Protein Kinase C Inhibitors Prevent Impairment of Endothelium-Dependent Relaxation by Oxidatively Modified LDL

Masamichi Ohgushi, Kiyotaka Kugiyama, Kohji Fukunaga, Toyoaki Murohara, Seigo Sugiyama, Eishichi Miyamoto, Hirofumi Yasue

The mechanism(s) of inhibition of endothelium-dependent relaxation (EDR) by oxidized low-density lipoprotein (Ox-LDL) was examined in isolated porcine coronary arteries and rabbit aortas. Incubation with Ox-LDL but not native LDL caused the inhibition of thrombin- or acetylcholine-induced EDR, whereas A23187-induced EDR was preserved after incubation with Ox-LDL. Lysophosphatidylcholine (lysoPC), which was abundant in Ox-LDL and was found to be transferred from Ox-LDL to endothelial cells, also caused the inhibition of EDR in response to thrombin or acetylcholine but not to A23187. Ox-LDL depleted of lysoPC, which was prepared by phospholipase B, failed to inhibit the vasorelaxation. Coincubation with staurosporine or calphostin C, potent inhibitors of protein kinase C, attenuated the EDR inhibition by Ox-LDL or lysoPC. Phorbol 12-myristate 13-acetate, a specific protein kinase C activator, caused the EDR inhibition, and its effect was attenuated by staurosporine or calphostin C. Furthermore, lysoPC was capable of activating protein kinase C purified from cultured porcine endothelial cells. In conclusion, protein kinase C activation plays a role in the inhibition of surface receptor-mediated EDR by Ox-LDL, and lysoPC transferred from Ox-LDL to endothelial cells may be involved in the activation of protein kinase C. (Arterioscler Thromb. 1993;13:1525-1532.)

KEY WORDS • protein kinase C • staurosporine • calphostin C • lysophosphatidylcholine oxidized LDL

Oxidatively modified low-density lipoprotein (Ox-LDL) is a potent atherogenic lipoprotein that accumulates in atherosclerotic arterial lesions.1-3 Ox-LDL has been shown to alter a variety of endothelial cellular functions4-7 and to inhibit endothelium-dependent arterial relaxation (EDR).8 We have reported that Ox-LDL inhibits EDR of rabbit aortas in response to endothelial surface receptor-mediated agonists (acetylcholine, ATP, or substance P) but not to calcium ionophore A23187, which bypasses a receptor-dependent membrane regulation.8 We have also clarified that lysophosphatidylcholine (lysoPC), which is abundant in Ox-LDL, is primarily responsible for the inhibitory action of Ox-LDL on EDR.8 Although one may expect that Ox-LDL could interfere with endothelial surface receptor-mediated signaling pathways, there are few reports focusing on the cell signal transduction underlying the mechanism of inhibitory effect of Ox-LDL on EDR.

LysoPC is known to regulate a number of membrane-associated enzymes such as guanylyl and adenylyl cyclases,9 GTPase,10 or protein kinase C (PKC),11 all of which play an important role in the intracellular signaling pathways. Recently, we have shown that lysoPC inhibits surface receptor-mediated intracellular signals in cultured human endothelial cells and that PKC activation by lysoPC negatively regulates receptor-coupled signal transduction.12 In the present study, on the basis of our previous observation, we further determined whether the modulation of PKC activity may be involved in the EDR inhibition by Ox-LDL.

Methods

LDL Preparations

LDL was isolated by sequential ultracentrifugation (1.019<d<1.063) from fresh human plasma after addition of 1 mg/mL EDTA (native LDL), sterilized by filtration (filter pore size, 0.22 μm; Millipore, Bedford, Mass), and then stored in a sterile tube at 4°C in darkness. Just before modification, LDL was extensively dialyzed against phosphate-buffered saline (PBS; 137 mmol/L NaCl, 2.7 mmol/L KCl, 1.4 mmol/L NaH2PO4, and 4.3 mmol/L Na2HPO4, pH 7.4) under a nitrogen stream for 24 hours at 4°C.

