Sex-based transmural differences in cardiac repolarization and ionic-current properties in canine left ventricles

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Xiao, Ling, Liming Zhang, Wei Han, Zhiguo Wang, and Stanley Nattel. Sex-based transmural differences in cardiac repolarization and ionic-current properties in canine left ventricles. Am J Physiol Heart Circ Physiol 291: H570–H580, 2006. First published February 24, 2006; doi:10.1152/ajpheart.01288.2005. —The female sex is associated with duration of QT intervals and increased proarrhythmic risks of QT-prolonging drugs. This study examined the hypothesis that sex differences in repolarization may be associated with differential transmural ion-current distribution. Whole cell patch-clamp and current-clamp were used to study ion currents and action potentials (APs) in isolated canine left ventricular cells from epicardium, midmyocardium, and endocardium. No sex differences in AP duration (APD) were found in cells from epicardium versus endocardium. In midmyocardium, APD was significantly longer in female dogs (e.g., at 1 Hz, female vs. male: 288 ± 21 vs. 237 ± 8 ms; P < 0.05), resulting in greater transmural APD heterogeneity in females. No sex differences in inward rectifier K+ current (I_K1) were observed. Transient outward K+ current (I_o) densities in epicardium and midmyocardium also showed no sex differences. In endocardium, female dogs had significantly smaller I_o (e.g., at +40 mV, female vs. male: 2.5 ± 0.2 vs. 3.5 ± 0.3 pA/pF; P < 0.05). Rapid delayed-rectifier K+ current (I_Kr) density and activation voltage-dependence showed no sex differences. Female dogs had significantly larger slow delayed-rectifier K+ current (I_Ks) in epicardium and endocardium (e.g., at +40 mV; tail densities, female vs. male: epicardium: 1.3 ± 0.1 vs. 0.8 ± 0.1 pA/pF; P < 0.001; endocardium: 1.2 ± 0.1 vs. 0.7 ± 0.1 pA/pF; P < 0.05), but there were no sex differences in midmyocardial I_Ks. Female dogs had larger L-type Ca2+ current (I_{Ca,L}) densities in all layers than male dogs (e.g., at −20 mV, female vs. male, epicardium: −4.2 ± 0.4 vs. −3.2 ± 0.2 pA/pF; midmyocardium: −4.5 ± 0.5 vs. −3.3 ± 0.3 pA/pF; endocardium: −4.5 ± 0.4 vs. −3.2 ± 0.3 pA/pF; P < 0.05 for each). We conclude that there are sex-based transmural differences in ionic currents that may underlie sex differences in transmural cardiac repolarization.

Methods

Cell preparation. All animal care and handling procedures were approved by the animal research ethics committee of the Montreal Heart Institute and followed the Guidelines of the Canadian Council for Animal Care. Adult mongrel dogs of both sexes [female, 26.4 ± 5.2 kg, n = 67; and male, 26.9 ± 5.9 kg, n = 68; P = not significant (NS)] were anesthetized with pentobarbital sodium (30 mg/kg iv) and ventilated with room air. A left lateral thoracotomy was performed, and hearts were quickly excised and immersed in oxygenated Tyrode solution at room temperature. The transmural free wall (∼3 × 5 cm) of the lateral left ventricle, which was irrigated by a coronary artery branching from the left circumflex coronary artery, was quickly dissected and the artery was cannulated. Cell isolation was performed as previously described (38) by perfusion with a solution containing collagenase (120–150 U/ml, Worthington type II). When the tissue was well digested, small tissue blocks were removed from the epicardial surface (1–1.5 mm thick) and midmyocardial layer (2–5 mm thick) and endocardial surface (1–1.5 mm thick). Cells were dispersed by gentle trituration with a Pasteur pipette and were kept in a high-K+ storage solution (see Solutions) at 4°C.

