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Albumin restores lysophosphatidylcholine-induced inhibition of vasodilation in rat aorta

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Albumin restores lysophosphatidylcholine-induced inhibition of vasodilation in the rat aorta.

Background. Impairment of vasodilation by oxidized low-density lipoprotein has been attributed to lysophosphatidylcholine (LPC). Albumin avidly binds LPC. Therefore, hypoalbuminemia may directly impair vasodilation and thus contribute to increased risk of atherosclerosis in nephrotic syndrome. The addition of albumin reduces LPC in erythrocytes and endothelial cells. We hypothesized that the addition of albumin will salvage vasodilation in aortic rings previously exposed to LPC. LPC increases superoxide production and disturbs L-arginine availability. Therefore, we also decreased superoxide with a superoxide dismutase mimic, $MnCl_2$, and supplemented L-arginine in an attempt to restore vasodilation.

Methods. Rat aorta rings, which had been incubated with various concentrations of LPC and human serum albumin (HSA), were mounted in organ chambers. Relaxation was studied with acetylcholine (0.01 to 100 μmol/L) after precontraction with phenylephrine (CON, 0.3 μmol/L; LPC, 0.03 μmol/L). In some studies $MnCl_2$ or L-arginine was added to the organ chamber.

Results. LPC had time- and dose-dependent inhibitory effects on acetylcholine-mediated vasodilation, but no effect on nitroprusside-mediated vasodilation. Preincubation with albumin (50 or 6 g/L) could protect vasodilation against very high levels of LPC. After preincubation with LPC, the addition of albumin to the incubation salvaged vasodilation. Albumin was more effective after short LPC incubation. $MnCl_2$ had no specific effect on the LPC-mediated disturbance in vasodilation. L-arginine completely salvaged vasodilation at low concentrations of LPC. However, even high concentrations of L-arginine (1 mmol/L) could not improve vasodilation at LPC levels at which vasodilation was restored by albumin.

Conclusions. LPC affects several pathways that inhibit vasodilation, all of which are salvaged by addition of albumin.

Key words: L-arginine, endothelium-dependent relaxation, superoxide, oxidized low-density lipoprotein, nephrotic syndrome, aortic rings.

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Oxidized low-density lipoprotein (ox-LDL) impairs endothelium-dependent relaxation (EDR) [1]. One change observed in ox-LDL is the marked increase in lysophosphatidylcholine (LPC) content [2, 3]. LPC has been implicated as the factor that is primarily responsible for disturbed EDR [2, 3], even at very low concentrations (0.1 to 10 μmol/L) [4]. A characteristic of LPC is that it is reversibly bound to albumin [5, 6], erythrocytes [7], or lipoproteins [3, 8] in the circulation. Albumin serves like a reservoir for LPC, effectively controlling LPC bioavailability. Recently, we showed that the LPC concentration was increased in very LDL (VLDL) and LDL fractions of normolipidemic patients with hypoalbuminemia, although the sum of LPC in all plasma fractions was normal [9]. Furthermore, Stoll, Oskarsson, and Spector have shown that albumin reduces LPC uptake in cultured endothelial cells and erythrocytes [10]. The LPC content was increased in erythrocytes from analbuminemic rats, which was normalized by the addition of albumin [11]. The addition of albumin to ox-LDL also decreases LPC content in the membrane of cultured endothelial cells [12].

Impairment in EDR caused by LPC is probably due to derangement of endothelium membrane structure because of transfer of LPC from ox-LDL to this compartment [13, 14]. This derangement in membrane structure can be responsible for the disturbance of several pathways affected by LPC. One of these pathways is the capability of LPC to increase superoxide production via activation of kinases. LPC can stimulate superoxide production in isolated human neutrophils and vascular smooth muscle cells by activating phosphatidylinositol 3 kinase [15] and protein kinase C [16], respectively. Another mechanism by which LPC can disturb EDR is by inhibiting the high-affinity component of the arginine transporter, causing depletion of the substrate for nitric oxide (NO) generation [17]. Finally, G proteins can be uncoupled from cell-surface receptors involved in the

activation of nitric oxide synthase (NOS) because of the derangement in endothelial membrane structure [18–20].

It was found that EDR was disturbed in patients [21] and in rats [22] with the nephrotic syndrome, where both hypoalbuminemia and hyperlipidemia were present. Furthermore, it was found that L-arginine could restore impaired EDR in patients with combined hyperlipidemia [23] and familial hypercholesterolemia [24, 25]. However, it is remarkable that L-arginine could not correct the impairment of EDR in the nephrotic syndrome [21]. This may be due to hypoalbuminemia over and above hypercholesterolemia, and implies that besides high cholesterol levels, hypoalbuminemia also may induce a specific intra-endothelial derangement. We hypothesized that hypoalbuminemia will result in diminished binding of LPC to albumin and hence sequestration of LPC in endothelial cells, which will directly impair EDR by one or more of the mechanisms mentioned previously in this article.

