FULL PAPER *Pharmacology*

Lysophosphatidylcholine Decreases Delayed Rectifier K^+ Current in Rabbit Coronary Smooth Muscle Cells

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ABSTRACT. Lysophosphatidylcholine (LPC), which exists abundantly in lipid fraction of oxidized low density lipoprotein, has been implicated in enhanced agonist-induced contraction and increase of intracellular Ca^{2+}. The effect of LPC on the activity of delayed rectifier K^+ current (I_{dK}), which is a major determinant of membrane potential and vascular tone under resting condition, was examined in rabbit coronary smooth muscle cells using whole cell patch clamping technique. Application of LPC to the bath solution caused a concentration-dependent inhibition of I_{dK}, and the concentration to produce half-maximal inhibition was 1.51 μM. This effect of LPC on I_{dK} was readily reversed after washout of LPC in the bath. The steady-state voltage dependence of I_{dK} was shifted to positive direction by both extra- and intracellular application of LPC. Staurosporine (100 nM) pretreatment significantly suppressed the LPC-induced inhibition of I_{dK}. These results suggest that LPC inhibits I_{dK} in rabbit coronary smooth muscle cells by a pathway that involves protein kinase C, and the LPC-induced inhibition of I_{dK} may be, at least in part, responsible for the abnormal vascular reactivity in atherosclerotic coronary artery.

KEY WORDS: coronary smooth muscle, delayed rectifier K^+ current, lysophosphatidylcholine, protein kinase C.

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Prolonged exposure to a high cholesterol diet elevated the plasma level of low-density lipoprotein (LDL) which has been associated with the development of atherosclerosis [8]. Especially, oxidative modification of LDL is a key factor for progression of atherosclerosis [19], and *in vitro* application of oxidized LDL mimicked the abnormal vascular reactivity, which was seen in atherosclerotic artery [4, 9, 13]. Several studies suggested that LPC might be the active component mediating the *in vitro* effect of oxidized LDL on vascular contraction. LPC enhances the agonist-induced contraction [22], increases intracellular Ca^{2+} [6, 26], inhibits endothelium dependent relaxation response [11, 22], and activates protein kinase C (PKC) [23, 27, 28].

Activation of PKC has a profound effect on vascular contractility via inhibition of nitric oxide synthase activity, changing Ca^{2+} sensitivity, and modulating ion channel activity [7, 17, 23]. Aiello *et al.* [3] reported that PKC inhibits delayed rectifier K^+ current (I_{dK}), which determines the resting membrane potential in the rabbit coronary smooth muscle cells [18]. Depolarization of membrane potential by inhibition of I_{dK} increased intracellular Ca^{2+} concentration through opening of the voltage dependent Ca^{2+} channels, and results in increased vascular tone [24]. These reports suggest the possibility that LPC may induce abnormal vascular reactivity through PKC-dependent inhibition of I_{dK} in coronary artery. However, there is no direct finding on the inhibiting effect of LPC on I_{dK} and the involvement of PKC on the LPC-induced inhibition of I_{dK} in rabbit coronary artery. Therefore, the aim of present study was to examine the effect of LPC on the activity of I_{dK} and to investigate the role of PKC in this LPC-induced change of I_{dK} using whole cell patch clamping technique in rabbit coronary smooth muscle cells.