Solutions. The standard Tyrode solution contained (in mM) 136 NaCl, 5.4 KCl, 1 MgCl2, 1 CaCl2, 0.33 NaH2PO4, 5 HEPES, and 10 dextrose (pH 7.35 with NaOH). The high-K+ storage solution contained (in mM) 20 KCl, 10 KH2PO4, 10 dextrose, 40 mannitol, 70

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L-glutamic acid, 10 β-OH-butyric acid, 20 taurine, and 10 EGTA and 0.1% BSA (pH 7.3 with KOH). The standard pipette solution used in most experiments contained (in mM) 110 K-aspartate, 20 KCl, 1 MgCl₂, 5 MgATP, 0.1 GTP, 10 HEPES, 5 Na-phosphocreatine, and 5 EGTA (for current recording) or 0.025 (for AP recording), with pH adjusted to 7.3 with KOH.

For AP recordings, external solutions contained 2 mM CaCl₂. For K⁺/H⁺-current recordings, atropine (1 μM) and CdCl₂ (200 μM) or nimodipine (5 μM, for I_K) was added to external solutions to eliminate muscarinic K⁺ currents and to block Ca²⁺ currents. Na⁺ current contamination was avoided by using a holding potential of −50 mV or by substitution of equimolar Tris-HCl for external NaCl. For currents other than I_to, 1 mM 4-aminopyridine was used to block I_to.

I_K was studied as 1 mM Ba²⁺-sensitive current. For studies of I_Kr and slow delayed-rectifier K⁺ current (I_Ks), chromanol 293B (50 μM) was added to record I_Kr (6), and E-4031 (5 μM) was used to record I_Ks, after verification that chromanol 293B-resistant tail current was strongly blocked by E-4031 and that E-4031-resistant current was blocked by 50 μM chromanol 293B.

For I_Ca,L studies, the external solution contained (in mM) 136 tetraethylammonium chloride, 5.4 CsCl, 2 CaCl₂, 0.8 MgCl₂, 10 HEPES, and 10 dextrose (pH 7.4 with CsOH). Niflumic acid (50 μM) was added to inhibit Ca²⁺-dependent Cl⁻ current (I_{Cl,Ca}). The pipette solution contained (in mM) 20 CsCl, 110 Cs-aspartate, 1 MgCl₂, 5 MgATP, 0.1 GTP, 5 Na₂ phosphocreatine, 10 EGTA, and 10 HEPES (pH 7.2 with CsOH).

Data acquisition and analysis. The whole cell patch-clamp technique was applied to record ionic currents and APs at 36°C, as previously described in detail (37, 38). Ionic currents were recorded in the voltage-clamp mode, and APs were recorded in current-clamp mode. Compensated series resistance and capacitive time constant (τ) values averaged 2.3 ± 0.1 MΩ and 294 ± 10 μs. Junction potentials (~10 mV) were corrected for AP recordings only. Leakage compensation was not used. Cell capacitance averaged as follows: epicardium, 118.1 ± 3.4 pF in female (n = 87) and 126.4 ± 3.9 pF in male (n = 86) cells, P = NS; midmyocardium, 123.2 ± 3.1 in female (n = 88) and 131.6 ± 3.2 pF in male (n = 93) cells, P = NS; and endocardium, 113 ± 2.9 in female (n = 77) and 116.7 ± 3.4 pF in male cells (n = 76), P = NS. Currents are expressed in terms of density (normalized to capacitance).

To obtain QT-interval data, 14 female dogs (weight, 25 ± 5 kg) and 14 male dogs (weight, 26 ± 4 kg) were studied in vivo. On study...
days, dogs were anesthetized with acepromazine (0.07 mg/kg iv), ketamine (5.3 mg/kg iv), Valium (6.25 mg/kg iv), and isoflurane (2%) and were mechanically ventilated with room air. Radiofrequency ablation of the atrioventricular node was performed to study the QT interval over a range of controlled basic cycle lengths (BCLs). Body temperature was maintained at 37°C. A median sternotomy was performed, and a bipolar Teflon-coated stainless steel electrode was hooked into the right ventricular free wall for stimulation. A programmable stimulator was used to deliver 2 ms twice-threshold pulses, and surface ECGs were recorded at BCLs of 300, 400, 600, and 1,000 ms. QT interval was measured from lead 2, and the mean of three QT interval measurements at each BCL for each dog was used for analysis.

