Gender Disparities in Cardiac Cellular Electrophysiology and Arrhythmia Susceptibility in Human Failing Ventricular Myocytes

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SUMMARY

Gender disparities in ECG variables and susceptibility to arrhythmia exist. The basis of these sex-related distinctions in cardiac electrophysiology has been extensively studied in various species, but is virtually unexplored in humans. The aim of this study was to clarify the cellular basis of electrophysiological gender disparities in human cardiac myocytes.

Human midmyocardial left ventricular myocytes were isolated from explanted hearts of male and female patients in end-stage heart failure at the time of cardiac transplantation. The action potentials, sarcolemmal ion currents, and susceptibility to the generation of early afterdepolarizations were studied using whole-cell patch-clamp methodology. The functional effects of gender disparities in sarcolemmal ion currents were assessed by computer simulations using the Priebe-Beuckelmann or the ten Tusscher-Noble-Noble-Panfilov human ventricular cell models.

Female myocytes had significantly longer action potentials and greater susceptibility to early afterdepolarizations than male myocytes. All other action potential parameters (resting membrane potential, amplitude, plateau level, upstroke velocity, maximal velocity of phase-1 and phase-3 repolarization) had similar values for both genders. In female myocytes, the transient outward potassium current (Ito1) tended to be smaller, while the L-type calcium current (ICa,L) and quasi-steady state current (IQSS) tended to be larger. Computer simulations showed that these subtle differences in sarcolemmal ion currents may conspire to cause the observed gender disparities in action potential properties.

Female failing myocytes have longer action potentials and a greater susceptibility to early afterdepolarizations than male failing myocytes. These gender disparities may be due to slightly larger depolarizing ICa,L in conjunction with slightly smaller repolarizing IQSS and Ito in female myocytes. (Int Heart J 2005; 46: 1105-1118)

Key words: Gender, Heart, Electrophysiology, Action potential, Membrane currents
Gender differences in human electrocardiogram (ECG) variables exist.\textsuperscript{1,2)} Women have faster resting heart rates and longer rate-corrected QT intervals (QTc) than men.\textsuperscript{3,4)} In women, the J-point, ST-angle, and T-wave amplitude are smaller, while the ascending/descending limbs of the T-wave are less steep at heart rates of 60-80 bpm.\textsuperscript{5,6)} Gender disparities in the QT-heart rate relationship (rate-adaptation) may contribute to the longer QTc duration in women. Women exhibit a greater lengthening of the QT interval at longer cycle lengths, rendering gender-related differences in QT intervals most prominent at slow heart rates.\textsuperscript{6-8)} Mirroring these gender disparities in ECG variables, there are striking gender disparities in the incidence of cardiac arrhythmias.\textsuperscript{1,2)} Among the most notable examples are inherited\textsuperscript{9)} and acquired (eg, secondary to drug use)\textsuperscript{10)} long QT syndrome (LQTS), where women are far more likely than men to sustain torsade de pointes (TdP) ventricular tachycardia.

The basis of this sex-related distinction in cardiac electrophysiology in humans is unknown. Animal studies highlight the role of differences in sarcolemmal ion current densities.\textsuperscript{1,2)} However, to date, data about gender disparities in membrane current densities and action potentials (APs) in humans are lacking. The aim of the present study was to examine such differences. Accordingly, we isolated human ventricular myocytes (VMs) of diseased male and female hearts from patients with dilated cardiomyopathy (DCM) that were explanted at the time of cardiac transplantation, and characterized the APs and membrane currents.

**Methods**

**Cell preparation and recording procedures:** Explanted hearts were obtained at the time of cardiac transplantation of 5 men and 5 women with end-stage heart failure from DCM. Both gender groups had similar clinical characteristics, ie, age, functional class, left ventricular ejection fraction (LVEF), and medication (Table I). Informed consent was obtained. The protocol complied with institutional guidelines and the ‘Declaration of Helsinki’.

