Lysophosphatidylcholine Increases Ca^{2+} Current via Activation of Protein Kinase C in Rabbit Portal Vein Smooth Muscle Cells

Seungsoo Jung, Youngho Lee, Sungsik Han, Youngwhan Kim, Taiksang Nam, and Ducksun Ahn

Department of Physiology, Yonsei University College of Medicine, Seoul 120-752, Korea

Lysophosphatidylcholine (LPC), a metabolite of membrane phospholipids by phospholipase A_2, has been considered responsible for the development of abnormal vascular reactivity during atherosclerosis. Ca^{2+} influx was shown to be augmented in atherosclerotic artery which might be responsible for abnormal vascular reactivity. However, the mechanism underlying Ca^{2+} influx change in atherosclerotic artery remains undetermined. The purpose of the present study was to examine the effects of LPC on L-type Ca^{2+} current ($I_{Ca(L)}$) activity and to elucidate the mechanism of LPC-induced change of $I_{Ca(L)}$ in rabbit portal vein smooth muscle cells using whole cell patch clamp. Extracellular application of LPC increased $I_{Ca(L)}$ through whole test potentials, and this effect was readily reversed by washout. Steady state voltage dependency of activation or inactivation properties of $I_{Ca(L)}$ was not significantly changed by LPC. Staurosporine (100 nM) or chelerythrine (3 μM), which is a potent inhibitor of PKC, significantly decreased basal $I_{Ca(L)}$, and LPC-induced increase of $I_{Ca(L)}$ was significantly suppressed in the presence of PKC inhibitors. On the other hand, application of PMA, an activator of PKC, increased basal $I_{Ca(L)}$ significantly, and LPC-induced enhancement of $I_{Ca(L)}$ was abolished by pretreatment of the cells with PMA. These findings suggest that LPC increased $I_{Ca(L)}$ in vascular smooth muscle cells by a pathway that involves PKC, and that LPC-induced increase of $I_{Ca(L)}$ might be, at least in part, responsible for increased Ca^{2+} influx in atherosclerotic artery.

Key Words: Lysophosphatidylcholine, Ca^{2+} current, Protein kinase C, Vascular smooth muscle

INTRODUCTION

Atherosclerosis is an arterial disease with a histopathologic changes, such as fatty streak and atheromatous plaque (Cox & Cohen, 1996a). In addition to this structural changes, the functional abnormalities including impairment of relaxation response and augmentation of contractile response to vasoconstrictors have also been reported in atherosclerotic arteries (Galle et al, 1990; Cox & Tulenko, 1995; Auge et al, 1996; Cox & Cohen, 1996b). These alterations in vascular reactivity are potentially important for the development of acute vasospasm, which is responsible for unstable angina or myocardial infarct. Lysophosphatidylcholine (LPC) is one of the candidates held to be responsible for the development of abnormal vascular reactivity in atherosclerotic artery for the following reasons. First, concentration of LPC in blood and within vessel wall increased profoundly during atherosclerosis (Murohara et al, 1994). Second, application of LPC mimicked abnormal vascular response of atherosclerotic artery and increased intracellular $[Ca^{2+}]_i$ in vascular smooth muscle cells which was blocked by L-type Ca^{2+} channel ($I_{Ca(L)}$) antagonist (Stoll & Spector, 1993; Chen et al, 1995; Eizawa et al, 1995). These findings suggest that LPC increases $I_{Ca(L)}$, thereby increasing intracellular $[Ca^{2+}]_i$ and vascular contractility which are seen in atherosclerotic artery. However, it is still uncertain whether LPC increases $I_{Ca(L)}$ or not.

