Inward Sodium Current at Resting Potentials in Single Cardiac Myocytes Induced by the Ischemic Metabolite Lysophosphatidylcholine

Albertas I. Undrovinas, Ilya A. Fleidervish, and Jonathan C. Makielski

To investigate possible ionic current mechanisms underlying ischemic arrhythmias, we studied single Na⁺ channel currents in rat and rabbit cardiac myocytes treated with the ischemic metabolite lysophosphatidylcholine (LPC) using the cell-attached and excised inside-out patch-clamp technique at 22°C. LPC has been reported previously to reduce open probability and to induce sustained open channel activity at depolarized potentials. We now report two new observations for Na⁺ currents in LPC-treated patches: 1) The activation–voltage relation of the peak of the ensemble currents is shifted to the negative (hyperpolarizing) direction by approximately 20 mV compared with control currents. This effect was observed in all patches for depolarizations from a holding potential of −150 mV to different test potentials. 2) In some LPC-treated patches, Na⁺ channels exhibited sustained bursting activity at potentials as negative as −150 mV, giving a nondecaying inward current. This bursting activity was accompanied by double and triple simultaneous openings and closings, suggesting tight cooperativity in channel gating. These LPC-modified channels were identified as Na⁺ channels, because their unitary conductance was the same as Na⁺ channels in control solutions, because the single channel current–voltage relation was extrapolated to reverse at the Na⁺ Nernst potential, and because the current was blocked by the local anesthetic QX-222. This novel depolarizing current may play a role in the electrophysiological abnormalities in ischemia, including abnormal automaticity and reentrant arrhythmias, and could be a target for antiarrhythmic drugs. (Circulation Research 1992;71:1231–1241)

KEY WORDS • cardiac sodium channel • gating modification • inactivation • activation shift • cooperativity • drug interaction • QX-222 • rat ventricular cells • rabbit ventricular cells • patch-clamp single-channel conductance • synchronous openings

Lysophosphatidylcholine (LPC) has long been suspected to be one of the mediators of ischemic injury of the heart.1,2 Experimentally, LPC induces electrophysiological changes and arrhythmias that closely parallel those observed in ischemia,3–6 but the underlying cellular mechanisms of the LPC effect are not completely understood. LPC decreases K⁺ conductance,6–8 but this effect opposes the well-described increased K⁺ conductance observed in ischemia. We have previously shown that LPC-induced long-lasting bursts of the Na⁺ channel in response to membrane depolarization above threshold while decreasing the overall probability of opening.9,10 We now show that LPC can also induce openings of a channel carrying an inward current at potentials negative to the usual Na⁺ channel threshold. This current would have the effect of depolarizing the cell membrane independent of other mechanisms such as the loss of the K⁺ gradient and, moreover, would provide a driving force for K⁺ efflux and accumulation in the extracellular space.

Materials and Methods

Experiments were performed both in Moscow and Chicago under slightly different experimental conditions. For experiments in Moscow, single cells were enzymatically dispersed from adult rat ventricle,11 which was placed in a chamber (120 μl) held on the stage of an inverted microscope (Diavert, Leitz). We recorded single channel currents using either the cell-attached (cytoplasm intact) or excised inside-out (cytoplasmic side of membrane exposed to bath solution) patch-clamp method12 at 22°C. Pipettes were made of borosilicate glass with resistances of 2–5 MΩ, coated by Sylgard, and filled with a solution containing (mM) NaCl 135, CsCl 5.4, MgCl₂ 1.2, CaCl₂ 0.3, HEPES buffer 5.0 (pH 7.4), and glucose 10. For cell-attached patches, a high bath KCl concentration was used to depolarize the cell; the bath contained (mM) KCl 135, MgCl₂ 1.2, CaCl₂ 0.3, HEPES buffer 5.0 (pH 7.4), and glucose 10. For inside-out patches, the bath solution (cytoplasmic surface) contained (mM) CsF 100, EGTA 10.0, KOH 44, and HEPES 10.0 (pH 7.2 with 3.6 NaOH). LPC (20 μM, Serva Biochemicals, Germany) was added directly to cell suspensions without solvent for 1–3 hours before

From the Cardiac Electrophysiology Laboratories, Department of Medicine, The University of Chicago (A.I.U., J.C.M.), and the Cardiology Research Center (A.I.U., I.A.F.), Moscow.

