Nonselective Cation Currents Regulate Membrane Potential of Rabbit Coronary Arterial Cell
Modulation by Lysophosphatidylcholine

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Background—The effects of lysophosphatidylcholine (LPC) on electrophysiological activities and intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) were investigated in coronary arterial smooth muscle cells (CASMCs).

Methods and Results—The patch clamp techniques and Ca\(^{2+}\) measurements were applied to cultured rabbit CASMCs. The membrane potential was −46.0±5.0 mV, and LPC depolarized it. Replacement of extracellular Na\(^{+}\) with NMDG\(^+\) hyperpolarized the membrane and antagonized the depolarizing effects of LPC. In Na\(^{+}\)-, K\(^{+}\)-, or Cs\(^{+}\)-containing solution, the voltage-independent background current with reversal potential (E\(_r\)) of approximately +0 mV was observed. Removal of Cl\(^−\) failed to affect it. When extracellular cations were replaced by NMDG\(^+\), E\(_r\) was shifted to negative potentials. La\(^3+\) and Gd\(^3+\) abolished the background current, but nicardipine and verapamil did not inhibit it. In Na\(^{+}\)-containing solution, LPC induced a voltage-independent current with E\(_r\) of approximately +0 mV concentration-dependently. Similar current was recorded in K\(^{+}\)- and Cs\(^{+}\)-containing solution. La\(^3+\) and Gd\(^3+\) inhibited LPC-induced current, but nicardipine and verapamil did not inhibit it. In cell-attached configurations, single-channel activities with single-channel conductance of ≈32pS were observed when patch pipettes were filled with LPC. LPC increased [Ca\(^{2+}\)]\(_i\), as the result of Ca\(^{2+}\) influx, and La\(^3+\) completely antagonized it.

Conclusions—These results suggest that (1) nonselective cation current (I\(_{NSC}\)) contributes to form membrane potentials of CASMCs and (2) LPC activates I\(_{NSC}\), resulting in an increase of [Ca\(^{2+}\)]\(_i\). Thus, LPC may affect CASMC tone under various pathophysiological conditions such as ischemia. (Circulation. 2002;106:3111-3119.)

Key Words: muscle, smooth muscle cells; ischemia; ion channels

Lysophosphatidylcholine (LPC), a major lysophospholipid in mammalian tissues, is formed from phosphatidylcholine by phospholipase A\(_2\). It accumulates in myocardial tissue during ischemia\(^1,2\) and has toxic effects on myocardium, which include electrophysiological disturbances such as a decrease in membrane potential, followed by the occurrence of arrhythmia during ischemia.\(^3\) LPC is also known as a vasoactive phospholipid that has biological effects on arterial walls, including coronary artery.\(^4\) The prominent mechanism for vascular effects of LPC is to impair endothelium-dependent relaxing factor–mediated vasodilatation, which inhibits nitric oxide production.\(^5\) In addition, LPC is reported to increase intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) in vascular smooth muscle cells (VSMCs) and increase vascular tone.\(^5,6\) However, the underlying mechanisms for LPC effects on coronary arterial smooth muscle cells (CASMCs) have not been investigated.

Smooth muscle tone of arteries is an important determinant of coronary circulation and vascular resistance. Coronary arterial tone is regulated by various physiological and pathological changes such as O\(_2\) tension and an increase of H\(^+\), resulting in autoregulation of local blood flow. Membrane potential plays additional crucial roles in regulating arterial tone and hence arterial diameter; depolarization activates voltage-gated Ca\(^{2+}\) channels, subsequently increasing Ca\(^{2+}\) entry, which regulates muscle contractility and leads to vasoconstriction. The voltage-dependent K\(^+\) channel (K\(_V\)) plays essential roles in forming membrane potential in VSMCs.\(^7\) ATP-sensitive K\(^+\) channels also regulate coronary tone under ischemia.\(^8\) However, the membrane potential of VSMCs including CASMCs is much less than K\(^+\) equilibrium potential,\(^9,10\) proposing that additional membrane channels such as Cl\(^−\) may contribute to form it.\(^11\) Alternatively, the background nonselective cation current (I\(_{NSC}\)), which forms membrane potential, is identified in cardiac myocytes\(^12-14\) and pulmonary smooth muscle cells,\(^15\) but it has not been reported in CASMCs.
Therefore, we have investigated the effects of LPC on electrophysiological activities and \([\text{Ca}^{2+}]_{i}\) mobilization in rabbit CASMCs. We found that the background \(I_{\text{NSC}}\) contributes to form membrane potential of CASMCs, and LPC further induces \(I_{\text{NSC}}\), followed by an increase in \([\text{Ca}^{2+}]_{i}\).

