Effects of Lysolipids and Oxidatively Modified Low Density Lipoprotein on Endothelium-Dependent Relaxation of Rabbit Aorta

Earl L. Mangin Jr., Kiyotaka Kugiyama, Judy H. Nguy, Scott A. Kerns, and Philip D. Henry

Exposure of isolated arteries to oxidatively modified low density lipoprotein (LDL) has been reported to suppress endothelium-dependent relaxation (EDR). To determine whether lipid degradation products in oxidized LDL contribute to impaired relaxation, we have tested the responsiveness of isolated rabbit aortas to endothelium-dependent relaxants (acetylcholine, ATP, and calcium ionophore A23187) and nitroglycerin before and after 2-hour incubations with selected lipids and LDL preparations. Concentrations (10 μM) of lecithin, phosphatidylserine, lysophosphatidylserine, sphingomyelin, phosphatidic acid, palmitate, arachidonate, and auto-oxidized arachidonate had no effect on EDR. Concentrations (10 μM) of lysocleithin, lysoplatelet activating factor, and sphingosine significantly suppressed endothelium-dependent relaxation. Native LDL (100 μg/ml incubation buffer) containing only small amounts of lysophosphatidylcholine exerted no effect on EDR. In contrast, LDL preparations oxidatively modified by exposure to cultured endothelial cells or copper inhibited EDR. When modified LDL was depleted of its lysocleithin by treatment with a selective phospholipase B (lysocleithinase), the inhibitory effects were attenuated. In contrast, native LDL accumulating lysocleithin under the influence of a phospholipase A₂ (lecithinase) exerted inhibitory effects mimicking those of oxidized LDL. Lipids and lipoproteins had no effect on the responsiveness to nitroglycerin, an endothelium-independent vasodilator. We conclude that lysocleithin in oxidatively modified LDL contributes importantly to its vasomotor effects. (Circulation Research 1993;72:161–166)

KEY WORDS • endothelium-dependent relaxation • oxidatively modified low density lipoprotein • lysocleithin • lysophosphatidylcholine • phospholipase A₂ • phospholipase B

Previous reports indicate that human low density lipoprotein (LDL) exposed to endothelial cells in culture or to transition metals undergoes a peroxidative degradation that confers cytotoxic properties to the lipoprotein.¹⁻⁴ Of interest is that isolated arteries incubated with preparations of oxidized LDL (O-LDL) become insensitive to endothelium-dependent vasodilators and acquire vasomotor properties resembling those of atherosclerotic arteries.⁵

Parthasarathy et al.⁶⁻⁷ have demonstrated that oxidative modification of LDL is associated with a substantial degradation of lecithin to lysocleithin, a reaction mediated by a phospholipase A₂ activity apparently closely associated with apolipoprotein B-100. Recently, we have observed that membrane-active effects of O-LDL were markedly attenuated after depletion of its lysocleithin by pretreatment with albumin, a potent lysocleithin scavenger.⁸ In addition, incubation of arteries with submicellar concentrations of palmitoyl lysocleithin produced changes in arterial reactivity mimicking those evoked by O-LDL.⁵ Although absorption of O-LDL with albumin is very effective in removing lysocleithin, the possibility must be considered that this procedure concomitantly extracts other lipids that might contribute to the activity of O-LDL. Although a report by Yokoyama et al.⁹ appeared to confirm the importance of lysocleithin in mediating vasoactive effects of O-LDL, others found that free and lipoprotein-bound lysocleithin had no or variable effects on endothelium-dependent arterial relaxation.¹⁰,¹¹ Others concluded that both native LDL (N-LDL) and O-LDL exerted their effects by inactivating endothelium-derived relaxing factor.¹² Therefore, mechanisms of action of LDL on vasomotor regulation remain controversial. In the present study, we have tested effects of N-LDL and O-LDL containing varying amounts of lysocleithin after pretreatment with phospholipase A₂ (lecithinase) or phospholipase B (lysocleithinase). In addition, we have determined the vasomotor effects of nonlysocleithin lipids that may be contained in O-LDL.

