A Combined Approach Using Patch-Clamp Study and Computer Simulation Study for Understanding Long QT Syndrome and TdP in Women

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Abstract: Female sex is an independent risk factor for development of torsade de pointes (TdP)-type arrhythmias in both congenital and acquired long QT syndrome (LQTS). In females, QTc interval and TdP risk vary during the menstrual cycle and around delivery. Biological experiments including single-cell current recordings with the patch-clamp technique and biochemical experiments show that progesterone modulates cardiac K⁺ current and Ca²⁺ current via the non-genomic pathway of the progesterone receptor, and thus the cardiac repolarization duration, in a concentration-dependent manner. Incorporation of these biological findings into a computer model of single-cell and coupled-cell cardiomyocytes simulates fluctuations in QTc interval during the menstrual cycle with reasonable accuracy. Based on this model, progesterone is predicted to have protective effects against sympathetic nervous system-induced arrhythmias in congenital LQTS and drug-induced TdP in acquired LQTS. A combined biological and computational approach may provide a powerful means to risk stratify TdP risk in women.

Key Words: Long QT syndrome, sex hormone, nitric oxide, arrhythmia, patch-clamp, non-genomic pathway

INTRODUCTION

A growing body of evidence suggests that clinical arrhythmia syndromes emerge as a result of complicated interactions of multiple endogenous and environmental factors. A combined approach using patch-clamp study and computer simulation study is a powerful means for investigating the influence of multiple interacting factors on the development of clinical symptoms. In this mini-review, we will discuss our recent work using a combined biological and computational approach to predict arrhythmic risks in women.

1. ARRHYTHMIAS IN LONG QT SYNDROME (LQTS) IN WOMEN

LQTS is a cardiac arrhythmia syndrome characterized by prolonged QT intervals on the 12-lead surface electrocardiogram, polymorphic ventricular tachyarrhythmias with unique morphology, called torsade de pointes (TdP), and syncope and sudden death. Experiments using multicellular wedge preparation indicate that TdP is triggered by early afterdepolarization (EAD) followed by intramural phase 2 reentry, which is based on heterogeneous prolongation myocardial action potential duration (APD) [3]. APD prolongation is caused by either suppression of outward currents including transient outward current (Iₒ), and rapidly-activating and slowly-activating delayed rectifier K⁺ current (Iₖᵣ and Iₖₐ), or/and enhancement of inward currents including L-type Ca²⁺ current (Iₖₗₐₗ) and persistent Na⁺ current (Iₙa).

LQTS occurs as a congenital form or an acquired form. In both congenital and acquired LQTS, female sex is an independent risk factor for the development of TdP [1, 2]. In females, there are dynamic fluctuations in QTc interval and the risk of TdP during the menstrual cycle [4]. Although several previous studies did not find QTc interval differences among the different menstrual phases [5, 6], a recent study analyzing various parameters of cardiac repolarization finds that repolarization duration is shorter in the luteal phase than in the follicular phase by about 10 msec [6]. Ibutilide is a class III antiarrhythmic agent that prolongs QTc interval in a dose-dependent manner, and is used for termination of atrial fibrillation and atrial flutter. QTc prolongation induced by ibutilide is the greatest during menses (63 msec), intermediate in ovulation (59 msec), and the least in the luteal phase (53 msec) [5]. In these studies [5, 7], serum sex hormone level was determined: serum progesterone level was higher in ovulation (59 msec), and the least in the luteal phase (53 msec) [5]. In these studies [5, 7], serum sex hormone level was determined: serum progesterone level was higher in the luteal phase than in the follicular phase, during menses, and in ovulation, while serum 17β-estradiol level was not significantly different between the luteal phase and the follicular phase. Thus, progesterone is suggested to be responsible for differences in cardiac repolarization duration and in ibutilide-induced QTc prolongation during the menstrual cycle.

In post-menopausal women, although earlier studies report conflicting data for effects of hormone replacement therapy on QTc interval [8–10], a recent study consisting of a large study population indicates that hormone replacement therapy with estrogen alone causes slight but significant prolongation of QTc interval by about 2 msec, while combination hormone replacement therapy with estrogen and progesterin consistently shortens QTc interval by about 1 msec [11]. Effects of pregnancy in LQTS patients were also examined [12, 13]. In careful survey of arrhythmia events in congenital LQTS patients around delivery, new-onset of arrhythmia events increased postpartum where progesterone level falls dramatically compared to before or during pregnancy [12]. Taken together, the luteal hormone, progester-
one, is strongly suggested to have protective effects against long QT-associated arrhythmias.

