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.

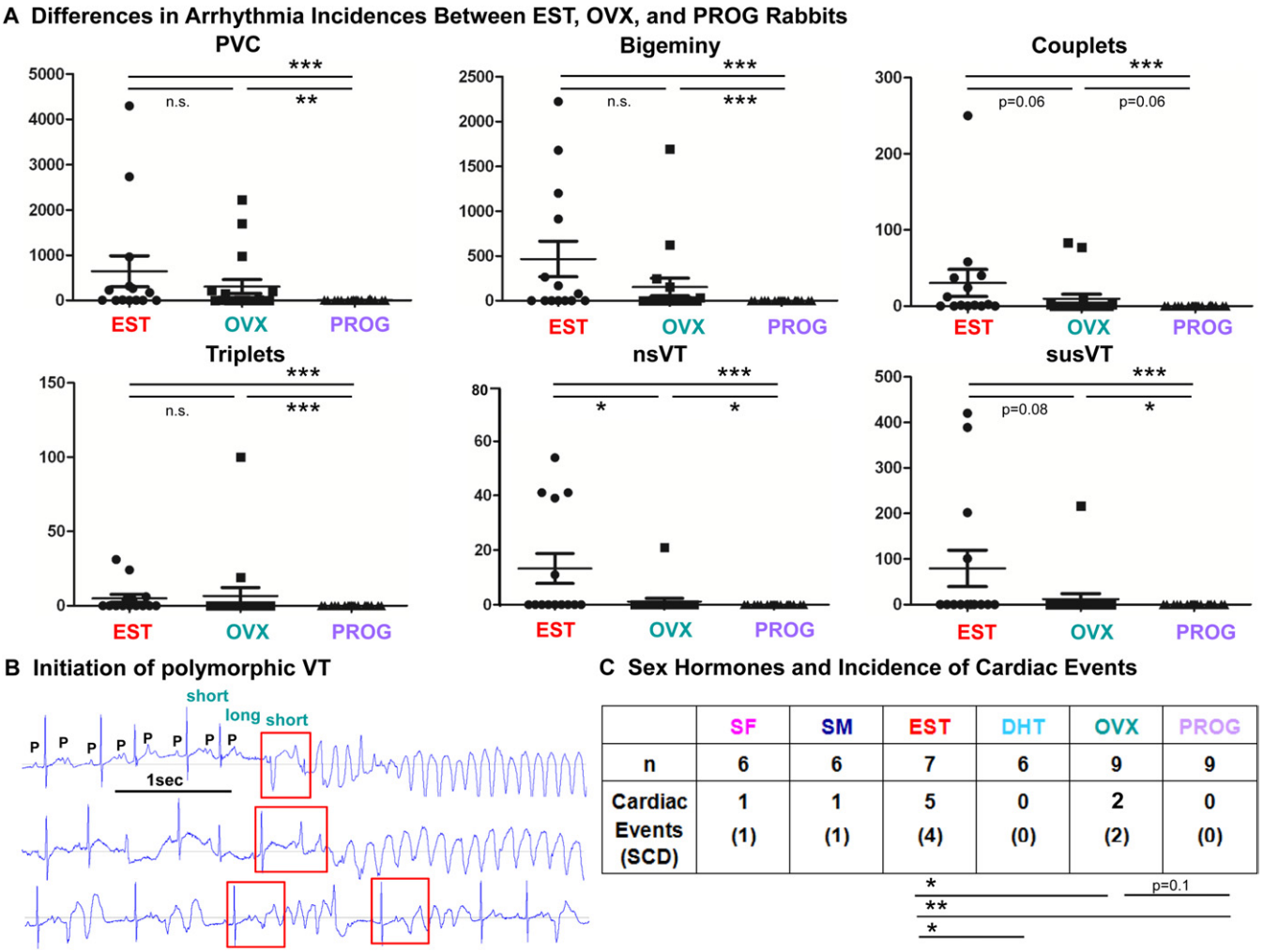
Statistical analysis

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-



	SF	SM	EST	DHT	OVX	PROG
n	6	6	7	6	9	9
Cardiac Events (SCD)	1 (1)	1 (1)	5 (4)	0 (0)	2 (2)	0 (0)

Figure 1 Effect of sex hormones on incidence of arrhythmias. **A:** Dot blots of differences in arrhythmia incidences (PVC, bigeminy, couplets, triplets, nsVT, all presented as beats per 2 hours; susVT, duration in seconds). Each dot represents a 2-hour interval of an individual rabbit (n = 14 in EST rabbits; n = 18 in OVX and PROG rabbits).*P <.05, **P <.01, ***P <.001. **B:** Telemetric ECG recordings of the initiation of lethal pVT in 2 EST rabbits (top 2 rows). Indicated are R-on-T (red square), short-long-short sequences, and P waves (P) during episodes of AV 2:1 block. Bottom row shows several episodes of nsVTs following couplets in an EST rabbit. **C:** Incidence of major cardiac events during 8 weeks of hormone treatment. Incidences of SCDs are indicated in parentheses. *P <.05, **P <.01. AV = atrioventricular; DHT = dihydrotestosterone; ECG = electrocardiographic; EST = estradiol; nsVT = nonsustained ventricular tachycardia; OVX = ovariectomy and placebo-treatment; PROG = progesterone; PVC = premature ventricular contraction; SCD = sudden cardiac death; susVT = sustained ventricular tachycardia.

