Progesterone Regulates Cardiac Repolarization Through a Nongenomic Pathway
An In Vitro Patch-Clamp and Computational Modeling Study

Hiroaki Nakamura, MD; Junko Kurokawa, PhD; Chang-Xi Bai, MD, PhD; Ken Asada, MS; Jun Xu, PhD; Ronit V. Oren, MS; Zheng I. Zhu, PhD; Colleen E. Clancy, PhD; Mitsuaki Isobe, MD, PhD; Tetsushi Furukawa, MD, PhD

Background—Female sex is an independent risk factor for torsade de pointes in long-QT syndrome. In women, QT interval and torsade de pointes risk fluctuate dynamically during the menstrual cycle and pregnancy. Accumulating clinical evidence suggests a role for progesterone; however, the effect of progesterone on cardiac repolarization remains undetermined.

Methods and Results—We investigated the effects of progesterone on action potential duration and membrane currents in isolated guinea pig ventricular myocytes. Progesterone rapidly shortened action potential duration, which was attributable mainly to enhancement of the slow delayed rectifier K⁺ current (I_{Ks}) under basal conditions and inhibition of L-type Ca²⁺ currents (I_{CaL}) under cAMP-stimulated conditions. The effects of progesterone were mediated by nitric oxide released via nongenomic activation of endothelial nitric oxide synthase; this signal transduction likely takes place in the caveolae because sucrose density gradient fractionation experiments showed colocalization of the progesterone receptor c-Src, phosphoinositide 3-kinase, Akt, and endothelial nitric oxide synthase with KCNQ1, KCNE1, and CaV1.2 in the caveolae fraction. We used computational single-cell and coupled-tissue action potential models incorporating the effects of progesterone on I_{Ks} and I_{CaL}; the model reproduces the fluctuations of cardiac repolarization during the menstrual cycle observed in women and predicts the protective effects of progesterone against rhythm disturbances in congenital and drug-induced long-QT syndrome.

Conclusions—Our data show that progesterone modulates cardiac repolarization by nitric oxide produced via a nongenomic pathway. A combination of experimental and computational analyses of progesterone effects provides a framework to understand complex fluctuations of QT interval and torsade de pointes risks in various hormonal states in women. (Circulation. 2007;116:2913-2922.)

Key Words: arrhythmia ■ computers ■ ion channel ■ long-QT syndrome ■ nitric oxide ■ progesterone

Female sex is an independent risk factor for the development of torsade de pointes (TdP) in both congenital and acquired long-QT syndrome (LQTS).¹ ² In females, there are dynamic fluctuations in QT interval and TdP risk during the menstrual cycle and pregnancy. Repolarization duration is shorter in the luteal phase than in the follicular phase by ≈10 ms.³ QTc prolongation induced by the class III antiarrhythmic agent ibutilide is greatest during menses, intermediate in ovulation, and least in the luteal phase.⁴ In congenital LQTS patients, TdP risk is low during pregnancy and suddenly increases postpartum.⁵ In postmenopausal women, hormone replacement therapy with estrogen alone causes slight but significant prolongation of the QTc interval, whereas combinational hormone replacement therapy with estrogen and progesterin consistently shortens QTc interval.⁶ Collectively, these clinical data suggest that the luteal hormone progesterone has protective effects against long QT–associated arrhythmias; however, very few studies have investigated the effects of progesterone on cardiac repolarization.

Clinical Perspective p 2922

We have recently reported that testosterone acutely affects cardiac repolarization by modulating slowly activating delayed rectifier K⁺ current (I_{Ks}) and L-type Ca²⁺ current (I_{CaL}) through the nongenomic pathway of the androgen receptor.⁷ Progesterone also exhibits various actions through a non-
genomic pathway in several cells and tissues. In the present study, therefore, we examined the rapid effects of progesterone on action potentials and membrane currents in cardiac myocytes. We found that progesterone acutely modulated both Ik and Ic, via a pathway involving phosphoinositide 3-kinase (PI3K)/Akt–dependent endothelial nitric oxide (NO) synthase (eNOS) activation. We also incorporated the effects of progesterone into the Faber-Rudy model of cardiac ventricular action potential. The model reproduces observed fluctuations of cardiac repolarization during the menstrual cycle in women. Simulations predict protective effects of progesterone against rhythm disturbance in both single-cell and coupled-tissue models of congenital and drug-induced LQTS.

Methods

Details of the methods can be found in the expanded Methods section of the online-only Data Supplement.