2. GENOMIC EFFECTS OF PROGESTERONE ON CARDIAC ION CHANNELS

Progestosterone belongs to lipophilic gonadal steroid hormone family, whose canonical pathway is to permeate into cell across surface membrane, binds to intracellular receptor, translocates into the nucleus as a ligand/receptor complex form, and binds to a gene containing a hormone responsive element (Fig. 1) [14-16]. In addition to this “genomic action”, for the last decade sex hormones have been shown to exhibit rapid actions which cannot be explained by genomic action and are referred to as “non-genomic action” (Fig. 1) [17-20]. Non-genomic action takes place in a membrane-delimited manner: PI3-kinase/Akt-dependent activation of endothelial nitric oxide synthase (eNOS) [21, 22] and activation of MAP-kinase [23, 24] are the two most well characterized signaling pathways.

Previous studies of effects of progesterone on cardiac ion channels have mostly dealt with its genomic actions. Song et al. [25] examined effects of gonadal steroids on expression of transient outward current channels, K\text{v}4.3, using a myometrium heterologous expression as a model system. They found that 4 days-injection of 17β-estradiol (50 μg/ml) decreased expression of K\text{v}4.3, whereas injection of progesterone (3 mg/ml) did not affect K\text{v}4.3 expression. The α1C subunit of the L-type Ca\textsuperscript{2+} current (I\textsubscript{Ca,L}) channel can be detected as a 240 kDa long form (α1C long) and a 190 kDa short form (α1C short). In myometrium, 17β-estradiol decreased the long α1C form/short α1C form (L/S ratio), while progesterone increased the L/S ratio; in brain or heart, either 17β-estradiol or progesterone did not change the L/S ratio [26]. Thus, the genomic effects of progesterone on cardiac repolarization are currently undefined and cannot explain a protective effect of progesterone against TdP risk.

3. NON-GENOMIC EFFECTS OF PROGESTERONE ON CARDIAC ION CURRENTS

Major currents determining cardiac repolarization are I\textsubscript{Ks}, I\textsubscript{Kr}, and I\textsubscript{Ca,L} in human and guinea-pig. I\textsubscript{Ks} and I\textsubscript{Ca,L} are critical in mouse and rat [27, 28]. Thus, we used cardiac myocytes isolated from guinea pig left ventricle to investigate acute effects of progesterone. Sympathetic nervous system (SNS) stimulation is a critical triggering factor for TdP in LQTS patients [29], and thus we examined both the basal condition and the SNS stimulation-mimicked condition with isoproterenol application or with intracellular dialysis of cAMP and okadaic acid (OA). Progesterone at a concentration of 100 nM shortened APD both in the basal condition and the SNS-stimulated condition. Progesterone-induced APD shortening is via the non-genomic pathway, since progesterone-induced APD shortening was observed within a few minutes, reached steady-state within 10 min, and was inhibited by a specific progesterone receptor inhibitor, mifepristone (1 μM).

The ionic mechanism underlying APD shortening by progesterone is to modulate I\textsubscript{Ks} and I\textsubscript{Ca,L}, but not I\textsubscript{Kr}. In the basal condition, progesterone enhanced I\textsubscript{Ks} in a concentration-dependent manner with an EC\textsubscript{50} value of 2.7 nM, while progesterone did not significantly affect I\textsubscript{Ca,L} (Fig. 2A) [30]. SNS-stimulation caused enhancement of both I\textsubscript{Ca,L} and I\textsubscript{Ks}. Further application of progesterone reduced I\textsubscript{Ca,L} to the level before cAMP and OA application, while it did not significantly change I\textsubscript{Ks} [30]. The IC\textsubscript{50} value for I\textsubscript{Ca,L} suppression was 29.9 nM (Fig. 2B).

The biophysical mechanism for regulation of I\textsubscript{Ks} and I\textsubscript{Ca,L} is different. The effects of progesterone on I\textsubscript{Ks} were frequency- and voltage-independent [30]. In contrast, progesterone caused a positive shift in the I\textsubscript{Ca,L} activation curve and a negative shift in the inactivation curve [30]. Computer simulation analysis showed that changes in current conduc-

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**Fig. (1).** Genomic and non-genomic pathway of sex hormones.

