Korean J Physiol Pharmacol
Vol 13: 27−32, February, 2009

Oxidized Low-density Lipoprotein- and Lysophosphatidylcholine-induced Ca^{2+} Mobilization in Human Endothelial Cells

Moon Young Kim[1], Guo Hua Liang[1], Ji Aee Kim[1], Soo Seung Choi[2], Shinku Choi[1], and Suk Hyo Suh[1]

Department of [1]Physiology and Medical Research Institute, [2]Thoracic and Cardiovascular Surgery, Schoolof Medicine, Ewha Womans University, Seoul 158-710, Korea

The effects of oxidized low-density lipoprotein (OxLDL) and its major lipid constituent lysophosphatidylcholine (LPC) on Ca^{2+} entry were investigated in cultured human umbilical endothelial cells (HUVECs) using fura-2 fluorescence and patch-clamp methods. OxLDL or LPC increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), and the increase of $[Ca^{2+}]_i$ by OxLDL or by LPC was inhibited by La^{3+} or heparin. LPC failed to increase $[Ca^{2+}]_i$ in the presence of an antioxidant tempol. In addition, store-operated Ca^{2+} entry (SOC), which was evoked by intracellular Ca^{2+} store depletion in Ca^{2+}-free solution using the sarcoplasmic reticulum Ca^{2+} pump blocker, 2, 5-di-t-butyl-1, 4-benzohydroquinone (BHQ), was further enhanced by OxLDL or by LPC. Increased SOC by OxLDL or by LPC was inhibited by U73122. In voltage-clamped cells, OxLDL or LPC increased $[Ca^{2+}]_i$ and simultaneously activated non-selective cation (NSC) currents. LPC-induced NSC currents were inhibited by 2-APB, La^{3+} or U73122, and NSC currents were not activated by LPC in the presence of tempol. Furthermore, in voltage-clamped HUVECs, OxLDL enhanced SOC and evoked outward currents simultaneously. Clamping intracellular Ca^{2+} to 1 μM activated large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) current spontaneously, and this activated BK_{Ca} current was further enhanced by OxLDL or by LPC. From these results, we concluded that OxLDL or its main component LPC activates Ca^{2+}-permeable Ca^{2+}-activated NSC current and BK_{Ca} current simultaneously, thereby increasing SOC.

Key Words: Oxidized LDL, Endothelial cell, Store-operated Ca^{2+} entry, Large conductance Ca^{2+}-activated K^+ channel, Nonselective cation current

INTRODUCTION

Low-density lipoprotein (LDL) can be oxidized in many types of cells, including monocytes, neutrophils, and fibroblasts, and macrophages, endothelial cells and smooth muscle cells among them are the most likely to contribute to LDL oxidation in vascular wall, thereby inducing atherosclerotic lesions (Young et al., 2001). Thus, oxidized LDL (OxLDL) plays a key role in atherosclerosis (Steinberg et al., 1989; Steinbrecher et al., 1990; Berliner et al., 1996). Taken up by macrophages, OxLDL leads to foam cell formation that is characteristic of the earliest atherosclerotic lesion (Ross, 1993; Steinberg, 1997).

OxLDL is known to evoke endothelial dysfunction by increasing oxidative stress (Ross, 1993; Mehta, 2004; Mehta et al., 2006). OxLDL impairs endothelium-dependent vasodilation (Itagaki et al., 2003), and damages endothelial cells to lose endothelial integrity (Hessler et al., 1983). The major lipid constituent of OxLDL, lysophosphatidylcholine (LPC), plays an important role in OxLDL-induced endothelial dysfunction (Takeshita et al., 2000; Matsubara et al., 2005). LPC increases oxidative stress by generating reactive oxygen species, and thereby, causes endothelial dysfunction (Muller et al., 2002; Zmijewski et al., 2005).

On the other hand, OxLDL or LPC has been reported to change electrophysiological properties of cells (Kuhlmann et al., 2003; Fearon, 2006) and increase intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in endothelial cells (Weisser et al., 1992; Thorin et al., 1995; Zhao et al., 1997; van Tits et al., 2000). LPC activates nonselective cation (NSC) current in vascular smooth muscle cells (Jabr et al., 2000; Terasawa et al., 2002), and OxLDL activates large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) current in human umbilical vein endothelial cells (HUVECs). In addition, OxLDL enhances L-type Ca^{2+} currents via LPC-induced mitochondrial reactive oxygen species production (Fearon, 2006). However, little is known about the mechanisms by which OxLDL or LPC increases $[Ca^{2+}]_i$ in endothelial cells.

