L-type calcium current recovery versus ventricular repolarization: preserved membrane-stabilizing mechanism for different QT intervals across species

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BACKGROUND Long QT syndrome is associated with early afterdepolarization (EAD) that may result in torsade de pointes (TdP). Interestingly, the corrected QT interval seems to be proportional to body mass across species under physiologic conditions.

OBJECTIVE The purpose of this study was to test whether recovery of L-type calcium current ($I_{Ca,L}$), the primary charge carrier for EADs, from its inactivated state matches ventricular repolarization time and whether impairment of this relationship leads to development of EAD and TdP.

METHODS Transmembrane action potentials from the epicardium, endocardium, or subendocardium were recorded simultaneously with a transmural ECG in arterially perfused left ventricular wedges isolated from cow, dog, rabbit, and guinea pig hearts. $I_{Ca,L}$ recovery was examined using action potential stimulation in isolated left ventricular myocytes.

RESULTS The ventricular repolarization time (action potential duration at 90% repolarization [APD_{90}]), ranging from 194.7 ± 1.8 ms in guinea pig to 370.2 ± 9.9 ms in cows, was linearly related to the thickness of the left ventricular wall among the species studied. The time constants (τ) of $I_{Ca,L}$ recovery were proportional to APD_{90}, making the ratios of τ to APD_{90} fall into a relatively narrow range among these species despite markedly different ventricular repolarization time. Drugs with risk for TdP in humans were shown to impair this intrinsic balance by either prolongation of the repolarization time and/or acceleration of $I_{Ca,L}$ recovery, leading to the appearance of EADs capable of initiating TdP.

CONCLUSION An adequate balance between $I_{Ca,L}$ recovery and ventricular repolarization serves as a "physiologic stabilizer" of ventricular action potentials in repolarization phases.

KEYWORDS Long QT syndrome; Arrhythmia; Ion channels; Myocytes

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Introduction

Long QT syndrome, either inherited or acquired (e.g., drug-induced), is a disease characterized by an excessively prolonged QT interval on body surface ECG. It often manifests clinically as recurrent syncope or sudden cardiac death as the result of atypical polymorphic ventricular tachycardia, known as torsade de pointes (TdP).[1–4] Interestingly, corrected QT intervals (i.e., QT intervals corrected on heart rate) in various species seem to be proportional to body mass under physiologic conditions, ranging from tens of milliseconds in mice to hundreds of milliseconds in large animals.[5] Prolongation of the action potential duration (APD) in small animals may lead to the occurrence of EADs and arrhythmia, whereas in larger species, the same length of or longer APDs can be physiologically normal. Apparently, there is a mechanism that stabilizes cell membrane potentials in large species so that a normal heart rhythm is maintained with a relatively longer QT interval.

It is widely accepted that TdP is triggered by the early afterdepolarization (EAD), that is, unstable and fluctuating membrane potentials in the plateau phase of the ventricular action potential, under conditions of an excessively prolonged QT interval.[1,6–8] Therefore, the occurrence of EADs in ventricular action potentials can be used as a marker to indicate whether a prolonged QT interval is physiologic or pathophysiologic. Because the L-type calcium current ($I_{Ca,L}$) is the primary charge carrier for EAD under delayed ventricular repolarization[9,10] and the key excitatory current responsible for excitation–contraction coupling in the heart, we speculated that the major contributor to stabilization of physiologically long action potentials might be the reduced availability of $I_{Ca,L}$ at action potential phases 2 and 3. To

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test this hypothesis, we examined the time course of $I_{Ca,L}$ recovery from inactivation in ventricular myocytes and compared it with the corresponding ventricular repolarization time in guinea pigs, rabbits, dogs, and cows. We found that an adequate ratio of $I_{Ca,L}$ recovery time constant (τ) to repolarization time serves as a "physiologic stabilizer" of the ventricular cell membrane potentials in repolarization phases 2 and 3.

Materials and methods

Isolated arterially perfused cardiac wedge preparation and electrophysiologic recordings. Use of animals in this project was approved by the Institutional Animal Care and Use Committee (IACUC) at the Lankenau Institute for Medical Research. Surgical preparation of the left or right ventricular wedge from the canine and rabbit has been described in detail previously.[7,11,12] The procedures for the bovine ventricular wedge preparation were essentially the same as those for the canine and rabbit wedges. For guinea pig ventricular wedges, because of the smaller sizes of the hearts, cannulation was performed via the left main coronary artery instead of its branches. After cannulation, the left anterior descending coronary artery was ligated so that the dissected left ventricular wedge would be perfused through the left circumflex artery, similar to the large animal wedges. For rabbit atrial wedge preparations, a branched artery that supplies the atria from the right or left coronary artery was used for cannulation. In all wedge preparations, the cannulation procedure required 4 to 5 minutes. Unperfused tissue was carefully removed using a razor blade.