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MATERIALS AND METHODS

Single coronary arterial smooth muscle cells were prepared using enzymatic digestion as described by Ahn *et al.* [2]. Whole cell experiments were performed at room temperature with an Axopatch 1-D patch clamp amplifier (Axon instruments, U.S.A.) using pipettes with a resistance from 2 to 3 MΩ. The pipette solution contained (mM): 100 K-gluconate, 30 KCl, 5 K_2ATP, 5.7 $MgSO_4$, 1 NaGTP, 10 BAPTA, 10 HEPES; pH was adjusted to 7.2 with Tris. The extracellular solution contained (mM): 140 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.5 $MnCl_2$, 10 glucose, 10 HEPES; pH was adjusted to 7.4 with Tris. To isolate I_{dK} from whole cell current, 10 mM BAPTA was added to the pipette solution to buffer intracellular Ca^{2+} and external Ca^{2+} was replaced with Mn^{2+}. Under these experimental conditions the contribution of Ca^{2+}-activated K^+ current ($I_{K\text{-}Ca}$) to total outward current was minimal, especially under our test potential ranges, as assessed by using iberiotoxin, a specific blocker of $I_{K\text{-}Ca}$ [1]. Low dose Gd^{3+} (10 μM) was also included in the bath solution to block the non-selective cation current (I_{ns}) which was activated by a prolonged exposure to LPC [20]. All chemicals used in this experiment were purchased from Sigma Co. (St. Louis, U.S.A.). 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 computer hard disk for further analysis.

RESULTS

In the first series of experiment, we determined the effect of LPC on I_{dK} in the rabbit coronary smooth muscle cells. The outward current activating with time and depolarizing command steps under our experimental conditions is mainly due to activation of I_{dK}, because contribution of $I_{K\text{-}Ca}$ and other Ca^{2+} activated current to outward current was minimized as described in method section. To monitor the time course of the effect on amplitude of I_{dK}, depolarizing steps to 0 mV were applied every 10 s from a holding potential of –80 mV. Figure 1A shows a typical result in which the effects of two concentration of LPC (1 and 3 μM) were tested on I_{dK} recorded in one myocyte. LPC inhibited I_{dK} in a concentration-dependent manner and the inhibition was almost completely reversible upon washout. Figure 1B shows the summarized results on different concentrations (0.01 to 10 μM) of LPC on amplitude of I_{dK}, and the concentration to produce a half-maximal inhibition was calculated to be 1.51 μM. This inhibitory effect of LPC on I_{dK} was dependent on the magnitude of test pulses. As shown in Figs. 1C and 2A, the magnitude of LPC-induced inhibition of I_{dK} was reached to a peak at around 0 mV of test potential and then gradually decreased with more positive potentials. To investigate whether this voltage dependency of LPC effect was due to the contamination of I_{ns} in the recording of I_{dK}, effect of LPC on I_{dK} was compared using two different holding potentials of –80 mV and 0 mV. Holding the membrane potentials to 0 mV more than 5 min inactivated the I_{dK}, and under this experimental condition, application of LPC had no apparent effect on the magnitude of current (Fig. 2B).

To investigate how LPC modulates I_{dK}, we examined the effects of LPC on the voltage dependence of steady-state activation of I_{dK} using a two pulse protocols. As shown in Fig. 3, externally applied 1 μM LPC significantly shifted the steady state activation curve to a positive direction. The change of half maximal activation potential ($V_{1/2}$) was –11.5 ± 0.7 mV and –0.7 ± 0.3 mV in control and external LPC, respectively (n=10). Internally applied low concentration of LPC (0.01 μM) showed a similar effect on the steady state activation curve ($V_{1/2}$=1.1 ± 0.3 mV, n=5).

We also tested the involvement of PKC on LPC-induced inhibition of I_{dK}. Figure 4A represents time course of the effect of LPC on amplitude of I_{dK} during control (absence of staurosporine) and after pretreatment with 100 nM stauro-