In the genomic pathway, sex steroid hormones penetrate into cells, and bind to receptors in the cytosol. The ligand/receptor complex then translocates into the nucleus, binds to the genes with hormone responsive element (HRE), and regulates gene expression. In the non-genomic pathway, sex hormones release nitric oxide via the PI3-kinase/Akt/eNOS pathway or activate MAP-kinase in a membrane-delimited manner.
tance without changes in current kinetics reproduced the effects of progesterone on $I_{Ks}$ observed in biological experiments. Changes in voltage dependency alone with no change in current conductance reproduced the effects of progesterone on $I_{Ca,L}$ with a high accuracy. Thus, effects of progesterone on $I_{Ks}$ are mainly to alter current conductance and modulate $I_{Ca,L}$ by affecting current kinetics.

Despite distinct biophysical mechanism for $I_{Ks}$ and $I_{Ca,L}$ regulation, the principal mediator for both $I_{Ks}$ enhancement in the basal condition and $I_{Ca,L}$ suppression in the SNS-stimulated condition appears to be nitric oxide (NO), since both were abolished by nitric oxide (NO) trappers and eNOS inhibitors [31, 32]. However, the mechanism by which NO modulates $I_{Ks}$ and $I_{Ca,L}$ appears to be different. $I_{Ca,L}$ suppression by progesterone was abolished by an inhibitor of soluble guanylyl cyclase (sGC), indicating that $I_{Ca,L}$ is regulated by progesterone via a NO/sGC/cGMP axis (Fig. 3) [33]. Antagonistic action of cAMP and cGMP for $I_{Ca,L}$ has been demonstrated, which appears to vary among species [34]. In rabbit and frog ventricular myocytes, cGMP antagonizes cAMP effects by promoting cAMP-dependent phosphodiesterase (PDE2) [34]. In guinea-pig and rat ventricular myocytes, cAMP-dependent protein kinase (PKA) phosphorylates the $\alpha$-subunit of $I_{Ca,L}$ and enhances $I_{Ca,L}$ only in the presence of A-kinase anchoring protein (AKAP) [34], cGMP-dependent protein kinase (PKG) phosphorylates both the $\alpha$-subunit and the $\beta$-subunit of $I_{Ca,L}$ [35]. Phosphorylation of the $\alpha$-subunit by PKG does not affect $I_{Ca,L}$, likely due to the absence of AKAP, while phosphorylation of the $\beta$-subunit antagonizes the effect of the $\alpha$-subunit phosphorylation by PKA [35]. In addition, the inhibition of PDE3 by cGMP to enhance the cAMP-induced activation and facilitation of $I_{Ca,L}$ and activation of protein phosphatase via cGMP-PKG signaling pathway may also be involved.

On the other hand, $I_{Ks}$ enhancement was not inhibited by a sGC inhibitor, but was inhibited by a thiol-alkylating reagent, $N$-ethylmaleimide, and a reducing reagent, di-thiothreitol [33]. These data suggest that cGMP-independent mechanisms, possibly protein $s$-nitrosylation, play a role for $I_{Ks}$ enhancement (Fig. 3) [33]. Protein $s$-nitrosylation is the direct NO transfer to the thiol residue of Cys, is highlighted as a novel mechanism of protein post-translational modification [36, 37], and occurs independent of cAMP. Thus, it is possible that progesterone regulates $I_{Ks}$ in the basal condition and $I_{Ca,L}$ only in the SNS-stimulated condition. However, it remains to be proven if the $I_{Ks}$ channel is indeed $s$-nitrosylated. If that is the case, it is also undetermined whether the $\alpha$-subunit, KCNQ1, or the $\beta$-subunit, KCNE1, is the target of $s$-nitrosylation, what is the underlying mechanism for specific $s$-nitrosylation of KCNQ1 or KCNE1, and how $s$-nitrosylation induces $I_{Ks}$ channel activation.

4. COMPUTATIONAL SIMULATION OF THE EFFECTS OF PROGESTERONE

$QT_c$ interval and TdP risk are regulated by various factors, including SNS status, heart rate, medications, serum electrolyte level, and others. Our biological experiments suggest progesterone as an additional major factor that modulates $QT_c$ interval and TdP risk. Since progesterone
level varies during the menstrual cycle and around delivery, progesterone effects may contribute to the fluctuation of QTc interval and TdP risk during the menstrual cycle and pregnancy. Since a computational approach is especially powerful to simulate these changes, our first challenge was to investigate if incorporating effects of progesterone in the cardiac APD computer model reproduces fluctuation of APD during the menstrual cycle.