Nonlinear least-square curve-fitting algorithms were performed for curve fitting. Unpaired Student’s t-tests were used for comparisons between female and male groups. P < 0.05 was taken to indicate statistical significance, and group data are expressed as means ± SE.

RESULTS

APs. Examples of APs recorded in isolated cells from various layers of female and male canine left ventricles (at 1 Hz, and 0.5 Hz) are shown in Fig. 1, A–C. In both female and male left ventricular cardiomyocytes, APs showed a prominent phase 1 and “spike-and-dome” configuration, with an intervening notch, in epicardial (Fig. 1A) and midmyocardial (Fig. 1B) cells. Endocardial cells showed more limited phase 1 repolarization and virtually no notch (Fig. 1C). APD was consistently longer in midmyocardial cells than in epicardial and endocardial cells. This pattern of transmural AP heterogeneity is consistent with the results of previous studies (2, 27).

APD was similar for female and male canine left ventricular cardiomyocytes in both epicardium and endocardium (1 Hz, Fig. 1D). In midmyocardium, female dogs had significantly longer APDs compared with male dogs (Fig. 1D). For example, at 1 Hz, APD to 90% repolarization averaged 292 ± 17 ms in females (n = 11) versus 235 ± 7 ms in males (n = 21, P < 0.05), whereas APD to 50% repolarization was 249 ± 19 ms in females (n = 11) versus 186 ± 6 ms in males (n = 21, P < 0.05). In agreement with the APD data, QT interval at matched cycle lengths was significantly larger in female versus male dogs (Fig. 1E, n = 14, for male and female dogs, respectively).

No sex differences were observed in resting membrane potentials. Resting potentials averaged −78.4 ± 0.7 mV (female, n = 20) versus −79.9 ± 0.7 mV (male, n = 19) in epicardium, −80.0 ± 0.6 mV (female, n = 15) versus −81.3 ± 0.6 mV (male, n = 21) in midmyocardium, and −79.8 ± 0.8 mV (female, n = 28) versus −81.2 ± 0.6 mV (male, n = 20) in endocardium (P = NS for all male/female comparisons).

IK1. IK1 was studied as 1 mM Ba2+-sensitive current. Figure 2A shows representative IK1 recordings from female and male epicardial cells. IK1 density was similar between female and male dogs for epicardium, midmyocardium, and endocardium as shown in Fig. 2, B–D, respectively.

Iio. A typical transmural gradient in Iio was present in both female and male left ventricles (Fig. 3, A, C, and E). Mean Iio density was similar for epicardial and midmyocardial cells in female and male dogs (Fig. 3, B and D). In endocardial cells, mean Iio density was significantly larger in male dogs than in female dogs (Fig. 3F), with mean current density at +30 mV, averaging 2.5 ± 0.2 pA/pF (female, n = 24 cells) and 3.5 ± 0.3 pA/pF (male, n = 28 cells, P < 0.05). No sex differences in the form of the current-voltage (Iio-V) relations were found for all transmural levels.
The voltage dependence of $I_{\text{Kr}}$ inactivation was studied with a two-pulse protocol, providing the results illustrated in Fig. 4, A, D, and G. Voltage for 50% activation and inactivation ($V_{1/2}$) and slope factors were not significantly different between cells from males versus females for any myocardial layer (Table 1). $I_{\text{Kr}}$ inactivation kinetics were well fitted by biexponential relations, and inactivation time constants were similar between female and male left ventricular cells (Fig. 4, B, E, and H). $I_{\text{Kr}}$ reactivation was assessed with the two-pulse protocol shown in Fig. 4C. Reactivation kinetics were well fitted by biexponential relations (Fig. 4, C, F, and I), with no apparent dependency from sex difference. A detailed presentation of $I_{\text{Kr}}$ recovery kinetics for each layer is shown in Table 1 and indicates no significant between-sex differences. $I_{\text{Kr}}$ frequency dependence, as determined by steady-state current at 0.1, 0.5, 1, 2, and 5 Hz upon 100-ms pulses from −80 to +50 mV, also showed no sex differences within any regions of the left ventricle (data not shown).

