Estradiol promotes sudden cardiac death in transgenic long QT type 2 rabbits while progesterone is protective

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BACKGROUND Postpubertal women with inherited long QT syndrome type 2 (LQT2) are at increased risk for polymorphic ventricular tachycardia (pVT) and sudden cardiac death (SCD), particularly during the postpartum period.

OBJECTIVE To investigate whether sex hormones directly modulate the arrhythmogenic risk in LQTS.

METHODS Prepubertal ovariectomized transgenic LQT2 rabbits were treated with estradiol (EST), progesterone (PROG), dihydrotestosterone (DHT), or placebo (OVX).

RESULTS During 8 weeks of treatment, major cardiac events—spontaneous pVT or SCD— occurred in 5 of the 7 EST rabbits and in 2 of the 9 OVX rabbits ($P < .05$); in contrast, no events occurred in 9 PROG rabbits and 6 DHT rabbits ($P < .01$ vs PROG; $P < .05$ vs DHT). Moreover, EST increased the incidence of pVT ($P < .05$ vs OVX), while PROG reduced premature ventricular contractions, bigeminy, couplets, triplets, and pVT ($P < .01$ vs OVX; $P < .001$ vs EST). In vivo electrocardiographic monitoring, in vivo electrophysiological studies, and ex vivo optical mapping studies revealed that EST promoted SCD by steepening the QT/RR slope ($P < .05$), by prolonging cardiac refractoriness ($P < .05$), and by altering the spatial pattern of action potential duration dispersion. Isoproterenol-induced Ca^{2+} oscillations resulted in early afterdepolarizations in EST-treated hearts (4 of 4), while PROG prevented SCD by eliminating this early afterdepolarization formation in 4 of the 7 hearts ($P = .058$ vs EST; $P < .05$ vs OVX). Analyses of ion currents demonstrated that EST increased the density of $I_{Ca,L}$ as compared with OVX ($P < .05$) while PROG decreased it ($P < .05$).

CONCLUSION This study reveals the proarrhythmic effect of EST and the antiarrhythmic effect of PROG in LQT2 in vivo, outlining a new potential antiarrhythmic therapy for LQTS.

KEYWORDS Long QT syndrome; Sex hormones; Arrhythmogenesis; Sudden cardiac death; Transgenic LQT2 rabbit model; Cardiac ion currents; Early afterdepolarization; In vivo electrophysiological study

ABBREVIATIONS **APD** = action potential duration; **AV** = atrioventricular; **DHT** = dihydrotestosterone; **EAD** = early afterdepolarization; **ECG** = electrocardiography; **EPS** = electrophysiological study; **EST** = estradiol; **ISO** = isoproterenol; **LQT2** = long QT syndrome type 2; **LQTS** = long QT syndrome; **LV** = left ventricular; **NCX** = sodium-calcium exchanger; **OVX** = ovariectomy and placebo-treatment; **PLN** = phospholamban; **PROG** = progesterone; **PVC** = premature ventricular contraction; **pVT** = polymorphic ventricular tachycardia; **RV** = right ventricular; **SCD** = sudden cardiac death; **SERCA2a** = sarcoplasmic reticulum calcium ATPase2a; **SF** = sham-operated female; **SM** = sham-operated male; **VERP** = ventricular effective refractory period; **VF** = ventricular fibrillation; **VT** = ventricular tachycardia

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Introduction

The inherited long QT syndrome (LQTS) is characterized by an impaired cardiac repolarization resulting in QT interval prolongation, polymorphic ventricular tachycardia (pVT), and sudden cardiac death (SCD).[1] Importantly, patients with LQTS exhibit pronounced gender differences in cardiac repolarization and their arrhythmogenic risk. Data from the international LQTS registry show longer QT intervals, a steeper QT/RR ratio, and a higher risk for pVT and SCD in postpubertal women with LQTS type 2 (loss of the rapidly activating delayed-rectifier potassium current I_{Kr}[2]).[3] In contrast, before puberty, the arrhythmia incidence is higher in boys.[4] Moreover, both the menstrual cycle and the postpartum period are associated with changes in the incidence of pVT. Patients with long QT syndrome type 2 (LQT2) have

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a reduced risk during pregnancy and a markedly increased risk during the postpartum period.[5,6] In addition, in the acquired drug-induced LQTS variant, the risk for pVT is higher during menses and the follicular phase with high serum estradiol (EST) levels than during the luteal phase with high progesterone (PROG) levels.[7] These observations strongly suggest a potential proarrhythmic effect of EST and an antiarrhythmic effect of PROG. However, these postulated proarrhythmic and antiarrhythmic sex hormone effects in LQTS have never been demonstrated in vivo and their underlying mechanisms are yet to be characterized.