**Methods**

**Cell Preparation**

Rabbit CASMCs were grown from explants of adult male Japanese White rabbit (2.5 to 3.0 kg, \(n=15\)) coronary arteries by explants methods. They exhibited typical "hill and valley" growth patterns and exhibited positive fluorescence with antibodies against \(\alpha\)-smooth muscle actin but no fluorescence with antibodies against factor VIII antigen. They were grown in Dulbecco’s modified Eagle’s medium (DMEM, Sigma) supplemented with 10% fetal bovine serum (FBS, Sigma), 100U/mL penicillin, 100 \(\mu\)g/mL gentamicin, and 0.25 \(\mu\)g/mL amphotericin B (GIBCO BRL) in a humidified atmosphere of 5% CO\(_2\) and 95% air at 37°C. When cells became confluent, they were subcultured in the same medium with 0.5% trypsin in 0.02% EDTA. Confluent cells at passages 3 to 6 were used for the experiments.

**Solutions and Drugs**

The control Tyrode solution contained (in mmol/L) NaCl 136.5, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 0.53, glucose 5.5, and N-2-hydroxyethylpiperazine-N'-ethane sulfonic acid (HEPES)-NaOH buffer 5.5 (pH 7.4). The \([\text{Ca}^{2+}]_{i}\)-free Tyrode solution contained EGTA (0.5 mmol/L). The high K\(^+\) bathing solution contained KCl 140, CaCl\(_2\) 1.8, MgCl\(_2\) 0.53, glucose 5.5, and HEPES-NaOH buffer 5.5 (pH 7.4). In NMDG\(^+\) solutions, Na\(^+\) was replaced with equimolar N-methyl-D-glucamine\(^+\) (NMDG\(^+\)). In low Cl\(^-\) solutions, Cl\(^-\) was replaced with aspartate, and external concentration of Cl\(^-\) was reduced to 10 mmol/L. The K\(^+\) internal solution in the patch pipette contained KCl 140, EGTA 5, MgCl\(_2\) 2, Na\_ATP 3, GTP 0.1, and HEPES-KOH buffer 5 (pH 7.2). The Cs\(^+\) internal solution contained CsCl 140, EGTA 5, MgCl\(_2\) 2, Na\_ATP 3, GTP 0.1, and HEPES-CsOH buffer 5 (pH 7.4). In cell-attached experiments, the Cs\(^+\)-pipette solution contained CsCl 140, CaCl\(_2\) 1.8, MgCl\(_2\) 0.53, HEPES 5 (pH 7.4).

Palmitoyl-\(\alpha\)-lysophosphatidylcholine (LPC), lanthanum (La\(^{3+}\)), gadolinium (Gd\(^{3+}\)), verapamil, nicardipine, 4-aminopyridine (4-AP), and tetraethylammonium (TEA) were purchased from Sigma. Fura-2 acetoxymethyl ester (fura-2/AM, molecular probes) was obtained from Dojin Chemicals.