Endothelial cells harvested from porcine aorta were cloned and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum at 37°C under a humidified atmosphere of 5% CO2-95% air. Confluent cultures of the cells exhibited the
typical cobblestone pattern, and most of the cells had factor VIII–related antigen as detected by indirect immunofluorescence. 13

Ox-LDL was prepared by incubating LDL (150 μg protein/mL) with confluent cultured porcine endothelial cells (endothelial cell–modified LDL [EC-LDL]) in serum-free F-10 medium under sterile conditions as reported elsewhere. 8 Thioharbituric acid–reactive substances in the culture medium after incubation of native LDL with endothelial cells averaged 2.4±0.3 nmol malondialdehyde equivalents/mL culture medium. After 24 hours of incubation, EC-LDL in the medium was reisolated by ultracentrifugation at d=1.21 for 24 hours, sterilized by filtration, and used for the bioassay experiments within 3 days. The electrophoretic mobility of EC-LDL on the agarose gel14 related to that of native LDL was 1.7±0.1-fold. Just before being used for the bioassay experiments, stored native LDL and EC-LDL were extensively dialyzed against PBS containing 50 μmol/L EDTA and 20 μmol/L butylated hydroxytoluene (BHT) for 24 hours at 4°C in separate bottles. No LDL preparation was contaminated by endotoxin (less than 10 pg/mL protein) as assessed by the chromogenic limulus lysate test (Toxicolor test, Seikagaku Kogyo, Tokyo, Japan) as described in our previous article. 15 To determine whether lysoPC is responsible for the biological effects of EC-LDL, aliquots of EC-LDL (4 mg protein/mL) were incubated for 2 hours at 37°C with phospholipase B (4 U/mL) in PBS. 16 The effect of phospholipase B–treated EC-LDL was always compared with the same batch of EC-LDL treated without phospholipase B but in the same preparative manipulation as phospholipase B–treated EC-LDL.

Protein concentration of LDL was determined by the method of Lowry et al. 17

Organ Chamber Experiments With Porcine Coronary Arteries

The left circumflex coronary arteries were isolated from domestic Yorkshire pigs within 10 minutes after death. All experiments were in accordance with the guidelines on experimental animals issued by our institution. These arteries were dissected free from adherent connective tissues and cut into ring segments 4 mm wide. During the preparation, any contacts with the luminal surface were avoided to preserve endothelial cell integrity.

Each segment was vertically suspended between stainless steel hooks and incubated in the organ chamber with Krebs-Henseleit solution. The solution was aerated with 15% O2–5% CO2–80% N2 (Po2=100 mm Hg) and maintained at 37°C and pH 7.4. The composition of Krebs-Henseleit solution was as follows (in mmol/L): Na+, 144.2; K+, 4.0; Ca2+, 1.5; Mg2+, 1.2; Cl–, 123.0; SO42–, 1.2; H2PO4–, 1.2; HCO3–, 25.0; and glucose, 10.0; EDTA (20 μmol/L) and BHT (20 μmol/L) were included in the buffer to avoid intraexperimental auto-oxidation of lipoprotein. The resting tension was adjusted to 3 g in porcine coronary arteries, and changes of isometric tension were monitored using a force transducer (UL-20GR; Minebea, Tokyo, Japan) and recorded by an ink-writing pen recorder (SR6211; Graphtec, Tokyo, Japan). Before the start of experiments, all ring segments were allowed to equilibrate for 2 hours, during which the incubation buffer was replaced every 20 minutes.

After the equilibration period, the contractile response to potassium chloride (60 mmol/L) was first obtained to confirm the contractile response of the vascular smooth muscle. The segments were then washed repeatedly and equilibrated for 30 minutes before the start of the next protocol. Arterial rings were exposed to LDL preparations (100 to 300 μg LDL protein/mL), phospholipids (10 μmol/L), or phorbol 12-myristate 13-acetate (PMA; 1 nmol/L) for 40 minutes in the presence or absence of staurosporine (20 nmol/L) or calphostin C (5 nmol/L). After the incubation, the rings were washed repeatedly. They were precontracted with 30 μmol/L prostaglandin F2α (PGF2α) for the porcine coronary arteries and tested with various vasodilators as indicated. Control studies were performed to expose the coronary arteries in the organ chamber to the same volume of PBS (less than 1% of chamber volume) and to the same period of incubation as the studies that examined the arteries exposed to EC-LDL or lysoPC. In some experiments, the rings were pretreated with indomethacin (10 μmol/L) for 30 minutes and then exposed to LDLs or phospholipids for an additional 40 minutes. Indomethacin remained present in the organ bath solution during the exposure of the rings to LDLs or phospholipids.

