Reduced repolarization reserve in ventricular myocytes from female mice

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Abstract

Objective: Cardiac repolarization is prolonged and repolarization reserve (RR) is diminished in female rabbits and humans, compared to males. Reduced RR is evidenced by the relatively greater increase in ventricular action potential duration (APD) in myocytes from females in response to drugs that block repolarizing K currents. Mice are an increasingly important experimental model animal for cardiovascular research, but gender-dependent differences have not been reported for repolarization in murine ventricular myocytes.

Methods: APD and repolarizing K currents were measured in isolated ventricular myocytes from adult littermate male and female mice. Repolarizing K currents were dissected into transient (\(I_{\text{to}}\)) and sustained (\(I_{\text{sus}}\)) components and the selective \(I_{\text{to}}\) antagonist FK506 was used to probe for differences in RR. Results: Under control conditions APD at 50\% (APD\(_{50}\)) and at 90\% (APD\(_{90}\)) repolarization was significantly longer in females (APD\(_{50}\) 15±3 ms, \(n=6\) and APD\(_{90}\) 63±6 ms, \(n=6\)) compared to males (APD\(_{50}\) 8±2 ms, \(n=7\) and APD\(_{90}\) 42±9 ms, \(n=7\)) at 1.0 Hz. At 0.3 Hz stimulation frequency APD\(_{90}\) was significantly longer in females (APD\(_{90}\) 12±2 ms and APD\(_{90}\) 54±5 ms, \(n=10\)) compared to males (APD\(_{90}\) 11±2 ms and APD\(_{90}\) 47±7 ms, \(n=10\)). FK506 treatment (25 \(\mu\)M) selectively and equally inhibited \(I_{\text{to}}\) in all cells, and significantly increased APD\(_{50}\) and APD\(_{90}\) in males and females at 0.3 and 1.0 Hz. However, increases in APD\(_{50}\) and APD\(_{90}\) (0.3 and 1.0 Hz) in response to FK506 were significantly greater in myocytes from females compared to males. Voltage clamp measurement of \(I_{\text{to}}\) and \(I_{\text{sus}}\) revealed that males had a relatively more prominent \(I_{\text{to}}\) while females exhibited a more prominent \(I_{\text{sus}}\). Conclusions: Ventricular action potential repolarization is prolonged in myocytes from female compared to male mice. Female mice have reduced RR that is unmasked by FK506. These findings suggest that gender is an important variable for cardiovascular studies using mice. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Gender; K-channel; Membrane potential; Myocytes; Repolarization

1. Introduction

Mice have become an increasingly important model for the study of cardiovascular diseases, in large part because of the development of technologies allowing for manipulation of the murine genome [1,2]. Despite the proliferation of reports using wild type and genetically modified mice for cardiovascular research, little attention has been focused on potential gender-linked differences in murine cardiac electrophysiology. Such differences, if found, may be of great importance for understanding the results of electrophysiologic experiments, and would necessarily prompt further investigations to determine the scope and mechanism of gender influence on murine cardiac physiology. Although the specific ion channels underlying cardiac membrane potential repolarization vary between species, K\(^{+}\) currents (\(I_{K}\)) are fundamental determinants of cardiac repolarization in all species. Baseline action potential duration (APD) is prolonged in women [3,4] and in female rabbits [5], but it is not known if a similar gender-determined difference is present in mice.