**Measurements of \([\text{Ca}^{2+}]_{i}\)**

Primarily cultured CASMCs on a glass culture dish were used. At confluence, CASMCs were loaded with 4 \(\mu\)mol/L fura-2/AM in DMEM for 60 minutes at 37°C. After loading, cells were washed 3 times, and the medium containing fura-2/AM was removed. The glass culture dish was mounted on an inverted microscope (Diaphot TMD, Nikon). Measurement of \([\text{Ca}^{2+}]_{i}\), was performed with a SPEX dual-wavelength fluorolog spectrometer (SPEX Industries, Inc). Excitation wavelengths of 340 and 380 nm and an emission...
Membrane potentials and effects of LPC

Membrane potentials and currents were recorded by using whole-cell clamp techniques. The patch electrode had the tip resistance of 3 MΩ and series resistance was compensated. The data were reproduced, low-pass filtered at 1 kHz (−3dB) with a Bessel filter (FV-625, NF,48dB/octave slope attenuation), and sampled at 5 kHz. All data were acquired and analyzed on a Power Macintosh 7100/80 A/D board for digitization. From videotape either to a chart recorder (2400S, Gould Inc), or to Igor PRO (Wave Metrics). Single-channel currents were replayed by using the PULSE PULSEFIT software (HEKA Electronic) and the A/D broad for digitization.

Statistical data are expressed as mean±SD; n represents the number of cells tested. A Student’s t test was used for statistical analysis, and a value of P<0.05 was considered significant.

Results

Membrane Potentials and Effects of LPC

With K⁺ internal solution, the membrane potential was −46.0±5.0 mV (n=30). 4-AP (Figure 1Aa,10 mmol/L) depolarized the membrane potential from −51.6±4 mV to −34±6 mV (Figure 1Aa, n=4, P<0.05). TEA (10 mmol/L, n=4, Figure 1Ab) also depolarized it. Figure 1B illustrates the effects of replacement of extracellular Na⁺ with membrane-impermeable NMDG⁺ on membrane potentials. It hyperpolarized the membrane potential from −43±3 mV to −69±6 mV (Figure 1Ba, 1Bb, n=5, P<0.05) reversibly. However, the reduction of [Cl⁻], failed to hyperpolarize it. The depolarizing effects of LPC were similarly observed at any cells of the passage numbers examined.

Background Current in Rabbit CASMCs

Figure 2 shows the effects of replacement of extracellular Na⁺ by NMDG⁺ on membrane currents. The patch pipette contained Cs⁺ internal solution with 5 mmol/L EGTA and 3 mmol/L ATP to block K⁺ currents and Ca²⁺-dependent currents. The cell was held at −40 mV and the voltage pulses were applied from −90 to +40 mV. The background current without any time-dependent activation and inactivation was observed. The current-voltage relation measured at steady state is illustrated in Figure 2B. It is almost linear and crosses zero at −10 mV in Na⁺-containing solution. Replacement of Na⁺ by NMDG⁺ reduced the inward current (Figure 2A) and shifted the reversal potential (E_r) toward more negative potentials. E_r is shifted from 0.05 to 0.05). Replace-

Effects of LPC on Membrane Currents

Figure 4 illustrates the effects of LPC on membrane currents. The patch pipette contained 140 mmol/L Cs⁺ internal solu-

wavelength of 505 nm were used. In assessment of [Ca²⁺], fluorescence intensity ratio of F_{340}/F_{380} was used as an indicator of [Ca²⁺].

Recording Technique and Data Analysis

Membrane potential and currents were recorded by using whole-cell clamp techniques. The patch electrode had the tip resistance of 3 MΩ, and series resistance was compensated. The data were reproduced, low-pass filtered at 1 kHz (−3dB) with a Bessel filter (FV-625, NF,48dB/octave slope attenuation), and sampled at 5 kHz. All data were acquired and analyzed on a Power Macintosh 7100/80 by using the PULSE+PULSEFIT software (HEKA Electronic) and Igor PRO (Wave Metrics). Single-channel currents were replayed from videotape either to a chart recorder (2400S, Gould Inc), or to the A/D board for digitization.