ing an antiarrhythmic PROG effect (Figure 1A). The incidences of PVC and bigeminy were similar in EST and OVX rabbits. However, couplets tended to occur more often in EST rabbits (P = .06) and nonsustained ventricular tachycardia (VT) occurred significantly more frequently in EST rabbits than in OVX rabbits, suggesting a proarrhythmic EST effect. Of note, all lethal pVTs were initiated by short-long-short sequences and an early PVC coinciding with the T wave (R-on-T phenomenon), as described in patients with LQTS,[17] suggesting that EADs likely underlie arrhythmia initiation (Figure 1B). Moreover, prior to the initiation of pVTs, the heart rate slowed down because of short-long-short sequences and AV block in 5 of the 8 rabbits (Figure 1B). The incidence of AV blocks, however, did not differ between groups.

We consecutively ranked the severity of arrhythmias by using Lown's classification[14]: 9 of the 9 PROG rabbits had either Lown 0 (no PVCs) or Lown 1 (fewer than 30 PVCs per hour) arrhythmias, while 5 of the 9 OVX rabbits demonstrated at least Lown 3 arrhythmias (eg, bigeminy) (P <.01 vs PROG), and 3 of the 9 even had Lown 4 arrhythmias (eg, couplets, triplets, or nonsustained VT) (P = .05 vs PROG). In EST rabbits, the rate of Lown 4 arrhythmias (5 of 7) was even higher (P <.01 vs PROG).

Finally, we compared the incidence of major cardiac events—defined as spontaneous pVT and SCD—between hormone groups. In EST rabbits, the incidence of major cardiac events (5 of 7) was significantly higher than in OVX (2 of 9; P <.05), PROG (0 of 9; P <.01), and DHT (0 of 6; P <.05) rabbits (Figure 1C). Most lethal pVT occurred within 96 hours after EPS in rabbits that were fully awake, similar to observations in previous studies.[9,15] In a follow-up study of rabbits that were not exposed to anesthesia, 3 of the 13 EST rabbits died of pVT, contrasted with no

SCD in any other hormone group (0 of the 9 DHT, 0 of the 12 PROG, and 0 of the 12 OVX rabbits). All these observations strongly indicate a proarrhythmic effect of EST and an antiarrhythmic effect of PROG in LQT2 rabbits.

Sex hormone–induced changes in cardiac repolarization

To investigate mechanisms that account for these proarrhythmic and antiarrhythmic hormone effects, we first compared hormone effects on QT duration and QT/RR ratio in free-moving ECG-monitored LQT2 rabbits. Heart rate–corrected QT indices were significantly longer in EST than in OVX, PROG, or DHT rabbits and tended to be longer in sham-operated female (SF) rabbits than in sham-operated male (SM) rabbits ($P = .1$) (Figure 2B). These differences in QT duration were particularly pronounced at slow heart rates. As demonstrated in representative ECG tracings in Figure 2A, at RR intervals of 300 ms, the QT duration in EST rabbits was similar to that in SF rabbits while in DHT rabbits the QT interval was shortened as observed in SM rabbits. In OVX and PROG rabbits, the QT duration was intermediate. Furthermore, EST steepened the QT/RR slope (Figure 2C), mimicking the adult LQT2 female phenotype (Figure 2G). In contrast, DHT decreased the steepness of the QT/RR slope (Figure 2D), thus mimicking the male phenotype (Figure 2G). Similarly, OVX decreased the steepness of the QT/RR slope (Figure 2E), while PROG did not alter the QT/RR ratio (Figure 2F).

To further examine the effects of hormones on rate-dependent changes in QT and VERP, we performed in vivo EPS. Figures 3A and 3B depict representative ECG recordings in EST and PROG rabbits during VERP determination in the RV apex at a stimulation cycle length of 300 ms (S1 train). In the top panel, the coupled extra stimulus (S2) was captured, whereas in the lower panel, the 10-ms shorter S2 stimulus failed to capture (at 240 ms in EST and 150 ms in PROG rabbit). In the RV apex, VERPs were longer in EST than in

