Plasmalogen-Derived Lysolipid Induces a Depolarizing Cation Current in Rabbit Ventricular Myocytes

Ray A. Caldwell, Clive M. Baumgarten

Abstract—Plasmalogen rather than diacyl phospholipids are the preferred substrate for the cardiac phospholipase A2 (PLA2) isoform activated during ischemia. The diacyl metabolite, lyso phosphatidylcholine, is arrhythmogenic, but the effects of the plasmalogen metabolite, lysoplasmaldehyde (LPLC), are essentially unknown. We found that 2.5 and 5 μmol/L LPLC induced spontaneous contractions of intact isolated rabbit ventricular myocytes (median times, 27.4 and 16.4 minutes, respectively) significantly faster than lyso phosphatidylcholine (>60 and 37.8 minutes, respectively). Whole-cell recordings revealed that LPLC depolarized the resting membrane potential from –83.5±0.2 to –21.5±1.0 mV. Depolarization was due to a guanidinium toxin–insensitive Na+ influx. The LPLC-induced current reversed at –18.5±0.9 mV and was shifted 26.7±4.2 mV negative by a 10-fold reduction of bath Na+ (Na+/K+ permeability ratio, ~ 0.12±0.06). In contrast, block of Ca2+ channels with Cd2+ and reducing bath Cl− failed to affect the current. The actions of LPLC were opposed by lanthanides. Gd3+ and La3+ were equally effective inhibitors of the LPLC-induced current and equally delayed the onset of spontaneous contractions. However, the characteristics of lanthanide block imply that Gd3+-sensitive, poorly selective, stretch-activated channels were not involved. Instead, the data are consistent with the view that lanthanides increase phospholipid ordering and may thereby oppose membrane perturbations caused by LPLC. Plasmalogens constitute a significant fraction of cardiac sarcolemmal choline phospholipids. In light of their subclass-specific catabolism by phospholipase A2 and the present results, it is suggested that LPLC accumulation may contribute to ventricular dysrhythmias during ischemia. (Circ Res. 1998;83:533-540.)

Key Words: plasmalogen • lyso phosphatidylcholine • ischemia • lanthanide • lysoplasmaldehyde

Alternated membrane phospholipid metabolism is one of the earliest manifestations of myocardial ischemia. Phospholipase A2 (PLA2) is activated,1,2 and lyso phosphatidylcholine (LPC), a product of diacyl phospholipid catabolism, increases in tissue4–6 and coronary effluents.7–9 In addition to lysophosphatidylcholine, a product of diacyl phospholipid catabolism, lyso phosphatidylcholine, is arrhythmogenic, but the effects of the plasmalogen metabolite, lysoplasmaldehyde (LPLC), are essentially unknown. We found that 2.5 and 5 μmol/L LPLC induced spontaneous contractions of intact isolated rabbit ventricular myocytes (median times, 27.4 and 16.4 minutes, respectively) significantly faster than lyso phosphatidylcholine (>60 and 37.8 minutes, respectively). Whole-cell recordings revealed that LPLC depolarized the resting membrane potential from –83.5±0.2 to –21.5±1.0 mV. Depolarization was due to a guanidinium toxin–insensitive Na+ influx. The LPLC-induced current reversed at –18.5±0.9 mV and was shifted 26.7±4.2 mV negative by a 10-fold reduction of bath Na+ (Na+/K+ permeability ratio, ~ 0.12±0.06). In contrast, block of Ca2+ channels with Cd2+ and reducing bath Cl− failed to affect the current. The actions of LPLC were opposed by lanthanides. Gd3+ and La3+ were equally effective inhibitors of the LPLC-induced current and equally delayed the onset of spontaneous contractions. However, the characteristics of lanthanide block imply that Gd3+-sensitive, poorly selective, stretch-activated channels were not involved. Instead, the data are consistent with the view that lanthanides increase phospholipid ordering and may thereby oppose membrane perturbations caused by LPLC. Plasmalogens constitute a significant fraction of cardiac sarcolemmal choline phospholipids. In light of their subclass-specific catabolism by phospholipase A2 and the present results, it is suggested that LPLC accumulation may contribute to ventricular dysrhythmias during ischemia. (Circ Res. 1998;83:533-540.)