L-type calcium channels are modulated by distinct vascular agonist known to activate different second messenger systems, including the signaling cascade that activates protein kinase C (PKC) (Keef et al, 2001). Several lines of evidence indicate that PKC activators stimulate $I_{Ca(L)}$ in vascular smooth muscle cells obtained from different vascular territories (Lepretre & Mironneau, 1993; Shimamura et al, 1994; Obejero-Paz et al, 1998). In vitro and in vivo studies demonstrated that low concentration (less than 10 μM) of LPC activates PKC in some cell types, including vascular tissue (Sugiyama et al, 1994; Murohara et al, 1996; Watson & Gold, 1997). We also reported previously that LPC decreased delayed rectifier K^+ current through activation of PKC in rabbit coronary smooth muscle cells (Yeon et al, 2001). Therefore, it is highly possible that LPC would increase $I_{Ca(L)}$ through activation of PKC in vascular smooth muscle cells, although this relationship has not yet been investigated in these cells. The aims of the present study were to examine the LPC-induced modulation of $I_{Ca(L)}$ in smooth muscle cells and to investigate the possible role of PKC in this modulation.

Address corresponds to; Ducksun Ahn, Department of Physiology, Yonsei University College of Medicine, 134, Shinchon-dong, Seodaemun-gu, Seoul 120-752, Korea. (Tel) 82-2-2228-1707, (Fax) 82-2-393-0203, (E-mail) dsahn@yumc.yonsei.ac.kr

ABBREVIATIONS: LPC, Lysophosphatidylcholine; $I_{Ca(L)}$, L-type Ca^{2+} current; PKC, protein kinase C; PMA, phorbol myristic acid.

METHODS

Cell Preparation

Single rabbit portal vein smooth muscle cells were prepared using enzymatic digestion as previously described (Lepretre et al, 1994). Briefly, portal vein segments were incubated at 37°C for 60 min in a Ca^{2+}-free buffer solution of the following composition (in mmol/L): NaCl 140, KCl 5, $MgCl_2$ 1, N-2-hydroxyethylpiperazine-N`-2-ethansulfonic aid (HEPES) 10, glucose 10, with pH adjusted to 7.4 with Tris. The tissue was then incubated in a buffer with collagenase (1 mg/ml, Wako) and papain (0.1 mg/ml, Sigma) for $15\sim20$ min. The tissue was gently agitated with Pasteur pipette, and released cells were kept at 4°C.
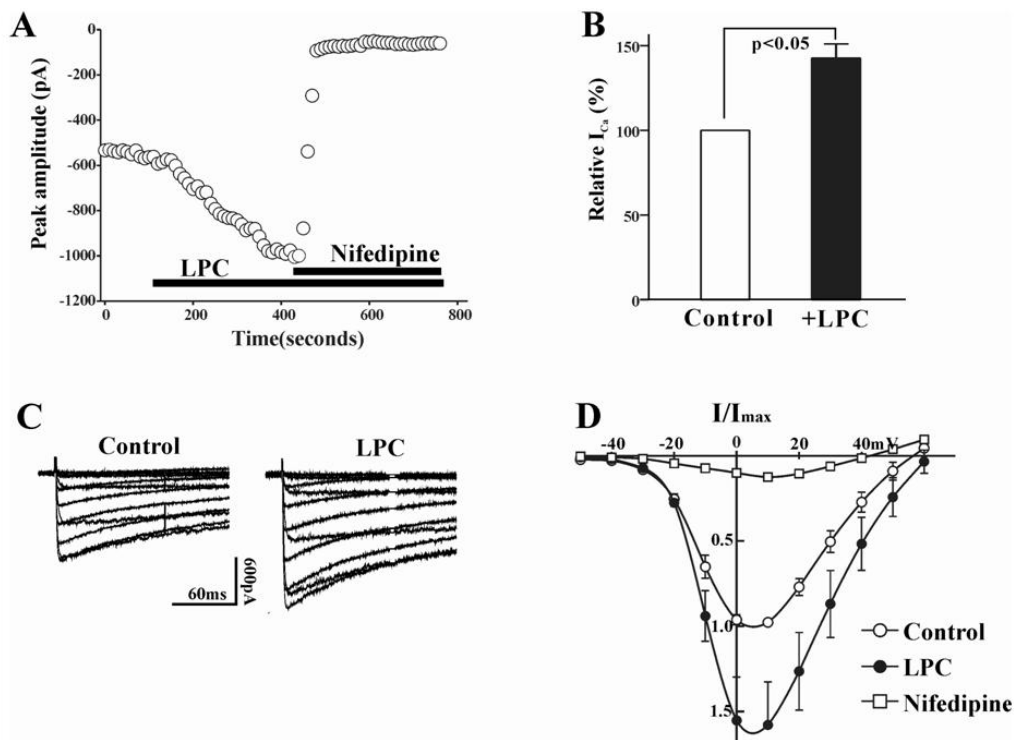
Electrophysiological recording