Left midmyocardial VMs were enzymatically dissociated using coronary artery perfusion methods\textsuperscript{11)} and superfused with solution (36 ± 0.5°C) containing (mM): NaCl 140, KCl 5.4, CaCl\textsubscript{2} 1.8, MgCl\textsubscript{2} 1.0, glucose 5.5, HEPES 5.0, at pH 7.4 (NaOH). Consistent with the laborious isolation method for human ventricular cells,\textsuperscript{11)} the percentage of viable VMs was typically low (< 5%). Nevertheless, the obtained Ca\textsuperscript{2+}-tolerant VMs were rod-shaped cells with clear cross striations, had smooth surfaces and were quiescent, even more than 8 hours after isolation. Membrane potentials and currents were recorded in the ruptured-patch whole-cell configuration of the patch-clamp technique of quiescent, rod-shaped VMs. Patch pipettes (3 ± 0.5) MΩ were filled with solution containing (mM): KCl 145, K\textsubscript{2}--
ATP 5, HEPES 10, at pH 7.2 (KOH). In some experiments, 125 mM KCl was replaced by K-gluconate. All potentials were corrected for the calculated liquid junction potential. Membrane currents and potentials were low-pass filtered online with a cut-off frequency of 1kHz, and digitized at 2kHz.
Stimulation protocols and data analysis: APs were elicited by approximately 50% suprathreshold current pulses (3-ms) applied via the patch pipette. AP characteristics were determined as shown in Figure 1A after 3-minute stimulation.

Figure 2. Membrane currents in male and female VMs. A: Top: generic voltage clamp protocol to elicit L-type calcium current (I_{Ca,L}). Bottom: representative superimposed current traces of a male and female VM following voltage steps from −40 to 0 mV. B: Average current-voltage (I-V) relationship of (peak) I_{Ca,L}. C: Top: generic voltage clamp protocol to elicit quasi-steady state current (I_{QSS}). Bottom: representative current traces of a male and female VM following voltage steps from −40 to −100 and +40 mV. I_{QSS} is measured at the end of the voltage steps (arrows). D: Average I-V relationship of I_{QSS}. Inset: current traces of a female VM elicited by voltage steps from −40 to +50 mV (duration 0.1–3 s) in the presence of 1 mM CdCl₂. E: Top: generic voltage clamp protocol to elicit transient outward potassium current (I_{to}). Bottom: representative current traces of a male and female VM following voltage steps from −80 to +50 mV. F: Average I-V relationship of (peak) I_{to}. Inset: I_{to} measured at +50 mV; note the number of VMs measured at this voltage.
Values presented are the averages of 10 consecutive APs. Voltage-clamp measurements were performed to characterize the membrane currents underlying APs. To ensure that the AP shapes and the remaining viable VMs in the recording chamber remained undistorted for biophysical analysis, these measurements were performed without specific channel blockers or modified solutions, except $I_{to}$ measurements. The L-type calcium current ($I_{\text{Ca,L}}$), the quasi-steady state current ($I_{\text{QSS}}$), and the transient outward potassium current ($I_{to}$) were determined by the voltage clamp protocols shown in Figure 2 and described previously.\(^{11,12}\) $I_{\text{Ca,L}}$ amplitude was calculated as the difference between peak inward current and $I_{\text{QSS}}$. $I_{\text{QSS}}$ amplitude was defined as the absolute current amplitude at 500ms after the onset of the voltage step. $I_{to}$ amplitude was measured in the presence of CdCl$_2$ (1 mM) to block calcium and sodium currents, and was calculated as the difference between peak outward current and $I_{\text{QSS}}$. All currents were normalized for cell size (capacitance, $C_m$).\(^{12}\) From each heart, 2-4 cells were used to measure APs and current characteristics.