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Materials and Methods

Cardiac Myocyte Isolation

Freshly isolated ventricular myocytes were obtained from New Zealand White rabbits (2.0 to 3.0 kg) by a collagenase-protease digestion procedure. Briefly, spontaneously beating hearts were perfused via the aorta with an oxygenated modified Tyrode’s solution containing (mmol/L) NaCl 140, KCl 5, CaCl2 1.8, MgCl2 1, and HEPES 5, titrated to pH 7.4 with 1 mol/L NaOH. Subsequently, the perfusate was switched to a Tyrode’s solution containing 1 mg/mL collagenase (type II, Worthington Biochemical Corp), 0.1 mg/mL pronase E (type XIV, Sigma Chemical Co), and 80 μmol/L CaCl2. After collection, myocytes were stored in a modified Kraft-Bühe solution containing (mmol/L) NaCl 140, KCl 5, CaCl2 1.8, MgCl2 1, and HEPES 5, titrated to pH 7.4 with 1 mol/L NaOH. The pipette (internal) solution contained (mmol/L) potassium aspartate 140, CaCl2 0.062, K2EGTA 5, MgCl2 5, Na-ATP 5, Na-GTP 0.4, and HEPES 5, titrated to pH 7.1 with 1 mol/L KOH. For experiments in low bath Na+, 126 mmol/L NaCl was replaced with equimolar N-methyl-d-glucamine (NMDG) chloride. For experiments in low bath Cl-, 135 mmol/L NaCl was replaced with equimolar sodium isethionate. Ca2+-free bath solution was made by omitting CaCl2 and adding 1.8 mmol/L Na2EGTA. Bath solution was made hypotonic by adding 150 mmol/L mannitol (relative osmolarity, 1.5). Tetrodotoxin (TTX), saxitoxin (STX), and the plasmalogen content provided by the supplier. Synthetic LPC (Sigma), prepared as a 10 mmol/L stock solution and kept frozen, was added to the bath solution and sonicated for 2 minutes. Concentrations indicated for LPLC are based on nominal formula weight calculated from the n-1 analysis and the plasmalogen content provided by the supplier. Synthetic LPC (Sigma) was prepared as a 10 mmol/L stock solution and kept frozen, was added to the bath solution and sonicated for 2 minutes. All experiments were conducted at room temperature (≈23°C).

Electrophysiology

For whole-cell recordings, pipettes were fabricated from 7740 or 7052 glass, coated with Sylgard 184 (Dow Corning), and fire-polished. The pipette resistance ranged from 1 to 3 MΩ. An Ag/AgCl pellet connected to the bath via a 0.15 or 3 mol/L KCl agar bridge served as the ground electrode. The diffusion potential between pipette and bath solution was 13.1±0.2 mV, and all voltages were corrected by this amount.

A List EP-7 amplifier (List-Medical) was used to patch-clamp myocytes. Voltage- and current-clamp protocols and data acquisition were controlled by custom programs written in ASYST (Keithly). Ionic currents were elicited by 200-ms voltage pulses from a holding potential of −83 mV to potentials ranging from −113 to +37 mV. The current output was filtered at 2 kHz (−3 dB, 8-pole Bessel, Frequency Devices) and digitized at 10 kHz (12 bits). The quasi-steady-state current, taken as the average of the last 16.7 ms of each voltage step, is plotted in the current-voltage (I-V) relationships. Series resistance averaged 7.7±2.5 MΩ (n=26), and ≥40% was compensated electronically.

The resting membrane and action potentials were recorded under current-clamp conditions. Membrane voltage was filtered at 0.5 or 1 kHz, digitized at 1 or 3 kHz, and reproduced on a strip-chart recorder.