An aliquot of cells was placed in a perfusion chamber mounted on an inverted microscope (Nikon Diaphot), allowed to adhere to the bottom of the glass, then superfused with HEPES-buffered solution. Cells were sealed to pipettes with gentle suction, and the patch membrane was ruptured with further negative pressure at a holding potential of -50 mV. All experiments were performed at room temperature. Membrane currents were recorded by whole cell clamp technique with voltage clamp amplifier (Axopatch 1-D, Axon) using pipettes with a resistance from 2 to 3 MΩ. Voltages were corrected for the liquid junction potential. Voltage command and data collections were controlled by pClamp 6.0 software (Axon Instruments). The data were filtered by an 8-pole low-pass Bessel filter at 5 KHz, digitized at a sampling frequency of 25 KHz and then stored at hard disk for further analysis.

Experimental solutions

The pipette solution contained (mmol/L) Cs-aspartate 100, CsCl 30, MgATP 5, NaGTP 1, BAPTA 10, HEPES 10, adjusted to pH 7.2 with CsOH. The extracellular solution contained (mmol/L) NaCl 130, TEA-Cl 5.6, $MgCl_2$ 1.2, $BaCl_2$ 10, glucose 10, HEPES 10, adjusted to pH 7.4 with Tris. Nifedipine, staurosporine, chelerythrine, phorbol myristic acid (PMA), and LPC were dissolved in dimethylsulfoxide (DMSO) in concentrations that allowed DMSO concentration to remain at $<1:10,000$ in all experiments. At these concentrations, DMSO had no effect on $I_{Ca(L)}$ compared with $I_{Ca(L)}$ amplitudes prior to DMSO application. All the chemicals listed above were added directly to the bath solution from a freshly made stock solution.

Fig. 1. (A) Plot of Ca^{2+} currents evoked by repetitive step depolarization from -80 mV to 0 mV versus time. Horizontal bar indicates the time of LPC (1 μM), and nifedipine (1 μM) application in the bath. (B) Summary of the LPC-induced change of $I_{Ca(L)}$. The value of LPC-induced change was expressed as % of control. Bath application of LPC increased the current amplitude to 142.6±8.4% (n=19). (C) Effect of LPC on current-voltage relations of $I_{Ca(L)}$. $I_{Ca(L)}$ was recorded at 10 mV increments between -50 and $+60$ mV from a holding potential of -80 mV. (D) Summary of voltage dependence of $I_{Ca(L)}$ for control ($\bigcirc$), 1 μM LPC ($\bullet$), and 1 μM nifedipine. At each voltage steps, peak values of current were determined, and averaged (n=8).

The results of the experiments are expressed as means ± S.E.M. Statistical evaluation of the data was performed by Student's t-test for comparison between two groups. A value of p less than 0.05 was taken as significant. The number of preparations taken from separate animals was indicated by n.

