Sex differences and sex hormone effects in long-QT syndrome: what can we learn from rabbit models?

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Published online: 19 February 2013
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Abstract Patients with inherited long-QT syndrome (LQTS) exhibit pronounced sex differences in cardiac repolarization and in the incidence of arrhythmias: Adult women have longer QT intervals and a higher risk to develop potentially lethal torsade-de-pointes (TdP) ventricular tachycardia and sudden cardiac death (SCD) than men. Rabbit models exhibit similar sex differences with longer QT intervals and a higher propensity to additional drug-induced QT prolongation and drug-induced TdP in females. Since rabbits also have similar cardiac repolarizing currents as human subjects, they provide a useful system to explore sex differences in cardiac repolarization and arrhythmogenesis in LQTS and to reveal the underlying molecular mechanisms. This review article summarizes clinical observations on sex differences and sex hormone effects on cardiac repolarization and arrhythmogenesis in patients with LQTS and recapitulates findings on underlying mechanisms (sex hormone effects on ion channels and Ca^{2+} -cycling proteins) and their clinical implications in drug-induced and transgenic rabbit models of LQTS.

Keywords Long-QT syndrome · Sex hormones · Sudden cardiac death · Ion channels · Rabbit models

Zusammenfassung Long-QT Syndrom (LQTS) Patienten zeigen ausgeprägte Geschlechtsunterschiede in der Dauer ihrer kardialen Repolarisationsphase und ihres Arrhythmierisikos. So haben erwachsene LQTS-Patientinnen nicht nur längere QT Intervalle im Oberflächen-EKG, sondern sind auch einem höheren Risiko für das Entstehen von ventrikulären torsade-de-pointes Tachykardien (TdP) und dem daraus resultierenden plötzlichen Herztod ausgesetzt als Männer. Kaninchen zeigen ähnliche Geschlechtsunterschiede: Weibliche Kaninchen haben längere QT Intervalle, ein höheres Risiko für zusätzliche, medikamenten-induzierte QT-Verlängerungen und insbesondere eine höhere Inzidenz medikamenten-induzierter TdP-Arrhythmien als männliche Kaninchen. Da in Kaninchen zudem die gleichen repolarisierenden Ionenströme für die kardiale Repolarization verantwortlich sind wie beim Menschen, eignen sich Kaninchenmodelle sehr gut, um Geschlechtsunterschiede in der kardialen Repolarizationsphase und bei long-QT-assoziierten Arrhythmien näher zu untersuchen und die zugrunde liegenden molekularen Mechanismen aufzudecken. Dieser Review-Artikel fasst 1. klinische Beobachtungen zu Geschlechtsunterschieden und Geschlechtshormon-Effekten auf die kardiale Repolarization und das arrhythmogene Risiko bei LQTS Patienten zusammen und stellt 2. Forschungsergebnisse zu den zugrunde liegenden Mechanismen (Hormon-Wirkungen auf Ionenkanäle und Calcium-Transport-proteine) in medikamenten-induzierten und transgenen LQTS Kaninchenmodellen vor.

Schlüsselwörter Long-QT Syndrom · Geschlechtshormone · Plötzlicher Herztod · Kardiale Ionenkanäle · (transgene) Kaninchenmodelle

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Introduction

The incidences of several ventricular and supraventricular types of arrhythmias—such as long-QT-related arrhythmias and paroxysmal supraventricular tachycardias—differ with gender and particularly, vary pronouncedly with different menstrual phases, pregnancy, and the postpartum phase [1–5]. These clinical observations strongly suggest that sex hormones can have an impact on arrhythmogenesis.

Since functionally active androgen, estradiol, and progesterone receptors are present in atrial and ventricular cardiomyocytes [6–9], sex hormones may influence arrhythmogenesis via sex hormone receptor-mediated genomic modulation of the expression of cardiac ion channels and calcium-handling proteins. Moreover, sex hormones can acutely alter electrical properties of ion currents via nongenomic pathways involving nitric oxide [10]. Sex hormones may also affect arrhythmogenesis indirectly, e.g., by their effects on basal body temperature [11] and on sympathetic activity [12] that can both alter electrophysiological properties of several cardiac ion currents such as sodium, calcium, and potassium currents [12–14].

