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# New Aspects for the Treatment of Cardiac Diseases Based on the Diversity of Functional Controls on Cardiac Muscles: Acute Effects of Female Hormones on Cardiac Ion Channels and Cardiac Repolarization

Junko Kurokawa[1,*], Takeshi Suzuki[2], and Tetsushi Furukawa[1]

[1]*Department of Bio-informational Pharmacology, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan*
[2]*Faculty of Pharmacy, Division of Basic Biological Sciences, Keio University, 1-5-30 Shiba-koen, Minato-ku, Tokyo 105-8512, Japan*

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**Abstract.** Regulation of cardiac ion channels by sex hormones accounts for gender differences in susceptibility to arrhythmias associated with QT prolongation (TdP). Women are more prone to develop TdP than men with either congenital or acquired long-QT syndrome. The risk of drug-induced TdP varies during the menstrual cycle, suggesting that dynamic changes in levels of ovarian steroids, estradiol and progesterone, have cyclical effects on cardiac repolarization. Although increasing evidence suggests that the mechanism of this involves effects of female hormones on cardiac repolarization, it has not been completely clarified. In addition to well-characterized transcriptional regulation of cardiac ion channels and their modifiers through nuclear hormone receptors, we recently reported that physiological levels of female hormones modify functions of cardiac ion channels in mammalian hearts. In this review, we introduce our recent findings showing that physiological levels of the two ovarian steroids have opposite effects on cardiac repolarization. These findings may explain the dynamic changes in risk of arrhythmia in women during the menstrual cycle and around delivery, and they provide clues to avoiding potentially lethal arrhythmias associated with QT prolongation.

***Keywords***: cardiac ion channel, arrhythmia, sex hormone, gender difference, menstrual cycle, cardiac disease

## Introduction

It is widely accepted that women are more prone to develop drug-induced arrhythmias (*torsades de pointes*, TdP) in association with prolongation of the QT interval, which corresponds to the duration of the ventricular action potential. The ventricular action potential is characterized by a long-lasting so-called “plateau” period in which a fair degree of balance is maintained between small inward and outward currents. Small changes in this balance can have severe functional consequences, mostly in the cardiac repolarization process. Although the underlying reasons for the gender disparity in incidence of TdP have yet to be completely clarified, it is believed that gonadal steroids play roles in gender differences in baseline QT intervals by affecting the cardiac repolarization process. Some clinical reports have indicated that ovarian steroids modify cardiac repolarization, and the chronic genomic effects of ovarian steroids on cardiac ion channels have been intensively studied. In addition to genomic regulation, we recently reported that physiological levels of female 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. In this review, our recent investigations regarding the acute effects of female hormones will be summarized and their implications will be discussed.

*Corresponding author. junkokuro.bip@mri.tmd.ac.jp
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## Drug-induced long-QT syndrome

It is now apparent that the life-threatening ventricular tachyarrhythmia termed *torsades de pointes* (TdP) can be induced by many commonly used drugs that delay cardiac repolarization (1 – 7), and such induction is the most common reason for withdrawal of medications from the market (8, 9). Drug-induced arrhythmia, one type of acquired long-QT syndrome (LQTS), is associated with prolonged rate-corrected QT ($QT_C$) intervals on the electrocardiogram (ECG), resulting 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 ($I_{Kr}$). The incidence of drug-induced arrhythmia is also affected by other risk factors such as gender (1 – 3) or/and sympathetic nervous system activity (10, 11). Because drug-induced QT prolongation is the most common reason for withdrawal of medications from the market, pharmaceutical companies and basic researchers are striving to improve ways to predict the risk of novel agents as early as possible (8, 12, 13). Thus, unraveling the molecular basis for the effects of such risk factors may be beneficial for avoiding this lethal side effect.

## Gender differences in drug-induced LQTS

Being female is an independent risk factor for development of TdP in the case of both congenital and acquired (drug-induced) LQTS (1 – 3, 6). In terms of drug-induced LQTS, women are more prone to develop TdP than men in response to QT-prolonging drugs, with 65% – 75% of cases of drug-induced TdP occurring in women (1 – 3, 14).

Although the mechanisms underlying the gender differences in development of TdP have not yet been clarified, the higher susceptibility of females to drug-induced TdP has been thought to be associated with prolonged baseline $QT_C$ intervals in women by about 20 ms in comparison with those in men (15). Although direct evidence from humans is still missing, it has been reported that virilized women have shorter JT intervals than castrated men and that orchiectomized men have longer JT intervals than before orchiectomy (16). These findings strongly suggest that gonadal steroid sex hormones have an impact on gender differences in the ionic processes underlying cardiac repolarization (17 – 21).

