Phospholipid Lysophosphatidylcholine as a Metabolic Trigger and HERG as an Ionic Pathway for Extracellular K⁺ Accumulation and “Short QT Syndrome” in Acute Myocardial Ischemia

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Key Words

Myocardial ischemia • Arrhythmias • 1-palmitoyl-lysophosphatidylcholine • Potassium • HERG, I_{Kr} • Action potential duration

Abstract

The most profound abnormalities during acute myocardial ischemia are extracellular K⁺ accumulation ([K⁺]_o—↑) and shortening of action potential duration or QT interval (APD—↓ or QT—↓), which are pivotal in the genesis of ischemic arrhythmias and sudden cardiac death. The ionic mechanisms however remained obscured. We performed studies in a rabbit model of acute global myocardial ischemia in order to explore ionic and metabolic mechanisms for ischemic [K⁺]_o—↑ and QT—↓. Exogenous 1-palmitoyl-lysophosphatidylcholine (LPC-16) mimicked the low-perfusion ischemia to produce significant [K⁺]_o—↑ and QT—↓. The [K⁺]_o—↑ and QT—↓ induced by either LPC-16 or ischemia were prevented by dofetilide, a blocker

of rapid delayed rectifier K⁺ current (I_{Kr}), but not by blockers for other K⁺ channels. Consistently, dofetilide efficiently abolished the ventricular tachy-arrhythmias induced by ischemia or LPC-16. LPC-16 remarkably shortened APD and enhanced the function of I_{Kr} and HERG (the pore-forming subunit of I_{Kr}). The effects of LPC-16 manifested with shorter APD (faster repolarization rate) and at more negative potential (membrane repolarization). Dofetilide abolished the I_{Kr}/HERG enhancing and APD shortening effects of LPC-16. Our results suggest that LPC-16 accumulation/HERG enhancement may be a link between metabolic trigger and ionic pathway for ischemic [K⁺]_o—↑ and QTc—↓. This represents the first documentation of I_{Kr}/HERG as the ionic mechanism in ischemic [K⁺]_o—↑ and QTc—↓. Inhibition of LPC-16 production and accumulation and/or of I_{Kr}/HERG may be a promising therapeutic strategy to attenuate the incidence of lethal arrhythmias associated with ischemic heart disease.

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Introduction

Extracellular K^+ accumulation ($[K^+]_o \uparrow$) in the myocardium following net cellular K^+ loss is one of the earliest consequences of myocardial hypoxia and ischemia, beginning within 15–30 s [1–6]. During the first 10–15 min of myocardial ischemia, $[K^+]_o$ (or potassium activity) rises 2–3 fold from its normoxic value (5 mM) to a plateau level of 6–11 mM above its initial value. $[K^+]_o \uparrow$ is related to profound modifications of the electrical behavior of ischemic cardiomyocytes. In particular, it contributes to depolarization of resting membrane potential, decrease in the maximum upstroke velocity of action potential (AP), and decrease in excitability. Associated with this $[K^+]_o \uparrow$ is the shortening of action potential duration (APD $\downarrow$) or QT interval (QT $\downarrow$). These electrophysiological alterations together form the substrates for the ischemic arrhythmogenesis and the consequent sudden cardiac death.

Ischemic arrhythmias are generally categorized into three phases based on the time when they appear. Phase I arrhythmias occur during the first 30 min of ischemia, phase II arrhythmias between 5 to 72 hours and phase III arrhythmias of the chronic stage after an infarct. The first burst of ventricular tachycardia occurs between 2 and 10 min of ischemia. In human, phase I arrhythmias often results in ventricular fibrillation and sudden cardiac death. Reentry [7, 8] and delayed afterdepolarizations (DADs) [1, 9] are the major mechanisms for phase I arrhythmias in ischemic myocardium. Noticeably, APD $\downarrow$ /QT $\downarrow$ favors the reentrant arrhythmias and DADs, a reproducible pattern of dysrhythmic activities during acute myocardial ischemia [10]. Here we refer to this typical pattern of ischemic electrical disorders and arrhythmias “short QT syndrome”. Indeed, pharmacological modulation of $[K^+]_o \uparrow$ and APD $\downarrow$ during acute myocardial ischemia has been considered as a promising new antiarrhythmic and cardioprotective strategy during myocardial ischemia and reperfusion. However, our current knowledge of the mechanisms for $[K^+]_o \uparrow$ and APD $\downarrow$ is still insufficient to allow us to benefit from the promise.

The cell surface membrane is primarily composed of phospholipid, cholesterol, and proteins which together form a complex dynamic structure which retains the intracellular contents, regulates ion homeostasis, governs nutrient transports, and transduces extracellular signals into the cells. The principal classes of sarcolemmal phospholipids are, according to their relative abundance, phosphatidylcholine, phosphatidylethanolamine,