$I_{\text{Ks}}$. Figure 5A shows representative recordings of chromanol 293B (50 μM)-resistant $I_{\text{Ks}}$ in epicardial cells from female and male dogs. $I_{\text{Ks}}$ activated with half-activation voltages of −1.9 (male) and +2.0 mV (female, $n = 8$ cells/group, $P = \text{NS}$), based on tail currents at −40 mV after 4-s depolarizations to various test voltages. There were no significant sex differences in $I_{\text{Ks}}$ tail-current density (Fig. 5, C, D, and E) and activation voltage dependencies in any regions. The kinetics of $I_{\text{Ks}}$ showed no differences between male and female dogs. Activation was well-fitted by biexponential kinetics. Detailed activation time-constant data upon depolarization to +40 mV are provided in Table 2 and indicate no significant sex differences for any layer. Deactivation was similarly biexponential, and detailed results obtained on repolarizing from +40 to −40 mV (Table 2) show no significant sex-based differences.

Representative recordings of E-4031 (5 μM)-resistant $I_{\text{Ks}}$ from epicardial cells of female and male dogs are shown in Fig. 6A. $I_{\text{Ks}}$ activation voltage dependence was assessed by normalizing tail-current amplitudes (obtained with the pulse protocol shown in Fig. 6A) to tail-current amplitude after depolarization to +70 mV. There were no differences in activation voltage dependence between female and male dogs in any regions, as illustrated by the mean data for epicardial cells shown in Fig. 6B. A transmural $I_{\text{Ks}}$ gradient has been implicated in the important and well-known transmural APD gradient (2, 27). $I_{\text{Ks}}$ showed a greater transmural density gradient in female than in male dogs (Fig. 6, C, D, and E). The $I_{\text{Ks}}$ density was larger in female than in male dog cells in both epicardium (Fig. 6C) and endocardium (Fig. 6D), e.g., at +40 mV, female vs. male $I_{\text{Ks}}$ tail-current density averaged as follows: epicardium, 1.3 ± 0.1 vs. 0.8 ± 0.1 pA/pF, $n = 25$ (female) and 20 (male) cells, $P < 0.001$; and endocardium, 1.1 ± 0.2 vs. 0.7 ± 0.1 pA/pF; $n = 16$ cells/group, $P < 0.05$. By contrast, $I_{\text{Ks}}$ density in midmyocardial cells was comparable for both female (0.5 ± 0.05 pA/pF, $n = 21$ cells) and male (0.4 ± 0.02 pA/pF, $n = 24$, $P = \text{NS}$) cells. As in the case of $I_{\text{Kr}}$, the kinetics of $I_{\text{Ks}}$ were similar for male and female dogs. $I_{\text{Ks}}$ activation was monoeXponential and showed no sex-dependent differences (Table 3).
Fig. 4. \(I_{\text{to}}\) voltage dependence and kinetics. A (Endo), D (Mid), and G (Epi): \(I_{\text{to}}\) inactivation and activation voltage dependence. Inactivation was evaluated with 1,000-ms prepulses followed by 200-ms test pulse to +50 mV (at 0.1 Hz). Activation voltage dependence was analyzed from data obtained with protocol in Fig. 3 (see equation in RESULTS, \(I_{\text{Ca,L}}\)). Data are means ± SE (\(n = 6\) cells/group, and inactivation; \(n = 6\) cells/group, activation); curves are best-fit Boltzmann relations. B (Endo), E (Mid), and H (Epi): data are means ± SE inactivation \(t\) values (\(n = 10\) cells/group). C (Endo), F (Mid), and I (Epi): \(I_{\text{to}}\) reactivation time course evaluated by ratio of current (\(I_2\)) during a 100-ms test pulse (P2, identical to P1) to current (\(I_1\)) during a conditioning pulse (P1) with varying P1-to-P2 interval [holding potential (HP) = −80 mV, step to +50 mV at 0.07 Hz]. Data are means ± SE (\(n = 6\) cells/group); curves are biexponential fits.