The gender differences in $QT_C$ intervals and susceptibility to TdP depend on age, which may correlate with changes in serum levels of sex hormones. At birth, the $QT_C$ interval is quite similar in men and women (22 – 24). As sex hormone levels increase during puberty, $QT_C$ intervals in boys 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 approximately 60 years, when it approaches that in women (22 – 24). The age-dependent changes in $QT_C$ intervals in men imply the involvement of testosterone in these gender differences. In fact, we have found that testosterone acutely modifies functions of cardiac ion channels via a non-genomic pathway involving androgen receptors and that this results in shortening of $QT_C$ intervals (18). The non-genomic pathway involving androgen receptors in the heart shares the signaling pathway downstream of cSrc used by progesterone and estrogen receptors (25, 26).

Female 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. In females, there are dynamic fluctuations in QT interval and TdP risk during the menstrual cycle and pregnancy, which may correlate with changes in serum levels of ovarian steroids (3). Several studies have evaluated the potential impact of hormone replacement therapy (HRT) on $QT_C$ intervals in postmenopausal women (17, 27). Although conflicting findings exist regarding HRT, these clinical findings imply that the dynamic changes in levels of female hormones have cyclical effects on action potential duration (APD). The effects of the female hormones progesterone and estrogen on cardiac ion channels and ventricular repolarization will be discussed in the following sections.

## Progesterone

There are some lines of clinical evidence suggesting that the luteal hormone progesterone exerts protective effects against prolonged QT–associated arrhythmias by shortening ventricular depolarization. Although several previous studies did not find $QT_C$-interval differences among the different menstrual phases (21, 28, 29), a recent study analyzing various parameters of cardiac repolarization found that repolarization duration is shorter in the luteal phase than in the follicular phase by about 10 ms (30). The susceptibility of drug-induced arrhythmias also fluctuates considerably during the menstrual cycle in women: the $QT_C$ prolongation induced by ibutilide, a class III antiarrhythmic agent, is greatest during menses, intermediate at ovulation, and least in the luteal phase, in which progesterone level is highest (28). In this study (28), the authors concluded that progesterone is a major determinant of the cyclical changes during the menstrual cycle in ibutilide-induced

$QT_C$ prolongation. As regards to the increased female hormone levels during pregnancy, the risk of TdP in congenital LQTS patients is significantly decreased during pregnancy, although cardiac events are increased postpartum, suggesting the involvement of changes in serum female hormone levels (31). In post-menopausal women, although earlier studies reported conflicting findings regarding the effects of HRT on $QT_C$ interval (17), a recent study with a large study population indicated that HRT with estrogen alone causes slight but significant prolongation of the $QT_C$ interval, while the combination of HRT with estrogen and progestin consistently shortens this interval (27). Collectively, these clinical findings suggest the tempting hypothesis that the luteal hormone progesterone shortens the duration of ventricular depolarization by regulating cardiac ion channels.

Progesterone belongs to the lipophilic gonadal steroid hormone family, whose canonical pathway is through nuclear receptors, resulting in types of transcriptional regulation referred to as genomic effects (32, 33). A few studies have investigated the chronic effects of progesterone on the expression of cardiac ion channels. Song et al. (34) found that 4-day administration of 17$\beta$-estradiol (50 $\mu$g/ml) decreased expression of a transient outward potassium channel, $K_V$4.3, whereas administration of progesterone (3 mg/ml) did not affect the expression of $K_V$4.3. Helguera et al. (35) found that ovarian steroids modify the ratio of isoforms for the $\alpha$1C subunit of the L-type $Ca^{2+}$ channel ($\alpha$1C-long/$\alpha$1C-short) in myometrium, but not in brain or heart. Thus, the genomic effects of progesterone on cardiac ion channels do not appear to be consistent, at least at present, with the clinically-supported conclusion that progesterone shortens the duration of ventricular depolarization.

In addition to genomic effects, in the last decade sex hormones have been shown to exhibit rapid effects that cannot be explained genomically and are referred to as "non-genomic effects" (32). Non-genomic effects take place in membrane-delimited fashion, with phosphoinositide 3-kinase (PI3K)/Akt-dependent activation of endothelial nitric oxide synthase (eNOS) and activation of mitogen-activated protein (MAP)-kinase as the two most well-characterized signaling pathways (36, 37).

