Lysophosphatidylcholine Stimulates the Release of Arachidonic Acid in Human Endothelial Cells*

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Lysophosphatidylcholine (lyso-PC) is a product of phosphatidylcholine hydrolysis by phospholipase A₂ (PLA₂) and is present in cell membranes, oxidized lipoproteins, and atherosclerotic tissues. It has the ability to alter endothelial functions and is regarded as a causal agent in atherogenesis. In this study, the modulation of arachidonate release by lyso-PC in human umbilical vein endothelial cells was examined. Incubation of endothelial cells with lyso-PC resulted in an enhanced release of arachidonate in a time- and concentration-dependent manner. Maximum arachidonate release was observed at 10 min of incubation with 50 μM lyso-PC. Lyso-PC species containing palmitoyl (C₁₆:₀) or stearoyl (C₁₈:₀) groups elicited the enhancement of arachidonate release, while other lysolipids such as lysophosphatidylethanolamine, lysophosphatidylserine, lysophosphatidylinositol, or lysophosphatidate were relatively ineffective. Lyso-PC-induced arachidonate release was decreased by treatment of cells with PLA₂ inhibitors such as para-bromophenacyl bromide and arachidonoyl trifluoromethyl ketone. Furthermore, arachidonate release was attenuated in cells grown in the presence of antisense oligodeoxynucleotides that specifically bind cytosolic PLA₂ mRNA. Treatment of cells with lyso-PC resulted in a translocation of PLA₂ activity from the cytosolic to the membrane fractions of cells. Lyso-PC induced a rapid influx of Ca²⁺ from the medium into the cells, with a simultaneous enhancement of protein kinase C (PKC) activity in the membrane fractions. The lyso-PC-induced arachidonate release was attenuated when cells were preincubated with specific inhibitors of PKC (staurosporine and Ro31–8220) or a specific inhibitor of mitogen-activated protein kinase/extracellular regulated kinase kinase (PD098059). Taken together, the results of this study show that lyso-PC caused the elevation of cellular Ca²⁺ and the activation of PKC, which stimulated cytosolic PLA₂ in an indirect manner and resulted in an enhanced release of arachidonate.

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The release of arachidonate from phospholipids is the rate-limiting step in the synthesis of eicosanoids via the arachidonate cascade (1). Arachidonate and its metabolites possess diverse biological properties, many of which are related to vascular homeostasis (1). In endothelial cells, arachidonate is converted to prostacyclin, a potent vasodilator and platelet antiaggregator (2). Although different mechanisms have been proposed for the release of arachidonate in mammalian cells, the hydrolysis of the acyl chain at the sn-2 position of glycerophospholipids by phospholipase A₂ (PLA₂)¹ is regarded as the primary pathway for this reaction (1, 3). In mammalian cells, several forms of PLA₂ have been identified. Those that have been purified and well characterized include the “type II” 14-kDa secretory PLA₂ (sPLA₂) and the “type IV” 85-kDa cytosolic PLA₂ (cPLA₂) (for reviews, see Refs. 3–5). These two isoforms are products of distinct genes (5) and have different properties. The cPLA₂ preferentially hydrolyzes phospholipid substrates containing arachidonate at the sn-2 position (6), while sPLA₂ does not exhibit any preference with respect to substrate acyl composition. The sPLA₂ requires millimolar concentrations of Ca²⁺ for maximum activity, while cPLA₂ contains a calcium-dependent lipid binding domain and requires submicromolar levels for translocation to cellular membranes (6, 7). In stimulated cells, cPLA₂ activity is enhanced by phosphorylation at serine 505 by mitogen-activated protein kinase (MAPK) (3, 8). Protein kinase C (PKC) also appears to play a role in the regulation of PLA₂ activity, although PKC is not thought to directly phosphorylate cPLA₂ in vivo (3, 9). Both isoforms are found in human endothelial cells and have been implicated in arachidonate release and prostacyclin production (10–13).

