Progesterone Impairs Human Ether-a-go-go-related Gene (HERG) Trafficking by Disruption of Intracellular Cholesterol Homeostasis

Zhi-Yuan Wu, De-Jie Yu, Tuck Wah Soong, Gavin S. Dawe, and Jin-Song Bian

From the Departments of Pharmacology and Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore

The prolongation of QT intervals in both mothers and fetuses during the later period of pregnancy implies that higher levels of progesterone may regulate the function of the human ether-a-go-go-related gene (HERG) potassium channel, a key ion channel responsible for controlling the length of QT intervals. Here, we studied the effect of progesterone on the expression, trafficking, and function of HERG channels and the underlying mechanism. Treatment with progesterone for 24 h decreased the abundance of the fully glycosylated form of the HERG channel in rat neonatal cardiac myocytes and HERG-HEK293 cells, a cell line stably expressing HERG channels. Progesterone also concentration-dependently decreased HERG channel current density, but had no effect on voltage-gated L-type Ca\(^{2+}\) and K\(^{+}\) channels. Immunofluorescence microscopy and Western blot analysis show that progesterone preferentially decreased HERG channel protein abundance in the plasma membrane, induced protein accumulation in the dilated endoplasmic reticulum (ER), and increased the protein expression of C/EBP homologous protein, a hallmark of ER stress. Application of 2-hydroxypropyl-\(\beta\)-cyclodextrin (a sterol-binding agent) or overexpression of Rab9 rescued the progesterone-induced HERG trafficking defect and ER stress. Disruption of intracellular cholesterol homeostasis with simvastatin, imipramine, or exogenous application of cholesterol mimicked the effect of progesterone on HERG channel trafficking. Progesterone may impair HERG channel folding in the ER and/or block its trafficking to the Golgi complex by disrupting intracellular cholesterol homeostasis. Our findings may reveal a novel molecular mechanism to explain the QT prolongation and high risk of developing arrhythmias during late pregnancy.

Progesterone (P4) is an important steroid hormone involved in the female menstrual cycle, pregnancy, and embryogenesis. In the normal menstrual cycle, P4 levels increase from 0.6 – 4.5 nmol/liter during the preovulatory phase to 10.5 – 80 nmol/liter during the luteal phase (1). It was reported that P4 at <100 nmol/liter, which is comparable with that occurring during the luteal phase, had no significant effect on the QT interval (2–4). However, recent studies show that P4 may shorten the action potential duration or QT interval (5). This is probably because P4 at this level enhances the rapid component of the delayed rectifier K\(^{+}\) current and inhibits L-type Ca\(^{2+}\) currents (6). If pregnancy occurs, P4 levels are initially maintained at the luteal level, but may increase to 1 \(\mu\)mol/liter at term (7). Elevated levels of P4 are important for the implantation of the embryo and the maintenance of a conducive environment for the embryo. At such a high level, the corrected QT interval is significantly prolonged (8, 9). This explains why pregnant women are more susceptible to ventricular arrhythmias during pregnancy, labor, and delivery (10–12). However, it is interesting to note that in the patients with inherited long QT syndrome (LQTS) the risk for cardiac events is not higher during pregnancy than during other periods in life (10, 13). These findings suggest that the mechanisms for pregnancy-induced cardiac events in normal healthy women are different from those in the patients with inherited LQTS.

In the fetus, the P4 level was reported to be very high (~4.5 \(\mu\)mol/liter) in umbilical cord vein during late stages of pregnancy (7). At this stage, longer QT and corrected QT intervals were reported in fetal magnetocardiography and noninvasive fetal electrocardiography (14, 15). More importantly, the rate of sudden death is higher in the uterus than at most other times in the human life cycle. Although the major reasons remain unknown, several studies of newborns suggest that QT prolongation and the increased risk of ventricular arrhythmias may account for the significant mortality (16, 17). This may be the same for stillbirth as several case reports indicate that LQTS is one cause for otherwise unexplained fetal demise (18).