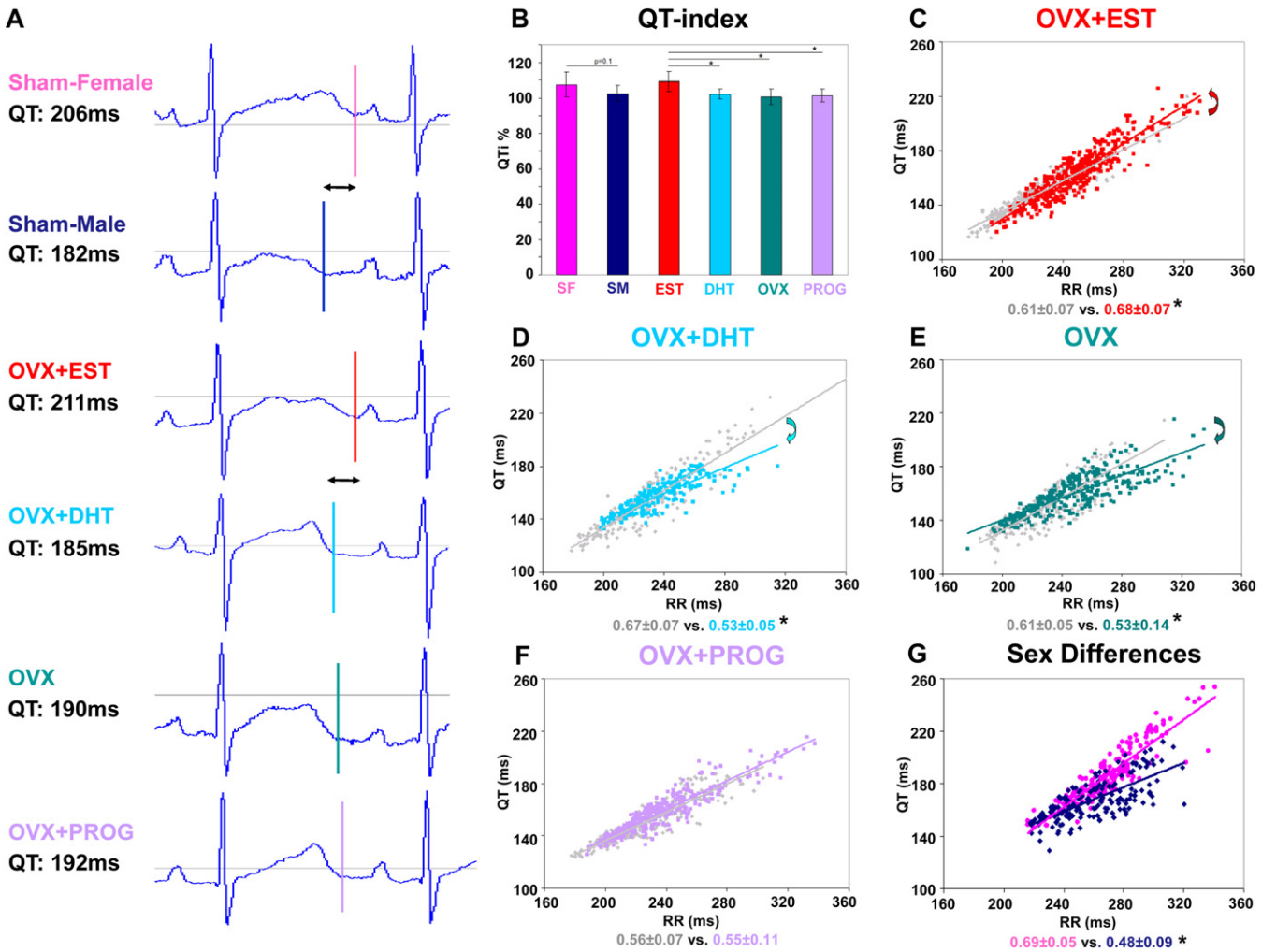


Figure 2 Effect of sex hormones on QT duration. **A:** Exemplary representative ECG traces of individual rabbits at 300-ms RR intervals. QT durations are indicated. **B:** QT indices in n = 6 rabbits after 4 weeks of hormone treatment calculated on the basis of QT and RR intervals acquired over 24 hours of ECG monitoring. *P <.05. **C–F:** QT/RR ratio in n = 6 rabbits at baseline (gray) and after 4 weeks of treatment (color). Arrows indicate the direction of changes in the QT/RR ratio. *P <.05. **G:** QT/RR ratio in n = 6 adult SF and SM rabbits. *P <.05. DHT = dihydrotestosterone; ECG = electrocardiographic; EST = estradiol; OVX = ovariectomy and placebo-treatment; PROG = progesterone; SF = sham-operated female; SM = sham-operated male.