We have recently reported that progesterone exhibits acute effects on cardiac repolarization by modulating cardiac slowly-activating delayed rectifier $K^+$ currents ($I_{Ks}$) and L-type $Ca^{2+}$ currents ($I_{Ca,L}$) through a non-genomic pathway involving progesterone receptors (36). The non-genomic pathway for acute effects of progesterone is the same as that for androgen receptors, which we previously reported as mediating the acute effects of testosterone (18). Non-genomic effects of progesterone have already been reported in several cells and tissues (38, 39). We therefore examined rapid effects of physiological circulating levels of progesterone on action potentials and membrane currents in cardiac myocytes isolated from guinea-pig ventricle (36). We found that progesterone acutely modulates either $I_{Ks}$ or $I_{Ca,L}$ via a pathway involving PI3K/Akt-dependent eNOS activation, resulting in shortening of APD (Fig. 1A). The NO produced enhances $I_{Ks}$ to a maximum extent of approximately 140%, and suppresses $I_{Ca,L}$ to a minimum extent of approximately 60%, and both of these effects were abolished by NO scavenger or inhibitors of signaling molecules in the non-genomic pathway. Interestingly, the suppression of $I_{Ca,L}$ was cGMP-dependent, as described previously (40), while the enhancement of $I_{Ks}$ was independent of the cGMP-soluble guanylate cyclase (sGC) pathway and may have involved protein *S*-nitrosylation (36). Since antagonistic effects of cGMP and cAMP on $I_{Ca,L}$ 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 (41), altered the target ion channel, resulting in regulation through non-genomic effects of progesterone, while progesterone shortened APD regardless of SNS stimulation (Fig. 1: B and C). In the basal condition, progesterone enhanced $I_{Ks}$ in dose-dependent fashion ($EC_{50}$ = 2.7 nM), although progesterone at 100 nM (higher than the progesterone level in the luteal phase, approximately 40.6 nM) (42) did not significantly affect $I_{Ca,L}$. In the presence of SNS stimulation (plus cAMP and okadaic acid), progesterone partially suppressed $I_{Ca,L}$ in a dose-dependent fashion ($IC_{50}$ = 29.9 nM), while 100 nM progesterone did not significantly affect $I_{Ks}$. Since the reported progesterone level in women is approximately 2.5 nM in the follicular phase and approximately 40.6 nM in the luteal phase (42), we hypothesized that non-genomic effects of progesterone contribute to the fluctuation of $QT_C$ interval and TdP risk during the menstrual cycle, and we tested this hypothesis using a systems-biological *in silico* approach. Our findings for the acute effects of progesterone have been incorporated into a computational model of cardiac action potential, the Faber-Rudy model for a guinea-pig ventricular myocyte (43). The model reproduces observed fluctuations of cardiac repolarization during the menstrual cycle in women and predicts protective effects of progesterone against rhythm disturbance in a cellular and a cell sheet model of congenital and drug-induced LQTS (36). These findings (36) suggest that nongenomic regulation of cardiac ion channels by progesterone has a major impact on fluctua-