Materials and Methods

Artery Preparation

Pharmacological experiments with isolated rabbit arterial strips were performed as previously described.⁵,¹³

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Male New Zealand White rabbits were anesthetized with pentobarbital (30 mg/kg i.v.), and the descending aorta was rapidly excised and placed in Krebs-Henseleit buffer of the following composition (mM): NaCl 116, KCl 4.0, NaHCO₃ 25, CaCl₂ 1.5, MgSO₄ 1.2, Na₂HPO₄ 1.2, glucose 5.0, and Na₃ EDTA 0.025 (25 μM). The isolated aorta was cleaned of periarterial tissue, and 2-mm-wide rings were cut from the midthoracic aorta. The rings were opened and mounted as transverse strips in a myograph for the monitoring of isometric tension. The myograph chamber was filled with Krebs’ buffer equilibrated at 37°C with 15% O₂–5% CO₂ to maintain PO₂ and pH at 100 mm Hg and 7.4, respectively. The strips were preloaded with an optimal preload of 2 g.¹³

Preparation of Native and Oxidatively Modified LDL

N-LDL (density [d]: 1.019–1.063 g/ml) was isolated by the ultracentrifugal method from pooled human normocholesterolemic plasma (total cholesterol, <4.7 mM) collected in Na₂ EDTA (=1 mg/ml). All preparative solutions contained Na₂ EDTA (0.1 mg/ml). O-LDL was produced by incubation of N-LDL with cultured rabbit aortic endothelial cells or with 5 μM copper, as reported by Steinberg and colleagues.¹⁻³ After incubation, LDL was reisolated by ultracentrifugation (flocculation at d=1.210 g/ml). In some experiments, reisolation was omitted. Preparations were dialyzed against Krebs’ buffer formulated as described above but containing no added Ca⁺.²⁵ Protein in LDL preparations was estimated by the Lowry method using bovine serum albumin as a standard.¹⁻³ Phospholipase-treated LDLs were prepared by incubation of N-LDL or O-LDL for 60 minutes at 37°C in nitrogenated Krebs’ buffer (=300–400 μg LDL protein/ml) containing phospholipase A₂ (from Naja naja atra, 4 IU/ml) or phospholipase B (from Vibrio parahemolyticus, 10 IU/ml). For incubations with phospholipase B, the Krebs’ buffer contained 2.5 mM EDTA and no added Ca⁺. After centrifugal reisolation in the presence of 2.5 mM EDTA, the preparations were dialyzed as described above. Lipoprotein-free incubation mixtures subjected to the same preparative manipulations as the lipoprotein-containing mixtures were used as controls. Oxidative modification of LDL was monitored by determining the lipoproteins’ electrophoretic mobility and content in thiobarbituric acid–reactive substances (expressed as nanomoles in “malondialdehyde equivalents” per milligram lipoprotein).¹⁻³⁵ Ray–Barbier acid–reactive substances are a useful estimate of a broad range of lipid peroxidation products (aldehydes and hydroperoxides).¹⁴ Final preparations of LDL were subjected to sequential extractions with chloroform/methanol (first 2:1; then 1:1 [vol/vol]). The combined extracts were evaporated under nitrogen and analyzed by one-dimensional thin-layer chromatography in silica G gel plates (solvent, chloroform/methanol/water at 25:10:1 [vol/vol/vol]), as previously reported.⁵ Separated lipids visualized with iodine vapor were identified by coderveloop appropriate phospholipids or fatty acid standards (see “Chemicals”). Lipid phosphorus in scraped-off silica gel was extracted and measured by the Bartlett procedure (Kugiyama et al).⁶

Pharmacological Protocol

After equilibration for 30 minutes, arteries were constricted with 1 μM phenylephrine and then relaxed by the cumulative addition of acetylcholine, ATP, or calcium ionophore A23187, agents that act as endothelium-dependent relaxants.¹⁵ Agonist concentrations producing maximal effects before exposure to exogenous lipids are presented. The direct (musculotropic) vasodilator nitroglycerin was added at the end of each experiment. The arteries were then washed with Krebs’ buffer and incubated for 2 hours with Krebs’ buffer containing selected lipids or preparations of LDL (or LDL-free control incubation media). The arteries were washed, reequilibrated in Krebs’ buffer, constricted with phenylephrine, and exposed to graded concentrations of the vasodilator as before. In some experiments, arachidonic acid in EDTA-free Krebs’ buffer gassed with 95% O₂–5% CO₂ was incubated for 24 hours at 37°C before use. Peroxidation was monitored spectrophotometrically, recording absorption at 233 nm as an index for the formation of conjugated dienes. Absorbance rose rapidly during the first hours and plateaued after 18 hours.¹⁶

Animals

Random-bred male New Zealand rabbits weighing 2.0–2.5 kg were purchased from a single local vendor.