Therefore, we tested whether albumin can protect endothelial cells against the deleterious effects of LPC and whether the addition of albumin will salvage EDR in rat aortic rings previously exposed to LPC. LPC concentrations used were similar to those found in total plasma and LDL in nephrotic subjects [9]. We also investigated the pathways by which LPC can disturb EDR, by testing whether addition of L-arginine or of $MnCl_2$, a superoxide dismutase mimic [26], could salvage EDR.

METHODS

Organ chamber experiments

Male Sprague-Dawley rats weighing 200 to 250 g were housed in a controlled environment and given free access to standard rat chow and tap water. All animal procedures were approved by our university animal care and use committee.

Aortas were dissected free during anesthesia with phenobarbital sodium (60 mg/kg IP) and immersed in a carbogenized Krebs-Ringer buffer composed of (in mmol/L) 118.3 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 25.0 $NaHCO_3$, and 11.1 glucose. The aortas were carefully cleaned of blood clots and peri-aortic tissue and were cut into rings (2 to 4 mm long). Care was taken not to damage the endothelium. The rings were mounted horizontally between two stainless steel hooks in organ chambers filled with 10 mL of Krebs-Ringer buffer (37°C) gassed with 95% O_2/5% CO_2. One hook was anchored in the organ chamber, and the other was connected to a strain gauge transducer for the measurement of isometric tension. The aortic rings were then progressively stretched to an optimal basal tension of 1 g, and the contraction ability of the rings was checked with KCl.

Rings were incubated with Krebs-Ringer buffer in the presence or absence of human serum albumin (HSA) and/or LPC. In the organ chamber, all rings were treated with indomethacin (10 μmol/L) to prevent the formation of endogenous prostaglandins. Relaxation with acetylcholine (0.01, 1, and 100 μmol/L) and sodium nitroprusside (separate dose-response curves and 10 μmol/L at the end of each acetylcholine experiment) was examined during precontraction with phenylephrine (0.3 μmol/L for control rings and 0.03 μmol/L for rings treated with LPC). At least three rats and three rings per rat were used in each condition. Note that HSA was present only during the incubation period, but not in the organ chamber. Foam formation did not occur because the buffer was gassed with 95% O_2/5% CO_2 before the incubation period and in the organ chamber after the incubation period.

The first experiment consisted of aortic rings exposed to LPC for 90 minutes at different concentrations (0 to 100 μmol/L) followed by contraction with phenylephrine (PE). In the second experiment, rings were exposed to LPC for 90 minutes at different concentrations (0 to 200 μmol/L) and preconstricted with PE, and the dose-response to sodium nitroprusside was measured. In the third experiment, the rings were exposed to LPC for 30 or 90 minutes at different concentrations (0 to 200 μmol/L) and preconstricted with PE, and the dose-response to acetylcholine was measured. In the fourth experiment, the rings were preincubated with LPC (100 μmol/L) for different incubation times (0, 5, 10, 20, 30, 60, or 90 min).

The fifth experiment consisted of preincubation with 50, 20, or 6 g/L HSA for 90 minutes. To display the capability of HSA to protect EDR by preventing uptake of LPC, we added LPC (0 to 2000 μmol/L) to the HAS solution after 30 minutes. In the sixth experiment, we wanted to demonstrate the ability of albumin to salvage LPC-induced impairment in EDR by binding of LPC that already had been taken up into the tissue. Rings were preincubated with LPC, for 30 or 90 minutes, followed by the addition of 50 g/L HSA after 10 or 30 minutes, respectively.

In the seventh and eighth experiments, rings exposed to LPC, but not incubated with albumin, were tested with $MnCl_2$ (dismutation of superoxide) or L-arginine (increasing NO availability), respectively. Twenty minutes before the addition of PE to the rings, $MnCl_2$ or L-arginine was added to the organ chambers. At the end of every experiment, the integrity of smooth muscle dilatory function was checked with sodium nitroprusside (10 μmol/L).

Lucigenin-enhanced chemiluminescence

Superoxide activity was measured using a LUMAT LB 9507 (Berthold, Wildbad, Germany) luminometer. In vitro enzymatic systems that produced little O_2^- have shown that lucigenin itself can act as a source of O_2^- via auto-oxidation of the lucigenin cation radical [27]. Dose-response curves with lucigenin were obtained (3, 10, 100, and 300 μmol/L) in rat aorta to find a concentration of lucigenin at which auto-oxidation does not occur [28].