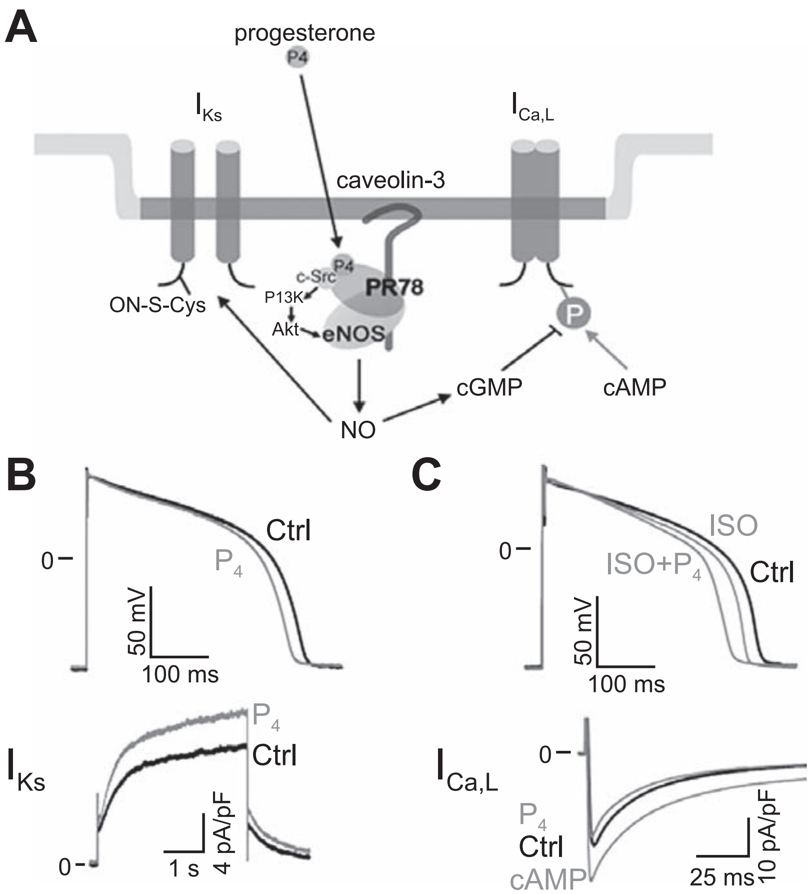


**Fig. 1.** Regulation of the L-type $Ca^{2+}$ channel and the $I_{Ks}$ channel via a non-genomic pathway involving the progesterone receptor (36). A: Schematic diagram of regulation of the L-type $Ca^{2+}$ channel and the $I_{Ks}$ channel through a non-genomic pathway involving the progesterone receptor. When progesterone binds to its receptor, PR78, c-Src, PI3-kinase (PI3K), and Akt are activated sequentially. Subsequently, Akt phosphorylates NOS3 (endothelial NO synthase) to increase its production of NO. The NO produced inhibits cAMP-stimulated $I_{Ca,L}$ in a cGMP-dependent fashion and enhances $I_{Ks}$ in a cGMP-independent fashion. B: Effects of progesterone on the action potential (upper) and $I_{Ks}$ (lower) under basal conditions. Shown are representative recordings before (Ctrl) and 10 min after ($P_4$) application of progesterone at 40.6 nM, the reported serum level in the luteal phase of adult women. $I_{Ca,L}$ was not modified by the application of $P_4$ under these conditions. C: Effects of progesterone on the action potential (upper) and $I_{Ca,L}$ (lower) under conditions mimicking sympathetic nervous system stimulation. Representative recordings of the action potential in the control condition (Ctrl), after administration of isoproterenol at 100 nM (ISO), and after additional application of progesterone ($P_4$) at 40.6 nM. Representative recordings of $I_{Ca,L}$ just after establishment of whole-cell patch configuration (Ctrl), after stabilization of effects of cAMP and okadaic acid (cAMP), and after subsequent application of progesterone at 40.6 nM ($P_4$). $I_{Ks}$ was not modified by $P_4$ under these conditions.

tion of female baseline $QT_C$ during the menstrual cycle of approximately 10 ms, consistent with previous clinical reports (21, 28, 30).

## Estrogen

Estrogen has a number of cardiovascular effects that may be arrhythmic or anti-arrhythmic. There is in fact much evidence to suggest that estrogen may reduce the risk of arrhythmias indirectly by protecting against cardiac ischemia–reperfusion injury as a consequence of vasodilation (44). Estrogen may have a profound impact in some cases of drug-induced LQTS (3, 17, 45). Hara et al. (45) found that chronic treatment with 17*β*-estradiol (E2) enhanced E4031-induced APD prolongation and the incidence and magnitude of EAD in rabbit papillary muscle. Although a chronic treatment of E2 dramatically enhanced the APD-prolongation induced by E4031, an hERG blocker, this treatment did not affect baseline electrocardiographic characteristics, suggesting that chronic E2 treatment reduces the repolarization reserve (45). In another cellular study, Pham et al. (20) showed that serum E2 levels were unrelated to the effects of dofetilide, although testosterone decreased the effect. These data suggest that the effects of sex hormones on hERG blocker sensitivity may be drug-specific. These chronic effects of E2 may be involved in transcriptional regulation of message levels of some $K^+$ channels, but not the hERG channel (19). It is difficult to clearly discuss the relative impact of estrogen-induced transcriptional regulation on cardiac repolarization, since there may be several unknown transcriptional targets and wide interspecies variation in it (19, 46).

Regarding effects on cardiac repolarization, although there are conflicting findings regarding the effects of estrogen on $QT_C$ intervals in women, a recent large-scale clinical study of post-menopausal women revealed very slight, but significant, $QT_C$ prolongation by a few milliseconds with estrogen-replacement menopausal therapy alone (27). Because $QT_C$ prolongation in women currently taking estrogen-only HRT is statistically significant compared with that in women with previous use of HRT (27), exogenous estrogen may affect cardiac repolarization in a reversible fashion, suggesting the existence of acute effects of estrogen. This clinical evidence suggests that acute effects of estrogen are likely to underlie the dynamic fluctuation in drug-induced $QT_C$ prolongation and arrhythmia development during the menstrual cycle (28), in turn suggesting the contribution of non-transcriptional, ‘acute’ effects of