The extent of agonist-induced relaxation was expressed as a percentage of the precontraction value evoked by PGF2α.

Organ Chamber Experiments With Rabbit Aortas

Aortic strips, as another arterial source, were obtained from male New Zealand White rabbits weighing 2.5 to 3.0 kg. The rabbit aortic strips of 4-mm width were prepared in a similar manner to the porcine coronary arteries, as shown in a previous article. 8 The Krebs-Henseleit solution for rabbit aortas was as follows (in mmol/L): Na+, 144.2; K+, 4.0; Ca2+, 1.5; Mg2+, 1.2; Cl–, 123.0; SO42–, 1.2; H2PO4–, 1.2; HCO3–, 25.0; and glucose, 5.0; EDTA, 0.02; and BHT, 0.02. The aortic strips were exposed to LDL preparations or phospholipids for 2 hours. Control studies were performed to expose the rabbit aortas in the organ chamber to the same volume of PBS (less than 1% of chamber volume) and to the same period of incubation as the studies that examined the arteries exposed to EC-LDL or lysoPC. Thereafter, the strips were repeatedly washed, precontracted with phenylephrine (1 μmol/L), and then tested with various vasodilators.

The extent of agonist-induced relaxation was expressed as a percentage of the precontraction value evoked by phenylephrine.

Measurement of LysoPC Content in LDL Preparations

The method for measurement of lysoPC content in LDL preparations was described previously. 8 Briefly, lipids were extracted from LDL preparations by the method of Bligh and Dyer 18 and were then analyzed by thin-layer chromatography (TLC) using silica gel G plates with a solvent mixture containing chloroform-methanol-water (25:10:1, vol/vol/vol). The lipid band representing lysoPC was eluted with a solvent mixture containing chloroform-methanol-water (5:5:1, vol/vol/
The reaction was initiated by the addition of 5 µL of the aliquots on phosphocellulose paper (Whatman P-81) squares to measure the PKC activity. 21 The phosphatidylserine-dependent and diolein-dependent PKC activities were calculated as the difference between the total count in the standard reagent mixture and the background count in the reagent mixture without phosphatidylserine and diolein and with a peptide inhibitor for PKC (PKC-inhibitor, 2 µmol/L). 22 In the experiments for the assay of PKC activity, protein concentrations were determined by the method of Bradford 23 using bovine serum albumin as a standard.

**Data Analysis**

All data are expressed as mean ± SEM in the text, figures, and tables. Statistical evaluation of the data was performed by Student's t test for unpaired observations. When more than three groups were compared, an analysis of variance was used. If a significant F value was found, a modified t test (Tukey's) was used for statistical evaluation between each group. Differences between values were considered to be statistically significant when P < .05.