Lysophosphatidylcholine (lyso-PC) is a product of phosphatidylcholine hydrolysis by PLAs. This lysophospholipid possesses detergent properties at high concentrations (14) but is quickly metabolized or reacylated within cells (15, 16). Lyso-PC is a normal constituent of blood plasma (17), vascular tissue (18), and lipoproteins (19, 20), but its levels are greatly elevated in hyperlipidemia (21), atherosclerotic tissue (18), oxidized lipoproteins (19, 20), and ischemic hearts (22). A growing body of evidence has implicated lyso-PC in the pathogenesis of cardiovascular diseases. For example, lyso-PC in oxidized low density lipoproteins impairs vascular relaxation (20, 23, 24) and induces mitogenesis of macrophages (25). Lyso-PC is chemotactic for monocytes (26) and T lymphocytes (27). In endothelial cells, lyso-PC can induce the expression of genes for various growth factors (28, 29) and cellular adhesion molecules (30, 31). The perturbation of vascular...
endothelial function and recruitment of various cell types to sites of lesion have been implicated as early events in atherogenesis (32, 33). Thus, given its many biological properties, lyso-PC has been postulated to be an important causal agent in inflammation and atherosclerosis (34, 35).

The interactions among phosphatidylcholine, fatty acids, lyso-PC, and sPLA₂ have been examined in *in vitro* kinetic studies (36). An abrupt increase in PLₐ₂ activity after an initial lag period was observed in these studies. This pattern of activity was attributed to the accumulation of fatty acid and lysophospholipids, which together altered the organization of substrate vesicles (36). In light of the many biological effects of lyso-PC, we hypothesize that it can modulate PLₐ₂ in intact cells. In the present study, the effects of lyso-PC on the release of arachidonate in endothelial cells was examined. The involvement of Ca²⁺, PKC, and MAPK in the modulation of PLₐ₂ activity was examined.

**EXPERIMENTAL PROCEDURES**

**Materials**—Medium 199 with Hanks’ salt and t-glutamate, heat-inactivated fetal calf serum, and other standard culture reagents were obtained from Life Technologies, Inc. Type I collagenase was obtained from Worthington. Endothelial cell growth supplement was obtained from Collaborative Biomedical Products (Bedford, MA). Phorbol 12-myristate 13-acetate, staurosporine, para-bromophenacyl bromide, and all other chemicals were purchased from Sigma. PD98059 was a product of Calbiochem. [5,6,8,11,12,14,15-³H]arachidonate (230.5 Ci/mmol) was obtained from NEN Life Science Products, and 1-stearoyl-2-[¹⁴C]arachidonoyl-L-3-phosphatidylcholine (55 mCi/mmol) was obtained from Amersham Corp. Arachidonoyl trifluoromethyl ketone (AACOCF₃) was obtained from Fisher. Anti-cPLA₂ polyclonal antibody was a generous gift from Drs. J. L. Knopf and L-L. Lin of the Genetics Institute (Boston, MA). Anti-human sPLA₂ monoclonal antibody was a product of Upstate Biotechnology Inc. (Lake Placid, NY).

**Cell Culture**—Endothelial cells were harvested from human umbilical veins using Type I collagenase as described previously (37, 38). The cells were grown in flasks or culture dishes pretreated with 0.2% calf serum, 100 units/ml penicillin, 100 units/ml streptomycin, and 1.25 g/ml aprotinin, and 10 μg/ml 2-mercaptoethanol in culture dishes pretreated with 0.2% gelatin, in medium 199 (pH 7.4) supplemented with 25 mM HEPES, 30 μg/ml endothelial cell growth supplement, 90 μg/ml heparin, 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1.25 μg/ml fungizone. The cells were subcultured at a 1:3 ratio using 0.05% trypsin to free the cells from the cultureware. Near-confluent cell monolayers from the third passage were used for all experiments.

**Binding of Lyso-PC to Endothelial Cells**—Endothelial cells were cultured on 60-mm plates and incubated with medium 199 containing 100 μM lyso-PC (57 nCi/ml) for 15 min. The medium was removed, and the cells were incubated for 15 min with medium 199 (control) or medium 199 containing 10 μM lyso-PC (a 100-fold excess of nonradioactive lyso-PC). The media were subsequently removed, and the cells were dislodged from the culture dish in HEPES-buffered saline. Samples were taken for protein determination or scintillation counting. 