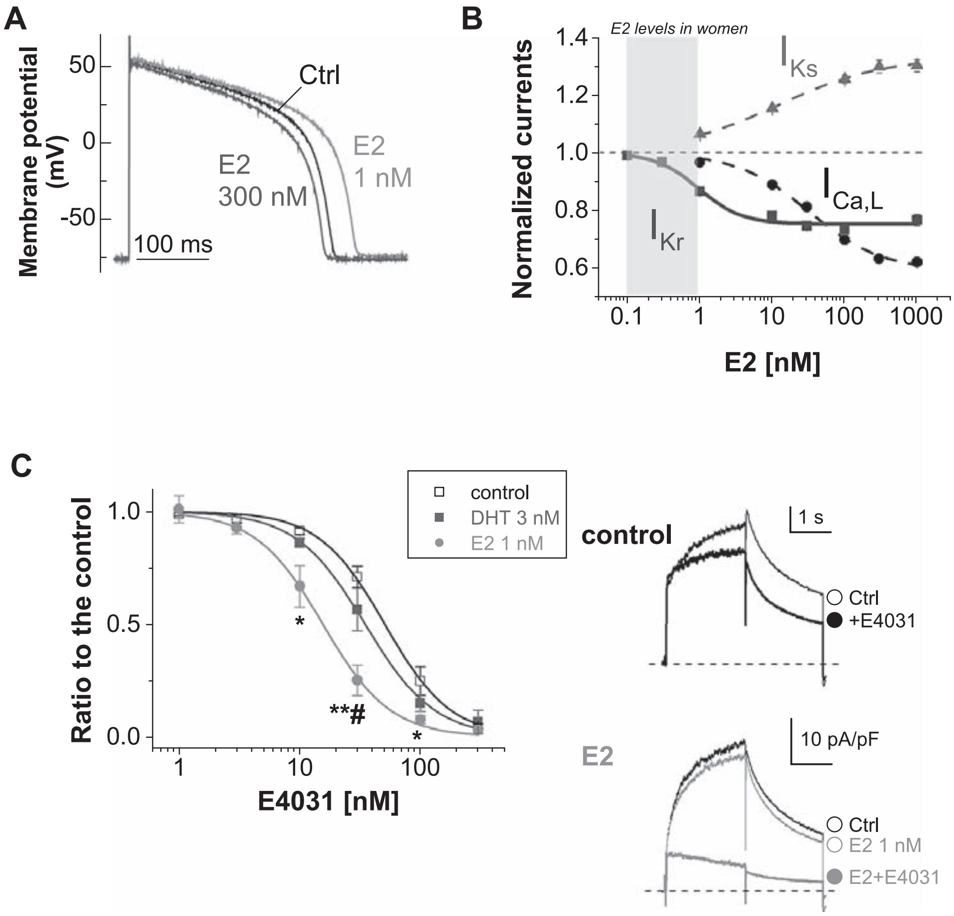


**Fig. 2.** Receptor-independent effects of 17$\beta$-estradiol (E2) on the $I_{Kr}$ channel in guinea-pig hearts and human hearts (47). A: Dual effects of E2 on action potentials. Shown are representative action potential traces recorded from an isolated guinea-pig ventricular myocyte without (control) or with E2. Action potential duration (APD) is significantly prolonged by E2 at 1 nM, while APD is shortened by E2 at 300 nM. B: Concentration-dependent effects of E2 on $I_{Ca,L}$, $I_{Kr}$, and $I_{Ks}$ recorded from guinea-pig ventricular myocytes. Curves represent the best fit of data points with Langmuir's isotherm, where $K_d$ is the dissociation equilibrium constant and A is the drug-sensitive component. $I_{Ca,L}$: $K_d = 29.5$ nM and A = 0.40, $I_{Kr}$: $K_d = 1.3$ nM and A = 0.27, $I_{Ks}$: $K_d = 39.4$ nM and A = 0.25. C: Effects of E2 and DHT on sensitivity to E4031 of hERG currents recorded from HEK293 cells. Concentration-dependent curves (left) are drawn with fits of normalized tail amplitudes relative to the values before application of E4031. $IC_{50}$ values are as follows: Control (open squares): 50.6 nM, E2 at 1 nM (closed circles): 15.5 nM, DHT at 3 nM (closed squares): 34.8 nM. *$P$<0.05, **$P$<0.01 vs. control, #$P$<0.05 vs. DHT, ANOVA. Representative traces (right) in the absence (control) or presence of E2 at 1 nM are shown by superimposing the traces before (open symbols) and after addition of E4031 at 30 nM (closed circles).

estrogen on ion channels to cardiac repolarization.