RESULTS

In the first series of experiment, we determined the effect of LPC on $I_{Ca(L)}$ in rabbit portal vein smooth muscle cells. The inward current activating with depolarizing command steps under our experimental conditions was due to activation of $I_{Ca(L)}$, because the application of nifedipine (1 μM), a specific blocker of $I_{Ca(L)}$, abolished the inward current (Fig. 1A). LPC produced a significant increase in $I_{Ca(L)}$ at 0 mV test pulse (p < 0.01, 142.6±8.4%, n=19). However, LPC had no apparent effect on $I_{Ca(L)}$ in some portal vein smooth muscle cells, which might be due to damage of cells during preparation of single cells or rundown of Ca^{2+} current. We excluded these LPC-unresponsive cells from analysis. Average values of $I_{Ca(L)}$ from LPC-responsive portal vein smooth muscle cells were significantly larger than those from controls for voltages positive to -40 mV. As shown in Fig. 1D, this was most apparent above the threshold for activation of $I_{Ca(L)}$. The reversal potential of $I_{Ca(L)}$ was 54.5 ±1.5 mV and 56.9±0.9 mV in control and LPC-treated group, respectively (n=8).
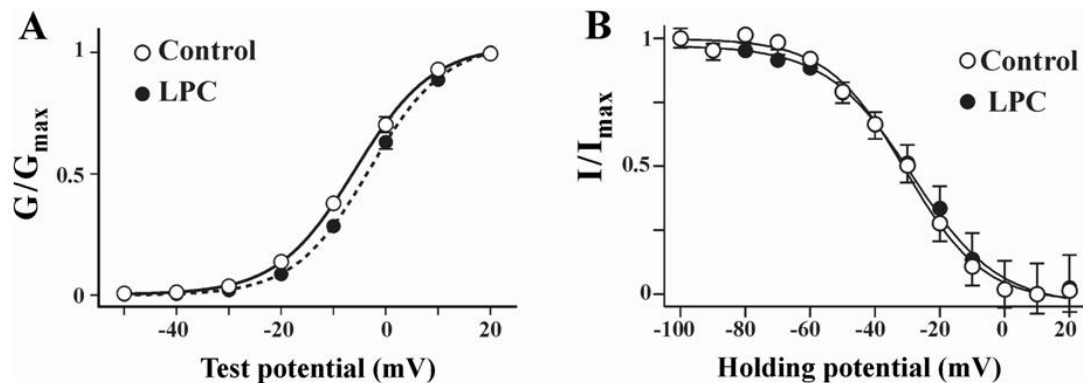
To investigate how LPC modulates $I_{Ca(L)}$, we examined the effects of LPC on the voltage dependence of steady state activation and inactivation of $I_{Ca(L)}$. The conductance values of $I_{Ca(L)}$ were calculated using estimated reversal potential and were normalized by peak conductance to produce activation curves. The voltage dependent activation curves of control and LPC treatment were constructed using Boltzman fitting. As shown in Fig. 2, the half maximal

