Cellular basis for the electrocardiographic and arrhythmic manifestations of Timothy syndrome: Effects of ranolazine

Serge Sicouri, MD,* Katherine W. Timothy,† Andrew C. Zygmunt, PhD,* Aaron Glass,* Robert J. Goodrow, BA,* Luiz Belardinelli, MD,‡ Charles Antzelevitch, PhD*

*From *Masonic Medical Research Laboratory, Utica, New York, †University of Utah, Salt Lake City, Utah, and ‡CV Therapeutics, Inc., Palo Alto, California.*

BACKGROUND Timothy syndrome is a multisystem disorder associated with QT interval prolongation and ventricular cardiac arrhythmias. The syndrome has been linked to mutations in $Ca_V1.2$ resulting in gain of function of the L-type calcium current ($I_{Ca,L}$). Ranolazine is an antianginal agent shown to exert an antiarrhythmic effect in experimental models of long QT syndrome.

OBJECTIVE The purpose of this study was to develop and characterize an experimental model of Timothy syndrome by using BayK8644 to mimic the gain of function of $I_{Ca,L}$ and to examine the effects of ranolazine.

METHODS Action potentials from epicardial and M regions and a pseudo-electrocardiogram (ECG) were simultaneously recorded from coronary-perfused left ventricular wedge preparations, before and after addition of BayK8644 (1 μM).

RESULTS BayK8644 preferentially prolonged action potential duration of the M cell, leading to prolongation of the QT interval and an increase in transmural dispersion of repolarization (from 44.3 $\pm$ 7 ms to 86.5 $\pm$ 25 ms). Stimulation at cycle lengths of 250–500 ms led to ST-T wave alternans due to alternation of the plateau voltage of the M cell action potential as well as development of delayed afterdepolarizations in epicardial and M cell action potentials. Ventricular extrasystoles and tachycardia (monomorphic, bidirectional, or torsades de pointes) developed spontaneously or after rapid pacing. Peak and late I_{Na} were unaffected by BayK8644. Clinically relevant concentrations of ranolazine (10 μM) suppressed all actions of BayK8644.

CONCLUSION A left ventricular wedge model of long QT syndrome created by augmentation of $I_{Ca,L}$ recapitulates the ECG and arrhythmic manifestations of Timothy syndrome, which can be suppressed by ranolazine.

KEYWORDS Long QT syndrome; Sudden cardiac death; Arrhythmias; Ion channelopathy; Syndactyly; Left ventricle; Inherited syndrome

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Introduction

Timothy syndrome, also referred to as syndactyly-associated long QT syndrome (LQTS) or LQT8, is a multisystem disorder characterized by developmental defects causing dysmorphic facial features including round face, flat nasal bridge, receding upper jaw, thin upper lip, and webbing of the toes and fingers (syndactyly).[1] The disorder also is associated with prolongation of the QT interval, development of ventricular arrhythmias, and sudden cardiac death. The syndrome has recently been linked to a missense mutation in $Ca_V1.2$, which encodes for the α subunit of the L-type calcium channel, resulting in a gain of function of the L-type calcium current ($I_{Ca,L}$).[1]

Calcium loading has been shown to contribute to the development of both the trigger [early afterdepolarizations [EADs] and delayed afterdepolarizations (DADs)] and substrate (transmural dispersion of repolarization [TDR]) for torsades de pointes (TdP). Thus, the gain of function in $I_{Ca,L}$ is expected to be associated with a high risk for TdP in this form of LQTS.

Ranolazine is a novel antianginal agent capable of producing anti-ischemic effects at plasma concentrations of approximately 1.5–8 μM with minimal or no changes in heart rate or blood pressure.[2] In addition to its anti-ischemic effects, ranolazine has been shown to be effective in suppressing arrhythmogenesis in experimental models of LQTS, particularly LQT2 and LQT3.[3–5] The antiarrhythmic efficacy of ranolazine has been attributed to its potent block of late sodium channel current (late I_{Na}).[4] Inhibition of late I_{Na} lessens the prolongation of the action potential (AP) of the M cell, the cell type in which late I_{Na} is most prominent, thus limiting the increase in TDR and the development of EADs.

In the present study, we used the calcium channel agonist BayK8644 to mimic the gain of function of $I_{Ca,L}$ in an attempt to create an experimental model of Timothy syndrome and to elucidate the cellular basis for the ECG fea-

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tures and arrhythmias responsible for sudden cardiac death in this variant of LQTS. We also tested the hypothesis that ranolazine can effectively suppress the arrhythmias observed in the BayK8644 model of Timothy syndrome.

Methods

Dogs weighing 20–35 kg were anticoagulated with heparin (180 IU/kg) and anesthetized with pentobarbital (35 mg/kg IV). The chest was opened via left thoracotomy, and the heart was excised and placed in a cold cardioplegic solution ($[K^+]_o$ = 8 mmol/L, 4°C). Fourteen animals were used in this study. Nine of 14 wedges were included in the study. Five wedge preparations displayed persistent ST-segment depression indicating the presence of ischemia somewhere in the preparation and were discarded. A total of nine left ventricular (LV) wedge preparations were exposed to BayK8644 to create a model of Timothy syndrome. All protocols were in conformance with guidelines established by the Institutional Animal Care and Use Committee.