The present study was designed to elucidate how OxLDL or its main component LPC increases $[Ca^{2+}]$i in endothelial cells, and evidence indicates that OxLDL or LPC activates Ca^{2+}-permeable Ca^{2+}-activated NSC current and BK_{Ca} current simultaneously, thereby increasing Ca^{2+} entry.

Corresponding to: Suk Hyo Suh, Department of Physiology, College of Medicine, Ewha Womans University, 911-1, Mok-6-dong, Yangchun-gu, Seoul 158-710, Korea. (Tel) 82-2-2650-5722, (Fax) 82-2-2650-5791, (E-mail) shsuh@ewha.ac.kr

ABBREVIATIONS: oxLDL, oxidized low-density lipoprotein; LPC, lysophosphatidylcholine; HUVECs, human umbilical endothelial cells; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; SOC, store-operated Ca^{2+} entry; BHQ, 2,5-di-t-butyl-1, 4-benzohydroquinone; NSC currents, non-selective cation currents; BK_{Ca} current, large-conductance Ca^{2+}-activated K^+ current; 2-APB, amino-ethoxybiphenyl borane.

METHODS

Endothelial cell culture

Human umbilical vein endothelial cells (HUVECs, CRL-1730) were purchased from the American Type Culture Collection and cultured as monolayer in Medium 199 (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin and 15 μg/ml endothelial cell growth supplement, or Eagle basal medium 2 (EBM2, Clonetics, Walkersville, MD) supplemented with 2% FBS, 0.1% vascular endothelial growth factor, 0.1% ascorbic acid, 0.1% gentamycin sulfate amphotericin-B, 0.04% hydrocortisone and 0.1% heparin. All cells were maintained at 37°C in humidified condition under 5% CO_2. The cells were then detached by exposure to trypsin, re-seeded on gelatine-coated cover slips, and maintained in culture for 2~4 days before use. Measurements were performed on non-confluent cells.

Electrophysiology

Electrophysiological methods and Ca^{2+} measurements have previously been described in detail (Nilius et al., 1994). Membrane potential was monitored in current clamp mode or controlled in voltage clamp mode with an EPC-9 (HEKA Elektronik, Lambrecht, Germany), using a nystatin-perforated patch (100 mg/ml). Whole-cell currents were measured using ruptured patches. Voltages were monitored in voltage clamp mode with an EPC-9 (HEKA Elektronik, Lambrecht, Germany, sampling rate 1 ms, 8-Pole Bessel filter 2.9 kHz). Holding potential of whole cell experiment was 0 mV or −60 mV. We applied a voltage ramp from −100 mV or −150 mV to +100 mV every 10 seconds with duration of 650 ms. Currents were recorded at a sampling rate of 1 kHz to 4 kHz.

Ca^{2+} measurement

Cells were loaded with Fura-2/AM (the acetoxymethyl ester form), and $[Ca^{2+}]_i$ was measured using a microfluorimeter consisting of an inverted microscope (DM IRB, Leica, Germany) and a PTI filter scan power illuminator system (Photon Technology International Inc, Canada). Fura-2/AM (2 μM) was added to the bath, and the cells were incubated for 25 min at 37°C. The cells were illuminated alternatively at wavelengths of 340 and 380 nm through a chopper wheel (frequency of 50 Hz). Fluorescence was measured at 510 nm and autofluorescence was subtracted from the signals obtained. The free Ca^{2+} concentration was calculated from the ratio of the fluorescence signals emitted at each excitation wavelength. The calibration procedure was identical to that described previously (Nilius et al., 1993; Nilius et al., 1994).

Solutions

The standard extracellular solution was an iso-osmolar Krebs solution, containing (in mM) 150 NaCl, 6 KCl, 1 $MgCl_2$, 1.5 $CaCl_2$, 10 glucose, 10 HEPES, pH 7.4 with NaOH. The osmolality of this solution, as measured with a vapour pressure osmometer (Wescor 5,500, Schlag, Gladbach, Germany), was 320±5 mOsm. For simultaneous measurement of membrane current and $[Ca^{2+}]_i$, we used this external solution and an internal high K^+ solution, unless mentioned otherwise. The internal high K^+ pipette solution contained (in mM) 40 KCl, 100 K-aspartate, 2 $MgCl_2$, 0.1 EGTA, 4 Na_2ATP, 10 HEPES, pH 7.2 with KOH (290 mOsm).