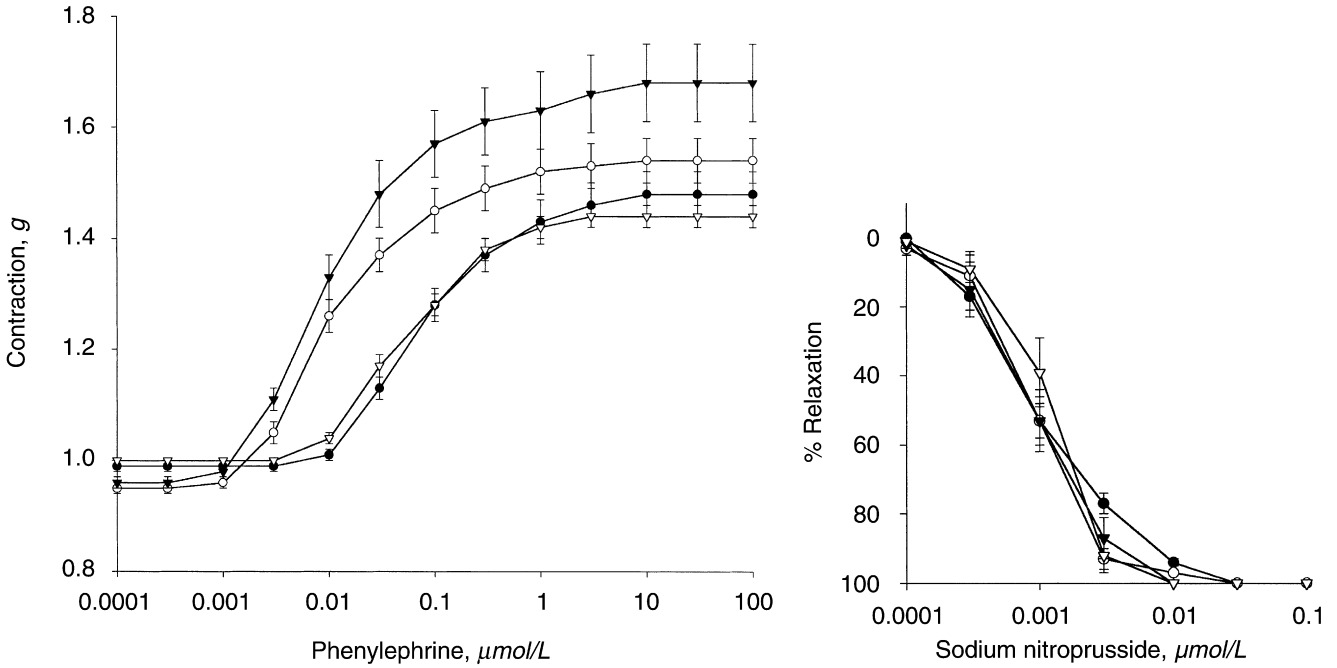

Fig. 1. Dose-response curves to phenylephrine (PE) were determined after 90-minute incubations with 0 (●), 60 (○), 100 (▼) μmol/L lysophosphatidylcholine (LPC) or 50 g/L human serum albumin (HSA; ▽). HSA did not affect contraction to PE, while rings incubated with LPC were more sensitive. (Inset) Dose-response curves to sodium nitroprusside (SNP) were determined after 90-minute incubations with 0 (●), 60 (○), 100 (▼), and 200 (■) μmol/L lysophosphatidylcholine (LPC). LPC did not affect relaxation to SNP.

This was 100 μmol/L. Rat aorta rings (~1 cm long) were incubated, in Krebs-Ringer buffer, for 90 minutes with or without LPC at room temperature. After incubation, the rings were put in a polystyrene tube (Sarstedt, Nümbrecht, Germany) with 300 μL Krebs-HEPES buffer composed of (in mmol/L) 10 HEPES, 135.3 NaCl, 4.7 KCl, 1.8 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, and 11.1 glucose. The tube was placed in the luminometer, and background lucigenin-enhanced chemiluminescence (LEC) was measured for four minutes. Lucigenin (bis-N-methyl acridinium nitrate, 300 μL) was injected, realizing a final concentration of 100 μmol/L, and LEC was measured during 10 minutes (sample rate 1 Hz). Finally, $MnCl_2$ was injected to reverse the superoxide production, and LEC was measured for two minutes. Rings were dried overnight and weighed. LEC was calculated as average counts measured during the last five minutes minus average background activity and was expressed as counts per 10 mg dry weight [29].

Agents

L-α-lysophosphatidylcholine, containing primarily palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids, phenylephrine, acetylcholine, KCl, sodium nitroprusside, L-arginine, $MnCl_2$, indomethacin, and lucigenin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). HSA was obtained from the Central Laboratory Blood bank (CLB; Amsterdam, The Netherlands). All drugs were dissolved and

Table 1. Effect of lysophosphatidylcholine (LPC) on acetylcholine (1 μmol/L)-mediated relaxation after phenylephrine contraction: Concentration-dependent effects at 30- and 90-minute incubations

	Incubation	
Concentration	30 min	90 min
0 μmol/L LPC	62 ± 9	65 ± 4
10 μmol/L LPC	ND	43 ± 15[b]
20 μmol/L LPC	ND	51 ± 7[b]
40 μmol/L LPC	45 ± 12[a]	35 ± 7[b]
60 μmol/L LPC	43 ± 6[a]	5 ± 2[b]
80 μmol/L LPC	11 ± 3[a]	2 ± 2[b]
100 μmol/L LPC	9 ± 2[a]	−1 ± 1[b]
200 μmol/L LPC	5 ± 2[a]	0 ± 0[b]