The human ether-a-go-go-related gene (HERG) K\(^{+}\) channel, which encodes the \(\alpha\)-subunit of the rapid component of the delayed rectifier K\(^{+}\) current (\(I_{Kr}\)), is largely responsible for the repolarization of action potentials in cardiac myocytes. Inherited mutations or drug-induced blockade of the HERG channel prolongs QT intervals and increases the risk of lethal arrhythmia. Trafficking to the plasma membrane is very important for HERG channel, and defects in trafficking, caused either by mutation of HERG gene or by drugs, can affect HERG function significantly.
The function of the HERG channel is important for embryo development because impaired HERG channel function not only induces LQTS in embryo, but also affects the structure of the heart (19, 20) and even causes embryonic lethality (20). However, the effect of P4 on HERG K⁺ channel function remains unclear. Because the P4 level during late pregnancy is very high, we hypothesized that P4 may contribute to prolonged QT intervals and the sudden cardiac death in the fetus and perhaps in pregnant women as well. This study was therefore designed to examine the effect of P4 on protein expression, trafficking, and function of HERG K⁺ channels in heterologous expression systems and rat neonatal cardiac myocytes.

EXPERIMENTAL PROCEDURES

**Reagents**—PD98059 and LY294002 were from Merck. Simvastatin was from Eurodrug Laboratories (Belgium). Sulfo-NHS-LC-Biotin and Pierce Streptavidin UltraLink® Resin were from Thermo Scientific. All other reagents were from Sigma. P4, cholesterol, and RU486 were dissolved in ethanol as stock solutions. Cycloheximide, PD98059, and LY294002 were dissolved in dimethyl sulfoxide as stock solutions. The final ethanol and dimethyl sulfoxide concentrations were <0.2%. The GFP-Rab9 plasmid was a gift from Dr Suzanne Pfeffer, Stanford University.

**Cell Culture and Transfection**—HERG-HEK293 cell line that stably expresses HERG K⁺ channels with a myc tag was used (21). CHO cells were transiently transfected with cDNAs of HERG and GFP by electroporation as described previously (21). To study the L-type Ca²⁺ current, HEK293 cells were transiently transfected with human Ca₁.2 cardiac splicing form Ca₁.2CM (1a-8a-32-33) constructs (1.25 g), together with β²a (1.25 µg) and α²6 (1.25 µg) using the calcium phosphate transfection method (22). Lipofectamine 2000 (Invitrogen) was used for the transfection of myc-K₁.5, FLAG-Mirp1, GFP, and GFP-Rab9 plasmids.

**Isolation of Rat Neonatal Cardiac Myocytes**—Rat neonatal cardiac myocytes were isolated from 1–2-day-old Sprague-Dawley rats via serial pancreatin (Sigma)/collagenase II (Worthington Biochemical) digestion. Briefly, rat hearts were minced in ice-cold dissociation buffer (116 mM NaCl, 20 mM Hepes, 0.8 mM Na₂HPO₄, 5.6 mM glucose, 5.4 mM KCl, 0.8 mM MgSO₄, pH 7.35) with pancreatin and collagenase II and then digested four times and then incubated with 1 mg/ml Sulfo-NHS-LC-Biotin for 30 min at 4 °C, followed by washing with PBS + 100 mM glucose for three times to quench and remove excess biotin reagent and byproducts. Cells were lysed as described previously (21). Whole cell lysates were incubated with Pierce Streptavidin UltraLink® Resin for 1 h at room temperature. The resin was washed with binding buffer (0.1 M NaCl, pH 7.2) four times. The collected samples were subjected for Western blotting analysis.

**Western Blot Analysis**—Western blot analysis was performed using whole cell lysates as described previously (21). Anti-myc mouse monoclonal 9E10 and anti-tubulin rabbit monoclonal antibodies were from Sigma. Other antibodies were from Santa Cruz Biotechnology. All gels illustrated in the figures are representative examples from four to eight independent experiments. β-Tubulin and hypoxanthine phosphoribosyltransferase were applied as internal controls to normalize protein loading. The intensity of bands was quantified using LabWorks™ Image Analysis software (UVP).

