabeled Lysophospholipids—In these studies broken cell preparations were prepared from prelabeled neutrophils containing either 1-O-['H]alkyl-2-arachidonoyl-GPC or 1-O-['H]alkyl-1'-enyl-2-arachidonoyl-GPE. The effects of unlabeled alkyl-2-lyso-GPC and alkyl-2-lyso-GPE on deacylation of the labeled substrates were then compared (Fig. 7). It was found that the 1-O-['H]alkyl-2-arachidonoyl-GPC was deacylated to a greater extent than the 1-O-['H]alkenyl-2-arachidonoyl-GPE in response to both unlabeled lysophospholipids. However, because the endogenous pool sizes and subcellular distribution of the labeled phospholipids likely influence the observed deacylation, it is not clear if the presence of the lysophospholipids favors the deacylation of the 1-O-alkyl-2-arachidonoyl-GPC over alkyl-2-arachidonoyl-GPE.

DISCUSSION

The present studies evolved from a number of recent findings. In an accompanying report (16), we concluded that the CoA-independent transacylase of human neutrophils can generate ['H]lyso-PAF (1-O-['H]hexadecyl-2-lyso-GPC) from membrane-bound 1-O-['H]alkyl-2-arachidonoyl-GPC in the presence of endogenous 1-O-alkyl-2-lyso-GPC. It has been recognized for a number of years that plasmalogens comprise approximately two-thirds of the PE fraction of human neutrophils (18, 31, 32). Moreover, from 70 to 80% of the arachidonate of PE is linked to the plasmalogen component (approximately 1 nmol/10^6 cells/min). Our recent studies (19) indicated that arachidonate-containing species of PE are hydrolyzed selectively by neutrophils upon stimulation, leading to the accumulation of lyso-PE that predominantly comprised the 1-O-alk-1'-enyl-2-lyso-GPE subclass (approximately 1 nmol/10^6 cells/min).

The observations prompted us to test the effects of exogenous alkyl-lyso-GPE on the generation of lyso-PAF from 1-O-['H]alkyl-2-arachidonoyl-GPC. In the present studies, addition of the alkyl-lyso-GPE to preparations containing membrane-bound 1-O-['H]alkyl-2-arachidonoyl-GPC was shown to elicit the rapid appearance of ['H]lyso-PAF. In preparations prelabeled with ['H]arachidonate, a major shift of ['H]arachidonate from 1-ralyl-2-['H]arachidonoyl-GPE to the PE fraction was observed in permeabilized cells in the presence of alkyl-lyso-PE. A similar, though less dramatic shift, was observed in disrupted neutrophil preparations.

When acetyl-CoA was added to the cell-free system, alkyl-lyso-GPE elicited synthesis of ['H]PAF from 1-O-['H]alkyl-2-arachidonoyl-GPC. Thus the CoA-independent transacylase was able to initiate PAF synthesis in the presence of the lyso-PAF. In further studies, we examined the time course of endogenous arachidonate release from PE and PC in intact stimulated neutrophils and found that PE is hydrolyzed rapidly upon stimulation. This opens the possibility that rapid accumulation of alkyl-lyso-GPE in stimulated neutrophils may trigger the deacylation of arachidonate from PC by the transacylase. In intact neutrophils stimulated with ionophore A_23187, little PAF synthesis is observed before 1 min but the synthesis then continues over a 15-min period (2).

Based on the present studies and earlier findings, we propose a series of reactions which can account for the observations in the cell-free system and intact cells and which may be operative in intact stimulated neutrophils (Fig. 8). In this model, stimulation of neutrophils triggers activation of phospholipase A_2 which extensively hydrolyzes arachidonate-linked species of PE and thus generates significant levels of alkyl-lyso-PE and free arachidonic acid (reaction 1),
which is then available for conversion to lipoxigenase products. The appearance of the lysosomal enzyme triggers the selective transfer of arachidonate from 1-acyl-2-arachidonoyl-GPE to the lysosomal enzyme and releases lyso-PAF (reaction 2) which can be acetylated to form PAF (reaction 3). Once the arachidonate of PE is transferred to PC, it could be hydrolyzed rapidly by phospholipase A2 acting on the PE.

Our findings have not eliminated a role of direct hydrolysis of 1-O-alkyl-2-arachidonoyl-GPE by phospholipase A2 in PAF synthesis by stimulated neutrophils. However, in view of the active deacylation of the membrane-bound substrate elicited by alkyl-lyso-GPE and the observed accumulation of arachidonyl-lyso-GPE in intact stimulated cells, it appears likely that the transacylase-mediated reaction may play an important, if not obligatory, role in promoting the synthesis of PAF in the intact cells. In very recent reported studies, Sugita and co-workers (33) found that both 1-O-alkyl-2-lyso-GPC and alkyl-lyso-GPE induced production of PAF in intact neutrophils without activating the acylation transferase. They further showed that transfer of 1-[H]arachidonate from PC to PE in the intact cells and demonstrated a CoA-independent transfer in membrane preparations. The investigators suggested the transacylase reaction may play a role in initiating PAF synthesis, but did not elaborate on possible mechanisms.