**Immunoblotting Analysis of Phospholipase A2**—Immunoblotting analysis of cPLA₂ or sPLA₂ was performed as described previously (39). Cell lysates containing approximately 50 μg of protein were subjected to sodium dodecylsulfate, 7.5% polyacrylamide gel electrophoresis. The protein fractions from the gels were transferred to nitrocellulose membranes and then allowed to react with a polyclonal anti-cPLA₂ antibody or with an anti-sPLA₂ antibody. The nitrocellulose membranes were then exposed to a goat anti-rabbit antibody that was coupled to horse-radish peroxidase. The cPLA₂ or sPLA₂ bands were detected on film using a Western blotting detection reagent kit (Amersham), which yields a fluorescent compound via a reaction catalyzed by the peroxidase.

**Oligonucleotide Treatment**—The antisense oligonucleotides for group II PLₐ₂ (ASₐ₂a, 5'-GAT CTT CCG CCA CCC ACA CC-3') (40) and for sPLA₂ (ASₐ₂a, 5'-GTA AGC ATG TAT AAA TGA CAT-3') (11) with phosphorothioate linkages were synthesized by the University Core DNA Services, University of Calgary (Alberta, Canada). Complementary sense oligonucleotides were used as controls. Seventy-two hours prior to challenge with lyso-PC, the cells were incubated with medium containing 10 μM oligonucleotides. The cells were supplied with fresh medium containing 10 μM oligonucleotides at 24-h intervals thereafter. The amount of oligonucleotides did not affect cell viability or arachidonate labeling.

**Determination of Phospholipase A2 Activity**—Cells were lysed by sonication in a buffer containing 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 10 μM leupeptin, 10 μM aprotinin, 20 mM NaF, and 10 mM NaHPO₄. Cell lysates were centrifuged at 100,000 × g for 60 min. The supernatant was designated as the cytosolic fraction, while the pellet was designated as the membrane fraction and resuspended in the buffer described above. PLₐ₂ activity in the subcellular fractions was determined by the hydrolysis of 1-stearoyl-2-[¹⁴C]arachidonoyl-sn-glycero-3-phosphocholine to yield free radiolabeled arachidonate. The assay mixture contained 50 mM Tris-HCl (pH 8.0), 1.5 mM CaCl₂, 0.9 nmol of 1-stearoyl-2-[¹⁴C]arachidonoyl-sn-glycero-3-phosphocholine (100,000 dpm/sample), and approximately 10 μg of protein in a final volume of 100 μl. The reaction mixtures were incubated at 37 °C for 30 min, and the reactions were terminated by the addition of 1.5 ml of chloroform/ methanol (2:1, by volume). Total lipid was extracted, and the radioactivity of the arachidonate released was determined as described above. The amounts of protein in the samples were determined by the bichinchonic acid method (41).

**Monitoring of Intracellular Ca²⁺**—Changes in cytosolic free Ca²⁺ were monitored using the fluorescent Ca²⁺ indicator fura-2 as described previously (42). Briefly, monolayers grown on glass coverslips were incubated in medium with 5 μM fura-2/AM for 30 min. Fura-2/AM is permeable to cells, and once inside the cells the compound is hydrolyzed by endogenous esterases to yield the cell-impermeable fura-2. The cells on the coverslip were transfused into a cuvette, rinsed with HEPES-buffered saline containing 0.025% bovine serum albumin, and immersed in the same buffer. Fluorescent signals were monitored on a SPEX fluorescence spectrophotometer at the excitation and emission wavelengths of 340 and 380 nm, respectively. Cells were then challenged with lyso-PC or A23187 for 10 min, and the ratio of the fluorescent wavelengths at the two wavelengths was monitored as an indicator of changes in intracellular free Ca²⁺ levels. The calibration curve (cross-over) point of fura-2 remained constant during lyso-PC treatment.

**Determination of PKC Activity**—Cells were sonicated in buffer B (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 10 mM EGTA, 0.25 mM sucrose, 0.3% β-mercaptoethanol, 10 μM benzamidine, 1 mM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) and were centrifuged at 1500 g for 10 min. The supernatants were subjected to ultracentrifugation at 100,000 × g for 60 min to obtain the soluble and membrane fractions. Approximately 15–30 μg of protein from these fractions were used to determine PKC activity using a PKC assay kit (Amersham), which is based on the incorporation of ³²P from [γ-³²P]ATP into a PKC-specific substrate peptide.

**Statistical Analysis**—The data were analyzed using a two-tailed independent Student’s *t* test. The level of statistical significance was defined as *p* < 0.05.