In some experiments, we abolished the activity of large conductance Ca^{2+} activated K^+ (BK_{Ca}) channels by substituting extra- and intracellular K^+ with Cs^+. The internal high Cs^+ pipette solution contained 145 mM Cs-glutamate, 8 mM NaCl, 2 mM $MgCl_2$, 10 mM HEPES, and 1 mM Na_2ATP, buffered with CsOH to 7.2. The osmolality of all external solutions was increased to 420±5 mosmol/kg with 100 mM mannitol (Voets et al., 1997) to eliminate volume activated Cl^- currents. For buffering free Ca^{2+}, the appropriate amount of Ca^{2+} (calculated by the program CaBuf, G. Droogmans) was added in the presence of 5 mM EGTA.

Chemicals

Amino-ethoxybiphenyl borane (2-APB), 2,5-di-t-butyl-1,4-benzohydroquinone (BHQ), L-α-lysophosphatidyl choline (LPC) from egg yolk, Tempol and U73312 were purchased from Sigma (St. Louis, MO). OxLDL was obtained from Intracel Inc (Frederick, MD), and Fura-2 AM from Molecular Probe (Eugene, OR). U73312, LPC, 2-APB and nystatin were applied from a stock solution in DMSO. The final concentration of DMSO was less than 0.05%.

All experiments were performed at 37°C. Pooled data are presented as means±SEM and significant differences were detected using the Student's t test ($p < 0.05$).

RESULTS

OxLDL- or LPC-induced increase of intracellular Ca^{2+}

Effect of OxLDL or of LPC on $[Ca^{2+}]_i$ is illustrated in Fig. 1. OxLDL (20 μg/ml) or LPC (3 μgM) increased $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+} (Fig. 1A and C). OxLDL reversibly increased $[Ca^{2+}]_i$, whereas increased $[Ca^{2+}]_i$ by LPC did not return to a resting level for more than one hours (Fig. 1A). This increase in $[Ca^{2+}]_i$ by OxLDL or LPC was inhibited by 10 μM La^{3+} (Fig. 1B), 50 IU/ml heparin (Fig. 1C) or depleting extracellular Ca^{2+} (data not shown). In addition, LPC failed to increase $[Ca^{2+}]_i$ in the presence of an antioxidant tempol (Fig. 1E). In Ca^{2+}-free solution, the increase of $[Ca^{2+}]$i by OxLDL (Fig. 1D) or by LPC (data not shown) was transient and there was no sustained Ca^{2+} plateau, indicating that the Ca^{2+} plateau is mainly due to influx of Ca^{2+} and that Ca^{2+}-entry pathway (s) is (are) activated by OxLDL or by LPC. Thus, we next examined whether OxLDL or LPC activated store-operated Ca^{2+} entry. Fig. 1F shows that store-operated Ca^{2+} entry was activated by emptying intracellular Ca^{2+} stores using BHQ, an inhibitor of sarcoplasmic reticulum Ca^{2+} pump. Exposure of HUVECs in Ca^{2+}-free solution to 20 μM BHQ transiently increased $[Ca^{2+}]_i$ and then $[Ca^{2+}]_i$ decreased slowly to a steady state within 10~15 min after BHQ application. Then, we added Ca^{2+} to the Ca^{2+}-free solution. The subsequent reapplication of extracellular Ca^{2+} caused an increase of $[Ca^{2+}]_i$ (Fig. 1E), and this increase of $[Ca^{2+}]_i$, dependent on extracellular Ca^{2+}, obviously proceeds via Ca^{2+} entry pathways which was activated during this protocol (store-operated Ca^{2+} entry). When OxLDL (Fig. 1E) or LPC (Fig. 1F) was applied after store-operated Ca^{2+} entry was evoked, store-operated Ca^{2+} entry was further enhanced by

OxLDL or LPC (Fig. 1F).

OxLDL- or LPC-induced activation of nonselective cation current

The increase of $[Ca^{2+}]_i$ via Ca^{2+} entry pathways, which were activated by the above protocol, could be modulated by changing membrane potential, which affects the driving force for Ca^{2+} entry. Thus, we clamped membrane potential of cells to 0 mV to eliminate the effect of membrane potential on Ca^{2+} entry, and then examined whether OxLDL enhances store-operated Ca^{2+} entry (Fig. 2). In voltage-clamped cells, we simultaneously recorded membrane current and $[Ca^{2+}]_i$. As shown in Fig. 2, store-operated Ca^{2+} entry was evoked by the reapplication of extracellular Ca^{2+} after exposure of HUVECs in Ca^{2+}-free solution to 20 μM BHQ. The reapplication of extracellular Ca^{2+} increased $[Ca^{2+}]_i$. When an increase of $[Ca^{2+}]_i$ reached a steady state, OxLDL was applied. As shown in the figure, OxLDL activated large inward and outward currents and further enhanced the evoked store-operated Ca^{2+} entry simultaneously (Fig. 2). Substitution of external monovalent cations by $NMDG^+$ completely abolished the inward currents (Fig. 3A), indicating that the current is carried by cations. These results suggest that OxLDL activates Ca^{2+}-permeable NSC currents in HUVECs, thereby increasing store-operated Ca^{2+} entry, independently of membrane potential.