phosphatidylinositol, and phosphatidylserine [8]. Under certain situations, for example, in ischemic tissues, the phospholipids (e.g. phosphatidylcholine) can be metabolized to generate lysophosphatidylcholines (LPCs). LPCs are amphiphiles possessing a charged headgroup like phospholipids but only a single aliphatic hydrocarbon chain as a consequence of the hydrolytic cleavage of one of the two aliphatic hydrocarbon groups of phosphatidylcholines. The major species of LPCs produced in ischemic myocardium is 1-palmitoyl-lysophosphatidylcholine (LPC-16) which possesses 16 hydrocarbons in its aliphatic chain. As an intermediate of metabolism of phosphatidylcholine, LPC-16 is present in a variety of mammalian tissues and accumulates rapidly in the heart during cardiac ischemia [8, 11, 12]. Accumulation of LPC-16 is thought to be a major contributor to the development of cardiac arrhythmias during myocardial ischemia [12–15], such as abnormal rhythmic activity, delayed afterdepolarizations (DAD), triggered activity and intramyocardial reentry, and is regarded as a biochemical trigger of ischemic arrhythmias. APD shortening by LPC-16 has been documented in rabbit atrial and ventricular cells [14], guinea pig ventricular cells [16], and canine ventricular cells [1] and Purkinje fibers [8]. LPC-16 levels correlate with the occurrence of arrhythmias in ischemic hearts [11, 13, 15]. Coincidentally, a recent study by Goldhaber *et al* [17] reported that LPC-16 decreases tissue K^+ content by ~15%, an effect associated with gradual APD $\downarrow$ and $[K^+]_o \uparrow$. However, the mechanisms underlying the deleterious actions of LPC-16 remained unclear.

The delayed rectifier K^+ current (I_{Kr}), encoded mainly by human *ether-a-go-go* related gene (*HERG*) [18], carries outward K^+ flow which is crucial for cardiac repolarization. I_{Kr} has the potential of being a contributor to ischemic $[K^+]_o \uparrow$ and APD $\downarrow$ and the associated arrhythmias, considering the following notes. First, previous studies concerning I_{Kr} and ischemia suggest that I_{Kr} is increased during ischemia and infarction. Shinmura *et al* [19] studied the effect of E-4031, a class III antiarrhythmic drug which specifically inhibits I_{Kr} , on ischemia- and reperfusion-induced arrhythmias. They found that E-4031 (100 nM) attenuated loss of K^+ from the ischemic myocardium and concomitant reduction of ischemia-induced arrhythmias. Another study reported by Pinto and Boyden [20] described an E-4031-sensitive K^+ current which was increased in arrhythmogenic subendocardial Purkinje myocytes from the infarct hearts of dogs. Although the nature of this E-4031 sensitive K^+ current remains to be clarified, it possesses some

characteristics of I_{K_r} . Second, HERG is highly susceptible to modulation by various extracellular and intracellular factors. We have recently found that LPC-16 significantly increased I_{HERG} amplitude by shifting the inactivation of the channels to more positive potentials and by slowing the inactivation kinetics [21, 22]. The effects took place within 1 min after LPC-16 reached the cells and were readily reversible upon removal of the drug. These data are in line with the fact that LPC-16 causes $[K^+]_o \uparrow$ and $APD \downarrow$ and thus suggest that I_{HERG}/I_{K_r} contribute to the early event of ischemia-induced alterations of cardiac electrical activities. Finally, HERG conductance is known to increase with increasing extracellular K^+ , a typical condition in ischemia. It is thus conceivable that I_{K_r} is both a cause for and a consequence of $[K^+]_o \uparrow$.

We proposed that ischemic electrical disorders are a consequence of interrelated metabolic stress and electrolyte disturbance. In other words, ischemic $[K^+]_o \uparrow$ and $APD \downarrow$ are an event involving the interplay between ionic channels and metabolic substrates. The present study was designed to examine this hypothesis. Our results provide supporting evidence for the role of LPC-16 and $I_{K_r}/HERG$ in ischemic $[K^+]_o \uparrow$ and $APD \downarrow$, and suggest that both LPC-16 and $I_{K_r}/HERG$ are the pharmacological targets for the treatment of ischemic cardiac electrical disturbance or arrhythmias.

Materials and Methods

Global Myocardial Ischemic Model

New Zealand white rabbits weighing 2.2–2.8 kg of either sex were sedated with a subcutaneous injection of ketamine (50 mg/kg) and xylazine (4 mg/kg) and anesthetized to a surgical plane with intravenous administration of sodium pentobarbital (30 mg/kg). Heparin (1,000 units/kg) was delivered intravenously. Heart was then rapidly excised after thoracotomy and placed in cold heparinized Tyrode's solution (4°C).

The aorta was immediately cannulated in a heated chamber filled with humidified room air and the hearts were perfused retrograde in the Langendorff perfusion apparatus at 75 mmHg constant perfusion pressure with modified Tyrode's solution of the following composition (in mM): 130 NaCl, 4 KCl, 1 CaCl₂, 1 MgCl₂, 24 NaHCO₃, 1.2 NaH₂PO₄, and 10 glucose. The perfusate was maintained at 35–37°C by a temperature transducer (Physitemp TH8) and oxygenated with 95% O₂ and 5% CO₂. The cava and pulmonary veins were sutured closed and the pulmonary artery was cannulated in order to obtain samples of venous effluent under anaerobic conditions. The perfusion rate was continuously monitored with a flow meter (transonic T206) and maintained at 30 ml/min for all baseline control periods. The pressure in the aorta was measured with a pressure transducer (WPI BLRP) connected to a strain gauge

amplifier (EMKA technologies).

Experimental Protocols

To mimic global acute ischemia, the perfusion flow was reduced from 30 ml/min to 5 ml/min (1/6 or 17% of baseline control) for 15 min under normothermic condition. The hearts were then subjected to the following experimental procedures.