Small increases in baseline QT interval or APD in females are magnified upon exposure to \(I_{K}\) antagonist

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agents due to reduced repolarization reserve (RR) [6,7].
RR refers to the redundancy of repolarizing currents in ventricular myocytes, and gender-dependent reduction in RR is manifested by greater relative APD prolongation in ventricular myocytes from females, compared to males, in response to blockade of repolarizing current. The total number and molecular identity of all murine cardiac $I_k$ components remain controversial. Recently four components of $I_k$ have been identified in mice [8]. The murine ventricular APD is primarily determined by two repolarizing $I_k$ components: the transient ($I_{to}$) and sustained ($I_{sus}$) outward currents [9], with contribution to both of these components by a third current with intermediate kinetic characteristics [10]. Recent studies have clarified the ion channel subunit families likely responsible for $I_{to}$ (Kv4) [11] and $I_{sus}$ (Kv1) [12] using dominant-negative transgenic approaches, but the effect of gender on these repolarizing currents is unknown. The immunosuppressant agent FK506 is a selective inhibitor of $I_{sus}$ in murine ventricular myocytes, at slow stimulation rates [10] and was used to test the hypothesis that female mice have reduced repolarization reserve, as occurs in other mammalian species, including humans.

2. Methods

2.1. Myocyte isolation

Mature left ventricular myocytes were prepared from 10 to 12-month-old $\geq$F4 offspring of C57BL/6J males crossed with 129SVE females as previously described [13]. The isolated cells were used within 8 h of isolation. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Table 1

<table>
<thead>
<tr>
<th>Action potential measurements at 0.3 Hz (A)</th>
<th>Female (–FK506)</th>
<th>Female (+FK506)</th>
<th>Male (–FK506)</th>
<th>Male (+FK506)</th>
</tr>
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<tr>
<td>(A) Action potential measurements (0.3 Hz)</td>
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<tr>
<td>Number of mice studied</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>RP (mV)</td>
<td>$-71 \pm 1$ ($n=10$)</td>
<td>$-71 \pm 1$ ($n=10$)</td>
<td>$-73 \pm 1$ ($n=10$)</td>
<td>$-73 \pm 1$ ($n=10$)</td>
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<tr>
<td>$APD_{50}$ (ms)</td>
<td>$12 \pm 2$ ($n=10$)</td>
<td>$47 \pm 13$ ($n=10$)</td>
<td>$11 \pm 2$ ($n=10$)</td>
<td>$20 \pm 6$ ($n=10$)***</td>
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<tr>
<td>$APD_{90}$ (ms)</td>
<td>$54 \pm 5$ ($n=10$)</td>
<td>$136 \pm 19$ ($n=10$)***</td>
<td>$47 \pm 7$ ($n=10$)</td>
<td>$70 \pm 13$ ($n=10$)***</td>
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<td>(B) Action potential measurements (1.0 Hz)</td>
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<tr>
<td>Number of mice studied</td>
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<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>RP (mV)</td>
<td>$-72 \pm 1$ ($n=6$)</td>
<td>$-72 \pm 1$ ($n=6$)</td>
<td>$-73 \pm 1$ ($n=7$)</td>
<td>$-72 \pm 1$ ($n=7$)</td>
</tr>
<tr>
<td>$APD_{50}$ (ms)</td>
<td>$15 \pm 3$ ($n=6$)</td>
<td>$85 \pm 24$ ($n=6$)***</td>
<td>$8 \pm 2$ ($n=7$)</td>
<td>$16 \pm 5$ ($n=7$)***</td>
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<tr>
<td>$APD_{90}$ (ms)</td>
<td>$63 \pm 6$ ($n=6$)</td>
<td>$158 \pm 26$ ($n=6$)***</td>
<td>$42 \pm 9$ ($n=7$)</td>
<td>$67 \pm 20$ ($n=7$)***</td>
</tr>
</tbody>
</table>

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to ADP in the absence of FK506; $\dagger$ $P<0.05$ compared to males in the absence of FK506; $\ddagger$ $P<0.05$, $\ddagger$ $P<0.01$ compared to males in the presence of FK506. $n$ = number of ventricular myocytes studied (experimental observations), RP, cell membrane resting potential; $APD_{50}$, action potential duration at 50% repolarization to baseline; $APD_{90}$, action potential duration at 90% repolarization to baseline.