LPC also evoked inward and outward currents (Fig. 3). The current was time-independent at each potential and was not inactivated during the voltage steps (data not shown). The reversal potentials of these currents were close to 15 mV, and the current-voltage relationships showed no rectification (Fig. 3). This LPC-activated NSC current was decreased by the inositol 1,4,5-triphosphate (IP_3) -gated Ca^{2+} channel blocker 2-APB (Fig. 3A), La^{3+} (Fig. 3C) or the phospholipase C inhibitor U-73122 (Fig. 3C). Furthermore, LPC failed to activate NSC currents in the presence of an antioxidant tempol (5 μM, Fig. 3E).

OxLDL activates BK_{Ca} current

In some cells, OxLDL or LPC augmented store-operated Ca^{2+} entry and evoked outward currents simultaneously (Fig. 4). In voltage-clamped cells, store-operated Ca^{2+} entry was activated by emptying intracellular Ca^{2+} stores using BHQ, and $[Ca^{2+}]_i$ was increased by adding Ca^{2+} to the Ca^{2+}-free solution. When the increase of $[Ca^{2+}]_i$ reached a steady

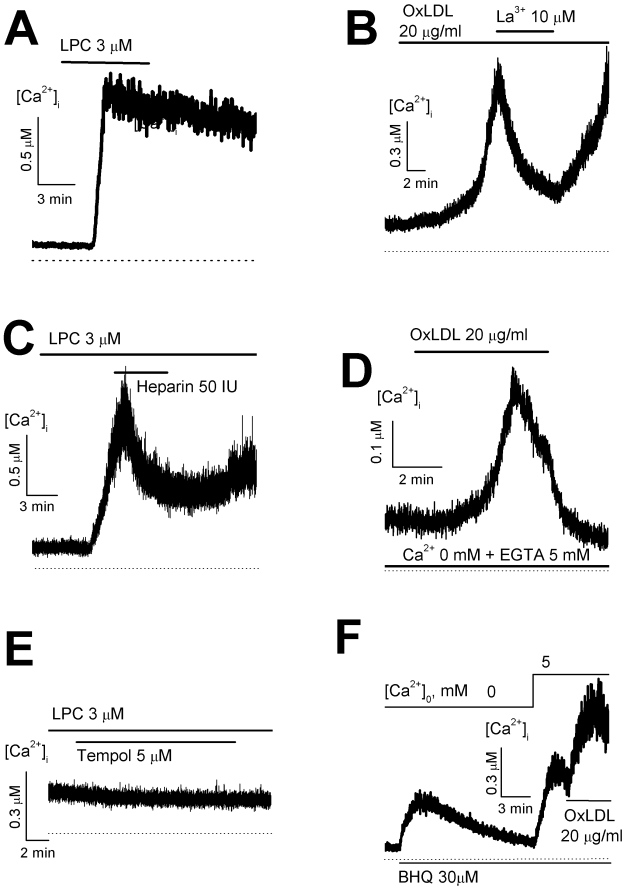

Fig. 1. Effect of OxLDL or LPC on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in HUVECs. (A, C and D) OxLDL-induced increase of $[Ca^{2+}]_i$ in the presence of (A, C) or in the absence of extracellular Ca^{2+} (D). (B) in the presence of an antioxidant tempol, LPC failed to increase $[Ca^{2+}]_i$. (E and F) The increase of store-operated Ca^{2+} entry by OxLDL (E) or LPC (F).