Table 1. \(I_{\text{to}}\) voltage dependence and kinetics

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<th>Inactivation</th>
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<th>Activation</th>
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<th>Recovery</th>
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<td></td>
<td>(V_{1/2}), mV</td>
<td>Slope, mV</td>
<td>(n)</td>
<td>(V_{1/2}), mV</td>
<td>Slope, mV</td>
<td>(n)</td>
<td>(\tau_{\text{fast}}, \text{ms})</td>
<td>(\tau_{\text{slow}}, \text{ms})</td>
<td>(n)</td>
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<td><strong>Epi</strong></td>
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<tr>
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<td>6</td>
<td>8.2±2.0</td>
<td>12.6±0.7</td>
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<td>12.7±5.0</td>
<td>103±17.0</td>
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<tr>
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<td>6</td>
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<td>88.0±13.8</td>
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<td><strong>Mid</strong></td>
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<tr>
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<td><strong>Endo</strong></td>
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<tr>
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Values are means ± SE; \(n\), number of dogs. \(I_{\text{to}}\), transient outward K⁺ current; \(V_{1/2}\), voltage for 50% activation or inactivation; \(\tau\), time constant; Epi, epicardium; Mid, midmyocardium; Endo, endocardium. There were no statistically significant differences between male and female results for corresponding regions.
Upon repolarization from $+40$ to $-30$ mV, deactivation was biexponential, and once again there were no significant sex differences (Table 3).

$I_{\text{Ca},L}$. Examples of representative $I_{\text{Ca},L}$ recordings from epicardial cells are shown in Fig. 7, A (female) and B (male). No transmural $I_{\text{Ca},L}$ density gradient was observed in either female or male left ventricles. Female dogs had significantly larger $I_{\text{Ca},L}$ density than male dogs for each transmural level (Fig. 7, C, D, and E). For example, at $+20$ mV, in epicardium, $I_{\text{Ca},L}$ density averaged $4.2 \pm 0.4$ in females and $3.2 \pm 0.2$ pA/pF in male cells, respectively ($P < 0.05, n = 15$ cells/group); in midmyocardium, mean $I_{\text{Ca},L}$ density was $4.5 \pm 0.5$ in females and $3.3 \pm 0.3$ pA/pF in males ($P < 0.05, n = 15$ female), 18 (male) cells; and in endocardium, current density was $4.5 \pm 0.4$ in females and $3.2 \pm 0.3$ pA/pF in males ($P < 0.05, n = 16$ female) or 8 (male) cells/group. The normalized $I_{\text{Ca},L}$ I-V relations were similar for female and male results in all regions (Fig. 7, D, F, and H).

$I_{\text{Ca},L}$ inactivation was fitted by monoexponential relations and showed no sex differences, as illustrated by the mean data for midmyocardium shown in Fig. 8A. The voltage dependence of $I_{\text{Ca},L}$ activation and inactivation was evaluated as illustrated in Fig. 8B and showed no sex-dependent differences. Activation voltage dependence was assessed according to the relation

$$I_{\text{TP}} \cdot a_{\text{TP}} \cdot G_{\text{max}}(V_{\text{TP}}) - V_{R},$$

where $I_{\text{TP}}$ is current at given test potential; $a_{\text{TP}}$ is activation variable at test potential; $G_{\text{max}}$ is maximum conductance; $V_{\text{TP}}$ is voltage of test potential; and $V_{R}$ is reversal potential, which was obtained from a linear fit to the ascending portion of the I-V relation. There were no significant sex-based differences in mean activation or inactivation $V_{1/2}$ values or slope factors in any layer (Table 4). Figure 8C shows $I_{\text{Ca},L}$ reactivation kinetics at a holding potential of $-80$ mV in