**Computer simulations:** To test the functional effects of differences in membrane currents, we conducted computer simulations using the comprehensive human ventricular cell models developed by Priebe and Beuckelmann (PB-model)\(^{13}\) and ten Tusscher, Noble, Noble, and Panfilov (TNNP-model).\(^{14}\) Both models are largely based on experimental data from single VMs isolated from explanted human hearts. Because these myocytes were generally isolated from midmyocardial areas of the left ventricle of male patients,\(^{13}\) the PB-model and the midmyocardial variant of the TNNP-model\(^{14}\) are, in effect, models of male midmyocardial VMs. Therefore, we scaled the ‘male’ current densities of the model cells by the observed female-to-male ratio in density of selected membrane currents to obtain models of female VMs. The TNNP-model has separate formulations for $I_{to}$ and the accompanying sustained outward current (‘plateau potassium current,’ $I_{Kp}$); $I_{Kp}$ formulations are based on experimental data in nonfailing human VMs.\(^{15}\) In the PB-model, $I_{Kp}$ is included in $I_{to}$ as a sustained component due to incomplete inactivation of $I_{to}$ at positive potentials. To allow independent scaling of $I_{to}$ and $I_{Kp}$ in the PB-model, we removed this incomplete inactivation of $I_{to}$ and incorporated the TNNP formulation of $I_{Kp}$. All computer simulations were set to generate at least 200 APs in order to obtain steady-state AP characteristics.

**Statistics:** Data are expressed as the mean ± SEM. Group comparisons were made using the unpaired Student’s $t$-test or two-way repeated measurements ANOVA (followed by Holm-Sidak post hoc testing). The susceptibility to early afterdepolarizations (EADs) of male and female cells was compared using Fisher’s exact test. Statistical significance was defined as $P < 0.05$.\(^{16}\)
**RESULTS**

**Action potential characteristics:** In 14 male and 15 female VMs, APs were recorded at a cycle length of 2 seconds and characterized as shown in Figure 1A. Figure 1B shows typical APs of a male and female VM. The average characteristics are summarized in Table II. There were no significant differences between male and female VMs in $C_m$, resting membrane potential (RMP), maximal AP amplitude ($A_{P_{\text{max}}}$), AP amplitude at 100ms after the upstroke indicating the plateau level ($A_{P_{100}}$), maximal velocity of phase-0 depolarization ($dV/dt_{\text{phase-0}}$), and maximal velocity of phase-1 and phase-3 repolarization ($dV/dt_{\text{phase-1}}$ and $dV/dt_{\text{phase-3}}$). However, there were clear gender differences in AP duration. Males exhibited significantly shorter AP durations at 20%, 50%, and 90% repolarization ($A_{P_{20}}, A_{P_{50}}, A_{P_{90}}$) than females. For instance, the $A_{P_{90}}$ was 670 ms in males and 870 ms in females. On average, the APs of female VMs were 29% longer than those of male VMs.

In 13 of 15 female and 11 of 14 male VMs, we attempted to stimulate at the more physiologic cycle length of 1 second. However, in 7 of 13 (54%) female and 2 of 11 (18%) male VMs, the membrane potential did not repolarize to its resting value. This disparity is most likely due to longer APs in women.

Since QTc duration is age-dependent, we studied whether the observed gender disparities in $A_{P_{90}}$ might be due to age-dependence. However, we found little or no age-dependence (Figure 1C).

**Susceptibility to early afterdepolarizations:** We studied the susceptibility to EAD formation because animal studies have indicated that female VMs are more susceptible to EADs than male VMs. EADs typically occur at slow heart rates. Increasing the cycle length from 2 to 5 seconds resulted in AP prolongation, along with a significantly higher incidence of EAD formation in females. EADs

