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Sex hormonal regulation of cardiac ion channels in drug-induced QT syndromes

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Abstract

Female sex is an independent risk factor for development of torsade de pointes (TdP) arrhythmias not only in congenital long QT syndromes but also in acquired long QT syndromes. Clinical and experimental evidences suggest that the gender differences may be due to, at least in part, gender differences in regulation of rate-corrected QT (QT_C) interval between men and women. In adult women, both QT_C interval and arrhythmic risks in TdP alter cyclically during menstrual cycle, suggesting a critical role of female sex hormones in cardiac repolarization process. These gender differences in fundamental cardiac electrophysiology result from variable ion channel expression and diverse sex hormonal regulation via long term genomic and acute non-genomic actions, and sex differences in drug responses and metabolisms. In particular, non-genomic actions of testosterone and progesterone on cardiac ion channels are likely to contribute to the gender differences in cardiac repolarization processes. This review summarizes current knowledge on sex hormonal regulation of cardiac ion channels which contribute to cardiac repolarization processes and its implication for gender differences in drug-induced long QT syndromes.

Keywords

sex hormones; cardiac repolarization; arrhythmias; gender difference

1. Introduction

Gender differences in electrocardiograms (ECGs) were reported for the first time in 1920(Bazett, 1920). In general, women have faster resting heart rates (Liu *et al.*, 1989) and

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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longer rate-corrected QT intervals in comparison with men (Di Diego *et al.*, 2002; Fish *et al.*, 2003; Hara *et al.*, 1998; Merri *et al.*, 1989; Pham *et al.*, 2002; Xiao *et al.*, 2006). Moreover, it is becoming clear that gender differences in cardiac electrophysiology play an important role in the prevalence of clinical arrhythmias.

It is widely accepted that women are more prone to develop drug-induced arrhythmia (specifically *Torsade de Pointes*, TdP) in association with prolongation of the QT interval, which corresponds to the duration of the ventricular action potential (Abi-Gerges *et al.*, 2004). Although the underlying reasons for the gender disparity in incidence of TdP have yet to be completely clarified, clinical evidences imply that sex steroid hormones appear to play important roles in gender differences by affecting the cardiac repolarization process of action potential (Bai *et al.*, 2005; Furukawa *et al.*, 2007; Korte *et al.*, 2005; Nakagawa *et al.*, 2005; Verkerk *et al.*, 2005; Xiao *et al.*, 2006). Shortening of QT interval after puberty in men have been cited as an evidence that male hormone is involved in cardiac repolarization process (Rautaharju *et al.*, 1992). Shorter QT intervals in women with virilization than normal women and castrated men (Bidoggia *et al.*, 2000) imply that androgens accelerate cardiac repolarization processes and shorten action potential durations (APD), which was demonstrated in rabbit models (Pham *et al.*, 2002). In terms of female hormones, it has been known that dynamic changes in levels of female sex hormones during women's menstrual cycle have cyclical effects on QT intervals, implying that serum concentrations of female sex hormones impact cardiac ion channel functions.

Sex steroid hormones have been traditionally considered to act via the regulation of transcriptional processes (Mangelsdorf *et al.*, 1995). In addition to genomic regulation, accumulating evidences, however, show that the sex hormones can also exhibit rapid effects involving intracellular signaling, and are therefore referred to as "non-genomic regulation" (Baron *et al.*, 2004; Furukawa *et al.*, 2007). Non-genomic acute effects of sex steroid hormones take place outside the cell nucleus via the activation of specific signaling pathways that include eNOS and MAP kinase. In addition to genomic regulation, we recently reported that physiological levels of sex hormones can acutely modify cardiac repolarization by regulating cardiac ion channels via either a non-genomic pathway involving hormone receptors or in a receptor-independent fashion. These non-genomic actions likely contribute to the gender differences in QT_C intervals and susceptibility of TdP. A computational approach revealed a significant impact of non-genomic regulations of sex hormones on susceptibility of long QT linked arrhythmias (Yang *et al.*, 2010a; Yang *et al.*, 2010b).

The main goal of this review article is to provide an overview of how cardiac repolarization is regulated by sex hormones, then to discuss the implication to occurrence of drug-induced long QT syndrome.