**Results**

**Effects of EC-LDL or LysoPC on Relaxation of Porcine Coronary Arteries**

Incubation with EC-LDL (100 µg protein/mL) significantly inhibited the relaxation in response to graded concentrations of thrombin (0.001 to 1.0 U/mL) in porcine coronary arteries, whereas native LDL (up to 300 µg protein/mL) had no inhibitory effect on the relaxation (Fig 1). The vasorelaxation in response to calcium ionophore A23187 (1 to 100 nM) was completely preserved after the incubation with native LDL or EC-LDL (Fig 1). Pretreatment with phospholipase B reduced lysoPC content in EC-LDL to the level of native LDL (Fig 2) and at the same time attenuated the inhibitory effect of EC-LDL on thrombin-induced vasorelaxation (Fig 1). Incubation with synthetic palmitoyl lysoPC (10 µmol/L) inhibited the vasorelaxation in response to thrombin but not to calcium ionophore A23187, whereas dipalmitoyl PC (10 µmol/L) had no effect on either thrombin- or A23187-induced vasorelaxation. The maximal relaxations in response to thrombin and A23187 were as follows: control, 84.3 ± 5.5% (n = 7) and 102.0 ± 1.7% (n = 8); lysoPC, 19.5 ± 2.7% (n = 10, P < .01 versus control) and 100.9 ± 0.6% (P > .05 versus control).
Incubation with endothelial cell-modified LDL (EC-LDL) significantly inhibited the maximal vasorelaxation in response to thrombin but not to A23187, whereas native LDL (N-LDL) and phospholipase B–treated EC-LDL (PLB-EC-LDL) failed to inhibit the vasorelaxation in response to thrombin and A23187 (**P < .01 vs control). Number of each experiment is indicated in parentheses.

(n = 6); and PC, 63.8±5.3% (n = 6) and 102.3±3.0% (n = 6). Sodium nitroprusside (0.01 to 10 μmol/L), an endothelium-independent vasodilator, produced a complete vasorelaxation after incubation with EC-LDL (100 μg protein/mL) or lysophosphatidylcholine (LysoPC) (10 μmol/L). The maximal relaxations were as follows: control, 98.7±3.0% (n = 5); EC-LDL, 100.2±2.8% (n = 5), P = not significant (NS); and lysoPC, 93.6±0.6% (n = 5); P = NS. Coincubation with indoethacin (10 μmol/L) did not significantly influence the inhibition of thrombin-induced vasorelaxation after incubation with EC-LDL or lysoPC (maximal relaxations: indoethacin+EC-LDL, 28.8±7.0% [n = 8] versus EC-LDL alone, 24.8±9.9% [n = 8], P = NS; indoethacin+lysoPC, 24.7±15.7% [n = 6] versus lysoPC alone, 19.5±6.7% [n = 6], P = NS).

To determine the possible involvement of PKC in the inhibitory effects of EC-LDL or lysoPC on vasorelaxation, we examined whether treatment of the arteries with staurosporine or calphostin C, the potent inhibitors of PKC, affected the inhibitory effects of EC-LDL or lysoPC. Coincubation with staurosporine (20 nmol/L) significantly attenuated the inhibitory effects of both EC-LDL and lysoPC on the vasorelaxation in response to thrombin (Figs 3 and 4). Coincubation with calphostin C (5 nmol/L) also significantly prevented the EC-LDL- and lysoPC-induced inhibition of vasorelaxation in response to thrombin (Table 1). Incubation with PMA (1 nmol/L), a specific activator of PKC, caused the inhibition of vasorelaxation in response to thrombin but not in response to calcium ionophore A23187 (Fig 5), which closely resembled the actions exerted by EC-LDL and lysoPC. Coincubation with staurosporine attenuated the inhibitory effect of PMA on the vasorelaxation in response to thrombin (Fig 5). Also, coincubation with calphostin C prevented the inhibitory effect of PMA on the thrombin-induced vasorelaxation (Table 1). These results suggested that PKC activation may at least in part be responsible for the inhibitory effect of EC-LDL or lysoPC on thrombin-induced vasorelaxation.

Incubation with LDL preparations or phospholipids had no significant influences on the PGF2α-induced precontraction values compared with control values (Table 2). Incubation with staurosporine alone exerted no significant effect on the vasorelaxation in response to thrombin (maximal relaxation: control, 77.6±5.6% [n = 8]; staurosporine, 75.1±4.2% [n = 7]; P = NS). Incubation with calphostin C alone exerted no effect on the thrombin-induced vasorelaxation (Table 2). Dimethyl sulfoxide at a concentration of less than 0.1% in the

![Fig 1](image1.png)  
**Fig 1.** Bar graph showing relaxations of porcine coronary arteries in response to thrombin or A23187 after incubation with low-density lipoprotein (LDLs) (100 μg protein/mL). Incubation with endothelial cell–modified LDL (EC-LDL) significantly inhibited the maximal vasorelaxation in response to thrombin but not to A23187, whereas native LDL (N-LDL) and phospholipase B–treated EC-LDL (PLB-EC-LDL) failed to inhibit the vasorelaxation in response to thrombin and A23187 (**P < .01 vs control). Number of each experiment is indicated in parentheses.