Statistical Analysis

All numerical values are presented as mean±SEM. Statistical significance between baseline and drug application experiment was evaluated by a nonparametric test (Wilcoxon signed-rank test), and that among repeated measures between baseline and various drugs was evaluated by an ANOVA with repeated measures followed by Bonferroni’s multiple-comparison test. The Bonferroni-adjusted probability values were used. Values of P<0.05 were taken as significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Figure 1. Effects of progesterone under basal condition. A, Effects of progesterone on APD. a and b, Representative recordings of action potential (a) and APD (b) before (open symbols) and 10 minutes after (closed symbols) application of 100 nmol/L progesterone and its washout (gray symbols), c, Effects of mifepristone (n=6). B, Effects of progesterone on Ik. a, Time course of the effects of progesterone on Ik tail. Inset, Representative recordings of Ik in baseline (open circle), 10 minutes after application of 100 nmol/L progesterone (closed circle), and 10 minutes after washout of progesterone (gray circle). Ik was elicited by 3.5-second depolarization pulses to 50 mV at 0.1 Hz. B, Dose-response curve for enhancement of Ik tail density by progesterone. Continuous line is the result of Hill fits (see Data Supplement Methods section). Numbers in parentheses indicate the number of data. c, I-V relationships of Ik tail in control (Cont; open symbols) and in the presence of 40.6 nmol/L progesterone (closed symbols) and its washout (WO; gray symbols). Ik was elicited by 400-ms depolarizing pulses to 50 mV at 1 Hz. C, Effects of progesterone on Ic (a) and peak Ic (b) before (open circles) and after (closed circles) 10 minutes after (closed symbols) application of 100 nmol/L progesterone. Statistical significance in A-b and A-c was analyzed with an ANOVA followed by Bonferroni’s test and in C-b with a nonparametric test (Wilcoxon signed-rank test). P4 indicates progesterone; mifepristone. **P<0.01.

Results

Effects of Progesterone Under Basal Conditions

To test whether the effects of progesterone vary depending on the stage of estrous cycle, we examined the effects of progesterone on action potential duration (APD) at 2 different estrous stages: at the estrous stage when the serum progesterone level is lowest and at the diestrous stage when the serum progesterone level is highest.11 We found no significant difference in progesterone effects on APD between the 2 estrous stages (supplementary Figure I); thus, we pooled data obtained from guinea pigs at different stages of the estrous cycle.

Progesterone at 100 nmol/L shortened APD (Figure 1A), and a 10-minute washout of progesterone did not reverse the effects of progesterone: APD at 100 nmol/L was 314.9±17.5 ms at baseline, 302.4±16.6 ms after progesterone (P<0.01 versus baseline), and 303.0±16.0 ms after washout (P<0.01 versus baseline; P=NS versus progesterone) (n=7). A specific progesterone receptor inhibitor, mifepristone (1 μmol/L), reversed APD shortening: APD at 100 nmol/L was 305.1±24.4 ms at baseline, 290.0±22.5 ms after progesterone (P<0.01 versus baseline), and 305.4±23.3 ms after mifepristone (P<0.01 versus progesterone; P=NS versus baseline) (n=6). Thus, we conclude that APD shortening by progesterone is a specific, receptor-dependent effect, and we assume that a 10-minute washout might not be long enough to achieve the reversal of progesterone, a highly lipophilic gonadal steroid.

To investigate the ionic mechanism underlying progesterone-induced APD shortening, we examined the
effects of progesterone on \( I_{\text{Kca}} \) and \( I_{\text{cal}} \). Progesterone (100 nmol/L) enhanced \( I_{\text{Kca}} \) elicited by 3.5-second depolarizing pulses at 0.1 Hz within 5 minutes after progesterone application and reached a pseudosteady state within 10 minutes (Figure 1B, a), suggesting that this effect is acute and nongenomic. A 10-minute washout of progesterone again did not reverse \( I_{\text{Kca}} \) enhancement (Figure 1B, a). \( I_{\text{Kca}} \) enhancement by progesterone is dose dependent, with an EC50 value of 2.7 nmol/L (Figure 1B, b). To test in more physiologically relevant conditions, we examined the effects of progesterone on \( I_{\text{Kca}} \) induced by a 400-ms depolarizing pulse at 1 Hz. We tested progesterone at 2.5 nmol/L (data not shown) and 40.6 nmol/L (Figure 1B, c) because the reported serum progesterone level in women is 2.5 nmol/L in the luteal phase and 40.6 nmol/L in the follicular phase.12 The fractional increase in \( I_{\text{Kca}} \) at 1 Hz at both concentrations (1.18 ± 0.06 at 2.5 nmol/L [n=7] and 1.35 ± 0.04 at 40.6 nmol/L [n=4]) was similar to the values at 0.1 Hz calculated with Hill fits (1.21 at 2.5 nmol/L and 1.34 at 40.6 nmol/L). The current-to-voltage (I-V) curves showed that \( I_{\text{Kca}} \) was uniformly enhanced at all potentials tested independent of membrane potential (Figure 1B, c). Thus, the effects of progesterone on \( I_{\text{Kca}} \) were frequency or voltage independent.