(1) Baseline recording (30 min, 30 ml/min) — Ischemia (15 min, 5 ml/min) — Reperfusion (30 ml/min);

(2) Baseline recording (30 min, 30 ml/min) — K^+ channel blockers (30 ml/min) — Ischemia (15 min, 5 ml/min) — Reperfusion (30 ml/min);

(3) Baseline recording (30 min, 30 ml/min) — LPC-16 (30 ml/min) — Reperfusion (30 ml/min);

(4) Baseline recording (30 min, 30 ml/min) — K^+ channel blockers (30 ml/min) — Blockers + LPC (3 μ M, 15 min, 30 ml/min) — Reperfusion (30 ml/min).

For experiments involving K^+ channel blockers, drugs were administered by a Harvard apparatus pump at a rate of 3 ml/min (1/10 dilution). Lysophosphatidylcholine (LPC-16) was purchased from Avanti Polar Lipid Inc (Alabaster, Alabama) and glibenclamide (GA) was purchased from Sigma. Dofetilide (Dof, $I_{K_r}/HERG$ blocker) is a gift from Pfizer, and chromanol 293B (293B, I_{K_s} blocker, 20 μ M) and HMR1556 (3R, 4S)-(+)-N-[-3-hydroxy-2,2-dimethyl-6-(4,4,4-trifluorobutoxy)chroman-4-yl]-N-methyl-ethanesulfonamide, I_{K_s} blocker, 1 μ M) were supplied by Aventis. The drug concentrations were set based on our previous and preliminary experiments, as well as on the studies from other laboratories, being around their respective IC_{50} values. LPC-16 was dissolved directly into the bath solution at the desired concentrations immediately before each experiment [21, 22].

Measurement of QT Interval

ECG was monitored on a data acquisition system (EMKA) continuously throughout the experiment. QT interval was measured on lead II (the negative lead positioned on the base of the right ventricle and the positive lead was inserted at the apex of the heart) from QRS onset to the end of the T wave; biphasic T waves were measured to the time of final baseline return. These QT measurements and simultaneously recorded RR interval were used to derive heart rate corrected QT interval using Bazett's formula ($QT_c = QT/(RR)^{0.5}$) [23]. ECG was recorded at a sampling rate of 1 kHz with a low pass filter of 0.5 kHz and a notch filter of 60 Hz. The software ECGauto 1.5.7 was used to analyze ECG and ECG parameters were averaged from measurements of three consecutive complexes.

Measurement of $[K^+]_o$

Effluent (8 ml) was collected every 5 min throughout the whole experimental period. K^+ content was measured by a K^+ sensitive electrode (FastFil Combination Model 44, Lazar Research Laboratory Inc). A standard curve was constructed with a series of concentrations of KCl solution. The readings (in mV) were obtained with a pH meter (Accumet Basic AB15, Fisher Scientific) and the values were converted to mM concentration in a semilogarithmic paper.

Myocyte Isolation

The enzymatic isolation procedures were the same as previously described [24-27]. Rabbit hearts were used for action potential recordings and guinea pig hearts were used for I_{Kr} recordings because I_{Kr} in rabbit myocytes was too small in its amplitude to allow for detailed analysis. The hearts were quickly removed and mounted to a Langendorff perfusion system. The preparation was perfused with Ca^{2+} -containing Tyrode solution at 37°C until the effluent being clear of blood, and then switched to Ca^{2+} -free Tyrode solution (mM: 136 NaCl, 4.7 KCl, 1 $MgCl_2$, 0.33 NaH_2PO_4 , 5 HEPES, 10 glucose and 1 $CaCl_2$; pH 7.4) for 20 min at a constant rate of 12 ml/min, followed by perfusion with the same solution containing collagenase (110 U/ml CLS II collagenase; Worthington Biochemical) and 0.1% bovine serum albumin for ~30 minutes. The dispersed cells from the left ventricular endocardium were stored at 4°C in the medium containing (mM): 20 KCl, 10 KH_2PO_4 , 25 glucose, 70 potassium glutamate, 10 β -hydroxybutyric acid, 20 taurine, 10 EGTA, 0.1% albumin and 40 mannitol (pH 7.4). Use of animals (rabbit and guinea pig) was in accordance with the regulations of the ethic committees of Harbin Medical University.

Cell Culture

HEK293 cells stably expressing HERG (a kind gift from Drs. Z Zhou and C January) [28] were seeded in a 25 cm² triangular cell-culture flask and grown in Dulbecco's Modified Eagle's Medium supplemented with 10% heat-inactivated FBS, 200- μ M G418, 100-U/ml penicillin and 100- μ g/ml streptomycin. The cells subcultured to ~85% confluency were harvested by trypsinization and stored in Tyrode solution containing 0.5% BSA at 4°C. Electrophysiological recordings were conducted within 10hrs of storage.