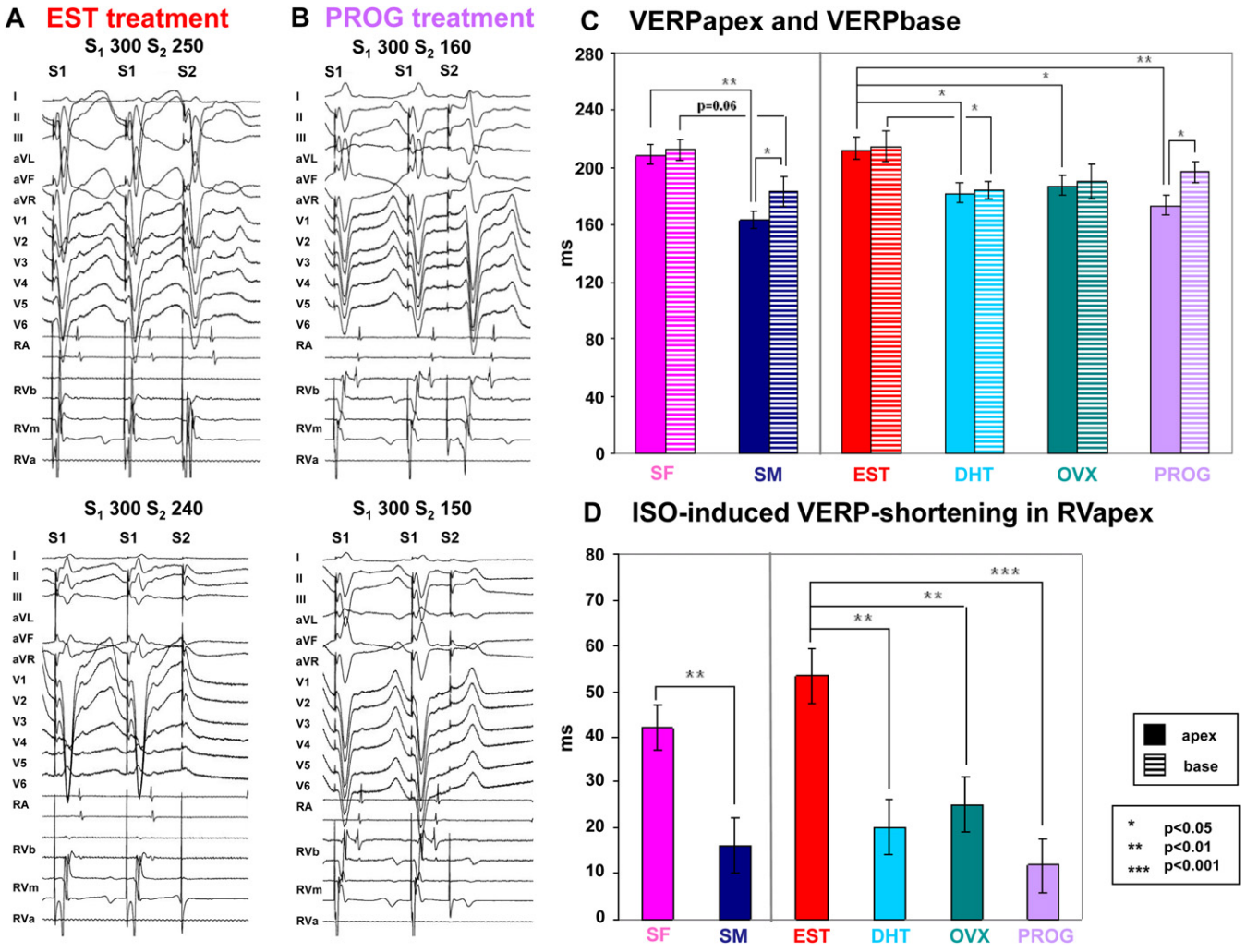


Figure 3 Effect of sex hormones on cardiac repolarization. **A–B:** Surface and intracardiac ECG in individual EST and PROG rabbits during VERP determination. Top to bottom: 12 surface ECG leads, RA (2 recordings), RV base (2 recordings), RV mid (2 recordings), and RV apex. Top panel shows stimulation with 300-ms cycle length (S1) and coupled extrastimuli (S2) that are captured. Lower panel shows shorter S2 extrastimuli that fail to capture. **C:** VERP in RV apex (filled bars) and base (hatched bars) in n = 6 rabbits per group. *P <.05, **P <.01. All values are shown as mean ± SD. **D:** ΔVERP baseline isoproterenol in the RV apex. **P <.01, ***P <.001. DHT = dihydrotestosterone; ECG = electrocardiographic; EST = estradiol; OVX = ovariectomy and placebo-treatment; PROG = progesterone; RV = right ventricular; SF = sham-operated female; SM = sham-operated male; RA = right atrium; VERP = ventricular effective refractory period.

OVX, PROG, or DHT rabbits (Figure 3C). In the RV base, however, the effects of hormones on the VERP were not as pronounced (Figure 3C). Continuous infusion of ISO shortened the VERP in SF rabbits more than in SM rabbits. Similarly, the ISO-induced VERP shortening was more pronounced in EST than in OVX, PROG, or DHT rabbits (Figure 3D).

Sex hormones and APD dispersion

We next investigated whether sex hormones modulate spatial APD dispersion—a known mechanism underlying arrhythmogenesis in LQT2 syndrome[11]—by using optical mapping. Figure 4A shows representative APD maps of the LV anterior surface of hormone-treated LQT2 rabbit hearts, with basal regions represented in the upper part of the image and apical regions in the lower part. Isolines of APD are drawn every 5 ms, and dark regions represent long APD while bright regions represent short APD. In all OVX, PROG, and DHT rabbits (n = 4−7 each), the region with the longest APD was found in the LV midseptal region and the shortest APD in the apex. Yet, EST altered the pattern of APD dispersion, and the longest APD region was shifted toward the LV apex. However, we found no significant differences in mean APD and APD dispersion among groups (Figure 4B). Programmed ventricular stimulation induced ventricular fibrillation (VF) in all 5 EST rabbits, contrasting with 3 of the 6 OVX (P <.05), 2 of the 7 PROG (P <.05), and 0 of the 4 DHT (P <.01) rabbits. In all EST rabbits, during VF, activation waves propagated around the apical region of prolonged APD. As illustrated in Figure 4C, activation waves encountered refractoriness in the apical region with prolonged APD, which caused unidirectional block and wave propagation around this region. Conduction velocity measured on the anterior surface of the left ventricle was similar in all groups (OVX: 0.55 ± 0.12 m/s; EST: 0.59 ± 0.2 m/s; PROG: 0.56 ± 0.1 m/s), indicating a lack of structural changes with hormone treatment.