![Fig 2](image2.png)  
**Fig 2.** Bar graph showing the content of lysophosphatidylcholine (LysoPC) in low-density lipoprotein (LDL) preparations. LysoPC content in endothelial cell–modified LDL (EC-LDL) significantly increased compared with native LDL (N-LDL) (P < .01). After treatment with phospholipase B (PLB-EC-LDL), lysoPC content in EC-LDL markedly decreased (P < .01 vs EC-LDL). The mean value in each group was determined by six independent experiments, respectively.

![Fig 3](image3.png)  
**Fig 3.** Line graph showing effect of endothelial cell–modified low-density lipoprotein (EC-LDL) (100 μg protein/mL) on the relaxations of porcine coronary arteries in response to graded concentrations of thrombin in the presence or absence of staurosporine (20 nmol/L). Coincubation with staurosporine (EC-LDL+Staurosporine, n = 8) prevented the inhibitory effect of EC-LDL on the vasorelaxation. *P < .05, **P < .01 vs control (n = 10); †P < .05, ††P < .01 vs EC-LDL (n = 8).
organ bath solution did not significantly influence the thrombin-induced relaxation of porcine coronary arteries after incubation with EC-LDL or lysoPC (data not shown). EDTA (20 μmol/L) and BHT (20 μmol/L) in the organ bath solution had no significant influence on the vasorelaxation in response to thrombin (maximal relaxation: control, 73.0±4.6% [n=5]; EDTA and BHT, 77.6±5.6% [n=8]; P=NS). Electron microscopy showed that the endothelial cell lining of the porcine coronary arteries in the organ chamber was almost completely preserved after exposure to EC-LDL.

### TABLE 1. Effects of Calphostin C on the EC-LDL-, LysoPC-, or PMA-Induced Inhibition of Relaxation in Response to Thrombin in Porcine Coronary Arteries

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(-) Calphostin C</th>
<th>(+) Calphostin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83.4±4.8 (7)</td>
<td>86.1±2.8 (6)</td>
</tr>
<tr>
<td>EC-LDL</td>
<td>53.7±6.6* (6)</td>
<td>78.9±5.8 (6)</td>
</tr>
<tr>
<td>LysoPC</td>
<td>46.7±7.3* (7)</td>
<td>71.6±14.3 (6)</td>
</tr>
<tr>
<td>PMA</td>
<td>57.6±4.9* (5)</td>
<td>86.4±4.1 (6)</td>
</tr>
</tbody>
</table>

Isolated porcine coronary arterial rings were incubated with endothelial cell-modified low-density lipoprotein (EC-LDL; 100 μg protein/mL), lysophosphatidylcholine (LysoPC; 10 μmol/L), or phorbol 12-myristate 13-acetate (PMA; 1 nmol/L) in the presence (+) or absence (-) of calphostin C (5 nmol/L) for 40 minutes. After the incubation, the rings were washed, precontracted with prostaglandin F$_{2alpha}$ (P gababa; 30 μmol/L), and tested with thrombin. The maximal relaxation is expressed as a percentage of precontraction value with prostaglandin F$_{2alpha}$. Values are expressed as mean±SEM; number of each experiment is indicated in parentheses. *P<.01 vs control in the absence of calphostin C; †P<.05, ‡P<.01.