Data are expressed as percentage relaxation. ND is not determined.
[a] $P < 0.05$ vs. 0 μmol/L LPC (incubation 30 min)
[b] $P < 0.05$ vs. 0 μmol/L LPC (incubation 90 min)

Table 2. Time-dependent effects of 100 μmol/L lysophosphatidylcholine (LPC)

Time	100 μmol/L LPC
0 min	65 ± 4
5 min	59 ± 7
10 min	20 ± 5[a]
20 min	5 ± 2[a]
30 min	9 ± 2[a]
60 min	2 ± 2[a]
90 min	−1 ± 1[a]

[a] $P < 0.05$ vs. 0 min

Table 3. Effect of human serum albumin (HSA) on lysophosphatidylcholine (LPC)-mediated inhibition of acetylcholine (1 μmol/L)-mediated relaxation after phenylephrine contraction, after a 90-minute incubation period

	HSA			
	50 g/L LPC	20 g/L	6 g/L	0 g/L
0 μmol/L LPC	61 ± 6	68 ± 5	62 ± 5	65 ± 4
100 μmol/L LPC	64 ± 1	63 ± 8	62 ± 6	−1 ± 1[d]
200 μmol/L LPC	55 ± 4	63 ± 4	44 ± 4[c]	0 ± 0[d]
400 μmol/L LPC	59 ± 3	60 ± 5	7 ± 2[c]	ND
600 μmol/L LPC	58 ± 6	54 ± 7	−1 ± 1[c]	ND
800 μmol/L LPC	24 ± 2[a]	5 ± 4[b]	ND	ND
1000 μmol/L LPC	23 ± 6[a]	0 ± 0[b]	0 ± 0[c]	ND
1400 μmol/L LPC	14 ± 4[a]	ND	ND	ND
2000 μmol/L LPC	14 ± 9[a]	0 ± 0[b]	ND	ND

Data are expressed as percentage relaxation. ND is not determined.
[a] $P < 0.05$ vs. 0 μmol/L LPC (50 g/L HSA)
[b] $P < 0.05$ vs. 0 μmol/L LPC (20 g/L HSA)
[c] $P < 0.05$ vs. 0 μmol/L LPC (6 g/L HSA)
[d] $P < 0.05$ vs. 0 μmol/L LPC (0 g/L HSA)

diluted in saline (0.9% NaCl), except LPC and indomethacin, which were dissolved in methanol (maximum final concentration 2.44%), and KCl, which was dissolved in demineralized water.

Data analysis

Results are expressed as mean ± SEM. The PE dose–response is expressed in gram contraction. In rings precontracted with PE, acetylcholine responses are expressed as percent relaxation. One-way (LPC concentration, incubation time) or two-way (LPC concentration × incubation time, LPC concentration × arginine or $MnCl_2$ concentration) analysis of variance was used to evaluate the statistical significance between values obtained in different groups. Values were considered statistically different at $P < 0.05$.

RESULTS

Phenylephrine and nitroprusside dose–response

The PE dose–response was determined in control rat aortic rings and rings incubated with LPC for 90 minutes. A low LPC concentration (20 μmol/L) had no effects on the PE response (data not shown). However, at higher LPC levels (60 and 100 μmol/L), the rings were more sensitive to PE, and the maximum contraction was increased (Fig. 1A). Therefore, different PE concentrations—0.3 μmol/L for controls and 0.03 μmol/L for rings incubated with LPC—were used for relaxation studies with acetylcholine (ACh). Precontraction obtained at these PE concentrations was approximately 70% of the maximum contraction. SNP dose response was determined in control rings and in rings incubated with LPC (60 to 200 μmol/L) for 90 minutes. Even at 200 μmol/L, LPC did not affect the SNP response (Fig. 1B).

Acetylcholine-mediated relaxation

Relaxation to ACh was determined after incubation of the rings with LPC for 30 or 90 minutes. Relaxation to sodium nitroprusside was complete at all LPC levels after either short or long incubation. Dose-response curves were obtained (data not shown), and the data were compared at an ACh concentration of 1 μmol/L. Increasing concentrations of LPC had an increasing inhibitory effect on EDR, which was more pronounced after a longer incubation time (Table 1). LPC-induced impairment in EDR was also time dependent. When rings were incubated with 100 μmol/L LPC, the concentration found in LDL fractions of normolipidemic patients with hypoalbuminemia [9], during a 10- to 90-minute incubation time, the EDR was progressively impaired (Table 2). Rings incubated in buffer with the vehicle (methanol 2.44%) showed normal EDR.