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Effects of LPC on Membrane Currents

Figure 4 illustrates the effects of LPC on membrane currents. The patch pipette contained 140 mmol/L Cs⁺ internal solu-
The cell was held at -60 mV and ramp pulses from -60 to +60 mV (200 ms duration) were applied. In Figure 4A, LPC (10 μmol/L) gradually increased the inward current at a holding potential within 1 to 2 minutes and La3+ (1 mmol/L) completely inhibited it. Figure 4Ab illustrates the current-voltage relations in control, in the presence of LPC, and LPC plus La3+. The current-voltage relation of the LPC-induced current was obtained by subtracting control current from the

**Figure 3.** Background La3+-sensitive currents. In A, current traces are shown in control, in the presence of La3+ (1 mmol/L), and after the washout. B, Current-voltage relations measured at steady state in control and in the presence of La3+. C and D, Effects of Ca2+-channel blockers on background current. Current traces are shown in control, in the presence of nicardipine (10 μmol/L, C), La3+ (1 mmol/L, C) and verapamil (50 μmol/L, D).

**Figure 4.** Effects of LPC on membrane currents. A (a, b), LPC (10 μmol/L) gradually increased the holding current into the inward direction and La3+ (1 mmol/L) completely inhibited it (Aa). Current-voltage relations are shown in control, in the presence of LPC, and after the addition of La3+ (Ab). B, Reversibility of LPC-induced current. Current-voltage relations are shown in control, in the presence of LPC, and after washout, and in the presence of La3+. C, Current-voltage relations in the presence of LPC (1 to 50 μmol/L). D, Current amplitude induced by 50 μmol/L LPC at -60 mV was defined as 1.0; relative current amplitude induced by LPC is plotted against each concentration. Mean±SD value obtained from 4 different cells is shown.
current in the presence of LPC. It was linear with $E_i$ of approximately $+0$ mV. The effects of LPC (10 $\mu$mol/L) were partly reversible as shown in Figure 4B. Similar effects of LPC were observed at any cells of the passage number examined.

Figure 4, C and D show the effects of LPC (1 to 50 $\mu$mol/L) on current-voltage relations obtained by ramp pulses and dose-dependent effects of LPC. The current amplitude induced by 50 $\mu$mol/L LPC at $-60$ mV was defined as 1.0, and the relative current amplitude induced by LPC is plotted against each concentration. LPC concentration-dependently induced the current.

Figure 5 illustrates the effects of La$^{3+}$, Gd$^{3+}$, and nicardipine on LPC-induced current. The voltage pulses were applied from $-90$ to $+40$ mV. The cells were held at $+0$ mV (Figure 5, A and B) and at $-40$ mV(Figure 5C). The current-voltage relations measured at steady state are shown in Figure 5, Ab through Bb. La$^{3+}$ (1 mmol/L,Figure 5A) and Gd$^{3+}$ (1 mmol/L,Figure 5B) abolished the LPC-induced current, whereas nicardipine (10 $\mu$mol/L, Figure 5C) and verapamil (50 $\mu$mol/L) only partly inhibited it at $-90$ mV by $-20 \pm 5\%$ and $-15 \pm 5\%$ (n=4), respectively.

Comparative Properties of the Background and LPC-Induced Current

Figure 6 compared ionic properties of the background current with those of LPC-induced current by using ramp pulses. The background current was obtained by subtracting the current in the presence of La$^{3+}$ from the control current. The LPC-induced current was obtained by subtracting the control current from the current in the presence of LPC (10 $\mu$mol/L).

Reduction of $[Cl^-]_o$ caused no significant change of $E_i$ on the background and LPC-induced current. $E_i$ of background current was $-6 \pm 5$ and $-5 \pm 5$ mV (n=4, $P>0.05$) in control and low Cl$^-$ solutions, respectively (Figure 6Aa). $E_i$ of LPC-induced current was $-2 \pm 3$ and $-1 \pm 3$ mV (n=4, $P>0.05$) in control and low Cl$^-$ solution, respectively (Figure 6Ab). The replacement of Na$^+$ by NMDG$^+$ shifted $E_i$ of the background current from $-6 \pm 5$ to $-31 \pm 10$ mV (Figure 6Ba, n=4, $P<0.05$) and shifted $E_i$ of the LPC-induced current from $-3 \pm 3$ to $-16 \pm 5$ mV (Figure 6Bb, n=4, $P<0.05$).