In contrast, progesterone (100 nmol/L) did not significantly affect \( I_{\text{cal}} \) under basal conditions (Figure 1C): current density was −7.7 ± 0.9 pA/pF at baseline and −7.6 ± 0.8 pA/pF after progesterone (P=NS) (n=5).

**Effects of Progesterone in cAMP-Stimulated Conditions**

Because sympathetic nervous system (SNS) stimulation is a critical triggering factor for TdP in LQTS patients,13 we examined the effects of progesterone on APD, \( I_{\text{Kca}} \), and \( I_{\text{cal}} \) in conditions mimicking SNS stimulation. Application of \((−)\)-isoproterenol hydrochloride (Isp; 100 nmol/L) to the bath (extracellular) solution shortened APD, which reached a pseudosteady state within 10 minutes (Figure 2A, gray symbols). Additional application of progesterone (100

---

**Figure 2.** Effects of progesterone under conditions mimicking SNS stimulation. A, Effects of progesterone on APD. a and b, Representative recordings of action potential (a) and APD50 (n=8; b) at baseline (open symbols), after Isp (gray symbols), after additional application of 100 nmol/L progesterone (black symbols), and after a washout of progesterone (hatched symbols). B, Effects of progesterone on \( I_{\text{Kca}} \), a through c, Representative recordings of \( I_{\text{Kca}} \) at 2.5 nmol/L progesterone (a), those at 40.6 nmol/L progesterone (b), and averaged \( I_{\text{Kca}} \) tail density (c) just after establishment of whole-cell patch-clamp configuration (open symbols), after stabilization of effects of cAMP and okadaic acid (gray symbols), and after subsequent application of 2.5 nmol/L (n=8) or 40.6 nmol/L (n=7) progesterone (black symbols). C, Effects of progesterone on \( I_{\text{cal}} \), a, Representative recordings of \( I_{\text{cal}} \) just after establishment of whole-cell patch-clamp configuration (open circle), after stabilization of effects of cAMP and okadaic acid (gray circle), after subsequent application of 40.6 nmol/L progesterone (black circle), and after a 10-minute washout (hatched circle). b, Dose-response curve for suppression of \( I_{\text{cal}} \) inward peak by progesterone. Continuous line is the result of fitting to Langmuir’s isotherm (see supplementary Methods). Numbers in parentheses indicate the number of data. c, Effects of progesterone (40.6 nmol/L) on I-V curves (c; n=7). In A and B, statistical significance was analyzed with an ANOVA followed by Bonferroni’s test. OA indicates okadaic acid. Other abbreviations as in Figure 1. *P<0.05; **P<0.01.
by guest on April 20, 2017 http://circ.ahajournals.org/ Downloaded from

eNOS.14 We performed a series of pharmacological experi-

ments to test whether a similar signaling pathway mediated
the progesterone-induced IKs enhancement. The reagents used
included inhibitors for PgR (mifepristone), Akt (TAT-Akt
inhibitor peptide [Akt-in]), PI3-kinase [2-(4-morpholino)-8-
phenyl-1(4H)-benzopyran-4 to 1 hydrochloride (LY294,002)],
and the NO scavenger 2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-
1H-imidazol-1-yloxy-3-oxide (carboxy-PTIO).

After preincubation with mifepristone (1 μmol/L; Figure
3A) or intracellular dialysis of Akt-in (500 μmol/L) in
the whole-cell patch-clamp configuration (Figure 3B), progester-

tone (100 nmol/L) significantly shortened APD (Figure 2A, black
symbols). In the presence of Isp, the effects of progesterone
were not reversed by a 10-minute washout of progesterone.
APD was 315.1±16.3 ms at baseline, 306.4±16.9 ms at 10
minutes after Isp (P=NS versus baseline), 284.9±15.3 ms
after Isp and progesterone (P<0.01 versus baseline; P<0.05
versus Isp), and 282.0±17.4 ms after washout of progesterone
(P<0.01 versus baseline; P<0.05 versus Isp; P=NS
versus Isp and progesterone) (n=8).