### Table II. Action Potential Properties

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<tr>
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<th>Female ($n = 15$)</th>
<th>Male ($n = 14$)</th>
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<tr>
<td>$C_m$ (pF)</td>
<td>292 ± 37</td>
<td>269 ± 31</td>
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<tr>
<td>RMP (mV)</td>
<td>-81.1 ± 0.9</td>
<td>-80.6 ± 1.5</td>
</tr>
<tr>
<td>$A_{P_{\text{max}}}$ (mV)</td>
<td>112.8 ± 2.1</td>
<td>112.0 ± 2.2</td>
</tr>
<tr>
<td>$A_{P_{100}}$ (mV)</td>
<td>108.9 ± 1.9</td>
<td>106.2 ± 3.6</td>
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<tr>
<td>$A_{P_{20}}$ (ms)</td>
<td>375 ± 29</td>
<td>271 ± 36*</td>
</tr>
<tr>
<td>$A_{P_{50}}$ (ms)</td>
<td>729 ± 57</td>
<td>529 ± 32*</td>
</tr>
<tr>
<td>$A_{P_{90}}$ (ms)</td>
<td>866 ± 60</td>
<td>672 ± 43*</td>
</tr>
<tr>
<td>$dV/dt_{\text{phase-0}}$ (V/s)</td>
<td>130 ± 10</td>
<td>131 ± 12</td>
</tr>
<tr>
<td>$dV/dt_{\text{phase-1}}$ (V/s)</td>
<td>3.60 ± 0.96</td>
<td>3.83 ± 1.15</td>
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<tr>
<td>$dV/dt_{\text{phase-3}}$ (V/s)</td>
<td>0.89 ± 0.05</td>
<td>1.07 ± 0.11</td>
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</table>

Values expressed as mean ± SEM and measured at 2-s cycle lengths. $n$, number of cells. See text for abbreviations. *$P < 0.05$. 
were observed in 80% of the female VMs \((n = 5)\) tested, but in none of the male VMs \((n = 5)\). Figure 1D shows a typical EAD in a female VM. The EADs occurred as single rather than multiple afterdepolarizations and had a ‘take-off potential’ - the potential where repolarization turns into depolarization - between \(-40\) and \(0\) mV.

**Membrane currents:** We examined \(\text{I}_{\text{Ca,L}}\), \(\text{I}_{\text{QSS}}\), and \(\text{I}_{\text{i0}}\) to determine which membrane currents may account for the observed gender differences in AP duration.

**L-type calcium current.** To measure \(\text{I}_{\text{Ca,L}}\), 500-ms depolarizing voltage steps were applied from a holding potential of \(-40\) mV (cycle length: 2 seconds). Figure 2A shows typical current traces following a step to 0 mV in a male and a female VM. The depolarizing step activated \(\text{I}_{\text{Ca,L}}\), which was larger in the female than the male VM. Figure 2B shows the mean current-voltage (I-V) relation for \(\text{I}_{\text{Ca,L}}\). Although gender differences did not reach statistical significance, \(\text{I}_{\text{Ca,L}}\) density was larger in female VMs at all voltages, eg, 129% of that in male VMs at 0 mV.

**Quasi-steady state current.** To measure \(\text{I}_{\text{QSS}}\), 500-ms hyperpolarizing and depolarizing voltage steps were applied from a holding potential of \(-40\) mV (cycle length: 2 seconds). Figure 2C shows typical current traces following a hyperpolarizing step \((-100\) mV) and a depolarizing step \((+40\) mV), in a male and a female VM. The amplitude of \(\text{I}_{\text{QSS}}\) at 500 ms after the onset of the voltage step was similar in male and female VMs at \(-100\) mV, but larger at \(+40\) mV in the male VMs than the female VM. Figure 2D shows the mean I-V relationship of \(\text{I}_{\text{QSS}}\). At potentials negative to \(-30\) mV, \(\text{I}_{\text{QSS}}\) overlapped in male and female VMs, indicating that the inward rectifier current \((\text{I}_{\text{K1}})\) did not differ between genders. At potentials positive to \(-30\) mV, \(\text{I}_{\text{QSS}}\) showed a linear I-V relationship with a smaller density in female than male VMs, but these differences did not reach statistical significance. At \(+40\) mV, \(\text{I}_{\text{QSS}}\) density was \(2.8 \pm 0.6 \text{ pA/pF}\) in males and \(1.8 \pm 0.4 \text{ pA/pF}(64\%)\) in females.