Data Analysis and Statistics

Current- and voltage-clamp data were analyzed with custom programs written in ASYST and plotted using SigmaPlot (SPSS). Except as noted, results are reported as mean±SEM, and n corresponds to the number of cells. For spontaneous activity experiments, multiple comparisons of median response times for each lysolipid and experimental protocol were made with Dunn’s method after a 1-way ANOVA on ranks. Comparisons of patch-clamp data were made by the Student-Newman-Keuls method after ANOVA or by a Student t test where indicated. Statistics were computed using SigmaStat 2.0 (SPSS), and P<0.05 was considered significant.

Results

Evaluation of the Arrhythmogenic Potential of LPLC

On exposure to exogenous LPC, myocytes contract spontaneously and die. To determine whether LPLC possesses potentially arrhythmogenic properties similar to those described for LPC, the effects of LPLC and LPC on ventricular myocytes were compared. Figure 1 is a survival plot of the fraction of myocytes remaining quiescent over time after exposure to different concentrations of LPLC or LPC in physiological bath solution. In this experiment, myocytes were intact; ie, they were not dialyzed by a patch pipette. Induction of spontaneous contractions depended on both the amount and type of lysolipid used. At the lowest concentrations, 2.5 and 5 μmol/L, LPLC induced spontaneous contractions significantly faster than LPC. Spontaneous activity developed with median times of 27.4 minutes (n=36) and 16.4 minutes (n=64) for 2.5 and 5 μmol/L LPC, respectively, compared with >60 minutes (n=29) and 37.8 minutes (n=48) for LPC. However, there were no significant differences in the median times to development of spontaneous activity with 10 μmol/L lysolipids (LPLC, 8.2 minutes, n=62; LPC, 8.7 minutes, n=15). The end point was 60...
Electrophysiological Effects of LPLC on Ventricular Myocytes

To investigate the mechanism underlying the spontaneous contractions observed after exposure to LPLC, Em was recorded under current-clamp conditions. Figure 2 shows results from a representative myocyte. Under control conditions, Em was stable and well-polarized at −83.5±0.2 mV (n=17). Within 7 minutes of exposure to 10 μmol/L LPLC, Em underwent a small depolarization (typically ≈5 mV) followed by an abrupt sustained depolarization to −21.5±1.0 mV (n=11). The mean time to the large depolarization in LPLC was dose dependent (31.1±7.4 [n=3], 9.0±1.5 [n=11], and 3.0±0.5 [n=6] minutes for 2.5, 5, and 10 μmol/L LPLC, respectively). In contrast, myocytes under control conditions remained well polarized for at least 60 minutes (n=5), the maximum time tested.

Applying 75-pA hyperpolarizing pulses initially caused partial repolarization after depolarization in LPLC (Figure 2, point b) demonstrating the bistability of Em. However, Em spontaneously depolarized again ≈1 minute later. With longer exposure to LPLC, hyperpolarizing current pulses failed to restore Em (Figure 2, point c), and contracture of the myocyte followed shortly thereafter. Dialysis with an EGTA-containing pipette solution prevented contraction on depolarization because myocytes were dialyzed with EGTA-containing pipette solution. Break at VC indicates suppression of voltage-clamp command pulses. Break in record (*) was for 2 minutes during control period.

Ultimately myocyte contracture depends on Ca2+. Therefore, the effect of a Ca2+-free bathing solution on the time to spontaneous contraction was evaluated for the 2 highest concentrations of LPLC. A Ca2+-free bathing solution prevented the occurrence of spontaneous contractions in 5 μmol/L LPLC, and cells remained quiescent for the entire 60-minute exposure (n=14). In contrast, adding 10 μmol/L LPLC to Ca2+-free bathing solution caused cells to promptly round up and die rather than simply contract (n=12). This suggests that the role of Ca2+ is complex.