Arterially perfused canine LV wedge preparations

Transmural LV wedges with dimensions of approximately 12 mm × 35 mm × 12 mm were dissected from the mid-to-basal anterior region of the LV wall, and a diagonal branch of the left anterior descending coronary artery was cannulated to deliver the perfusate (Tyrode's solution). The composition of the Tyrode's solution was as follows (in mM): NaCl 129, KCl 2 or 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, and D-glucose 5.5. Intracellular APs were recorded from epicardial and subendocardial M cell regions using floating microelectrodes. A transmural pseudo-electrocardiogram (ECG) was recorded using two Ag/AgCl half cells (2 mm diameter × 4 mm) placed approximately 1 cm from the epicardial (+) and endocardial (−) surfaces of the preparation and along the same axis as the intracellular recordings. Four intramural unipolar electrograms were recorded using stainless steel wires (120-μm diameter). Ventricular wedges were allowed to equilibrate in the chamber for 2 hours while pacing was performed at basic cycle length (BCL) of 2,000 ms using silver bipolar electrodes contacting the endocardial surface. Perfusion pressure was maintained at 40–50 mmHg and temperature at 37° ± 0.5°C. The preparations were fully immersed in the extracellular solution throughout the course of the experiment.

Epicardial APs were recorded from the epicardial surface of the wedge preparation, and the reported M cell is the one with the longest AP obtained along the same axis as the ECG electrodes. *Transmural dispersion of repolarization* was defined as the difference between the longest and the shortest repolarization times [activation time plus action potential duration (APD) measured at 90% repolarization (APD_{90})] of intracellular APs recorded across the wall (typically M cell minus epicardial cell repolarization time). *QT interval* was defined as the time interval between QRS onset and the point at which the line of maximal downslope of the T wave crossed the isoelectric line.

Canine LV myocyte dissociation

Myocytes were isolated by enzymatic dissociation.[6] Adult mongrel dogs of either sex (≥1 year old, weight 20–25 kg) were anesthetized with sodium pentobarbital (35 mg/kg IV), and their hearts were quickly removed and placed in HEPES-buffered Tyrode's solution containing 2 mM $CaCl_2$. All procedures followed Institutional Animal Care and Use Committee guidelines. The left descending circumflex artery was cannulated and flushed with Ca-free HEPES-buffered Tyrode's solution supplemented with 0.1% bovine serum albumin (fraction V, Sigma-Aldrich Co, St. Louis MO) and gassed with 100% O_2 for 5 minutes at a rate of 12 mL/min. Perfusion was then switched to Tyrode's solution containing 1 mg/ml bovine serum albumin and 0.1 mg/ml collagenase (CLS 2, Worthington, Lakewood, NJ, USA) for 5–11 minutes at 37°C (100% O_2, with recirculation). Following perfusion, thin slices of tissues were dissected from the midmyocardium using a dermatome. Midmyocardial cells were stored in Tyrode's solution (see following) containing 0.5 mM Ca^{2+} and 1.5% bovine serum albumin at room temperature for later use. HEPES-buffered Tyrode's solution contained the following (in mM): NaCl 145, KCl 4, $MgCl_2$ 1, HEPES 10, and glucose 10; pH adjusted with NaOH to 7.4.

Whole-cell patch clamp experiments

APs were measured using the perforated patch technique.[7] Experiments were performed on cells that had been allowed to adhere to the polylysine-coated floor of a 0.5-mL chamber mounted in a stage heater (model PDMI-2, Harvard Apparatus, Holliston, MA, USA) on a Nikon Eclipse microscope. The chamber was perfused with Tyrode's solution at 37°C at a rate of 2–3 mL/min before and during gigaseal formation, after which the appropriate experimental solution replaced the Tyrode's solution. APs were recorded using a Multiclamp 700A amplifier with a CV-7A head stage (Axon Instruments–Molecular Devices Corp., Union City, CA, USA). Stimulations were delivered and data acquired using a Digi-Data 1322A computer interface and the pClamp 9 program suite (Axon Instruments). Data were acquired at 10 kHz and filtered at 2 kHz.

Cells were stimulated with 3-ms square current pulses of 2.5–2.8 nA. Cells were rested for 15 seconds before evoking a train of 30 APs at 800-ms BCL, followed immediately by five APs at a faster rate, picked to produce stable DADs or alternans of APD in the presence of 1 μM BayK8644. In eight cells in which either DADs >2 mV or alternans of APD were observed, this BCL ranged from 290–450 ms. Once these rates were established for an individual midmyocardial myocyte, the protocol was repeated in the presence of 1 μM BayK8644 and 10 μM ranolazine.

Voltage clamp

Whole-cell currents were measured using the ruptured membrane patch technique. Experiments were performed on cells that had been allowed to adhere to the polylysine-