activation potential ($V_{1/2}$) was -5.8 ± 0.2 mV and -3.1 ± 0.1 mV in control and LPC-treated group, respectively (n=8). Average values of half-maximal activation potentials in LPC treated groups slightly positively shifted compared to control. The voltage dependence of $I_{Ca(L)}$ availability was determined with 10 sec conditioning voltage pulses stepped from -100 mV to 20 mV in 10 mV increments, followed by a 500 ms test pulse to the voltage of 10 mV. Conditioning potentials positive to -70 mV produced a decrease of $I_{Ca(L)}$, reaching full inactivation near 0mV. No difference was observed in half maximal steady state inactivation potential (-31.4 ± 1.2 mV and -30.2 ± 1.6 mV in control and LPC-treated group, n=6). These data suggest that, although LPC significantly increased the activity of $I_{Ca(L)}$, it did not depend on the change in the voltage-dependent characteristics of $I_{Ca(L)}$.
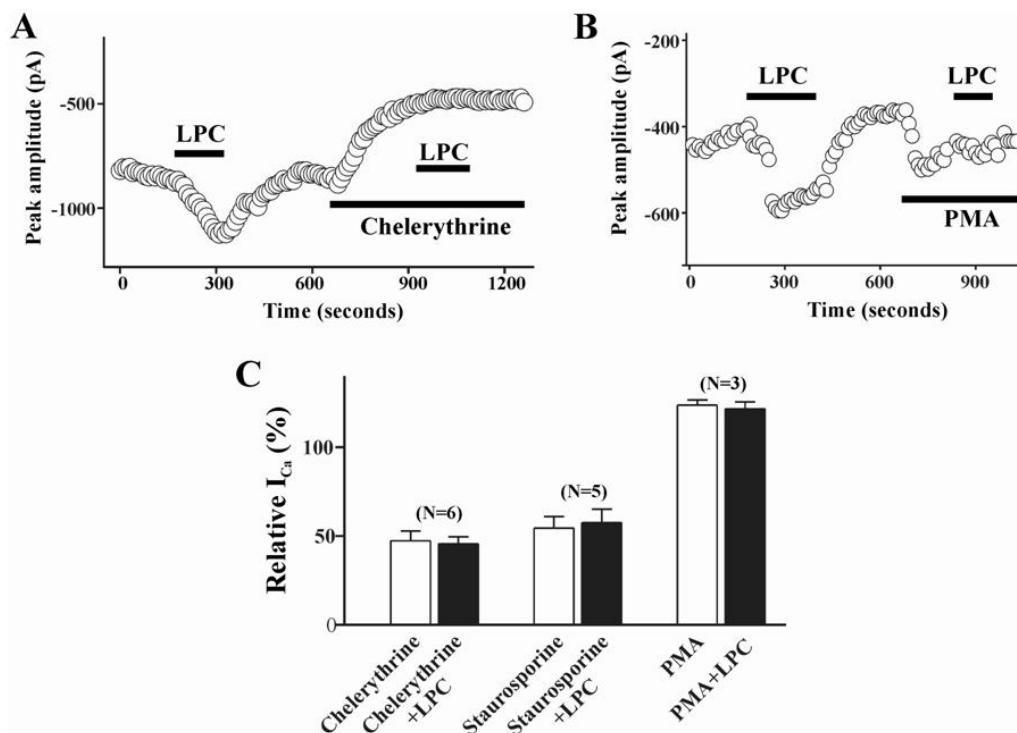
To test the possibility that LPC stimulates $I_{Ca(L)}$ through activation of a protein kinase C, we examined the effect of PKC inhibitor or activator on LPC-induced increase of $I_{Ca(L)}$. Thus, staurosporine (100 nM) was applied to the bath after acquiring a control $I_{Ca(L)}$. Staurosporine produced a significant inhibition of basal I_{Ca} (57.6±7.5% of control), suggesting that a basal activity of protein kinase C is critical to maintain the amplitude of $I_{Ca(L)}$ under our recording conditions. Application of chelerythrine (3 μM), a more specific inhibitor of PKC, showed similar effect on basal $I_{Ca(L)}$ (47.4±5.5% of control). After the effect of PKC inhibitors reached to steady state, LPC-induced enhancement of $I_{Ca(L)}$ was completely abolished (Fig. 3). On the other hand, pretreatment of the cells with 100 nM PMA, a PKC activator, enhanced the basal $I_{Ca(L)}$ activity (123.5±3.1% of control) and significantly suppressed the LPC-induced enhancement of I_{Ca} (% of I_{Ca} change in PMA treated group vs. PMA+LPC treated group; 123.5±3.1% vs 121.4±4.0%, n=3).

DISCUSSION

We report herein a novel property of LPC to activate a

Fig. 2. Steady state activation and inactivation curves of $I_{Ca(L)}$ obtained before and after LPC application. (A) Steady state activation curves of $I_{Ca(L)}$ in control and LPC. Chord conductance (G) was measured at different membrane potentials and normalized to the maximal chord conductance (G_{max}). Each data points were the means of 8 experiments and fitted using a following form of Boltzman equation; $Y=\{1+\exp[(V_{1/2} - V)/k]\}^{-1}$, where $V_{1/2}$ represents half maximal activation potential and k is slope factor. Measured $V_{1/2}$ was -5.8 ± 0.2 mV and -3.1 ± 0.1 mV in control and LPC, respectively. (B) Steady state inactivation curves of $I_{Ca(L)}$ in control and LPC. Current amplitudes (I) of test potential to 0 mV from different pretest potentials were normalized to the maximal current (I_{max}). Each data points were the means of 6 experiments. Curves were obtained from following form of Boltzman equation; $Y=\{1+\exp[(V - V_{1/2})/k]\}^{-1}$, where $V_{1/2}$ represents half maximal inactivation potential and k is slope factor. $V_{1/2}$ was -31.4 ± 1.2 mV and -30.2 ± 1.6 mV in control and LPC, respectively.