### Effects of EC-LDL or LysoPC on Relaxation of Rabbit Aortas

To examine whether species differences may alter the effect of staurosporine, rabbit aortic strips were used to examine the effect of staurosporine on the inhibitory actions of EC-LDL and LysoPC. Incubations with EC-LDL (100 μg protein/mL) and LysoPC (10 μmol/L) significantly inhibited vasorelaxation in response to acetylcholine (0.01 to 10 μmol/L; Figs 6 and 7) and as previously reported. The inhibitory effects of EC-LDL or LysoPC on the vasorelaxation in response to acetylcholine were studied in the presence of staurosporine (STS; 20 nmol/L). EDTA (20 μmol/L) and BHT (20 nmol/L) in the organ bath solution had no significant influence on the vasorelaxation in response to thrombin (maximal relaxation: control, 73.0±4.6% [n=5]; EDTA and BHT, 77.6±5.6% [n=8]; P=NS). Electron microscopy showed that the endothelial cell lining of the porcine coronary arteries in the organ chamber was almost completely preserved after exposure to EC-LDL.
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**Figure 6.** Line graph showing effect of endothelial cell-modified low-density lipoprotein (EC-LDL) (100 μg protein/mL) on the relaxation of rabbit aortas in response to graded concentrations of acetylcholine in the presence or absence of staurosporine (20 nmol/L). Incubation with EC-LDL significantly inhibited the vasorelaxation in response to acetylcholine compared with control. However, coincubation with staurosporine prevented the inhibitory effect of EC-LDL on the vasorelaxation. Each experiment was repeated six times. *P<.05, **P<.01 vs control; †P<.05 vs EC-LDL.

**Figure 7.** Line graph showing effect of lysophosphatidylcholine (LysoPC) (10 μmol/L) on the relaxation of rabbit aortas in response to graded concentrations of acetylcholine in the presence or absence of staurosporine (20 nmol/L). Incubation with lysoPC significantly inhibited the vasorelaxation in response to acetylcholine compared with control. However, coincubation with staurosporine prevented the inhibitory effect of lysoPC on the vasorelaxation. Each experiment was repeated six times. *P<.05, **P<.01 vs control; †P<.05 vs lysoPC.

**Table 3.** Transfer of LysoPC From EC-LDL to Endothelial Cells

| Transferred LysoPC, nmol/mg Cell Protein per 2 hours |
|---------------------------------|---------------|
| Cytosol | Particulate |
| EC-LDL, 10 μg/mL | 7.0±2.7 | 27.0±2.4 |
| EC-LDL, 50 μg/mL | 15.0±0.5 | 65.4±6.3 |
| EC-LDL, 100 μg/mL | 13.8±0.9 | 104.8±1.7 |

Cultured porcine endothelial cells (5×10^6 cells) were incubated for 2 hours with endothelial cell-modified low-density lipoprotein (EC-LDL) labeled with [14C]lysophosphatidylcholine (LysoPC) in 7 mL serum-free medium. Protein concentration of the cytosol fraction was 281±14 μg/5×10^6 cells, and that of the particulate fraction was 225±18 μg/5×10^6 cells. For details, see "Methods." The values in each group were determined by five experiments; data are expressed as mean±SEM.

**Discussion**

The present study demonstrated that EC-LDL inhibited EDR in response to thrombin and acetylcholine. Both thrombin and acetylcholine are vasodilator agonists that act through endothelial surface receptors. The
Fig 8. Bar graph showing effect of lyso-phosphatidylcholine (LysoPC) on the activity of protein kinase C (PKC) purified from cultured porcine endothelial cells. The activity of PKC was assayed by measuring phosphatidylserine-dependent and diolein-dependent phosphorylation of the PKC substrate as described in "Methods." LysoPC (0.5 μmol/L) enhanced the PKC activity compared with control (**P<.01; n=4), but the lysoPC at concentrations higher than 5 μmol/L suppressed the activity (*P<.05; n=4). Each experiment consisted of quadruplicate determinants, and identical results were obtained in three separate sets of experiments.

Oxidative modification of LDL is associated with the inhibitory effect of EC-LDL on the EDR in response to calcium ionophore A23187, which is an endothelium-dependent vasodilator that bypasses a receptor-coupled membrane regulation. Vasorelaxation in response to sodium nitroprusside, an endothelium-independent vasodilator, was completely preserved after the incubation with EC-LDL. Furthermore, the study using electron microscopy showed that the morphological appearance of the endothelium of the arteries was well preserved after exposure to EC-LDL. These results indicated that the inhibition of EDR is not due to the nonspecific cytotoxic effects of EC-LDL on endothelium but that the surface receptor-mediated EDR is selectively inhibited by EC-LDL.