Characterization of Ionic Current Underlying LPLC-Induced Membrane Depolarization

The effects of LPLC on the ionic currents are illustrated in Figure 3A and 3B. The steady-state I-V relationships under control conditions (A) and after a 9-minute exposure to 5 μmol/L LPLC (B) are plotted in Figure 3C. Exposure to LPLC caused a counterclockwise rotation of the control I-V relationship, and a pronounced inward current was observed at the physiological resting potential (~−83 mV). Figure 3D shows the I-V relationship of the LPLC-induced current obtained by subtracting the steady-state current under control conditions from that after LPLC exposure, reversed at −18.5±0.9 mV, indicating a poorly selective ionic current.

Hyperpolarizations elicited by 75-pA pulses after LPLC-induced depolarization (Figure 2, point c) were greater than those under control conditions (Figure 2, point a). This indicates that input resistance was greater after the LPLC-induced depolarization than before and was consistent with the voltage dependence of membrane resistance rather than loss of the pipette-membrane seal.

The sustained depolarization in LPLC usually was irreversible. Up to 25 minutes of washout of LPLC in bath solution containing 5 mg/mL albumin, which hastens lysolipid extraction from the membrane,23 failed to restore the resting potential in 4 of 5 cells.

Ionic Basis of the LPLC-Induced Current

To identify the charge carriers responsible for the LPLC-induced current, ion channels were blocked pharmacologi-
lympholympcholine-Induced Current

The LPLC-induced current was also insensitive to changes in bath Cl−. After the sustained membrane depolarization in LPLC, a 10-fold reduction in bath Cl− (from 150 to 15 mmol/L) failed to inhibit the current or shift $E_{\text{rev}}$ of the LPLC-induced current ($\Delta E_{\text{rev}}=1.4 \pm 1.8 \text{ mV}$, $n=3$, $P=0.547$).

Lanthanides Inhibit the LPLC-Induced Current

Ion substitution studies indicated that the LPLC-induced current was a poorly selective cationic current. This raised the possibility that LPLC modulates cationic stretch-activated ion channels (SACs), which poorly distinguish between Na+ and K+.26,27 Moreover, recent preliminary studies have shown that cardiac cell volume regulation is disrupted by lysolipids, including LPLC.28 To investigate the possibility that SACs contributed to the LPLC-induced current, Gd3+ and La3+ were used. Gd3+ is a moderately selective and potent (IC50 = 23.5 mol/L) blocker of SACs that exhibits cooperative binding,26,27 whereas La3+ is devoid of SAC-blocking activity in this concentration range.28 In rabbit ventricular myocytes, Gd3+ blocks all of the cation SAC current elicited by cell swelling.26 Figure 5 shows the effect of both lanthanides on the LPLC-induced current. Gd3+ (100 μmol/L) inhibited the LPLC-induced current by 80.2 ± 8.3% ($n=7$) (A) and La3+ (100 μmol/L) inhibited 80.7 ± 8.3% ($n=6$) (B) of the LPLC-induced current at $-83 \text{ mV}$. The effect of the lanthanides was indistinguishable.

The LPLC-induced current was also insensitive to changes in bath Na+. After the sustained membrane depolarization in LPLC, a 10-fold reduction in bath Na+ (from 150 to 15 mmol/L) failed to inhibit the current or shift $E_{\text{rev}}$ of the LPLC-induced current ($\Delta E_{\text{rev}}=1.4 \pm 1.8 \text{ mV}$, $n=3$, $P=0.547$).

Figure 4. LPLC-induced depolarization was [Na+]o dependent but insensitive to guanidinium toxins. A, After depolarization in 5 μmol/L LPLC, reducing [Na+]o from 140 to 14 mmol/L reversibly restored $E_{\text{m}}$ ($n=3$). In contrast, 1 μmol/L STX ($n=3$) and 10 μmol/L TTX ($n=3$, not shown) failed to restore $E_{\text{m}}$ or prevent LPLC-induced depolarization. B, LPLC-induced currents from the same cell as in panel A are shown. The LPLC-induced current was [Na+]o dependent but insensitive to STX. Low [Na+]o caused a $-26.7 \pm 4.2 \text{ mV}$ ($n=6$) shift in $E_{\text{m}}$ and reduced the inward current at negative potentials. Similar results were obtained with 10 μmol/L TTX ($n=4$, data not shown). The shift in $E_{\text{m}}$ of the LPLC-induced current is equivalent to that expected for a channel with a $P_{\text{Na}}/P_{\text{K}}$ ratio of 0.12 ± 0.06 ($n=6$).