To determine cationic selectivity of the background and LPC-induced current, Na$^+$ was replaced by K$^+$ and Cs$^+$ (Figure 6C). The background current showed linear current-voltage relations (Figure 6Ca). $E_i$ was $+5 \pm 3$ mV (n=4) in 140 mmol/L K$^+$ solution, $+1 \pm 5$ mV (n=4) in 140 mmol/L Cs$^+$ solution, and $-8 \pm 6$ mV (n=4) in 140 mmol/L Na$^+$ solution. The values of the slope conductance were $3.3 \pm 0.4$ nS (n=4), $2.9 \pm 0.5$ nS (n=4), and $2.2 \pm 0.5$ nS (n=4) for K$^+$, Cs$^+$, and Na$^+$, respectively. The permeability ratios were calculated according to the Goldman-Hodgkin-Katz equation $P_{X^-}/P_{Cl^-}[X^-]/[Cl^-]$, where $X^-_i$ is Na$^+$, K$^+$, or NMDG$^+$ and F, R, T, Z have their usual meanings. $[X^-]_o$, a concentration of extracellular $X^-$ ion, is 140 mmol/L and $[Cl^-]_o$, a concentration of internal Cs$^+$ ion, is 140 mmol/L. The value of $P_{Na}/P_{Cl^-}$, $P_{K^+}/P_{Cl^-}$, and $P_{NMDG}/P_{Cl^-}$ was 1.21, 0.73, and 0.30, respectively. The LPC-induced current also showed linear current-voltage relations (Figure 6Cb). $E_i$ was $+1 \pm 4$ mV (n=4), $+0 \pm 3$ mV (n=4), and $-1 \pm 4$ mV (n=4) for K$^+$, Cs$^+$, and Na$^+$ bathing solution, respectively. The values of the slope conductance were $40 \pm 8$ nS (n=4), $35 \pm 2$ nS (n=4), $30 \pm 2$ nS (n=4).
and 33 ± 6 nS (n = 4) for K⁺, Cs⁺, and Na⁺, respectively. The values of P₀/P₉, P₉/P₀, and P₉/P₉₉₉ were 1.04, 0.96, and 0.54, respectively.

LPC-Induced Channels in Rabbit CASMCs

Figure 7 shows the results of single-channel recordings, using cell-attached methods. The patch pipette was filled with 140 mmol/L CsCl-pipette solution. The bath was perfused with high K⁺ bathing solution to settle membrane potential to approximately 0 mV. Under the conditions with LPC (10 μmol/L) in the patch pipette, marked channel activities were observed at a holding potential of −100 mV (lower trace), as compared with the control (upper trace). Figure 7B shows the amplitude histogram of LPC-induced channel, which was fitted by a sum of gaussian distribution using the least-squares method. The single channel amplitude measured at −100 mV was −3.4 pA. The current-voltage relations of LPC-induced channel are illustrated in Figure 7, C and D. The relations show linearity and slope conductance of 34 pS in this cell, and E₀ was approximately +0 mV. The mean slope conductance was 32.5 ± 5 pS (n = 4).

Effects of LPC on [Ca²⁺]

Figure 8 shows the effects of LPC on [Ca²⁺]. In Ca²⁺-free solution, LPC (50 μmol/L) did not affect [Ca²⁺], significantly (Figure 8A). The addition of Ca²⁺ induced a sustained rise of [Ca²⁺], which was abolished by the removal of Ca²⁺ from the extracellular solution. The effects of LPC on [Ca²⁺] were
partly reversible (Figure 8B). La\(^{3+}\) (0.5 mmol/L, Figure 8C) blocked LPC-induced, sustained \([\text{Ca}^{2+}]_i\) rise. However, verapamil (50 \(\mu\)mol/L, Figure 8D) and nicardipine (10 \(\mu\)mol/L, Figure 8E) partly inhibited it by 30\% (n=3) and 19\% (n=3), respectively. LPC (1 to 50 \(\mu\)mol/L) concentration-dependently increased \([\text{Ca}^{2+}]_i\) (Figure 8F).