2. Gender differences in drug-induced long QT syndrome

It is now apparent that TdP, a life-threatening ventricular tachyarrhythmia can be induced by common medications which prolong duration of the cardiac action potential (Drici *et al.*, 1998; Hashimoto, 2007; Hreiche *et al.*, 2008; James *et al.*, 2007; Lehmann *et al.*, 1996;

Makkar *et al.*, 1993; Tamargo, 2000). Acquired long QT syndrome (LQTS) can be associated with drug-induced prolonged QT_C intervals and arrhythmia, and results from delay of cardiac repolarization caused mostly by inhibition of the *human ether-a-go-go-related gene* (hERG) channel, which conducts the rapid component of the delayed rectifier K⁺ current (I_{Kr}). Thus, regulatory agencies require pre-clinical hERG inhibition data for any drug candidate (Abriel *et al.*, 2004; Fermini *et al.*, 2003; Satoh *et al.*, 2000; Takahara *et al.*, 2007). However, the predictability of clinical arrhythmias with hERG test is often debated because of too many false positives. Actually, the incidence of drug-induced arrhythmia is also affected by other risk factors such as other channel inhibition, gender (Drici *et al.*, 1998; Lehmann *et al.*, 1996; Makkar *et al.*, 1993) or/and sympathetic nervous system activity (Kurokawa, 2007; Marx *et al.*, 2002). Thus, unraveling of the molecular basis of effects of such risk factors may be beneficial for improving predictability of this lethal arrhythmia caused by medication.

It has been shown that female sex is an independent risk factor for development of drug-induced TdP in patients with 65–75% of cases occurring in women (Drici *et al.*, 1998; Lehmann *et al.*, 1996; Locati *et al.*, 1998; Makkar *et al.*, 1993). The higher risks in women have been thought to be associated with the 20-ms prolonged baseline QT_C intervals in women in comparison with those in men (Bazett, 1920). The gender differences in QT_C intervals appear to correlate with age-dependent changes in serum levels of sex hormones. There is no statistical difference in the QT_C interval between men and women before puberty (Merri *et al.*, 1989; Rautaharju *et al.*, 1992; Stramba-Badiale *et al.*, 1995). As sex hormone levels increase during puberty, QT_C intervals in men are shortened, leaving adult women with longer QT_C intervals than adult men. The QT_C interval in men then gradually increases until the age of ~60 years when the intervals approach that of women (Merri *et al.*, 1989; Rautaharju *et al.*, 1992; Stramba-Badiale *et al.*, 1995). The age-dependent changes in QT_C intervals in men imply the involvement of androgens in these gender differences. Although we had reported that testosterone shortens APD in guinea pig by decreasing the L-type calcium current (I_{Ca,L}; an inward depolarizing current) and increasing slow delayed rectifier potassium current (I_{Ks}; an outward repolarizing current) (Bai *et al.*, 2005), recent simulation study showed that the early repolarization changes in men were most influenced by the effect of testosterone on calcium currents (Vicente *et al.*, 2014).

Female sex hormones are also involved in the gender differences both in QT_C intervals and in the susceptibility to TdP, although the situation is more complex than that for androgens. During the menstrual cycle and pregnancy in females, there are dynamic fluctuations in QT interval and TdP risk which may correlate with changes in serum levels of ovarian steroids (Nakagawa *et al.*, 2006). Several studies have evaluated the potential impact of hormone replacement therapy (HRT) on QT_C intervals in postmenopausal women (Abi-Gerges *et al.*, 2004; Kadish *et al.*, 2004). Although conflicting findings exist regarding HRT, these clinical evidences imply that the dynamic changes in levels of female sex hormones, progesterone and estrogen, have cyclical effects on action potential duration. The effects of female hormones on ventricular repolarization will be discussed in the following sections.