Fig. 3. Effect of PKC on LPC-induced change of $I_{Ca(L)}$. Plot of Ca^{2+} currents evoked by repetitive step depolarization from -80 mV to 0 mV versus time. Temporal course of change of $I_{Ca(L)}$ during treatment of LPC ($1\,\mu$M) alone and LPC with $3\,\mu$M chelerythrine (A), or with 100 nM PMA (B). Horizontal bars indicate the time of drugs application. (C) Summary of the effect of pretreatment of staurosporine (100 nM), chelerythrine ($3\,\mu$M), and PMA (100 nM) on LPC-induced change of $I_{Ca(L)}$. Data are expressed as the % of control. Number of cells in each experimental condition is indicated in parenthesis.

L-type Ca^{2+} current ($I_{Ca(L)}$) in the rabbit portal vein smooth muscle cells. To the best of our knowledge, this is the first study to show that LPC can increase $I_{Ca(L)}$ in vascular smooth muscle cells and its effect is mediated by activation of PKC. The enhancement of $I_{Ca(L)}$ by LPC may be involved in augmented vascular contractility which is seen in atherosclerotic artery, because the activity of Ca^{2+} channel determines the amount of Ca^{2+} influx and intracellular Ca^{2+} concentration.

It has earlier been reported that LPC affects several kinds of ionic current, including Na^+ current in cardiac myocytes and nonselective cation current in canine renal arterial cells (Magishi et al, 1996; Watson & Gold, 1997; Jabr et al, 2000). In the present study, nonselective cation current evoked by a prolonged exposure to LPC might contaminate to the recording of $I_{Ca(L)}$ and involve in LPC-induced increase of $I_{Ca(L)}$. However, this does not seem to be the case because 1) we used very low concentration of LPC ($1\,\mu$M) compared with that used for activation of nonselective cation current ($10\,\mu$M LPC; Magishi et al, 1996), and 2) application of nifedipine, which selectively blocks $I_{Ca(L)}$, completely inhibited LPC-induced $I_{Ca(L)}$ increase (Fig. 1A). These results suggest that LPC-induced enhancement of inward current in our experimental condition was due to mainly activation of Ca^{2+} current, but not a modification of other current, such as nonselective cation currents.

There are several possible mechanisms by which $I_{Ca(L)}$ could be increased by LPC. One possibility is that LPC indirectly increased $I_{Ca(L)}$ activity via destabilizing sarco-

lemma. At relatively high concentrations ($>10\,\mu$M), LPC may induce a change of phospholipid packing when it is inserted into the lipid bilayer and this alone can modulate the activity of membrane proteins (Epand & Lester, 1990; McHowat et al, 1993). In the present experiment, the activity of $I_{Ca(L)}$ was significantly enhanced by LPC at a concentration of $1\,\mu$M. Furthermore, pretreatment of the muscle cells with PKC inhibitors effectively inhibited LPC-induced change of $I_{Ca(L)}$. These results suggest that LPC-induced increase of $I_{Ca(L)}$ may not be due to alteration of membrane properties.

Another possibility is that LPC can increase $I_{Ca(L)}$ through activation of PKC. In vitro as well as in vivo studies demonstrated that LPC activates PKC in some cell types, including vascular tissues (Sugiyama et al, 1994; Murohara et al, 1996; Watson & Gold, 1997). Activation of PKC has been implicated in regulation of various ion channel activities, such as Ca^{2+} channels, in vascular smooth muscle cells (Cole et al, 1996; Keef et al, 2001). Theses reports suggest the possibility that LPC increased the $I_{Ca(L)}$ in portal vein smooth muscle cells through activation of PKC. As shown in Fig 3, the LPC-induced activation of $I_{Ca(L)}$ was effectively antagonized by pretreatment with 100 nM staurosporine, indicating the involvement of PKC in the LPC-induced activation of $I_{Ca(L)}$. It has been reported that staurosporine lacks specificity against PKC action, and staurosporine-induced inhibition of LPC activity might be due to inhibition of protein kinases other than PKC (Ruegg & Burgess, 1989). However, this was not the case in the

present study because 1) pretreatment of chelerythrine, which is more specific for inhibition of PKC other than protein kinases (Zheng et al, 2001), showed results similar to those of staurosporine pretreatment, and 2) LPC-induced increase of $I_{Ca(L)}$ was also effectively antagonized by pretreatment of PMA, an activator of PKC. Theses results suggest that LPC increased the $I_{Ca(L)}$ through activation of PKC in rabbit portal vein smooth muscle cells.