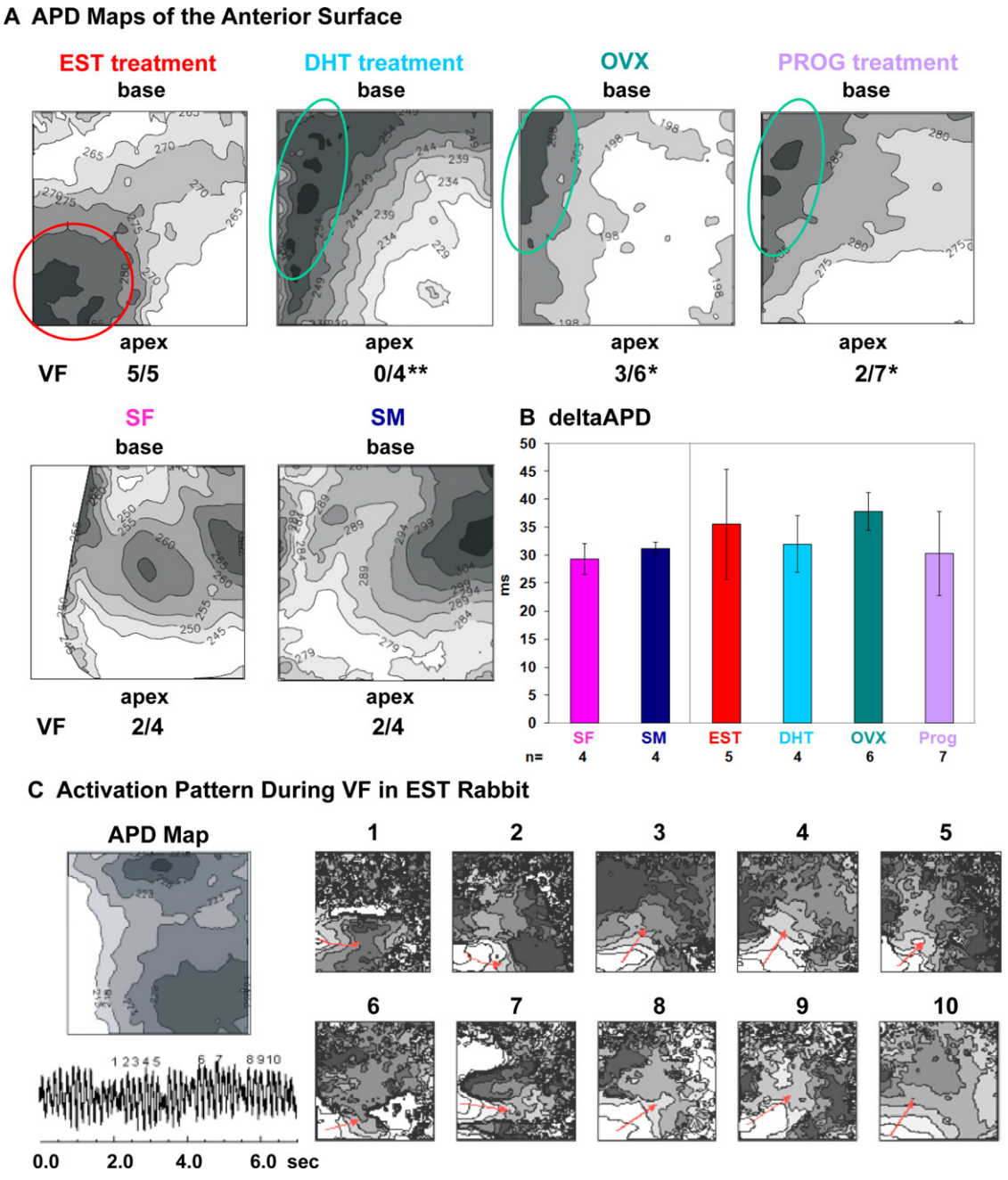


Figure 4 Effect of sex hormones on APD dispersion. **A:** Representative APD maps of the anterior surface of the left ventricle (field of view 1.5 × 1.5 cm) of individual rabbits. Isolines of APD are drawn every 5 ms; darker regions represent longer APD. Indicated are regions of long APD in the LV midbase region (green circle) and LV apex (red circle). Rates of VF inducibility are listed. *P <.05, **P <.01. **B:** ΔAPD defined as longest – shortest APD. All values are shown as mean ± SD. **C:** Activation pattern during VF in an EST rabbit. Displayed are APD map, ECG trace of VF (bottom left), and consecutive maps (1−10) of the activation pattern during VF. Red arrows indicate the direction of activation waves rotating around the apical region of prolonged APD. APD = action potential duration; DHT = dihydrotestosterone; ECG = electrocardiographic; EST = estradiol; LV = left ventricular; OVX = ovariectomy and placebo-treatment; PROG = progesterone; VF = ventricular fibrillation.

Sex hormones and EAD formation

Since it is well known that pVT is initiated by EADs triggered by sympathetic surge,[18] we examined the response to a bolus of ISO by using dual voltage-calcium optical mapping. In all EST (4 of 4), OVX (5 of 5), SF (4 of 4), and SM (4 of 4) rabbits, both membrane potentials and Ca^{2+} transients began to oscillate within 5−10 seconds after ISO bolus (Figure 5A). Ca^{2+} started to rise prior to changes in the membrane potential during the early phase of the EAD formation, yet in later EADs with larger amplitude the membrane potential changes led to Ca^{2+} rise (Figure 5A), suggesting that Ca^{2+} oscillations may be an important trigger for the EAD formation in this LQT2 model. In contrast, in 4 of the 7 PROG and 2 of the 4 DHT rabbits, Ca^{2+} transient oscillations failed to initiate EADs, resulting in a significantly reduced EAD formation rate in PROG rabbits (P = .058 vs EST; P <.05 vs OVX) (Figure 5B).