We recently found that physiological concentrations of E2 acutely delayed cardiac repolarization, resulting in prolongation of the $QT_C$ interval and APD (Fig. 2A) (47). A wide range of concentrations of E2 had various effects on at least 3 important cardiac ion channels in guinea-pig ventricles. Lower concentrations of E2 inhibited $I_{Kr}$ channel currents ($I_{Kr}$) ($K_d = 1.3$ nM), resulting in $QT_C$ prolongation, while higher 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), resulting in $QT_C$ shortening (Fig. 2: A and B) (47). Since 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 (6, 21, 30), the effects of E2 on $I_{Kr}$ can have a major impact on the cyclical changes in cardiac repolarization and TdP risk during the menstrual cycle (28). The magnitude of $I_{Kr}$ suppression by physiological levels of E2 was statistically significant but relatively small (<30%), and such partial suppression may be due to a shift in voltage-dependence of $I_{Kr}$ activation (47). This is consistent with the less clear impact of circulating estrogen on fluctuation of female baseline $QT_C$ intervals during the menstrual cycle than that of progesterone (21, 28, 30).

Very recently, we found that estrone 3-sulfate at physiological concentrations in both women and men at any ages can suppress hERG currents with the maximal extent of effectiveness (48).

The $I_{Kr}$ suppression by E2 has been proposed to be a type of receptor-independent regulation because an estrogen receptor inhibitor did not antagonize the E2-induced $I_{Kr}$ suppression and E2 suppressed hERG currents in estrogen-negative culture cell lines. A mutagenesis study of the common drug-binding sites of the hERG channel (49, 50) revealed that aromaticity of $Phe^{656}$ 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 (47). In fact, as shown in Fig. 2C, E2 augments the hERG blockade by E4031 whose binding site includes $Phe^{656}$ and enhances the sensitivity of E4031 to $QT_C$ prolongation in Langendorff-perfused guinea-pig hearts (47). Although the mechanism responsible for it has not been clarified, E2 enhances the E4031-induced hERG suppression, in line with the increase in ibutilide-induced $QT_C$ prolongation in the late follicular phase (28), which could underlie the enhanced susceptibility of women to acquired LQTS (1 – 3, 6).

## Closing remarks

We have summarized here recent progress in examination of gender differences in susceptibility to arrhythmias including drug-induced long-QT syndrome. In addition to well-characterized genomic effects of ovarian steroids, non-genomic effects mediated via the progesterone receptor (36) and receptor-independent regulation by estrogen (47) have recently been introduced as novel possible causes of the higher susceptibility to drug-induced long-QT syndrome in women. These new findings on the acute effects of female hormones will have to be taken into account in assessing the risk of drug-induced QT prolongation in women during the menstrual cycle.

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## References

1 Lehmann MH, Hardy S, Archibald D, quart B, MacNeil DJ. Sex difference in risk of torsade de pointes with d,l-sotalol. Circulation. 1996;94:2535–2541.
2 Makkar RR, Fromm BS, Steinman RT, Meissner MD, Lehmann MH. Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs. JAMA. 1993;270:2590–2597.
3 Drici MD, Knollmann BC, Wang WX, Woosley RL. Cardiac actions of erythromycin: influence of female sex. JAMA. 1998; 280:1774–1776.
4 Tamargo J. Drug-induced torsade de pointes: from molecular biology to bedside. Jpn J Pharmacol. 2000;83:1–19.
5 Hashimoto K. Arrhythmia models for drug research: classification of antiarrhythmic drugs. J Pharmacol Sci. 2007;103:333–346.
6 James AF, Choisy SC, Hancox JC. Recent advances in understanding sex differences in cardiac repolarization. Prog Biophys Mol Biol. 2007; 94:265–319.
7 Hreiche R, Morissette P, Turgeon J. Drug-induced long QT syndrome in women: review of current evidence and remaining gaps. Gend Med. 2008;5:124–135.
8 Fermini B, Fossa AA. The impact of drug-induced QT interval prolongation on drug discovery and development. Nature Rev Drug Discovery. 2003;2:439–447.
9 Abriel H, Schlapfer J, Keller DI, Gavillet B, Buclin T, Biollaz J, et al. Molecular and clinical determinants of drug-induced long QT syndrome: an iatrogenic channelopathy. Swiss Med Wkly. 2004;134:685–694.
10 Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, et al. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. Science. 2002;295:496–499.
11 Kurokawa J. Compartmentalized regulations of ion channels in the heart. Biol Pharm Bull. 2007;30:2231–2237.
12 Satoh Y, Sugiyama A, Tamura K, Hashimoto K. Effects of mexiletine on the canine cardiovascular system complicating cisapride overdose: potential utility of mexiletine for the treatment of drug-induced long QT syndrome. Jpn J Pharmacol. 2000;83:327–334.
13 Takahara A, Nakamura H, Nouchi H, Tamura T, Tanaka T, Shimada H, et al. Analysis of arrhythmogenic profile in a canine model of chronic atrioventricular block by comparing in vitro effects of the class III antiarrhythmic drug nifekalant on the ventricular action potential indices between normal heart and atrioventricular block heart. J Pharmacol Sci. 2007;103:181–188.
14 Locati EH, Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Lehmann MH, et al. Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome: findings from the International LQTS registry. Circulation. 1998;97:2237–2244.
15 Bazett HC. The time relations of the blood-pressure changes after excision of the adrenal glands, with some observations on blood volume changes. J Physiol. 1920;53:320–339.
16 Bidoggia H, Maciel JP, Capalozza N, Mosca S, Blaksley EJ, Valverde E, et al. Sex differences on the electrocardiographic pattern of cardiac repolarization: possible role of testosterone. Am Heart J. 2000;140:678–683.
17 Abi-Gerges N, Philp K, Pollard C, Wakefield I, Hammond TG, Valentin JP. Sex differences in ventricular repolarization: from cardiac electrophysiology to Torsades de Pointes. Fundam Clin Pharmacol. 2004;18:139–151.

