Cellular basis for electrocardiographic and arrhythmic manifestations of Andersen-Tawil syndrome (LQT7)

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BACKGROUND Andersen-Tawil syndrome, a skeletal muscle syndrome associated with periodic paralysis and long QT intervals on the ECG, has been linked to defects in KCNJ2, the gene encoding for the inward rectifier potassium channel (I_{K1}.)

OBJECTIVES The purpose of this study was to examine the cellular mechanisms underlying the ECG and arrhythmic manifestations of Andersen-Tawil syndrome.

METHODS To investigate the effects of KCNJ2 loss-of-function mutations responsible for Andersen-Tawil syndrome, we used barium chloride ($BaCl_2$) to inhibit I_{K1} in arterially perfused wedge preparation. Transmembrane action potentials (APs) were simultaneously recorded from endocardial, midmyocardial, and epicardial cells, together with a transmural ECG.

RESULTS $BaCl_2$ (1 to 30 μM) produced a concentration-dependent prolongation of the QT interval, secondary to a homogeneous prolongation of AP duration of the three cell types. QT interval was prolonged without an increase in transmural dispersion of repolarization (TDR). Low extracellular potassium (2.0 mM), isoproterenol (20–50 nM), and an abrupt increase in temperature (36°C–39°C) in the presence of 10 μM $BaCl_2$ did not significantly increase TDR but increased ectopic extrasystolic activity. Early afterdepolarizations were not observed under any condition. Spontaneous torsades de pointes arrhythmias were never observed, nor could they be induced with programmed electrical stimulation under any of the conditions studied.

CONCLUSION Our results provide an understanding of why QT prolongation associated with Andersen-Tawil syndrome is relatively benign in the clinic and provide further support for the hypothesis that the increase in TDR, rather than QT interval, is responsible for development of torsades de pointes.

KEYWORDS Long QT syndrome; Sudden cardiac death; Arrhythmias; Ion channelopathy; Ventricle

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Introduction

Studies have linked mutations in KCNJ2, which encodes the inward rectifier potassium channel I_{K1}, to Andersen-Tawil syndrome, also referred to as Andersen syndrome of the LQT7 form of the long QT syndrome.[1-6] This skeletal muscle syndrome is associated with periodic paralysis often linked to fluctuations in plasma potassium levels.[2,7,8] Clinical studies indicate that Andersen-Tawil syndrome may be associated with arrhythmias, particularly when aggravated by other health problems such as infection.[3]

The cellular basis for the ECG and arrhythmogenic manifestations of Andersen-Tawil syndrome are not well defined. The present study was designed to develop and characterize an experimental model of Andersen-Tawil syndrome using canine left ventricular wedge preparations. Our protocols are designed to define the effects of reduced I_{K1} (using barium chloride [$BaCl_2$]) on transmural repolarization gradients, the morphology of the ECG T wave, the appearance of early afterdepolarization (EAD)-induced triggered activity, and the development of spontaneous and programmed electrical stimulation-induced torsades de pointes arrhythmias. We also assessed the modulatory influence of rate, temperature, adrenergic stimulation, and extracellular potassium levels.

Methods

Dogs weighing 20 to 30 kg were anticoagulated with heparin and anesthetized with pentobarbital 30 to 35 mg/kg IV.