Patch-Clamp Techniques

The procedure was the same as described in detail elsewhere [25-27, 29]. Briefly, a small aliquot of the solution containing the isolated cells were placed in a 1-ml chamber mounted on the stage of an inverted microscope. The cells were perfused at 5ml/min with the standard extracellular solution (36°C). Ionic currents and action potentials (APs) were recorded with whole-cell patch-clamp methods in the voltage-clamp mode and the current-clamp mode, respectively, using an Axopatch-200B amplifier (Axon Instruments). Borosilicate glass electrodes (1 mm o.d.) had tip resistances of 1-3 M Ω when filled with pipette solution of the following composition (mM): 110 potassium aspartate, 20 KCl, 1 $MgCl_2$, 5 Mg-ATP, 0.1 GTP, 10 EGTA, 5 phosphocreatine, 10 HEPES, pH adjusted to 7.3 with KOH. Command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP6 software (Axon Instruments). Junction potentials were zeroed before formation of the membrane-pipette seal in Tyrode solution containing (in mM): 136 NaCl, 4.7 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 HEPES (pH 7.4). The capacitance and series resistance were both electrically compensated to minimize the duration of the capacitive surge on the current recording and the voltage drop across the clamped cell membrane.

For myocyte experiments, recordings were made under conditions which minimized the potentially contaminating

currents. Contamination by sodium current was prevented by holding the cell at -50 mV. Cadmium chloride (200 μ M) was used to inhibit Ca^{2+} current as well as Ca^{2+} -activated chloride current. K_{ATP} , if any, was suppressed by glibenclamide (10 μ M) in the perfusate and by 5 mM Mg-ATP in the pipette. I_{Kr} was recorded in the continuous presence of chromanol 293 (10 μ M) block I_{Ks} . Acute application of drugs was achieved by bath perfusion. Current recordings taken under control conditions were repeated every 5 minutes up to a total of 15min after drug superfusion, followed by another 15min after addition of dofetilide (1 μ M). I_{Kr} amplitude was measured as dofetilide-sensitive currents. For group comparisons, data were expressed as current density calculated by dividing the current amplitude by the cell capacitance.

Data Analysis

Group data are expressed as mean $\pm$ S.E. Statistical comparisons (performed using ANOVA followed by Dunnett's method) were carried out, and paired or unpaired *t*-test was used, as appropriate, for single comparisons, using Microsoft Excel. A two-tailed *p* < 0.05 was taken to indicate a statistically significant difference. Nonlinear least square curve fitting was performed with CLAMPFIT in pCLAMP 8.0 or GraphPad Prism.

Results

Role of I_{Kr} /HERG in $[K^+]_o$ - $\uparrow$ Induced by Ischemia or by Exogenous LPC-16

The K^+ concentration in the perfusate ($[K^+]_o$) was 4 mM and the $[K^+]_o$ measured from the effluent collected 30min after stabilization with normal perfusion rate (30ml/min) was 3.9 ± 0.1 mM. $[K^+]_o$ was elevated when the perfusion rate was reduced to 5ml/min to mimic global myocardial ischemia and this $[K^+]_o$ - $\uparrow$ developed rapidly and reached a steady-state (10.5 mM) within 10min of ischemia, as shown in Fig. 1A. Administration with dofetilide (20 nM) did not significantly alter $[K^+]_o$ under control conditions, but prevented ischemic $[K^+]_o$ - $\uparrow$ (Fig. 1B). By comparison, blockers for I_{Ks} and K_{ATP} , 293B (50 μ M) and GB (glibenclamide, 10 μ M), respectively, both failed to affect the ischemic $[K^+]_o$ - $\uparrow$. The efficacies of these blockers were validated in isolated single rabbit ventricular myocytes; 293B at 50 μ M produced 92% reduction of I_{Ks} and GB at 10 μ M abolished K_{ATP} (data not shown).

Coincidentally, perfusion with LPC-16 produced concentration-dependent $[K^+]_o$ - $\uparrow$, similar to that seen with ischemia. The EC_{50} calculated with the Hill equation was 2.3 μ M (Fig. 1C). Hence, 3 μ M LPC-16 was used for the rest of the Langendorff study. LPC-16 caused a time-dependent $[K^+]_o$ - $\uparrow$ with a maximum effect occurring 10min after perfusion; $[K^+]_o$ was increased from 4 mM

Fig. 1. Role of I_{Kr} enhancement in extracellular K^+ concentration ($[K^+]_o$ -↑) induced by global ischemia or by lyso-phosphatidylcholine (LPC-16). $[K^+]_o$ was measured with a K^+ -sensitive electrode with the effluent of Langendorff-perfused rabbit hearts. (A) Time course of $[K^+]_o$ -↑ when perfusion rate was reduced to 5ml/min from the normal perfusion rate (30 ml/min). (B) Effects of K^+ channel blockers on ischemic $[K^+]_o$ -↑. Dof: dofetilide (20 nM, I_{Kr} /HERG inhibitor); 293B: chromanol 293B (50 μ M, I_{Ks} inhibitor); GB: glibenclamide (10 μ M, K_{ATP} inhibitor). Note that $[K^+]_o$ -↑ was prevented only in the hearts pretreated with Dof but not with other blockers studied. * p <0.05 vs. Control (Ctl); n =4 hearts for each group. $[K^+]_o$ -↑ induced. (C) Concentration-dependent $[K^+]_o$ -↑ induced by LPC-16 with normal perfusion rate. (D) $[K^+]_o$ -↑ as a function of time in the presence of LPC-16 (3 μ M). (E) Effects of K^+ channel blockers on $[K^+]_o$ -↑ induced by LPC-16. Dof: dofetilide (20 nM); 293B: chromanol 293B (50 μ M); GB: glibenclamide (10 μ M). Note that $[K^+]_o$ -↑ was prevented only in the hearts pretreated with Dof but not with other blockers studied. * p <0.05 vs. Control (Ctl); n =5 hearts for each group.

under baseline control to 7.3 mM in the presence of LPC-16 (Fig. 1D). Application of I_{Kr} blocker dofetilide but not I_{Ks} and K_{ATP} blockers prevented the $[K^+]_o$ -↑ induced by LPC-16 (Fig. 1E).