Table 2. $I_{\text{Kr}}$ kinetics

<table>
<thead>
<tr>
<th>Region</th>
<th>$\tau_{\text{act}}$ (mS)</th>
<th>$\tau_{\text{decr}}$ (mS)</th>
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<tbody>
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<td>Epi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>81±23</td>
<td>1,143±199</td>
<td>7</td>
</tr>
<tr>
<td>Male</td>
<td>81±34</td>
<td>921±296</td>
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<td>129±58</td>
<td>2,121±755</td>
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<td>109±50</td>
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<td>Male</td>
<td>112±37</td>
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<td>Female</td>
<td>109±38</td>
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<tr>
<td></td>
<td>105±37</td>
<td>758±313</td>
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</table>

Values are means ± SE; $n$, number of dogs. $I_{\text{Kr}}$, rapid delayed-rectifier K⁺ current. There were no statistically significant differences between male and female results for corresponding regions.

Fig. 5. A: representative chromanol 293B (50 μM)-resistant $I_{\text{Kr}}$ recordings in female (left) and male (right) Epi cells. B: means ± SE normalized $I_{\text{Kr}}$ tail currents ($n = 8$ cells/group from Epi) and best-fit Boltzmann relations. C, D, and E: data are means ± SE $I_{\text{Kr}}$ density-voltage relations in female and male Epi (C), Mid (D), and Endo (E) cells.

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midmyocardium. Reactivation time constants were similar between cells from female and male dogs in all layers (Table 4). Similarly, no sex differences in $I_{Ca,L}$ frequency dependence were found. Figure 8D shows mean data for midmyocardium.

Similar results were obtained for epicardium and endocardium.

**DISCUSSION**

In the present study, we analyzed in detail transmural ionic current function in left ventricular cardiomyocytes from male versus female dogs. We found male/female differences in three ionic-current systems: $I_{to}$, $I_{Ks}$, and $I_{Ca,L}$. For $I_{to}$ and $I_{Ks}$, the male/female differences varied transmurally, in a way that may have functional significance. In addition, we observed statistically significant longer APDs in females for M cells only, increasing transmural APD heterogeneity.

**Relation to previous findings regarding sex-related ionic current differences in the literature.**

A variety of differences in ionic current properties have been described between male and female animals, and there are numerous discrepancies in the literature, possibly related to interspecies and interstrain differences. Trepanier-Boulay et al. (34) showed similar $I_{to}$ and reduced $I_{Kur}$ in female mice, whereas Wu and Anderson (36) reported that female mice have smaller $I_{to}$ and larger sustained depolarization-induced outward current, which has a major contribution from $I_{Kur}$. In contrast to Trepanier-Boulay et al. (34), who observed longer APDs and smaller $I_{to}$ in female mice, Brunet et al. (8) could not identify sex differences in $K_{11001}$ currents and ventricular repolarization in mice. Previous studies (20) on rabbit hearts pointed to smaller $I_{Kr}$ in female rabbits, associated with longer QT intervals. We found no sex differences in $I_{Ks}$, at any level of the canine left ventricle. Liu et al. (20) also reported smaller outward, but not inward, $I_{K1}$ in female rabbits, whereas James et al. (14) observed smaller inward, but not outward, $I_{K1}$ in female guinea pigs. We did not observe any sex-dependent $I_{K1}$ differences in dogs. Unlike our observation of larger $I_{Ca,L}$ in females, Trepanier-Boulay et al. (34) did not note an $I_{Ca,L}$ difference between male and female mice. We were unable to identify previous studies of sex-dependent differences in $I_{Ks}$.