Protection by albumin against LPC effect

To test the hypothesis that albumin could bind LPC and therefore prevent its uptake into the endothelium, preincubation with 50 g/L HSA, as a model for normoalbuminemia, was done for 90 minutes. After 30 minutes, LPC was added. HSA alone did not affect EDR compared with controls. Higher LPC levels were needed to block EDR than without HSA (Table 3). HSA (50 g/L) completely protected EDR up to 600 μmol/L LPC. Partial protection was present at 1 mmol/L, and slight protection was even seen at 2 mmol/L. On the other hand, after preincubation with 20, 6, and 0 g/L HSA, as a model for mild and severe hypoalbuminemia and analbuminemia, respectively, progressively less LPC, as compared with 50 g/L HSA, was needed to disturb EDR (Table 3). These results show that albumin was very effective in binding LPC and preventing LPC-induced impairment in EDR.

Salvaging EDR with albumin after exposure to LPC

Next, we studied whether 50 g/L albumin could scavenge LPC that was already present in the tissue. Rings were incubated with LPC (100 μmol/L) for 30 or 90 minutes. Albumin was added at 10 or 30 minutes, respectively. HSA (50 g/L) restored ACh-mediated relaxation completely in the short incubation and to 25% in the long incubation ($P < 0.05$ vs. LPC alone; Fig. 2). Thus, albumin could salvage LPC-induced impairment in EDR by binding to both LPC in the solution and to LPC that presumably had already been taken up in the tissue.

Superoxide production increased by LPC did not impair EDR

Lysophosphatidylcholine dose-dependently increased superoxide production, measured with LEC (Fig. 3, inset). Superoxide activity was abolished after addition of $MnCl_2$

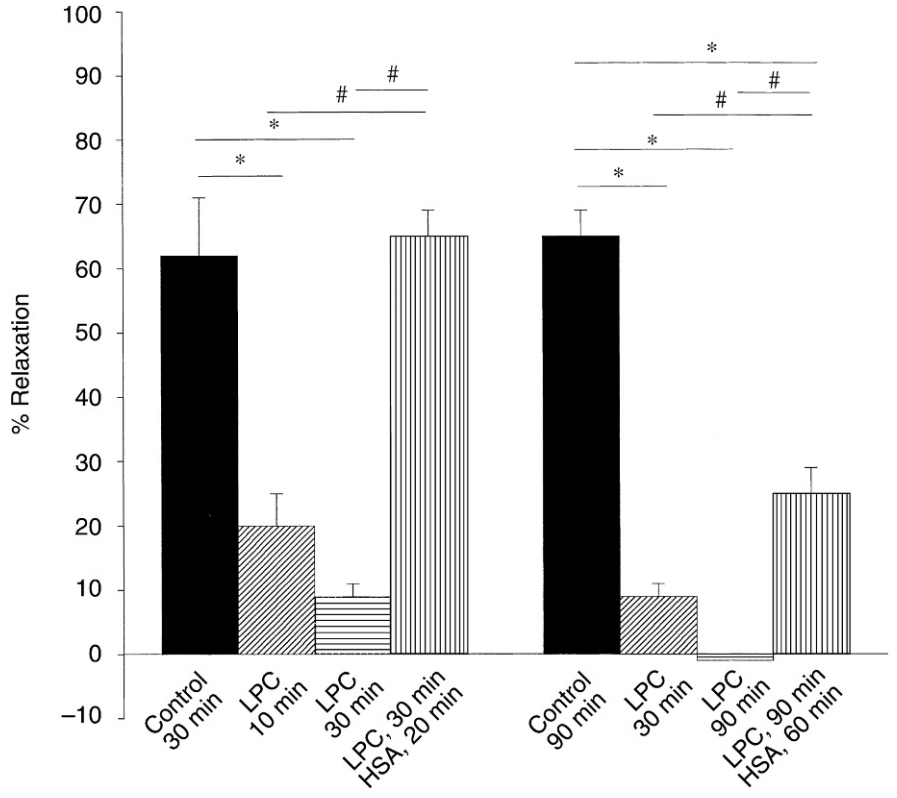

Fig. 2. Both 30- and 90-minute incubations with 100 μmol/L LPC blocked acetylcholine (1 μmol/L)-mediated relaxation, and later, the addition of human serum albumin (HSA; 50 g/L) after 10 and 30 minutes, respectively, completely restored relaxation in the short incubation and partially restored relaxation in the long incubation. $*P < 0.05$ vs. control; $\#P < 0.05$ vs. LPC.

(100 μmol/L). However, although $MnCl_2$ always increased EDR approximately 10%, it had no specific effect on the dose-dependent LPC-mediated disturbance (Fig. 3).

L-arginine restored EDR after exposure to LPC

L-arginine did not affect the vascular function of CON rings; however, L-arginine (10 μmol/L; Fig. 4) completely restored the disturbance in EDR induced by 20 μmol/L LPC, and also dose-dependently restored EDR after 40 μmol/L LPC. Complete restoration of EDR was seen by the addition of 1 mmol/L L-arginine to rings exposed to 40 μmol/L LPC. However, 1 mmol/L L-arginine only partially restored EDR after 60 μmol/L LPC. In contrast to albumin, not even the highest concentration of L-arginine could improve EDR at LPC levels higher than 60 μmol/L.