Role of I_{Kr} /HERG in QTc-? Induced by Ischemia or by Exogenous LPC-16

Parallel to $[K^+]_o$ -↑ during ischemia or in the presence of exogenous LPC-16, QT interval and the heart-rate corrected QT interval (QTc) were consistently shortened (Figs. 2 and 3). The extent of QTc-↓ depended upon LPC-16 concentration (Fig. 3C). Intriguingly, QTc-↓ failed to reach a steady-state within 15min low-rate perfusion (5ml/min, Fig. 3A) or 15min with LPC-16 perfusion (Fig. 3D). Perfusion with 20 nM dofetilide prevented the QTc-? induced by either ischemia (Fig. 3B) or LPC-16 (Fig. 3E). In contrast, the I_{Ks} and K_{ATP} blockers did not affect the ischemic QTc-? (Fig. 3B) and LPC-16-induced QTc-↓ (Fig. 3E).

I_{Kr} /HERG blocker Suppresses Arrhythmias Induced by Ischemia or Exogenous LPC-16

Arrhythmias were induced by either global ischemia (low perfusion rate of 5 ml/min) or LPC-16 (3 μ M) and they were mainly ventricular tachycardia (VT), defined as runs of at least five consecutive QRS complex on ECG, occurring within the first 10min of low rate perfusion or LPC-16 application. VT was observed in all 4 hearts undergone global ischemia and in 3 out of 4 hearts treated with LPC-16 (Fig. 4). VT occurred primarily within the

first 10min after ischemia (average of 10 episodes) or LPC-16 perfusion (4 episodes), and within the next 10min VT incidence considerably decreased with only one episode in ischemic hearts and none in LPC-16-treated hearts. When pretreated with dofetilide (20 nM), only two out of four ischemic hearts had an average of one incidence of VT, and none of the five hearts treated LPC-16 showed any arrhythmias.

Enhancement of I_{Kr} /HERG by LPC-16 Accounts for APD-↓

To investigate the cellular mechanisms for the observed QTc-↓, we assessed the effects of LPC-16 on single cell AP in isolated rabbit ventricular myocytes. As illustrated in Fig. 5, LPC-16 (3 μ M) applied to the superfusion solution substantially shortened APD and the shortening was prevented by co-application with dofetilide (20 nM) that when applied alone caused striking prolongation of APD.

The above data suggest that LPC-16 shortens APD by enhancing the function of I_{Kr} /HERG. To examine this notion, we performed whole-cell action potential-clamp (AP-clamp) experiments in HERG expressing HEK293

Fig. 2. Shortening of QT interval (QT-↓) induced by global ischemia or by LPC-16. Shown are typical examples of ECG recordings showing QT-↓ during ischemia (slow perfusion rate, 5 ml/min) or in the presence of LPC-16 (3 μ M) and abolition of QT-↓ by dofetilide (Dof, 20 nM).

cells. AP waveforms, recorded in myocytes from various regions of the canine heart, including ventricular endocardium, mid-myocardium and right atrium, were used as command pulses to elicit HERG current (I_{HERG}). As depicted in Figure 6A-6C, LPC-16 (3 μ M) increased I_{HERG} with various AP waveforms. Noticeably, the LPC-16 effect was greatest in myocytes with the shortest action potential duration, in an order of atrial AP > endocardial AP > mid-myocardial AP. Intriguingly, APD is shortest in atrial AP, followed by endocardial AP and then by mid-myocardial AP. This implies that the shorter the APD, the greater the enhancement of I_{HERG} by LPC-16. If it is true then more pronounced I_{HERG} enhancement is expected with briefer APD of APs from the same cell type. This was indeed verified by applying two endocardial APs in tandem, one recorded at 2.5 Hz with shorter APD and the other at 0.5 Hz with longer APD. As shown in Figure 6D, I_{HERG} enhancement was markedly greater with the shorter AP than with the longer AP. This point was further addressed by the data in Figure 6E where APD₅₀ and APD₉₀ (APD to 50% and 90% full repolarization, respectively) and percent increases in I_{HERG} are shown. Correlation between I_{HERG} increase and APD was analyzed by plotting the former as a function of the latter and by the linear regression. The goodness of fit was

0.997 for APD₅₀ and 0.992 for APD₉₀, with a 95% confidence range of 41 to 85 for APD₅₀ and of 25 to 141 for APD₉₀.