2.2. Electrophysiology

2.2.1. Current clamp

Cells were stimulated in current clamp mode (Axopatch 200B amplifier, Axon Instruments) at 0.3 or 1.0 Hz with 1.0–2.0 nA pulses of depolarizing current (1.25× threshold) for 3–4 ms at 35±1°C using a thermostatic heated stage (Warner Instruments) [14]. These slow stimulation frequencies were used to reduce $I_{to}$ inactivation and to preserve the $I_{sus}$ antagonist specificity of FK506 [10]. Action potential duration at 50 ($APD_{50}$) and 90% ($APD_{90}$) repolarization was measured as previously described [15].

2.2.2. Voltage clamp

Isolated myocytes were studied with whole cell mode voltage clamp configuration at 35±1°C and cell capacitance was measured by integrating the current transient after a 10-mV hyperpolarizing step from −80 mV using pClamp 6.01 software. $I_{sus}$ was defined as the residual current at the end of a 450- or 1300-ms depolarizing pulse and $I_{to}$ was the difference between peak outward K+ current and $I_{sus}$ (Fig. 2) [9,10,16].

2.3. Solutions

Current clamp experiments used a pipette solution that contained (mM): K aspartate 120.0, HEPES 5.0, KCl 25.0, Na2ATP 4.0, MgCl2 1.0, Na2phosphocreatine 2.0, NaGTP 2.0, CaCl2 1.0, and EGTA 10; the bath solution contained (mM): NaCl 140.0, HEPES 5.0, glucose 10.0, KCl 5.4, CaCl2 2.5, MgCl2 1.0. The pH was adjusted to 7.4 with 0.1 N NaOH. The bath solution for $I_k$ voltage clamp studies was (mM): N-methyl-D-glucamine 149, HEPES 5.0, glucose 5.0, KCl 1.0, MgCl2 5.0, and the pH was adjusted to 7.4 with 12 N HCl [15], and the pipette solution was identical to that used in the current clamp measurements. Na+ and Ca2+ were omitted from the voltage clamp bath.
solution to avoid contamination by $Na^+$, $Ca^{2+}$, and $Na^+/Ca^{2+}$ exchanger currents. Cells were dialyzed for $\geq 5$ min prior to initiating experimental protocols. Unless otherwise noted, all chemicals were from Sigma.

2.4. FK506

FK506 was added from a DMSO stock solution for a final bath concentration of 25 $\mu$M. The maximum final

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Fig. 1. Reduced repolarization reserve in ventricular myocytes from female mice is unmasked by FK506. Panels A and B show representative action potential recordings (0.3 Hz) from myocytes from female (A) and male (B) mice before (Control) and after addition of FK506. The horizontal line at the left of each panel marks 0 mV. Panels C–F show summary data for the action potential duration at 50% ($APD_{50}$) and 90% ($APD_{90}$) repolarization to baseline during stimulation at 0.3 Hz (C and D) and 1.0 Hz (E and F). $APD_{50}$ at 0.3 Hz and $APD_{90}$ at 1.0 Hz were significantly longer in females than males. The increases in APD following FK506 were significant for $APD_{50}$ and $APD_{90}$ for both males and females at 0.3 Hz and 1.0 Hz (Table 1), but the increases in APD following FK506 were significantly greater ($P<0.05$) in females than in males (indicated by the asterisks). Data in panels C–F are the same as in Table 1.
concentration of DMSO was 0.001 vol% and was found to have no effect on K⁺ currents in previous control experiments [15]. FK506 was a generous gift from Dr. Ihor Bekersky (Fujisawa Healthcare, Inc., Deerfield, IL, USA).

2.5. Statistics

Statistical analysis was performed using Student’s t-test, or two-way ANOVA with Bonferroni’s corrected t-test (SPSS Science, Chicago, IL, USA), as appropriate. The null hypothesis was rejected for P<0.05. Data are presented as means±standard error.