**RESULTS**

**Lyso-PC Stimulates Arachidonate Release in Endothelial Cells**—To determine the effect of lyso-PC on arachidonate re-
release, human umbilical vein endothelial cells were labeled with \[^{[3H]} \text{arachidonate}\] in medium containing 10% fetal calf serum for 24 h. Cells were washed three times with HEPES-buffered saline containing 0.025% bovine serum albumin prior to challenge. A, cells were challenged with 0 \(\mu\text{M}\) (○) or 50 \(\mu\text{M}\) (■) lyso-PC in HEPES-buffered saline containing 0.025% bovine serum albumin for the indicated times. B, cells were challenged for 10 min with the indicated concentrations of lyso-PC in HEPES-buffered saline containing 0.025% (▲), 0.05% (▲), or 0.10% (●) bovine serum albumin (w/v). Arachidonate release was determined as described under “Experimental Procedures.” Values represent means ± S.D. of three separate experiments.

**Fig. 1. Lyso-PC stimulates arachidonate release in endothelial cells.** Cells were incubated with 1 \(\mu\text{Ci}/\text{ml}\) of \[^{[3H]} \text{arachidonate}\] in medium containing 10% fetal calf serum for 24 h. Cells were washed three times with HEPES-buffered saline containing 0.025% bovine serum albumin and 0 or 50 \(\mu\text{M}\) lyso-PC for various time periods (Fig. 1A). Lyso-PC elicited a time-dependent arachidonate release, which reached a maximum at 10 min of incubation, after which arachidonate release was slightly diminished. A nominal amount of bovine serum albumin was required to bind the arachidonate that is released into the buffer. The optimal concentration of lyso-PC for the induction of arachidonate release was determined at bovine serum albumin concentrations ranging from 0.025 to 0.1% (w/v) (4–16 \(\mu\text{M}\) albumin). The effect of lyso-PC on arachidonate release was affected by the albumin concentration (Fig. 1B). Higher concentrations of lyso-PC was required to elicit a stimulation of arachidonate release at higher albumin concentrations. For example, at 0.025% albumin, the maximal stimulation of arachidonate release was observed at 50 \(\mu\text{M}\) lyso-PC. Lyso-PC at this concentration has been found to be nonlethal to endothelial cells (43), and we confirmed cell viability under the incubation conditions by the exclusion of trypan blue dye. Hence, these conditions were routinely used in subsequent experiments.

**Release of Arachidonate by Long Chain Lyso-PC and Other Lysolipids**—Initial experiments on the effect of lyso-PC on arachidonate release were performed using lyso-PC derived from egg lecithin. Since egg lysolecithin contains mainly saturated acyl species, we tested the ability of palmitoyl (16:0)- and stearoyl (18:0)-lyso-PC to stimulate arachidonate release. Fig. 2 shows that lyso-PC containing palmitoyl and stearoyl chains induced a high release of arachidonate. To determine if the stimulation of arachidonate release is specific to lyso-PC or if it is a property common to all lysolipids, we tested the effect of other lysophospholipids such as lysophosphatidylethanolamine, lysophosphatidylserine, lysophosphatidylglycerol, and lysophosphatidic acid on arachidonate release. As shown in Fig. 2, lysophospholipids with head groups other than choline were minimally effective in the stimulation of arachidonate release. Based on these results, lyso-PC containing a palmitoyl (16:0) chain was used in subsequent experiments.

Lyso-PC is an amphiphilic molecule and can incorporate into lipid membranes. Thus, we performed binding studies as described under “Experimental Procedures” to determine the nature of the association of lyso-PC with the endothelial cells. Cells were labeled with \[^{[14C]} \text{lyso-PC}\] (100 nm, 57 nCi/nmol), followed by incubation with control medium (without lyso-PC) or medium containing 10 \(\mu\text{M}\) nonradioactive lyso-PC. The majority of radioactivity remained associated with cells in the presence of excess lyso-PC (results not shown), indicating that the association of lyso-PC with cells is mainly nonspecific.

**Involvement of Phospholipase A2 in Lyso-PC-induced Arachidonate Release**—To determine whether the release of arachidonate is mediated by PLA2, we examined the effects of the PLA2 inhibitors para-bromophenacyl bromide (pBPB) and arachidonoyl trifluoromethyl ketone (AACOCF3), the latter of which specifically inhibits the cPLA2 (44). As shown in Table I, arachidonate release was significantly inhibited in those cells that were preincubated with these inhibitors prior to challenge with lyso-PC. The inhibition of arachidonate release by up to 62% by AACOCF3 indicates that the cPLA2 may be involved in the arachidonate release induced by lyso-PC. However, sPLA2 is also present in endothelial cells and may also participate in
arachidonate release (10).