Rabbits have similar cardiac repolarizing currents as humans [15, 16] and exhibit similar sex differences in cardiac repolarization and long-QT-related arrhythmias [17, 18], making the rabbit a useful model system to explore sex differences in cardiac repolarization and arrhythmogenesis and their underlying molecular mechanisms.

This article focuses on the arrhythmogenic disease long-QT syndrome (LQTS), and summarizes sex differences and sex hormone effects on cardiac repolarization and arrhythmogenesis in patients with LQTS. Moreover, it recapitulates findings on underlying mechanisms and their clinical implications in drug-induced and transgenic rabbit models of LQTS.

Clinical observations: sex differences and sex hormone effects in long-QT syndrome

The inherited LQTS is a heterogeneous arrhythmogenic disease, in which an impaired cardiac repolarization predisposes patients to torsade-de-pointes (TdP) tachycardia, syncope, and sudden cardiac death (SCD) [19]. Data from the international LQTS registry demonstrate pronounced sex differences in cardiac repolarization and the incidence of arrhythmias: adult women with LQT type 1 (mutation in $KvLQT1/I_{Ks}$) and type 2 (mutation in $HERG/I_{Kr}$) have longer QT intervals and a higher risk for TdP and SCD than men [20–22]. Moreover, during adulthood, female gender is a major independent risk factor for SCD independent of genotype or the location of the mutation within the gene [21–23].

In contrast, before puberty, the incidence of arrhythmias is higher in boys than in girls [24].

Several observations suggest that sex hormones confer these sex differences: different phases of menstrual cycle, pregnancy, and postpartum period are associated with changes in QT duration and incidence of TdP. In acquired LQTS, drug-induced QT prolongations and the arrhythmogenic risk are more pronounced during menses and follicular phase (with high estradiol levels) than during luteal phase (with relatively higher progesterone levels) [1]. Pregnant LQT2 patients have a reduced arrhythmogenic risk, while the risk markedly increases during the 9-month postpartum period [2]. These observations strongly suggest a proarrhythmic effect of estradiol and an antiarrhythmic effect of progesterone in LQTS.

LQTS rabbit models: insights into sex differences and sex hormone effects on cardiac repolarization and arrhythmogenesis

Rabbits exhibit similar sex differences as human subjects: female rabbits have longer QT intervals [17], a more pronounced drug-induced QT prolongation, and a higher incidence of drug-induced TdP than males [18] (Table 1). Since rabbits also have similar repolarizing currents as humans [15, 16], they provide a useful system to explore these sex differences in cardiac repolarization and arrhythmogenesis and to reveal the underlying molecular mechanisms.

Castration and subsequent exposure to sex hormones revealed hormone-effects on cardiac repolarization and arrhythmogenesis. Pham et al. [25] found similar action potential duration (APD) in castrated male and female rabbits. Moreover, castration reverses the normal sex differences in drug-sensitivity: orchiectomy prolongs APD and increases the incidence of I_{Kr} -blocker induced early afterdepolarizations (EAD), while ovariectomy reduces drug-induced APD prolongation and EADs [25] (Table 1).

The normal female phenotype with an increased risk for drug-induced TdP can be mimicked by treating ovariectomized or orchiectomized rabbits with estradiol [18, 25, 26], while a combined treatment with estradiol and progesterone reduces the risk for drug-induced arrhythmias [27]. Exposing castrated rabbits to testosterone shortens QT duration and reduces drug-induced QT prolongation and EADs [18, 28], suggesting that sex hormones—and not only the genetic sex—account for sex differences in cardiac repolarization.