To investigate the effects of maximal SNS activation on IKs
and ICa,L, we used the whole-cell configuration of the patch-

clamp technique with a pipette (intracellular) solution contain-
ing 3’,5’-cAMP (cAMP; 0.2 mmol/L) and okadaic acid
(0.2 μmol/L). On establishment of the whole-cell configuration,
IKs started to increase in amplitude and reached a
pseudosteady state within 10 minutes (Figure 2B, gray
symbols). Subsequently applied progesterone at 2.5 and 40.6
nmol/L did not significantly enhance IKs tail density (Figure
2B); current density was 6.6±0.8 and 7.1±0.7 pA/pF before
and after application of 2.5 nmol/L progesterone (P=NS)
(n=8) and 6.6±0.9 and 7.2±0.9 pA/pF before and after
application of 40.6 nmol/L progesterone (P=NS) (n=7). In
contrast, progesterone (100 nmol/L) significantly suppressed
cAMP-enhanced IKs (Figure 2C, a, black circles). Progesterone-induced IKs suppression was dose dependent,
with an IC50 value of 29.9 nmol/L (Figure 2C, b). The effects
of progesterone on the I-V curve, activation curve, and
inactivation curve were obtained at 2.5 nmol/L (data not
shown) and at 40.6 nmol/L (Figures 2C, c and Data Supple-
ment Figure II). Progesterone caused a positive shift in the
activation curve (Data Supplement Figure IIA); V1/2 was
−24.0±2.0 mV in cAMP, −19.6±2.5 mV after progesterone
(P<0.05 versus cAMP), and −18.5±2.5 mV after washout
(P<0.05 versus cAMP; P=NS versus progesterone).
Progesterone caused a negative shift in the inactivation curve (supple-
mentary Figure IIIB); V1/2 was −27.0±0.9 mV in cAMP,
−30.9±1.2 mV after progesterone (P<0.05 versus cAMP),
and −33.5±1.0 mV after washout (P<0.05 versus cAMP;
P=NS versus progesterone) (n=7). Thus, APD shortening
by progesterone under SNS-stimulated conditions is due mainly
to the modulation of gating kinetics of ICa,L, which results in
a net reduction in depolarizing Ca2+ current.

Signal Pathway for Progesterone Effects
We have previously reported that phytoestrogen, ginsenoside
Re, enhanced IKs through the nongenomic pathway of progester-

tone receptor (PgR) involving c-Src, PI3-kinase, Akt, and
eNOS.
We performed a series of pharmacological experi-

ments to test whether a similar signaling pathway mediated
the progesterone-induced IKs enhancement. The reagents used
included inhibitors for PgR (mifepristone), Akt (TAT-Akt
inhibitor peptide [Akt-in]), PI3-kinase [2-(4-morpholino)-8-
phenyl-1(4H)-benzopyran-4 to 1 hydrochloride (LY294,002)],
and eNOS (L-N-(1-lminoethy)ornithine [L-NIO]) and the NO
scavenger 2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-
1H-imidazol-1-yloxy-3-oxide (carboxy-PTIO).

After preincubation with mifepristone (1 μmol/L; Figure
3A) or intracellular dialysis of Akt-in (500 μmol/L) in
the whole-cell patch-clamp configuration (Figure 3B), progester-