18 Bai CX, Kurokawa J, Tamagawa M, Nakaya H, Furukawa T. Nontranscriptional regulation of cardiac repolarization currents by testosterone. Circulation. 2005;112:1701–1710.

19 Drici MD, Burklow TR, Haridasse V, Glazer RI, Woosley RL. Sex hormones prolong the QT interval and downregulate potassium channel expression in the rabbit heart. Circulation. 1996;94:1471–1474.

20 Pham TV, Sosunov EA, Gainullin RZ, Danilo P Jr, Rosen MR. Impact of sex and gonadal steroids on prolongation of ventricular repolarization and arrhythmias induced by $I_K$-blocking drugs. Circulation. 2001;103:2207–2212.

21 Hulot J-S, Demolis J-L, Riviere R, Strabach S, Christin-Maitre S, Funck-Brentano C. Influence of endogenous oestrogens on QT interval duration. Eur Heart J. 2003;24:1663–1667.

22 Merri M, Benhorin J, Alberti M, Locati E, Moss AJ. Electrocardiographic quantitation of ventricular repolarization. Circulation. 1989;80:1301–1308.

23 Rautaharju PM, Zhou SH, Wong S, Calhoun HP, Berenson GS, Prineas R, et al. Sex differences in the evolution of the electrocardiographic QT interval with age. Can J Cardiol. 1992;8:690–695.

24 Stramba-Badiale M, Spagnolo D, Bosi G, Schwartz PJ. Are gender differences in QTc present at birth? MISNES Investigators. Multicenter Italian Study on Neonatal Electrocardiography and Sudden Infant Death Syndrome. Am J Cardiol. 1995;75:1277–1278.

25 Furukawa T, Bai CX, Kaihara A, Ozaki E, Kawano T, Nakaya Y, et al. Ginsenoside Re, a main phytosterol of Panax ginseng, activates cardiac potassium channels via a nongenomic pathway of sex hormones. Mol Pharmacol. 2006;70:1916–1924.

26 Kurokawa J, Furukawa T. [Protein modification of cardiac potassium channels and lethal arrhythmias.] Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi). 2005;26:273–279. (in Japanese)

27 Kadish AH, Greenland P, Limacher MC, Frishman WH, Daugherty SA, Schwartz JB. Estrogen and progestin use and the QT interval in postmenopausal women. Ann Noninvasive Electrocardiol. 2004;9:366–374.

28 Rodriguez I, Kilborn MJ, Liu XK, Pezzullo JC, Woosley RL. Drug-induced QT prolongation in women during the menstrual cycle. JAMA. 2001;285:1322–1326.

29 Burke JH, Ehlert FA, Kruse JT, Parker MA, Goldberger JJ, Kadish AH. Gender-specific differences in the QT interval and the effect of autonomic tone and menstrual cycle in healthy adults. Am J Cardiol. 1997;79:178–181.

30 Nakagawa M, Ooie T, Takahashi N, Taniguchi Y, Anan F, Yonemochi H, et al. Influence of menstrual cycle on QT interval dynamics. Pacing Cclin Electrophysiol. 2006;29:607–613.