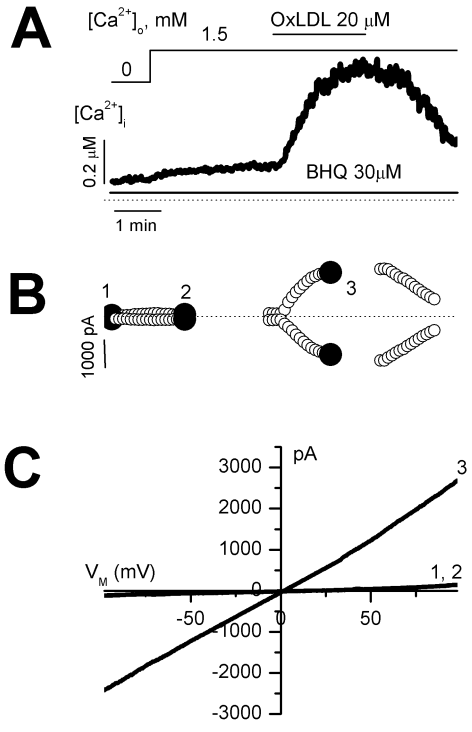

Fig. 2. Effect of OxLDL on store-operated Ca^{2+} entry in a voltage-clamped cell. Membrane currents and $[Ca^{2+}]_i$ were measured simultaneously at a holding potential of 0 mV in nystatin-perforated mode. (A) further activation of store-operated Ca^{2+} entry (SOC) by OxLDL. SOC was activated by depletion of the intracellular Ca^{2+} store and re-exposure to Ca^{2+}. (B) time course of membrane currents at +50 and −50 mV activated by a rise in $[Ca^{2+}]_i$ (A). Membrane currents were activated by repetitive ramps from −100 to +100 mV. (C) current-voltage relations obtained at points indicated 1∼3 in (B).

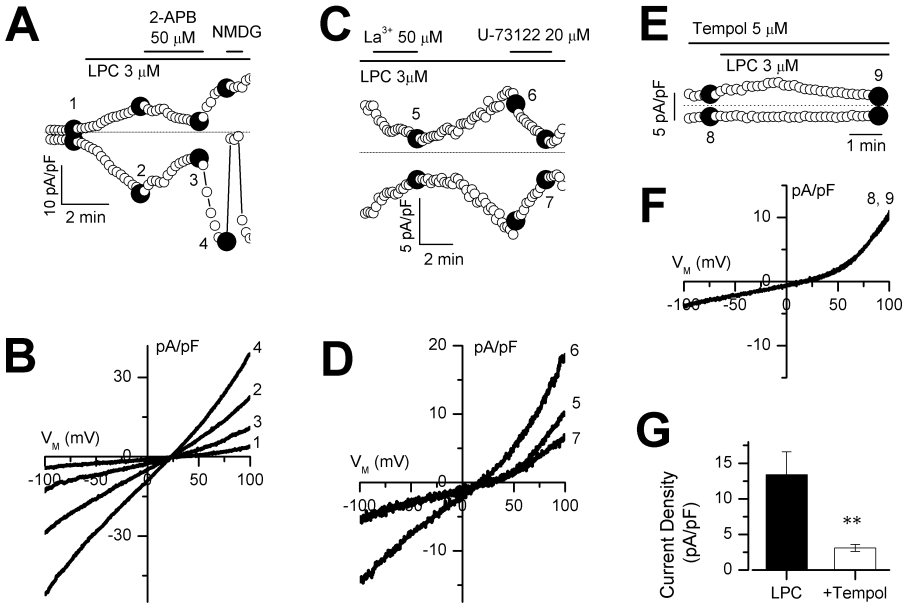

Fig. 3. Effect of LPC on nonselective cation (NSC) currents in HUVECs. Membrane potential was held at 0 mV. (A, C and E) time courses of membrane currents obtained at +50 and −50 mV during repetitive ramps from −100 to +100 mV. (B, D and F) current-voltage relations obtained at points indicated 1∼9 in (A), (C) and (E), respectively. (G) bar graph showing current density at +50 mV. n=8, *p<0.001.

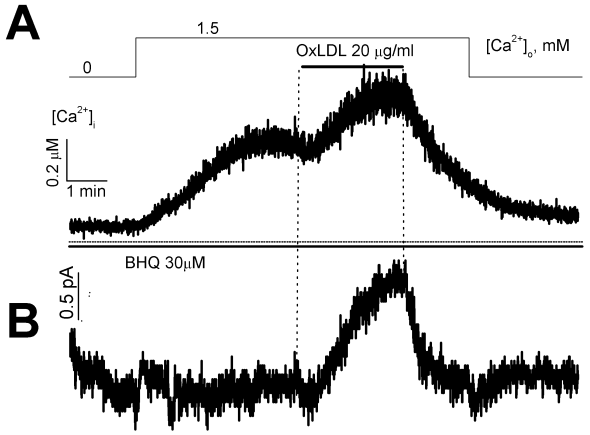

Fig. 4. Effect of OxLDL on store-operated Ca^{2+} entry and membrane currents in a voltage-clamped cell. Membrane currents (B) and $[Ca^{2+}]_i$ (A) were measured simultaneously at a holding potential of 0 mV in nystatin-perforated mode.

state and OxLDL was applied, the increased $[Ca^{2+}]_i$ was further augmented by OxLDL application and outward currents was simultaneously developed (Fig. 4).