Using a transgenic LQT2 rabbit model that mimics the human LQT2 phenotype [29, 30], we investigated the role of sex hormones in cardiac repolarization and arrhythmogenesis *in vivo*. We demonstrated that estradiol steepens QT/RR ratio by prolonging QT duration, particularly at slow heart

Table 1 Sex hormone effects on cardiac repolarization and arrhythmogenesis in rabbits

Sex	Intervention	APD	Drug-induced QT prolongation	Drug-induced EADs	QT/RR in LQT rabbits	EADs in LQT rabbits	TdP/SCD in LQT rabbits	Ion currents	Ca ²⁺ -handling proteins
Males									
		↓ APD shorter than in females [9]	↓ QT prolongation less pronounced than in females [9]	↓ EADs fewer than in females	↓ QT/RR less steep than in females [17]			↑ IKr, ↑ IKs, ↑ IK1 [9]	
	ORCH	↑ APD prolongation compared with males [12]	↑ QT prolongation more pronounced than in males [12]	↑ EADs more than in males [12]					
	ORCH+DHT	↓ APD shortening compared with ORCH [10, 12]	↓ QT prolongation less pronounced than in ORCH [10, 12]	↓ EADs reduced compared with ORCH [10, 12]				↑ IKr, ↑ IKs, ↑ IK1, [25, 26]	
Females									
		↑ APD longer than in males [9]	↑ QT prolongation more pronounced than in males [9]	↑ EADs more than in males [9]	↑ QT/RR steeper than in males [17]			↓ IKr, ↓ IK1, ↑ ICa _L (gradient) [9, 19]	
	OVX	↓ APD shortening compared with females [12]	↓ QT prolongation less pronounced than in females [12]	↓ EADs reduced compared with females [12]	↓ QT/RR less steep than in females [18]				
	OVX+EST	↑ APD prolongation compared with OVX [10, 12]	↑ QT prolongation more pronounced than in OVX [10, 12, 14]	↑ EADs more than in OVX [10, 12]	↑ QT/RR steeper than in OVX [18]		↑ TdP/SCD, increased incidence of arrhythmias [18]	↑ IKs, ↓ IsK, ↑ ICa _L [13, 18, 21–24]	↑ NCX [27]
	OVX+DHT	↓ APD shortening compared with females	↓ QT prolongation less pronounced than in females	↓ EADs reduced compared with females	↓ QT/RR less steep than in females [18]	↓ EADs reduced compared with females and OVX [18]	No TdP, no SCD, decreased incidence of arrhythmias [18]	↑ IKs [18]	
	OVX+PROG					↓ EADs reduced compared with females and OVX [18]	No TdP, no SCD, decreased incidence of arrhythmias [18]	↑ IKs, ↓ ICa _L [18, 26]	↑ SERCA [18]

APD action potential duration, EAD early afterdepolarization, TdP torsade-de-pointes tachycardia, SCD sudden cardiac death, ORCH orchietomized, OVX ovariectomized, DHT dihydrotestosterone, EST estradiol, PROG progesterone, I_{Kr} rapidly delayed rectifier K⁺ current, I_{Ks} slow delayed rectifier K⁺ current, I_{K1} inward rectifier K⁺ current, I_{CaL} L-type Ca²⁺ current, NCX sodium–calcium exchanger, SERCA sarcoplasmic reticulum ATPase, ↑ indicates an increase, ↓ indicates a reduction

rates, while testosterone decreases QT/RR slope steepness [31], similarly as observed in human LQT2 patients. Moreover, we could demonstrate a proarrhythmic effect of estradiol and an antiarrhythmic, protective effect of progesterone, which completely abolishes the occurrence of TdP or SCD. On the organ level, we identified a reduced rate of EADs in response to sympathetic stimulation as underlying mechanism of this protective progesterone effect [31] (Table 1).

Cellular studies: sex hormone effects on ion currents and Ca^{2+} -cycling proteins

Current densities of rapid delayed rectifier current I_{Kr} and inward rectifier current I_{K1} are lower in female than in male rabbits' ventricular cardiomyocytes [17]. This lower I_{Kr} is thought to account for the higher sensitivity of females to I_{Kr} -blocking drugs [17]. Moreover, female but not male rabbits exhibit a transmural gradient of $I_{Ca,L}$ with a higher epi- than endocardial $I_{Ca,L}$ [32]. Ovariectomy eliminates this transmural gradient, which reappears upon estradiol treatment. In contrast, orchietomized rabbits have no transmural gradient of $I_{Ca,L}$ even after exposure to estradiol, suggesting a potential genetic difference in the mechanism of action of sex hormones (Table 1).