tone (100 nmol/L) failed to increase IKs tail currents. In the
presence of mifepristone, IKs density was 2.4±0.3 and
2.3±0.3 pA/pF before and after application of progesterone
(P=NS) (n=5); in the presence of Akt-in, IKs density was
3.1±0.4 and 3.0±0.4 pA/pF before and after application of
progesterone (P=NS) (n=5). After progesterone (100
nmol/L) enhanced IKs tail currents, subsequent application of
LY294,002 (30 μmol/L; Figure 3C), L-NIO (1 μmol/L; Figure
3D), or carboxy-PTIO (100 μmol/L; Figure 3E) decreased
IKs tail current density to the level before proges-
terone application. IKs density was 1.8±0.2 pA/pF at baseline,
2.6±0.5 pA/pF after progesterone (P<0.05 versus baseline),
and 1.9±0.3 pA/pF after progesterone and LY294,002
(P=NS versus baseline; P<0.05 versus progesterone) (n=5);
it was 2.8±0.1 pA/pF at baseline, 4.8±0.5 pA/pF after
progesterone (P<0.05 versus baseline), and 3.0±0.2 pA/pF
after progesterone and L-NIO (P=NS versus baseline;
Figure 4. Sucrose density gradient fractionation. Progesterone-free condition (A) and progesterone-treated condition (B). a, Representative data for immunoblot analysis of membrane fractions 1 to 11 and the cytosolic fraction. b, Immunodensity in each fraction was normalized to the sum of immunodensities from fraction 1 to fraction 11 (n=5).
P<0.05 versus progesterone) (n=5); and those values were 4.1±0.8 pA/pF at baseline, 6.4±0.5 pA/pF after progesterone (P<0.05 versus baseline), and 4.1±0.7 pA/pF after progesterone and carboxy-PTIO (P=NS versus baseline; P<0.05 versus progesterone) (n=5). These data indicate that progesterone-induced IKs enhancement was caused by NO, which was released via the c-Src/PI3-kinase/Akt–dependent eNOS activation. We also found that progesterone suppressed cAMP-induced ICa,L via eNOS-induced NO release (supplementary Figure III).

Caveolae Localization of Molecules Involved in the Nongenomic Pathway of Progesterone

We next determined the localization of molecules involved in the nongenomic pathway of progesterone using sucrose density gradient fractionation experiments in the absence (Figure 4A) and presence (Figure 4B) of progesterone. In the absence of progesterone, caveolin-3, a caveolae maker, migrated mainly to fraction 5, indicating that fraction 5 is the caveolae fraction in our experimental condition. A majority of KCNQ1 (α subunit of the IKs channel) and eNOS comigrated with caveolin-3 to fraction 5. Cav1.2 (α subunit of the ICa,L channel), KCNE1 (β subunit of the IKs channel), c-Src, p85 (a regulatory subunit of PI3-kinase), and Akt localized rather broadly between fractions 4 and 11 and/or cytoplasmic fraction; however, a substantial fraction of these molecules localized to the caveolae fraction (fraction 5). Although neither the full-length PgR-A (94 kDa) nor PgR-B (116 kDa) was detected in any membrane fractions or the cytosolic fraction, an antibody against the C terminus of PgR detected truncated forms of the PgR at 78 kDa (PR78) and 54 kDa (PR54) that migrated mainly to the caveolae fraction. Thus, a substantial fraction of each of the molecules involved in the nongenomic pathway of progesterone clustered in the caveolae fraction.

In hearts perfused with progesterone (100 nmol/L) for 10 minutes, although localization of most of the molecules was not affected, PR78 and PR54 exhibited changes in localization (Figure 4B, a). After a 10-minute incubation with progesterone, PR78 and PR54 migrated broadly between fractions 5 and 11. Densitometric analysis revealed that the fraction of PR78 and PR54 localized in fraction 5 was
Computational Modeling for the Effects of Progesterone on IKs, ICa,L, APD, and QT

We next investigated the physiological effects of progesterone on cardiac action potentials by carrying out simulations in the Faber-Rudy model of the guinea pig myocyte. Figure 5A shows the effects of cAMP alone and with progesterone (40.6 nmol/L) on IKs and ICa,L on the basis of our experimental data. cAMP results in a large increase in IKs (Figure 5A, a, gray circle). Progesterone application causes an additional, albeit more subtle, increase (Figure 5A, b, black circle). cAMP application increases ICa,L (Figure 5A, b, gray circle), but additional application of progesterone abolishes the effect (Figure 5A, b, black circle). Notice the agreement between the simulation and experiments (shown in Figure 2B, b, and 2C, a).

We next investigated how the effects of progesterone on IKs and ICa,L influence APD. Both in the absence (Figure 5B, a) and presence (Figure 5B, b) of SNS stimulation, the model predicts the progesterone-induced APD shortening in a dose-dependent manner. The magnitude of progesterone-induced APD shortening in patch-clamp experiments (6.3%) is in good agreement with the APD shortening in the simulation study (7.2%).