To further delineate the type of PLA2 that was involved in the lyso-PC-induced arachidonate release, we used antisense oligonucleotides toward cPLA2 and sPLA2. These oligonucleotides were designed to bind specifically to the respective mRNAs and prevent the translation and synthesis of the enzyme protein (11, 40). Complementary sense oligonucleotides were used as negative controls. Cells were grown in the presence of sense or antisense oligonucleotides to either PLA2 isoform for 3 days prior to challenge with lyso-PC. Treatment of the cells with either sense or antisense oligonucleotides did not alter the total incorporation of [3H]arachidonate. However, lyso-PC-induced arachidonate release was significantly attenuated in cells grown in the presence of antisense oligonucleotides for cPLA2, compared with cells grown without oligonucleotides or with sense oligonucleotides (Fig. 3). The level of cPLA2 protein after the treatment with antisense cPLA2 oligonucleotides was determined by immunoblotting analysis with a polyclonal antibody for cPLA2. The level of cPLA2 protein was decreased (40% reduction) by the antisense oligonucleotide treatment (Fig. 4A). In cells treated with antisense sPLA2 oligonucleotides, the lyso-PC-induced arachidonate release was not significantly affected (Fig. 3), despite a 35% decrease in the sPLA2 protein level in those cells (Fig. 4B).

Table I

| Treatment          | Arachidonate release | Inhibition |
|--------------------|----------------------|------------|
|                    | dpm x 10^-3/dish     | %          |
| Control            | 5.85 ± 0.40          |            |
| 50 μM lyso-PC      | 31.05 ± 2.35         |            |
| AACOCF3 + lyso-PC  | 1 μM                 | 19.11 ± 0.60a | 39          |
|                    | 25 μM                | 11.93 ± 1.55a | 62          |
| pBPB + lyso-PC     | 5 μM                 | 22.09 ± 1.79a | 29          |
|                    | 25 μM                | 16.44 ± 2.61a | 47          |

*p < 0.05.

Fig. 3. Effect of antisense oligonucleotides for PLA2 on arachidonate release. Cells were cultured in the presence of oligonucleotides for 72 h as described under “Experimental Procedures.” Cells were labeled with [3H]arachidonate for 20 h prior to challenge with 50 μM lyso-PC for 10 min. Control, cells cultured without oligonucleotides and not challenged with lyso-PC; No oligo, cells cultured without oligonucleotide treatment; ScA2, cells cultured in the presence of sense oligonucleotide for cPLA2; AScA2, cells cultured in the presence of antisense oligonucleotide for cPLA2; ScsA2, cells cultured in the presence of sense oligonucleotide for sPLA2; AssA2, cells cultured in the presence of antisense oligonucleotide for sPLA2. Values represent means ± S.D. of three separate experiments.

Fig. 4. Immunoblots of cPLA2 and sPLA2 in cells grown in the presence of sense or antisense oligonucleotides to cPLA2. Cells were cultured in the absence or presence of 10 μM sense or antisense oligonucleotides to cPLA2 or sPLA2 for 72 h. The levels of cPLA2 (A) or sPLA2 (B) in the cell lysates were quantitated by immunoblotting as described under “Experimental Procedures.” A, control, cells cultured without oligonucleotides; ScA2, cells cultured in the presence of sense oligonucleotides for cPLA2; AScA2, cells cultured in the presence of antisense oligonucleotides for cPLA2; B, control, cells cultured without oligonucleotides; ScsA2, cells cultured in the presence of sense oligonucleotides for sPLA2; AssA2, cells cultured in the presence of antisense oligonucleotides for sPLA2.
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**Table II**

| Subcellular fraction | Lyso-PC treatment | PLA2 activity pmol/min/mg protein |
|----------------------|-------------------|---------------------------------|
| Cytosol              | +                 | 29.4 ± 0.2                      |
| Membrane             | +                 | 13.9 ± 2.6                      |
| Membrane             | −                 | 10.5 ± 0.8                      |

*Significant at p < 0.05.