Chemicals

The following grade A chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.; acetycholine chloride (No. A6500); ATP, disodium (No. A3377); arachidionate, sodium (No. A9548); calcimycin (A23187, No. C7887); indomethacin (No. 17378); lysophosphatidylcholine, palmitoyl (No. L5521); lysophosphatidylserine, stearate (No. S772); lysoplatelet activating factor (No. L7890); palmitate, sodium (No. P9767); phosphatidic acid, (No. P4013); phosphatidylcholine, dipalmitoyl (No. P5911); phosphatidylserine, dipalmitoyl (No. P1902); platelet activating factor (No. 9525); phosphatidylserine (No. P4034); phospholipase B (No. P8914); silica gel TLC plates (No. T5649); sphingomyelin (No. S704); sphingosine (No. S6879); and thiobarbituric acid (No. T3263). Lipids were dispersed by sonication in nitrogenated Krebs’ buffer immediately before use.

Data Analysis

Relaxation responses were expressed as a percentage of stable constrictor tone developed in response to phenylephrine.⁵¹³ Differences between group means before and after incubations with lipids/lipoproteins were analyzed by the Wilcoxon signed rank test for paired observations. Values of p<0.05 were defined as statistically significant.

Results

Effects of Phospholipids

Two-hour incubations with 10-μM concentrations of lecithin, phosphatidylserine, sphingomyelin, lysophosphatidylserine, and phosphatidic acid had no effect on endothelium-dependent relaxation (Table 1). This indicates that not all phospholipid amphiphile with detergent effects impairs endothelium-dependent relaxation. Attempts at testing platelet activating factor at concentrations of 1–10 μM were not possible because of the instability (insolubility) of this phospholipid in Krebs’ buffer.
TABLE 1. Phospholipids Without Supressant Effects on Endothelium-Dependent Relaxation

<table>
<thead>
<tr>
<th>Lipid (10 μM)</th>
<th>ACh (1 μM)</th>
<th>ATP (100 μM)</th>
<th>A23187 (0.1 μM)</th>
<th>TNG (1 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin (%)</td>
<td>n=16</td>
<td>100±3</td>
<td>100±3</td>
<td>100±3</td>
</tr>
<tr>
<td>Phosphatidylserine (%)</td>
<td>11</td>
<td>108±7</td>
<td>108±7</td>
<td>108±7</td>
</tr>
<tr>
<td>Phosphatidic acid (%)</td>
<td>10</td>
<td>106±4</td>
<td>106±4</td>
<td>106±4</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; TNG, trinitroglycerin. Values are mean±SEM and express the percentage of corresponding values before incubation with lipids. No value differed significantly from preincubation controls.

Incubations with 10-μM concentrations of lyssolecithin, lyso–platelet activating factor, and sphingosine exerted similar inhibitory effects on endothelium-dependent relaxation (Table 2). Incubations with 1 μM lyssolecithin, lyso–platelet activating factor, and sphingosine produced only modest suppressions in the response to acetylcholine. Lyssolecithin (1 μM) had a minor effect on the response to ATP. None of the lysolipids produced significant effects at concentrations <1 μM. Incubations with phospholipids (10 μM) had no influence on responses to nitroglycerin (Table 2), nor did it influence phenylephrine-induced constrictor tone (data not shown).

Effects of Fatty Acid

Fatty acids may exert direct membrane effects. In addition, vessels may metabolize fatty acids (arachidonic acid) to potent vasoactive lipids (prostaglandins, leukotrienes, and 20-hydroxyicosatetraenoic acid derivatives). Peroxides of fatty acid could likewise exert vasoactive effects. Therefore, we have tested effects of incubations with palmitate and arachidonate with and without indomethacin as a blocker of cyclooxygenase activity (Table 3). Under the conditions of our experiments (PO2 ≈100 mm Hg; 25 μM EDTA), the fatty acids had no effect on subsequent relaxation responses. Further, extensive autodissociative degradation products of arachidonate (exposure of 10 μM arachidonate to an oxygenated EDTA-free buffer for 24 hours at 37°C) did not significantly affect the responsiveness to endothelium-dependent vasodilators.

Lecithin and Lysolipids Contents of Modified LDLs

The lecithin and lysolipids contents of N-LDL, O-LDL, N-LDL treated with phospholipase A2, and O-LDL treated with phospholipase B are shown in Table 4.