Similar results were obtained from 4 different cells. To investigate whether LPC-induced \([\text{Ca}^{2+}]_i\) rise is related to membrane potential, the bathing solution was changed from 5.4 to 140 mmol/L K\(^{+}\) solution to settle membrane potential to approximately −56 mV. LPC-induced \([\text{Ca}^{2+}]_i\) rise was decreased (Figure 8F).

**Discussion**

Voltage-dependent K\(^{+}\) current (I\(_{\text{K}}\)) regulates vascular tone and forms membrane potential in VSMCs, including CASMCs. In our experimental conditions, 4-AP and TEA, K\(^{+}\) channel blockers that produce coronary spasm, inhibited I\(_{\text{K}}\) and resulted in depolarizing the membrane. The membrane potential has been reported to be −56±2 mV and to 40.4±4.9 mV in canine and porcine CASMCs. It was −46.0±5.0 mV in cultured CASMCs. These values were less than K\(^{+}\) equilibrium potential of approximately −80 mV, suggesting that additional membrane channels contribute to form membrane potential. The contribution of Cl\(^{−}\) currents has been reported in VSMCs. In our study, however, reduction of \([\text{Cl}^{−}]_e\) failed to affect the membrane potential, whereas replacement of Na\(^{+}\) by NMDG\(^{−}\) markedly hyperpolarized it. These results suggest that the contribution of Cl\(^{−}\) current is minimal, and the background I\(_{\text{NSC}}\) contributes to form membrane potential in rabbit CASMCs.

The role of I\(_{\text{NSC}}\) has been investigated in pacemaking cells of hearts and cardiac myocytes, where the background Na\(^{+}\) current is important to generate action potentials by raising resting membrane potential to the threshold for activation of Ca\(^{2+}\) current. The background I\(_{\text{NSC}}\) of rabbit CASMCs had the same tendencies of linear current-voltage relations and permeability sequences (K\(^{+}\)>Cs\(^{+}\)>Na\(^{+}\)) as the properties of the background I\(_{\text{NSC}}\) described previously and may play important roles in forming membrane potentials as reported in pulmonary VSMCs. Depolarization through I\(_{\text{NSC}}\) may open voltage-operated Ca\(^{2+}\) channels and subsequently increase \([\text{Ca}^{2+}]_i\), which regulates muscle contractility and leads to vasoconstriction.

The activation of the background I\(_{\text{NSC}}\) was not mediated by \([\text{Ca}^{2+}]_i\) rise because it was still observed under the conditions with high EGTA in the patch pipette. Similar Ca\(^{2+}\)-independent properties were observed in the background I\(_{\text{NSC}}\) of cardiac myocytes and pulmonary VSMCs, though they were different from that reported in other cells.
I_{\text{NSC}} has been reported to be activated by membrane stretch.22 Here, we provided evidence that LPC was a potent activator of I_{\text{NSC}} in rabbit CASMCs, which was consistent with previous studies of whole-cell clamp conditions showing that LPC induced I_{\text{NSC}} in guinea pig ventricular myocytes and dog renal VSMCs.23,24 The effects of LPC on I_{\text{NSC}} were not dependent on the passage number, and similar effects were observed in cultured cells of any passage number and freshly isolated CASMCs (data not shown). In our study, the ionic permeability ratio of K⁺, Cs⁺, Na⁺, and NMDG⁺ of the LPC-induced current was 1.04, 1.00, 0.96, and 0.54, which showed the same tendencies as the previous study using renal VSMCs.24 However, it does not seem to be the same I_{\text{NSC}} reported in cardiac myocytes by Magishi et al.,23 because NMDG⁺ passed the channel similarly as Na⁺ and K⁺, and Gd³⁺ did not inhibit it. Thus, it is likely that there are several types of LPC-induced I_{\text{NSC}}, depending on cell types investigated. Actually, the present study provided direct evidence showing that LPC activated a specific type of channels. However, further studies with single-channel analysis are needed to clarify the characteristic and molecular mechanisms underlying the activation of I_{\text{NSC}} by LPC.