We recently generated transgenic LQT2 rabbits overexpressing a loss-of-function pore mutation of the hERG channel (HERG-G628S) in the heart, mimicking the human LQT2 phenotype with QT-interval prolongation, steeper QT/RR ratio in female rabbits, spontaneous pVT, and SCD—with a particularly high incidence in the postpartum period.[8,9] Mechanisms underlying these arrhythmias include a pronounced spatial dispersion of action potential duration (APD) and dynamic APD changes that lead to discordant alternans,[8,10] as observed in patients with LQTS.[11] Here we demonstrate in prepubertal ovariectomized female LQT2 rabbits chronically treated with different sex hormones that EST and PROG have direct and contrasting effects on arrhythmias and SCD by modulating the arrhythmogenic substrate and the generation of triggered activity.

Methods

A detailed description of the methods that were used can be found in an accompanying online supplement.

Ovariectomy and hormone treatment

Prepubertal LQT2 rabbits underwent ovariectomy or sham surgeries, and 90-day release pellets (Innovative Research of America, Sarasota, Florida) containing 17β-EST, dihydrotestosterone (DHT), PROG, or placebo (OVX) were implanted subcutaneously, resulting in similar EST levels as during the follicular phase, PROG levels as in pregnant rabbits, and DHT levels as in male rabbits[12,13] (see online supplement Figure 1).

Telemetric electrocardiographic monitoring: QT/RR ratio and arrhythmia screening

Using telemetric electrocardiographic (ECG) devices (F70-EEE, Data Sciences International, St Paul, Minnesota), QT/RR ratio and heart rate–corrected QT indices were calculated.[8,9] Arrhythmias and major cardiac events—pVT and SCD—within corresponding 2-hour intervals were analyzed and classified by using Lown's classification.[14]

In vivo electrophysiological studies

Catheter-based in vivo electrophysiological studies (EPS) were performed to assess ventricular effective refractory periods (VERPs) in right ventricular apex (RV apex) and base at baseline and during isoproterenol (ISO) infusion (0.10−0.25 μg/min).[15]

Optical mapping

Dual voltage-calcium optical mapping (100 × 100 pixels, Ultima-L, Scimedia, Costa Mesa, California)[16] was performed by using fluorescence probes PGH1 for membrane potential (generously provided by Dr Guy Salama, University of Pittsburgh) and rhod-2 for Ca_i (Invitrogen, Grand Island, New York). Images were acquired from the left ventricular (LV) anterior surface, and the field of view was set to 1.5 × 1.5 cm with a spatial resolution of 150 × 150 μm.[28,16] To investigate the effects of hormones on early afterdepolarization (EAD) formation, hearts were exposed to an intracoronary ISO bolus (140 nM) after atrioventricular (AV) ablation.

Patch clamp

Whole-cell recordings in cardiomyocytes isolated from the LV apex were obtained with an Axopatch-200B amplifier (Axon Instruments, Sunnyvale, California) with standard patch-clamp techniques.[8]

Western blot

Western blot experiments on crude membrane preparations of the LV apex were performed[8] by using the following antibodies: anti–sarcoplasmic reticulum calcium ATPase2a (SERCA2a; Thermo Scientific [Waltham, Massachusetts], MA3-919), anti–phospholamban (PLN; Thermo Scientific, MA3-922), and anti–sodium-calcium exchanger (NCX; Thermo Scientific, MA3-926) as primary antibodies and Horseradish peroxidase (HRP)-conjugated goat-anti-mouse (immunoglobulin G polyclonal, Thermo Scientific) as secondary antibodies.

Statistical analysis

For normally distributed values, we used the Student *t* test (paired and unpaired). The χ^2 test was used for categorical variables. Analysis was performed with Prism 4.03 (Graphpad, La Jolla, California), and a P value of $\leq$.05 was considered significant.

Results

Sex hormone effects on arrhythmogenesis

To investigate the effects of hormones on arrhythmogenesis in LQTS, we treated prepubertal ovariectomized transgenic LQT2 rabbits with EST, PROG, DHT, or placebo (OVX) for 8 weeks. We first compared arrhythmia incidences within corresponding 2-hour intervals 1 week before and within 96 hours following EPS by using telemetric ECG monitoring (Figure 1A). In the week before EPS, no arrhythmias besides isolated sinus pauses occurred in either group. In the 96 hours after EPS, however, arrhythmia incidences were higher in all groups but varied significantly among groups. PROG significantly reduced the incidence of premature ventricular contractions (PVCs) and couplets compared with OVX and EST, and importantly, bigeminy and triplets did not occur in any PROG rabbit (Figure 1A), strongly indicating an antiarrhythmic effect in PROG rabbits. Moreover, no single episode of nonsustained or sustained pVT occurred in any PROG rabbit, further underlin-