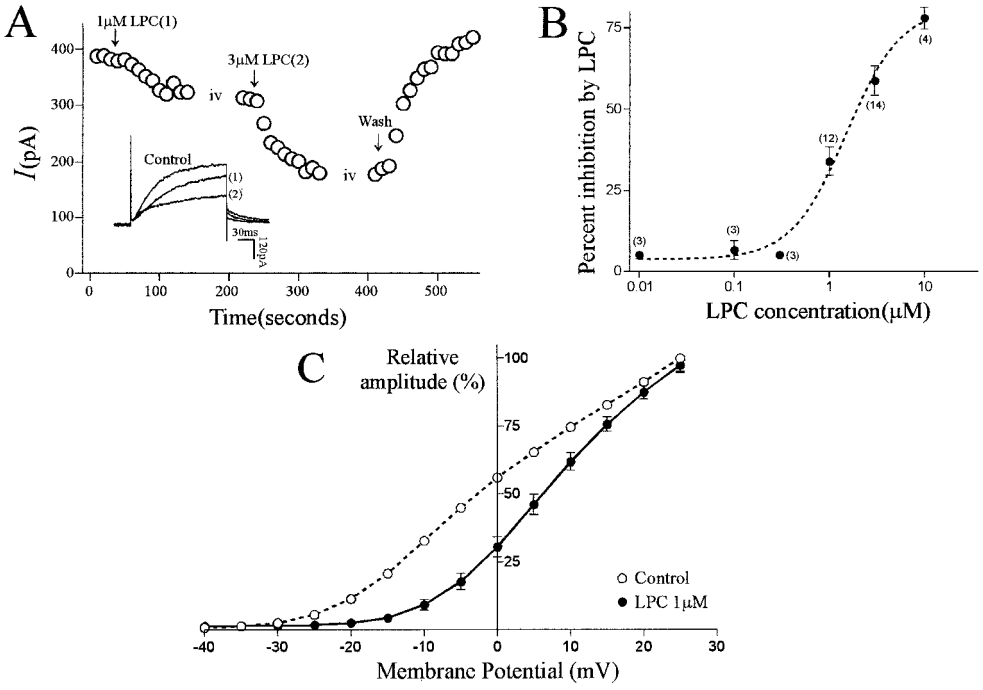

Fig. 1. (A) Plot of K^+ currents evoked by repetitive step depolarization from –80 mV to 0 mV versus time. Arrows indicate the time of LPC application in the bath solution. Inset shows current traces in control, 1 μM LPC (1), and 3 μM LPC (2). (B) Concentration response curve for LPC on I_{dK}. Each data point represents normalized mean from different number of cells as indicated in parenthesis. The points are fitted by 4 parameter logistic function described by following equation: $Y=I_{min} + (I_{max} – I_{min})/1+10^{\wedge} [(\log X_{1/2}\text{-}X)]k]$, where I_{min} and I_{max} represent normalized minimal and maximal currents, respectively, and X represents logarithmic concentration of LPC. $X_{1/2}$ and k represent concentration of LPC producing half-maximal inhibition of I_{dK} and slope factor, respectively. In our experiments, $X_{1/2}$ was 1.51 μM. (C) Summary of voltage dependence of I_{dK} for control and LPC. At each voltage step, peak values of current were determined, and averaged (n=14).