**Confocal Microscopy**—HERG-HEK293 cells grown on glass coverslips were subject to formaldehyde fixation before indirect immunofluorescent staining. The method was described in our previous publication (21). To study the plasma membrane HERG K⁺ channel distribution, cells were incubated with anti-K₁.1.1 (HERG extracellular epitope, residues 430–445, between S1 and S2 loop) antibody (Alomone Labs, Jerusalem, Israel) without permeabilization, and the primary antibody was detected with Alexa Fluor 488-conjugated anti-rabbit Ig (Invitrogen).

**Filipin Staining**—HERG-HEK293 cells were fixed with formaldehyde, and glycine was used to quench extra formaldehyde. Cells were then stained with filipin (Sigma-Aldrich) and viewed by fluorescence microscopy at magnification of ×20 or ×40 times using a UV filter set.

**Cellular Cholesterol Measurement**—An Amplex® Red Cholesterol Assay kit (Molecular Probes) was used to measure the cellular cholesterol level following the manufacturer’s instructions.

**Patch Clamp Recording**—Cells grown on coverslips were placed in an acrylic/polystyrene perfusion chamber (Warner Instruments) for electrophysiological measurements and are described in our previous publications (21, 22). For whole cell voltage clamp, the pipette solution consisted of 126 mM KCl, 2 mM MgSO₄, 0.5 mM CaCl₂, 5 mM EGTA, 4 mM MgATP, and 25 mM Hepes (pH 7.2, osmolality: 280 ± 10 mOsm). External bath solution consisted of 150 mM NaCl, 1.8 mM CaCl₂, 4 mM KCl, 1 mM MgCl₂, 5 mM glucose, and 10 mM Hepes (pH 7.4, osmolality: 320 ± 10 mOsm).

**Statistics**—Values presented are as means ± S.E. ANOVA and Tukey’s post hoc tests were employed to assess statistical significance, and p values of < 0.05 were considered to be significant.

RESULTS

**P4 Impairs the Maturation of HERG K⁺ Channels**—There are two forms of the HERG protein in HERG-HEK293 cells. The 135-kDa form represents the core-glycosylated protein located mainly in the ER, and the 155-kDa form represents the fully glycosylated protein located mainly in the Golgi complex.
and plasma membrane. P4 at 0.5 μmol/liter or higher significantly decreased the amount of the mature (fully glycosylated) form, but had no significant effect on the immature (core-glycosylated) form of the HERG channel (Fig. 1A). HERG co-assembles with its β-subunit Mirp1 to conduct I\textsubscript{Kr}. Similar results were also found in the HERG-HEK293 cells transfected with Mirp1, the β-subunit of HERG/I\textsubscript{kr} (supplemental Fig. 1). These data suggest that P4 may impair HERG trafficking in the presence or absence of its β-subunit.

In a subsequent time course study, the significant inhibitory effect on channel maturation was observed after treatment with P4 for 8 h (Fig. 1B). The effect of P4 was reversible as removal of P4 for 6 h successfully rescued the mature form of HERG (Fig. 1C). The above data suggest that the effect of P4 on HERG maturation is both concentration- and time-dependent.

P4 Significantly Decreases HERG Current Intensity—We also tested whether P4 can affect HERG function by recording K\textsuperscript{+} currents by whole cell patch clamping. Current protocols are stated in figure legends. As shown in Fig. 2, A and B, treatment with P4 (0.5–5 μmol/liter, 24 h) concentration-dependently decreased current density at the peak of tail current in HERG-HEK293 cells. Acute P4 treatment (0.05–5 μmol/liter) for 15 min failed to affect HERG K\textsuperscript{+} current density (data not shown), which is in agreement with the previous study on I\textsubscript{kr} (6). These data suggest that the long term effect of P4 was not secondary to its direct interaction with HERG channels. Similar results were observed in the CHO cells overexpressing HERG K\textsuperscript{+} channels (supplemental Fig. 2). P4 also significantly reduced HERG current density in the HERG293 cells expressing both HERG and Mirp1 (Fig. 2C). These data suggest that P4 may also inhibit the function of HERG/I\textsubscript{kr}.