In vascular smooth muscle, 11 isoforms of PKC have been reported, and they are divided to 3 groups: 1) classic or conventional PKCs that are activated by diacylglycerol or phorbol ester and are Ca^{2+} sensitive (including α, βI, βII, γ), 2) novel or new PKCs that are activated by diacylglycerol or phorbol ester but are not Ca^{2+} dependent (δ, ε, θ, η, Λ, μ), and 3) atypical PKCs that are not activated by either diacylglycerol or Ca^{2+} (λ/ι, ζ) (Hoffman, 1997). In portal vein smooth muscle cells, 3 groups of PKCs (α, ε, ζ) have been reported (Clement-Chomienne et al, 1996). In our present experiment, we did not directly measure which type of PKC isoforms was involved in LPC-induced increase in $I_{Ca(L)}$. However, in our experimental condition, novel PKC in portal vein smooth muscle cells was most likely activated by LPC, because 1) we used 10 mM BAPTA-containing internal solution (see Method), and the involvement of conventional PKCs, which needs a [Ca^{2+}]$_i$ for its activation, could be excluded, and 2) enhancement of basal $I_{Ca(L)}$ by PMA also ruled out the involvement of atypical PKCs in LPC-induced increase of $I_{Ca(L)}$. Nevertheless, the activation of other type of PKC isoforms by LPC can not still be excluded.

Thus, it may be concluded that LPC may increase the activity of $I_{Ca(L)}$ via the activation of PKC in portal vein smooth muscle cells. And these LPC-induced increases in $I_{Ca(L)}$ may be responsible for the increase of intracellular Ca^{2+} concentration and for the LPC-induced abnormal vascular reactivity in atherosclerotic artery.

ACKNOWLEDEGMENT

REFERENCES

Auge N, Fitoussi G, Bascand JL, Pieraggi M, Junquero D, Valet P. Mildly oxidized LDL evokes a sustained Ca^{2+}-dependent retraction of vascular smooth muscle cells. *Circ Res* 79: 871−880, 1996

Chen Y, Morimoto S, Kitano S, Koh E, Fukuo K, Jiang B, Chen S, Yasuda O, Hirotani A, Ogihara T. Lysophosphatidylcholine causes Ca^{2+} influx, enhanced DNA synthesis and cytotoxicity in cultured vascular smooth muscle cells. *Atherosclerosis* 112: 69−76, 1995

Clement-Chomienne O, Walsh MP, Cole WC. Antiotensin II activation of protein kinase C decreases delayed rectifier K$^+$ current in rabbit vascular myocytes. *J Physiol* 495: 689−700, 1996

Cole WC, Clement-Chomienne O, Aiello EA. Regulation of 4-aminopyridine-sensitive, delayed rectifier K$^+$ channels in vascular smooth muscle by phosphorylation. *Biochem Cell Biol* 74: 439−447, 1996

Cox DA, Cohen ML. Effects of oxidized low-density lipoprotein on vascular contraction and relaxation: clinical and pharmacological implications in atherosclerosis. *Pharmacol Rev* 48: 3−19, 1996a

Cox DA, Cohen ML. Selective enhancement of 5-hyroxytryptamine-induced contraction of porcine coronary artery by oxidized low density lipoprotein. *J Pharmacol Exp Ther* 276: 1095−1103, 1996b

Cox RH, Tulenko TN. Altered contractile and ion channel function in rabbit protal vein with dietary atherosclerosis. *Am J Physiol* 268: H2522−H2530, 1995

Eizawa H, Yui Y, Inoue R, Kosuga K, Hattori R, Aoyama T. Lysophosphatidylcholine inhibits endothelium-dependent hyperpolarization and N-omega-nitro-L-arginine/indomethacin-resistant endothelium-dependent relaxation in the porcine coronary artery. *Circulation* 92: 3520−3526, 1995