**Table III**

| Treatment               | Arachidonate release pmol/min/mg protein | Inhibition % |
|-------------------------|----------------------------------------|-------------|
| Control                 | 3.13 ± 0.63                             |             |
| 50 µM lyso-PC           | 32.66 ± 2.19                            |             |
| Staurosporine + lyso-PC | 18.28 ± 0.06*                          | 44          |
| 0.1 µM                  | 10.31 ± 0.10*                           | 68          |
| 10 µM                   | 15.63 ± 0.20*                           | 52          |
| Ro31–8220 + lyso-PC     | 11.25 ± 0.05*                           | 66          |
| H89 + lyso-PC           | 29.70 ± 1.25                            | 9           |
| 0.1 µM                  | 29.40 ± 2.19                            | 10          |

*Significant at p < 0.05.

**Fig. 5. Requirement of extracellular Ca2+ for lyso-PC-induced arachidonate release.** Cells were labeled with [3H]arachidonate for 20 h and then challenged with 0 µM (○) or 50 µM (■) lyso-PC for 10 min in the presence of the indicated Ca2+ concentrations. Arachidonate release was determined as described in Fig. 1. Values represent means ± S.D. of three separate experiments.

**DISCUSSION**

The present study was conducted to study the effects of lyso-PC on the release of arachidonate in endothelial cells. We...
found that exposure of the cells to lyso-PC containing long saturated acyl chains induced a dose-dependent increase in the release of arachidonate and that the effect was mediated through cPLA$_2$. Our findings support a model in which the induction of arachidonate release by lyso-PC is dependent on Ca$^{2+}$ influx and the activation of PKC. These processes result in the stimulation of cPLA$_2$ activity to give rise to an enhanced arachidonate release.

A major pathway for arachidonic acid release from agonist-stimulated cells is via hydrolysis of phospholipids by PLA$_2$ (4). Within the PLA$_2$ subtypes, the preference for arachidonate-containing substrates and the low (intracellular concentrations) requirement for Ca$^{2+}$ of cPLA$_2$ have led many investigators to believe that this isoform is the main enzyme responsible for arachidonate release (3). The present study shows that the cPLA$_2$ is involved in the lyso-PC-stimulated arachidonate release.

The contribution of sPLA$_2$ was also considered. Although antisense oligonucleotides for this isoform caused a reduction in sPLA$_2$ protein, a corresponding attenuation of the lyso-PC-induced arachidonate release was not detected in those cells. This result suggests that the sPLA$_2$ does not contribute significantly to the arachidonate release stimulated by lyso-PC. This finding is in accord with studies in which hormone-stimulated arachidonate release and eicosanoid production was attributed to cPLA$_2$, but not sPLA$_2$ (11–13). It is interesting to note that the involvement of both the cytosolic and secretory PLA$_2$ subtypes in the release of arachidonate for prostacyclin synthesis has also been reported (10).

Lyso-PC, with the participation of diacylglycerol, phosphatidylserine, and Ca$^{2+}$ (50–52), has been shown to modulate PKC activity both in vitro and in vivo. The increase in intracellular Ca$^{2+}$ caused by lyso-PC may contribute to the enhancement of membrane-associated PKC activity. Although cPLA$_2$ is an in vitro substrate for PKC, the direct phosphorylation of cPLA$_2$ by PKC does not result in enhanced phospholipase activity (8), nor has PKC been shown to directly phosphorylate cPLA$_2$ in vivo. However, PKC is a known activator of the p42/44 MAPK signaling cascade via phosphorylation of Raf-1 (62). Our results with the MAPK/extracellular regulated kinase kinase 1 inhibitor PD098059 implicates the involvement of the p42/p44 MAPK cascade in the arachidonate release by lyso-PC. The concentrations of PD098059 used in this study (up to 30 $\mu$M) were similar to those used to almost completely inhibit the activation of MAPK/extracellular regulated kinase kinase 1 and to inhibit the activation of p42 MAPK by up to 80% (53, 63). The partial inhibition of arachidonate release by PD098059 suggests that lyso-PC may also act through pathways other than the recruitment of p42/p44 MAPK. For example, the p38 MAPK is thought to participate in the activation of cPLA$_2$ by agonists (64, 65).