P4 Preferentially Decreases HERG Channel Protein in the Plasma Membrane—We further investigated the effect of P4 on HERG K\textsuperscript{+} channel subcellular compartmentalization. To detect the surface expression, cells were incubated with anti-Kv11.1 raised against the extracellular loop between S1 and S2 domains. Confocal microscopic examination showed the fluorescent HERG signals at the cell surface (Fig. 3A). The signals were quantified with the software ImageJ as shown in the right panel. It was found that P4 significantly reduced HERG expression on the plasma membrane (Control: 50.5 ± 7.7 versus P4: 10.4 ± 5.0, p < 0.05). To confirm further the preferential effect of P4, cells were permeabilized and double-stained with ant MYC and anti-calnexin (ER marker) or anti-GM130 (Golgi complex marker) antibodies. P4 caused ER dilation (dotted green
signal, anti-calnexin), and HERG K⁺ channels (red signal) accumulated in the dilated ER (Fig. 3B). In contrast, there is no co-localization between the accumulated HERG K⁺ channels (red signal) and the Golgi complex (green signal, anti-GM130) (Fig. 3C). This result suggests that P4 treatment may cause ER stress and affect HERG K⁺ channel folding and trafficking.

To confirm our findings, we labeled plasma membrane protein with biotinylation. Fig. 4A shows that the fully glycosylated form of HERG protein is the predominant form present in the plasma membrane. P4 treatment significantly reduced biotinylation-labeled plasma membrane HERG protein. These data suggest that P4 may mainly reduce the plasma membrane protein.

To study P4-induced ER stress that may impair HERG trafficking from the ER, we further examined the protein expression of C/EBP homologous protein (CHOP), a hallmark of ER stress. We found that P4 increased CHOP protein expression starting from 4 h of treatment in a time-dependent manner (Fig. 4B, left panels). This is mimicked by thapsigargin, which induces ER stress via blocking ER Ca²⁺-ATPase and disturbing ER Ca²⁺ homeostasis (Fig. 4B, right panels). These data clearly suggest that P4 may induce a HERG trafficking defect via promoting ER stress.
Progesterone Impairs HERG Trafficking

The effect of P4 on cholesterol content was also examined. As shown in Fig. 6C, P4 had no significant effect, whereas simvastatin (10 μM, an inhibitor of de novo cholesterol synthesis) significantly decreased free and total cholesterol levels in HERG-HEK293 cells. These data suggest that P4 can only affect the distribution of cholesterol, but had no effect on the amount of total and free forms of cholesterol.

Effect of P4 Is Neither P4 Receptor-mediated nor via de Novo Protein Synthesis—RU486, a P4 receptor antagonist, decreased the mature form of HERG K+ channel slightly, but failed to block the inhibitory effect of P4 on HERG maturation (Fig. 5A). These data suggest that the effect of P4 is not mediated by the nuclear P4 receptor. This is also supported by our functional data that P4 decreased HERG K+ current density in CHO cells (supplemental Fig. 2), which do not express P4 receptors (23).

Effect of P4 Is Reversed by a HERG Channel Blocker and Low Culture Temperature—Both E-4031, a HERG channel blocker, and low temperature (27 °C) improve the proper folding of HERG channel in ER (24). As shown in Fig. 5, C and D, both maneuvers rescued the HERG channel trafficking-defect caused by P4. These data imply that P4 may affect HERG channel folding in ER and/or block its trafficking to Golgi complex.

Effect of P4 on Cholesterol Level and Distribution—Protein folding/trafficking can be directly or indirectly affected by cholesterol (25–28). We examined the role of cholesterol in the regulatory effect of P4 on HERG K+ channel maturation. The free cholesterol in the cells was stained with filipin. As shown in Fig. 6A, P4 caused free cholesterol accumulation in the cytosol in a concentration-dependent manner (1–5 μM). 2-Hydroxypropyl-β-cyclodextrin (HPCD), a sterol-binding agent, redistributed cholesterol in the compartments of cells (Fig. 6B), which is consistent with previous reports (29).

We further tested the involvement of cholesterol in P4-induced ER stress. As shown in Fig. 7D, HPCD reversed P4-induced up-regulation of CHOP expression, but failed to affect thapsigargin-induced CHOP expression. These data further indicate that P4 may impair HERG maturation by disturbing intracellular cholesterol homeostasis and the subsequent ER stress.