Epand RM, Lester DS. The role of membrane biophysical properties in the regulation of protein kinase C activity. *Trends Pharmacol Sci* 11: 317−320, 1990

Galle J, Bassenge E, Busse R. Oxidized low density lipoproteins potentiate vasoconstriction to various agonists by direct interaction with vascular smooth muscle. *Circ Res* 66: 1287−1293, 1990

Hoffmann J. The potential for isoenzyme-selective modulation of protein kinase C. *FASEB J* 11: 649−669, 1997

Jabr RI, Yamazaki J, Hume JR. Lysophosphatidylcholine triggers intracellular calcium release and activation of non-selective cation channels in renal arterial smooth muscle cells. *Pflu Arch Eur J Physiol* 439: 495−500, 2000

Keef KD, Hume JR, Zhong J. Regulation of cardiac and smooth muscle Ca^{2+} channels by protein kinases. *Am J Physiol* 281: C1743−C1756, 2001

Lepretre N, Mironneau J. Alpha 2-adrenoreceptors activate dihydropyridine-sensitive calcium channels via Gi-proteins and protein kinase C in rat portal vein myocytes. *Pflu Arch* 429: 253−261, 1994

Magishi K, Kimura J, Kubo Y, Abiko Y. Exogenous lysophosphatidylcholine increases non-selective cation current in guinea-pig ventricular myocytes. *Pflügers Arch Eur J Physiol* 432: 345−350, 1996

McHowat J, Yamada KA, Wu J, Yan GX, Corr BB. Recent insights pertaining to sarcolemmal phospholipid alterations underlying arrhythmogenesis in the ischemic heart. *J Cardiovasc Electrophysiol* 4: 288−310, 1993

Murohara T, Kugiyama K, Ohgushi M, Sugiyama S, Ohta Y, Yasue H. LPC in oxidized LDL elicits vasocontraction and inhibits endothelium-dependent relaxation. *Am J Physiol* 267: H2441−H2449, 1994

Murohara T, Scalia R, Lefer AM. Lysophosphatidylcholine promotes P-selectin expression in platelets and endothelial cells: possible involvement of protein kinase C activation and its inhibition by nitric oxide donors. *Circ Res* 78: 780−789, 1996

Obejero-Paz CA, Auslender M, Scarpa A. PKC activity modulates availability and long openings of L-type Ca^{2+} channels in A7r5 cells. *Am J Physiol* 275: C535−C543, 1998

Ruegg UT, Burgess GN. Staurosporine, K-252 and NCN-01: potent but nonspecific inhibitors of protein kinases. *Trends Pharmacol Sci* 10: 218−220, 1989

Shimamura K, Kusaka M, Sperelakis N. Protein kinase C stimulates Ca^{2+} current in pregnant rat myometrial cells. *Can J Physiol Pharmacol* 72: 1304−1307, 1994

Stoll LL, Spector AA. Lysophosphatidylcholine causes cGMP-dependent verapamil-sensitive Ca^{2+} influx in vascular smooth muscle cells. *Am J Physiol* 264: C885−C893, 1993

Sugiyama S, Kugiyama K, Ohgushi M, Fujimoto K, Yasue H. Lysophosphatidylcholine in oxidized low-density lipoprotein increases endothelial susceptibility to polymorphonuclear leukocyte-induced endothelial dysfunction in porcine coronary arteries: Role of protein kinase C. *Circ Res* 74: 565−575, 1994

Watson CL, Gold MR. Lysophosphatidylcholine modulates cardiac I_{Na} via multiple protein kinase pathways. *Circ Res* 81: 387−395, 1997

Yeon DS, Kwon SC, Nam TS, Ahn DS. Lysophosphatidylcholine decreases delayed rectifier K current in rabbit coronary smooth muscle cells. *J Vet Med Sci* 63: 395−399, 2001

Zheng T, Li W, Altura BT, Altura BM. Use of protein kinase C inhibitors in rapid [Mg^{2+}]$_i$ mobilization in primary cultured rat aortic smooth muscle cells: are certain protein kinase C isoforms natural homeostatic regulators of cytosolic free Mg^{2+}? *Eur J Pharmacol* 413: R1−R3, 2001