At potentials positive to \(-30\) mV, \(\text{I}_{\text{QSS}}\) may be composed of various repolarizing currents, including the delayed rectifier current \((\text{I}_K)\), which consists of two components: a rapidly activating component showing inward rectification \((\text{I}_{K_\text{r}})\) and a slowly activating component without rectification \((\text{I}_{K_\text{s}})\). While some investigators report that human ventricular \(\text{I}_K\) consists of both \(\text{I}_{K_\text{r}}\) and \(\text{I}_{K_\text{s}}\), others suggest that only \(\text{I}_{K_\text{r}}\) is present, or that \(\text{I}_K\) is absent or hardly detectable at all. In some female VMs, we assessed the nature of \(\text{I}_{\text{QSS}}\) in more detail. In the presence of 1 mM CdCl\(_2\) to block calcium current and inward sodium-calcium exchange current upon repolarization, we applied 0.1 to 3-second depolarizing steps from \(-40\) to \(+50\) mV. In only a few VMs did we find a small slow time-dependent current, most likely reflecting \(\text{I}_{K_\text{s}}\) (Figure 2D, inset). However, tail currents were absent. The absence of clear time-dependent currents and tail currents
suggest that the contribution of $I_{Ks}$ and $I_{Kr}$ to $I_{QSS}$ was small. The absence of significant $I_{Ks}$ and $I_{Kr}$ may be due to heart failure, cell isolation procedures, or experimental techniques.\textsuperscript{20,21} The nature of the gender difference in repolarizing current at potentials $> -30$ mV thus remains unclear. We propose that it may be caused by differences in time-independent background K$^+$ currents,\textsuperscript{25} eg, the ‘plateau potassium current’ ($I_{Kp}$),\textsuperscript{15} because the density and I-V relationship of $I_{QSS}$ compares well with previously reported $I_{Kp}$ characteristics in nonfailing human subepicardial VMs.\textsuperscript{15}

**Transient outward current.** To measure $I_{to}$, 500-ms depolarizing voltage steps were applied from a holding potential of $-80$ mV (cycle length: 5 seconds). Figure 2E shows typical current traces following a step to $+50$ mV in a male and a female VM. This step activated $I_{to}$, which was smaller in female than male VMs. Figure 2F shows the I-V relation for $I_{to}$. While gender differences did not reach statistical significance, $I_{to}$ density was smaller at all voltages in female VMs, eg., 84% of that in male VMs at $+50$ mV (inset).

![Figure 3](image)

**Figure 3.** A: Superimposed APs (cycle length, 2 seconds) of the midmyocardial ten Tusscher-Noble-Noble-Panfilov (TNNP) model cell (left) and the Priebe-Beuckelmann (PB) model cell (right) at default parameter settings and after implementation of experimentally observed disparities in $I_{to}$, $I_{Kp}$, and $I_{Ca,L}$ densities, both individually and combined. B: Cycle length dependence of APD$_{90}$ of TNNP (left) and PB (right) model cells.
Computer simulations: Although the gender differences in $I_{\text{Ca,L}}$, $I_{\text{QSS}}$, and $I_{\text{to}}$ densities did not reach statistical significance due to the large cell-to-cell variability, the average differences were of the same magnitude as in animal studies. To explore whether the small differences in current densities may be responsible for the longer APs in female VMs, we implemented them in the PB-model\(^{13}\) and the midmyocardial TNNP-model.\(^{14}\) We tested the effects of the experimentally observed difference in $I_{\text{QSS}}$ density by scaling the current density of $I_{\text{Kp}}$.

Figure 3A shows that a decrease of $I_{\text{to}}$ to 84\% hardly affected AP duration in the TNNP- (left) and PB-model (right) at a cycle length of 2 seconds. However, AP was prolonged in both models by increasing $I_{\text{Ca,L}}$ to 129\%. In both models, a decrease of $I_{\text{Kp}}$ to 64\% resulted in slight AP prolongation, most prominently in the TNNP-model. A combination of decreased $I_{\text{to}}$, decreased $I_{\text{Kp}}$, and increased $I_{\text{Ca,L}}$ resulted in significant AP prolongation from 299 to 335 ms (12\% increase) in the TNNP-model, and from 355 to 392 ms (10\% increase) in the PB-model. Figure 3B shows that such observations were made at all cycle lengths.