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Address for correspondence: A.I. Undrovinas and J.C. Makielski, University of Chicago Medical Center, 5841 S. Maryland Ave. MC 4057, Chicago, IL 60637.

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making seals and recording currents. During the time of LPC exposure, approximately 30% of the cells undergo degeneration, as evidenced by the appearance of granulation and membrane bleb formation. This cell loss in LPC is comparable to untreated control cells. The remaining cells appeared the same as control “healthy” cells; i.e., they had clear cross striations and distinct membranes. We studied only these latter cells. A total of 28 LPC-treated patches in both the cell-attached and inside-out modes were studied. Single channel data were acquired and analyzed as previously described.9,10 Unless noted otherwise in the figure legends, all Moscow records were low-pass-filtered at a corner frequency of 2.5 kHz (−3 dB) and digitally sampled at 5 kHz.

Experiments done in Chicago were performed on rat ventricular cells isolated in the same way as in Moscow and on ventricular cells and atrial cells isolated from rabbit hearts.13 These cells were isolated in calcium-free Eagle’s minimal essential media with 10 mM HEPES (pH 7.3 with KOH). After isolation, the cells were stored at 22°C in the same solution with 0.3 mM CaCl2 added. Those cells to be studied with LPC were pretreated as above with 20 μM t-α-LPC palmitoyl or dL-α-LPC palmitoyl (Sigma Chemical Co., St. Louis, Mo.) added to the storage solution. Manufacture of pipettes was similar to that stated above. The pipettes were filled with a solution containing (mM) NaCl 280, CaCl2 1.0, MgCl2 1.0, tetraethylammonium 10, and HEPES 10 (pH 7.4 with NaOH). The bath solution for attached patches contained (mM) potassium t-aspartate 150, MgCl2 2.0, HEPES 10 (pH 7.2 with KOH), and glucose 5. The bath solution for inside-out patches contained (mM) CsF 120, NaF 30, EGTA 10, and HEPES 10 (pH 7.2). QX-222-containing solutions (QX-222, a local anesthetic, was kindly provided by Astra Pharmaceuticals) were made by adding 100-mM stock solutions in distilled water to the bath solution. The electrophysiological apparatus included a Nikon inverted microscope, an Axopatch 200 patch clamp (Axon Instruments, Burlingame, Calif.), and a 386-SX IBM-compatible computer running pCLAMP 5.1 (Axon Instruments) software for acquisition. A total of 34 LPC-treated patches (13 rat ventricle cell-attached, seven rat ventricle inside-out, six rabbit ventricle cell-attached, seven rabbit ventricle inside-out, and one rabbit atrial cell-attached) were studied. Unless noted otherwise in the figure legends, all Chicago records were low-pass (−3 dB) filtered at a corner frequency of 5 kHz and digitally sampled at 10 kHz.

Results

Four distinct types of LPC modification of Na+ current were observed, and these are summarized in Table 1. Type I modification, an overall decrease in peak open probability, has been reported previously.9,10,14 Type II modification, sustained channel bursting at depolarized potentials, has also been reported previously and was ascribed to a defect in the inactivation mechanism.9,10 In the present study, we report changes in the activation properties of Na+ current, type III and type IV modifications.