We incorporated effects of progesterone obtained in our biological experiments in the Faber-Rudy model of the guinea pig myocyte [38]. Since reported progesterone level in women is ~2.5 nM in the follicular phase and ~40.6 nM in the luteal phase [39], we incorporated effects of progesterone at 2.5 nM and at 40.6 nM. The model predicts that progesterone at 40.6 nM shortens APD by 3.7% under basal conditions and 4.6% under SNS-stimulated conditions compared to APD at 2.5 nM progesterone (Fig. 4) [30]. Clinically observed QT intervals are shorter by about 2.4%-2.8% in the luteal phase than in follicular phase [5], and so the APD shortening predicted in the model (3.7-4.6%) fits well with the observed fluctuation in QT interval during the menstrual cycle in women.

Effects of progesterone in a single cell do not necessarily predict the effect at the multi-cell level, organ level, or in vivo level. As a first step to simulate effects of progesterone in higher dimensions, we constructed a coupled-cells model, in which 100 cardiomyocytes are electrotonically connected with simulated resistances between them to represent gap-junctions. We then investigated the effects of progesterone and SNS in simulated coupled tissue and computed virtual electrograms from simulated gradients of depolarization and repolarization. Simulations suggest that during the luteal phase when progesterone = 40.6 nM, maximal SNS may additionally shorten QT interval by 12.2% (Fig. 4) [30]. These simulations support the notion that progesterone may exert protective QT shortening effects under conditions on SNS.

5. PREDICTED EFFECTS OF PROGESTERONE AGAINST ARRHYTHMIA

Since the model reproduces the effects of progesterone on APD in patch-clamp experiments and QTc variation during the menstrual cycle in women with a good accuracy, our next step was to utilize this model to predict the effects of progesterone on LQTS-associated arrhythmia susceptibility. To examine the effects on SNS-induced arrhythmias, we used the D76N KCNE1 mutation linked to congenital LQTS5. I_{Ks} exhibits accumulation in the pre-open state during the rapid heart rates, resulting in action potential adaptation [40]. SNS stimulation enhances I_{Ca,L} to increase Ca2+ influx [41]. SNS stimulation also enhances I_{Ks} [42, 43] that counter-balances I_{Ca,L} enhancement and maintains APD within a certain range [44]. In LQTS1 and LQTS5, I_{Ks} channel disturbance results in dysfunction of action potential adaptation to rapid heart rates and response to SNS stimulation.
The D76N KCNE1 mutation reduces the current and renders the IKs channel insensitive to β-adrenergic stimulation [45], thus probands carrying D76N KCNE1 mutation readily develop TdP with SNS stimulation at rapid heart rates [46]. In the absence of progesterone, the mutant model cells are unable to adapt to the fast pacing frequency because IKs fails to increase in response to the SNS stimulation (Fig. 5A) [30]. Interestingly, both in the single-cell and coupled-cell model in the presence of progesterone at 2.5 nM, some improvement is observed; in the presence of 40.6 nM, a failed SNS stimulation response is compensated for by the action of progesterone alone to increase IKs (Fig. 5A) [30]. Thus, enhancement of IKs in the absence of SNS stimulation, and inhibition of cAMP-induced ICa,L by progesterone improve action potential adaptation, which is dependent on progesterone level. These simulations suggest a mechanism for SNS-related arrhythmic risk varies during the menstrual cycle in women.

Drug-induced TdP is believed to occur by blockade of the human ether-a-go-go related gene (hERG) channel by drugs with various structures [47], and at slow heart rates. In a simulation, severe EADs were induced by 50% block of IKr at a slow heart rate (30 bpm) (Fig. 5B). At 2.5 nM of progesterone, some improvement is observed (middle panel); at 40.6 nM of progesterone, the EADs are abolished and the action potential morphology is normalized (Fig. 5B). Thus, progesterone is predicted to have protective effects against drug-induced arrhythmias, which also fluctuate during the menstrual cycle. Progesterone does not have apparent effects on IKr (data not shown), and thus predicted protection against drug-induced EAD may be attributed to an increase in repolarization reserve by IKs enhancement [48].

