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SPECIAL REPORT

Evidence for selective inhibition by lysophosphatidylcholine of acetylcholine-induced endothelium-dependent hyperpolarization and relaxation in rat mesenteric artery

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The effects of lysophosphatidylcholine (LPC) on acetylcholine-induced hyperpolarization and relaxation were examined in rat mesenteric arteries. LPC (3–10 μM) reversibly inhibited endothelium-dependent hyperpolarization by acetylcholine in a concentration-dependent manner. LPC (10 μM) inhibited only partially endothelium-dependent relaxation by acetylcholine. However, acetylcholine-induced relaxation obtained in the presence of 100 μM N^G-nitro-L-arginine was almost completely eliminated by 10 μM LPC. These results indicate that LPC inhibits hyperpolarization and relaxation due to endothelium-derived hyperpolarizing factor more specifically than the relaxation due to endothelium-derived nitric oxide.

Keywords: Acetylcholine; endothelium-dependent hyperpolarization; endothelium-dependent relaxation; lysophosphatidylcholine; rat mesenteric artery

Introduction Endothelium-dependent relaxation is impaired in the atherosclerotic arteries (Bossaller *et al.*, 1987). Oxidatively modified low density lipoproteins (LDL), which accumulate in the atherosclerotic lesions of arteries (Ylä-Herttuala *et al.*, 1989), play a pathogenically important role in the impairment of endothelium-dependent relaxation, since normal arteries exposed to oxidized LDL mimic the impaired endothelium-dependent relaxant property of the atherosclerotic arteries (Kugiyama *et al.*, 1990; Yokoyama *et al.*, 1990). Lysophosphatidylcholine (LPC), which is generated during oxidative modification of LDL (Parthasarathy *et al.*, 1985), appears to be a principal substance responsible for the impairment of endothelium-dependent relaxations in these atherosclerotic arteries (Kugiyama *et al.*, 1990; Inoue *et al.*, 1992).

Endothelium-dependent relaxations are mediated by an endothelium-derived relaxing factor, which is thought to be identical to nitric oxide (NO), and also by an endothelium-derived hyperpolarizing factor (EDHF), which relaxes vascular smooth muscle cells through hyperpolarization as a result of opening of potassium channels (Suzuki *et al.*, 1992). In the present study, we examined the effect of LPC on endothelium-dependent hyperpolarization by acetylcholine in rat mesenteric arteries in order to assess the role of EDHF in the diminished endothelium-dependent relaxation of atherosclerotic arteries.

Methods The main trunk of the superior mesenteric artery was obtained from male Wistar rats, weighing 250–380 g, anaesthetized with diethyl ether. The arteries were cut into rings (3 mm) or transverse strips. The latter were pinned down, intimal side upward, on the bottom of an organ chamber (capacity 3 ml). The preparation was superfused with warmed (37°C) physiological salt solution (PSS) aerated with 95% O_2 and 5% CO_2 at a constant flow rate (7 ml min^{-1}). PSS contained the following (in mM): NaCl 118.2, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 10.0. Glass microelectrodes filled with 3 M KCl (tip resistances 40–80 MΩ) were inserted into the smooth muscle cells from the intimal side. Membrane potentials were displayed continuously on an oscilloscope and recorded on a chart recorder.

For the mechanical experiments, the arterial ring was attached to a pair of stainless steel hooks and suspended in a water-jacked bath filled with 25 ml of oxygenated PSS at 37°C. Isometric tension, under a resting tension of 1g, was monitored with a transducer and recorded on a pen recorder. The experiments were performed in the presence of 10 μM indomethacin. The rings were precontracted with 1 μM phenylephrine, and then acetylcholine was applied in a cumulative manner. When N^G-nitro-L-arginine (L-NOARG) was used, it was added 15 min before application of phenylephrine. In the experiments with L-NOARG, the tissues were contracted with 0.1 μM phenylephrine, in order to match the contractions induced by phenylephrine in control rings and the pretreated rings. Relaxations were expressed as a percentage of the height of contraction induced by phenylephrine. LPC was dissolved in a mixture (1:1 [vol/vol]) of methanol and chloroform, and appropriate aliquots of the solution were dried with a stream of N_2 gas, followed by sonication in distilled water. All values are given as means ± s.e.mean. Analysis by Student's *t* test was performed using unpaired observations. *P* values less than 0.05 were considered significant.