Type III Modification: Activation Shift

Ensemble currents from LPC-treated patches (Figure 1) typically resembled those of untreated patches, with the exception that the decay of the current was slowed and current could be detected at more negative potentials. Single open-channel current–voltage relations were fitted by linear regression to yield slope conductances and reversal potentials for control and LPC-treated patches in both the cell-attached (Figure 2A) and inside-out (Figure 2B) configurations. For cell-attached patches, the single channel conductance was 19 pS in both control and LPC-treated cells (Figure 2A), and for inside-out patches, it was 20 pS in control cells and 21 pS in LPC-treated cells (Figure 2B). The differences in slope conductances were not statistically significant. In cell-attached patches, the cytoplasmic Na+ is not under direct experimental control. The extrapolated reversal potential in the cell-attached patches was 75 mV in both control and LPC-treated

<table>
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<th>Type</th>
<th>Description</th>
<th>Estimated frequency</th>
<th>Reversibility</th>
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<tr>
<td>I</td>
<td>Decreased peak current</td>
<td>100%</td>
<td>Yes</td>
<td>9,10,14</td>
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<tr>
<td>II</td>
<td>Bursting (&gt;−70 mV)</td>
<td>50%</td>
<td>Yes</td>
<td>9,10, this study</td>
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<tr>
<td>III</td>
<td>Activation shift</td>
<td>100%</td>
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<td>This study</td>
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<td>IV</td>
<td>Bursting (&lt;−70 mV)</td>
<td>5−14%</td>
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TABLE 1. Lyosphosphatidylcholine Modification of Na+ Channels
cells (Figure 2A), which by the Nernst equation predicted an internal permeant ion activity of 14 mM. For inside-out patches, the bath solution contained 30 mM Na⁺, and the pipette solution contained 280 mM Na⁺, which predicts a reversal of 54 mV by the Nernst equation (allowing for 3% Cs⁺ permeability). This is in close agreement with the extrapolated values of 61 mV in control and 54 mV in LPC-treated cells. The difference between control and LPC interfaces was not statistically significant. Thus, the LPC modification that caused a shift in activation did not affect single channel conductance or reversal potential.

Whereas in control patches openings at or negative to −80 mV were never observed, in 34 of 34 patches treated with LPC, openings at or negative to −80 mV were frequently recorded. The average peak of the ensemble (not corrected for the number of channels in the patch) was 6.83 ± 1.88 (mean ± SEM) pA (n=7) at −80 mV and 2.4 ± 0.98 pA (n=4) at −90 mV. Summary data for the normalized peak current–voltage relation for LPC-treated cell-attached (Figure 2C) and inside-out (Figure 2D) patches show that for both configurations the activation threshold was shifted to more negative potentials. The conductance transforms (Figures 2E and 2F) of the data clearly demonstrated this shift. The midpoint of the Boltzmann relation (solid line) was shifted by 26 mV for the cell-attached patches and 17 mV for the inside-out patches.