Enhancement of I_{Kr} by LPC-16 was verified in native myocytes isolated from guinea pig ventricular endocardium. A series of voltage steps ranging from -40 mV to +60 mV were delivered to inactivate I_{Kr} , followed by a repolarizing pulse to -40 mV to reactivate I_{Kr} so as to observe I_{Kr} tail currents. The dofetilide-sensitive current when plotted as a function of inactivation pulses (depolarizing prepulses) shows characteristics of I_{Kr} (Fig. 7A and 7B). The current reached a steady-state (maximum) level from potentials positive to -10 mV. Inclusion of LPC-16 (3 μ M) in the bathing solution for 10-15 min caused a pronounced increase in the tail current that was reversible upon subsequent washout of LPC-16. When the I_{Kr} tail currents were normalized to the maximum value to obtain conductance (G) curve, an approximately 5 mV negative shift of I_{Kr} activation in the presence of LPC-16 was revealed. This negative shift resulted in greater increases in I_{Kr} at more negative potentials or at the potentials closer to resting membrane potentials in ischemic myocardium. For example, at -30 mV, LPC-16 produced 136% enhancement of I_{Kr} , but the effect decreased to

Fig. 3. Role of I_{Kr} enhancement in shortening of heart rate-corrected QT interval (QTc ↓) during global ischemia (IS) or induced by LPC-16. (A) Time course of QTc ↓ when perfusion rate was reduced to 5 ml/min from the normal perfusion rate (30ml/min). (B) Effects of K^+ channel blockers on ischemic QTc ↓. Dof: dofetilide (20 nM, I_{Kr} /HERG inhibitor); 293B: chromanol 293B (50 μ M, I_{Ks} inhibitor); GB: glibenclamide (10 μ M, K_{ATP} inhibitor). Note that QTc ↓ was prevented only in the hearts pretreated with Dof but not with other blockers studied. * $p < 0.05$ vs. Control (Ctl); $n = 4$ hearts for each group. (C) QTc ↓ in the presence of various concentrations of LPC-16. (D) QTc ↓ as a function of time with 3 μ M LPC-16. (E) Effects of K^+ channel blockers on LPC-16-induced QTc ↓. Dof: dofetilide (20 nM, I_{Kr} /HERG inhibitor); 293B: chromanol 293B (50 μ M, I_{Ks} inhibitor); GB: glibenclamide (10 μ M, K_{ATP} inhibitor). Note that QTc ↓ was prevented only in the hearts pretreated with Dof but not with other blockers studied. * $p < 0.05$ vs. Control (Ctl); $n = 5$ hearts for each group.

Fig. 4. Arrhythmias induced by ischemia or LPC-16 (3 μ M) and the effects of I_{Kr} /HERG blocker dofetilide (20 nM). Ventricular tachycardia (VT), defined as runs of at least five consecutive QRS complex on ECG, occurring within the first 10 min of low rate perfusion (5 ml/min) (A) or LPC-16 application (B). Upper panels are examples of VT during ischemia or in presence of LPC-16 and lower panels show averaged data of VT episodes. Note that in the hearts pretreated with dofetilide (Dof), arrhythmias were abolished. * $p < 0.05$ vs. Control (Ctl); + $p < 0.05$ vs. ischemia or LPC-16; $n = 4$ hearts for each group.

only 35% at +20 mV. The same pattern of actions of LPC-16 was consistently seen with the cloned HERG channel expressed in HEK293 cells (Fig. 7C): negative shift of activation and voltage-dependent increase with markedly more pronounced enhancement at the potentials near the resting potential of ischemic heart (145% increase at -40 mV vs. 34% increase at +20 mV).

Discussion

Extracellular K^+ accumulation in myocardium and APD shortening due to abnormally enhanced K^+ efflux are a critical cause of arrhythmogenesis during the early stage of acute myocardial ischemia. Despite the paramount importance of understanding $[K^+]_o$ ↑ and

Fig. 5. Shortening of action potential duration (APD $\downarrow$) induced by LPC-16 and potential role of I_{K_r} . Action potentials were recorded in isolated single ventricular myocytes from left endocardial wall of rabbits. (A) APD $\downarrow$ induced by LPC-16 (3 μ M). Measurements made under baseline control conditions (Ctl) were repeated 10min after superfusion with LPC-16. Upper panels: typical examples of action potentials; lower panels: averaged data from 10 cells. APD $_{50}$ and APD $_{90}$: action potential duration at 50% and 90% repolarization, respectively. (B) Effect of dofetilide (Dof, 20 nM) on APD $\downarrow$ induced by LPC-16 (3 μ M). Cells were pretreated with Dof for 10min before co-application with LPC-16. Note Dof alone prolonged APD and prevented APD $\downarrow$ induced by LPC-16. Dash lines indicate 0 mV potential. * p <0.05 vs. Ctl.

APD $\downarrow$ for the treatment of ischemic electrical disorders, the intrinsic pathways through which K^+ flows out of the cell remained incompletely defined and the ionic mechanism for ischemic $[K^+]_o\uparrow$ and APD $\downarrow$ is still a puzzle to cardiologists and scientists. Similarly, metabolic perturbation of ischemic myocardium is known to have a great impact on electrical disturbance. Among the various metabolic insults, the rapid accumulation of phospholipid metabolite LPC in the ischemic myocardium is of pivotal importance. However, while LPC has long been recognized as a biochemical trigger of ischemic arrhythmias, how LPC causes $[K^+]_o\uparrow$ and APD $\downarrow$ remained poorly understood. The results of the present study may improve our understanding of these issues. First, exogenous LPC-16 causes $[K^+]_o\uparrow$ and APD $\downarrow$ /QTc $\downarrow$ and the associated arrhythmias as seen in the low-perfusion ischemia and the effects are reversed by I_{K_r} /HERG block. Second, I_{K_r} /HERG blocker dofetilide is highly effective in preventing ventricular tachyarrhythmias induced by low-perfusion ischemia or by exogenous LPC-16. Third, LPC-16 enhances I_{K_r}/I_{HERG} and the effects are greater with shorter APD and at more negative potentials. These results indicate that enhancement of I_{K_r}/I_{HERG} by LPC-16 contributes to $[K^+]_o\uparrow$ and APD $\downarrow$ /QTc $\downarrow$ and the associated arrhythmias during acute myocardial ischemia. In other

words, HERG K^+ channel forms one of the pathways and LPC-16 acts as a trigger for abnormal intracellular K^+ loss or K^+ efflux in ischemic myocardium.