Results Resting membrane potentials of the mesenteric arterial smooth muscle cells were −52.1 ± 0.6 mV ($n=33$). Acetylcholine (1 μM) hyperpolarized the membrane potential by −16.4 ± 0.4 mV ($n=22$). The hyperpolarization was not observed in tissues without endothelium. LPC at concentrations up to 10 μM had no effect on the resting membrane potentials (before: −51.4 ± 0.8 mV, after; −52.1 ± 0.8 mV, $n=7$). However, the hyperpolarizing response to 1 μM acetylcholine was almost completely abolished by pretreatment with 10 μM LPC (Figure 1a). In contrast, pinacidil-induced hyperpolarization was unaffected by LPC (Figure 1b): pinacidil (1 μM) hyperpolarized the membrane potential by −19.0 ± 0.9 ($n=5$) and −19.2 ± 1.4 mV ($n=5$) in the absence and presence of 10 μM LPC, respectively. Figure 1c shows the concentration-dependent inhibition by LPC of acetylcholine-induced hyperpolarization. A significant inhibition was observed at concentrations of 3 μM and over. In the presence of 10 μM LPC, 1 μM acetylcholine failed to cause a significant hyperpolarizing effect. The inhibitory effect of LPC was reversible: the hyperpolarization produced by 1 μM acetylcholine 45 min after washing out of 10 μM LPC was −16.0 ± 0.6 mV ($n=5$).

Acetylcholine caused concentration-dependent relaxations of the arterial rings precontracted with phenylephrine when the

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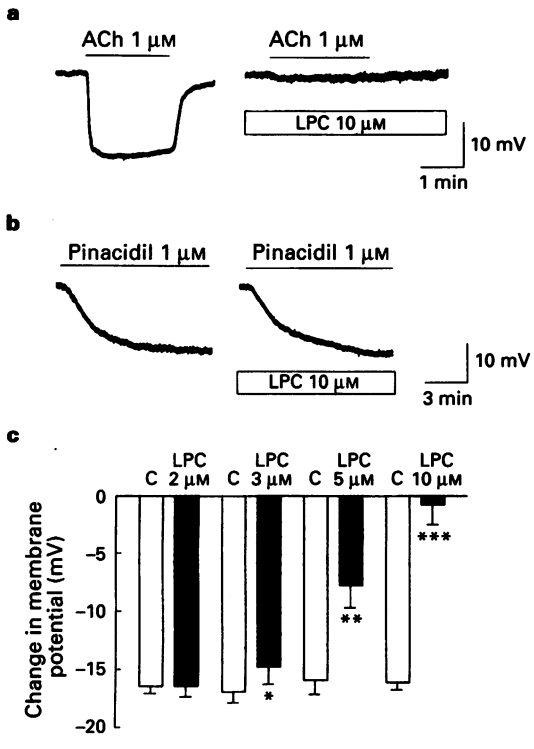

Figure 1 Effect of lysophosphatidylcholine (LPC) on hyperpolarizing responses to acetylcholine (ACh) and pinacidil. (a and b) Actual tracings of the membrane potential. ACh (a) and pinacidil (b) were present for the periods indicated by the horizontal bars. LPC (10 μM) was added 20–30 min before exposure to ACh (1 μM) or pinacidil (1 μM). (c) Concentration-dependent inhibitory effect of LPC on ACh-induced hyperpolarization. After control responses to 1 μM ACh (indicated by C) had been obtained, LPC at different concentrations was applied 20–30 min before a second challenge with ACh. Columns are means ± s.e.mean of 4–5 experiments. $*P<0.05$, $**P<0.01$, $***P<0.001$ *vs.* the corresponding control values.