Corr et al.31 reported that LPC was increased during ischemia, and the concentration of LPC corresponded to 990 μmol/L. In addition, atherosclerotic arteries have been reported to be chronically exposed to high concentrations of LPC as compared with normal arteries.25,26 Thus, LPC may accumulate under pathophysiological conditions such as ischemia and atherosclerosis. Our results indicate that LPC at concentrations of 1 to 50 μmol/L induced I_{\text{NSC}}, depolarized the membrane, and resulted in [Ca²⁺]₀ rise. Thus, the effects of LPC observed in this study may play significant roles in pathophysiological conditions such as ischemia.

LPC increased [Ca²⁺]₀ in rabbit CASMCs, which was consistent with the previous studies in ASMCs or cardiac myocytes.27,28 LPC may increase [Ca²⁺]₀ through an increase of Ca²⁺ entry or release from Ca²⁺ storage sites. In the absence of extracellular Ca²⁺, it failed to increase [Ca²⁺]₀, suggesting that LPC increased Ca²⁺ influx as reported in rat ASMCs.29,30 Several mechanisms by which LPC might influence [Ca²⁺]₀ have been proposed. The [Ca²⁺]₀ increase induced by LPC may relate to detergent or toxic effects of this amphiphile.29 However, we used low concentrations of LPC (<50 μmol/L), and cell viability measured by trypan blue exclusion was not changed in between control and LPC-treated cells. Moreover, La³⁺ and Gd³⁺ completely abolished it, suggesting that detergent action of LPC is not likely. LPC depolarized the membrane, proposing that LPC increased [Ca²⁺]₀ indirectly through voltage-dependent L-type Ca²⁺ channels. Actually, nicardipine and verapamil partly inhibited LPC-induced [Ca²⁺]₀ rise. However, La³⁺ and Gd³⁺ abolished it, suggesting that LPC increased [Ca²⁺]₀ mainly by activating Ca²⁺ entry pathways other than L-type Ca²⁺ channels. In our study, including single-channel analysis, LPC activated I_{\text{NSC}} with a large amplitude of approximately ~1 nA at around -50 mV near resting membrane potential. La³⁺ and Gd³⁺ inhibited both the activation of I_{\text{NSC}} by LPC and LPC-induced [Ca²⁺]₀ rise. In addition, when cells were bathed with high K⁺ solution to settle the membrane potential to approximately +0 mV, LPC-induced [Ca²⁺]₀ rise was decreased. These observations also support that LPC increased [Ca²⁺]₀, through activation of I_{\text{NSC}}.

LPC has been reported to increase cGMP-dependent vaso- relaxation-sensitive [Ca²⁺]₀ in ASMCs.29 However, verapamil (50 μmol/L) and nicardipine (10 μmol/L) only partly inhibited LPC-induced [Ca²⁺]₀ rise, proposing that vaso- relaxation-sensitive Ca²⁺ influx pathways do not largely contribute to [Ca²⁺]₀ rise induced by LPC. The discrepancy among these results may depend on different concentrations of LPC or different cell types. LPC enhances cell proliferation and migration of CASMCs.29,30 Since [Ca²⁺]₀ is known to be related to cell proliferation and migration, activation of I_{\text{NSC}} by LPC may play essential roles in these events as well as CASMC tone.

In conclusion, I_{\text{NSC}} plays important roles in forming membrane potentials of CASMCs and LPC induces I_{\text{NSC}} and then depolarizes the membrane, resulting in an increase of [Ca²⁺]₀. Thus, LPC may affect CASMCs tone under various pathophysiological conditions such as ischemia.

References
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