Type IV Modification: Sustained Currents at Negative Potentials

In the majority of LPC-treated patches, the openings at potentials negative to threshold (−80 to −100 mV) clustered near the beginning of the depolarization, as evident by the decay in the ensembles (Figure 1). Also, in the majority of LPC-treated patches, no openings were apparent at the holding potential (−120 or −150 mV), although in a minority of LPC-treated patches (10 total) channels opened repetitively at potentials as negative as −150 mV. An example of such activity is shown in Figure 3A, where open activity persisted after clamping back to −120 mV in this inside-out patch. An immediately preceding depolarization was not required to show this activity; persistent open channel activity was evident at fixed holding potentials (Figure 3B). The open times for channels showing this modification (Fig-
Figure 2. Graphs showing open channel current–voltage relations (panels A and B), normalized peak ensemble current–voltage relations (panels C and D), and peak ensemble conductance (g\text{max})–voltage relations (panels E and F) for channels with type III lysophosphatidylethanolamine (LPC) modification. Open symbols represent control data; filled symbols data from LPC-treated patches. Data from cell-attached patches are shown on the left (panels A, C, and E) and from inside-out patches on the right (panels B, D, and F). Data points are mean ± SD for data from up to six patches. Data points without error bars are single observations except for panels E and F, where variability is the same as panels C and D, respectively. The open channel current–voltage data (panels A and B) are mean open channel currents from 200–500 single channel openings selected from up to six patches in which openings with overlapping events were excluded. The patch was depolarized from −150 mV to the indicated membrane potential. The relations were fit by linear regression to give slope conductance (γ) and extrapolated reversal potential. The reversal potential for control cell-attached patches is 74 mV; for control inside-out patches, 61 mV; for LPC cell-attached patches, 76 mV; and for LPC inside-out patches, 56 mV. The differences between the single channel conductance and reversal potentials in control and LPC were not significant (by paired t test, p > 0.24, 0.68, 0.48, and 0.44 for cell-attached slope, cell-attached intercept, inside-out slope, and inside-out intercept, respectively). For the peak ensemble current plots (panels C and D), each patch was analyzed by averaging between 20 and 186 recordings of depolarizations from −150 mV to the indicated membrane potential and normalized for the number of channels in the patch as described in “Materials and Methods.” The solid lines in panels A and B represent a nonlinear regression fit of the data to a Boltzmann equation: I_{\text{peak}} = g_{\text{max}}(V_m - V_r)/(1 + \exp((V_m - V_{1/2})/\text{slope})) for panel A and g_{\text{max}} = (1 + \exp((V_m - V_{1/2})/\text{slope})) for panel B, where I_{\text{peak}} is the peak ensemble current at each voltage V_m, g_{\text{max}} is a maximal conductance, V_{1/2} is the half point of the Boltzmann distribution.
ure 3C) were quite long (>60 msec at −60 mV) compared with those reported previously\(^\text{15,16}\) for unmodified channels (usually <1 msec), although a direct comparison at −120 mV cannot be made because unmodified sodium channels do not open at this potential. Channels cycled to closed states more often at the more negative holding potential, as evidenced by a shorter mean open time. Holding at various potentials for long periods resulted in persistent currents without any apparent decay in the time-averaged currents (Figure 4A). A plot of the potential dependence of the time-averaged currents (Figure 4B) was nonohmic, suggesting that some voltage-dependent gating remains. This finding is related to the strong voltage dependence of the mean open times (Figure 3C). This type of modification with prolonged bursting at very negative potentials was also found in inside-out patches (Figure 5). Single, double, and triple opening amplitudes at different potentials had linear regression slope conductances of 15, 32, and 48 pS, respectively. The open channel current–voltage relations were nearly ohmic over −150 to +30 mV. Extrapolated regression lines intercept the potential axis near +80 mV. The reversal potential for Na\(^+\) channels according to the Nernst equation for the transmembrane Na\(^+\) gradient and allowing for the known Cs\(^+\) permeability of the channel\(^\text{17}\) is calculated to be +70 mV for the conditions of this experiment. Thus, this type of modification does not appear to affect single channel conductance or reversal potential.

Synchronous Gating of LPC-Modified Channels

Multilevel openings were recorded in both cell-attached (Figure 3) and inside-out (Figure 5) patches. Each level presumably represents the superimposed opening of another channel. From the current tracings, it was apparent that channels tended to open and close synchronously such that an opening to the single channel conductance level was rarely seen. This is reflected in the near absence of a single channel peak in the amplitude histograms (Figures 5A and 5B). If channels open independently, the peaks of the amplitude histograms should form a binomial distribution, but the peaks in Figures 5A and 5B were clearly nonbinomial and indicated that channels were not gating independently. At the more depolarized potential, channel activity was nearly continuous, resulting in an absence of a zero conductance level in the amplitude histogram (Figure 5A).

Frequency and Interdependence of Different LPC Modifications

Type I modification, and overall reduction in open probability, was not looked for in this study because it has been well described previously.\(^\text{9,10,14}\) The type II modification, bursting at depolarized potentials, was observed in this study just as in previous reports\(^\text{9,10}\) and it occurred in 50% of the patches (17 of 34). The patches studied in Moscow were selected for type II modification and were thus excluded from this analysis of the overall frequency of the modifications. As mentioned earlier, the type III modification was present in every patch examined. Type IV modification, sustained bursting at negative potentials, was observed in just two of 34 patches, both of which also exhibited type II modification. Type IV modification occurred in eight of 28 patches that were selected for type II activity. Therefore, it would appear that type IV modification only occurs in cells that have type II modification and that the frequency of this modification may be between 5% (two of 34 patches) and 14% (eight of 56 patches, assuming a 50% type II modification rate). More exact estimates of the frequency of these modifications, as well as the sequence and timing of their development and reversibility, await further study. There was no apparent dependence of the frequency or type of modification on species or patch technique (cell-attached versus inside-out patches).