DISCUSSION

The present study shows that acetylcholine mediated endothelium-dependent relaxation (EDR), which was dose- and time-dependently blocked after incubation with LPC, could be protected and salvaged by a physiological concentration of HSA. In fact, preincubation with 50 g/L HSA completely protected EDR against LPC levels that were sixfold higher than those found in LDL of nephrotic subjects [9]. However, preincubation with 6 g/L HSA only partially protected EDR against a much lower LPC concentration. After a shorter incubation with LPC, EDR was less disturbed, and 50 g/L HSA completely salvaged EDR. L-arginine could only completely restore EDR impairment when caused by a low concentration of LPC (20 μmol/L), while the superoxide dismutase-mimic $MnCl_2$ had no specific effect on the LPC-mediated impairment in EDR.

Lysophosphatidylcholine content is increased in ox-LDL as compared with native LDL [2, 3]. LPC is also avidly bound by albumin. Albumin can stimulate the release of LPC from cultured rat hepatocytes [30], and a loss of albumin binding of LPC may affect membrane-linked cell function by deranging membrane structures [13, 14]. Such changes in the membrane could influence the function of membrane-bound receptors [18] and, therefore, vascular function by disrupting signal transduction [19, 20]. Bradykinin-induced EDR in porcine coronary arteries is dose-dependently inhibited by low concentrations of LPC (0 to 20 μmol/L) [31]. In the latter study, albumin was used to bind LPC to prevent damage to the endothelial cells [31]. However, organ chamber studies to find out whether the addition of albumin can salvage EDR in vascular tissue previously exposed to LPC have not yet been done. The results of the present study show that albumin could both protect EDR against the inhibitory effect of LPC and salvage EDR after exposure to LPC, presumably by preventing the uptake of LPC or by ex-