**Potential relevance to sex differences in electrophysiology.**

We noted rather complex sex-based differences in ionic currents across the canine ventricular wall. A smaller $I_{to}$ was found in females only in the endocardial layer. Females had
larger $I_{Ca,L}$ across the ventricular wall, but $I_{Ks}$ was larger in females only in the epicardium and endocardium but not midmyocardium. These differences would be expected to cause transmurally based differences in APD, which we observed. The absence of significant male/female APD differences in the endocardium and epicardium could be due to offsetting differences in inward $I_{Ca,L}$ and outward $I_{Ks}$. The larger female $I_{Ca,L}$ in the face of similar $I_{Ks}$ in the midmyocardium could account for the larger midmyocardial APD in females. The larger APD observed in female midmyocardium relative to male, in the face of similar endocardial and epicardial APD, increased the transmural dispersion of repolarization in females versus males. Despite smaller $I_{to}$ in female endocardium, we did not observe any associated APD differences. The lack of appreciable impact of the endocardial male/female $I_{to}$ difference in overall APD may have been due to the fact that $I_{to}$ flows primarily during the very early phases of the AP and inactivates well before the onset of phase 3. Variations in $I_{to}$ over the range that we observed (3–6 pA/pF) had no effect on canine endocardial APD in a recently published study (32) using an elegant dynamic clamp technique.

The presence of significant sex-based differences in cardiac repolarization is well recognized. The QT interval is longer in women (4, 24), and women clearly have an increased sensitivity to drug-induced QT-interval prolongation and TdP (5, 11, 16, 17, 22). The basis for these differences remains poorly understood. The present results point to male/female differences in transmural ion-channel function. The transmural distribution of cardiac ion channels is complex, and this complex distribution plays a key role in cardiac electrophysiology (1, 2, 27, 30). There is evidence for greater repolarization heterogeneity in women compared with men (31). Pham et al. (25)
observed greater transmural repolarization heterogeneity in female dogs upon exposure to $I_{Ks}$ blockers. Despite the importance of transmural ion-channel function in repolarization heterogeneity and arrhythmias, we were unable to find detailed studies of transmural ion-current properties in female compared with male subjects. Pham et al. (23) found somewhat larger $I_{Ca}$ densities in the epicardium of female versus male rabbits, in accordance with our results, but no substantial endocardial differences. Midmyocardial cells were not studied, nor were other ionic currents. Greater midmyocardial APD has been attributed to a variety of factors, including smaller $I_{Ks}$ density in the midmyocardium (1, 12, 18). Consistent with this notion, we observed smaller and similar $I_{Ks}$ in midmyocardial versus endocardial or epicardial cells for both male and female dogs. Because $I_{Ks}$ was larger in female than male dogs in the epicardium and endocardium, female dogs had a larger transmural $I_{Ks}$ gradient compared with male dogs, potentially contributing to longer midmyocardial APDs and a larger repolarization gradient in females. These in turn may contribute to increased QT intervals and greater risks of TdP.

Sex differences in currents governing transmural repolarization might be expected to produce differences in QT interval and T-wave morphology, as well as in arrhythmia susceptibility. Women do have longer corrected QT intervals than men (4, 24). Young men have larger T-wave offset dispersion than young and old women, whereas women have greater T-wave complexity after exercise and with autonomic blockade (33). Susceptibility to drug-induced TdP is clearly greater in women than in men (11, 22). Women might be less prone to reentrant arrhythmias for which M-cell repolarization is limiting because of longer APDs; however, the enhanced transmural repolarization gradient we observed could promote reentry in women by favoring the establishment of unidirectional block at the M-cell border. This complex area clearly requires further study and analysis.

The principal arrhythmic risk known to be enhanced in women is drug-induced long QT syndrome, almost uniformly by $I_{Ks}$ blocking drugs. Our data suggest reduced repolarization reserve in the M-cell layer, because unlike the other two transmural layers, the larger $I_{Ca,L}$ in females was not offset by larger $I_{Ks}$. The notion of repolarization reserve implies an ability of the heart to minimize the effects of agents impairing repolarization, in particular $I_{Ks}$ blocking drugs, by enhancing outward current carried by other channels, in particular $I_{Ca,L}$.