Spontaneous Modification

On one occasion, we observed sustained open channel activity very similar to LPC-modified channels (Figures 4 and 5) in a cell not treated with LPC (Figure 6). The reason for this spontaneous modification is unknown, but it is interesting to speculate that it may share a common mechanism with LPC-induced modification.

Local Anesthetics Block LPC-Modified Channels

The permanently charged local anesthetic QX-222 blocked all openings of LPC-modified channels. Before drug application, LPC-modified activity is evident in the prolonged bursting of the channels at both −30 and −80 mV, suggesting type II and type IV modification, respectively (Figure 7). These openings were nearly completely suppressed by 250 μM QX-222. This patch was lost before washout of the drug effect was achieved, but in a second patch with type IV modification, 500 μM QX-222 applied to the inside of the cell completely eliminated all channel activity, and full activity was restored upon washing QX-222 from the bath.

Discussion

LPC-Induced Currents Are Modified Na\(^+\) Channels

LPC induced a novel ionic current that in the extreme showed open channel activity at all potentials between −180 and +30 mV. We have identified this channel to be an LPC-modified Na\(^+\) channel because of the following supporting evidence. The single channel conductance is the same as for unmodified Na\(^+\) channels, and the extrapolated reversal potential is near the Na\(^+\) reversal potential, indicating Na\(^+\) selectivity. They are unlikely to be K\(^+\) channels, because current persisted in

\[^{*}\text{V}_\text{m}\text{ is the reversal potential, and slope is the Boltzmann slope factor, fixed to }-6. The reversal potential was set to 75 mV for cell-attached patches and 57 mV for inside-out patches, as predicted by panel A. The half points of the Boltzmann fit with 95% confidence interval of the parameter estimates for the cell-attached patches were } V_{1/2} = -57\pm1.2 \text{ mV for control patches and } V_{1/2} = -78\pm2.4 \text{ mV for LPC patches. For inside-out patches, } V_{1/2} = -57\pm1.0 \text{ mV for control patches and } V_{1/2} = -74\pm1.1 \text{ mV for LPC patches. Statistical analysis and curve fitting were done using SAS (SAS Institute, Inc., Cary, N.C.) on a Sun SPARC station. Summary data are from both rat and rabbit ventricular cells. Experiments done in Chicago.}\]
FIGURE 3. Noninactivating and nondeactivating sodium channels (type IV modification) recorded in lysophosphatidylcholine (LPC)-treated (20 μM) cell-attached membrane patches. Panel A: Consecutive sweeps in response to 90-mV, 100-msec depolarizations from a holding potential of −120 mV are shown (protocol as diagrammed in the figure). Channel activity remained after patches were clamped back to the holding potential. Panel B: Samples of continuous single current recordings at three potentials after 1 hour of LPC treatment show continuous activity in the absence of prior depolarizations. The numbers on the left of each tracing indicate the holding potential. Bars on the right indicate the number of open channels. The increased "noise" apparent when a channel is open probably reflects closures not resolved by the recording system. Panel C: Overall distribution of open times for sustained channel openings at a fixed potential (V_m) of −60 mV (top graph) and −120 mV (bottom graph) is plotted. The curves represent a monoexponential fit to the open-time distribution, with the time constants (τ_o) given on the figure. The recordings in panels A and B were low-pass filtered at 300 Hz and displayed on a paper recorder (model 2800S, Gould, Cleveland, Ohio). Data in panels A, B, and C are from different cell-attached patches. The dotted lines mark zero current levels, and inward current is shown as a downward deflection. The frequency of open times is plotted on the ordinate as the probability density (pdf) in sec⁻¹. These data were obtained in Moscow on rat ventricular cells.
The absence of K+ and in the presence of Cs+ or tetrathyramonium, blockers of most K+ channels, on both sides of the membrane. If it had been a K+ channel that became nonselective for cations, then the reversal potential would be expected to have been closer to 0 mV rather than +80 mV. It is also unlikely to be the calcium-induced cation-nonspecific channel because the gating was strongly potential dependent, unlike cation-nonspecific channels. Also, we observed channel activity for more than 30 minutes for inside-out patches (Figure 5) with very low and fixed internal Ca2+ (10 mM EGTA). Finally, QX-222, a relatively selective blocker of Na+ channels, rapidly and reversibly blocked this current.