Since large conductance Ca^{2+}-activated K^+ (BK_{Ca}) current produces outward currents in HUVECs, we examined whether OxLDL or LPC modulates BK_{Ca} current. Loading cells with a Ca^{2+} solution buffered at 1 μM activated outwardly rectifying current (Fig. 5). This outwardly rectifying current was inhibited by iberiotoxin (data not shown), suggesting that this outwardly rectifying current is BK_{Ca} current. Then, OxLDL or LPC was applied. With the application of OxLDL, this activated BK_{Ca} current of 62.7±4.8 pA/pF was further enhanced to 126.4±34.2 pA/pF (Fig. 5A−C). Like OxLDL, LPC also augmented BK_{Ca} currents (Fig. 5D).

DISCUSSION

Present study suggests that OxLDL or its major component LPC activates Ca^{2+}-permeable NSC current and BK_{Ca} current, thereby activating store-operated Ca^{2+} entry. OxLDL or LPC might activate NSC current by releasing Ca^{2+} from intracellular stores. This Ca^{2+}-activated NSC might be Ca^{2+}-permeable, therefore acts as Ca^{2+} entry pathway. In addition, OxLDL or LPC activates BK_{Ca} current. BK_{Ca} current activation hyperpolarizes membrane, thereby increasing the driving force for Ca^{2+} entry. Thus, OxLDL or LPC promotes Ca^{2+} entry through NSC channel via activating BK_{Ca} current.

The present results suggest that OxLDL- or LPC-activated NSC channel in HUVECs is virtually permeable to Ca^{2+}, inasmuch as an enhancement of store-operated Ca^{2+} entry is accompanied by the activation of this NSC currents. This is consistent with previous findings that Ca^{2+}-activated NSC channel in endothelial cell line derived from human umbilical vein (EA cells) is permeable to Ca^{2+} (Kamouchi et al., 1999; Suh et al., 2002). Kamouchi et al. reported that the relative permeability for Ca^{2+} over Na^+ in the NSC in EA cells is 0.07±0.01. In addition, Nilius et al. (1990) reported that the relative permeability in histamine-activated NSC current is 0.2. Thus, it is highly likely that Ca^{2+} entry pathway to evoke store-operated Ca^{2+} entry is NSC channel.

Our present results suggest that NSC current activation by OxLDL or LPC is induced by a $[Ca^{2+}]_i$-dependent mechanism. NSC current in EA cells is activated by an increase in $[Ca^{2+}]_i$ (Suh et al., 2002). Thus, vasoactive agonists, such as ATP, histamine and bradykinin, which can increase $[Ca^{2+}]_i$, activate NSC current in EA cells (Kamouchi et al., 1999). Like NSC current in EA cells, NSC current in HUVECs was also activated by increasing $[Ca^{2+}]_i$ and by vasoactive agents to increase $[Ca^{2+}]_i$. Therefore, OxLDL, LPC and ATP might activate NSC current via releasing Ca^{2+} from intracellular stores in HUVECs.