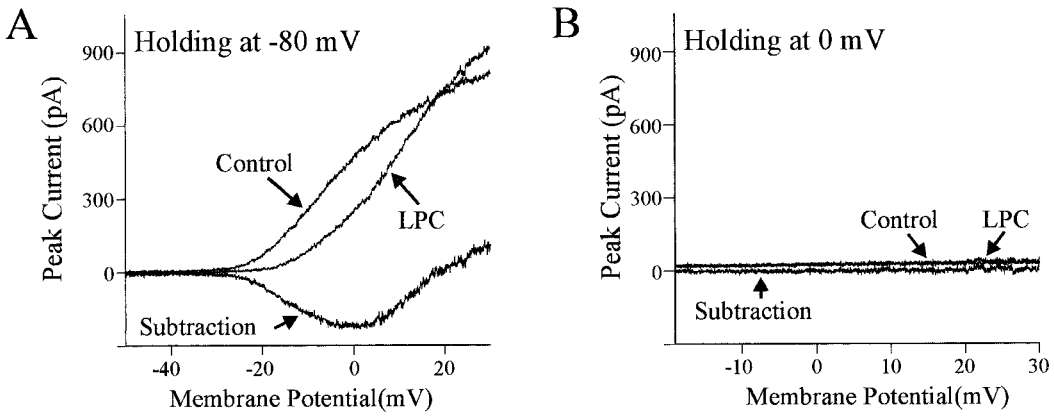

Fig. 2. Effect of depolarizing holding potential on LPC-induced change of I_{dK}. Current traces to voltage ramp pulses for 2 s from a holding potential of –80 mV (A) and 0 mV (B) in the absence (control) and in the presence of 1 μM LPC. Difference in the current traces is also shown (subtraction).

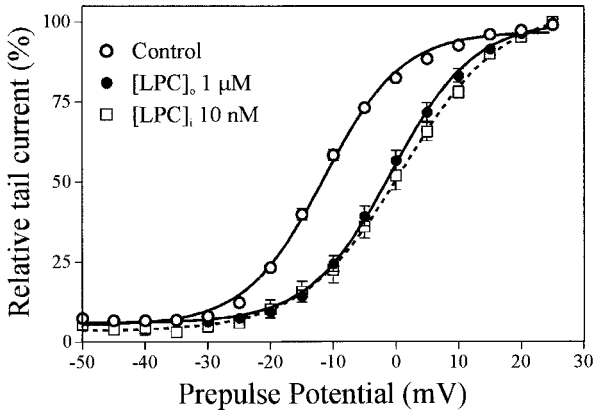

Fig. 3. Effect of LPC on the steady-state activation of I_{dK}. K^+ currents were elicited by pulsing from –50 mV to 25 mV in 5 mV increments from a holding potential of –80 mV and tail currents evoked upon repolarization to –50 mV were measured. Each data points were fitted using a following form of Boltzman equation; $Y = 1 + \exp[(V_{1/2} - V)/k]^{-1}$, where $V_{1/2}$ represents half maximal activation potential and k is a slope factor. $V_{1/2}$ was – 11.5 ± 0.7 mV, –0.7 ± 0.3 mV, and 1.1 ± 0.3 mV in control (open circle), external LPC (closed circle), and internal LPC (open square), respectively.

sporine in the same myocyte. The pretreatment with staurosporine significantly blocked the effect of LPC on the amplitude of I_{dK} in response to 0 mV test pulses. Figure 4B summarizes results of percent inhibition of I_{dK} by LPC during control and after pretreatment with staurosporine (n=12).

DISCUSSION

We report here a novel property of LPC to inhibit a delayed rectifier K^+ current in the rabbit coronary smooth muscle cells. The inhibition of I_{dK} by LPC may be involved in augmented vascular smooth muscle contractility or coronary spasm in atherosclerotic coronary artery, because the resting tone of coronary artery depends on the activity of I_{dK} [8, 9, 22]. In the previous electrophysiological studies, it has been reported that LPC affects several kinds of ionic channels, including Na^+ current, Ca^{2+} current, and I_{ns} in cardiac myocytes [11, 20, 28]. I_{ns} evoked by a prolonged exposure to LPC might have contaminated to the recording of I_{dK} and decreased the recorded amplitude of I_{dK}. However, this does not seem to be the case because 1) we used a 10 μM gadolinium-containing bath solution to block the I_{ns} during a whole experiment [15], and 2) change of holding potential to 0 mV, which inactivates the I_{dK} [5], prevented the development of LPC-induced change of outward current. 3) Furthermore, the inhibitory effect of LPC on I_{dK} was apparent at 0 mV test potential, at which little current is developed through the nonselective cation channel [15]. These results suggest that LPC-induced inhibition of outward current in our experimental condition were mainly due to inhibition of I_{dK}. It is still uncertain how voltage dependence of LPC-induced inhibition of I_{dK} occurred. However, the positive shift of steady-state activation by LPC might be responsible for the voltage dependence of LPC-induced inhibition of I_{dK}.