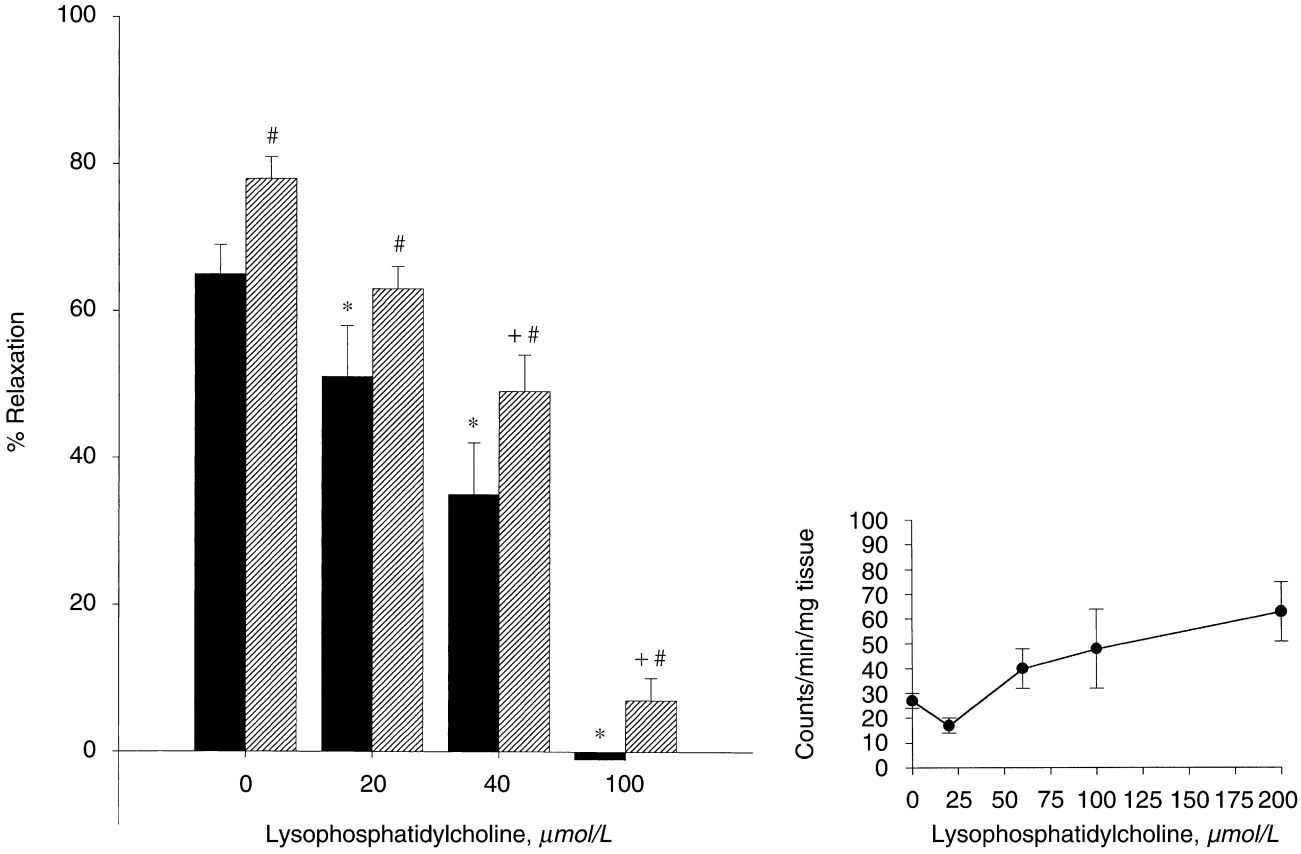

Fig. 3. The superoxide dismutase-mimic, $MnCl_2$ (100 μmol/L), consistently increased acetylcholine (ACh; 1 μmol/L)-mediated relaxation, but had no specific effects on the lysophosphatidylcholine (LPC)-mediated disturbance. Symbols are: (■) no $MnCl_2$; (▨) 100 μmol/L $MnCl_2$; $*P < 0.05$ vs. 0 μmol/L LPC without $MnCl_2$; $+P < 0.05$ vs. 0 μmol/L LPC with $MnCl_2$; $\#P < 0.05$ $MnCl_2$ vs. no $MnCl_2$ at each LPC concentration. (Inset) LPC dose-dependently increased superoxide activity, measured with LEC.

tracting LPC, respectively. After a long (90 min) incubation period with LPC, albumin was not able to restore EDR fully. Whether this has implications for hypoalbuminemia in vivo cannot be determined from these experiments.

In human plasma, albumin is the most abundant protein with a normal concentration of approximately 40 g/L [32]. It is well known that a rapid exchange of LPC occurs between red cell membranes and either albumin [33, 34] or lipoproteins [35]. Furthermore, it has been shown that albumin can reduce the uptake of LPC by cultured endothelial cells [10]. Thus, it is conceivable that in the presence of a normal or increased production of LPC during hypoalbuminemia, endothelial function will be disturbed. Previously we found that red blood cells from analbuminemic rats showed a marked increase in LPC content with a concomitant reduction in deformability. These changes were reversed by the addition of albumin [11]. Hypoalbuminemia and hyperlipidemia that usually occur in conjunction in the nephrotic syndrome can both disturb endothelial function. Hyperlipidemia may increase superoxide production [36], resulting in enhanced degradation of NO [37], whereas hypoalbuminemia results in diminished binding of LPC to albumin and sequestration of LPC in lipoproteins and possibly in other compartments. Indeed, high LPC levels in VLDL, intermediate density lipoprotein (IDL), and LDL were related to hypoalbuminemia rather than to hyperlipidemia in a selected group of normolipidemic nephrotic patients with severe hypoalbuminemia. Moreover, in hyperlipidemic nephrotic subjects, LPC levels in lipoproteins were even higher [9].

Cell membranes have a high affinity for LPC [30]. Several pathways are described by which LPC can exert its effect on the endothelium. One of these pathways is the activation of kinases, resulting in superoxide anion production [15, 16, 38]. The present study confirms that superoxide activity, measured with LEC, is increased in rat aortic rings incubated with LPC. The increase of superoxide activity could be reversed with $MnCl_2$, a superoxide dismutase mimic [26]. However, $MnCl_2$ could not restore LPC-mediated impairment in EDR. This shows that LPC-induced superoxide activity, in as far as