TABLE 3. Effects of Fatty Acids on Endothelium-Dependent Relaxation

<table>
<thead>
<tr>
<th>Lipid (10 μM)</th>
<th>ACh (1 μM)</th>
<th>ATP (100 μM)</th>
<th>A23187 (0.1 μM)</th>
<th>TNG (1 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonate (%)</td>
<td>7</td>
<td>105±8</td>
<td>108±8</td>
<td>110±4</td>
</tr>
<tr>
<td>Arachidonate+INDO (%)</td>
<td>7</td>
<td>96±6</td>
<td>103±7</td>
<td>101±7</td>
</tr>
<tr>
<td>Palmitate (%)</td>
<td>7</td>
<td>103±4</td>
<td>98±6</td>
<td>110±4</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; TNG, trinitroglycerin; INDO, 10 μM indomethacin. Values are mean±SEM and express percentages of corresponding values before incubation with lipids. No value differed significantly from preincubation controls.

TABLE 2. Phospholipids With Suppressant Effects on Endothelium-Dependent Relaxation

<table>
<thead>
<tr>
<th>Lipid (1 μM)</th>
<th>ACh (1 μM)</th>
<th>ATP (100 μM)</th>
<th>A23187 (0.1 μM)</th>
<th>TNG (1 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyssolecithin (%) (n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μM</td>
<td>92±8*</td>
<td>98±4</td>
<td>103±4</td>
<td>98±4</td>
</tr>
<tr>
<td>1.0 μM</td>
<td>66±5*</td>
<td>81±4*</td>
<td>97±4</td>
<td>108±7</td>
</tr>
<tr>
<td>10 μM</td>
<td>18±4*</td>
<td>28±2*</td>
<td>58±4*</td>
<td>101±6</td>
</tr>
<tr>
<td>Lyso-PAF (%) (n=14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μM</td>
<td>95±7</td>
<td>98±3</td>
<td>101±5</td>
<td>96±8</td>
</tr>
<tr>
<td>1.0 μM</td>
<td>82±4*</td>
<td>96±7</td>
<td>98±8</td>
<td>103±4</td>
</tr>
<tr>
<td>10 μM</td>
<td>34±1*</td>
<td>45±8*</td>
<td>65±17*</td>
<td>96±7</td>
</tr>
<tr>
<td>Sphingosine (%) (n=12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 μM</td>
<td>103±9</td>
<td>101±6</td>
<td>97±4</td>
<td>104±6</td>
</tr>
<tr>
<td>1.0 μM</td>
<td>84±4*</td>
<td>98±5</td>
<td>105±12</td>
<td>103±5</td>
</tr>
<tr>
<td>10 μM</td>
<td>15±12*</td>
<td>27±7*</td>
<td>51±11*</td>
<td>93±8</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; TNG, trinitroglycerin; PAF, platelet activating factor. Values are mean±SEM and express percentages of corresponding values before incubation with lipids.

*p<0.05 vs. preincubation values.

Effects of Modified LDLs

Incubations with N-LDL had no effect on subsequent relaxation responses. In contrast, after pretreatment with phospholipase A2, a procedure that degraded a substantial fraction of the lipoprotein lecithin to its lysoform, the enzymatically modified lipoprotein evoked a marked unresponsiveness to acetylcholine. Unresolated O-LDL was as potent as phospholipase A2-treated N-LDL in inhibiting relaxations. When lyssolecithin in O-LDL was degraded to approximately one third of its original value, the lipoprotein’s inhibitory effects were attenuated (Table 5). Lipoprotein-free incubation mixtures had no effect on endothelium-dependent relaxations (Table 5, figures in parentheses).
Relation Between LDL-Phospholipid and Endothelium-Dependent Relaxation

The relations between lyssolecithin in various LDL preparations and endothelium-dependent relaxation after exposure of arteries to these LDLs are depicted in Figure 1. The graph suggests that increasing lyssolecithin contents are associated with increasing impairment in cholinergic relaxation.