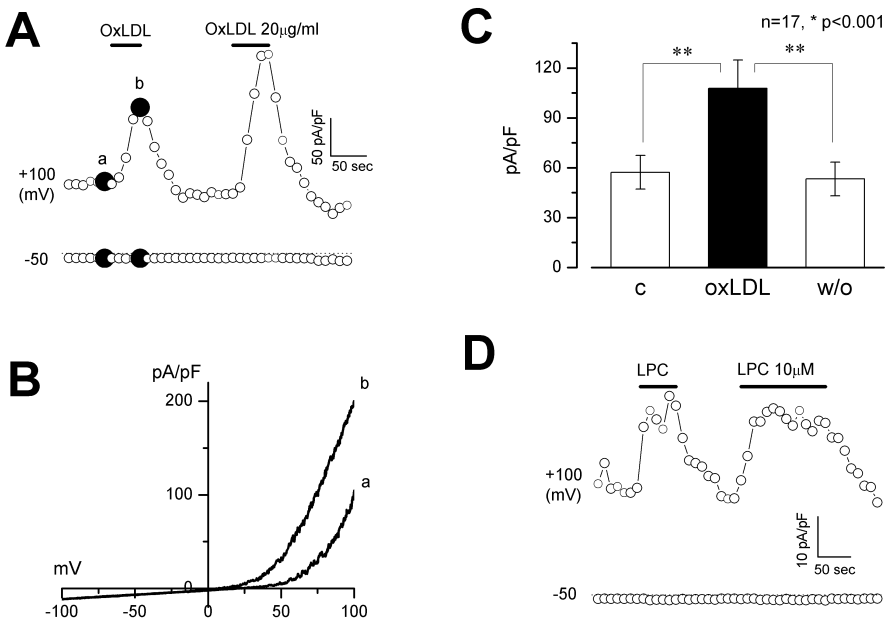

Fig. 5. Effect of OxLDL and LPC on BK_{Ca} currents. BK_{Ca} currents were activated by clamping $[Ca^{2+}]_i$ at 1 (A−C) or 0.5 μM (D). Membrane potential was held at 0 mV. (A and D) time course of membrane current change by OxLDL (A) or LPC (D) measured at −50 and +100 mV. (B) current-voltage relationship obtained from the voltage ramps from −100 to +100 mV labeled as a and b in (A). (C) bar graph showing current density at +100 mV. (C) control. OxLDL, maximal current density obtained during OxLDL (20 μg/ml) application; w/o, wash-out. n=17, *p $<$0.001.

OxLDL or LPC increases $[Ca^{2+}]_i$ in HUVECs and the increase of $[Ca^{2+}]_i$ activate BK_{Ca} current, because BK_{Ca} currents are activated dependently of intracellular Ca^{2+}. This explains the mechanism of how OxLDL (Kuhlmann et al., 2003) or LPC activates BK_{Ca} current. In addition, OxLDL or LPC might increase the sensitivity of BK_{Ca} channels to Ca^{2+}, because OxLDL or LPC augmented BK_{Ca} current further which was already activated by clamping $[Ca^{2+}]_i$ to 1 μM. BK_{Ca} current activation can hyperpolarize membrane, thereby increasing the driving force for Ca^{2+} (Kamouchi et al., 1999; Kim et al., 2006). Thus, OxLDL or LPC might facilitate Ca^{2+} entry through Ca^{2+}-permeable Ca^{2+}-activated NSC channel via activating BK_{Ca} current.

Furthermore, OxLDL and LPC might increase the sensitivity of NSC channel to Ca^{2+}. In the present study, the increase of Ca^{2+} entry might have been caused by increased driving force for Ca^{2+} and/or by further activation of Ca^{2+}-activated NSC channel. Since OxLDL increased Ca^{2+} entry in voltage-clamped cells, further activation of Ca^{2+}-activated NSC channel by OxLDL or by LPC might evoke increased Ca^{2+} entry, suggesting that OxLDL or LPC increases the sensitivity of Ca^{2+}-activated NSC channel to intracellular Ca^{2+}.

In conclusion, we have shown that OxLDL activates NSC current and BK_{Ca} current, thereby increasing $[Ca^{2+}]_i$. These effects of OxLDL on HUVECs are mimicked by its main lipid constituent LPC, and therefore, LPC mediates these responses on endothelial cells. These data explain the mechanisms involved in physiological and pathological regulation of endothelial cells when exposed to OxLDL.

ACKNOWLEDGEMENTS

This work was supported by the Korea Research Foundation Grant funded by the Korea Government (MOEHRD) (KRF-2005-041-E00020).

REFERENCES

Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherogenesis. *Free Radic Biol Med* 20: 707−727, 1996.

Fearon IM. OxLDL enhances L-type Ca^{2+} currents via lysophosphatidylcholine-induced mitochondrial reactive oxygen species (ROS) production. *Cardiovasc Res* 69: 855−864, 2006.

Hessler JR, Morel DW, Lewis LJ, Chisolm GM. Lipoprotein oxidation and lipoprotein-induced cytotoxicity. *Arteriosclerosis* 3: 215−222, 1983.

Itagaki K, Hauser CJ. Sphingosine 1-phosphate, a diffusible Ca^{2+} influx factor mediating store-operated Ca^{2+} entry. *J Biol Chem* 278: 27540−27547, 2003.

Jabr RI, Yamazaki J, Hume JR. Lysophosphatidylcholine triggers intracellular Ca^{2+} release and activation of non-selective cation channels in renal arterial smooth muscle cells. *Pflugers Arch* 439: 495−500, 2000.

Kamouchi M, Droogmans G, Nilius B. Membrane potential as a modulator of the free intracellular Ca^{2+} concentration in agonist-activated endothelial cells. *Gen Physiol Biophys* 18: 199−208, 1999.

Kamouchi M, Mamin A, Droogmans G, Nilius B. Nonselective cation channels in endothelial cells derived from human umbilical vein. *J Membr Biol* 169: 29−38, 1999.

Kim MY, Liang GH, Kim JA, Kim YJ, Oh S, Suh SH. Sphingosine-1-phosphate activates BKCa channels independently of G protein-coupled receptor in human endothelial cells. *Am J Physiol Cell Physiol* 290: C1000−1008, 2006.

Kuhlmann CR, Schafer M, Li F, Sawamura T, Tillmanns H, Waldecker B, Wiecha J. Modulation of endothelial Ca^{2+}-activated K^+ channels by oxidized LDL and its contribution to endothelial proliferation. *Cardiovasc Res* 60: 626−634, 2003.