3. Results

3.1. Baseline APD is increased in female mice

APD₉₀, but not APD₅₀, was significantly increased in female compared to male mice (Table 1A) during stimulation at 0.3 Hz, under control conditions. Stimulation at 1.0 Hz resulted in significant prolongation of both APD₅₀ and APD₉₀ in female compared to male mice (Table 1B). These gender-determined differences in repolarizing currents in mice (Table 1) are comparable, on a percentage-wise basis, with gender differences reported in human QT interval measurement studies [3].

3.2. FK506 disproportionately prolongs the APD in female mice

Both APD₅₀ and APD₉₀ were significantly increased by FK506 in males and females at 0.3 and 1.0 Hz (Table 1 and Fig. 1). However, the increases in APD₅₀ and APD₉₀ in response to FK506 were both significantly greater in females compared to males at 0.3 and 1.0 Hz (Table 1 and Fig. 1C–D). Thus, ventricular myocytes from female mice exhibited a reduced RR [17] after FK506 that mirrors the exaggerated QT interval response seen in female patients [6,18,19] and rabbits [7] exposed to drugs that block I_{Kr}.

3.3. Iₜₒ is diminished and Iₘₚ is increased in female compared to male mice

We measured Iₜₒ and Iₘₚ components of I_K (Fig. 2) in mice to test the hypothesis that these I_K components were different in females and males, given the gender-dependence for baseline APD measurements (Table 1). Cell capacitance measurements revealed that female cardiomyocytes (172±6 pF, n=41) were smaller than male counterparts (199±7 pF, n=39, P=0.004). Iₜₒ was significantly (P<0.001) less in females than in males, independent of cell membrane potential (Fig. 3A), while Iₘₚ recorded after a 450-ms (Fig. 3B, P<0.001) or a 1300-ms depolarizing pulse (Fig. 3C, P<0.001), were also significantly greater in female mice than in male mice. For 1300-ms depolarizing steps, the effect of gender was increased by more positive cell membrane potentials. These findings showed that repolarizing K⁺ currents were not consistently diminished in female mice, but instead followed a more complex pattern where Iₜₒ and Iₘₚ amplitudes were reciprocally related according to gender.

3.4. FK506 selectively inhibits Iₘₚ

Excessive QT interval prolongation in women is most often evident in response to I_K antagonist drugs [19], but Iₜₒ is unlikely to significantly contribute to cardiac repolarization in mice [9]. Our measurements of I_K components indicated that female mice might be relatively more

Fig. 2. Repolarizing K⁺ currents in murine ventricular myocytes. (A) Schematic depiction of the voltage command steps applied at 0.3 Hz used to elicit K⁺ currents. (B) A family of currents resulting from these commands steps (panel A) is shown. Two current components are clearly identifiable: An initial outward current (Iₜₒ) that rapidly peaks and then decays to a sustained (Iₘₚ) plateau current.
under these conditions (Fig. 4C and D), consistent with a previous report [10]. The finding that FK506 antagonist actions are specific for $I_{\text{to}}$ over $I_{\text{us}}$ (Fig. 4) suggests that the relative increase in $I_{\text{us}}$ in female mice (Fig. 3) is important for reduced RR seen in female mice with FK506 (Table 1 and Fig. 1).

4. Discussion

4.1. Gender influence on ventricular action potential repolarization

Female gender is a recognized determinant of cardiac arrhythmias related to prolongation of cardiac repolarization in humans [3–5,19]. The increased tendency of cardiac myocytes from female rabbits to have longer APDs than male counterparts is likely related, in part, to a reduction in the amplitude of the rapid component of the delayed rectifier K⁺ current ($I_{\text{Kr}}$) [20,21]. In contrast to humans and rabbits, $I_{\text{Kr}}$ does not appear to significantly contribute to murine action potential repolarization [9]. Our experiments focused on $I_{\text{us}}$ and $I_{\text{to}}$ because of the demonstrated importance of these currents in determining murine APD [9]. We found gender-linked differences in both $I_{\text{to}}$ and $I_{\text{us}}$ (Figs. 3 and 4) in mice, and relative baseline action potential prolongation in females (Table 1A). While many ionic currents (including the Na⁺/Ca²⁺ exchanger that was not measured during the voltage clamp experiments) are important for action potential repolarization, the differences in $I_{\text{us}}$ and $I_{\text{to}}$ between males and females suggests that reciprocal, gender-associated differences in these currents may be compensatory for determining APD at low stimulation rates.