3. Sex hormonal regulations of cardiac ion channels

The gender differences in drug-induced long QT syndrome is partly based on the electrical differences resulting from expression of various ion channels. Analysis with human male and female showed decrease expression for several repolarizing channels including hERG (Gaborit *et al.*, 2010). Sex hormones bind to sex hormone receptors, translocate into nucleus, and regulate the transcription of target genes. There are some reports the transcriptional regulation of cardiac ion channels by sex hormones. In cardiomyocytes, testosterone increases expression of the L-type Ca^{2+} channels and the $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger in mRNA (Golden *et al.*, 2004). Androgen enhances expression of I_{Kur} and $\text{K}_{\text{V}1.5}$ resulting in short ventricular repolarization (Brouillette *et al.*, 2005). The chronic effects of testosterone and E2 include the transcriptional regulation of some K^{+} channels, but not the hERG channel (Drici *et al.*, 1996; Song *et al.*, 2001). In addition to conventional genomic pathway of sex hormone, E2 has also reported to augment membrane trafficking of hERG by enhancing interaction to heat shock proteins in non-genomic pathway (Anneken *et al.*, 2016). Chronic application of progesterone in hearts did not change protein expression of L-type Ca^{2+} channels (Helguera *et al.*, 2002) and the $\text{Kv}4.3$ channel (Song *et al.*, 2001).

Several reports have shown that sex steroid hormones rapidly inhibit various cardiac ion currents, including L-type Ca^{2+} currents ($\text{I}_{\text{Ca,L}}$), T-type Ca^{2+} currents ($\text{I}_{\text{Ca,T}}$), slowly- and rapidly-activating delayed rectifier K^{+} currents (I_{Ks} and I_{Kr}), transient outward current (I_{to}), and inwardly rectifying K^{+} current (I_{K1}) in isolated cardiomyocytes (Abi-Gerges *et al.*, 2004). However, to elicit these rapid inhibitions, estrogen and androgens were applied at levels (several μM) higher than their physiological levels. At these concentrations, sex steroids exhibit a non-specific binding to various molecules, and the resulting signaling pathways are also not known.

Reports of the effects of sex hormones within the physiological range are limited. We found in guinea pig ventricular myocytes that acute applications of physiological serum levels of testosterone and progesterone suppress a depolarizing current, $\text{I}_{\text{Ca,L}}$, and enhance a repolarizing current, I_{Ks} , resulting in shortening of the duration of ventricular action potentials. Thus, testosterone and progesterone shortens the QT_{C} interval and likely exhibit anti-arrhythmic effects (Bai *et al.*, 2005; Nakamura *et al.*, 2007). The regulation of $\text{I}_{\text{Ca,L}}$ and I_{Ks} occurs within 10 to 15 min via a non-genomic pathway involving sequential activation of c-Src, PI3-kinase, Akt, and eNOS and resultant release of nitric oxide (NO) (Bai *et al.*, 2005; Nakamura *et al.*, 2007). Pharmacological assays using inhibitors of the signaling molecules and sucrose density gradient fractionation experiments strongly suggest that the non-genomic pathway occurs in the caveolae/lipid raft domain (Nakamura *et al.*, 2007). In contrast to progesterone and testosterone, 17β -estradiol, a potent estrogen, shortens the duration of ventricular action potentials only at levels higher than physiological serum concentrations, suggesting a minimal impact on the regulation of $\text{I}_{\text{Ca,L}}$ and I_{Ks} (Bai *et al.*, 2005; Kurokawa *et al.*, 2008). The difference between progesterone and estrogen likely contributes differently to dynamic changes of the action potential duration during the menstrual cycle in females.

4. Non-genomic action of progesterone

Progesterone has robust non-genomic effects on cardiac ion channels. Progesterone at maximal effective concentration enhances I_{Ks} by ~140%, and suppresses $I_{Ca,L}$ by ~60%. Both of these effects were abolished by NO scavenger or inhibitors of signaling molecules in the non-genomic pathway. The suppression of $I_{Ca,L}$ by progesterone was cGMP-dependent, as described previously (Fischmeister *et al.*, 2005), while the enhancement of I_{Ks} was independent of the cGMP-soluble guanylate cyclase (sGC) pathway, and may involve protein *s*-nitrosylation (Asada *et al.*, 2009). Since antagonistic effects of cGMP on cAMP-stimulated $I_{Ca,L}$ have been demonstrated, it is tempting to consider the possibility of crosstalk with signaling mediated by cAMP. Actually, sympathetic nervous system (SNS) stimulation, a critical triggering factor for TdP in LQTS (Kass *et al.*, 2003), altered the properties of the target ion channels. (Figure 1). Under basal conditions without cAMP stimulation, progesterone enhanced I_{Ks} in a dose-dependent fashion ($EC_{50} = 2.7$ nM, the progesterone level in the luteal phase, ~40.6 nM) without modifying $I_{Ca,L}$ in this dose range (Janse de Jonge *et al.*, 2001). In the presence of SNS stimulation, progesterone partially suppressed $I_{Ca,L}$ in a dose-dependent fashion ($IC_{50} = 29.9$ nM), while 100 nM progesterone did not significantly affect I_{Ks} . Because the reported progesterone level in women is ~2.5 nM in the follicular phase and ~40.6 nM in the luteal phase (Janse de Jonge *et al.*, 2001), cyclical fluctuation of serum progesterone during the menstrual cycle may affect modulation of $I_{Ca,L}$ and I_{Ks} .