In field-stimulated isolated cardiac myocytes, LPC caused hypercontracture, which could be prevented by the application of the specific Na+ channel blocker tetrodotoxin.29 The authors speculated that Ca2+ overload occurred because of Na+ accumulation through LPC-modified Na+ channels10 and the resulting increase in internal Ca2+ by the effect on Na+-Ca2+ exchange. This study demonstrates a pathophysiological relevant consequence of LPC action and also supports the contention that LPC modifies the tetrodotoxin-sensitive Na+ channel.

A recent study of LPC action on whole-cell Na+ currents in the guinea pig4 confirmed previous reports from single channel studies9,10 that peak current progressively declines and decay slows (type I and II modifications). Unfortunately, in that study the cells died within minutes, and the effect could not be reversed. In our preparation, cells survived LPC treatment for at least several hours, and a partial reversal of the effect on peak current and altered inactivation have been demonstrated.9,10 Reversibility of type III and IV modifications, however, has not been demonstrated. Although we have given an estimate of the frequency of observing the different stages of LPC-modified gating, the exact sequence, timing, and extent of LPC-induced modification after exposure to LPC remain to be determined.

**Gating of LPC-Modified Na+ Channels**

The results described in this report along with previously described effects of LPC-modified Na+ channels (Table 1) suggest profound effects on gating of the channel. Although a detailed and quantitative kinetic model of LPC-modified channels is not yet possible, it is instructive to interpret the results in terms of a simplified Markov gating model:

\[
\begin{align*}
C & \rightarrow O \\
C & \rightarrow I
\end{align*}
\]

where normal gating is represented by three states: a closed but ready to open state (C), an open state (O), and an inactivated state (I). More detailed gating models would include multiple closed states and multiple inactivated states, but for simplicity they are lumped together. A fourth absorbing state (A) is introduced to explain type I modification. This state is nonconducting, and return from this state to the other states is unlikely in the presence of LPC. LPC favors transition into this absorbing state and accounts for the overall decrease in channel open probability (type I modification). Bursting at depolarized potentials (type II modification) implies an elimination of inactivation or, at least, a greatly reduced rate of inactivation (O→I). The shift in activation (type III modification) could be accounted for by a shift in the voltage dependence of the C→O and O→C transitions. Bursting at negative potentials implies for this model a profound decrease in the activation (C→O) and deactivation (O→C) rate constants. This would account for the long open times observed in type IV modification.

**Synchronized Gating**

A very interesting aspect of the behavior of the channels with type IV modification is the tendency for them to open (and close) to (and from) levels that are two or three times the conductance level of the unmodified Na+ channel. The mechanism for this phenomenon is completely unknown. It can be speculated that this synchronous behavior might result from a very tight cooperativity in the gating of separate channels. The possibility that Na+ channels form dimers and trimers has been mentioned by several investigators,21-24 and negative cooperativity has been suggested for batrachotoxin-treated channels.25 How this cooperativity is achieved is difficult to visualize without a close physical clustering of the channel. Na+ channels are normally anchored in the membrane,26 but membrane structure is altered in ischemia, possibly because of cytoskeleton