4.2. Reduced repolarization reserve in female murine ventricular myocytes

Baseline APD were prolonged and RR [17] was markedly less in females than in males in response to FK506 (Table 1; Fig. 1). FK506 specifically and equally inhibited $I_{\text{us}}$ in males and females under our experimental conditions (Fig. 4), but FK506 increased APD significantly more in myocytes from females than males (Table 1; Fig. 1). The relatively greater increase in APD in female mice, following exposure to FK506, supports the hypothesis that $I_{\text{us}}$ was more important than $I_{\text{to}}$ for determining APD and RR in female mice.

4.3. The mechanism of FK506 action on $I_{\text{us}}$

Recent studies have suggested that FK506 modulates $I_{K}$ through a novel signaling pathway in cardiac ventricular myocytes [10,16]. While the mechanism of FK506 actions on $I_{K}$ are not fully understood, they are independent of calcineurin activity [10,16]. One possibility is that FK506...
Fig. 4. FK506 selectively and equally inhibits $I_{\text{ss}}$ in ventricular myocytes from female and male mice. Panels A and B show a family of K+ currents in response to the voltage commands (Fig. 2) before (A) and after (B) addition of FK506 (25 μM). Panels C and D show summary data for $I_{\text{ss}}$ recorded from ventricular myocytes obtained from male (C) and female (D) mice. The magnitude of $I_{\text{ss}}$ is not changed by FK506 in either females or males. Panels E and F show summary data for $I_{\text{ss}}$ recorded from the same cells as were used in panels C and D. FK506 significantly and equally inhibited $I_{\text{ss}}$ recorded from male (E) and female (F) ventricular myocytes. * $P<0.05$ and ** $P<0.005$.

exerts $I_{\text{K}}$ antagonist actions through a FK binding protein (FKBP)-dependent mechanism. Such a mechanism is suggested by the finding that rapamycin, another agent that binds FKBP but has no activity against calcineurin, also is an $I_{\text{K}}$ antagonist [10]. A viable alternative explanation is that FK506 directly inhibits $I_{\text{ss}}$, independent of FKBP.

4.4. Mice as models in cardiovascular research

Wild type and genetically modified mice are increasing-ly important models for cardiac electrophysiology, but previous studies have not examined gender as a possible determinant of murine cardiac repolarization. It will now be important to extend the present findings to other mouse strains and to examine the possible effects of age and hormonal status [5] on cardiac repolarization and RR. Recent work has highlighted differences in populations of repolarizing K+ currents in myocytes derived from the left ventricular septum, apex, and free wall [8], but the potential effect of gender on these cell populations is
unknown. Perhaps the most important question is how APD prolongation causes ventricular arrhythmias. Reduced RR in women following \( K_r^- \) antagonists may account for the markedly increased incidence of sudden death in women in response to these drugs [18,19,7]. Studies in rabbits [14,15] support findings from quantitative AP models [22,23] that \( Ca^{2+} \)-dependent currents and signaling molecules activated by APD prolongation cause arrhythmia-initiating afterdepolarizations. Preliminary findings suggest that similar mechanisms for arrhythmias related to excessive APD are also present in mice [24,25]. Thus, it will be important to extend the present experimental findings to examine potential gender-dependence of other ionic currents and signaling molecules and to develop quantitative models of murine cardiac electrophysiology and electropharmacology.

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