Figure 5. Lanthanides (Gd3+ and La3+). □ blocked the LPLC-induced current (A). Gd3+ (100 μmol/L) inhibited 80.2 ± 8.3% ($n=7$) (A) and La3+ (100 μmol/L) inhibited 80.7 ± 8.3% ($n=6$) (B) of the LPLC-induced current at $-83 \text{ mV}$. The effect of the lanthanides was indistinguishable.
membrane depolarization (n=5) observed in LPLC (data not shown). Osmotic shrinkage itself does not appear to activate a mechanosensitive current in rabbit ventricular myocytes.26

**Lanthanides Delay Spontaneous Activity in LPLC**

Consistent with electrophysiological studies, pretreatment of myocytes with 100 μmol/L Gd3+ or La3+ significantly delayed the median time to development of spontaneous contractions in LPLC (Figure 7). In contrast, Ca2+ channel blockade with 100 μmol/L Cd2+ failed to delay spontaneous activity in LPLC. Although spontaneous activity was not completely inhibited with Gd3+ or La3+ pretreatment, neither was the LPLC-induced current. The unblocked current may have contributed to the eventual development of spontaneous activity.

**Effects of LPLC on the Ventricular Action Potential**

The LPLC-induced alterations in membrane current were expected to have profound effects on the action potential. Figure 8 shows representative recordings from 2 different cells under control conditions and within 5 minutes of exposure to 1 μmol/L LPLC. In control myocytes, the resting $E_m$ was $-82.1 \pm 0.7$ mV (n=7), and action potential duration, measured to 90% repolarization (APD90), was $319.2 \pm 19.2$ ms (n=7). After a switch to bathing media supplemented with LPLC, APD90 increased to $505.0 \pm 32.5$ ms (n=7) when measured just before induction of the sustained depolarization described earlier (see Figure 2). LPLC also caused a small but statistically significant reduction in action potential amplitude from $128.3 \pm 1.4$ to $122.5 \pm 1.6$ mV (n=7, $P=0.009$), predominantly resulting from a modest depolarization of resting $E_m$. Longer exposure to LPLC resulted in depolarization and usually loss of excitability. In some cases, a Ca2+-dependent upstroke could be elicited (arrow). In 2 of 7 myocytes, the effects of LPLC were reversible on washout (Wash), although APD90 became shorter than control.

![Figure 6](http://circres.ahajournals.org/)

**Figure 6.** Dose-response relationship for inhibition of the LPLC-induced current by Gd3+ (■). Inhibition was measured at –83 mV by using the average LPLC-induced current before and after lanthanide exposure. The data were well described by a 1:1 binding relationship with an IC50 of 23.5 μmol/L. Data for inhibition by 100 μmol/L La3+ (○) is also shown.

![Figure 8](http://circres.ahajournals.org/)

**Figure 8.** Effect of LPLC on ventricular action potentials from 2 cells. Within 3 to 5 minutes of exposure to 1 μmol/L LPLC, APD90 increased from $349.7 \pm 43.6$ ms (control) to $505.0 \pm 32.5$ ms (LPLC) (n=7). Action potential amplitude also was reduced from $128.3 \pm 1.4$ to $122.5 \pm 1.6$ mV (n=7); this reduction was primarily due to a 4.2-mV depolarization of resting $E_m$. Longer exposure to LPLC resulted in depolarization and usually loss of excitability. In some cases, a Ca2+-dependent upstroke could be elicited (arrow). In 2 of 7 myocytes, the effects of LPLC were reversible on washout (Wash), although APD90 became shorter than control.