It has been reported that LPC can disturb the normal biological function of proteins by destabilizing sarcolemma. At relatively high concentrations (>10 μM), LPC may cause a change in phospholipid packing when it is inserted into the lipid bilayer and this alone can modulate the activity of membrane proteins [12, 21]. In the present experiment, the activity of I_{dK} was significantly inhibited by LPC at a concentration of 1 μM. Furthermore, intracellular application of a low concentration of LPC (0.01 μM) effectively inhibited I_{dK} and shifted its steady-state voltage dependence. These results suggest that LPC-induced inhibition may not be due to alteration of membrane properties.

The ability of LPC to activate PKC has been documented in many cell types, including vascular tissues [16, 25], and the activation of PKC results in inhibition of I_{dK} in vascular

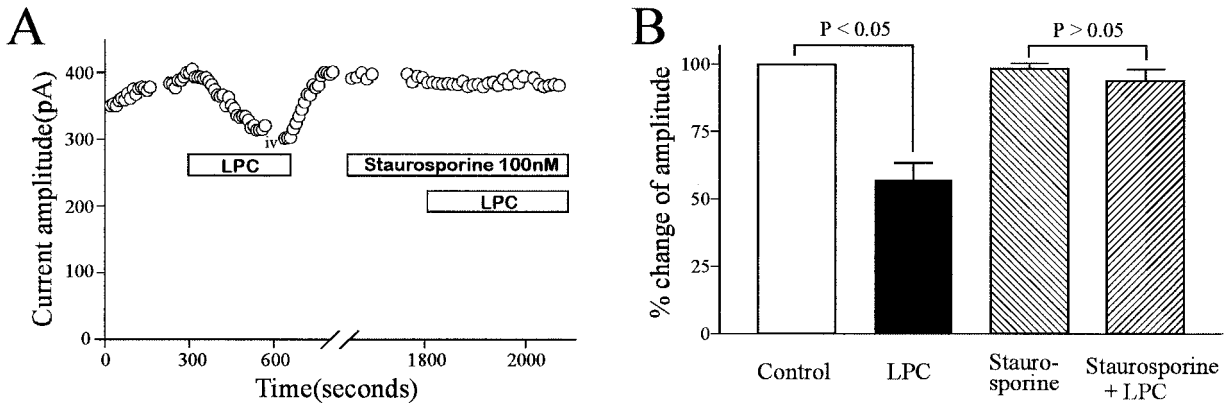

Fig. 4. The effect of pretreatment with staurosporine on the LPC-induced change of I_{dK}. (A) Plot of K^+ currents evoked by repetitive step depolarizations from –80 mV to 0 mV versus time. Horizontal boxes represent time during which the cell was exposed to 1 μM LPC and 100 nM staurosporine. (B) Summary of I_{dK} current amplitude recorded at 0 mV. 1 μM LPC reduced the I_{dK} to 56.6 ± 6.9% of control (n=14). Pretreatment with 100 nM staurosporine had a little effect on amplitude of I_{dK} (98.3 ± 6.9% of control, n=12), and LPC reduced I_{dK} insignificantly to 94 ± 4.1% of control after pretreatment with 100 nM staurosporine (n=12).

tissues. Thus, it is possible that LPC may inhibit I_{dK} via the activation of PKC. To test this possibility, we examined the effects of pretreatment with staurosporine, a potent PKC inhibitor [14], on LPC-induced inhibition of I_{dK}. As shown in Fig. 4, the LPC-induced inhibition of I_{dK} was effectively antagonized by pretreatment with 100 nM staurosporine, which suggests the involvement of PKC in the LPC-induced inhibition of I_{dK}.

We conclude that LPC inhibited the I_{dK} by changing its steady-state voltage dependency and through the activation of PKC, and the inhibition of I_{dK} may be responsible, at least in part, for the LPC-induced abnormal vascular reactivity in atherosclerotic artery.

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