We also computed the effects of progesterone and SNS stimulation in 1-dimensional strands of coupled cells (100 cells; 1 cm) (Figure 5C, a, no progesterone; Figure 5C, b, 40.6 nmol/L progesterone; and Figure 5C, c, 40.6 nmol/L progesterone and SNS stimulation) (details of simulations are contained in the supplementary Methods section) and computed signal averages (pseudo-ECGs) of gradients of depolarization and repolarization (Figure 5C, right). As shown in Figure 5C, the shortening of the APDs as a result of the effect of progesterone alone and SNS stimulation and progesterone in the simulated tissue results in a reduction of QT interval (from 225 ms in control conditions) to 205 ms (8.8%) and 180 ms (20.0%), respectively.

Protective Effects Against Arrhythmia in a Computer Modeling

Because the model reproduces the effects of progesterone on APD in patch-clamp experiments with good accuracy, we next used 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. The D76N KCNE1 mutation reduces the current and renders the IKs channel insensitive to β-adrenergic stimulation; thus, proabls carrying the D76N KCNE1 mutation readily develop TdP with SNS stimulation.

In the absence of progesterone, the mutant cells are unable to adapt to the fast pacing frequency because IKs fails to increase in response to the SNS stimulation, leading to action potentials marked by stimulus artifacts resulting from insufficient repolarization (Figure 6A, top). Interestingly, in the presence of 2.5 nmol/L progesterone, which corresponds to the serum level in the follicular phase in women, little improvement is observed (Figure 6A, middle); in the presence of 40.6 nmol/L progesterone (the luteal phase), a failed SNS stimulation response is compensated for by the action of progesterone alone to increase IKs (Figure 6A, bottom). It should be noted that with continued pacing, 2.5 nmol/L progesterone also eventually causes sufficient APD shortening via buildup of IKs channels in the open state to allow the simulated cell to respond to each stimulus. In simulated 1-dimensional tissue (Figure 6A, a and 6B, b), propagation of action potentials is affected by progesterone application. In Figure 6A, the pacing artifacts that are apparent in single cells in the absence of progesterone (Figure 6A, left) are rapidly extinguished in the fiber (Figure 6A, a) near the stimulus end and fail to propagate (gray circle). Interestingly, in the presence of 40.6 nmol/L progesterone (Figure 6A, b), the fiber readily responds to the rapid pacing frequency, as seen in the single cells. Computed electrograms (pseudo-ECGs) from the fibers in Figure 6A, a and 6A, b clearly show the generation of 2 QRS and T complexes when 40.6 nmol/L progesterone is applied. In the absence of progesterone, the second stimulus fails to propagate and is observed as a notch in the tail of the T wave of the computed electrogram.

We next investigated the effects of progesterone on drug-induced arrhythmias. Severe early afterdepolarizations were induced by 50% block of IKr at a slow heart rate (30 bpm) (Figure 6B, top). At 2.5 nmol/L progesterone, some improvement is observed (Figure 6B, middle); at 40.6 nmol/L progesterone, the early afterdepolarizations are abolished, and the action potential morphology is normalized (Figure 6B, bottom). When we ran the same simulations in coupled tissue (Figure 6B, right), we observed preservation of the abnormalities and propagation of early afterdepolarizations in the tissue. The simulation suggested that even in coupled tissue, 40.6 nmol/L progesterone is sufficient to normalize action potential morphology in the presence of 50% IKr block.

Discussion

Accumulating clinical data suggest protective effects of progesterone against LQTS-associated arrhythmias, but to the best of our knowledge, its effects on cardiac repolarization had not previously been reported. In the present article, we report 4 findings regarding the effects of progesterone on cardiac repolarization that we believe to be novel: (1) progesterone modulates IKs and ICa,L through a nongenomic pathway involving c-Src/PI3K/Akt–dependent eNOS activation; (2) progesterone enhances IKs under basal condition, whereas it inhibits ICa,L only when ICa,L is enhanced by cAMP; (3) substantial fractions of molecules involved in the nongenomic pathway of progesterone colocalize in the caveolae fraction; and (4) incorporating the effects of progesterone into a simulated myocyte and a coupled-tissue strand reproduces the observed fluctuations of cardiac repolarization duration during the menstrual cycle in women and predicts the protective effects of progesterone against rhythm disturbance in both single-cell and coupled-tissue models of congenital and drug-induced LQTS.
Although nongenomic actions of progesterone have been reported in various cells and tissues,8,9 we believe the present study to be the first to demonstrate nongenomic actions of progesterone in cardiac myocytes. Progesterone-induced $I_{Ks}$ enhancement was inhibited by LY294002, Akt-inhibitor peptide, L-NIO, and carboxy-PTIO, and progesterone-induced suppression of cAMP-enhanced $I_{Ca,L}$ was inhibited by L-NIO and carboxy-PTIO. Thus, progesterone exhibits its effects via NO produced through the nongenomic pathway involving PI3-kinase and eNOS. Our sucrose density gradient fractionation experiments demonstrate that substantial fractions of c-Src, Akt, eNOS, PR78, PR54, KCNQ1, KCNE1, and CaV1.2 are colocalized in the caveolae fraction.