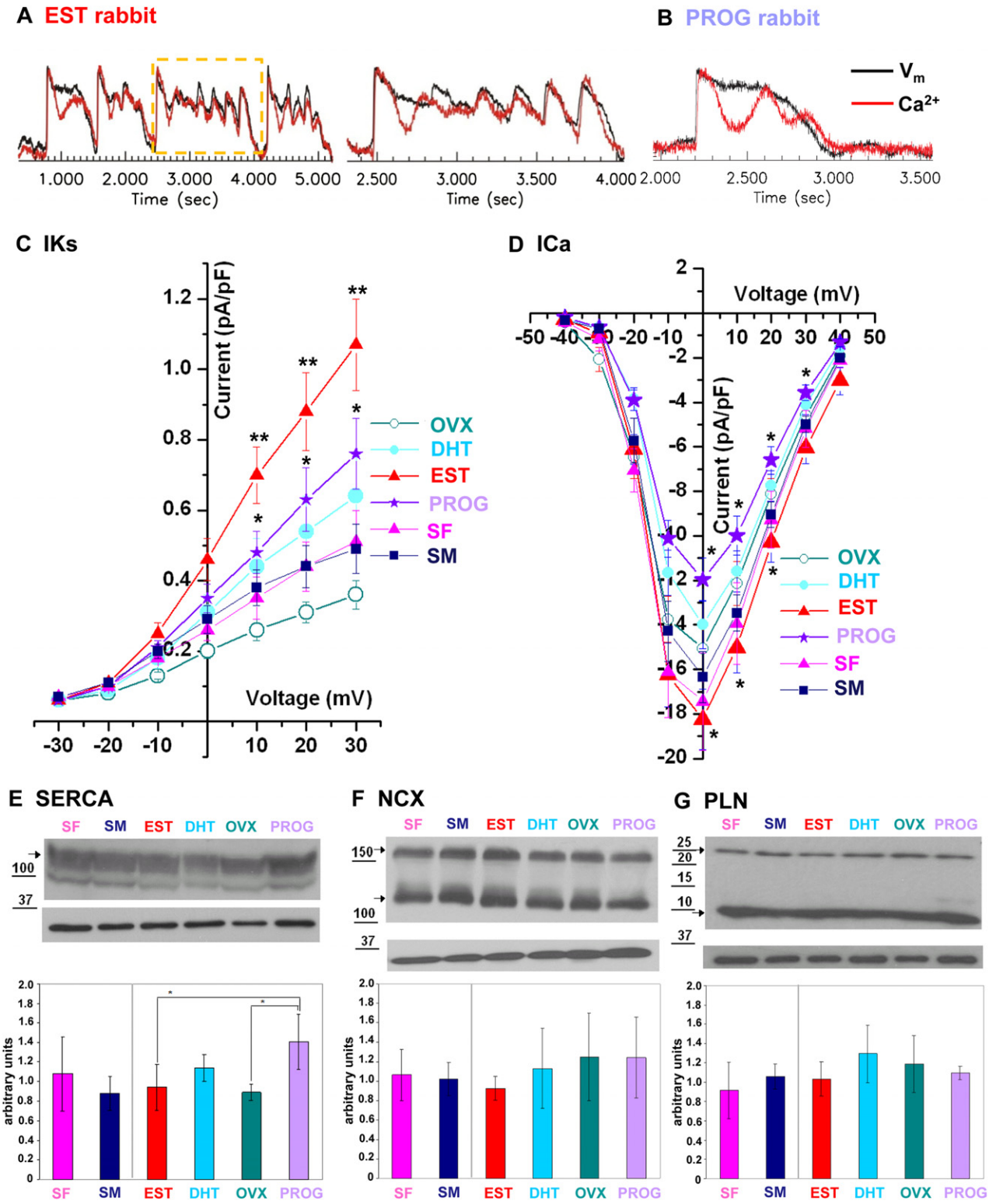


Figure 5 Effect of sex hormones on Ca^{2+} oscillations, EADs, ion currents, and Ca^{2+} cycling proteins. **A:** Representative trace of Ca^{2+} oscillations and EADs in an EST rabbit after ISO bolus. Black line indicates changes in the voltage fluorescence signal (V_m); red line indicates changes in the Ca^{2+}signal. The region shown in higher magnification in the right column is indicated by a yellow rectangle. **B:** Representative trace of Ca^{2+} oscillations and lack of EAD formation in PROG rabbit after ISO bolus. **C–D:** Hormones effects on I_{Ks} and $I_{Ca,L}$ current densities measured in cardiomyocytes harvested from the LV apex of EST (n = 15 cardiomyocytes), DHT (n = 14), OVX (n = 15), PROG (n = 18), SF (n = 6), and SM (n = 6) rabbits. All values are shown as mean ± SEM. I_{Ks}—EST vs OVX: $P < .01$; EST vs PROG, PROG and DHT vs OVX: $P < .05$. $I_{Ca,L}$—EST vs OVX, PROG vs OVX: $P < .05$; EST vs PROG: $P < .01$. **E–G:** Representative Western blots of SERCA2a, NCX, and PLN. Bar graphs indicate the expression levels of 3 independent experiments in 3 different rabbits per group in arbitrary units. All values are shown as mean ± SD. *$P < .05$. DHT = dihydrotestosterone; EAD = early afterdepolarization; EST = estradiol; ISO = isoproterenol; LV = left ventricular; NCX = sodium-calcium exchanger; OVX = ovariectomy and placebo-treatment; PLN = phospholamban; PROG = progesterone; SERCA2a = sarcoplasmic reticulum calcium ATPase2a; SF = sham-operated female; SM = sham-operated male.