Discussion

Recently, several authors have reported that exposure of isolated arteries to preparations of oxidatively modified LDL impairs their relaxation in response to endothelium-dependent vasodilators and/or evokes slow increases in resting arterial tone.9-12,22-24 In 1978, we had noted that LDL, but not very low density lipoprotein, intermediate density lipoprotein, or high density lipoprotein, produced slow constrictions in isolated canine arteries, but recognizing that the effects were related to degradation of LDL in vitro, we discarded the experiments as preparative artifacts.25 Subsequently, we reported that ultrasonically dispersed lipid extracts from O-LDL, but not from N-LDL, impaired endothelium-dependent relaxation.5 Removal of lyssolecithin from O-LDL by treatment with albumin, a

<table>
<thead>
<tr>
<th>Lipoprotein or lipid</th>
<th>n</th>
<th>Lecithin</th>
<th>Lyssolecithin</th>
<th>Lecithin plus lyssolecithin</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-LDL</td>
<td>6</td>
<td>1,510±123</td>
<td>8±3</td>
<td>1,518</td>
</tr>
<tr>
<td>N-LDL with PLA2 (%)</td>
<td>6</td>
<td>713±38</td>
<td>511±8</td>
<td>1,224</td>
</tr>
<tr>
<td>O-LDL-EC (NON-ISO) (%)</td>
<td>10</td>
<td>595±51</td>
<td>567±41</td>
<td>1,162</td>
</tr>
<tr>
<td>O-LDL-EC (ISO) (%)</td>
<td>4</td>
<td>503±63</td>
<td>343±34</td>
<td>736</td>
</tr>
<tr>
<td>O-LDL-EC with PLB (ISO)</td>
<td>5</td>
<td>499±49</td>
<td>190±21</td>
<td>689</td>
</tr>
<tr>
<td>O-LDL-Cu (NON-ISO) (%)</td>
<td>5</td>
<td>790±63</td>
<td>408±43</td>
<td>1,198</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; N-LDL, native LDL; PLA2, phospholipase A2; O-LDL-EC, endothelial cell-oxidized LDL; ISO, reisolation of LDL; PLB, phospholipase B; O-LDL-Cu, copper-oxidized LDL. Values are mean±SEM.

Preparations of N-LDL, O-LDL-EC, and O-LDL-Cu with or without subsequent treatment with PLA2 or PLB were performed as indicated in “Materials and Methods.” LDL was reisolated (ISO) or was not reisolated (NON-ISO) ultracentrifugally after oxidation and/or treatment with lipase. Thiobarbituric acid–reactive substances (see “Materials and Methods”) for preparations N-LDL, N-LDL with PLA2, O-LDL-EC (NON-ISO), O-LDL-EC (ISO), O-LDL-EC with PLB (ISO), and O-LDL-Cu (NON-ISO) averaged 3.8±0.5, 3.3±0.3, 48±8, 41±0, 38±4, and 58±5 nmol malondialdehyde/mg LDL protein, respectively.

Table 5. Effects of Low Density Lipoprotein Preparations With or Without Pretreatment With Lipases on Endothelium-Dependent Relaxation

<table>
<thead>
<tr>
<th>Lipoprotein or lipid</th>
<th>n</th>
<th>Acetylcholine (1 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-LDL* (%)</td>
<td>6</td>
<td>98±2</td>
</tr>
<tr>
<td>N-LDL with PLA2 (%)</td>
<td>6</td>
<td>10±3† (105±6)</td>
</tr>
<tr>
<td>O-LDL-EC (NON-ISO) (%)</td>
<td>10</td>
<td>14±4† (97±4)</td>
</tr>
<tr>
<td>O-LDL-EC (ISO) (%)</td>
<td>4</td>
<td>24±2† (103±5)</td>
</tr>
<tr>
<td>O-LDL-EC with PLB (ISO)</td>
<td>4</td>
<td>61±1†</td>
</tr>
<tr>
<td>O-LDL-Cu (NON-ISO) (%)</td>
<td>5</td>
<td>18±3† (98±7)</td>
</tr>
<tr>
<td>Lyssolecithin (%)</td>
<td>8</td>
<td>17±1</td>
</tr>
<tr>
<td>Lyssolecithin with PLB (%)</td>
<td>8</td>
<td>96±2 (110±5)</td>
</tr>
</tbody>
</table>

N-LDL, native low density lipoprotein (LDL); PLA2, phospholipase A2; O-LDL-EC, endothelial cell–oxidized LDL; ISO, reisolation of LDL; PLB, phospholipase B; O-LDL-Cu, copper-oxidized LDL. Values are mean±SEM and express percentages of corresponding values before incubation with lipoproteins.

Arteries were incubated for 2 hours with buffer containing LDL preparations (100 μg protein/ml). Numbers in parentheses correspond to LDL-free incubation media subjected to the same preparative procedures as the media containing LDL.

