Lysophosphatidylcholine Decreases Single Channel Conductance of Inward Rectifier K Channel in Mammalian Ventricular Myocytes

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Summary Effect of lysophosphatidylcholine (LPC) on the inward rectifier K channel of the isolated guinea pig ventricular cell was studied using patch clamp technique. In that LPC (100 μM) decreased the magnitude of the single channel conductance from 48±5 pS (mean and S.D., n=5) to 12±9 pS (n=8), this event may be the prime factor related to the alleged LPC-induced depolarization of cardiac tissues.

Key Words: lysophosphatidylcholine, inward rectifier K channel, patch clamp.

Evidence has accumulated that lysophosphoglycerides, such as lysophosphatidylcholine (LPC), could be one of the trigger substances of arrhythmias, as encountered in ischemia (for references see Corr et al., 1982). Two prominent electrophysiological effects of LPC have been suggested. (1) LPC increases the cellular level of cAMP, which may potentiate Ca influx via Ca channels (Ahumada et al., 1979). (2) LPC reduces transmembrane resting potential of cardiac tissues (Corr et al., 1979). Clarkson and Ten Eick (1983) showed that the depolarizing effect of LPC results from a decrease in potassium conductance near the normal resting potential which is known as an inward rectifier (Cleemann and Morad, 1979).

We attempted to elucidate the molecular mechanism involved in the effect of LPC on the inward rectifier channel. For this purpose, we recorded single channel currents using patch clamp technique. Guinea pig hearts were isolated and perfused with Ca-free Tyrode’s solution containing collagenase (0.3 mg/ml, Sigma type I) for 60 min at 35°C in a Langendorff’s perfusion system. The heart was then immersed into the “storage solution” which contained (in mM): glutamic acid 70, KCl 5, taurine 10, oxalic acid 10, KH2PO4 5, HEPES 5, glucose 11, and EGTA 0.5 (pH 7.4 by adding KOH). Cells were dispersed in a recording chamber.
and perfused with modified Tyrode's solution the composition of which was: NaCl 137, NaHCO₃ 3.0, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, NaH₂PO₄ 0.16, glucose 5.5, and HEPES 5 mM (pH 7.4).

Single channel currents were recorded in a "cell attached" mode with the use of fire-polished glass micro-pipettes filled with solution containing 150 mM K-aspartate and 5 mM HEPES (pH 7.4) with or without LPC (L-α-lysophosphatidylcholine, palmitoyl Sigma). The seal resistance of the patch membrane was 8 to 60 GΩ (30±19 GΩ, n=10). To minimize the effect of diffusion of LPC from the pipette, the chamber (0.8 ml in volume) was continuously perfused with normal Tyrode’s solution at a rate of 1 to 1.2 ml/min. The current signals were stored on magnetic tape (TEAC R-71) with a frequency response of 0–1.2 kHz which were processed by a computer (Nihon Kohden ATAC-450). The whole system had a band width of less than 1 kHz. All experiments were done at room temperature (24–25°C).

Normal activity of the inward rectifier K channel of guinea pig ventricular cell is shown in Fig. 1A. The channel had characteristics similar to those of rabbit ventricular cells (KAMEYAMA et al., 1983) as follows: (1) The channel has a long open time with bursting activity (Fig. 1A). (2) The current-voltage relationship of the single channel showed inward-going rectification (Fig. 2, control). (3) The calculated slope conductance was 48 pS, which is comparable to 47 pS reported in rabbit ventricular cells (KAMEYAMA et al., 1983). As it is highly likely that this channel has a high probability to open near the equilibrium potential of the potassium ions, E_K (KAMEYAMA et al., 1983), this channel probably plays an important role in maintaining the resting potential near E_K (−90 mV) in the ventricular cells.