5. Estrogen

Estrogen has a number of cardiovascular effects. There is much evidence to suggest that estrogen may reduce the risk of arrhythmias indirectly by protecting against cardiac ischemic injuries as a consequence of vasodilation (Murphy *et al.*, 2008). Although clinical impacts of estrogen on drug-induced LQTS are still debated, chronic application of estrogen has some impact in some cases of drug-induced LQTS (Abi-Gerges *et al.*, 2004; Drici *et al.*, 1998; Hara *et al.*, 1998). Chronic treatment with 17 β -estradiol (E2) enhanced action potential duration prolongation in rabbit papillary muscle and the incidence and magnitude of early afterdepolarizations (EADs) induced by E4031, a hERG blocker (Hara *et al.*, 1998). Because the E2 treatment did not affect baseline electrocardiographic characteristics, chronic E2 treatment appears to reduce the repolarization reserve (Hara *et al.*, 1998) which indicates the existence of redundancy of repolarizing currents in cardiomyocytes. But, it is difficult to clearly discuss the relative impact of estrogen-induced regulation on cardiac repolarization because of the existence of several unknown transcriptional targets and wide interspecies variation (Drici *et al.*, 1996; Fulop *et al.*, 2006).

There are conflicting evidences regarding the effects of estrogen- or hormone-replacement therapy (ERT or HRT) on QT_C intervals in post-menopausal women. Although it has been reported that ERT did not alter QT_C intervals (Abi-Gerges *et al.*, 2004), a large-scale clinical study of post-menopausal women revealed very slight (a few msec), but significant, QT_C prolongation with ERT alone (Kadish *et al.*, 2004). This study suggests that exogenous estrogen moderately delays cardiac repolarization in a reversible fashion, suggesting the existence of acute effects of estrogen (Rodriguez *et al.*, 2001). Consistent with impacts of

ERT on ECG in a large-scale clinical study, our recent data from guinea pig ventricular myocytes revealed that physiological concentrations of E2 acutely delayed cardiac repolarization, resulting in a slight prolongation of the QT_C interval and action potential duration (APD)(Kurokawa *et al.*, 2008). Circulating physiological concentrations of E2 vary from 0.1 to 1 nM during the menstrual cycle (<0.1 nM in men), and rise to as high as several hundred nM only during pregnancy (Hulot *et al.*, 2003; James *et al.*, 2007; Nakagawa *et al.*, 2006; Rodriguez *et al.*, 2001).

E2 has dual effects on electric function in guinea pig ventricles. Physiological concentrations of E2 inhibit I_{Kr} (K_d = 1.3 nM) and prolong QT_C interval and APD, while higher (non-physiological) concentrations of E2 yielded not only I_{Kr} inhibition, but also non-genomic regulation enhancing I_{Ks} (K_d = 39.4 nM) and suppressing I_{Ca,L} (K_d = 29.5 nM) as described above (Kurokawa *et al.*, 2008). The magnitude of E2-induced I_{Kr} suppression was statistically significant, but relatively small (<30%) (Kurokawa *et al.*, 2008). This is consistent with the evidences implying that estrogen has less clear impacts on baseline QT_C intervals (Hulot *et al.*, 2003; Nakagawa *et al.*, 2006; Rodriguez *et al.*, 2001). Another estrogen, estrone 3-sulfate at physiological concentrations in both women and men at any age has been reported to suppress hERG currents to about 80% (Kakusaka *et al.*, 2009).