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The chest was opened via left thoracotomy. The heart was excised, placed in cold (4°C–10°C) Tyrode's solution, and transported to a dissection tray. Preparations with dimensions of 25 × 10 × 10 mm to 32 × 17 × 15 mm were dissected from the left ventricle. The tissue was cannulated via a diagonal branch of the left anterior descending coronary artery and perfused with cold Tyrode's solution. Unperfused tissue was removed carefully using a razor blade or fine dissecting scissors. The preparation was placed in a small tissue bath and perfused with Tyrode's solution of the following composition (in mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 1, and glucose 5.5, bubbled with 95% O_2 and 5% CO_2 (37°C ±0.2°C). The perfusate was delivered to the artery by a roller pump (Cole Parmer Instrument Co., Niles, IL, USA). Perfusion pressure was monitored with a pressure transducer (World Precision Instruments, Sarasota, FL, USA) and maintained at 50 ± 5 mmHg by adjusting the perfusion flow rate. The preparations remained immersed in the perfusate, which was allowed to rise to a level at least 5 mm above the tissue surface to avoid a temperature gradient between the cut surface and epicardial and endocardial surfaces of the preparation. Ventricular wedge preparations were allowed to equilibrate in the tissue bath until they were electrically stable for 1 hour and stimulated with bipolar silver electrodes applied to the endocardial surface at a basic cycle length of 2,000 ms. A transmural ECG was recorded using silver electrodes placed in the Tyrode's solution bathing the preparation, 10 to 15 mm from the epicardial and endocardial surfaces, along the same axis as the transmembrane recordings. The QT interval was measured as the time between QRS onset and the point at which the line of maximal slope of the final segment of the T wave crossed the isoelectric line. Transmembrane APs were recorded simultaneously from the endocardial, midmyocardial, and epicardial sites using three separate floating microelectrodes (DC resistance 10–20 MΩ; 3 mM KCl). Endocardial and epicardial APs were recorded from the endocardial and epicardial surfaces of the preparations at positions approximating the transmural axis of the ECG recording. Midmyocardial AP was recorded from the cut surface 20 ± 5% from the endocardium.[9] Action potential duration (APD) was measured at 50% repolarization (APD_{50}) and 90% (APD_{90}) repolarization. Activation time was measured as the interval between the stimulus artifact and upstroke of AP. Transmural dispersion of repolarization (TDR) was defined as the difference between the longest and shortest repolarization times (activation time + APD_{90}) of transmembrane APs recorded across the wall.

Study protocols

We initially examined the concentration-dependent effects of $BaCl_2$ (1–30 μM), which led us to a $BaCl_2$ concentration of 10 μM for the Andersen-Tawil syndrome model. Baseline recordings were obtained after 1 hour of equilibration. AP and ECG characteristics were evaluated at cycle lengths of 4,000, 2,000, 1,000, and 500 ms in the absence and presence of 10 μM $BaCl_2$. We attempted to induce ventricular tachycardia (VT) with programmed electrical stimulation, up to two extrastimuli applied to the epicardial surface site of briefest refractoriness. In the case of single extrastimuli, S1-S2 was abbreviated under the refractory period was reached (roughly equivalent to APD_{90}). In the case of double extrastimuli, we used an S1-S2 of 180 or 200 ms and then abbreviated S2-S3 until the refractory period was reached.

To examine the influence of hyperthermia, we abruptly increased the temperature of the perfusate from 36°C to 39°C within 5 to 6 minutes in the presence of 10 μM $BaCl_2$. We continuously recorded the ECG and APs of three cell types for at least 15 minutes at a basic cycle length of 2,000 ms.

To examine the influence of adrenergic modulation, we applied isoproterenol (20–50 nM) for a 10-minute period to preparations pretreated with 10 μM $BaCl_2$. In most preparations, transient increase in T-wave amplitude was observed 3 to 4 minutes after isoproterenol administration, just before the increase in frequency of ectopic premature beats or development of monomorphic tachycardia. We report data of the ECG and APDs of the tissue layers recorded 3 to 4 minutes after isoproterenol administration as the dynamic state and those recorded 8 to 10 minutes after isoproterenol administration as steady state.

To examine the influence of $[K^+]_o$, we altered the concentration of potassium from 2.0 mM (low) through 4.0 mM (normal) to 6.0 mM (high) in the presence of 10 μM $BaCl_2$. Data were collected 8 to 10 minutes after introduction of low, normal, or high concentration of potassium at a basic cycle length of 2,000 ms.

Statistical analysis

Statistical analysis of the data was performed using the Student's t-test for paired data or one-way repeated analysis of variance (ANOVA) followed by Bonferroni test, as appropriate. Data are given as mean ± SEM.

Results

Figure 1A shows the concentration-dependent effects of barium chloride. The QT interval and APD of three cell types display a similar concentration-dependent prolongation. I_{K1} block caused very significant slowing of phase 3 of the AP, resulting in flattening and widening of the T wave. Under control conditions, epicardial repolarization was always coincident with the peak of the T wave, full repolarization of the M cell coincided with the end of the T wave, and Tpeak-Tend correlated with TDR. This relationship was disrupted following exposure to $BaCl_2$.