Molecular identity for the PgR responsible for the nongenomic action is currently undetermined, but several candidates have been reported. One candidate is PR78 because injection of PR78 mRNA into oocytes rapidly accelerates progesterone-induced mitogen-activated protein kinase activation and oocyte maturation.17 In the present experiments, we demonstrate the presence of PR78 in cardiac myocytes and its localization to the caveolae with KCNQ1, KCNE1, CaV1.2, and eNOS. Immunocytochemical experiments revealed that PgR located mainly to the T tubule and, at least in part, colocalized with caveolin-3 (Data Supplement Figure IV). Sucrose gradient experiments also showed that progesterone application shifted the localization of PR78 and PR54 to broad membrane fractions but not to the cytosolic or nuclear fraction. Immunocytochemical experiments showed that progesterone did not induce translocation of PgR into nucleus. These findings are in line with the idea that PR78 is

Figure 6. Progesterone may protect against long QT-related arrhythmia in a computer modeling. A, Effects of progesterone on arrhythmic rhythms 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 $I_{Ks}$ $\beta$-subunit KCNE1 that disrupts regulation of $I_{Ks}$ by protein kinase A. Left, Six action potentials (15th to 20th) elicited from cells with D76N $I_{Ks}$ at a fast rate (cycle length, 150 ms) during SNS stimulation in the absence (top) and presence (middle) of 2.5 or 40.6 nmol/L (bottom) progesterone. Right, Simulated propagation of action potentials in paced (150 ms; 29th and 30th beats are shown) 1-dimensional tissue in the absence of progesterone (a) and in the presence of 40.6 nmol/L progesterone (b) and the corresponding computed electrogram. The gray circle in A-a highlights failure of propagation of the second stimulus, which is applied during the mutation-induced extended refractory period. B, Effects of progesterone on early afterdepolarizations resulting from acquired LQTS simulated by $I_{Ks}$ block. Traces of the 9th and 10th action potentials during 50% $I_{Ks}$ block in the absence (top) and presence (middle) of 2.5 or 40.6 nmol/L (bottom) progesterone. Cycle length is 2000 ms. Left, single cells; right, results in corresponding fibers under the same conditions.
responsible for nongenomic actions at least in cardiomyocytes. However, further studies are certainly required to determine whether PR78 and/or newly identified PR54 is the receptor specific for the nongenomic pathway in cardiac myocytes and to clarify the physiological implication of ligand-induced translocation of PR78 and PR54 within plasma membrane.

We found that SNS stimulation differentially modulated the progesterone effects on \( I_{Ks} \) and \( I_{Ca,L} \). Under basal conditions, progesterone affected only \( I_{Ks} \), whereas under cAMP-stimulated conditions, progesterone clearly reversed cAMP-induced enhancement of \( I_{Ca,L} \). This difference could be explained by differential mechanisms of NO to modulate \( I_{Ks} \) and \( I_{Ca,L} \). We have previously suggested that NO-induced \( I_{ca,l} \) suppression was attributable to cGMP-dependent pathway, whereas NO-induced \( I_{Ks} \) enhancement was via a cGMP-independent pathway. The former agrees with the previous finding that cGMP counteracts cAMP-induced \( I_{Ca,L} \) enhancement.

We incorporated the effects of progesterone on \( I_{Ks} \) and \( I_{Ca,L} \) in the Faber-Rudy model of the guinea pig ventricular myocyte. The model predicted that 40.6 nmol/L progesterone, a serum level corresponding to that of the luteal phase in women, shortens APD by 3.7% under basal conditions and 4.6% under SNS-stimulated conditions compared with APD at 2.5 nmol/L progesterone (the level in the follicular phase). Clinically observed QT intervals are shorter by \( \approx 2.4\% \) to 2.8% in the luteal phase than in follicular phase, so the APD shortening predicted in the model (3.7% to 4.6%) fits well with the observed fluctuation in QT interval during the menstrual cycle in women. We also investigated the effects of progesterone and SNS stimulation in simulated coupled tissue and computed virtual electrograms from simulated gradients of depolarization and repolarization. Simulations suggest that during the luteal phase when progesterone is 40.6 nmol/L, maximal SNS stimulation may additionally shorten the QT interval by 12.2%. These simulations support the notion that progesterone may exert protective QT shortening effects under conditions of SNS stimulation.