Specificity of P4 on HERG K+ Channel Trafficking—In this series of experiments, we first studied whether P4 has a similar effect on L-type Ca2+ calcium channels. The human cardiac Ca2+1.2CM (22), and its accessory subunits, β2a and αδ, were transfected into HEK293 cells. Two protocols, IV protocol (Fig. 8A) and tail protocol (Fig. 8B), were employed to record ICa that flowed through the Ca2+1.2CM channels. As shown in the right panels of Fig. 8, A and B, the current densities recorded with both protocols were not affected by P4 (5 μM, 24 h). The effect of P4 on endogenous voltage-gated K+ current was also examined. As shown in Fig. 8C, P4 had no significant effect on current density of endogenous voltage-gated K+ current. K_1.5 channel undergoes similar glycosylation and trafficking to HERG. The fully glycosylated form (75 kDa) mainly represents the plasma membrane proteins.
channel, whereas the core-glycosylated form (68 KDa) mainly represents the immature form in ER (31). As shown in Fig. 8D, treatment with P4 (5 μM) for 24 h only impaired the trafficking of HERG, but had no effect on that of K,1.5 K⁺ channels. Taken together, the above data clearly demonstrated that P4 specifically induced a HERG K⁺ channel trafficking defect.

**P4 Impaired Maturation of ERG K⁺ Channels in Rat Neonatal Cardiac Myocytes**—There are also two forms of ERG protein (160 and 120 kDa) in rat neonatal cardiac myocytes, which are consistent with the mature and immature forms of rat ERG1a as previously reported (32). We found that treatment with 5 μM P4 for 24 h significantly decreased the mature form of the ERG K⁺ channel (Fig. 9A) and the current density of ERG K⁺ current (Fig. 9B), suggesting that P4 may also impair the maturation and function of ERG channels in the native cardiac tissue.

**DISCUSSION**

HERG trafficking defects are one of the main causes of LQTS. Trafficking can be impaired by mutations of the channel (33) or induced by drugs such as probucol, cardiac glycosides, fluoxetine, norfluoxetine, pentamidine, arsenic trioxide, and celastrol (31, 34). We report here that a HERG trafficking defect can also be induced by excessive P4 hormone. The effects of P4 on Iₖᵣ current density and the channel protein trafficking were further confirmed in HERG-HEK293 cells transfected with Mirp1 and in neonatal cardiac myocytes. The impaired ERG/Iₖᵣ may, in turn, induce imbalance of heart electrical stability and therefore development of ventricular arrhythmias. This may suggest a mechanism to explain why the corrected QT intervals are longer in pregnancy and why women at late pregnancy are more susceptible to ventricular arrhythmias. However, for the LQTS patients, the trafficking of the mutated HERG is already blocked. Thus, P4 may not be able to further impair the blocked trafficking of the mutated HERG channels in the inherited LQTS patients. For this reason, our results may also explain why the incidence of arrhythmias is not higher in pregnancy than in other periods in the life of the LQTS patients (3, 13).

During the late phase of pregnancy, the P4 level in fetal circulation (~4.5 μmol/liter) is much higher than that in maternal blood (1 μmol/liter). This may imply that P4 has a greater impact on the fetal heart and could explain the higher rate of

---

**FIGURE 5. Western blotting analysis showing the effect of P4 (5 μmol/liter, 24 h) on HERG channel maturation at different situations in HERG-HEK293 cells.** A, receptor-independence of the effect of P4. RU486 (10 μmol/liter) failed to reverse the effect of P4. B, effect of P4 in the presence or absence of cycloheximide (40 μmol/liter). C and D, impaired HERG maturation rescued by either treatment with 5 μmol/liter E-4031 (C) or low temperature (27 °C) incubation (D). Mean ± S.E. (error bars) are shown. n = 4–5, * p < 0.05; n.s., not significant.
Progesterone Impairs HERG Trafficking