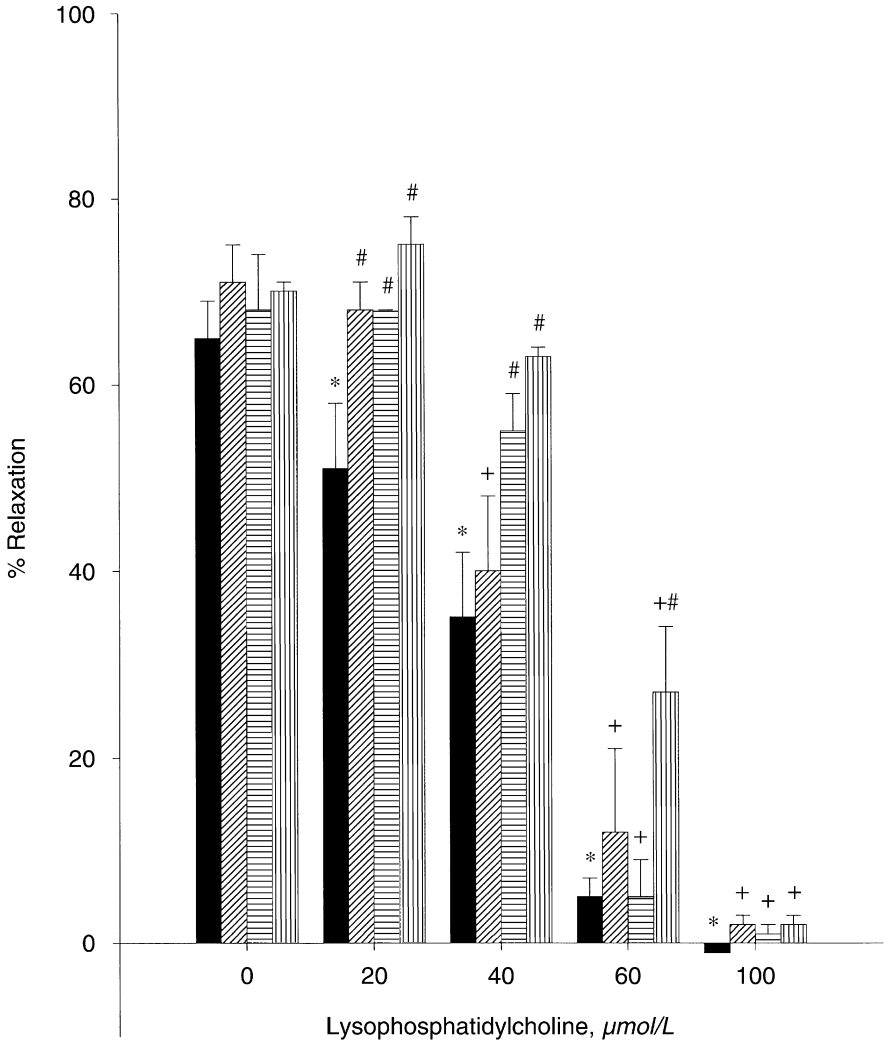

Fig. 4. At low concentrations of LPC (≤40 μmol/L), L-arginine (L-arg) completely restored acetylcholine (1 μmol/L)-mediated relaxation. At higher concentrations of LPC (≥60 μmol/L), even large amounts of L-arg could not affect full restoration. Symbols are: (■) no L-arg; (▨) 10 μmol/L L-arg; (▤) 100 μmol/L L-arg; (▥) 1000 μmol/L L-arg; $*P < 0.05$ vs. 0 μmol/L LPC without L-arg; $+P < 0.05$ vs. 0 μmol/L LPC with L-arg; $\#P < 0.05$ L-arg vs. no L-arg at each LPC concentration.

this can be assessed by the antioxidant effect of $MnCl_2$, is not responsible for the impairment in EDR.

Albumin has been identified as an antioxidant [39, 40], possibly due to binding of transition metals [32, 39, 40], LPC, or both. If albumin is an antioxidant, then SOD-mimics should salvage LPC-mediated EDR impairment. Although we found that LPC dose-dependently increased superoxide production and that $MnCl_2$ abolished LPC-mediated superoxide production, $MnCl_2$ could not restore ACh-mediated relaxation after exposure to LPC. Therefore, we assume that albumin had other effects than merely scavenging superoxide anions.

Another pathway by which LPC could impair EDR is via inhibition of the high-affinity component of the arginine transporter by LPC [17], resulting in an impaired NO production. Our results show that L-arginine can dose-dependently restore LPC-mediated disturbance in EDR. At low concentrations of LPC (≤40 μmol/L), complete restoration was seen. This is analogous with the finding that L-arginine restores EDR in normoalbuminemic patients with combined hyperlipidemia [23] or familial hypercholesterolemia [24, 25]. However, we found that only very high concentrations of L-arginine could partially restore EDR at 60 μmol/L LPC, and no restoration was seen at 100 μmol/L. Note that this was not due to an effect on vascular smooth muscle function because nitroprusside-mediated vasodilation was normal. A concentration of 100 μmol/L is close to the LPC concentration found in LDL fractions in plasma of nephrotic syndrome patients [9]. Previously, we failed to restore EDR with L-arginine in nephrotic syndrome patients, at a dose that did restore EDR in normoalbuminemic hypercholesterolemic subjects [23, 24]. This suggests that, although LPC reduces the availability of L-arginine for nitric oxide synthase (NOS), this is not the only LPC-effect that inhibits EDR.

In conclusion, in the absence of albumin, LPC levels that occur in normal plasma completely block EDR. Albumin can protect EDR against even higher LPC concentrations and can salvage EDR after exposure to LPC

levels that occur in circulating LDL of nephrotic subjects. Albumin could restore EDR at high concentrations of LPC at which L-arginine had no effect. At low LPC concentrations, the restoration of L-arginine transport appears to produce enough NO for complete recovery of EDR. Albumin was more effective than L-arginine because it probably reduced the primary effect, that is, uptake of LPC into the cell membrane. Thus, LPC affects several pathways that inhibit EDR, all of which are salvaged by addition of albumin.

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APPENDIX

Abbreviations used in this article are: ACh, acetylcholine; CON, control rat aortic rings; EDR, endothelium-dependent relaxation; HSA, human serum albumin; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LEC, lucigenin-enhanced chemiluminescence; LPC, lysophosphatidylcholine; NO, nitric oxide; NOS, nitric oxide synthase; ox-LDL, oxidized low density lipoprotein; PE, phenylephrine; SNP, sodium nitroprusside; VLDL, very low density lipoprotein.

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