Matsubara M, Hasegawa K. Benidipine, a dihydropyridine-Ca^{2+} channel blocker, prevents lysophosphatidylcholine-induced injury and reactive oxygen species production in human aortic endothelial cells. *Atherosclerosis* 178: 57−66, 2005.

Mehta JL. The role of LOX-1, a novel lectin-like receptor for oxidized low density lipoprotein, in atherosclerosis. *Canadian J Cardiol* 20 Suppl B: 32B−36B, 2004.

Mehta JL, Chen J, Hermonat PL, Romeo F, Novelli G. Lectin-like, oxidized low-density lipoprotein receptor-1 (LOX-1): a critical player in the development of atherosclerosis and related

disorders. *Cardiovasc Res* 69: 36−45, 2006.

Muller J, Petkovic M, Schiller J, Arnold K, Reichl S, Arnhold J. Effects of lysophospholipids on the generation of reactive oxygen species by fMLP- and PMA-stimulated human neutrophils. *Luminescence* 17: 141−149, 2002.

Nilius B. Permeation properties of a non-selective cation channel in human vascular endothelial cells. *Pflugers Arch* 416: 609−611, 1990.

Nilius B, Oike M, Zahradnik I, Droogmans G. Activation of a Cl^- current by hypotonic volume increase in human endothelial cells. *J Gen Physiol* 103: 787−805, 1994.

Nilius B, Schwarz G, Oike M, Droogmans G. Histamine- activated, non-selective cation currents and Ca^{2+} transients in endothelial cells from human umbilical vein. *Pfluers Archiv* 424: 285−293, 1993.

Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362: 801−809, 1993.

Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 272: 20963−20966, 1997.

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 320: 915−924, 1989.

Steinbrecher UP, Zhang HF, Lougheed M. Role of oxidatively modified LDL in atherosclerosis. *Free Radic Biol Med* 9: 155−168, 1990.

Suh SH, Watanabe H, Droogmans G, Nilius B. ATP and nitric oxide modulate a Ca^{2+}-activated non-selective cation current in macrovascular endothelial cells. *Pflugers Arch* 444: 438−445, 2002.

Takeshita S, Inoue N, Gao D, Rikitake Y, Kawashima S, Tawa R, Sakurai H, Yokoyama M. Lysophosphatidylcholine enhances superoxide anions production via endothelial NADH/NADPH oxidase. *J Atheroscler Thromb* 7: 238−246, 2000.

Terasawa K, Nakajima T, Iida H, Iwasawa K, Oonuma H, Jo T, Morita T, Nakamura F, Fujimori Y, Toyo-oka T, Nagai R. Nonselective cation currents regulate membrane potential of rabbit coronary arterial cell: modulation by lysophosphatidylcholine. *Circulation* 106: 3111−3119, 2002.

Thorin E, Hamilton C, Dominiczak AF, Dominiczak MH, Reid JL. Oxidized-LDL induced changes in membrane physico- chemical properties and $[Ca^{2+}]_i$ of bovine aortic endothelial cells. Influence of vitamin E. *Atherosclerosis* 114: 185−195, 1995.

van Tits LJ, Hak-Lemmers HL, Demacker PN, Stalenhoef AF, Willems PH. Oxidized low-density lipoprotein induces Ca^{2+} influx in polymorphonuclear leukocytes. *Free Radic Biol Med* 29: 747−755, 2000.

Voets T, Wei L, De Smet P, van Driessche W, Eggermont J, Droogmans G, Nilius B. Downregulation of volume-activated Cl^- currents during muscle differentiation. *Am J Physiol* 272: C667−674, 1997.

Weisser B, Locher R, Mengden T, Vetter W. Oxidation of low density lipoprotein enhances its potential to increase intracellular free calcium concentration in vascular smooth muscle cells. *Arterioscler Thromb* 12: 231−236, 1992.

Young IS, McEneny J. Lipoprotein oxidation and atherosclerosis. *Biochem Soc Trans* 29: 358−362, 2001.

Zhao B, Ehringer WD, Dierichs R, Miller FN. Oxidized low-density lipoprotein increases endothelial intracellular calcium and alters cytoskeletal f-actin distribution. *Eur J Clin Invest* 27: 48−54, 1997.

Zmijewski JW, Landar A, Watanabe N, Dickinson DA, Noguchi N, Darley-Usmar VM. Cell signalling by oxidized lipids and the role of reactive oxygen species in the endothelium. *Biocheml Soc Trans* 33: 1385−1389, 2005.