E2 suppresses I_{Kr} in a receptor-independent manner. A mutagenesis study of common drug-binding sites of the hERG channel (Clancy *et al.*, 2003; Fernandez *et al.*, 2004; Sanguinetti *et al.*, 2005) revealed that aromaticity of Phe⁶⁵⁶ is important for E2-induced hERG suppression, suggesting that the aromatic-centroid of E2, which exists only in estrogen and not in other sex steroids, may be responsible for modulation of the hERG channel (Kurokawa *et al.*, 2008) (Figure 2A). Although the mechanism responsible for it has not been clarified, E2 enhances that E4031-induced hERG suppression (Figure 2B), in line with the increase in ibutilide-induced QT_C prolongation in the late follicular phase (Rodriguez *et al.*, 2001), which could underlie the enhanced susceptibility of women to acquired LQTS (Drici *et al.*, 1998; James *et al.*, 2007; Lehmann *et al.*, 1996; Makkar *et al.*, 1993).

Recently, employment of an aromatase knockout mouse as an *in vivo* estrogen-null model supports the effects of estrogen on cardiac electrophysiology(Kurokawa *et al.*, 2015). The knockout mice were generated by targeted disruption of the *CYP19* gene coding aromatase cytochrome P450 (Honda *et al.*, 1998). The effects of E-4031 on electrocardiogram parameters were compared between wild-type mice (C57/BL6J) and the aromatase knockout mice. The ablation of circulating estrogens blunted the effects of E-4031 on heart rate and QT interval in mice under a denervation condition. The result provides *in vivo* proof of principle and demonstrates that endogenous estrogens increase the sensitivity of cardiac repolarization to hERG block by E-4031 (Figure 2C)..

6. Computational modeling and simulation approaches

Based on clinical and experimental studies suggesting that differences in arrhythmia vulnerability may stem from sex steroid hormones, mathematical modeling and simulation approaches have been developed to try to reveal plausible mechanisms of sex steroid

hormone linked arrhythmia vulnerability (Yang *et al.*, 2010a; Yang *et al.*, 2011)(Roberts *et al.*, 2012)(Grace *et al.*, 2012).

The sex steroid hormones estrogen and progesterone and their acute interactions with multiple subcellular targets in the heart have been incorporated into computationally based models of cardiac ion channels to predict how they alter the emergent electrical activity of guinea pig cells and tissues (Faber *et al.*, 2000; Yang *et al.*, 2010b). Models allow for simulation of the effects of individual components or components in combination and so have been used to tease out the individual contributions of estrogen, progesterone and testosterone on cardiac electrical behavior and then make predictions about their effects in combination and in the presence of drugs. The guinea pig model simulations reproduced observed fluctuations of cardiac repolarization during the menstrual cycle in females, and predicted protective effects of progesterone against rhythm disturbance in a cellular and a tissue model of congenital and drug-induced LQTS(Nakamura *et al.*, 2007). These findings (Nakamura *et al.*, 2007) suggested that non-genomic regulation of cardiac ion channels by progesterone may influence fluctuation of baseline QT_C during the menstrual cycle in females, consistent with previous clinical reports (Hulot *et al.*, 2003; Nakagawa *et al.*, 2006; Rodriguez *et al.*, 2001). Non-genomic effects of progesterone on I_{Ca,L} and I_{Ks} depict well how mathematical models of cardiac electrophysiology help to understand the mechanism of development of cardiac arrhythmias.

The computational models in guinea pig virtual cells have also shown that simulation of sex steroid hormones during the menstrual cycle in females can reproduce observed fluctuations of QT intervals as recorded on the ECG. The effects of testosterone on ECG parameters were also reproducibly simulated. Modeling predictions suggested that testosterone and progesterone are protective against drug-induced arrhythmias, while estrogen likely exacerbates the breakdown of normal cardiac electrical activity in the presence of QT-prolonging drugs (Yang *et al.*, 2010b).

Studies utilizing complementary computational and experimental approaches have also been used to inform human in silico models to determine if sex based differences in physiologically relevant concentrations of sex steroid hormones will *exacerbate or protect* against initiation of human self-sustaining reentrant arrhythmias, a clinically significant precedent event to lethal arrhythmias that has been observed with significantly higher incidence in women in the setting of acquired LQTS (Yang *et al.*, 2012).