Table 4. $I_{Ca,L}$ voltage dependence and kinetics

<table>
<thead>
<tr>
<th>region</th>
<th>$V_{1/2} \text{mV}$</th>
<th>Slope $\text{mV}$</th>
<th>$n$</th>
<th>$V_{1/2} \text{mV}$</th>
<th>Slope $\text{mV}$</th>
<th>$n$</th>
<th>$\tau \text{ms}$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>$-40.5 \pm 0.9$</td>
<td>$-5.8 \pm 0.3$</td>
<td>7</td>
<td>$-11.4 \pm 1.5$</td>
<td>$3.4 \pm 0.4$</td>
<td>5</td>
<td>69.7 $\pm 6.8$</td>
<td>5</td>
</tr>
<tr>
<td>Male</td>
<td>$-41.5 \pm 0.8$</td>
<td>$-6.2 \pm 0.2$</td>
<td>7</td>
<td>$-16.5 \pm 1.5$</td>
<td>$2.5 \pm 0.9$</td>
<td>5</td>
<td>85.4 $\pm 6.5$</td>
<td>6</td>
</tr>
<tr>
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<td></td>
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<td></td>
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<td></td>
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<tr>
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<td>$-41.4 \pm 0.6$</td>
<td>$-7.1 \pm 0.6$</td>
<td>6</td>
<td>$7.0 \pm 0.6$</td>
<td>$6.0 \pm 0.5$</td>
<td>6</td>
<td>54.4 $\pm 5.4$</td>
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<tr>
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<td>6</td>
<td>$5.4 \pm 0.9$</td>
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<td>53.7 $\pm 5.1$</td>
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<tr>
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<td>68.3 $\pm 3.9$</td>
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<tr>
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<td>$6.8 \pm 0.8$</td>
<td>6</td>
<td>73.2 $\pm 2.5$</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; $n$, number of dogs. $I_{Ca,L}$, L-type Ca$^{2+}$ channel current. There were no statistically significant differences between male and female results for corresponding regions.

Fig. 8. $I_{Ca,L}$ kinetics and voltage dependence from Mid (means $\pm$ SE). Similar results were obtained in all regions. A: data are means $\pm$ SE; $I_{Ca,L}$ inactivation time constant ($n = 10$ cells/group), obtained with the protocol in Fig. 7A. B: voltage dependence of $I_{Ca,L}$ inactivation and activation. Steady-state inactivation was assessed with 1,000-ms conditioning pulses, followed by a 300-ms test pulse to $+10 \text{mV}$ (0.1 Hz). Activation was assessed from data obtained with the protocol in Fig. 7A (see equation in RESULTS, $I_{Ca,L}$). Data are means $\pm$ SE ($n = 6$ cells/group); curves are best-fit Boltzmann relations. C: $I_{Ca,L}$ reactivation time course, studied with paired 100-ms pulses delivered with varying interpulse intervals at 0.1 Hz. Curves are monoeponential fits ($n = 6$ cells/group). D: $I_{Ca,L}$ frequency dependence, determined from the ratio of current during the 15th pulse to current during the first pulse of a train of 100-ms depolarizations from $-80 \text{mV}$ to $+10 \text{mV}$ at frequencies indicated ($n = 6$ cells/group).
(28). When repolarization reserve is reduced, as in female M cells in the present study, the effect of IKr blockers would be expected to be enhanced, leading to excessive delay and destabilization of M-cell repolarization and potentially early afterdepolarizations, transmural reentry, and TdP.

Potential limitations. We performed detailed studies of a wide range of K⁺ currents and of L-type Ca²⁺ currents at three transmural levels of male and female canine myocardium. This is, to our knowledge, a much more broad and detailed comparison than in previous comparisons between male and female cardiois, to our knowledge, a much more broad and detailed comparison transmural levels of male and female canine myocardium. This study was supported by the Canadian Institutes for Health Research, and the Mathematics of Information and Chantal St.-Cyr for technical assistance and France The´riault for secre-

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