*In independent experiments, thiobarbituric acid–reactive substances for N-LDL before and after 2 hour coincubations with an aortic strip in Krebs’ buffer averaged 4.0±0.5 and 3.8±0.4 nmol malondialdehyde/mg LDL protein, respectively (n=6, p>0.1). †P<0.05 vs. preincubation values.

Figure 1. Plot of data from Table 4 (lyssolecithin content of low density lipoproteins [LDLs]) vs. data from Table 5 (cholinergic relaxation after incubation with corresponding LDLs). N-LDL, native LDL; O-LDL, oxidized LDL; ALB, pretreatment with albumin; O-LDL-EC, endothelial cell–oxidized LDL; PLB, subsequent treatment with phospholipase B; O-LDL-Cu, copper-oxidized LDL; ISO and NON-ISO, presence and absence, respectively, of LDL reisolation after oxidation and/or treatment with lipase; PLA2, subsequent treatment with phospholipase A2. The data point referring to O-LDL-ALB was taken from a previous study in which lyssolecithin was removed from LDL by pretreatment with albumin.5
potent lyssolecithin scavenger,9 markedly attenuated vaso-active effects of LDL. Also, palmitoyl lyssolecithin exerted effects similar to those observed with O-LDL.5 In the present study, we have further investigated effects of lysolipids on endothelium-dependent relaxation. Results suggest a relation between the abundance of lyssolecithin accumulated in modified LDLs and vaso-active effects of the lipoproteins (Figure 1). The action of lyssolecithin on endothelium-dependent relaxation was, however, not specific, since both sphingosine and lys–platelet activating factor produced similar changes in cholinergic responsiveness. Compared with lyssolecithin, these compounds are present in only very small amounts in oxidatively modified LDL (attempts at demonstrating these compounds in our preparations of LDL were unsuccessful [results not shown]). Of greater importance are hydrolytic products generated from abundant phospholipids other than lyssolecithin. Interestingly, lysophosphatidylserine, an aminophospholipid that occurs physiologically predominantly in the inner leaflets of surface membranes, exerted no apparent inhibitory effects on endothelium-dependent relaxation. Yokoyama et al.9 who observed inhibitory effects of copper-oxidized LDL on endothelium-dependent relaxation, extracted the lipoprotein lipids and separated lyssolecithin from the rest of the extracts. Whereas separated lyssolecithin was active, the nonlyssolecithin fraction was inactive. These results appear to corroborate our findings.

We have previously proposed that atherosclerotic arteries27 or arteries exposed to O-LDL in vitro respond abnormally to endothelium-dependent agonists because of an impaired membrane-delimited regulation. In the present study, micromolar concentrations of phospholipids that affected responsiveness to acetylcholine and ATP did not attenuate responses to A23187 (Table 2). This relative preservation of the responsiveness to the ionophore, an agonist that bypasses membrane regulation, is consistent with the hypothesis that phospholipids influence transmembrane signaling.26

Although lyssolecithin in our experiments can account for vasoactive effects of oxidatively modified lipoproteins, they do not rule out other possible molecular mechanisms of action. One major difficulty is that peroxidation produces a multiplicity of lipid peroxidation and hydrolysis products that may be lost during preparative manipulations of the lipoproteins. As shown in Table 4, it is, for instance, not possible to reisolate ultracentrifugally O-LDL without concomitantly losing some polar lipids (lyssolecithin). Nevertheless, two independent interventions depleting LDLs of lyssolecithin (absorption with albumin5 and treatment with lysophospholipase B) exerted similar effects, and synthetic lyssolecithin substituted with a saturated fatty acid mimicked the effects of O-LDL. On the other hand, exposure to arachidonate (authentic or oxidized) appeared to exert little effects. This study was not designed to determine mechanisms by which polar lipids (lyssolecithin) may be transferred from O-LDL to endothelial cells. Although polar lipids are transferred from donors (LDL) to acceptors (endothelial cell membrane) independent of donor/acceptor collision events,27 we cannot rule out the possibility that binding and/or internalization via receptor mechanisms (LDL [apolipoproteins B and E] or scavenger receptors) contribute to amphiphile transfer. Lyssolecithin effects could play an important role in vivo, since the concentration of this lipid in atherosclerotic arterial walls may be increased severalfold compared with that found in normal arteries.28,29 Because lyssolecithin in high concentrations is cytotoxic, it may contribute to cell death occurring in atheromatous lesions.30

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References

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