31 Rashba EJ, Zareba W, Moss AJ, Hall WJ, Robinson J, Locati EH, et al. Influence of pregnancy on the risk for cardiac events in patients with hereditary long QT syndrome. Circulation. 1998; 97:451–456.

32 Furukawa T, Kurokawa J. Regulation of cardiac ion channels via non-genomic action of sex steroid hormones: Implication for the gender difference in cardiac arrhythmias. Pharmacol Ther. 2007;115:106–115.

33 Aranda A, Pascual A. Nuclear hormone receptors and gene expression. Physiol Rev. 2001;81:1269–1304.

34 Song M, Helguera G, Eghbali M, Zhu N, Zarei MM, Olcese R, et al. Remodeling of Kv4.3 potassium channel gene expression under the control of sex hormones. J Biol Chem. 2001; 276:31883–31890.

35 Helguera G, Olcese R, Song M, Toro L, Stefani E. Tissue-specific regulation of $Ca^{2+}$ channel protein expression by sex hormones. Biochim Biophys Acta. 2002;1569:59–66.

36 Nakamura H, Kurokawa J, Bai CX, Asada K, Xu J, Oren RV, et al. Progesterone regulates cardiac repolarization through a nongenomic pathway: an in vitro patch-clamp and computational modeling study. Circulation. 2007;116:2913–2922.

37 Simoncini T, Genazzani AR. Non-genomic actions of sex steroid hormones. Eur J Endocrinol. 2003;148:281–292.

38 Ehring GR, Kerschbaum HH, Eder C, Neben AL, Fanger CM, Khoury RM, et al. A nongenomic mechanism for progesterone-mediated immunosuppression: inhibition of $K^+$ channels, $Ca^{2+}$ signaling, and gene expression in T lymphocytes. J Exp Med. 1998;188:1593–1602.

39 Mendiberri J, Rauschemberger MB, Selles J, Massheimer V. Involvement of phosphoinositide-3-kinase and phospholipase C transduction systems in the non-genomic action of progesterone in vascular tissue. Int J Biochem Cell Biol. 2006;38:288–296.

40 Fischmeister R, Castro L, Abi-Gerges A, Rochais F, Vandecasteele G. Species- and tissue-dependent effects of NO and cyclic GMP on cardiac ion channels. Comp Biochem Physiol A Mol Integr Physiol. 2005;142:136–143.

41 Kass RS, Kurokawa J, Marx SO, Marks AR. Leucine/isoleucine zipper coordination of ion channel macromolecular signaling complexes in the heart. Roles in inherited arrhythmias. Trends Cardiovasc Med. 2003;13:52–56.

42 Janse de Jonge XA, Boot CR, Thom JM, Ruell PA, Thompson MW. The influence of menstrual cycle phase on skeletal muscle contractile characteristics in humans. J Physiol. 2001;530:161–166.

43 Faber GM, Rudy Y. Action potential and contractility changes in $[Na^+]_i$ overloaded cardiac myocytes: a simulation study. Biophys J. 2000;78:2392–2404.

44 Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev. 2008;88:581–609.

45 Hara M, Danilo P Jr, Rosen MR. Effects of gonadal steroids on ventricular repolarization and on the response to E4031. J Pharmacol Exp Ther. 1998;285:1068–1072.

46 Fulop L, Banyasz T, Szabo G, Toth IB, Biro T, Lorincz I, et al. Effects of sex hormones on ECG parameters and expression of cardiac ion channels in dogs. Acta Physiol (Oxf). 2006;188:163–171.

47 Kurokawa J, Tamagawa M, Harada N, Honda SI, Bai CX, Nakaya H, et al. Acute effects of estrogen on the guinea pig and human $I_{Kr}$ channels and drug-induced prolongation of cardiac repolarization. J Physiol. 2008;586:2961–2973.

48 Kakusaka S, Asayama M, Kaihara A, Sasano T, Suzuki T, Kurokawa J, et al. A receptor-independent effect of estrone sulfate on the hERG channel. J Pharmacol Sci. 2009;109:152–156.

49 Sanguinetti MC, Mitcheson JS. Predicting drug-hERG channel interactions that cause acquired long QT syndrome. Trends Pharmacol Sci. 2005;26:119–124.

50 Clancy CE, Kurokawa J, Tateyama M, Wehrens XH, Kass RS. $K^+$ channel structure-activity relationships and mechanisms of drug-induced QT prolongation. Annu Rev Pharmacol Toxicol. 2003;43:441–461.