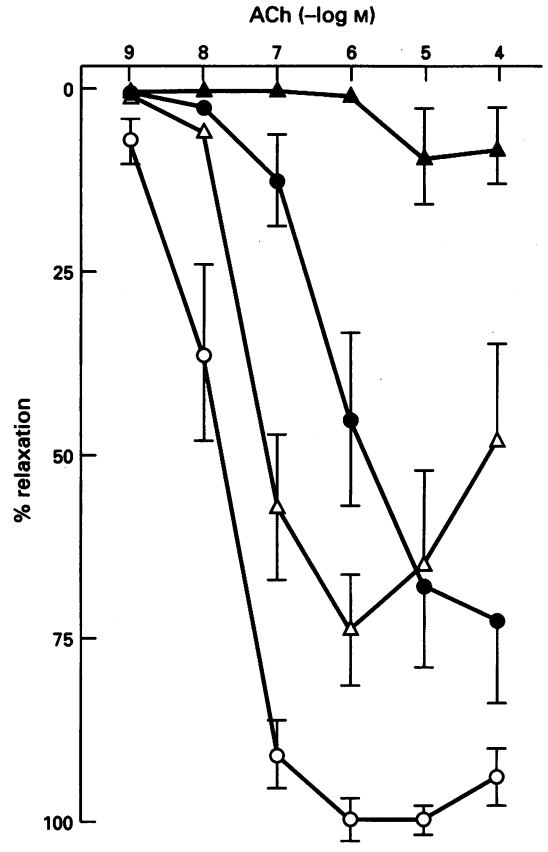

Figure 2 Concentration-response curves for relaxations produced by acetylcholine (ACh) in the absence (○) and presence of 100 μM N^G-nitro-L-arginine (L-NOARG, △), 10 μM lysophosphatidylcholine (LPC, ●) and 100 μM L-NOARG plus 10 μM LPC (▲). The rings were contracted with 1 and 0.1 μM phenylephrine in the absence and presence of L-NOARG, respectively. L-NOARG and LPC were added 15 and 20 min before application of phenylephrine, respectively. The points are shown as means ± s.e.mean of 6 experiments.

endothelium was intact (Figure 2). Pretreatment with 10 μM LPC partially inhibited the relaxant effect of acetylcholine. The maximal response to acetylcholine was significantly reduced by pretreatment with LPC (control: 100.3 ± 3.0%, LPC treated: 72.8 ± 11.1%; $P<0.001$). In the presence of 100 μM L-NOARG, the concentration-response curve for acetylcholine-induced relaxations was shifted to the right and the maximal relaxation was significantly reduced from 100.3 ± 3.0% to 73.7 ± 7.8% ($P<0.001$). LPC (10 μM) suppressed almost completely the relaxant response to acetylcholine after treatment with L-NOARG.

Discussion This study is the first report that LPC inhibits acetylcholine-induced endothelium-dependent hyperpolarization in rat mesenteric artery. Recent evidence suggests that acetylcholine-induced hyperpolarization in rat mesenteric artery is mainly due to EDHF-induced activation of a certain type of K^+ channel (Fujii *et al.*, 1992). It seems unlikely that the inhibition by LPC of endothelium-dependent hyperpolarization resulted from its action on K^+ channels responsible for EDHF-mediated hyperpolarization. First, LPC did not interfere with hyperpolarization induced by pinacidil, an ATP-sensitive K^+ channel opener. Second, LPC did not alter the resting membrane potentials of the smooth muscle cells.

Yokoyama *et al.* (1990) have reported that LPC at a relatively high concentration (about 20 μM) markedly inhibits acetylcholine-induced endothelium-dependent relaxations, which are largely mediated by NO, in rabbit aorta. In our study, 10 μM LPC inhibited endothelium-dependent relaxations by acetylcholine in rat mesenteric artery, but the extent of the inhibition was far from complete. However, in the presence of L-NOARG, acetylcholine-induced relaxation was almost completely prevented by LPC. In rat mesenteric artery pretreated with indomethacin, endothelium-dependent relaxant responses to acetylcholine are mediated by both NO and hyperpolarization (Fujii *et al.*, 1992). Thus, the endothelium-dependent relaxant response to acetylcholine that was resistant to L-NOARG is probably due to hyperpolarization of smooth muscle cells. Hence, it seems to be reasonable to conclude that LPC inhibits more potently relaxations caused by EDHF-mediated hyperpolarization than by endothelium-derived NO.