Mathematical models have been developed to represent in silico male and female ventricular human heart cells by incorporating experimentally determined genomic differences and effects of sex steroid hormones into the O'Hara-Rudy human virtual myocyte model (O'Hara *et al.*, 2011) (Yang *et al.*, 2012). The model incorporated genome-scale measurements of differences between human males and females. For example, human females have been shown to have lower expression of genes encoding key cardiac repolarizing potassium currents and connexin43, the primary ventricular gap-junction subunit. Human males and females also have distinct sex steroid hormones. The "male" and "female" model cells and tissues then were used to predict how various sex-based differences underlie arrhythmia risk. Genomic-based differences in ion channel expression

were alone sufficient to determine longer female cardiac APDs in both epicardial and endocardial cells compared to males. Addition of sex steroid hormones exacerbated these differences, as testosterone was predicted to further shorten APD, while estrogen and progesterone application resulted in disparate effects on APD.

Computational modeling studies predict that during the menstrual follicular phase (prior to ovulation), QT interval is longer than in the luteal phase (following ovulation) when progesterone (Pg) is increased. Moreover, susceptibility to drug-induced arrhythmias was predicted to be most likely in the late follicular phase where estrogen (E2) level is the highest (Yang *et al.*, 2012).

Model predictions suggested that males have redundant protective mechanisms including testosterone and more prominent connexin43, which may contribute to shorter rate corrected QT intervals compared to females. With application of 35 nM DHT, the model simulations suggested that a reentrant wave cannot be sustained, even in the presence of a hERG blocking drug (Yang *et al.*, 2012).

The increase in connexin43 was predicted to play a key factor for preventing sustained reentry in males. Indeed, we made some targeted swaps of model components to show this directly (Yang *et al.*, 2012). When female hormones and an I_{Kr} blocker was added to the “genetic” male model and tested for vulnerability to reentry, the male was still protected. Although the addition of drug and female hormones had the expected effect to increase APD, the faster male conduction velocity resulted in a rapid collision of the wave front with the wave tail after a single rotation. Similar behavior was observed when the genetic female model with female hormones corresponding to the late follicular phase was combined with the I_{Kr} blocker, but male connexin 43 level was swapped into the model alone. These simulations suggests that sex-based differences in conduction play an important role in arrhythmia vulnerability.

In the future, modeling and simulation approaches should be carried out to examine mechanisms of arrhythmic events associated with acquired and inherited LQTS during phases where progesterone is high, especially in the setting of sympathetic stimulation, a major factor in triggering TdP.

7. Summary

We have summarized here recent progress in examination of gender differences in susceptibility to arrhythmias including drug-induced LQTS (Figure 1). In addition to well-characterized genomic effects of ovarian steroids, non-genomic effects mediated via the progesterone receptor (Nakamura *et al.*, 2007) and receptor-independent regulation by estrogen (Kurokawa *et al.*, 2008) have recently been introduced as novel possible causes of the higher susceptibility to drug-induced LQTS in women. The acute effects of female hormones could be taken into account in assessing the risk of drug-induced QT prolongation especially in women.

Major remaining issues that need attention could be collection of human data and integration of emergent effects of sex hormones on multiple intersecting processes. Employments of

human iPS cell-derived cardiomyocytes and mathematical simulation would be possible solutions to translate knowledge of these basic researches into toxicological assessments.

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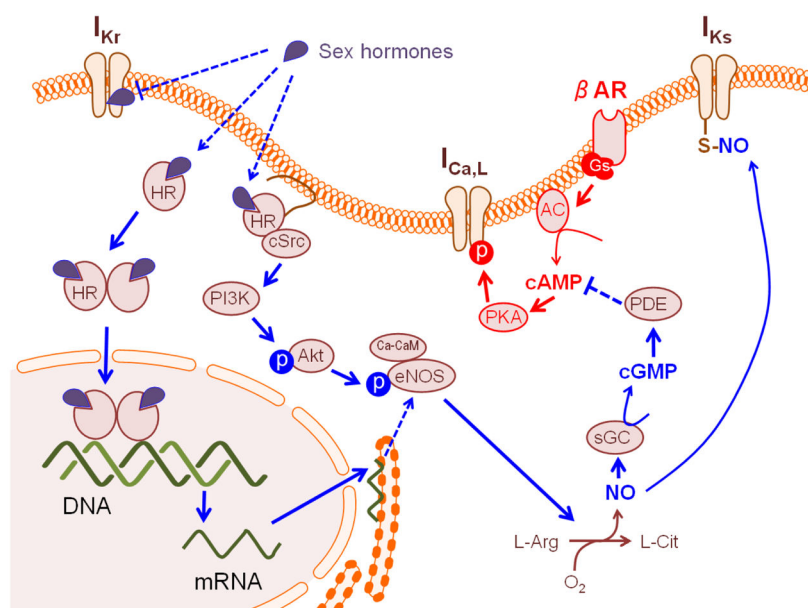