ATP-sensitive K^+ current (K_{ATP}) is gated by intracellular ATP and regulated by a variety of cellular metabolites, and it is naturally considered a sensor of the metabolic state of a myocardial cell, and activation of these channels is considered an indicator of cellular metabolic compromise [30-32]. However, K_{ATP} channels do not open unless intracellular ATP level ($[ATP]_i$) drops down to less than 100 μ M; the IC_{50} for K_{ATP} inhibition is <100 μ M. This implies that K_{ATP} channels normally stay in closed state because the physiological $[ATP]_i$ is in the order of millimolar range (5 mM). This is perhaps why the existing data regarding the role of K_{ATP} in ischemic $[K^+]_o\uparrow$ have been conflicting. Evidence exists that K_{ATP} channel mediates ischemic K^+ efflux and APD $\downarrow$; K_{ATP} inhibitors such as glibenclamide have been found to effectively reverse ischemia/hypoxia APD $\downarrow$ and the rate of $[K^+]_o\uparrow$ [6, 31-33]. However, there are also experimental findings against K_{ATP} channel as a mediator of ischemic $[K^+]_o\uparrow$ and APD $\downarrow$ [7, 34-38]. First, the measured $[ATP]_i$ at the time of metabolic blockade of acute ischemia is still in the millimolar range which is far too high to induce massive K_{ATP} [39, 40]. Second, the time-course of K_{ATP} channel opening is far too slow to

Fig. 6. Enhancement of HERG current (I_{HERG}) by LPC-16 in HERG-expressing HEK293 cells. I_{HERG} was recorded by action potential (AP)-clamp techniques with AP waveforms obtained from single canine myocytes of different regions. (A-C) Typical examples of APs and the respective I_{HERG} from endocardium (Endo), mid-myocardium (Mid) and atrium (A), showing enhancement of I_{HERG} by LPC-16 (3 μM). Note LPC-16 increases I_{HERG} more with AP waveforms of shorter duration. (D) Direct comparison of LPC-16-induced I_{HERG} enhancement between short and long APs. Note LPC-16 increases I_{HERG} more with shorter APD. (E) Comparison between APD_{50} and APD_{90} (Upper) and I_{HERG} enhancement (Lower). * $p < 0.05$ vs. atrium (A); $n = 5$ cells. (F) Correlation between I_{HERG} enhancement by LPC-16 and APD_{50} (Upper) or APD_{90} (Lower).

explain the start of $[\text{K}^+]_o \uparrow$ within the first minute of ischemia. Third, the results from K_{ATP} openers and inhibitors have been conflicting; early ischemic $[\text{K}^+]_o \uparrow$ and surface acidification are relatively insensitive to K_{ATP} channel inhibition or activation prior to the ischemic insult [36] and K_{ATP} opener pinacidil [41]. Finally, some blockers of voltage-gated K^+ channels are effective in preventing and reversing ischemic $[\text{K}^+]_o \uparrow$ and $\text{APD} \downarrow$; these include diltiazem [42]. Moreover, K_{ATP} was found to be inhibited by a variety of lipid metabolites such as lysophospholipids and arachidonic, linoleic, linolenic, and eicosatrienoic free fatty acids, which accumulate in ischemic myocardium [43]. The role of K_{ATP} channel in response to myocardial ischemia, therefore, has been uncertain. It is thus conceivable that other mechanisms or more specifically other K^+ currents, in addition to K_{ATP} , may also contribute to $[\text{K}^+]_o \uparrow$ and $\text{APD} \downarrow$ in ischemic myocardium.

Conductance of $\text{K}_{\text{Kr}}/\text{HERG}$ is known to critically depend upon extracellular K^+ activity, being enhanced with increasing extracellular K^+ activity [18, 44]. It is conceivable that an increase in $\text{K}_{\text{Kr}}/\text{HERG}$ in response to LPC-16 during ischemia results in increase in K^+ efflux. The increased extracellular K^+ activity as a result of

enhanced $\text{K}_{\text{Kr}}/\text{HERG}$ in turn promotes $\text{K}_{\text{Kr}}/\text{HERG}$ by enhancing the channel conductance, a positive feedback mechanism. Alternatively, the enhancement of K_{Kr} and the consequent shortening of APD result in abnormal electrical activities such as ventricular tachycardia, which in turn contributes to elevated K^+ efflux from the cells.