CONCLUSION

Our patch-clamp experiment demonstrates that the nongenomic effect of the sex hormone progesterone constitutes a novel regulatory mechanism of cardiac repolarization. Serum progesterone level fluctuates during the menstrual cycle: within this level, progesterone modulates IKs and ICa,L and, therefore, is partly responsible for the cyclic changes in QTC interval and TdP risk during the menstrual cycle. A computational approach allows for simulation of multi-factorial and periodical phenomenon. Incorporation of progesterone effects observed in our biological study into the computational model reproduces cyclic changes in QTC interval, and predicts dose-dependent protective effects of progesterone against SNS-stimulation-induced and drug-induced arrhythmias. This approach provides a first step to risk stratify TdP...
Fig. (5). Progesterone may protect against long QT-related arrhythmia.

A) The effects of progesterone on arrhythmia in congenital LQTS. Progesterone improves action potential adaptation in congenital LQTS (LQTS5) at fast heart rates during SNS stimulation. We simulated the D76N mutation in the IKr β-subunit KCNE1 that disrupts regulation of IKr by protein kinase A. Left panel: Shown are 6 action potentials (15th - 20th) elicited from cells with D76N IKr at a fast rate (CL = 150 ms) during the SNS stimulation in the absence (top panel), and in the presence of 2.5 nM (middle panel) or 40.6 nM (bottom panel) progesterone. Right Panel: Simulated propagation of action potentials in paced (150 ms, 29th and 30th beats are shown) one-dimensional tissue in the absence of progesterone (a), in the presence of 40.6 nM progesterone (b) and the corresponding computed electrogram. A gray circle in panel a highlights failure of propagation of the second stimulus, which is applied during the mutation induced extended refractory period.

B) The effects of progesterone on EADs resulting from acquired LQTS simulated by IKr block. Traces of the 9th and 10th action potentials during 50% IKr block in the absence (top panel), and in the presence of 2.5 nM (middle panel) or 40.6 nM (bottom panel) progesterone. The cycle length is 2000 ms. The left panel shows the single cells and the right panel shows results in corresponding fibers under the same conditions.

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references
[1] 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.
[2] Locati EH, Zareba W, Moss AJ, 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.
[3] Antzelevitch C, Shimizu W, Yan GX, Sicouri S. Cellular basis for QT dispersion. J Electrocardiol 1998; 30(supple): 168-175.
[4] 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.
[5] 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.
[6] Hulot JS, Demolis JL, Riviere R, Strbach S, Christin-Maire S, Funck-Brentano C. Influence of endogenous oestrogens on QT interval duration. Eur Heart J 2003; 24: 1663-1667.
[7] Nakagawa M, Osie T, Takahashi N, et al. Influence of menstrual cycle on QT interval dynamics. Pacing Clin Electrophysiol 2006; 29: 607-613.
[8] Larsen JA, Tung RH, Sadananda R, et al. Effects of hormone replacement therapy on QT interval. Am J Cardiol 1998; 82: 995-999.
[9] Sharoumi E, Zarvalis E, Kyriakides ZS, Kremastinos DT. Absence of effects of short-term estrogen replacement therapy on resting and exertional QT and QTc dispersion in postmenopausal women with coronary artery disease. Pacing Clin Electrophysiol 1998; 21: 2392-2395.
[10] Hasekorn K, Seyffart K, Weihe M, Christ M. Effects of progestin-estrogen replacement therapy on QT-dispersion in postmenopausal women. Internat J Cardiol 2000; 75: 161-165.
[11] Kadish AH, Greenland P, Limacher MC, Fruhsm WH, Daugherty SA, Schwartz JB. Estrogen and progestin use and the QT interval in postmenopausal women. Ann Noninvasive Electrocardiol 2004; 9: 366-374.
[12] Rashiha EJ, Zareba W, Moss AJ, et al. Influence of pregnancy on the risk for cardiac events in patients with hereditary long QT syndrome. LQTS Investigators. Circulation 1998; 97: 451-456.
[13] Heradien MJ, Goosen A, Crotti L, et al. Does pregnancy increase cardiac risk for LQT1 patients with the KCNQ1-A341M mutation? J Am Coll Cardiol 2006; 48: 1410-1415.
[14] Beato M, Klug J. Steroid hormone receptors: an update. Hum Reprod Update 2000; 6: 224-236.
[15] Aranda A, Pascual A. Nuclear hormone receptors and gene expression. Physiol Rev 2001; 81: 1269-1304.
[16] Levin ER. Cellular functions of plasma membrane estrogen receptors. Steroids 2002; 1-15.
[17] Losel RM, Hulot JS, Feuring M, et al. Nongenomic steroid action: controversies, questions, and answer. Physiol Rev 2003; 83: 965-1016.
[19] Watson CS, Gametchu B. Proteins of multiple classes may participate in nongenomic steroid actions. Exp Biol Med 2003; 228: 1272-1281.