FIGURE 6. Effect of P4 on the distribution and level of cholesterol in HEK293 cells. A and B, filipin staining of intracellular free cholesterol is shown. A, P4 induced accumulation of cholesterol in a dose-dependent manner. B, sterol-binding agent HPCD (10 mg/ml) abolished P4 (5 μM/liter, 24 h)-induced cholesterol accumulation. Photomicrographs were taken on an inverted microscope. This result represents three independent experiments. Scale bar, 15 μm. C, cellular cholesterol levels were measured with Amplex®Red Cholesterol assay. Cells were treated with simvastatin (10 μM/liter) or P4 (5 μM/liter) for 24 h. Mean ± S.E. (error bars) are shown. n = 6. *, p < 0.05 versus the corresponding value in the control group.

FIGURE 7. P4 impaired HERG maturation via disrupting intracellular cholesterol homeostasis. A, HPCD (10 mg/ml) rescued the HERG trafficking defect caused by P4 (5 μM/liter, 24 h), but not that caused by the V630A HERG mutant. n = 4. B, overexpression of Rab9 partially rescued the P4 (5 μM/liter, 24 h)-induced HERG trafficking defect. n = 6. C, effect of simvas-tatin (Statin, 10 μM/liter), cholesterol (Chol, 20 μg/ml) or imipramine (Imip, 30 μM/liter) on HERG maturation is shown. n = 5. D, HPCD (10 mg/ml) abolished the up-regulated CHOP expression induced by P4 (5 μM/liter), but not by thapsigargin (Thap, 200 nM/liter). The data represent three independent experiments. Mean ± S.E. are shown. *, p < 0.05. n.s., not significant.

The role of protein kinases was also investigated. We found that the effect of P4 is not secondary to activation of MAPK, PI3K/Akt, cAMP, or PKA.

Cholesterol homeostasis is very important for protein folding and trafficking. Cholesterol may affect membrane protein folding either directly or indirectly. For example, nicotinic acetylcholine receptor contains internal binding sites for cholesterol, and cholesterol binding stabilizes nicotinic acetylcholine receptor protein structure. Overloading of free cholesterol may also directly cause ER stress and ER dilation (35) and therefore impairs protein folding. We therefore examined whether the P4-induced HERG trafficking defect involves cholesterol. We found in the present study that P4 induced intracellular free cholesterol accumulation, which further induces ER stress and ER dilation. Because P4 can insert into lipid bilayers and perturb membrane function and lipid mobility (36), direct interaction between P4 and lipids may be responsible for the action of P4 on intracellular cholesterol homeostasis. HPCD is a sterol-binding agent, which redistributes cholesterol in the cellular compartments. HPCD also can disrupt membrane lipid rafts and affect functions of membrane receptors, ion channels, transporters, and protein kinases located in/related to lipid rafts. We found that HPCD abolished the effect of P4 on cholesterol homeostasis, ER stress, and HERG trafficking. Rab9 is important for cholesterol trafficking. Overexpression of Rab9 prevents cholesterol accumulation (30). We found in this study that Rab9 also reversed HERG trafficking defects. These data confirm that the P4-induced HERG trafficking defect is secondary to the accumulation of cholesterol. In addition, disrupting intracellular cholesterol homeostasis with simvastatin, imipramine, or exogenous delivery of cholesterol mimicked the effect of P4 on HERG K⁺ channel trafficking. Thus, impaired cholesterol

sudden death in the uterus than at most other times in the human life cycle.

Apart from the Western blots and patch clamp data, the impaired HERG protein trafficking by P4 was further confirmed with the confocal microscopy. P4 preferentially decreased total protein expression but failed to block the P4 effect. These results indicate that the effect of P4 is not via altering protein synthesis.
Progesterone Impairs HERG Trafficking