**Discussion**

Accumulation of lysolipids after activation of PLA2 during ischemia has been recognized as an arrhythmogenic factor for over 3 decades. Previous efforts have focused on the actions of LPC, a product of diacyl lipid catabolism. The present study is the first to characterize and compare the effects of plasmalogen-derived LPLC with those of LPC in intact ventricular myocytes. We found that 2.5 and 5 μmol/L LPLC induced spontaneous contractions faster than LPC. Electrophysiological studies demonstrated that the contractions were the result of an LPLC-induced depolarization due to Na+ influx via a poorly selective pathway. Replacement of Na+ with NMDG hyperpolarized $E_m$ and caused a negative shift in $E_m$ of the LPLC-induced current. On the other hand, lowering bath Cl– failed to affect $E_m$. The LPLC-induced current

![Figure 7](http://circres.ahajournals.org/)

**Figure 7.** Consistent with patch-clamp studies, pretreatment with 100 μmol/L La3+ or Gd3+, but not Cd3+, delayed development of spontaneous activity in 5 μmol/L LPLC. Times for 25%, 50%, and 75% of myocytes to develop spontaneous activity are plotted. Median times were 16.4 minutes (n=36) for LPLC alone and 17.4 (n=19), 53.0 (n=38), and 55.4 (n=35) minutes for cells treated with LPLC+Cd2+, LPLC+La3+, and LPLC+Gd3+, respectively. The 75th percentile (not shown) was >60 minutes for both lanthanides.
was not the result of altered Na\(^+\) channel properties. The guanidinium toxins, TTX and STX, did not affect the I-V relation in LPLC. In contrast, both Gd\(^{3+}\) and La\(^{3+}\) substantially inhibited the LPLC-induced current. These lanthanides did not act through blockade of SACs or Ca\(^{2+}\) channels. Closing SACs by osmotic shrinkage and blockade of Ca\(^{2+}\) channels with Cd\(^{2+}\) failed to inhibit the LPLC-induced current. Consistent with electrophysiological studies, both Gd\(^{3+}\) and La\(^{3+}\), but not blockade of Ca\(^{2+}\) channels with Cd\(^{2+}\), significantly delayed the time to development of spontaneous activity in LPLC.

**Differences Between LPLC and LPC**

Although LPLC and LPC are both choline phospholipids, important differences in the actions of these lysolipids have emerged. LPC does not induce a guanidinium toxin-sensitivesteady-state current. In contrast, exposure to 9 to 25 \(\mu\)mol/L LPC for up to 2 hours modifies cardiac Na\(^+\) channel gating and causes long-lasting bursts of openings far negative to the normal voltage range for Na\(^+\) channel activation.\(^{16}\) This generates a sustained depolarizing current and substantial Na\(^+\) influx at the resting potential. More acute effects of LPC noted in whole-cell studies include a modest depolarizing shift in the peak amplitude of macroscopic Na\(^+\) current within 16 minutes of exposure to 10 \(\mu\)mol/L LPC.\(^{18}\) This differs from the hyperpolarizing shift reported with prolonged LPC exposure in the single-channel studies.\(^{16}\) However, a slowing of macroscopic inactivation kinetics is consistent with the effect if LPC on single Na\(^+\) channels.\(^{18}\) A similar study evaluating the effects of 10 \(\mu\)mol/L LPC on Na\(^+\) current in guinea pig ventricular myocytes also reported a slowing of inactivation kinetics within 3 minutes.\(^{17}\) However, the effects of LPC on the voltage dependence of activation were not reported in that study.

Other laboratories have shown that LPC induces a nonslective current in guinea pig ventricular myocytes.\(^{22,30}\) As in the present case, the conductance induced by LPC increased with time and was insensitive to bath Cl\(^-\). The LPC-induced current reversed near 0 mV, \(\sim 20\) mV positive to that observed for LPLC. This difference may be explained by the higher \(P_{Na}/P_K\) ratio estimated for the LPC-induced current\(^{30}\) (0.79) compared with that estimated in the present study for the LPLC-induced current (0.12). Interestingly, membrane permeability was greater for NMDG than for Na\(^+\) (NMDG/Na\(^+\) permeability ratio, 1.13) after LPC treatment.\(^{30}\) This sharply contrasts with the results of the present study. Equimolar replacement of Na\(^+\) by NMDG shifted \(E_{rev}\) of the LPLC-induced current >25 mV in a negative direction. The negative shift of \(E_{rev}\) precludes the possibility that NMDG is more permeant than Na\(^+\) in the present case. However, an independent determination of the NMDG permeability was not made.