Sex hormone effects on ion currents

To elucidate underlying mechanisms on the cellular level, we performed patch-clamp experiments with cells harvested from the LV apex, where we observed the most pronounced differences in APD maps and Ca^{2+} oscillations. Current density of the slow delayed-rectifier potassium current I_{Ks} increased significantly in all hormone groups, SF rabbits, and SM rabbits as compared with that in OVX rabbits. However, this increase was most pronounced in EST rabbits, resulting in a significantly higher I_{Ks} current in EST than in PROG- or DHT-treated cardiomyocytes (Figure 5C). Importantly, EST increased L-type calcium current ($I_{Ca,L}$), while PROG decreased $I_{Ca,L}$ as compared with OVX, resulting in a significantly higher $I_{Ca,L}$ in EST than in PROG rabbits (Figure 5D). No differences were observed in the transient outward potassium current (I_{to}) and the inward rectifier potassium current (I_{K1}). Because of the dominant-negative effect of the hERG pore mutant in LQT2 rabbits, I_{Kr} was absent in all groups.[8]

Sex hormone effects on Ca^{2+} cycling proteins

To test whether changes in Ca^{2+} cycling proteins may account for the differences in the propensity to develop EADs, we compared their expression in the LV apex. We observed a significantly increased expression of the SERCA2a polypeptides in PROG rabbits as compared to those in EST and OVX rabbits ($P < .05$; Figure 5E) which may contribute to an increased Ca^{2+} reuptake into the sarcoplasmic reticulum (SR), thereby shortening Ca^{2+} transient duration. No hormone-induced differences were apparent in NCX (Figure 5F) and PLN (Figure 5G).

Discussion

Proarrhythmic and antiarrhythmic effects of sex hormones in vivo

Sex differences in long QT–related arrhythmias with a higher risk for pVT and SCD in women than in men and a particularly increased risk during the postpartum period have been well documented in the clinical setting.[3,6] This study takes advantage of a transgenic LQT2 rabbit model that develops spontaneous pVTs and SCD[8] to investigate the role of sex hormones in arrhythmogenesis in vivo.

Here we show for the first time a direct link between sex hormones and the incidence of arrhythmias and SCD. Our telemetry recordings of hormone-treated LQT2 rabbits demonstrate that PROG significantly reduces potential triggers for pVTs—such as bigeminy and couplets—and completely abolishes the occurrence of pVT. Moreover, we show that PROG is protective and prevents SCD, suggesting that high PROG levels during pregnancy likely account for the reduced risk in pregnant patients with LQT2.[5] In addition, the marked reduction in PROG during the postpartum period likely promotes postpartal arrhythmias and SCD in patients with LQT2.[6] EST, in contrast, increases both the triggers and the sustainability of pVT and thereby promotes SCD, indicating that EST likely underlies the increased arrhythmogenic risk in postpubertal women with LQT2.[3]

As in patients with LQT2,[3] SCD is a rare event in LQT2 rabbits. However, similar to observations in previous studies,[9,15] lethal pVT occurred more frequently within 96 hours after isoflurane anesthesia. It may be possible that anesthesia-induced slowing of the heart rate[15] or the I_{Ks}-blocking properties of isoflurane[9] may contribute to the overall increased risk. However, all rabbits had recovered from anesthesia and were fully awake at the time of SCD. Since similar anesthetic dosages were used, it is likely that anesthesia increased the likelihood of events similarly in all groups and therefore enabled us to uncover the protective effect of PROG and the proarrhythmic effect of EST. Moreover, in a group of rabbits not exposed to anesthesia, we observed 3 SCDs in 13 EST rabbits while no SCD was observed in any other group, further underlining the proarrhythmic effect of EST.

Hormone effects on the arrhythmogenic substrate

Enhanced APD dispersion due to a spatially heterogeneous prolongation of cardiac repolarization is considered a major contributor to LQT-related arrhythmias.[11] Moreover, we have previously identified an increased spatial APD dispersion across the anterior surface of the left ventricle as a major mechanism underlying arrhythmias in transgenic LQT2 rabbits.[8,10] We thus further explored whether sex hormones alter the dispersion of repolarization in the right ventricle and the left ventricle. In EST rabbits, the longest APD region was shifted toward the LV apex, in line with the more pronounced VERP prolongation in the RV apex, suggesting that EST may exert its proarrhythmic effects, at least partly, by differentially changing APD in different regions of the right ventricle and the left ventricle, thus modifying the arrhythmogenic substrate. Indeed, programmed ventricular stimulation induced VF in all 5 EST rabbits but only rarely and significantly less frequently in OVX, PROG, and DHT rabbits, thus indicating the proarrhythmic significance of these EST-induced changes. Moreover, during VF the activation waves propagated around the apical island of prolonged APD.