[20] Bai CX, Kurokawa J, Tamagawa M, Nakaya H, Furukawa T. Non-transcriptional regulation of cardiac repolarization currents by testosterone. Circulation 2005; 112: 1701-1710.

[21] Simioncini T, Mannella P, Forini L, Caruso A, Varone G, Genazzi AR. Genomic and non-genomic effects of estrogens on endothelial cells. Steroids 2004; 69: 537-542.

[22] Haynes MP, Sinha D, Russell KS, et al. Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the PI3-kinase-Akt pathway in human endothelial cells. Circ Res 2000; 87: 677-682.

[23] Endoh H, Sasaki H, Maruyama K, et al. Rapid activation of MAP kinase by estrogen in the bone cell line. Biochem Biophys Res Commun 1997; 235: 99-102.

[24] Watters JJ, Campbell JS, Cunningham MJ, Krebs EG, Dorsa D. Rapid membrane effects of steroids in neuroblastoma cells: effects of estrogen on mitogen activated protein kinase signaling cascade and c-fos immediate early gene transcription. Endocrinology 1997; 138: 4030-4033.

[25] Song M, Helguera G, Eghbali M, et al. Remodeling of Kv4.3 potassium channel gene expression under the control of sex hormones. J Biol Chem 2001; 276: 31883-31890.

[26] Helguera G, Olcese R, Song M, Toro L, Stefani E. Tissue-specific regulation of Ca2+ channel protein expression by sex hormones. Biochim Biophys Acta 2002; 1569: 59-66.

[27] Varró A, Lathrop DA, Hester SB, Nánásiová OÖ, Papp JG. Ionic currents and action potentials in rabbit, rat and guinea pig ventricular myocytes. Basic Res Cardiol 1993; 88: 93-102.

[28] Furukawa T, Kurokawa J. Potassium channel remodeling in cardiac hypertrophy. J Mol Cell Cardiol 2006; 41: 753-761.

[29] Schwartz PJ, Priori SG, Cerrone M, et al. Progesterone regulates cardiac repolarization through a nongenomic pathway. An in vitro patch-clamp and computational modeling study. Circulation 2007; 116: 2913-2922.

[30] Nakamura H, Kurokawa J, Bai C-X, et al. Progesterone regulates cardiac repolarization via a nongenomic pathway of sex hormones. Mol Pharmacol 2006; 70: 1916-1924.

[31] 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 - Part A: Mol Integ Physiol 2005; 142: 136-143.

[32] Yang L, Liu G, Zakhavor SI, Bellinger AM, Mongillo M, Marx SO. Protein kinase G phosphorylates Cav1.2 α1c and β2 subunits. Circ Res 2007; 101: 465-474.

[33] Furukawa T, Bai C-X, Kaihara A, et al. Ginsenoside Re, a main phytosterol of Panax ginseng, activates cardiac potassium channels via a nongenomic pathway of sex hormones. Mol Pharmacol 2007; 70: 1916-1924.

[34] Splawski I, Tristani-Firouzi M, Lehmann MH, Sanguinetti MC, Keating MT. Mutations in the hminK gene cause long QT syndrome and suppress IKs function. Nat Genet 1997; 17: 338-340.

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

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

[37] 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 (Lond) 2001; 530: 161-166.

[38] 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.

[39] Terrenoire C, Clancy CE, Cormier JW, Sampson KJ, Kass RS. Autonomic control of cardiac action potentials: role of potassium channel kinetics in response to sympathetic stimulation. Circ Res 2005; 96: e25-34.

[40] Kurokawa J, Chen L, Kass RS. Requirement of subunit expression for CAM-mediated regulation of a heart potassium channel. Proc Natl Acad Sci USA 2003; 100: 2122-2127.