**Figure 4. Time-averaged stationary current-voltage plots of type IV lysophosphatidylcholine (LPC)-modified Na+ currents.** Panel A: Average Na+ currents from 250 traces were plotted against time at the constant holding potentials indicated. The current recordings for this figure were reconstructed by averaging from between 200 and 250 segments of 100-msec duration randomly chosen from a 1-minute continuous recording at a given potential. Over time, the channel maintains a nondecaying open activity. Panel B: Current-voltage relation for the mean values of time-averaged currents from panel A showed a decrease at more negative potentials (nonohmic relation), indicating that these channels with type IV modification maintain potential-dependent gating, although greatly altered from normal Na+ channels. These results were from a cell-attached patch from a rat pretreated with LPC (20 μM) for 2 hours. This experiment was done in Moscow.
breakdown. Clusters of membrane particles have been observed after LPC treatment. Even if LPC induces physical clustering of channels, the problem of linking the gating mechanisms of the separate channels remains. Alternatively, this activity might represent a single LPC-modified Na channel exhibiting supraconductance levels that are only coincidentally the same as integral multiples of the unitary conductance of unmodified channels. For the open-time analysis of these channels (Figure 3C), we assumed a Markov process, i.e., history-independent transition rates and channel gating independent of neighboring channels. We found monoeXponential fits to open-time histograms (Figure 3C), which suggests a single open state, even for channel clusters. On the other hand, modeling cooperativity will require going beyond simple Markov process models. It is interesting to speculate that LPC may induce multiple channel protein conformation changes leading to modal gating or perhaps fractal behavior. Determining the mechanism(s) of the synchronous gating behavior, in

FIGURE 5. Amplitude analysis of type IV lysophosphatidylcholine-modified Na channel current from an inside-out patch. Panels A and B: Examples of channel activity at a membrane potential (V_m) of -70 mV (panel A) and -180 mV (panel B). The numbers 1, 2, and 3 to the left of each panel note openings to single, double, and triple unitary conductance levels. On the right of each panel, the channel current amplitude distribution histograms are shown. At V_m = -70 mV, openings with current amplitude triple the single channel amplitude were more frequent than double amplitude openings. The probability of a triple amplitude opening (P_3) was 0.775 and of a double amplitude opening (P_2) was 0.216. At the more negative potential, V_m = -180 mV, double amplitude events were more frequent than triple amplitude openings (P_3 = 0.054, P_2 = 0.229). The zero current peak of the histogram was truncated at 6,000 counts. At both potentials, single amplitude events were observed rarely; no single opening peak was discernible in the amplitude distribution histogram. Also, it was apparent, especially in panel B, that openings and closings to double and triple levels were synchronized. Panel C: Relation between channel current amplitude and membrane potential. Data were obtained on the same inside-out patch as in panels A and B. The lines represent linear regression through the data points, with slope conductances of 15, 32, and 48 pS for single, double, and triple openings, respectively. Reversal potentials were 70, 67, and 68 mV, respectively. The channel activity was observed after 15 minutes of patch excision and continued for more than 30 minutes. This experiment was done in Moscow on a rat ventricular cell.
phenomenon, and the changes in gating induced by LPC, in general, requires further study.