Another difference is that the median time for development of LPLC-induced spontaneous activity was less than half that required for LPC at 2.5 and 5 \(\mu\)mol/L. We did not determine the time required for LPC to induce depolarization of myocytes. Nevertheless, the mean time to depolarization in 5 \(\mu\)mol/L LPLC was also less than half that reported previously for LPC under similar conditions.\(^{22}\) The mecha-nism responsible for the faster onset of action of LPLC compared with LPC is unknown. One possibility is that LPLC may partition into the sarcolemma faster than LPC. Although no data are available comparing the partitioning kinetics of these amphiphiles, the sn-1 vinyl-ether bond in plasmalogens is more lipophilic than the acyl ester contained in LPC. However, the remainder of the molecules are identical. Differences in the rates of lysolipid catabolism must also be considered. Catabolism of LPLC is substantially slower than that of LPC and requires distinct enzyme isoforms.\(^{31}\) Thus, even with equal partitioning into the sarcolemma, slower catabolism is expected to lead to higher sarcolemmal levels of LPLC.

**Effects of Lysolipids and Lanthanides on Membranes**

The electrophysiological effects of LPLC could result directly from lysolipid interaction with individual proteins or secondarily from changes in the membrane biophysical properties. Lysolipids increase membrane fluidity in model membranes\(^{31}\) and biomembranes.\(^{32}\) An increase in membrane fluidity is correlated with an increased membrane permeability to a number of solutes\(^{33}\) and could result in nonspecific ion fluxes. Interestingly, plasmalogen-derived lysolipids are more potent effectors of membrane fluidity than are sn-1 acyl ester lysolipids.\(^{31}\) This difference in potency may help explain the shorter time to onset and lower bath concentrations required for the effects of LPC.

Inhibition of the effects of LPC by lanthanides is consist-ent with the idea that LPC acts through changes in membrane fluidity. Lanthanides prevent increases in membrane fluidity, such as those caused by lysolipids.\(^{31,32}\) At concentrations comparable to those used in the present study, La\(^{3+}\) was previously found to be maximally effective at reducing membrane fluidity in cardiac sarcolemma.\(^{30}\) In another study,\(^{21}\) Gd\(^{3+}\) was comparable to La\(^{3+}\) in reducing membrane fluidity in model membrane systems. However, details of the membrane phospholipid composition may alter the sensitivity to lanthanides.\(^{34}\) It should be noted that lanthanides exert a number of other effects on membranes and their proteins. Consequently, the inhibition of the LPLC-induced current by lanthanides is not sufficient to establish that the LPLC-induced current results from an increase in membrane fluidity.

Other effects of lanthanides should also be considered. Lanthanide inhibition of the LPLC-induced current cannot be explained by inhibition of the Ca\(^{2+}\) current. Although lanthanides block the Ca\(^{2+}\) current, Cd\(^{2+}\), a more selective inhibitor of the Ca\(^{2+}\) current, failed to block the LPLC-induced current. Moreover, pretreating myocytes with Gd\(^{3+}\) or La\(^{3+}\), but not Cd\(^{2+}\), significantly delayed the median time for development of spontaneous contractions in LPLC.