Our data demonstrate that cardiac membrane repolarization facilitates the $\text{K}_{\text{Kr}}/\text{HERG}$ -enhancing effect of LPC-16. This notion is supported by the following data. First, the $\text{K}_{\text{Kr}}/\text{HERG}$ -enhancing effect of LPC-16 is ~2-fold greater with shorter AP or faster repolarization rate (Fig. 6). Considering that APD is shortened during acute ischemia or in the presence of LPC-16, the $\text{K}_{\text{Kr}}/\text{HERG}$ -enhancing effect of LPC-16 is expected to be further promoted, likely being another positive feedback mechanism. Second, also significant is the fact that the $\text{K}_{\text{Kr}}/\text{HERG}$ -enhancing effect of LPC-16 is ~3-fold greater at negative or repolarized potentials near the resting membrane potential of the ischemic heart, relative to depolarized potentials positive to 0 mV (Fig. 7). This would imply that LPC-16 can cause an increase in K^+ efflux not only during an excitation but also during diastolic period. The concentrations of LPC-16 used in this study are within

Fig. 7. Enhancement of native rapid delayed rectifier K^+ current (I_{Kr}) in left ventricular endocardial myocytes isolated from guinea pigs, and cloned I_{HERG} in HEK293 cells, by LPC-16. I_{Kr} was elicited by the voltage protocol shown in the inset. (A) Typical examples of I_{Kr} traces recorded under control conditions, in presence of LPC-16 (5 μ M), and in presence of LPC-16 + dofetilide (Dof, 20 nM). Chromanol 293B (10 μ M) was continuously present in the superfusate to block I_{Ks} . The enhancement of I_{Kr} by LPC-16 is revealed by the LPC-16-sensitive currents obtained by digital subtraction between the current with and without LPC-16. Dofetilide, a specific I_{Kr} /HERG blocker was used to verify the native I_{Kr} . (B) Averaged data ($n=8$ cells) showing the reversible increase in I_{Kr} tail amplitude (left panel), the negative shift of activation curves (middle panel), and the voltage-dependence of percent increase in I_{Kr} (right panel), by LPC-16. The activation curves were constructed by plotting the conductance G as a function of depolarizing potentials. G was calculated by normalizing the tail currents at -40 mV by dividing the amplitude of the tail currents measured at various antecedent depolarizing potentials by that of the tail current at +60 mV. Symbols are mean of experimental data and lines represent the Boltzmann fit: $G/G_{max} = 1/[1 + \exp[(V_{1/2} - V)/k]]$, where G_{max} represents the maximal conductance at +60 mV, $V_{1/2}$ is a half-maximal activation voltage, and k is a slope factor. W/O: washout. $*p < 0.05$ LPC-16 vs. Ctl at potentials from -30 mV to +60 mV. (C) Averaged data ($n=6$ cells) showing the increase in I_{HERG} tail amplitude (left panel), the negative shift of activation curves (middle panel), and the voltage-dependence of percent increase in I_{HERG} (right panel), by LPC-16. $*p < 0.05$ LPC-16 vs. Ctl at potentials from -40 mV to +40 mV.

the range of its level found in ischemic myocardium [21, 22] and the effects of LPC-16 are readily reversible upon washout of LPC-16 or by co-application with dofetilide.

Based on our data, we are tempted to propose that there is a rapid ionic remodeling process for ischemic

$[K^+]_o \uparrow$ and $APD \downarrow / QTc \downarrow$. Enhanced function of HERG and other ion channels such as K_{ATP} , due to metabolic perturbations (such as accumulation of LPC) during acute ischemia, causes rapid ischemic $[K^+]_o \uparrow$ and $APD \downarrow / QTc \downarrow$, and the latter in turn further enhances

the function of HERG channel by directly increasing HERG conductance and by indirectly increasing the effect of LPC-16 on HERG amplitude. And this subsequent increase in HERG function will then go on to amplify $[K^+]_o$ - $\uparrow$ and APD - $\downarrow$ / QTc - $\downarrow$. Therefore, in addition to the paired ATP depletion/ K_{ATP} activation, LPC accumulation/HERG enhancement may be considered as another link between metabolic trigger and ionic pathway, for ischemic $[K^+]_o$ - $\uparrow$ and QTc - $\downarrow$.

Our findings that LPC-16 acts as one of the metabolic triggers, and HERG as one of ionic pathways, for ischemic $[K^+]_o$ - $\uparrow$ and QTc - $\downarrow$ and that ischemic arrhythmias is preventable and convertible by I_{Kr} /HERG blocker dofetilide suggest that inhibition of LPC-16 production and accumulation and/or of I_{Kr} /HERG may be a promising therapeutic strategy to attenuate the incidence of lethal arrhythmias associated with ischemic heart disease [45–47]. This notion warrants further investigation. However, it should be noted that our study does not provide definite link between LPC-16 and I_{Kr} /HERG due to current lack of LPC-16 inhibitors. The global ischemic model used in this study may not applicable to myocardial infarction in patients and to animal models of local ischemia.

Abbreviations

APD (action potential duration); APD_{50} and APD_{90} (APD to 50% and 90% full repolarization); APD - $\downarrow$ (APD shortening); QT - $\downarrow$ (QT interval shortening); LPC (lysophosphatidylcholine); LPC-16 (1-palmitoyl-lysophosphatidylcholine); HERG (human *ether-a-go-go* related gene encoding a K^+ channel); I_{HERG} (current generated by HERG channels); $[K^+]_o$ - $\uparrow$ (extracellular K^+ accumulation); I_{Kr} (rapid delayed rectifier K^+ current).

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