From these results, we then used the model to predict the effects of progesterone on SNS-induced rhythm disturbance in single-cell and coupled-tissue models of congenital LQTS and drug-induced LQTS. \( I_{Ks} \) exhibits accumulation in the preopen state during rapid heart rates, resulting in action potential adaptation.20 SNS stimulation enhances \( I_{Ca,L} \) to increase \( \text{Ca}^{2+} \) influx.21 SNS stimulation also enhances \( I_{Ks} \)22,23 which counterbalances \( I_{Ca,L} \) enhancement and maintains APD within a certain range.24 In LQTS1 and LQTS5, \( I_{Ks} \) channel disturbance results in dysfunction of action potential adaptation to rapid heart rates and response to SNS stimulation. Enhancement of \( I_{Ks} \) in the absence of SNS stimulation and inhibition of cAMP-induced \( I_{Ca,L} \) by progesterone improve action potential adaptation in our simulations in single cells and coupled tissue. 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.25 Progesterone does not have apparent effects on \( I_{Ks} \) (data not shown); thus, the predicted protection against drug-induced early afterdepolarizations may be attributed to an increase in repolarization reserve by \( I_{Ks} \) enhancement.26

Our electrophysiological and molecular biological studies demonstrate a novel effect of progesterone on cardiac repolarization. The biological experimental analysis, combined with a computational analysis of effects of progesterone on cardiac repolarization, provides a framework to understand the dynamic natures of QT interval and TdP risks in various hormonal states, particularly given the complex interaction with multiple factors, including SNS status, heart rate, QT-prolonging drugs, and gene mutations.

**Acknowledgments**

We thank S. Oishi for collecting data and E. Ozaki, M. Hayashi, and K. Watanabe for technical assistance.

**Sources of Funding**

This work was supported in part by grant-in-aid 17081007 (Dr Furukawa) for scientific research on priority areas; grants 18390231 (Dr Furukawa) and 19689006 (Dr Kurokawa) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; and a research grant from the Mitsui Kousei Foundation (Dr Furukawa) and the Naito Foundation (Dr Kurokawa). The research also was supported by grants from the American Heart Association, National Institutes of Health NHLBI RO1-HL-085592, and Alfred P. Sloan Foundation to Dr Clancy.

**Disclosures**

None.

**References**

CLINICAL PERSPECTIVE

Female sex is a widely known risk factor for life-threatening torsade de pointes (TdP) arrhythmias in long-QT syndrome. Thus, establishing a strategy to prevent TdP in women is clinically significant. In females, QT interval and TdP risk fluctuate dynamically. In the follicular phase of the menstrual cycle, the basal QT interval is longer, drug-induced QT prolongation is more severe, and TdP risk is increased compared with other phases. TdP risk also is higher postpartum when serum progesterone suddenly drops. Thus, QT interval prolongation/TdP risk and serum progesterone levels are inversely correlated, suggesting a protective effect of progesterone against QT prolongation. Here, we have demonstrated in in vitro experiments that progesterone acutely shortens cardiac repolarization both in the basal condition and in the sympathetic nervous system–stimulated condition by enhancing the K⁺ current in the basal condition and suppressing the Ca²⁺ current in the sympathetic nervous system–stimulated condition. We have incorporated these basic research data into a computer model of the cardiac action potential in a single-cell model and a coupled multicell model. The models show that progesterone shortens the repolarization time of cardiac action potentials and that progesterone extinguishes arrhythmias in simulations of congenital and drug-induced long-QT syndrome. A combined approach using wet experiments and computational analysis may provide a framework to establish an improved strategy to lower the TdP risk in women with complex hormone homeostasis.

Progesterone Regulates Cardiac Repolarization Through a Nongenomic Pathway: An In Vitro Patch-Clamp and Computational Modeling Study

Circulation. 2007;116:2913-2922; originally published online December 3, 2007;
doi: 10.1161/CIRCULATIONAHA.107.702407

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/116/25/2913

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2007/12/03/CIRCULATIONAHA.107.702407.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/