Recently, these findings on sex differences in cardiac ion channels have been corroborated in human cardiac tissue derived from nondiseased transplant donors. Using a high-throughput approach, Gaborit et al. [33] could demonstrate a reduced expression of several repolarizing K^+ -channel subunits such as HERG, KCNE1, and Kir2.3 in female hearts.

To increase our understanding of these sex differences, sex hormone effects on cardiac ion currents/channel proteins have been investigated. Estradiol downregulates KCNE1 (β -subunit to KvLQT1 to form I_{Ks}) [26], directly inhibits I_{Kr} [34, 35], and indirectly reduces I_{Kr} by upregulating the β -subunit KCNE2 [36]. Moreover, estradiol increases $I_{Ca,L}$ [31] and accentuates the base-to-apex gradient of $I_{Ca,L}$ in rabbits [37] (Fig. 1). All these effects result in an APD and QT prolongation and an increased dispersion of repolarization. Moreover, a synergistic effect of estradiol and I_{Kr} -blocking drugs on I_{Kr} currents was recently reported [35]. In contrast, testosterone increases I_{Kr} and I_{K1} in rabbits' cardiomyocytes [38], and increases I_{Ks} and decreases $I_{Ca,L}$ in guinea pigs' cardiomyocytes [39], thus shortening APD and QT duration. Progesterone also shortens APD by decreasing $I_{Ca,L}$ and increasing I_{Ks} [31, 39] (Table 1, Fig. 1).

In addition, sex hormone effects on expression and function of Ca^{2+} -cycling proteins have been investigated. Estradiol increases the expression and function of sodium-

Fig. 1 (a) Schematic figure of cardiac action potential and depolarizing and repolarizing ion currents. $\uparrow$ Arrows indicate outward currents, $\downarrow$ arrows indicate inward currents. I_{Na} Depolarizing Na^+ current, I_{to} transient outward K^+ current, $I_{Ca,L}$ L-type Ca^{2+} current, I_{Kr} rapidly delayed rectifier K^+ current, I_{Ks} slowly delayed rectifier K^+ current, I_{K1} inward rectifier K^+ current. (b–d) Schematic figure of estradiol's (b),

testosterone's (c), and progesterone's (d) effects on cardiac ion currents and on action potential duration. +++ Increase, --- decrease, +/- differential effects on alpha- and beta-subunits. e Sex differences and sex hormone effects on QT duration in transgenic LQT2 rabbits. (e) Modified and adapted from [31]

calcium exchanger NCX [40] that may contribute to an increased EAD formation in female rabbits. We reported a progesterone-induced increased SERCA2a expression in transgenic LQT2 rabbits, which may contribute to the antiarrhythmic effect of progesterone by shortening Ca^{2+} transient duration [39].

Clinical implications

As women with LQT1 and LQT2 have a higher risk for TdP and SCD than men and are particularly prone to develop lethal TdP during the postpartum [20, 22], a particularly close clinical monitoring of female LQT patients is mandatory, especially in phases of changing hormone levels. Understanding the exact mechanisms that underlie sex hormone effects on cardiac repolarization and arrhythmogenesis in inherited LQTS will help to better discern the individual risk of LQT patients. Moreover, as studies in transgenic LQT2 rabbits indicate an antiarrhythmic, protective effect of progesterone [31], clinical studies investigating new hormone-based therapeutic options in LQTS are clearly warranted.

Acknowledgments This article is part of a supplement sponsored by Lilly Deutschland GmbH and Daiichi Sankyo Deutschland GmbH.

Conflict of interest The authors declare that there is no actual or potential conflicts of interest in relation to this article.

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