Lanthanides were also not likely to be acting through blockade of SACs. Gd\(^{3+}\) and La\(^{3+}\) were equally effective at inhibiting the LPLC-induced current, even though Gd\(^{3+}\) is more potent than La\(^{3+}\) at blocking SACs. Gd\(^{3+}\) inhibited the LPLC-induced current with an IC\(_{50}\) of 23.5 \(\mu\)mol/L, nearly 14-fold higher than the IC\(_{50}\) for block of SACs in this preparation.\(^{26}\) Gd\(^{3+}\) appears to block all of the cation SAC
current elicited by cell swelling in this preparation. In addition, raising bath osmolarity to close SACs failed to affect $E_{rev}$ of the LPLC-induced current. On the other hand, the possibility that lysolipids activate poorly selective mechanosensitive channels that are insensitive to Gd$^{3+}$ and unaffected by cell swelling or shrinkage cannot be excluded. Such channels might explain the Na$^+$-dependent depolarization and provide a Ca$^{2+}$ influx pathway.

In contrast to the present results, it was reported that the poorly selective LPC-induced current in guinea pig myocytes is not inhibited by 15 μmol/L Gd$^{3+}$. However, these experiments were performed in a PO$_4^-$-containing bathing solution. Published stability constants for gadolinium phosphate indicate that virtually no free Gd$^{3+}$ is available in phosphate-buffered media. As a result, the ability of Gd$^{3+}$ to block the LPC-induced nonselective current in guinea pig myocytes remains an open question.

**Pathophysiological Relevance**

The kinetics of onset and the dose dependence of LPLC underscore its importance as an ischemic metabolite that induced a potentially arrhythmogenic inward current and prolonged APD$_{90}$ by extending the plateau at a partially depolarized level. The highest concentration of LPLC used in the present study (10 μmol/L) was less than or equal to the lowest LPC concentrations reported to have effects on ion channels. For instance, 20 to 100 μmol/L LPC$^{15}$ or up to 200 μmol/L LPC$^{16}$ was used to inhibit inward rectifier K$^+$ current. Effects on Na$^+$ current have been described using 10 to 50 μmol/L LPC, and exposure to between 9 and 25 μmol/L for up to 2 hours has been used for single-channel studies. Inhibition of the Na$^+$–K$^+$-ATPase was reported with a similar range of LPC concentrations.

Although LPLC induced spontaneous depolarization and contraction at lower concentrations than LPC, the extent of myocardial LPLC accumulation during myocardial ischemia remains uncertain. Many studies examining lysolipid content after ischemia did not report membrane LPLC content. In rats, rabbits, dogs, and humans, no increases in total LPLC were found for up to 24 hours after myocardial ischemia. This is not surprising for the rat, in which choline plasmalogen content is <2% of choline phospholipids compared with 36% in humans. The remaining studies may not shed light on the question at hand because lysoplasmalogens were normalized to total myocardial phospholipid content. Sarcolemma represents only 2% to 8% of the total myocardial lysoplasmalogen content. Nevertheless, substantial amounts of lysolipids are found in venous and lymphatic effluents and have access to the outer surface of the sarcolemma as do lysolipids added to the bath. Plasma lysolipid levels in coronary sinus effluents in humans are $\approx$100 μmol/L within 2 minutes of ischemia induced by rapid atrial pacing and reach 178 μmol/L. Similar values for lysolipids have been reported in plasma from cats and cardiac lymph from dogs during the first 10 to 15 minutes of ischemia. Plasma lysolipids are likely derived from both ischemic myocytes and vascular endothelium, although the contribution from each lysolipid subclass is unknown. The concentrations of lysolipid in extracellular fluid are much higher than those used in the present study. However, the majority of lysolipids in plasma are bound to protein. In a protein-free bathing solution, the uptake of 10 μmol/L [14C]LPC in rat ventricular myocytes after a 60-minute incubation period was comparable to tissue levels found during ischemia in cats, rabbits, dogs, and humans.

Electrophysiological studies focusing on LPC have ignored the potential contribution of lysolipids derived from plasmalogens. Nevertheless, plasmalogens are abundant in heart and are selectively degraded to lysolipids by PLA$_2$ during ischemia, and LPLC is slowly catabolized after its production. In view of the LPLC-induced current, membrane depolarization, and changes in action potential configuration observed in the present study, it is reasonable to propose that sarcolemmal accumulation of LPLC may contribute to ventricular dysrhythmias during ischemia.

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