The effect of LPC on the inward rectifier channel was studied in other cells using pipettes filled with 150 mM K-aspartate solution containing 100 μM LPC. As shown in Fig. 1B, just after the establishment of GΩ seal, the single channel current with normal amplitude at resting potential could be recorded. However, the amplitude progressively decreased with time (Fig. 1C) and it became all but undetectable at the resting potential levels within 10 min (Fig. 1D).

Such a decrease in the current amplitude shown in Fig. 1B–D would not be secondary to a depolarization of the cell. After recording the current traces in Fig. 1D, the patch membrane was broken by application of strong negative pressure to the pipette, and the intracellular potential was recorded using an amplifier which had common head stage with the patch clamp amplifier (OHARA et al., 1983). The measured resting potential was −82 mV after compensating the liquid junction potential (−9 mV). This value was comparable to those (−79±3 mV, n=5) obtained from other cells where the resting potential was measured with the pipette containing no LPC. Thus, the depolarization due to the leak of LPC into the bulk solution or by some mechanical injury may not be the cause of marked decrease in the current amplitude.

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Fig. 1. Single channel currents of inward rectifier K channel and reduction of single channel conductance by LPC. A: series of patch currents recorded in cell-attached mode under control condition (150 mM K-aspartate within the pipette and 5.4 mM [K] in the bulk solution). Single channel conductance was calculated to be 45 pS. B-D: records from different cell from A. When LPC (100 μM) was in the pipette, the unit current amplitude decreased as time elapsed from 1.7 pA in B to 0.9 pA in C within 5 min after formation of GΩ seal. The single channel conductance calculated from a series of current records obtained after 10 min (D) was 5 pS. The downward deflection from the baseline (dotted line) denotes inward direction of the current. Numerals to the left of each panel indicate the voltage deviations from resting potential (mV).
The effect of LPC on the single channel conductance of the inward rectifier K channel is summarized in Fig. 2. When LPC was in the pipette at the concentration of 100 µM, the single channel conductance was calculated to be 12±9 pS (mean and S.D., n=8), which is significantly (p<0.001) lower than that obtained in the absence of LPC (48±5 pS, n=5).

Inward rectifier channel is blocked also by inorganic cations such as Cs and Ba. However, they did not change the slope conductance but rather increased the probability that the channel closes (KAMEYAMA et al., 1983). This is in contrast to the LPC action on the same channel reported in the present study. However, we cannot completely rule out the possibility that limited time resolution of the present recording system (0-1.0 kHz) prevented us from detecting very rapid interruptions (flickering) of the single channel current such as reported for the blocking action of local anesthetics on the nicotinic ACh-receptor channel (NEHER and STEINBACH, 1978). If this is the case, the frequency of rapid closure must be higher than 2 kHz since LPC decreased, on average, the current amplitude by about 80% (−14 dB) as was estimated from Fig. 2. This frequency (≥2 kHz) seemed much greater than the flickering induced by Cs or Ba. Inspection of the flickering produced by 0.04 mM Cs (KAMEYAMA et al., 1983) or 0.2 mM Ba (BECHEM et al., 1983) revealed that the average frequency might be less than 1 kHz. This is well within the time resolution of our recording system. In addition, even much smaller concentrations of LPC (20 and 5 µM, 2 experiments each) did not
produce the flickering phenomenon. The flickering produced by local anesthetics in the ACh channel was decreased in the frequency with reduction of the drug concentration (NEHER and STEINBACH, 1978). Thus, we conclude that LPC decreased the current carried by inward rectifier K channel via reduction of the single channel conductance.

LPC has amphiphilic properties and is readily incorporated into sarcolemma (SNYDER et al., 1981). Relatively slow development of the blockade (Fig. 1B-D) may be related to the time required for LPC to produce some conformational changes in the phospholipids bilayer (CORR et al., 1979; SNYDER et al., 1981).

The LPC-induced reduction of the unitary K current may explain the drug-induced depolarization of the resting potential in cardiac tissues reported elsewhere (CORR et al., 1979; CLARKSON and TEN EICK, 1983).

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REFERENCES


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