The preparation was placed in a small tissue bath and perfused with Tyrode's solution (35.7°C ± 0.1°C, perfusion pressure 35–45 mmHg). Approximately 1 hour of perfusion was allowed for the tissue to equilibrate. The preparation was stimulated at the endocardial surface at a basic cycle length (BCL) of 1,000 ms or 2,000 ms via bipolar silver electrodes. Transmembrane action potentials were recorded simultaneously from epicardial, subendocardial, and endocardial sites using two or three intracellular floating microelectrodes. For rabbit atrial and right ventricular wedges, action potentials were recorded only from the epicardium and endocardium because of the thin walls. Action potential duration at 90% repolarization (APD_{90}) was measured to reflect the repolarization time. A transmural ECG signal was recorded in all experiments using extracellular Ag/AgCl electrodes placed in the bath solution. Electrodes were placed 1.0 to 1.5 cm from the epicardial and endocardial surfaces, along with the same vector as the transmembrane recordings.

Left and right ventricular wall thicknesses were measured at the base, middle portion, and apex of the lateral wall. The values then were averaged. Atrial thickness was measured from the lateral wall.

Myocyte isolation and recording of $I_{Ca,L}$

Subendocardial myocytes from left ventricles of cows and dogs and endocardial myocytes from left ventricles of rabbits an guinea pigs were isolated using enzymologic methods as described previously.[13] Cells were aliquoted into a recording chamber and superfused with a bath solution of the following composition (in mM): 140 NaCl, 5 CsCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, and 10 HEPES; pH adjusted to 7.4 with NaOH. $I_{Ca,L}$ was recorded at 35.7°C ± 0.1°C, a temperature identical to that at which action potentials were recorded in wedge preparations, using the whole-cell patch clamp technique. Axopatch-1C (Axon Instruments, Burlingame, CA, USA) was interfaced with a personal computer through a TL-1 DMA interface. pClamp-8 software (Axon Instruments) was used for data acquisition and analysis. Electrodes were prepared from borosilicate glass capillaries with tip resistance of 2 to 3 MΩ when filled with pipette solution of the following composition (in mM): 80 CsCl, 40 CsOH, 1 $MgCl_2$, 20 tetraethylammonium chloride, 10 EGTA, and 10 HEPES; pH adjusted to 7.25 with CsOH. Currents were filtered at 1 kHz and sampled at 2.5 kHz. In all experiments, junction potentials were adjusted to zero before seal formation and checked again at the end of each experiment. Series resistances were compensated electronically to the maximal extent before oscillation would occur.

The time course of $I_{Ca,L}$ recovery was examined using a double-pulse protocol similar to previous methods.[14–16] Because $I_{Ca,L}$ recovery from inactivation not only is time dependent but also voltage dependent,[16,17] the configuration of action potential phases 1 and 2 may influence the inactivation and recovery processes of the channel. Therefore, assessment of the kinetics of $I_{Ca,L}$ recovery was performed using conventional square-pulse voltage clamp technique as well as action potential stimulation method.[15,16] In conventional voltage clamp experiments, cells were held at −80 mV and then depolarized to −40 mV for 25 ms to inactivate I_{Na} and T-type calcium channels. The first test pulse (P_1) of 150 ms at +25 mV then was given, followed by the second pulses (P_2) after various intervals. The action potential stimulating protocol was similarly designed, except that an action potential tracing, which was obtained from the left ventricular wedge preparation of the respective species, served as the first command pulse (P_1).

In order to record $I_{Ca,L}$ without contamination from other membrane currents, K^+ currents were minimized by replacing K^+ with Cs^+ in both bath and pipette solutions and adding 20 mM tetraethylammonium chloride to the pipette solution (see reference Xu et al[18]). Na^+ and T-type calcium current were inactivated during initial depolarization at −40 mV prior to P_1.[15] The inward currents elicited by P_1 and P_2 could be completely blocked by the calcium channel blocker nicardipine (5 μmol/L), supporting that potential contaminations of other ionic currents had been minimized under these experimental conditions. To investigate $I_{Ca,L}$ recovery kinetics, interpulse intervals (P_1–P_2) were ranged from 5 to 665 ms or longer to allow P_2-elicited $I_{Ca,L}$ to reach a steady state. The ratios of peak current I_{P2} to I_{P1} were plotted on the ordinate as a function of the interpulse inter-