Oxidative modification of LDL is associated with the substantial hydrolysis of PC to lysoPC. We demonstrated that the decreased content of lysoPC in EC-LDL after treatment with phospholipase B was associated with attenuation of the inhibitory effect of EC-LDL on EDR in response to thrombin. The present study also demonstrated that the sonicated lysoPC but not PC exerted an inhibitory effect on the EDR in response to thrombin and acetylcholine, mimicking those exhibited by EC-LDL. These results suggested that lysoPC is primarily responsible for the inhibitory effect of EC-LDL on EDR in response to thrombin or acetylcholine. LysoPC is transferable to accessible membranes or macromolecular acceptors through the aqueous phase. The present study showed that lysoPC was transferred from EC-LDL to endothelial cell membrane and cytosol. These results indicated that lysoPC, which is transferred from EC-LDL to endothelial cells, may be involved in the mechanism of the inhibitory effect of EC-LDL on the EDR in response to endothelial surface receptor-mediated agonists such as thrombin or acetylcholine.

PKC has a crucial role in the signal transduction pathways involving functional alterations of various cells. Activated PKC is also known to inhibit the cell surface receptor–coupled signal transduction in many types of cells, including endothelial cells. Recently, we clearly showed that lysoPC inhibits thrombin- or histamine-mediated intracellular signals and that PKC activation is involved in the negative regulation by lysoPC. The present study demonstrated that stauroporine or calphostin C, the potent inhibitors of PKC, prevented the inhibition of EDR by EC-LDL or lysoPC in porcine coronary arteries and rabbit aortas. PMA, a specific activator of PKC, inhibited EDR in response to thrombin but not to A23187, and its inhibitory effect was prevented by stauroporine and calphostin C, which reflected the similar actions obtained by EC-LDL or lysoPC. As shown in the present study, lysoPC at the lower concentration stimulates the activity of PKC purified from porcine endothelial cells. Furthermore, we have reported that in intact endothelial cells lysoPC increases the membrane-associated PKC activity and decreases the cytosolic PKC activity. Thus, at the concentrations of EC-LDL and lysoPC used in the present organ chamber experiments, PKC activated by lysoPC inhibited endothelial surface receptor–coupled signal transduction and at least in part may be responsible for the inhibitory effect of EC-LDL on EDR in response to surface receptor–mediated agonists. However, the present study showed that lysoPC modulates the activity of purified PKC in a biphasic manner, i.e., lower concentrations of lysoPC activated the PKC activity, whereas higher concentrations of lysoPC suppressed the activity. We also demonstrated that approximately 1.8 μmol lysoPC was transferred from EC-LDL (100 μg protein/mL) in 7 mL serum-free medium to endothelial cells (5×10⁵ cells). However, the concentration of the transferred lysoPC in the subcellular fractions of the intact endothelial cells remained undetermined. Therefore, it remains unclear whether the concentration of the transferred lysoPC is comparable to the concentration of lysoPC that activated PKC in the test tube experiment in the present study. The intracellular process of PKC activation by EC-LDL and lysoPC and its endogenous substrates in the intact endothelial cells are now under investigation in our laboratory.

A recent article shows that stimulation of PKC in endothelial cells induces production of vasoconstrictive prostanooids that may counteract EDR. However, this possibility is less likely in this study because EDR was tested after repeated washing at the end of the incubation and coinubation with indomethacin had no effects on inhibition of EDR by EC-LDL or lysoPC. LysoPC is also known to regulate other membrane-associated enzymes such as guanylyl and adenylyl cyclase or GTPase, all of which play an important role in intracellular signaling pathways. We need further studies to clarify whether the effects of lysoPC on the other membrane-associated enzymes may be involved in the inhibition of surface receptor–mediated EDR by Ox-LDL.

In conclusion, PKC activated by lysoPC, which is transferred from EC-LDL to endothelial cells, may play...
an important role in the mechanism(s) of the inhibition of surface receptor-mediated EDR by Ox-LDL.

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References


Protein kinase C inhibitors prevent impairment of endothelium-dependent relaxation by oxidatively modified LDL.

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