Possible Mechanisms for Modified Gating

The mechanism for LPC-induced altered gating is unknown. LPC partitions into the membrane, and it can be speculated that the effects may result from changes in membrane fluidity and/or changes in lipid–protein interaction. G protein–dependent mechanisms can reduce sodium current peak (like type I modification). A slowing of the decay of sodium current (type II modification) can occur rarely but spontaneously in control patches, and both a decrease in peak sodium current and a slow decay (like type I and II modifications) can be induced by cAMP-dependent mechanisms. These similarities suggest that LPC might be acting through mechanisms present but activated infrequently in non–LPC-treated cells. In support of this idea is that LPC has been shown to increase cAMP content in the heart. The finding that LPC-modified channels are found in both cell-attached and inside-out patches suggests that the modification is neither dependent on nor prevented by soluble regulatory machinery that is lost with patch excision. Changes in activation, like type III and IV LPC modifications, are less frequently reported. We report for the first time an observation of spontaneously altered activation causing sustained bursting at negative potentials (Figure 6). Angiotensin II modulation of cardiac Na⁺ channels increased the current at negative potentials (−70 mV), resembling type II LPC modification, but it also increased peak current and current decay. Metabolites of the glycolytic pathway have been shown to reduce peak Na⁺ current and slow decay of Na⁺ current, but effects on activation were not reported. Disruption of the cytoskeleton has been shown to cause bursting of epithelial Na⁺ channels. Such a disruption of attachments by LPC could conceivably underlie the altered gating in LPC modification. Finally, it is interesting to speculate that low concentrations of LPC contaminating isolated cell preparations may be the trigger for spontaneous modification. The negative shift in activation (type III modification) resembles the time-dependent negative voltage shift in the gating of Na⁺ channels in whole-cell voltage clamp and patch clamp. Although a common underlying mechanism cannot be excluded, the entire LPC effect is unlikely to be simply an artifact of this previously observed shift. Also, the other three LPC-induced Na⁺ channel properties (decreased open probability, burst openings at depolarized
Implications of LPC-Modified Na⁺ Channels for Cardiac Ischemia

Lyosphosphoglycerides, the products of phospholipid catabolism, including LPC, accumulate in ischemic myocardium and may be a major factor causing the electrophysiological alterations contributing to arrhythmogenesis. LPC causes depolarization, reduction in maximal upstroke velocity of action potential, sustained abnormal rhythmic activity of Purkinje fibers, and delayed afterpotentials in isolated tissue even in the presence of high [K⁺], and reduced pH. Our data do not allow extrapolation to the density of LPC-modified channels under conditions of ischemia in vivo, but judging by the frequency with which we find them in our patch pipettes, it is possible that the density may be high (several per square micron). Moreover, at resting potentials the effect of an open Na⁺ channel is amplified by the large driving force for Na⁺, and during the plateau of the action potential, membrane resistance is sufficiently high that even a small amount of current can have a large effect. If LPC induces altered activation of a sufficient number of Na⁺ channels in ischemia, then puzzling aspects of the electrophysiological changes in ischemia (see Reference 50 for review) can be explained. Acute ischemia induces a K⁺ efflux and a loss of the normal resting potential. Extracellular K⁺ accumulation likely results from an enhanced K⁺ conductance probably through the ATP-inhibited K⁺ channel rather than by a decrease in K⁺ influx from depression of the Na⁺-K⁺ pump. The increased K⁺ conductance, however, would cause the membrane potential to more closely approximate the K⁺ equilibrium potential, which at least initially should cause hyperpolarization and reduce the driving force for K⁺ efflux. Therefore, why is hyperpolarization not a feature of early ischemia, and what provides the driving force for K⁺ efflux? K⁺ coe flux with lactate has been proposed as a driving force, and this may be part of the answer. The persistent inward current described in this report could answer both of these questions, because it would independently depolarize the membrane and at the same time augment K⁺ accumulation by increasing the electrical driving force for K⁺ efflux.

Inward current from LPC-modified Na⁺ channels would also theoretically contribute to proposed mechanisms underlying automaticity in ischemia. Abnormal automaticity is induced by LPC in Purkinje fibers. CsCl did not block abnormal automaticity of subendocardial Purkinje cells in infarcted dogs, indicating that K⁺ channels do not play a major role in this activity. Moreover, in a 24-hour infarct model, the rate of sustained abnormal automaticity in subendocardial Purkinje fibers linearly depended on external Na⁺ and was blocked by tetrodotoxin, supporting the case for involvement of a Na⁺ channel. The LPC-modified Na⁺ channels we have described might play the role of “ischemic pacemaker” channels in ventricular tissue.

Finally, these modified channels are a logical target for antiarrhythmic drugs. We have demonstrated that QX-222, a lidocaine analogue, blocks the modified channel. Some antiarrhythmic drugs bind preferentially to the open state of the Na⁺ channel; therefore, LPC-modified channels may be more susceptible to block than unmodified channels.

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