Reported plasma concentrations of lyso-PC range from approximately 130–150 $\mu$M in healthy subjects (66, 67) to 1.7 mM in hyperlipidemic patients (21), while reported concentrations of human serum albumin range from approximately 185 to 850 $\mu$M (68, 69). These figures would correspond to theoretical molar lyso-PC:albumin ratios in serum that range from 0.15 to 9.2. In our studies, arachidonate release was stimulated by lyso-PC at concentrations that correspond to lyso-PC:albumin ratios of 6.2–25. We selected a lyso-PC:albumin ratio of 12.5 ($50 \mu$M lyso-PC and 0.025% or approximately 4 $\mu$L of lyso-PC at concentrations that correspond to lyso-PC:albumin ratios of 0.15–2.5. We selected a lyso-PC:albumin ratio of 12.5 ($50 \mu$M lyso-PC and 0.025% or approximately 4 $\mu$L of lyso-PC for the subsequent experiments, because this ratio represented the lowest lyso-PC concentration that elicited the maximum effect on arachidonate release. Although the complexities of other serum components would probably complicate the in vivo situation, the lyso-PC:albumin ratios used in this study may mimic conditions found in physiological or pathophysiological situations.

Lyso-PC is a natural amphiphile and incorporates into lipid membranes and affects membrane fluidity and permeability (14, 70, 71). Indeed, lyso-PC (at concentrations higher than those used in this study) has been used as an agent for permeabilizing cells (72). However, the detergent properties of lyso-PC do not fully account for its myriad biological effects. For example, lyso-PC increases intracellular Ca$^{2+}$ levels (43, 73, 74) and yet inhibits receptor-mediated Ca$^{2+}$ mobilization (52, 74). It exhibits both vasorelaxant (75) and vasoconstrictive (23) properties. It perturbs nitric oxide synthase mRNA and protein levels in endothelial cells, and up- or down-regulation is dependent on the concentration and incubation conditions used in each study (76–78). Lyso-PC increases the expression of various growth factors and adhesive molecules in endothelial cells.

### Table IV
**Effect of lyso-PC on PKC activity**

| Treatment | PKC activity (pmol/min/mg protein) | Increase |
|-----------|------------------------------------|----------|
| Control   | 80 ± 7                             |          |
| Lyso-PC   | 126 ± 5†                           | 57.5     |
| PMA       | 242 ± 22†                          | 202.5    |

* $p < 0.05$.  

### Table V
**Effect of PD098059 on lyso-PC-induced arachidonate release**

| Treatment | Arachidonate release (dpm/dish) | Inhibition |
|-----------|---------------------------------|------------|
| Control   | 2813 ± 149                      |            |
| 50 $\mu$L lyso-PC | 11,689 ± 63                | 83         |
| 10 $\mu$L PD098059 + lyso-PC | 8018 ± 184$^a$               | 31.4       |
| 30 $\mu$L PD098059 + lyso-PC | 7270 ± 461$^a$               | 37.8       |

* $p < 0.05$.  

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*(This text continues with more detailed analysis and conclusions regarding the role of lyso-PC in endothelial cells, including the involvement of specific enzymes, pathways, and cellular responses)*
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(28–31), and it was shown recently that lysophosphatidylcholine can modulate gene expression independently of PKC and MAPK (31, 79, 80). In our study, lyso-PC was the only lysolipid that stimulates arachidonate release, despite the fact that other lysolipids also possess detergent properties (81, 82). Furthermore, the stimulation of arachidonate release by lyso-PC parallels earlier observations that the ability of lyso-PC to stimulate PKC is unique among lysolipids (50, 51). The findings of the current study demonstrate a novel role of lyso-PC in the modulation of endothelial cell functions.

It is clear from this study that lyso-PC may modulate a pathway that is responsible for its generation in vivo. It is therefore tempting to speculate that the activation of phosphotidylinositol hydrolysis by PLA₂ could be regulated via a positive feedback mechanism that is mediated by its product lyso-PC.

Lyso-PC to stimulate PKC is unique among lysolipids (50, 51). Lyso-PC parallels earlier observations that the ability of other lysolipids also possess detergent properties (81, 82).

The physiological consequences of the stimulation of arachidonate release in endothelial cells by lyso-PC will be an interesting area for further study. Since arachidonate and its metabolites have many biological properties related to vascular homeostasis, the perturbation of arachidonate release by lyso-PC may be a further mechanism whereby this lysolipid could contribute to vascular dysfunction.

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