Some of the strongest evidence for the direct action of phospholipase A2 on 1-O-alkyl-2-arachidonoyl-GPE in neutrophils was reported by Leslie and co-workers (34, 35) who found a phospholipase A2 activity that appears to be specific for arachidonate-containing species of PC. This activity did not appear to distinguish between diacyl and alkylacyl species; the preference of this enzyme for PC versus PE does not appear to have been established. Diez and Mong (36) have purified a similar arachidonate-specific phospholipase A2 from differentiated human monocytes U937 cells and shown that it hydrolyzes 1-O-alkyl-2-arachidonoyl-GPC. In other studies (37–39), the phospholipase A2 activity of neutrophils that exhibits a neutral pH optimum was found to prefer PE over PC as a substrate. In addition to these findings in neutrophils, Angle and co-workers (40) described a phospholipase A2 activity in rabbit lung and other tissues that has a preference for 1-O-alkyl-2-arachidonoyl-GPC over the corresponding acyl- or oleoyl-linked substrates.

Other evidence presented in support of a direct role of phospholipase A2 in the conversion of 1-O-alkyl-2-acyl-GPC is based on the use of inhibitors (9, 11, 12). However, inhibition of phospholipase A2 would not allow one to distinguish between direct hydrolysis and indirect hydrolysis coupled through the transacylase reaction as proposed here. Additionally, p-bromophenacyl bromide was used as a phospholipase A2 inhibitor in several of the studies (9, 11, 12, 41), yet this reagent inhibits numerous enzymes and has been deemed unsuitable as a specific phospholipase A2 inhibitor in crude systems (42, 43). The reagent was observed to inhibit the neutrophil transacylase activity (16) and earlier was found to block the active acylation of 1-O-alkyl-2-lyso-GPC observed in the absence of CoA or acyl-CoA in washed liver membranes (44).
of its arachidonate-linked substrate resulting in low release of lyso-PAF for the synthesis of PAF (4, 8). The model accounts for the release of arachidonic acid and formation of lipoxigenase products accompanying PAF synthesis (1–3). It also accounts for the formation of 1,2-acyl-GPC (50, 51) and 1-O-alk-1'-enyl-2-acetyl-GPE (52), which are formed along with PAF by stimulated neutrophils, because the transacylase can transfer arachidonate from diacyl-GPC and the phospholipase A₂ releases arachidonyl-lyso-GPE, as demonstrated with lyso-PAF as a transacylase substrate and allow the difference in the transacylase activity was observed in resting and stimulated neutrophils (16). In the proposed model it is assumed that the transacylase is in an active state at all times. Thus far, we have been unable to observe significant hydrolysis of [¹H]alkyl-2-arachidonoyl-GPC and its conversion to lyso-PAF and PAF. The overall scheme outlined would be under regulatory control through the phospholipase A₂. A very rapid (15 s) and striking shift of labeled arachidonoyl-GPC and its conversion to lyso-PAF and PAF in intact cells should provide a good system for further studies. Similar shifts have been observed in platelets (54).

In stimulated neutrophils it might be expected that a greater transfer of labeled arachidonate from PC to PE would be observed if the proposed interactions take place. However, the labeled arachidonate that is transferred may be particularly vulnerable to hydrolysis by phospholipase A₂ and could be hydrolyzed immediately after it is transferred. The permeabilized cells should provide a good system for further studies of the transfer and phospholipase A₂-catalyzed release of free arachidonic acid. The hydrolysis of arachidonate from PE by phospholipase A₂ appears to merit a closer examination. Thus far, we have been unable to observe significant hydrolysis of [¹H]arachidonate from membrane-bound PE or PC in cell-free assays.

In summary, the findings reported here demonstrate that alkenyl-lyso-GPE can trigger the deacylation of 1-O-alkyl-2-arachidonoyl-GPC and its conversion to lyso-PAF and PAF in the membrane system. The model proposed fits a number of observations. Although phospholipase A₂ plays a critical role in the model, the CoA-independent transacylase is the enzyme directly responsible for formation of lyso-PAF. If the transacylase proves to play an important role in PAF production, the enzyme could be a promising target for pharmacological agents. A preliminary report of these findings was presented recently (55). Further studies will be required to establish the relative importance of the direct hydrolysis of 1-O-alkyl-2-acetyl-GPC by phospholipase A₂ versus the transacylase reaction in PAF synthesis in intact neutrophils.

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References

Plasmalogen Hydrolysis and PAF Synthesis