**DISCUSSION**

We have presented for the first time data on the gender disparity in APs and sarcolemmal ion currents in humans. In VMs isolated from explanted hearts of patients with end-stage heart failure from DCM, we identified gender disparities in AP duration, sarcolemmal ion current densities, and susceptibility to EADs.

**Gender disparity in action potential duration and membrane currents:** We found that human failing female VMs have longer APs than their male counterparts (Figure 1, Table II). No other experimental findings of gender disparity in AP duration in humans are available, neither in healthy subjects, nor in patients in heart failure. In clinical and animal studies of hypertrophic or failing hearts, ECG parameters have not been stratified by gender. However, our findings are in agreement with studies in healthy animals. Female APs are significantly longer than male APs in mouse subepicardial VMs,\(^{27,28}\) rabbit left ventricular subendocardial VMs,\(^{29}\) dog left ventricular midmyocardial VMs,\(^{30}\) and guinea pig right and left ventricular VMs at the day of estrus.\(^{31}\) Furthermore, our findings are in accordance with the clinical observation that women have longer QTc intervals.\(^{3-6}\)

The gender disparity in QTc duration may be age-dependent.\(^{4-6}\) The QT interval is similar in boys and girls until puberty, but the QT interval of the male shortens with the growth spurt associated with puberty, a time of increased male hormone production.\(^{41}\) The gender disparity in QTc duration again becomes smaller with advancing age, as QTc lengthens in males. However, this issue remains controversial, as the age at which this gender difference is lost has been
reported differently, varying from > 50 years\textsuperscript{4) to > 76 years.\textsuperscript{5)} One study even reported that JTC intervals remain different between men and women aged 70-80 years.\textsuperscript{6)} We found no age-dependence in APD\textsubscript{90} (Figure 1C), indicating that age plays a minor role in our principal findings. Additionally, we found no differences in APD\textsubscript{90} between premenopausal and postmenopausal women (Figure 1C), suggesting that the role of gonadal backgrounds is limited, as reported previously.\textsuperscript{32-34} From the present study, however, we cannot draw firm conclusions regarding age and menopausal effects on APD differences due to the small num-

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<td>(I_{Ca,L})</td>
<td>dog</td>
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\(I_{Ca,L}\) indicates L-type calcium current; \(I_{K1}\), inward rectifier current; \(I_{Kr}\), rapid component of delayed rectifier potassium current; \(I_{Kr}\), slow component of delayed rectifier potassium current; \(I_{Kur}\), ultrarapid component of delayed potassium rectifier current; \(I_{Na}\), sodium current; \(I_{L}\), transient outward potassium current; LV, left ventricle; RV, right ventricle; endo, subendocardium; epi, subepicardium; and mid, midmyocardium.
number of hearts, particularly those of subjects in their teens and twenties.

The cardiac AP results from a complex interplay of several time- and voltage-dependent ion currents. In animal studies, various ion currents exhibited gender disparities, but these findings were strongly dependent on species and origin of the studied VM within the ventricular wall (Table III). Here, we demonstrate that the longer APs in female human VMs are caused by a longer plateau phase rather than differences in phase-1 or phase-3 repolarization (Figure 1, Table II). Voltage-clamp experiments suggest that $I_{to}$ and $I_{QSS}$ densities may be smaller in females than in males, while $I_{Ca,L}$ density may be larger (Figure 2). Given that $I_{Ca,L}$ plays a key role during the AP plateau where net current flow is small, even mild increases in $I_{Ca,L}$ are expected to produce longer APs in females. This expectation was supported by computer simulations (Figure 3). These simulations also showed that, in isolation, the effects of the smaller $I_{to}$ on AP duration are limited, while reduced $I_{QSS}$ results in further AP prolongation (Figure 3).