We further investigated how sex hormones differentially affect rate-dependent repolarization (restitution). Our in vivo ECG monitoring studies are the first to demonstrate that EST steepens the QT/RR slope in LQT2 by prolonging QT duration at slow heart rates and shortening QT interval at fast heart rates, whereas DHT decreases the steepness of the QT/RR slope. EST and DHT treatments thus mimic sex differences in cardiac repolarization in LQT2 rabbits,[9] similar to observations in patients with LQT2.[3] $I_{Ca,L}$ and I_{Ks} play an important role in conferring these hormone effects on the QT/RR ratio: EST rabbits have a higher density of $I_{Ca,L}$ than do any other groups, resulting in the prolongation of the QT interval at slow heart rates, while the higher density of I_{Ks} in EST rabbits shortens the QT interval at fast heart rates, thus contributing to EST-induced steepening of the QT/RR ratio.

Hormone effects on the susceptibility to proarrhythmic triggers

The initiation of pVTs in LQTS is known to be linked to EAD formation.[18] Moreover, previous animal studies demonstrated that hormones alter the EAD formation in isolated cardiomyocytes.[13,19] Here we demonstrate that sex hormones exert different effects on EAD formation in response to sympathetic stimuli at the organ level. In EST and OVX rabbits, ISO triggered both Ca^{2+} oscillations and EAD formation, while in PROG and DHT rabbits, ISO triggered Ca^{2+} oscillations without EAD formation. Thus, PROG altered the response of the membrane potential to Ca^{2+} oscillations.

Experimental and simulation studies show that the reactivation of $I_{Ca,L}$ plays an important role in EAD formation[20,21] and that reducing $I_{Ca,L}$ by Ca^{2+}-blocking drugs such as verapamil is most effective in preventing pVT formation in animal models of LQTS.[22] Previous experimental and simulation data strongly suggest that $I_{Ca,L}$ is a key player in the formation and propagation of EADs.[23,24] Here we demonstrate that EST increased $I_{Ca,L}$ while PROG and DHT decreased $I_{Ca,L}$ as previously described.[23,25] Thus, EST-induced increase in $I_{Ca,L}$ likely contributes to the higher propensity to develop EADs, contributing to the proarrhythmic effect of EST. By contrast, the PROG-induced decrease in $I_{Ca,L}$ reduces the likelihood of triggered activity. Our studies also demonstrate an increase in I_{Ks} in EST rabbits. Recent computer modeling studies show that both inward Ca^{2+} and outward K^+ currents are essential to generate the oscillatory behavior of the membrane potential during the plateau phase,[24] suggesting that the EST-induced increase in $I_{Ca,L}$ and I_{Ks} facilitates the triggered activity.

To further delineate molecular mechanisms underlying these proarrhythmic and antiarrhythmic effects on the susceptibility to triggers, we investigated hormone effects on Ca^{2+} cycling proteins, which may contribute to arrhythmogenesis by altering cytoplasmic and SR Ca^{2+} concentrations, and the initiation of EADs. While previous studies in rats reported a DHT- and EST-induced increase in NCX,[26,27] we observed no hormone effects on NCX or PLN expression in LQT2 rabbits. Yet, PROG increased SERCA2a expression in LQT2 rabbits, which may contribute to an increased Ca^{2+} reuptake into the SR, thereby shortening Ca^{2+} transient duration. An increased expression of SERCA2a has previously been reported to reduce VT/VF in ischemia-reperfusion models[28] and to reduce the susceptibility to alternans-mediated ventricular arrhythmias.[29] Consequently, this increase in SERCA2a and the decrease in $I_{Ca,L}$ currents contribute to the antiarrhythmic effect of PROG.

Study limitations

Transgenic LQT2 rabbits overexpress the dominant-negative loss-of-function mutation HERG-G628S that is localized in the pore region and leads to a complete loss of I_{Kr}.[30] Many missense mutations in HERG channels that are found in patients with LQT2, however, lead to a substantial decrease rather than a complete loss of I_{Kr}. The findings on hormone effects on arrhythmogenesis in this transgenic LQT2 rabbit model likely recapitulate the findings in human patients with pore mutations or other hERG mutations with a loss of functional I_{Kr} currents in the heart.

Conclusions

In this study, we demonstrated that EST promotes pVTs and SCD while PROG prevents arrhythmias and SCD in LQT2 in vivo. EST exerts this proarrhythmic effect by changing the arrhythmogenic substrate by steepening the QT/RR ratio, prolonging cardiac refractoriness, and altering the spatial pattern of APD dispersion. The underlying mechanisms are an increased I_{Ks} current that contributes to the steepening of the QT/RR ratio by shortening the QT interval at fast heart rates and notably a substantially increased $I_{Ca,L}$ that contributes to both a longer refractoriness and a higher propensity to depolarize the membrane in response to Ca^{2+} oscillations. PROG, in contrast, exerts an antiarrhythmic effect by preventing EAD formation in response to Ca^{2+} oscillations, likely owing to an increase in SERCA2a and a decrease in the oscillatory $I_{Ca,L}$.

Clinical implications

To date, standard treatment of patients with LQT2 consists of beta-blockade and implantation of implantable cardioverter-defibrillators.[1] Understanding the mechanisms that underlie sex hormones' deleterious or protective effects could help to develop specific, hormone-based therapies. The experimental observation of an antiarrhythmic PROG effect in transgenic LQT2 rabbits suggests a potential use of oral progestins as a new class of antiarrhythmic treatment in LQTS. Further prospective studies are needed to provide evidence-based data to support this treatment option in LQTS.

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Appendix

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hrthm.2012.01.009.

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