FIGURE 8. Effects of P4 (5 μmol/liter, 24 h) on currents of voltage-gated L-type Ca^{2+} channels, endogenous K^{+} channels, and trafficking of K_{1.5} channels. A and B, effect of P4 on L-type Ca^{2+} current density. A, current-density (i-V) relation curve plotted from peak currents at different voltages. Cells were activated from the holding potential (−100 mV) via a series of depolarizing (I-V) pulses (−60 to 40 mV) for 900 ms. n = 9–12. B, I-V curve plotted from the peak tail currents. Cells were activated via a series of depolarizing pulses (−60 to 100 mV) for 20 ms, and the tail currents were recorded at the voltage of −50 mV. n = 7–12. C, effect of P4 on endogenous voltage-gated K^{+} currents of HEK293 cells. I-V relation curve was plotted from the currents measured at the end of depolarizing test pulses. Cells were activated from the holding potential (−70 mV) via a series of depolarizing pulses (−60 to 120 mV) for 5 s. n = 9. Current and time scales are shown in the insets of different representative tracings (A–C). D, effect of P4 on the trafficking of K_{1.5} and HERG. Both myc-K_{1.5} and myc-HERG were transiently transfected into HEK293 cells. Cells were treated with P4 or vehicle for 24 h. Anti-myc antibody was used to detect both myc-K_{1.5} and myc-HERG. For these two potassium channels, the upper bands (arrows) indicate the mature or fully glycosylated form, and the lower bands (arrowheads) indicate the immature or core-glycosylated form. Mean ± S.E. (error bars) are shown. n = 5. *, p < 0.05.

processing (high levels, low levels, or disrupted distribution) may contribute to HERG K^{+} channel defects caused by P4.

The mechanisms underlying cholesterol-induced ER stress are still not clear. It has been reported that free cholesterol accumulation in the ER membrane activates the unfolded protein response (37). The unfolded protein response may induce ER-phagy, which selectively sequesters and tightly packs ER membranes into autophagosomes (38). In ER, cholesterol is also an important factor for ER-Golgi membrane transport. Sterol depletion in the ER inhibits the ER-to-Golgi transport of secretory membrane proteins (25). In addition, inhibition of the early stage of de novo cholesterol synthesis can also affect isoprenoid intermediates, which are very important for the membrane anchoring and activation of small G proteins such as Ras, Rho, and Rab. The dysfunction of these G proteins can affect protein trafficking (27, 28).

Interestingly, we found in the present study that the trafficking of K_{1.5} channels was not affected by P4. These data suggest that the mechanism for the effect of P4 may not be relevant to K_{1.5} trafficking. More studies are warranted to examine how exactly cholesterol regulates ER function/stress and its specificity on protein trafficking.

In summary, P4 may disturb intracellular cholesterol homeostasis and block HERG channel trafficking, which may prolong the QT intervals of both mother and fetus and increase the risk of developing ventricular arrhythmias. Our study may reveal a new mechanism for pregnancy-associated LQTS and provide new approaches to prevent ventricular arrhythmias and sudden death.

Acknowledgments—We thank Tan Junping for technical support. Part of the confocal images was acquired using Nikon A1R Confocal system (SBIC-Nikon Imaging Centre, Biopolis).
REFERENCES

1. Chiron Diagnostics ACS (1998) Centaur Progesterone Assay Manual, Chiron Diagnostics, Emmeryville, CA

2. Burke, J. H., Ehrlert, F. A., Kruse, J. T., Parker, M. A., Goldberger, J. J., and Kadish, A. H. (1997) Am. J. Cardiol. 79, 178–181

3. Rodriguez, J., Kilborn, M. J., Liu, X. K., Pezzullo, J. C., and Woosley, R. L. (2001) JAMA 285, 1322–1326

4. Huot, J. S., Démolis, J. L., Rivière, R., Strabach, S., Christin-Maitre, S., and Funck-Brentano, C. (2003) Eur. Heart J. 24, 1663–1667

5. Nakagawa, M., Osie, T., Takahashi, N., Taniguchi, Y., Anan, F., Yonemochi, H., and Saikawa, T. (2006) Pacing Clin. Electrophysiol. 29, 607–613

6. Nakamura, H., Kurokawa, J., Bai, C. X., Asada, K., Xu, J., Oren, R. V., Zhu, Z. L., Clancy, C. E., Isobe, M., and Furukawa, T. (2007) Circulation 116, 2913–2922