Elevation of cytosolic Ca^{2+} in endothelial cells is a key step in the synthesis or release of EDHF as well as NO (Lückoff *et al.*, 1988; Chen & Suzuki, 1990). In bovine cultured aortic endothelial cells, LPC has been found to inhibit both phosphoinositide hydrolysis and increase in cytosolic Ca^{2+} levels evoked by bradykinin (Inoue *et al.*, 1992). Therefore, the inhibitory action of LPC on EDHF-mediated hyperpolarization and relaxation may be related to the inhibition of phosphoinositide hydrolysis and the subsequent increase in cytosolic Ca^{2+} in endothelial cells.

In summary, the present findings indicate that LPC markedly attenuates EDHF-mediated hyperpolarization and relaxation probably due to the inhibition of the production or release of EDHF. LPC accumulates in oxidized LDL and atherosclerotic arteries (Parthasarathy *et al.*, 1985). Our results thus suggest that reduced endothelium-dependent hyperpolarization may account, at least in part, for the impaired endothelium-dependent relaxations in atherosclerotic arteries.

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References

BOSSALLER, C., HABIB, G.B., YAMAMOTO, H., WILLIAMS, C., WELLS, S. & HENRY, P.D. (1987). Impaired muscarinic endothelium-dependent relaxation & cyclic guanosine 5'-monophosphate formation in atherosclerotic human coronary artery and rabbit aorta. *J. Clin. Invest.*, **79**, 170–174.

CHEN, G. & SUZUKI, H. (1990). Calcium dependency of the endothelium-dependent hyperpolarization in smooth muscle cells of the rabbit carotid artery. *J. Physiol.*, **421**, 521–534.

FUJII, K., TOMINAGA, M., OHMORI, S., KOBAYASHI, K., KOGA, T., TAKATA, Y. & FUJISHIMA, M. (1992). Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ. Res.*, **70**, 660–669.

INOUE, N., HIRATA, K., YAMADA, M., HAMAMORI, Y., MATSUDA, Y., AKITA, H. & YOKOYAMA, M. (1992). Lysophosphatidylcholine inhibits bradykinin-induced phosphoinositide hydrolysis and calcium transients in cultured bovine aortic endothelial cells. *Circ. Res.*, **71**, 1410–1421.

KUGIYAMA, K., KERNS, S.A., MORRISETT, J.D., ROBERTS, R. & HENRY, P.D. (1990). Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low-density lipoproteins. *Nature*, **344**, 160–162.

LÜCKHOFF, A., POHL, U., MÜLSCH, A. & BUSSE, R. (1988). Differential role of extra- and intracellular calcium in the release of EDRF and prostacyclin from cultured endothelial cells. *Br. J. Pharmacol.*, **95**, 189–196.

PARTHASARATHY, S., STEINBRECHER, U.P., BARNETT, J., WITZTUM, J.L. & STEINBERG, D. (1985). Essential role of phospholipase A_2 activity in endothelial cell-induced modification of low density lipoprotein. *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 3000–3004.

SUZUKI, H., CHEN, G. & YAMAMOTO, Y. (1992). Endothelium-derived hyperpolarizing factor (EDHF). *Jpn. Circ. J.*, **56**, 170–174.

YLÄ-HERTTUALA, S., PALINSKI, W., ROSENFIELD, M.E., PARTHASARATHY, S., CAREW, T.E., BUTLER, S., WITZTUM, J.L. & STEINBERG, D. (1989). Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J. Clin. Invest.*, **84**, 1086–1095.

YOKOYAMA, M., HIRATA, K., MIYAKE, R., AKITA, H., ISHIKAWA, Y. & FUKUZAKI, H. (1990). Lysophosphatidylcholine: Essential role in the inhibition of endothelium-dependent vasorelaxation by oxidized low density lipoprotein. *Biochem. Biophys. Res. Commun.*, **168**, 301–308.

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