Figure 1. Scheme for mechanism underlying sex hormonal regulation of the L-type Ca^{2+} ($\text{I}_{\text{Ca,L}}$) channel, the rapid delayed rectifier K^{+} (I_{Kr}) channel and the slow delayed rectifier K^{+} (I_{Ks}) channel. HR; hormone receptor, βAR ; beta-adrenergic receptor, AC; adenylate cyclase, PDE; phosphodiesterase, L-Arg; L-arginine, L-Cit; L-citrulline, sGC; soluble guanylyl cyclase. βAR stimulation involves in modulation of $\text{I}_{\text{Ca,L}}$ through a non-genomic pathway of progesterone receptor (Nakamura *et al.*, 2007).

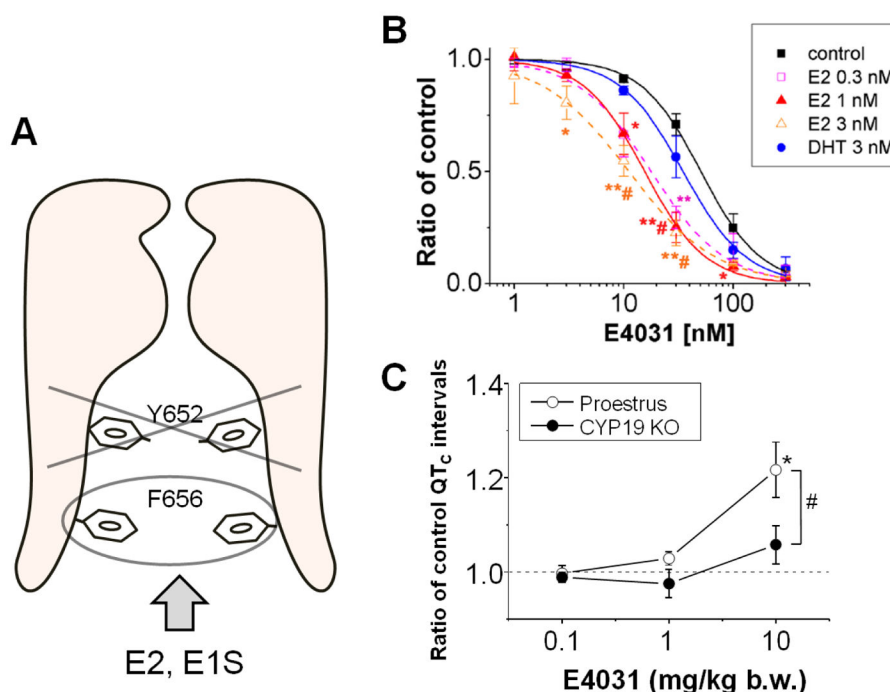


Figure 2.

Site specific effect of estrogen alters sensitivity of a selective HERG blocker, E4031. A. A scheme shows that Phe⁶⁵⁶ (F656) but not Tyr⁶⁵² (Y652), a common drug-binding site for the hERG, is important for the suppression by some estrogens (E2 and E1S)(Kakusaka *et al.*, 2009; Kurokawa *et al.*, 2008). B. E2 but not DHT (dihydrotestosterone) shifted concentration-dependent curves of HERG inhibition by E4031.. HERG currents were recorded from HEK293 cells overexpressing the hERG channel. E2 at 0.3, 1, 3 nM increased the E4031-induced fractional inhibition (* $P < 0.05$ vs. control, and vs. DHT), while DHT did not (n.s. vs. control)(Kurokawa *et al.*, 2008). C. Estrogen-null in CYP19 KO mice reduced the E-4031-induced QT_c prolongation. Prolongation of QT_c intervals in response to the cumulative administration of E-4031 were compared between late-proestrus female mice (open circles) and aromatase knockout mice (CYP19 KO, closed circles). Modified from(Kurokawa *et al.*, 2015).