7. Aisien, A. O., Towobola, O. A., Otubu, J. A., and Imade, G. E. (1994) J. Epidemiol. Commun. Health 48, 167–171

8. Nakagawa, M., Ooie, T., Takahashi, N., Taniguchi, Y., Anan, F., Yonemochi, H., and Saikawa, T. (2006) Pacing Clin. Electrophysiol. 29, 607–613

9. Aisien, A. O., Towobola, O. A., Otubu, J. A., and Imade, G. E. (1994) J. Epidemiol. Commun. Health 48, 167–171

10. Rashba, E. J., Zareba, W., Moss, A. J., Hall, W. J., Robinson, J., Goldenberg, I., Ackerman, M. J., Benhorin, J., Kaufman, D. J., Ehlert, F. A., Kruse, J. T., Parker, M. A., Goldberger, J. J., and Towbin, J. A. (2001) Circulation 104, 373–380

11. Wolbrette, D., Naccarelli, G., Curtis, A., Lehmann, M., and Kadish, A. (2002) Clin. Cardiol. 25, 49–56

12. Wisniewski, M., Bajaj, R., Choudhary, A., Dominguez, M., Puri, V., Sharma, D. K., Narita, K., and Wible, B. A., Brown, A. M., Kang, J., Chen, X. L., Sawamura, K., Reynolds, W., and Rampe, D. (2005) J. Pharmacol. Exp. Ther. 312, 316–323

13. Seth, R., Moss, A. J., Mcnitt, S., Zareba, W., Andrews, M. L., Qi, M., Robinson, J. L., Goldenberg, I., Ackerman, M. J., Benhorin, J., Kaufman, E. S., Locati, E. H., Napoli, C., Priori, S. G., Schwartz, P. J., Towbin, J. A., Vincent, G. M., and Zhang, L. (2007) Am. Coll. Cardiol. 49, 1092–1098

14. Chia, E. L., Ho, T. F., Rauff, M., and Yip, W. C. (2005) Prenat. Diagn. 25, 546–552

15. Kähler, C., Schleussner, E., Grönem, B., Schneider, A., Schneider, U., Nowak, H., and Seewald, H. J. (2002) Prenat. Diagn. 22, 408–414

16. Schwartz, P. J., Stramba-Badiale, M., Segantini, A., Austoni, P., Bosi, G., Giorgetti, R., Grancini, F., Marni, E. D., Perticone, F., Rosti, D., and Salice, P. (1998) N. Engl. J. Med. 338, 1709–1714

17. Ackerman, M. J., Siu, B. L., Sturmer, W. Q., Tester, D. J., Valdivia, C. R., Makielski, J. C., and Towbin, J. A. (2001) JAMA 286, 2264–2269

18. Beinder, E., Buheitel, G., and Hoffbeck, M. (2003) Prenat. Diagn. 23, 1097–1098

19. Bhuiyan, Z. A., Momenah, T. S., Gong, Q., Amin, A. S., Ghamdi, S. A., Carvalho, J. S., Homfray, T., Mannens, M. M., Zhou, Z., and Wilde, A. A. (2008) Heart Rhythm 5, 553–561

20. Teng, G. Q., Zhao, X., Lees-Miller, J. P., Quinn, F. R., Li, P., Rancourt, D. E., London, B., Cross, J. C., and Duff, H. J. (2008) Circ. Res. 103, 1483–1491

21. Wu, Z. Y., Chen, K., Haendler, B., McDonald, T. V., and Bian, J. S. (2008) Endocrinology 149, 5061–5069

22. Bhuiyan, Z. A., Momenah, T. S., Gong, Q., Amin, A. S., Ghamdi, S. A., Carvalho, J. S., Homfray, T., Mannens, M. M., Zhou, Z., and Wilde, A. A. (2008) Heart Rhythm 5, 553–561

23. Feng, B., Yao, P. M., Li, Y., Devlin, C. M., Zhang, D., Harding, H. P., Sweeney, M., Rong, J. X., Kuriakose, G., Fisher, E. A., Marks, A. R., Ron, D., and Tabas, I. (2003) Nat. Cell Biol. 5, 781–792

24. Bernales, S., McDonald, K. L., and Walter, P. (2006) PLoS Biol. 4, e423