At a 2-second cycle length, the patch-clamp experiments demonstrated a 29% longer APD$_{90}$ in female than male VMs, while the combined changes of $I_{Ca,L}$, $I_{to}$, and $I_{QSS}$ caused a 10-12% increase in APD$_{90}$ in the computer models. This APD$_{90}$ increase is larger than observed electrocardiographically, as healthy women have QTc durations only 2-6% longer than healthy men. The basis of these discrepancies is unknown, but may reside in the fact that we studied VMs of failing hearts, in which gender-related differences in the pathophysiological basis of heart failure existed, or the fact that patch-clamp studies and model studies were conducted in single cells.

**Gender disparities in susceptibility to early afterdepolarizations:** We found that human female failing VMs are more susceptible to EADs than male VMs in response to an increase in cycle length. This result corresponds with experimental findings in healthy animals and supports the clinical observation that female gender is an independent risk factor for TdP.

Several cellular mechanisms have been implicated in EAD formation in ventricular muscle, including $I_{Ca,L}$ reactivation, Na-Ca exchange current enhancement, and $I_{na}$ reactivation. In human VMs, we previously demonstrated that VMs with longer APs are predisposed to develop EADs. During AP prolongation, the membrane potential may remain long enough at a plateau level to permit recovery from inactivation of $I_{Ca,L}$ during early repolarization. The subsequent reactivation of $I_{Ca,L}$ results in transient depolarization, which may trigger EADs. In female VMs, APs were longer and $I_{Ca,L}$ density larger. Both factors may conspire to produce the greater susceptibility to EADs and TdP in inherited and acquired LQTS in females.

**Study limitations:** In addition to limitations inherent in studying cells isolated from their native milieu, this study has the limitation of having been conducted in...
VMs of failing human hearts. We cannot exclude significant modulating effects of heart failure, given that heart failure may cause down-regulation of various cationic currents. However, because the functional class and LVEF are comparable between the gender groups, it is likely that the observed gender disparities are not due to differences in heart failure severity. Thus, although the results must be appreciated in the context of heart failure and may not necessarily apply to healthy subjects, our findings may explain clinically observed gender disparities in arrhythmia susceptibility. Still, further studies are required to clarify gender disparities in nonfailing human hearts.

It has been reported that gender differences in membrane currents in dogs may be determined by the origin of the VMs from the various transmural layers (Table III). To eliminate this variable, we studied only midmyocardial VMs. Further studies are required to clarify gender disparities in various transmural layers of human hearts.

In each gender group, 2 of 5 patients received amiodarone, which might alter cardiac electrophysiology. However, amiodarone treatment had no prominent effects on AP duration in either group (not shown). Furthermore, the numbers of amiodarone-treated cells used for analysis of membrane currents and AP duration were comparable between the two groups. With regard to EAD susceptibility at long cycle lengths, all 5 female VMs, in which EAD inducibility was investigated, were obtained from patients treated with amiodarone, while this proportion was 2 of 5 among male VMs. However, because amiodarone did not prolong AP duration significantly and amiodarone induces TdP and EADs only very rarely in patients and animal studies, it is likely that amiodarone use did not strongly affect EAD susceptibility in our study.

Conclusions: Our study provides the first insights into the cellular basis of sex-related distinctions in human cardiac electrophysiology. Human failing female VMs have longer APs and an enhanced susceptibility to EADs, which may be explained by gender differences in $I_{Ca,L}$, $I_{QSS}$, and $I_{to}$ densities.

**ACKNOWLEDGEMENTS**

The authors thank Dr. Antonius Baartscheer and Cees Schumacher for their excellent technical assistance. Dr. Tan received financial support in the form of fellowships from the Royal Netherlands Academy of Arts and Sciences (KNAW), the Netherlands Heart Foundation (NHS-2002B191), and the Bekales Foundation.
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